Toxicol.appl.Pharmacol. 25, 54-59 (1973).



TOXICOLOGY AND APPLIED PHARMACOLOGY 25, 54–59 (1973)

2476.

# Effects of LSD on the Development, Histology and Fine Structure of the Chick Embryo[1]

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*Received July 5, 1972*

Effects of LSD on the Development, Histology and Fine Structure of the Chick Embryo. MESSIER, P.-E. (1973). *Toxicol. Appl. Pharmacol.* **25**, 54–59. Chick embryos cultured in vitro were exposed to LSD in concentrations of 0.0005, 0.005, 0.05, 0.5 and 2.5 μg/ml. The two highest concentrations retarded the segmentation of mesoderm into somites and caused a collapse of the roof of the neural tube. Electron microscopy showed that nucleolar components were segregated and that mitochondria were altered following the treatment. Doses of 0.005 and 0.05 μg/ml produced similar abnormalities but in fewer cases. The lowest concentration used produced no changes in the embryos.

Lysergic acid diethylamide (LSD) has been reported to be teratogenic in hamsters (Gerber, 1967) and rats (Alexander *et al.*, 1967), and preliminary information on lower primates is suggestive of a teratogenic effect (Kato *et al.*, 1970). However, other investigations on hamsters (DiPaolo *et al.*, 1968; Roux *et al.*, 1970) and rats (Roux *et al.*, 1970; Warkany and Takacs, 1968) have failed to substantiate teratogenicity. Only in mice (Auerbach and Rugowski, 1967; DiPaolo *et al.*, 1968; Hanaway, 1969) has the teratogenic effect of LSD been confirmed. In man, case reports of malformed children born to users of illicit LSD are rare, yet, they give some indication of an increase risk of spontaneous abortion (Dishotsky *et al.*, 1971). On the other hand, there is no evidence that *pure* LSD causes birth defects or "fetal wastage" in man (Dishotsky *et al.*, 1971).

Available information on the effect of the drug on laboratory animals is quite controversial and deserves further investigation. Along that line, we report here observations from preliminary work dealing with the effect of LSD on chick embryos cultured in vitro. Three lines of evidence will be presented. One relates to the growth response of chick embryos, exposed to LSD in varying concentrations. Another deals with the light microscopic aspect of the neural tube of those young embryos. And finally, we report on what fine structural alterations were observed when our specimens were subjected to LSD treatments.

[1] This work was supported by the Ministère de la Santé Nationale et du Bien-être Social (programme sur l'usage non-médical des drogues) and the Medical Research Council of Canada.

[2] Scholar of the Medical Research Council of Canada.

## METHODS

Chick embryos (*Gallus domesticus*) were explanted at stages 1–9 somites. A total of 30 control embryos were incubated at 38°C for 5.5 hr on 3 ml of Spratt's (1950) culture media. Embryos to be exposed to LSD were placed on identical media to which LSD-25 (lysergic acid diethylamide, Sandoz) was added to give final concentrations of 0.0005, 0.005, 0.05, 0.5 and 2.5 μg/ml. Fifteen embryos were used with each of the first 4 concentrations and 60 were subjected to 2.5 μg/ml. Control and treated embryos were incubated at the same time in identical conditions.

Following incubation, all embryos were fixed in 1.25% phosphate buffered glutaraldehyde and postfixed in 1% osmium tetroxide. Specimens were dehydrated and embedded in Epon (Luft, 1961). One-micron-thick sections stained with toluidine blue (pH 8.4) were used for light microscopy. Thin sections for electron microscopy were cut on an LKB Ultrotome I and stained with 1% uranyl acetate and lead citrate (Reynolds, 1963). The sections were examined in a Siemens Elmiskop IA electron microscope.

## RESULTS

Our morphological study is, at this point, restricted to the neural tube cells because the drug was tested at that time in organogenesis when neuralation (closure of the neural plate and formation of the neural tube) is occurring. A normal embryo is shown in Fig. 1.

All embryos withstood the 5.5 hr LSD treatment, irrespective of the concentration used. The segmentation of mesoderm into somites was used as an indication of the growth rate. Embryos of different stages, from 1 to 9 somites, all responded in the same way to the highest concentration used. The drug effect appears to be independent of the stage reached by the embryo. LSD at 2.5 μg/ml retarded growth; embryos of varying stages subjected to this concentration repeatedly developed two full pairs of somites less than the controls. Similar results were obtained with 0.5 μg/ml where treated embryos lagged by at least one pair of somites.

