distributed throughout the body. Evidence has been presented to indicate that the intestine is a site where carotene is converted to vit. A(2). The finding that the uptake of C^{14} in the CFNS fraction was higher at the end of 6 hours than after 24 hours may be considered as presumptive evidence that the small intestine is one of the sites for this conversion process.

Summary. The feeding of C^{14} labelled carotene resulted in the deposition of all of the absorbed C^{14} in the carotene-free, non-saponifiable fraction from the total animal. The highest concentration of C^{14} was found in the extrahepato-intestinal tissues, and the least amount was recovered in the liver.

1. Hjarde, W., *Acta Chemica Scandinavia*, 1950, v4, 628.

2. Glover, J., Goodwin, T. W., and Morton, R. A., *Biochem. J.*, 1948, v43, 512.

Received January 25, 1954. P.S.E.B.M., 1954, v85.

Congenital Malformations Produced by Injecting Azo Blue into Pregnant Rats.* (20867)

JAMES G. WILSON.

From the Department of Anatomy, College of Medicine, University of Cincinnati, Cincinnati, O.

Gillman and his coworkers(1) have shown that the injection of trypan blue into female rats before and during pregnancy causes maldevelopment in the offspring. A single dose of 1 cc of 1% aqueous solution during the first 9 days of pregnancy caused anomalies in 8.6% of the young, but when treatment was given before as well as during pregnancy, 32.4% of the young were congenitally defective.

As an extension of this significant observation the present author has undertaken to study the teratogenic activity of other azo compounds more or less closely related to trypan blue. The original experimental procedure of Gillman and collaborators has been simplified and standardized to facilitate a comparison of the results of trypan blue with those of other compounds tested. One cc of 1% aqueous solution was injected subcutaneously into pregnant rats on the 7th, 8th and 9th days of gestation. (Pregnancy was considered to be of 1 day's duration 24 hours after sperm were found in the vaginal smear.) On the first day of treatment (7th day of gestation) the females were laparotomized to verify pregnancy and to determine the number of implantation sites. Control females were similarly laparotomized and injected with distilled water during pregnancy. No further treatment was given after the last injection on the 9th day. The animals were killed on the 20th day, one day prior to expected delivery, in order that living fetuses as well as resorption sites might be observed *in situ*. The living offspring were removed, weighed and examined externally for malformations. They were then preserved in Bouin's fluid for later dissection or histologic sectioning. Dissection consisted of making serial transverse slices with a razor blade at 1-2 mm intervals through the head and trunk. The slices were examined and further dismembered, if necessary, under a dissecting microscope. This made possible the recognition of a wide range of visceral and other internal malformations without the tedium of preparing and studying serial histologic sections.

Results. Of the compounds tested to date, azo blue has proven the most effective in producing developmental abnormalities. Its effects on both pregnancy and on the offspring are summarized and compared in Table I with the effects of trypan blue tested under similar conditions. The data suggest that azo blue is less likely than trypan blue to cause early termination of pregnancy as a result of ma-

* This investigation was supported by a research grant from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, Public Health Service.

TABLE I. Effects on Gestation and on Offspring of Azo Blue and Trypan Blue Injected in 1% Aqueous Solution on the 7th, 8th and 9th Days of Pregnancy.

	Pregnancies terminating before 20th day			Pregnancies continuing to 20th day			
Treatment	No. of mothers	% maternal death	% complete resorption	%	No. implantations	% dead or resorbed	% survivors malformed
Azo blue	13	0	15	85	134	53	59
Trypan blue	20	10	25	65	151	38	49
Control	6	0	0	100	57	9	0

ternal death or complete resorption, but more likely to cause abnormal development of the offspring. Although the validity of these differences cannot be established until larger numbers of animals have been accumulated, there is no doubt that azo blue is a potent teratogenic agent.

The types of malformations, as well as their incidence, were generally similar following treatment with azo blue or trypan blue (Table II). Despite a dissimilar incidence of cardiovascular, genitourinary and appendicular defects, probably attributable to the small number of cases, the pattern of abnormality appears to be essentially the same after treatment with either dye. The pattern is also approximately that described by Gillman *et al.* for trypan blue except that these authors did not report cardiovascular and genitourinary anomalies.

The most frequent specific defect after treatment with either azo blue or trypan blue was hydrocephalus. This condition was not always readily recognizable on external examination of the 20-day fetus but was clearly visible in all degrees when razor-blade slices were made through the brain region (Fig. 2 and 3). Several other malformations, however, were conspicuous on external examination. The trunk was often seen to be shortened due to reduction of the number of vertebrae in the lumbar and sacral regions, and in many of these instances the tail was entirely missing (Fig. 5). Sometimes associated with shortened trunk were anal atresia, absent genital tubercle and urethral orifice, or

TABLE II. Frequency of Malformations in Various Organs and Regions (Expressed as % of Total Living Offspring).

