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LSD: Autoradiographic Study on the Placental Transfer and Tissue Distribution in Mice

Abstract. *^{14}C-lysergic acid diethylamide administered intravenously passed in a few minutes from the blood into the tissues. In addition to the brain, the adrenals, hypophysis, kidneys, liver, and lungs showed the highest uptake, much higher than the blood concentration. Excretion into the bile started immediately; this was the most important elimination route. In the early stage of pregnancy, 2.5 percent (and in the late stage, 0.5 percent) of the radioactive dose passed the placental barrier into the fetus in 5 minutes. Over 70 percent of this fetal radioactivity was unchanged ^{14}C-lysergic acid diethylamide.*

Lysergic acid diethylamide (LSD) may lead to chromosome damage (*1*) and may have teratogenic properties. Increased rates of abortion and malformations in fetuses have been reported after administration of LSD in early pregnancy in mice (*2*), rats (*3*), hamsters (*4*), and rabbits (*5*). The possible teratogenic effect on two human embryos has been suggested (*6*).

Lysergic acid diethylamide seems to stimulate adrenal activity, to lower metabolism, and to inhibit both thyroid and gonadal function (*7*). Despite the many studies on the behavioral and pharmacological actions of this agent, its kinetics and distribution in the body have had little study (*8*). We used an autoradiographic method to study the transplacental penetration, tissue distribution, and the rate and route of excretion of ^{14}C-LSD in the whole body of mice.

d-Lysergic acid diethylamide tartrate (Sandoz Pharmaceuticals, Hanover, New Jersey), labeled with ^{14}C in the side chain (specific activity, 5.96 μc/mg, 0.5 mg/ml) was concentrated under a stream of nitrogen gas in a water bath (70°C) to 1.7 mg/ml. Its chemical and radiochemical purity was determined with thin-layer chromatography (silica-gel G). Two-way chromatograms, developed first in chloroform and acetone (1:4) and then in methanol, had one spot with an R_F corresponding to that of authentic LSD. Ultraviolet light, fluorescent light, van Urk's reagent (*9*), and autoradiography were used to detect ^{14}C-LSD on the plates.

Four male (20 to 22 g) and six pregnant female mice (Yale Swiss) were injected intravenously with ^{14}C-LSD (19.8 and 9.9 μg/g, respectively). Three females (28 to 32 g) were in the first trimester of pregnancy, and the others (44 to 50 g) were in the last week of pregnancy. The pregnant mice were killed 5, 30, 60, and 120 minutes after injection, by being dropped into hexane cooled to about −70°C with solid carbon dioxide. The male mice were killed at 5 and 30 minutes and 6 and 24 hours after injection. Sagittal sections (30 to 60 μ), through the whole frozen animal, were cut with a model "K" microtome (R. Jung AG, Heidelberg) in a cold room (−10°C). The specimens were picked up with Scotch tape and dried for 24 hours at −10°C (*10*). The sections were then pressed onto Kodak RP/S X-omat medical x-ray film and exposed for 22 to 24 days. Radioactivity in the various animals and tissues was measured (*11*). Fetuses were first homogenized in five volumes of methanol. Unchanged ^{14}C-LSD was then

