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Interference of Niagara Blue 2B with the Teratogenic Action of Trypan Blue.* (27315)

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The fact that the disazo dye trypan blue is a potent teratogen to the developing mammalian embryo is now well documented(1). Available evidence suggests a causal relationship between altered physiological states of the mother and embryo and the teratogenicity of trypan blue. It has been clearly demonstrated that injection of trypan blue results in an alteration in the electrophoretic pattern of the maternal serum proteins and the proteins of the yolk sac fluid(2,3,4). It now is of interest to learn whether or not it is possible to interfere with this teratogenic action. Assuming that trypan blue (or a metabolic by-product) reacts at a specific site(s), it is possible that a non-teratogenic substance of similar structure might also react at these sites and thereby interfere with the action of trypan blue. The non-teratogenic disazo dye Niagara blue 2B was selected to test this hypothesis because it differs structurally from trypan blue only in the absence of 2 methyl groups and because it apparently has the same fate following injection; appearing in cells of the maternal macrophage system, kidney tubules, and visceral epithelial cells of the yolk sac placenta(5).

Materials and methods. All experiments were carried out using the same sample of specially purified Niagara blue 2B and trypan blue.‡ The dyes were prepared as 2% solutions in distilled water and injected intraperitoneally into Sherman rats on the 6th and 7th, or 8th day of gestation, considering the morning on which sperm was found in the vaginal smear as day zero. Fetuses were recovered on the 20th day, weighed, examined for gross malformations, and fixed in Bouin's fluid for subsequent free-hand serial sectioning of the head. The trunk was not sectioned.

Results. Table I illustrates the results obtained when equivalent amounts of Niagara blue 2B and trypan blue were injected. At the highest dose used there was no protective or sparing effect. However, at each lower concentration the injection of Niagara blue 2B prior to injection of trypan blue afforded some measure of protection to the embryo with respect to number of resorptions and malformations produced.

Table II indicates the results obtained after varying the relative amounts of the 2 dyes administered. In each group the prior injection of Niagara blue 2B reduced the number of resorptions and malformed survivors. On the other hand, when trypan blue was injected prior to Niagara blue 2B there was no significant reduction in its teratogenic activity. These figures also suggest a direct correlation between number of implantation sites affected and amount of trypan blue administered, irrespective of the amount of Niagara blue 2B administered.

Discussion. These results indicate that Niagara blue 2B can effectively interfere with the teratogenic and lethal action of trypan blue in Sherman rats, over a selected range in dye concentration. This interference is

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‡ Obtained from Matheson, Coleman and Bell Co., Norwood, Ohio.

seen when Niagara blue 2B is injected prior to trypan blue. This may mean that a competition exists between the 2 dyes at the reaction site.

A possible explanation of the action of trypan blue has been set forth by Langman and van Drunen(2). They were able to show, in the rabbit, disturbances in the normal proportion of the maternal serum proteins following administration of this dye. The disturbance was characterized by an increase in total proteins, albumin and alpha and beta globulin over their normal values, during the first 10 days of pregnancy. This alteration occurred during a time critical to embryogenesis. These observations have been extended, in the rabbit, to include the yolk sac fluid(3,4). In addition, Yamada(6) observed an abnor-

TABLE I. Effect of Injecting a 2% Solution of Niagara Blue 2B Intraperitoneally into Female Rats on the 6th and 7th Days of Pregnancy Followed by an Equivalent Amount of a 2% Solution of Trypan Blue on the 8th Day.

Treatment	No. mothers	No. implantation sites	% resorbed	% survivors malformed	% sites resorbed and/or malformed
20 mg per 100 g rat					
Trypan blue control	11	122	70	62	89
Niagara blue 2B control	8	85	16	0	16
" " " followed by Trypan blue (20 mg of each)	10	105	87	50	98
14 mg per 100 g rat					
Trypan blue control	19	181	57	54	80
Niagara blue 2B control	16	165	24	0	24
" " " followed by Trypan blue (14 mg of each)	25	248	33	26	50
10 mg per 100 g rat					
Trypan blue control	16	156	44	36	64
Niagara blue 2B control	15	145	7	0	7
" " " followed by Trypan blue (10 mg of each)	25	272	32	15	42

TABLE II. Effect of Intraperitoneal Injections of Varying Proportions of Niagara Blue 2B (Days 6 & 7) and Trypan Blue (Day 8) into Pregnant Rats.

