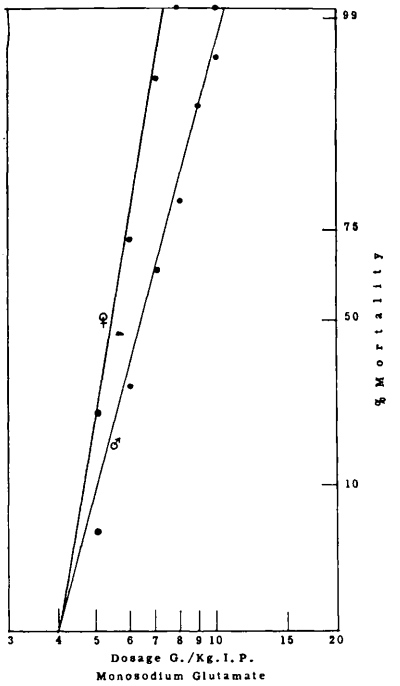

FIG. 1. Sex difference in toxicity of monosodium glutamate in mice.

implicated in the action of the phenothiazine-type of tranquilizer(8). Furthermore Lamson *et al.* has demonstrated that the administration of glutamate will reanesthetize an animal that has awakened from barbiturate hypnosis(9).

Summary. Glutamate moderately increased the mean sleeping time in mice that received hexobarbital. Reserpine markedly potentiated the action of the barbiturate. Administration of glutamate prior to reserpine-hexobarbital combinations caused a further and pronounced increase in the sleeping time. This "potentiation of the potentiation" by MSG was not observed when proclorperazine is substituted for the reserpine although proclorperazine also increased the duration of hexobarbital-hypnosis. This suggests that the specificity of this effect of monosodium glutamate is probably dependent upon the type of tranquilizer. Regardless of which of the 2 ataraxics was used there was an overall sex difference in response to the combination of these drugs, male mice being more sensitive to the depressant drugs.

1. Waelsch, H., *Advance Protein Chem.*, 1951, v6, 299.

2. Himwich, H. E., Wolff, K., Hunsicker, A. L., Himwich, W. A., *J. Nerv. Ment. Dis.*, 1955, v121, 40.

3. Brodie, B. B., Shore, P. A., Silver, S. L., *Nature*, 1955, v175, 1133.

4. Holck, H. G. O., Kenan, M. A., Mills, L. M., Smith, E. L., *J. Pharm. Exp. Ther.*, 1937, v60, 323.

5. Hoff, H., Arnold, O. H., *Wien. klin. Wsch.*, 1954, v66, 345.

6. Arnold, O. H., *Wien. Z. Nervenh.*, 1955, v10, 85.

7. Gaddum, J. H., *J. Physiol.*, 1953, v121, 15P.

8. Brodie, B. B., Shore, P. A., Pletscher, A., *Science*, 1956, v123, 992.

9. Lamson, P. D., Greig, M. F., Hobdy, C. J., *J. Pharm. Exp. Ther.*, 1951, v103, 460.

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Teratogenic Effect of Trypan Blue on the Developing Chick.* (23652)

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Gillman, Gilbert and Gillman(1) first reported the teratogenic action of trypan blue after injections into pregnant rats. Several workers have since employed this method to produce a variety of anomalies in the offspring of mice(2,3,4,5), rabbits(6,7), as well as rats (8,9,10). Recently Waddington and Perry (11) have reported a teratogenic action of trypan blue on amphibian embryos. The malformations reported have primarily involved the neural axis, skeleton, and heart and major vessels. Despite these relatively extensive studies, little is known of the underlying mechanism or even the site of action of the dye. It has been postulated that one or more

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TABLE I. Effects of Trypan Blue on Developing Chick Embryo when Injected into Yolk-Sac and Subgerminal Cavity.

	24 hr yolk-sac inj.*		24 hr subgerminal inj.†		36 hr subgerminal inj.‡		
	Trypan blue	Saline control	Trypan blue	Saline control	Trypan blue	Saline control	Noninj. control
Total treated	90	35	30	10	101	43	12
% mortality	74	20	73	40	45	11	1
% survivors malformed	52	0	75	33	76	13	0
			% survivors showing specific defects				
Rumplessness	43	0	62	33	69	2	0
Eye defects	4	0	0	0	16	2	0
Beak "	0	0	0	0	18	0	0
Gastroschisis	0	0	25	0	5	2	0
Hind limb defects	0	0	37	0	16	5	0
Others (see text)	8	0	0	0	0	5	0

* Recovered on day 10. † Recovered on day 12. ‡ Recovered on days 10 and 12.

of 3 general types of action may be involved: trypan blue may 1) produce a change in the maternal metabolism which secondarily affects the embryo, 2) cause a blockage or alteration of the placental transfer mechanism, or 3) have a direct action on the embryo. The recent work of Ferm(7) in the rabbit and Waddington and Perry(11) in amphibia has lent support to the likelihood of direct action of the dye on the embryo.