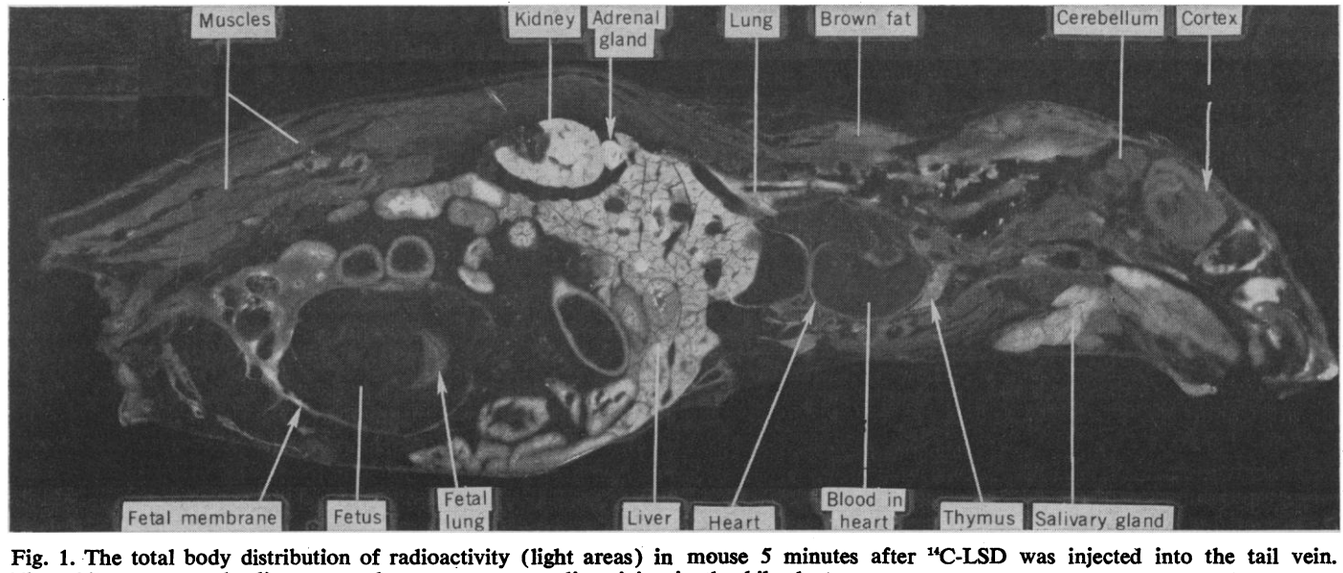

Fig. 1. The total body distribution of radioactivity (light areas) in mouse 5 minutes after ^{14}C-LSD was injected into the tail vein. The white spots on the liver parenchyma represent radioactivity in the bile ducts.

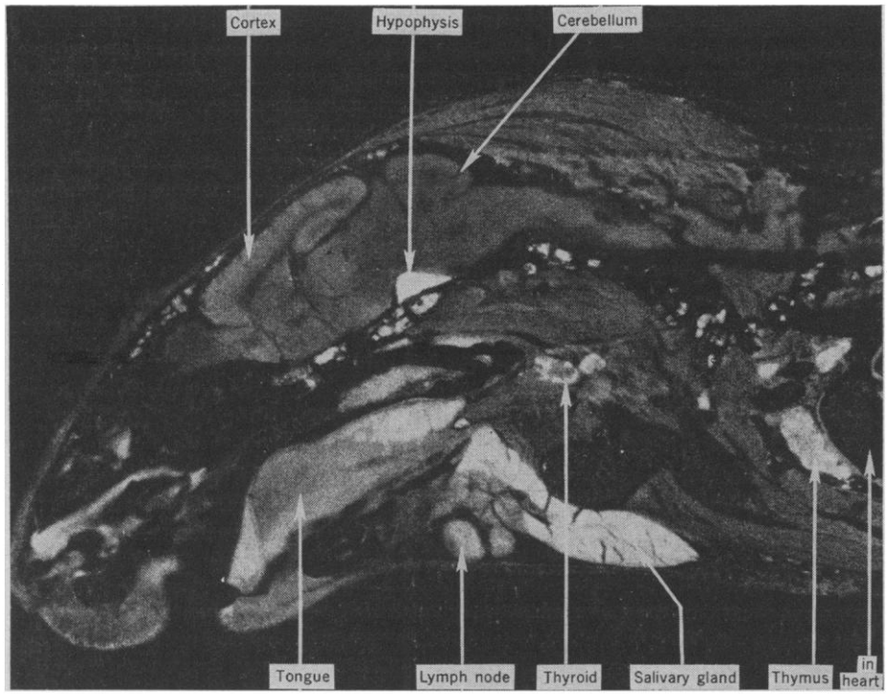

Fig. 2. Radioactivity (light areas) in mouse brain and in adjacent organs 5 minutes after injection of ^{14}C-LSD (× 4.9).

separated on thin-layer plates as described above, and its radioactivity was counted by liquid scintillation.

Five minutes after injection, radioactivity in the blood had rapidly decreased; that in the parenchymatous tissue and muscle had increased (Fig. 1). Compared to the blood, radioactivity in the adrenals, hypophysis, kidneys, liver, lungs, bone marrow, and lymphatic tissue was especially high. The amount of radioactivity in the brain and myocardium was also higher than that in the blood. These observations indicate LSD easily diffuses and penetrates cell membranes.