Concentrations of 0.05 and 0.005 μg/ml produced ambiguous results. The limited number of embryos treated at such low concentrations does not permit to ascertain whether or not LSD had an effect on the developmental rate. Finally, embryos exposed to 0.0005 μg/ml LSD behaved identically to controls. Therefore, it may be said that increasing concentrations of LSD will slow down development in chick embryos at stages 1–9 somites.

Light microscopy examinations were made for possible cytotoxic effects of LSD. Even at the highest concentrations used none of the treated embryos showed necrotic cells or pycnotic nuclei. No increase in mitotic figures was noted and interkinetic nuclear migration appeared to proceed normally. However, most embryos exposed to 2.5 and 0.5 μg/ml LSD showed a collapse of the roof of the neural tube concomitant with a lateral enlargement of that structure (Fig. 2). In younger embryos the folding up of the neural plate was not inhibited but neural crests, dragging along the ectoderm, followed an abnormal inward movement, sinking in the neurocoele and occasionally ending up in contact with the cells of the outer edge of the would be neural tube (Fig. 3). We have observed such collapsed neural tubes in over 80% of the embryos treated at the two highest LSD concentrations. At 0.005 and 0.05 μg/ml, just as pronounced abnormalities were observed but the occurrence was noted in fewer embryos. No effect