The purpose of the present investigation is to further explore the possibility of a direct action of trypan blue on an embryo, using the developing chick as a test animal.

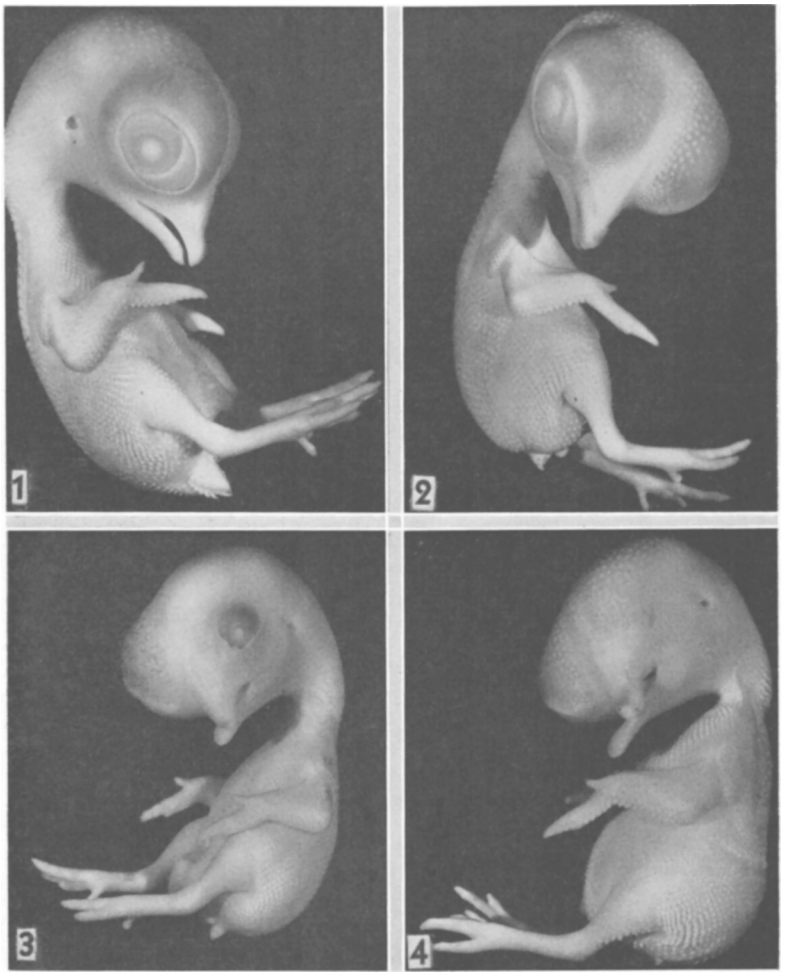
Materials and methods. Fertile eggs of White Leghorn chickens were obtained from a commercial hatchery.† The eggs were injected under aseptic conditions with a 0.1% solution of purified trypan blue prepared in sterile saline.‡ Ninety eggs received injections of 0.1 cc into the yolk sac and 131 eggs received 0.05 cc into the subgerminal cavity during the 24th or 36th hour of incubation. Dye injected into the yolk sac was deposited in the center of the yolk with the expectation that it would float upward to underlie the developing embryo. However, the quantity of dye reaching the embryo by this method was found to be highly variable when its location was checked in hard-boiled eggs. Injections into the subgerminal cavity were made through a finely drawn glass needle by way of a window sawed in the shell over the embryo. The needle was introduced into the subgerminal cavity by piercing the area pellucida close to its boundary with the area opaca. Embryos were recovered on the 10th or 12th days of incubation, fixed, weighed and examined for abnormalities. Eighty-eight control eggs were treated in the same manner as the experimentals, except that sterile saline was injected. In addition, 12 control eggs were subjected to all handling procedures, including insertion of the needle, but no solution was injected.

