The Effect of LSD on Somite Number in Explanted Chick Embryos

AUERBACH and RUGOWSKI[1] and HANAWAY[2] reported disturbances in the development of ectodermal derivatives in mice following administration of lysergic acid diethylamide (LSD). Findings by DIPAOLO et al.[3] and ROUX et al.[4] failed to support these observations. Malformations of brain and spinal cord were noted by GEBER[5] in hamsters following the injection of a single dose of LSD. These results could not be repeated by ROUX et al.[4]. HART and GREENE[6] reported that chick embryos cultured in vitro with 50 µg of LSD showed disturbances in neural fold fusion and in segmentation of the paraxial mesoderm. The examination of the literature on LSD clearly indicates that the embryotoxicity and teratogenicity of this drug remain an open question and hence a source for further investigation.

The present study was undertaken to determine the effect of selected doses of lysergic acid diethylamide (LSD-25) on somite number in chick embryos and to correlate drug dose and teratogenic response as measured by mean somite number.

Materials and methods. Chick embryos were cultured on a Ringer-agar plus yolk-albumen extract medium (basic medium) according to the technique of SPRATT[7]. LSD-25 (Delysid; Sandoz Batch No. 53032) was dissolved in Puck saline G (1.5×) and sterilized with a Swinny filter (0.45 µm pore size). The amount of LSD-25 used was such that 1 ml aliquots of basic medium contained 10, 25 and 100 µg of the drug. These aliquots were pipetted into sterilized watch crystals supported by glass rings in prehumidified Petri dish culture assemblies. Control embryos, run concurrently with drug-treated embryos, were cultured on media lacking the drug.

Fertile eggs of the White Leghorn variety were incubated at 37.5 °C for 26–27 h to obtain embryos at stages 8-8+[8]. Following explanation to medium with or without LSD-25, embryos were incubated at 37.5 °C for 20 h or until the majority of control embryos had reached stage 12[8]. Embryos were then removed from the media, placed in warm Ringer's solution, and examined under the dissecting microscope. Embryos classified as runts, i.e. small and stunted in appearance, were noted and then discarded without further consideration. The remaining control and experimental embryos showed normally-fused, pulsatile hearts and little evidence of gross morphological abnormalities. These were classified as normal and the appearance, arrangement, and number of somites recorded. For each treatment condition, the range of somite pairs, the mean ($\bar{X}$) number of somite pairs per embryo, and the deviation of the treatment mean as estimated by the standard error ($S_{\bar{x}}$) were determined. The mean number of somite pairs per embryo for each drug dose was plotted at 95% confidence intervals (C.I.). Where appropriate the significance of the difference of 2 treatment means was established using the Student's t-test at the 0.05 level.

Results. LSD did not appreciably increase the incidence of embryolethality as determined by the number of runt embryos over the dose range employed. All normal drug-treated embryos showed visible contractile activity of a well-organized tubular heart; blood islands were present in all embryos.

Embryos explanted at stage 8-8+ had developed 4–5 pairs of compact, block-shaped somites, symmetrically arranged on either side of the neural axis. Somite number in control and drug-treated embryos is summarized in the Table. The mean somite number in control embryos was 16.5 ± 0.31, a figure similar to that obtained in both in vitro and in ovo studies[6,8] carried out for a comparable period of incubation. At 10 µg/ml of the drug, somites were similar in appearance and organization to that of the control series; the mean somite number per embryo was 16.7 ± 0.54. Increasing the dose to 25 µg/ml yielded 14.6 ± 0.47 somite pairs per embryo. Variations in somite shape (triangular to block-shaped) were observed with posterior pairs often diffuse and less compact than anterior pairs. 5 embryos exposed to 100 µg/ml of LSD had a mean somite number of 17.4 ± 0.60; this did not statistically differ from that previously obtained[6].

The dose-response curve for LSD and mean number of somite pairs plotted at 95% confidence intervals is shown in the Figure. The mean number of somite pairs in embryos exposed to 10 µg/ml and 100 µg/ml did not statistically differ from the control condition. Although the confidence interval limits at 25 µg/ml overlap those for control embryos, the average number of somite pairs at this dose was significantly less than the control level ($p < 0.05$). The drug-related effect on somite number was even more pronounced at 50 µg/ml.