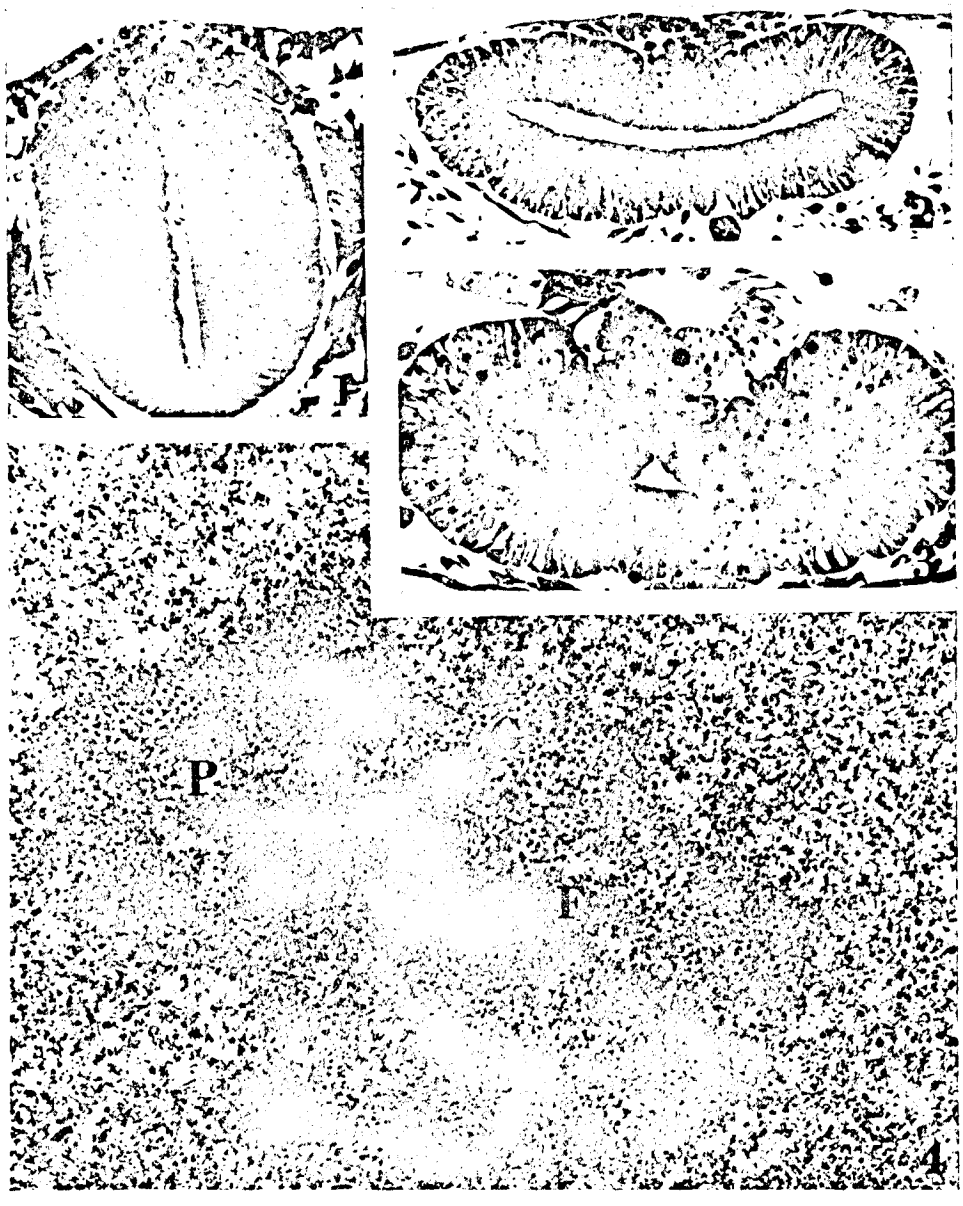


FIG. 1. Light micrograph of a section cut transversally in the first somite region of a normal 9-somite *in vitro* incubated chicken embryo. Neurulation occurred normally. × 100.

FIG. 2. Collapse of the roof of the neural tube and its lateral enlargement, as seen in chicken embryos cultured on media to which was added 2.5 μg/ml of LSD. Nine-somite embryo. × 65.

FIG. 3. Sinking of the neural crests and of attached ectoderm into the neurocoele of an 8-somite chicken embryo exposed to 2.5 μg/ml LSD. × 100.

FIG. 4. Nucleolus of neural tube cell of an untreated chicken embryo showing prominent nucleonema (N). Fibrillar (F) and granular (G) components of the nucleolus are seen loosely intermingled and interspaced with pars amorpha (P). × 24,000.

