

LSD: Teratogenic Action in Chick Blastoderms (35579)NATHAN H. HART AND MICHAEL GREENE
(Introduced by J. H. Leatham)

Department of Zoology, Rutgers University, New Brunswick, New Jersey 08903

Studies using lysergic acid diethylamide (LSD) have yet to provide conclusive evidence as to the teratogenicity of this drug. The administration of 5 $\mu\text{g}/\text{kg}$ of body weight to pregnant rats on day 4 of gestation increased the number of stillborn and stunted offspring (1), but the LSD was not teratogenic (2). Single or multiple doses of LSD were not teratogenic in pregnant mice or hamsters (3). Where specific malformations have been described with LSD treatment, the drug appears to affect structures chiefly derived from the embryonic ectoderm, *i.e.*, brain (4) and lens (5) formation in mice.

The purpose of this paper is to report on the effect of administering 50- and 100- μg doses of LSD to chick embryos cultured *in vitro* on 1 ml of medium. These particular doses were selected because basic information on the teratogenic and cytotoxic activity of catecholamines (6) and other compounds (7) has been obtained at these concentrations for chick embryos in culture. An effective period in the chick for evaluating the potential for LSD to cause teratogenesis is between Hamburger and Hamilton stages 8-12. During this time, three major morphogenetic events occur: fusion and regionalization of the neural tube, segmentation of the paraxial mesoderm, erythropoiesis and fusion of the paired heart primordia.

Materials and Methods. Chick blastoderms were cultured on a semisolid saline-agar plus whole egg extract medium (8) modified specifically for use with LSD. An egg extract component was prepared by homogenizing and centrifuging three whole eggs. A 3% agar mixture was then made to which was added 20 ml of modified Puck saline G (1.5 $\times$) solution buffered with phosphate salts and sterilized with negative pressure through a Millipore filter (0.45 μ pore size). The pH

was adjusted to 6.8, thereby reducing possible auto-oxidation of the LSD. Equal volumes of the agar-saline mixture and the egg extract component were combined at 50°. To this was added LSD-25 (Delysid; Sandoz Batch No. 53032),¹ dissolved in 1 ml of saline and sterilized with a Swinny filter. The amount of LSD used was such that 1-ml aliquots of medium, pipetted into sterilized watch crystals supported in prehumidified, petri dish culture chambers, gave final concentrations of 50 and 100 μg . Control embryos were cultured on media to which only saline was added. All cultures were prepared immediately prior to use.

Fertile chick eggs of the White Leghorn variety were incubated at 37.5° until H & H stage 8-8⁺ (approx 26 hr). The blastoderms were quickly excised in cold, sterilized chick Ringer's, confirmed as to age, and then transferred to appropriate watch crystals containing media. After incubation *in vitro* for a period of 20 hr, embryos were removed from the media and examined under the dissecting microscope.

Results. Embryos explanted to media at H & H stage 8-8⁺ showed: (i) the neural folds in the head region, meeting approximately at the level of the future midbrain; (ii) four to five pairs of compact, rectangular-shaped somites; and (iii) an absence of fusion of the heart primordia. There was no evidence of blood island formation.

A number of harvested embryos were abnormally small and stunted in appearance after 20 hr incubation *in vitro* (Stage 12), but the effect was not drug related (Table I). Fusion of the paired cardiac primordia into a tubular heart, generally bent to the right,

¹ The LSD-25 was obtained through the courtesy of the Department of Health, Education, and Welfare.

TABLE I. Effect of Selected Doses of Lysergic Acid Diethylamide upon the Chick Blastoderm Following Culture *in Vitro*.

Treatment (μ g)	No. of embryos H & H 8-8*	No. of embryos H & H 12		Cardiovascular		Nervous system			
		Normal	Runts	Heart fused	Blood islands	Brain open	SC LO*	SC AC-PO*	SC AO-PC*
Control	28	23	5	23	23	0	1	6	0
LSD, 50	32	24	8	24	24	5	14	5	5
100	25	19	6	19	19	5	6	9	3

* Spinal cord generally open along its length.

* Anterior end of spinal cord generally closed, posterior end open.

* Posterior end of spinal cord generally closed, anterior end open.

was observed in all control embryos. At recovery, each heart showed visible contractile activity. All drug-treated embryos manifested a tubular heart similar in position and organization to the control condition. One embryo of each dose level lacked contractility of the heart at recovery. Blood islands were present in all embryos after 20 hr incubation *in vitro* (Table I).

