is tissue specific. Well-differentiated tissues, such as liver or spleen, turn over their histone-bound ^{32}P faster than the similar fractions in regenerating liver or in hepatoma where a less-differentiated environment is found. The uncertainty of when maximum incorporation of ^{32}P occurs in regenerating liver imposes certain limitations. A twofold increase in the level of phosphate in the F1 fraction has been shown to occur 16 to 24 hours after partial hepatectomy (*13*). Since DNA and histone biosynthesis exhibit two peaks, one occurring about 20 hours and the other approximately 28 to 43 hours after hepatectomy (*14*), it was assumed that 36 hours of regeneration selected for these experiments were most likely in the range of maximum phosphorylation of histones.

Also, it appears that the active phosphorylation of histones is associated with metabolic functions of the cells and not with the growth, since the relative extent of ^{32}P incorporation decreases with increasing mitotic rates. More histone is synthesized by regenerating liver or hepatoma cells than by normal liver, but a greater amount of histone is phosphorylated in liver than in other tissues. Variability with age was observed; very young rats exhibited a marked increase (approximately twofold) in phosphorylation.

Ord and Stocken (*13*) demonstrated the sensitivity of changes in phosphorylation to irradiation and partial hepatectomy. Irradiation significantly depressed the incorporation of ^{32}P into the F1 fraction by thymus and regenerating rat liver; on the other hand, nonirradiated regenerating livers showed a twofold increase in ^{32}P incorporation 16 to 24 hours after partial hepatectomy. Another study by Kleinsmith and co-workers revealed that in isolated rat thymus nuclei (*6*) the phosphoserine content of the F1 fraction is three times greater than that of the F3 fraction. The results reported here tend to support the concept that histone phosphorylation may function in genetic derepression, as was suggested by Allfrey *et al.* (*15*).

Further evidence supporting the presence of phosphate in histones as phosphoserine was recently obtained. Isolated tryptic peptides of histones which have been fractionated by gel filtration on Sephadex G-75 were analyzed after partial hydrolysis with the aid of an automatic amino acid analyzer, and ninhydrin-positive, ^{32}P-containing peaks were identified by means of a scintillation flow detector. The results indicate that serine-O-phosphate, the first peak to appear during the analysis, was responsible for the ^{32}P activity in the peptides.

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Lysergic Acid Diethylamide: Effect on Embryos

Abstract. *Injection of lysergic acid diethylamide tartrate into mice in early pregnancy caused a 57 percent incidence of grossly abnormal embryos.*

A variety of agents, such as x-irradiation, viruses, drugs, vitamins, hormones, and hypoxia, are potent inducers of developmental malformations when administered to pregnant mice. Generally, the type and extent of abnormalities that may be produced depend on the stage of development reached at the time of experimental insult as well as on the nature and concentration of the agent used (*1*). These studies represent extension of an individual student project originally carried out as part of a class in developmental biology. The results of this project (experiment 1, Table 1) were sufficiently provocative to warrant more systematic examination.

Mice of strains BALB/CAu, C57BL6/Au, C3H/HeAu, and F_1 (BALB/C × 57BL) were used. Mice were checked for vaginal plugs daily, the day of appearance of the plug being taken as day 0 of development. Lysergic acid diethylamide (LSD-25) was obtained from the National Institutes of Health as 0.1 mg of lysergic acid diethylamide tartrate (Sandoz, batch No. 65002) per milliliter. Before injection, the LSD was diluted in Ty-

Table 1. Embryonic abnormalities induced by LSD-25 injected into mice on day 7 of pregnancy.

Experiment	LSD-25 (g)	Litters (No.)	Embryos (No.)	Normal (No.)	Abnormal (No.)
		C3H			
1	5×10^{-7}	4	29	6	23
	5×10^{-8}	6	43	14	29
2	2×10^{-7}	3	29	16	13
3	1×10^{-6}	1	10	5	5
		C × C57BL			
2	5×10^{-7}	1	8	5	3
	3×10^{-7}	1	8	5	3
	1×10^{-7}	1	9	9	0
		C57BL			
3	1×10^{-6}	1	7	1	6
		BALB/C			
2	5×10^{-7}	2	15	6	9
		Control mice			
2*		4	31	28	3
3†		5	33	31	2

* Tyrode's solution injected as control. † Sodium potassium tartrate injected as control (see text).

rode's solution to a concentration such that 0.1 ml to 0.5 ml could be injected, yielding final amounts indicated in Table 1.

In experiment 1, uninjected colony controls were used. In experiment 2, control pregnant mice were injected with an equivalent amount of Tyrode's solution. In experiment 3, 0.1 mg of sodium potassium tartrate was dissolved in 1 ml of H_2O containing 0.0087 mg of tartaric acid and 7 mg of NaCl (suspending fluid for LSD) and then diluted in Tyrode's solution. Controls for experiments 2 and 3 are included in Table 1; they do not differ significantly from the 10 percent abnormality found at this age in our colony. In all experiments, mice were injected intraperitoneally. Four days later, embryos were removed and examined by gross inspection. Mice were injected on day 7 of pregnancy (Table 1); 57 percent of the resultant embryos were deformed, while fewer than 10 percent of the control embryos had malformations. In all cases the malformations involved characteristic brain defects (Fig. 1). The midbrain was frequently enlarged or shifted, and the mid- and hindbrain regions showed improper closure. The fourth ventricle was correspondingly modified. In addition, abnormalities of the lower jaw, shifts in the position of the eyes, and modifications of facial contour frequently were associated with these defects.