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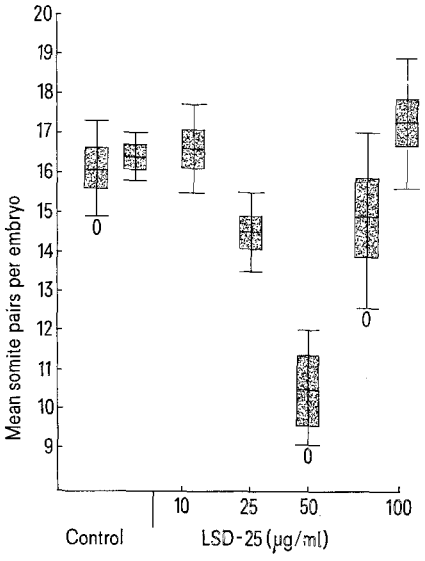
Effect of selected doses of LSD-25 on somite number in chick embryos explanted at stage 8-8+ and cultured for 20 h

LSD dose (µg/ml)	No. embryos stage 8–8+	No. embryos stage 12		No. somite pairs in normal embryos			
		Normal	Runt	Range	$\bar{X}$	$S_{\bar{x}}$	95% C.I.
0	34	31	3	14–21	16.5	0.31	16.5 ± 0.63
0[a]	28	23	5	11–20	16.2	0.60	16.2 ± 1.20
10	18	17	1	12–21	16.7	0.54	16.7 ± 1.14
25	29	25	4	11–19	14.6	0.47	14.6 ± 0.96
50[a]	32	24	8	5–17	10.6	0.70	10.6 ± 1.50
100	5	5	0	16–19	17.4	0.60	17.4 ± 1.67
100[a]	25	19	6	8–22	15.0	1.02	15.0 ± 2.20

[a] Figures from previous study[6].



Relationships between selected doses of LSD-25 and mean number of somite pairs per embryo at 95% confidence intervals. The open circle denotes data from a previous report[6]. The mid-horizontal bar = mean; the stippled area = standard error of the mean.

Discussion. This study indicates that lysergic acid diethylamide disturbs the orderly formation of somites over a dose range of 25 to 50 μg, with the minimum teratogenic dose being somewhere between 10 and 25 μg. Although both 25 and 50 μg doses inhibited somite formation, no appreciable effect on the macroscopic arrangement of those somites present could be detected. Since in the chick embryo regression movements of the primitive streak play a vital role in the segmentation of the paraxial mesoderm[9], this study also suggests that the LSD over the teratogenic dose range may act to retard these cellular movements, thereby resulting in fewer somite pairs.

Ten and 100 μg dose of LSD, being non-effective in their action on somite number, give the dose-response curve a biphasic appearance. The basis for this apparently unusual effect is presently not known. However, GEBER[5] reported that another hallucinogenic drug (mescaline) produced a greater number of defects at lower concentrations than at higher concentrations.

Résumé. L'administration de 10 μg d'acide lysergique diéthylamide (LSD-25) aux embryons de poulet en culture n'a pas eu d'effet sur le nombre des somites. Une réduction du nombre des somites a été observée avec 25 μg de LSD, ce qui confirme notre première observation sur la tératogénie de cette drogue. Il semble exister une relation directe entre la dose de LSD et le nombre de paires des somites dans chaque embryon au niveau de 25 et 50 μg.

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Glycogenolytic Effect of Adipose Tissue Extract

ANTONIADES et al.[1] have demonstrated that an aqueous extract of adipose tissue (ATE) of rat or bovine origin produces prolonged hypoglycemia after parenteral administration to rats and mice. LENTI et al.[2] and PERRI et al.[3] have reported that ATE-induced hypoglycemia is preceded by a brief period of marked hyperglycemia. It is our finding that the administration of ATE to intact and adrenalectomized rats produces hepatic glycogenolysis accompanying the hypoglycemic effect.

Materials and methods. The animals used were male rats of the Sprague-Dawley strain (Laboratory Supply Co.) weighing approximately 150 g. Adrenalectomy was performed by Hormone Assay Labs, Chicago, 7 days prior to the experiments. Food was available ad libitum until 2 h prior to the experimental period. To obviate the effect of diurnal variation in endogenous carbohydrate metabolism all animals were prepared and used for study at the same daily hours. ATE of bovine origin (kindly provided by Dr. G. C. PERRI) was dissolved in saline and injected via the femoral vein; control animals were given saline alone. At the time of sacrifice the animals were anesthetized with Metofane® and bled via cardiac puncture; heparin was used as anticoagulant. The liver was quickly excised, immersed in liquid nitrogen, and stored frozen until analysis for glycogen content. Plasma glucose was determined by the glucose oxidase method[4]. Liver glycogen was isolated[5], acid hydrolyzed and determined as glucose with glucose oxidase.

Results and discussion. The effects of ATE on plasma glucose levels and hepatic glycogen content are summarized in the Table. Within 1 h following ATE treatment, both intact and adrenalectomized animals became hyperglycemic; subsequently this effect was reversed and hypoglycemia developed. Continuous mobilization of hepatic glycogen was seen in ATE-treated animals; this effect led to an almost complete depletion of the glycogen stores. Adrenalectomized animals were more sensitive to ATE than intact animals (effective dose 5 mg/kg vs. 75 mg/kg) and glycogenolysis and hypoglycemia occurred earlier in the adrenalectomized groups. The glycogenolytic response was apparently independent of epinephrine as evidenced by nearly total depletion of hepatic glycogen in adrenalectomized as well as intact animals.

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