Treatment	Brain	Eye	Vertebral column	Cardiovascular	Urogenital	Gastroschisis	Appendicular	Other
Azo blue	29	14	19	16	5	5	1	4
Trypan blue	34	12	22	3	1	3	4	5

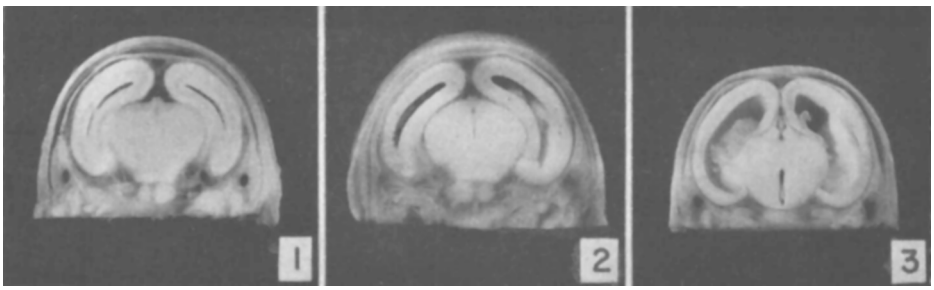


FIG. 1. Section through the ventricles of the brain of a normal 20-day rat fetus. The section was made free-hand with a razor blade.

FIG. 2. Mild degree of hydrocephalus in the lateral ventricles of the brain of a 20-day fetus from a mother injected with azo blue. This degree of hydrocephalus was not recognizable in the *intact* animal.

FIG. 3. Advanced hydrocephalus in the lateral ventricles of the brain of a 20-day fetus from a mother injected with azo blue. The 3rd ventricle is mildly distended.

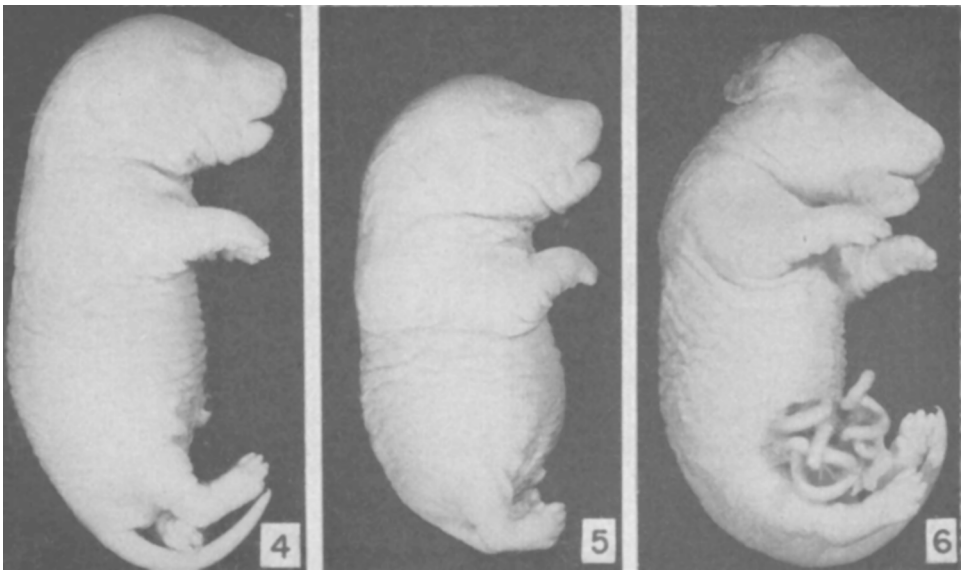


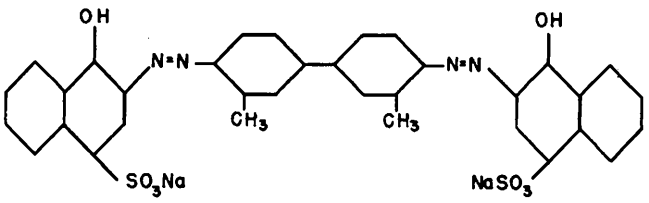
FIG. 4. Normal 20-day rat fetus from a mother injected with distilled water.

FIG. 5. Shortening of the vertebral column with concomitant shortening of the trunk in a 20-day fetus from a mother injected with azo blue. The tail and genital tubercle were missing and the anus and urethral orifices were atretic. The hind limbs were directed more toward the caudal than the ventral aspect of the body.