Results. Table I summarizes the incidence as well as the types of malformations after trypan blue injection. The same types of anomalies were produced by trypan blue injection subgerminally at 24 and 36 hours, indicating that the induction of these malformations is not sharply limited in time. The marked increase in mortality in control and experimental groups treated at 24 hours, as compared with those treated at 36 hours, is attributed to the greater sensitivity of the younger embryos to the operation.

It is apparent that the most common defect produced was rumplessness (Fig. 2), seen in 88% of all abnormal experimental embryos, and also in 2 of 7 abnormal control embryos. Rumplessness occurred alone or in association with other types of malformations (Fig. 3-8). Also observed but with lesser frequency were micropthalmia, anopthalmia, crossed beak, de-

† Florida State Hatcheries, Gainesville.

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



All embryos pictured were recovered during 10th day of incubation.

FIG. 1. Normal embryo.

FIG. 2. Treated embryo showing a degree of the rumpless condition in which the stub of a tail remains. Note shortening of entire trunk, indicating that other than caudal vertebrae are also affected.

FIG. 3. Treated embryo with left micropthalmia, crossed beak and rumplessness.

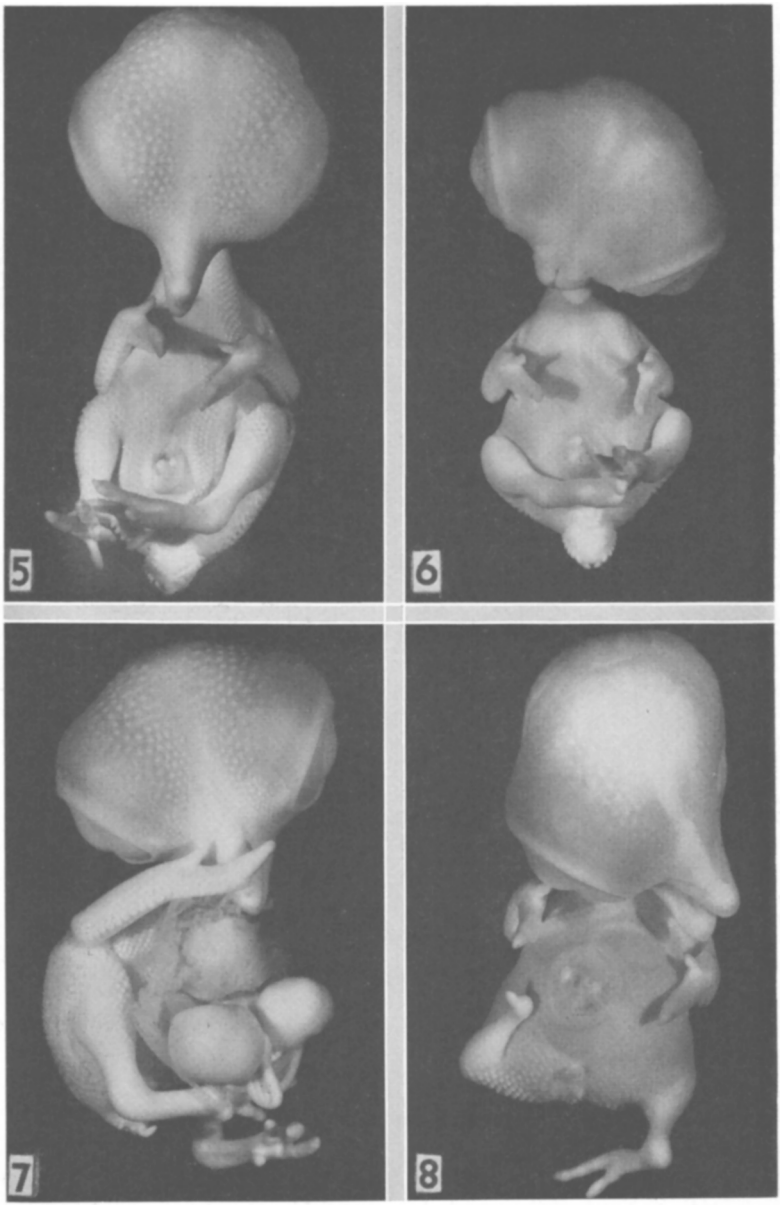
FIG. 4. Treated embryo with left anopthalmia, crossed beak, upper beak deficient and rumplessness.

ficient beak, gastroschisis, rudimentary limbs, defective feet and malposition of the hind limbs. One case was recorded of ectopia cordis and anencephaly. Not regarded as malformations were 5 embryos with generalized edema and 4 with retarded growth. The defects in 7 abnormal control embryos included 2 cases of rumplessness, 1 of exopthalmia, 1 of gastroschisis, 1 of malposition of the hind limb, 1 of defective feet and 2 of spina bifida. The abnormalities common to both the experimental and control embryos were rumplessness, gastroschisis and skeletal defects of the hind limbs.