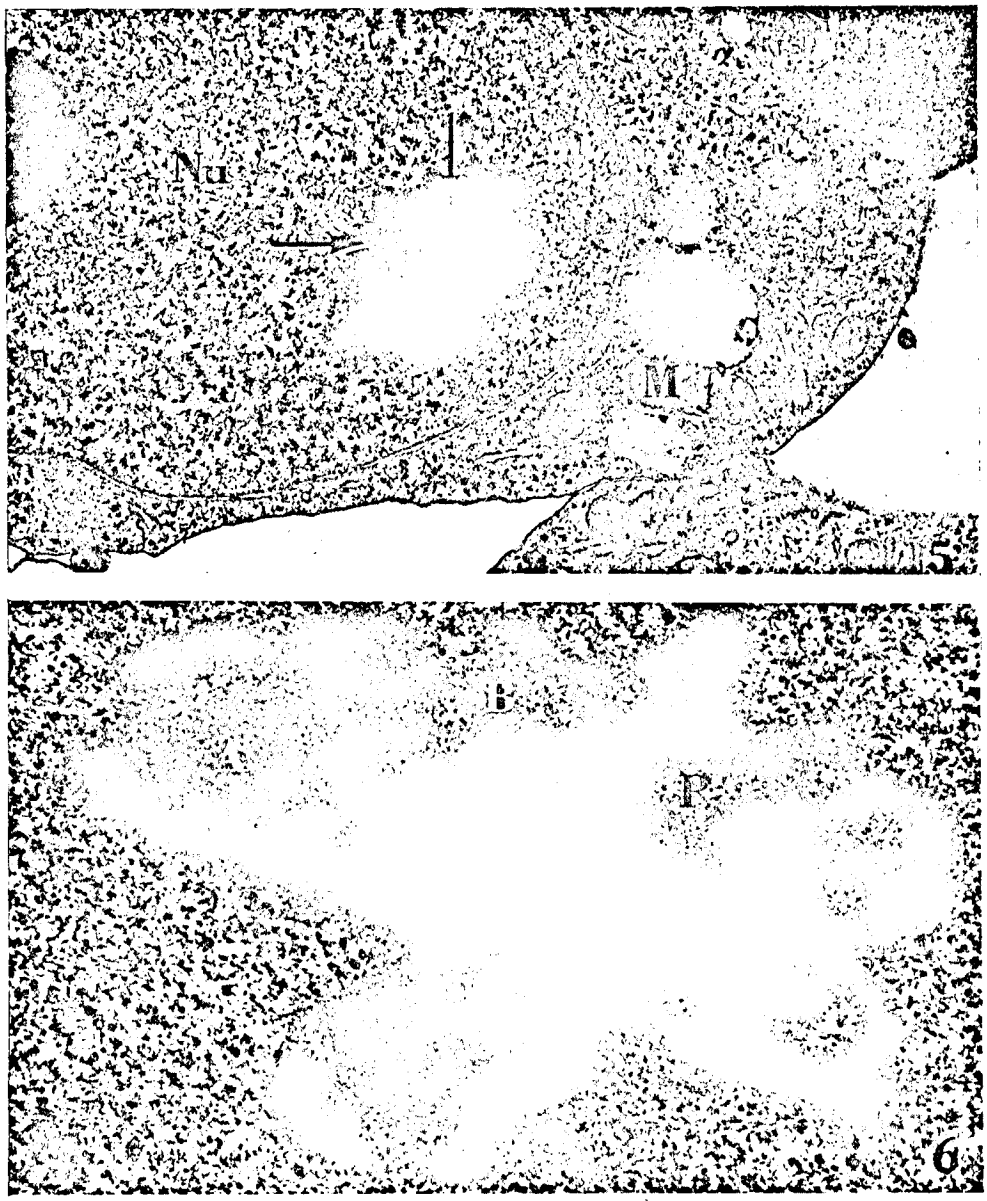


FIG. 5. Low-power electron micrograph showing portions of neural tube cells of an LSD-treated embryo (2.5 μg/ml). Nucleus (Nu) and nucleolar components are seen. In the nucleolus, the less electron-dense material (horizontal arrow) is pushed to one side. The remaining electron opaque material is interspaced by pars amorpha (vertical arrow). Slightly swollen mitochondria are seen (M). × 6000.

FIG. 6. Typical aspect of nucleolus in neural tube cell of LSD-treated chicken embryo (2.5 μg/ml) where the lighter, less electron-dense material (A) is pushed to one side while the remaining electron opaque material (B) is interspaced by pars amorpha (P). × 24,000.

on neural tube morphogenesis was ever detected in embryos treated with 0.0005 μg/ml LSD. Finally, those embryos exhibiting impaired neural tube development occasionally showed malformed notochords.

Ultrastructural analysis of collapsed neural tubes revealed constant and prominent nucleolar alterations. Indeed, in neural tube cells of normal early chick embryos the nucleolus was in the form of a large polymorphous structure containing an elaborate electron dense reticulum (nucleonema) interspaced by canals of the pars amorpha (Fig. 4). In the embryos treated with highest (2.5 μg/ml) LSD concentration the less electron-dense component of the nucleolus was characteristically located at the periphery of the organelle, where it took the aspect of a relatively homogeneous area deprived of pars amorpha (Figs. 5 and 6). The rest of the nucleolus appeared in the form of a closely packed electron-opaque material interspaced by the usual canals of the pars amorpha (Figs. 5 and 6).

It was also found that mitochondria were occasionally swollen. These organelles exhibited empty matrices and sometimes a complete loss of cristae.

## DISCUSSION

Many biological models, including plants (Kalia *et al.*, 1970; Sturelid and Kihlman, 1969), insects (Grace *et al.*, 1968; Vann, 1969) and a variety of rodents (DiPaolo *et al.*, 1968; Egozcue and Irwin, 1968; Fabro and Sieber, 1968; Gerber, 1967) have been used to study whether LSD is carcinogenic, mutagenic or teratogenic.

Here, we introduce chick embryos incubated in vitro as a model allowing for the study of the effects of LSD. Our preliminary work shows that the drug slows down development and is detrimental to neural tube morphogenesis in the early embryo. Ultrastructurally, nucleolar segregation was found to occur in high frequency and the only other cellular alteration thus far noted was in the form of occasionally swollen mitochondria, an effect that could, perhaps, be secondary to changes in nucleic acids.

Changes at the nuclear level have been observed with the light microscope in circulating lymphocytes of LSD-treated monkeys where variations in basophilia and nuclear reorganization have been reported (Ruffie *et al.*, 1971). Furthermore, it has been reported (Gayer and Pribys, 1970) that nucleoli in a human larynx cancerous cell line are transformed in shape and size, and sometimes disappear under the influence of LSD. On the other hand, Simard and Bernhard (1966) and Simard (1970) have studied the ultrastructural changes induced in nucleoli following the administration of various groups of antimetabolites. Among these, a group including actinomycin D, when used in small doses, brings about nucleolar segregation causing selective inhibition of DNA-dependent RNA synthesis. As a consequence, protein synthesis and proliferative activity are arrested.

Intercalation of lysergic acid diethylamide molecules between the bases of nucleic acids has been shown in circular dichroism experiments (Wagner, 1969; Smythies and Antun, 1969). This binding of the drug to nucleic acids, causing conformational changes, may provide the physical mechanism whereby the genetic code is altered, thus impeding normal growth and morphogenesis. We are now investigating these points while completing our study of the morphogenetic effects of LSD on embryos at different stages.

## ACKNOWLEDGMENTS

We are indebted to Mr. A. Comtois for his collaboration in the initial part of this work and to Mrs. G. Anglade for her technical assistance.

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