FIG. 6. Exencephaly, anophthalmia and gastroschisis in a 20-day fetus from a mother injected with azo blue. These particular defects did not regularly occur together.

displaced hind limbs. Also easily detected externally, but somewhat less frequent in occurrence, were exencephaly (Fig. 6), meningocele, spina bifida, gastrochisis (Fig. 6), and clubbed feet. Anophthalmia was visible externally (Fig. 6), but other ocular abnormali-

AZO BLUE (c.i. 463)



TRYPAN BLUE (c.i. 477)

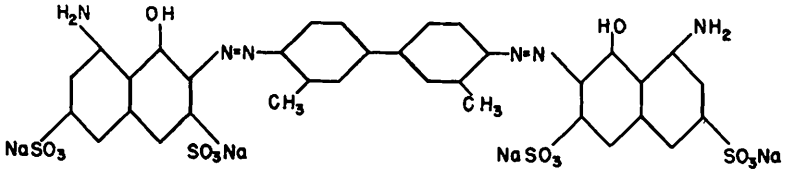
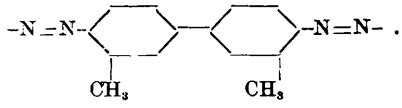


FIG. 7. Structural formula of azo blue and trypan blue. It is noteworthy that both compounds possess the diazotized ortho-tolidine grouping 

.

ties such as microphthalmia and colaboma were identified positively only in dissections or histologic preparations. The latter technics were also required to identify cardiovascular and internal genitourinary defects.

Comment. All azo dyes do not possess teratogenic properties. Gillman and collaborators have tested trypan red, Sudan IV, Bismarck brown and Niagara blue and found these compounds inactive as teratogenic agents. The present author has tested Niagara blue 4B and Congo red and likewise found both ineffective (unpublished data). Although several of these are closely related to trypan blue and azo blue, it is probably significant that of the substances tested only the latter two contain the diazotized ortho-tolidine group (3,3′-dimethyl-4,4′ diazobiphenyl) (Fig. 7).

Summary. The injection of 1 cc of a 1% aqueous solution of the diazo dye azo blue into female rats on the 7th, 8th and 9th days of pregnancy resulted in congenital malformations in 59% of living fetuses removed on the 20th day of gestation. The incidence was higher than that obtained with trypan blue (49%) under the same conditions, but the types of malformations were similar.

1. Gillman, J., Gilbert, C., Spence, I., and Gillman, T., *S. Afr. J. Med. Sci.*, 1951, v16, 125.

Received January 26, 1954. P.S.E.B.M., 1954, v85.

Reagents Modifying Stimulating Action of 2, 4 Dinitrophenol.* (20868)

JOSEPH HALL BODINE AND WILLIAM LIONEL WEST

From the Zoological Laboratory, State University of Iowa, Iowa City.

That 2-4 dinitrophenol (DNP) stimulates endogenous O_2 uptake and inhibits phosphorylation in living intact cells and their homogenates seems fairly well established(1). A wide variety of biological materials has been employed in arriving at such a conclusion. Certain differences in reaction of *intact cells* and their *homogenates* to the reagent, however, suggest the possibility of additional mechanisms entering into the above reactions. The present results have to do with a study of the action of DNP on the endogenous O_2 uptake of intact embryonic cells of the grasshopper (*Melanoplus differentialis*) and the effects of various reagents on such a reaction.

As previously shown from this laboratory (2), mitotically blocked intact embryonic cells respond to a greater degree than similar mitotically active ones. Such differential reactions to the reagent have also been pointed out for other tissues showing different degrees of metabolic activity(1). It has also been shown for intact embryos of the grasshopper that the concentration (5×10^{-5} M) of DNP producing maximal stimulation of endogenous O_2 uptake when added to the homogenate of the embryos produces no change whatever in its endogenous O_2 uptake. A somewhat similar situation has also been reported for the ova of the sea urchin(3). Since detergents are highly surface active compounds and have also been suggested to interfere with phosphorylation phenomena(1) it seemed desirable to determine their effects on the endogenous O_2 uptake of embryos and homogenates and also to see to what extent they might modify the stimulating action of DNP.

Results. In Table I are shown data for the effect of duponol and aerosol OT on the stimulating action of DNP on the endogenous O_2 uptake of intact diapause embryos. An inspection of this table shows that both detergents in appropriate concentrations completely antagonize the stimulating action of DNP. Determinations of phosphates have been carried out by the method of Sumner(4) and it is of some interest to note differences in the reactions of DNP and detergents on the labile phosphorus. With concentrations of DNP (5×10^{-5} M) the labile phosphorus

* Aided by grant from National Institutes of Health.