Sixteen of 23 control embryos exhibited fusion of the neural folds throughout the primordial brain and spinal cord. Where the neural tube remained open, this was visible only in the spinal cord region and in 6 of 7 embryos only at the posterior end (Table I). The brain was closed and generally regionalized into the three divisions typical of the early development of this structure. In all but two embryos, the prosencephalon showed a pair of lateral dilations, the primary optic vesicles. Embryos exposed to LSD showed a failure of the neural tube to fuse properly, evidenced in both brain and spinal cord (Table I). In five embryos of each drug-treated group, neural folds had failed to fuse in a portion of the brain, usually the rhombencephalon. Following exposure to 50 μ g of LSD, more than 50% of the embryos showed the spinal cord to be open along its length. The remaining 10 embryos exhibited some portion of the spinal cord open. In 5 embryos the anterior end of the spinal cord was open, but the posterior end closed. Increasing the LSD dosage to 100 μ g resulted in a delay in spinal cord closure in more than 90% of the embryos. Drug treatment did not appear to interfere with regionalization

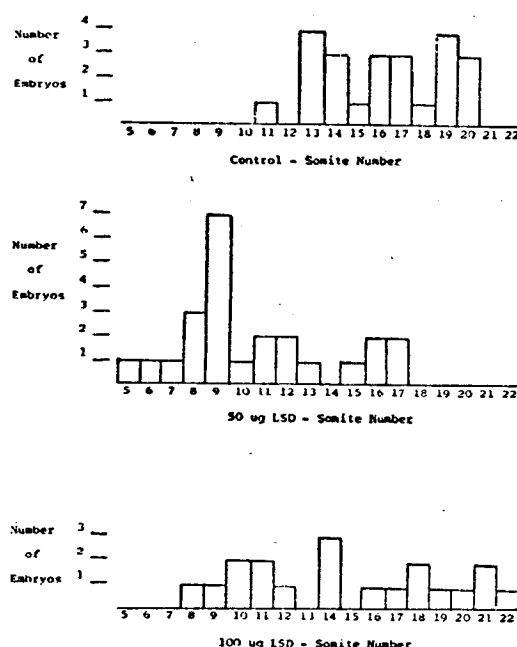


FIG. 1. Somite counts for embryos with and without LSD following 20-hr incubation *in vitro*. Embryos possessed 4-5 pairs of somites at explantation.

of the brain or primary optic vesicle formation.

Somite pairs in control embryos following 20 hr incubation *in vitro* ranged from 11 to 20 (Fig. 1). The mean somite number per embryo was 16.2 ± 0.6 . Individual somites appeared rectangular in surface view and often closely packed, particularly at the anterior end of the somite sequence. Treatment with 50 μ g of LSD resulted in a range of 5-17 somite pairs (Fig. 1) and a mean somite number of 10.6 ± 0.7 . The mean somite number

of embryos exposed to 100 μ g of LSD was 15.0 ± 1.0 . Results of the Tukey multiple range comparison test (95% confidence level) determined that the mean somite number for 50- μ g embryos was statistically different from both control and 100- μ g embryos. There were no significant differences in mean somite number between control and 100- μ g embryos.

Discussion. Exposure to LSD per se did not increase mortality or abnormally depress the growth of chick embryos cultured *in vitro* between H & H stages 8–12. Similar results were obtained in the rat, mouse, and hamster (3). Also, the drug did not appear to interfere with erythropoiesis or the migration of precardiac cells to form the tubular heart.

However, disturbances were observed after treatment with LSD in the fusion of the neural folds and in the segmentation of the paraxial mesoderm. Two types of disturbance in neural tube formation were evident: (i) retardation in neural fold fusion; (ii) improper fusion of the neural folds. In nearly half of the experimental embryos cultured with the drug, the spinal cord was visibly open along its length at recovery. This condition was particularly evident in blastoderms exposed to 50 μ g of LSD. Observations on control embryos indicated that closure of the neural folds proceeds progressively posteriorly. Improper neural fold fusion was present only in embryos cultured with the drug where the anterior end of the spinal cord was open but the posterior end normally closed. Failure of proper closure of the neural folds was also visible in the posterior region of the brain. These observations support previous findings in mice in which a high incidence of brain defects were noted when LSD was administered during the period when neural structures are known to be formed (4). Brain and spinal defects observed in the hamster following LSD treatment (9) may have been induced during the period of neural fold fusion. These data warrant further investigation and evaluation of LSD during this critical period in the development of the central nervous system.

To the knowledge of the authors, no specific defects in structures derived from mesoderm have been described following LSD

treatment. In this study, however, this drug, administered at 50 μ g/ml of medium, retarded the segmentation of the paraxial mesoderm as determined by the mean number of somite pairs per embryo. Since somite segmentation in the chick is related to regression movements of the primitive streak (10), LSD may be influencing this process. No correlation between dosage of drug administered and number of somites per embryo could be demonstrated since somite number in embryos treated with 100 μ g did not statistically differ from the controls. The absence of a direct dose-response relationship is not unusual for hallucinogenic drugs. Geber (9) observed that lower doses of mescaline were more teratogenic than higher doses in hamsters. Costa (11) reported that serotonin-induced uterine contractions were facilitated by low doses of LSD, but inhibited by high doses of the same drug.

Summary. Chick blastoderms at H & H stage 8–8+ were cultured *in vitro* on 1 ml aliquots of medium containing either 50 or 100 μ g of lysergic acid diethylamide. Following 20 hr incubation, drug-treated embryos failed to demonstrate any increase in mortality or growth-depressing effects. At both dose levels, however, disturbances were observed in fusion of the neural folds. A significant reduction in the mean number of somites per embryo was observed in embryos exposed to 50 μ g of LSD.

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