To determine stage specificity of the observed abnormalities, mice were injected at days 6, 8, or 9 of pregnancy. While brain abnormalities were observed in embryos of animals injected at the earliest stage, there were no gross observable effects when injection occurred later than day 7 of pregnancy.

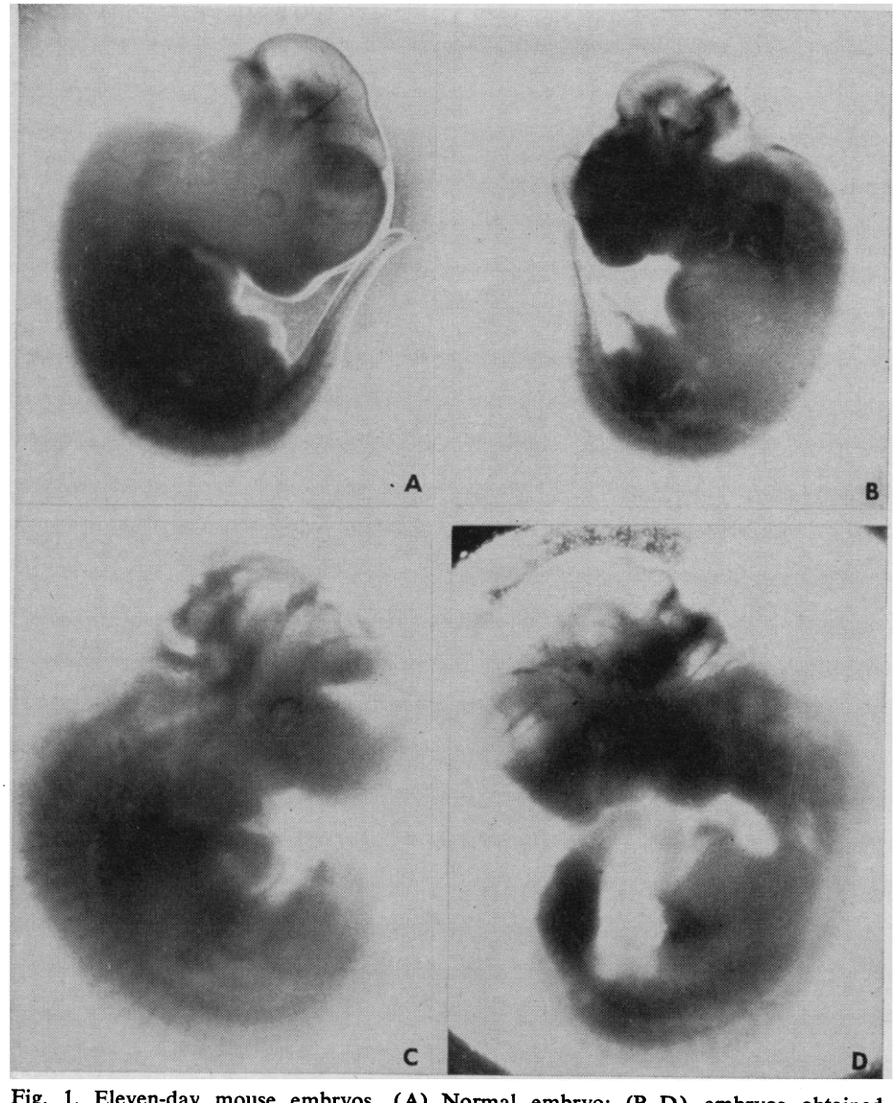

Fig. 1. Eleven-day mouse embryos. (A) Normal embryo; (B–D) embryos obtained from mice injected on day 7 with 5×10^{-7} g of LSD-25.

Since this is the stage where neural structures are first being formed, it is a stage uniquely sensitive to induced abnormalities of the brain (*1*); thus we can draw no conclusions concerning the correlation between the observed abnormalities and the behavioral or pharmacological effects of LSD (*2*). Similarly, we cannot determine whether the ineffectiveness of the drug when administered in later stages of pregnancy is due to a highly specific action of LSD, or whether it simply reflects increased resistance to teratogenesis in general (*1*). In this connection, tissue culture studies may prove useful (*3*).

The dose of LSD used in the present study is sufficiently low to warrant emphasis. On the basis of body weight, the effective dose is below that equivalent to an average single exposure in man. This calculation, moreover, appears conservative, since embryonic weights at neurulation are similar for mice and humans. Finally, one must point out that the stage of pregnancy in mice found to be sensitive to embryonic malformations mediated by LSD is a stage equivalent to human pregnancy of 16 to 22 days (*4*). This represents a period of embryonic development where pregnancy is frequently unsuspected.

Note added in proof: In vivo confirmation of the chromosome effects noted by Cohen *et al.* (*3*) has been reported recently (*5*). The relationship between chromosome damage and developmental malformation has not been determined in our study. Our experiments support similar findings obtained in rat embryos by Alexander *et al.* (*6*).

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