All experimental and control animals were weighed after fixation. No significant difference in weight was noted.

Discussion. Our results indicate that trypan blue can act upon the embryo of the developing hen's egg to produce abnormalities similar to those already described in the rat. This is particularly true in the case of ocular and vertebral (rumpless) defects. Perhaps the most significant aspect of the results is

the demonstration that no placental organ or maternal reactions are necessary for production of malformations with trypan blue. These observations together with those of Waddington and Perry(11) on frogs indicate a direct action of trypan blue on developing embryonic systems. Unfortunately, however, Waddington and Perry do not report control animals for their amphibian experiments. We have observed that decapsulation of frog



All embryos pictured were recovered during 10th day of incubation.

FIG. 5. Normal embryo.

FIG. 6. Treated embryo with crossed beak and deficient upper beak. Size and appearance are more nearly those of an 8-day than a 10-day embryo and indicate considerable growth retardation.

FIG. 7. Treated embryo with ectopia cordis, gastroschisis and rumplessness.

FIG. 8. Treated embryo with left anopthalmia, crossed beak, rudimentary right leg, rumplessness, and malposition of left leg with ectrodactyly.

TABLE II. Comparison of Incidence of Spontaneous Malformations Occurring in White Leghorn Chicks with Incidence of Malformations Produced by Trypan Blue Injected at 36 Hr into the Subgerminal Cavity.

Defect	Spontaneous,* %	Trypan blue induced, % in survivors
Rumplessness	1.00	69.0
Anopthalmia	.48	3.6
Micropthalmia	.28	3.6
Cross beak	.28	9.1
Upper beak rudimentary or missing	.07	9.1
% total No. of deformed chicks	.14	70.0

* Defect and spontaneous incidence selected from a Table by Landauer and Baumann(12).

eggs, as practiced in their investigation, can produce many of the abnormalities attributed to the action of the dye.

A comparison of the incidence of malformations produced in the present study with those occurring spontaneously in the White Leghorn chicken yields interesting similarities and differences (Table II). Many of the same types of defects occurred in both groups but it is noteworthy that other abnormalities did not. The incidence of certain defects that occur spontaneously appears to be greatly increased by application of the dye. Landauer and Baumann(12) report a seasonal variation in frequency of appearance of spontaneous rumplessness with the incidence higher in the late spring and summer than at other times of the year. To what extent such seasonal variation has affected the results reported here is not known, but most of the injections were made during the late spring and early summer.

Considerable variation was observed in the reaction of different batches of eggs to the same type of treatment. Eggs were injected in lots of 2 to 3 dozen at a time. In all injection lots rumplessness was the most common malformation but the concomitant abnormalities varied noticeably in type and incidence from lot to lot of eggs. A possible source of such variation may be the fact that the eggs were obtained from different flocks and, therefore, were not genetically consistent. In the same flock different hens are known to produce offspring with varying tendency toward such defects as rumplessness, whether spontaneously occurring or experimentally induced (12).

The production of rumplessness and defects of the appendicular skeleton, beak, and eyes in the chick is by no means unique to trypan blue. Landauer(13) has shown that many chemical agents produce similar defects when introduced into the yolk sac. The underlying mechanism of action is not known for any of the several agents. Landauer has postulated interference with metabolic functions at the cellular level but no adequate explanation is available for the peculiar susceptibility of such tissues as the tail, for example.

Summary. 1. Eggs of White Leghorn chickens were injected with saline solution of trypan blue into the yolk sac at 24 hours and into the subgerminal cavity at 24 and 36 hours. 2. Yolk sac injections caused a mortality of 74% and resulted in malformation of 52% of the survivors on the 10th day. 3. Subgerminal cavity injections caused 73% mortality at 24 hours and 45% mortality at 36 hours. Of the surviving embryos at 10 and 12 days, approximately 75% in each group were abnormal. 4. The abnormalities were essentially the same after both types of treatment and included, in order of incidence, rumplessness, hind limb defects, beak defects, eye defects, and gastroschisis. 5. It is concluded that, at least in the chick, trypan blue is capable of teratogenic action directly on the embryo and does not require as an intermediary site the maternal organism or a placenta.