Treatment	No. mothers	No. implantation sites	% resorbed		% survivors malformed		% sites resorbed and/or malformed	
			Actual	Expec.*	Actual	Expec.*	Actual	Expec.*
Niagara blue 2B, 7 mg/100 g followed by trypan blue, 7 mg/100 g	12	127	25	43	13	37	34	64
Niagara blue 2B, 14 mg/100 g followed by trypan blue, 7 mg/100 g	11	121	29	"	12	"	37	"
Niagara blue 2B, 7 mg/100 g followed by trypan blue, 14 mg/100 g	12	140	41	57	22	54	54	80
Niagara blue 2B, 14 mg/100 g followed by trypan blue, 14 mg/100 g	25	248	33	"	26	"	51	"
Trypan blue, 14 mg/100 g followed by Niagara blue 2B, 14 mg/100 g†	6	77	58	"	46	"	72	"

* Expec. = Expected following treatment with indicated dose of trypan blue alone.
† Note trypan blue inj. prior to Niagara blue 2B.

mal protein present between the alpha 1 and alpha 2 globulins in the serum electrophoretic pattern of male rats following treatment with trypan blue. It is plausible that a causal relationship exists between the alteration of proteins in the maternal serum and yolk sac fluid and embryonic malformations.

Other observations have shown that trypan blue can compete for protein binding sites in plasma(7). Therefore, it would not be unreasonable to presume that Niagara blue 2B could act in a similar manner, and thus compete with trypan blue for a protein binding site. If this is true, it could explain the interference of Niagara blue 2B with the lethal and teratogenic activity of trypan blue as described here.

Summary. The results demonstrate that the non-teratogenic disazo dye, Niagara blue 2B, can afford some measure of protection to the embryo against the lethal and teratogenic action of trypan blue when injected prior to trypan blue in Sherman rats, over a selected range in dye concentration. This protection was not complete.

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Interaction of Lysates of Histiocytes and Tubercle Bacilli.* (27316)

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Studies of the interaction of tubercle bacillus and phagocyte have resulted in a large body of evidence which suggested that, under certain circumstances, phagocytes may exhibit antibacterial activity, either independently or in conjunction with humoral factors (1,2). Although these observations have indicated suppression of growth of tubercle bacilli by phagocytic cells derived from various animals, the underlying mechanisms still remain to be elucidated.

In view of various suggestions(3-5) that cellular enzymes or other native, biologically active intracellular substances may contribute toward the antimycobacterial activity of these cells, the present investigation was undertaken to determine whether elements derived from disrupted histiocytes would in some manner exert a deleterious effect on the tubercle bacillus.

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Materials and methods. Histiocytes were obtained from the peritoneal cavity of adult normal or BCG-immunized rabbits or guinea pigs which had been injected 5 days previously with sterile Klearol using methods previously described(6). Immunized animals used as sources of cells or serum had received 2 to 3 subcutaneous injections of BCG over a period of 2 to 3 months. Each immunizing dose consisted of approximately 5×10^8 viable organisms which had been cultivated on Sauton's potato plug medium for 3 weeks. Only animals giving a positive tuberculin test (0.2 ml of 1:100 dil. of O.T., Eli Lilly Co., Indianapolis) were used as donors of immune histiocytes.

Lysates of histiocytes were prepared in the following manner: A suspension of washed cells of known concentration was sedimented by centrifugation at 250 *g* for 10 minutes; the sedimented cells were resuspended to a predetermined volume with sterile, chilled distilled water, mixed thoroughly by pipet-