1. Gillman, J. C., Gilbert, C., Gillman, T., *S. Afr. J. Med. Sci.,* 1948, v13, 47.

2. Hamburgh, M., *Nature,* 1952, v169, 27.

3. ———, *Anat. Rec.,* 1953, v119, 409.

4. Waddington, C. H., Carter, T. C., *Nature,* 1952, v169, 27.

5. Murakami, V., Kameyama, Y., *Jap. J. Genetics,* 1955, v30, 51.

6. Harm, H., *Z. f. Naturforch.,* 1954, v96, 536.

7. Ferm, V. H., *Anat. Rec.,* 1956, v125, 745.

8. Gunberg, D. L., *ibid.,* 1956, v126, 343.

9. Wilson, J. G., *ibid.,* 1955, v123, 313.

10. Fox, M. H., Goss, C. M., *ibid.,* 1956, v124, 189.

11. Waddington, C. A., Perry, M. M., *J. Embryol. Exp. Morph.,* 1956, v4, 110.

12. Landauer, W., Baumann, L., *J. Exp. Zool.*, 1943, v93, 51.
13. Landauer, W., *J. Cell. and Comp. Physiol.*, 1954, v43, 261, Suppl. 1.

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An Effect of Pyridine-2-Aldoxime Methiodide (2-PAM) on Cholinesterase at Motor End-Plates. (23653)

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Certain oximes have an effect on the end-plate potential of isolated nerve-muscle preparations(1,2). Therefore, the following experiments were done to study the action of the oxime, pyridine-2-aldoxime methiodide (2-PAM), on cholinesterase at motor end-plates in striated muscle.

Materials and methods. The oxime 2-PAM was dissolved in concentrations of 7.6 x 10^{-3} *M* and 1 x 10^{-3} *M* either in frog Ringer solution or in incubation medium for the Koelle technic(3) immediately before use. Tetraethylpyrophosphate (TEPP) was used in frog Ringer solution in approximately 1.5 x 10^{-5} *M* concentration for inactivation of cholinesterase (ChE). The *iliofibularis* muscle of *R. pipiens* was freed of surrounding connective tissue and separated into small portions lengthwise. Thus many of the muscle fibers and their motor end-plates were in intimate contact with the solutions. During each of the two 30-minute exposure periods the fibers were immersed in Ringer solution or in a solution of 2-PAM or TEPP in the pH range indicated in Table I and at temperatures of 24°-28°C. These preparations were rinsed in Ringer solution after each exposure period. Each was then transferred to the incubation medium containing acetylthiocholine as substrate either with or without 2-PAM and incubated for 30 minutes at 24°-28°C. The tissues were then mounted in glycerine on slides and examined microscopically. ChE activity was assessed by the relative amounts of copper sulfide (CuS) precipitated on the subneural apparatus and recorded quasi-quantitatively; ++++ indicating maximum activity and 0, too little activity to be detectable by this method.

Results. Photographs of representative end-plate areas showing various degrees of ChE activity are illustrated in Fig. 1. The

TABLE I. Histochemical Demonstration of Interactions between 2-PAM, TEPP and ChE in Frog Muscle.

	Exposure periods,* pH 7.75-7.10		Incubation period pH 5.35-5.30	ChE activity
	30 min.	30 min.	30 min.	
2-PAM = 7.6 × 10^{-3} *M*				
(1)	Ringer sol	Ringer sol	Medium + 2-PAM	+ ‡
(2)	2-PAM in Ringer sol †	2-PAM in Ringer sol †	Medium only	+++
(3)	TEPP *Idem*	Ringer sol	Medium "	0
(4)	TEPP "	2-PAM in Ringer sol	Medium "	+++
(5)	TEPP "	2-PAM *Idem*	Medium + 2-PAM	+
(6)	2-PAM "	TEPP "	Medium only	0
2-PAM = 1 × 10^{-3} *M*				
(7)	Ringer sol	Ringer sol	Medium + 2-PAM	+++
(8)	TEPP in Ringer sol	2-PAM in Ringer sol	Medium *Idem*	++
(9)	2-PAM *Idem*	TEPP *Idem*	Medium only	0

* After each exposure period the preparation was rinsed in Ringer solution.
† These exposures were made at pH 5.35-5.30 also with identical results.
‡ Control preparation, incubated without 2-PAM, showed maximum (++++) deposition of CuS.