Excretion of the radioactivity in the kidneys and liver was evident by 5 minutes (Fig.1). High uptake was found in salivary and lacrimal glands, which suggests an excretory function of these organs for LSD (Fig. 1). Gastric excretion is evident, for radioactivity crossed the stomach wall into the contents. Radioactivity had gradually accumulated in the gut contents of animals killed 30 to 120 minutes after injection, first in the oral and then in the distal part of the small and large intestines. At the same time, the kidneys had less activity than the liver or, more especially, the gall bladder. Elimination of ^{14}C-LSD and its metabolites is primarly through the liver by way of the bile and gastrointestinal tract. After 24 hours, radioactivity was detected only in the liver and gut contents, thus indicating a rather long elimination time. However, by that time the major part of the radioactivity recorded represents the degradation products of the ^{14}C-LSD molecule, as reported by others (*8*) and as confirmed by our own studies. Like many other compounds, LSD and its metabolites may be reabsorbed from the gastrointestinal tract into the enterohepatic circulation. This recirculation may lengthen the pharmacological effects of LSD in animals and humans.

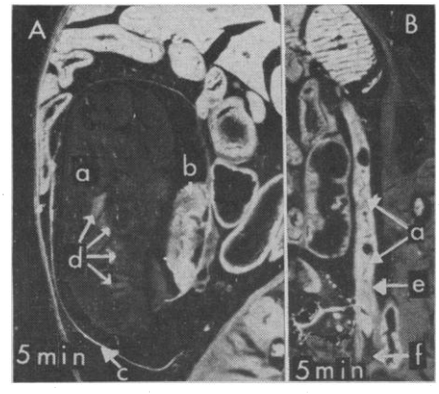

Fig. 3. Distribution of radioactivity in fetus (light areas) 5 minutes after injection of ^{14}C-LSD into tail vein of the mother. Animal A was in the last week of pregnancy and animal B in the first trimester of pregnancy. There is a high accumulation of radioactivity in the embryos of animal B. (*a*) Fetus; (*b*) placenta; (*c*) fetal membrane; (*d*) fetal lung, heart, liver, and intestine; (*e*) uterus; and (*f*) vagina.

The distribution of radioactivity in the brain was rather diffuse (Fig. 2). During the first 30 minutes, there was more radioactivity in the cortex than in the white matter. Thereafter (up to 24 hours), activities in gray and white matter were not significantly different. Slower penetration of white matter may be accounted for by the multiple membranes of the laminated myelin sheath (*12*).

Hippocampus and thalamus exhibited highest activity in the brain, and the cerebellum, medulla, and spinal cord showed about the same activity. The hypophysis, thyroid, and adrenal glands—and to a lesser degree the thymus, ovaries, and testes—had a great affinity for ^{14}C-LSD (Figs. 1 and 2). Piloerection, tachycardia, lacrimation, restlessness, and hyperreflexia were observed in all mice beginning 3 to 4 minutes after injection of ^{14}C-LSD and lasting about 40 to 60 minutes. The onset of these effects is correlated with a very high accumulation of radioactivity in the adrenals (Fig. 1). This fact suggests that the sympathomimetic efects of LSD occurring soon after administration in humans and animals may be caused not only by central sympathetic stimulation (*13*) but also by the liberation of catecholamines from the adrenal medulla. Whether the dense radioactive zone in the middle part of the adrenal cortex is causally related to the high cortical secretion of 17-ketosteroids and 17-hydroxycorticoids caused by LSD (*7*) is questionable.

Next to the adrenals, the hypophysis showed the highest accumulation of radioactivity (Fig. 2). More detailed localization of radioactivity in the hypophysis was not possible without microscopic autoradiography. The prolonged administration of LSD causes reduction in thyroid, uterine, and seminal vesicle weight, with corresponding hypothyroidism and hypogonadal function, but increases the adrenal weight and accordingly stimulates adrenal activity (*7*).

In the last week of pregnancy, about 0.5 percent of the administered radioactivity traversed the placenta and was found in the fetus by 5 minutes after injection. Of this radioactivity, over 70 percent was unchanged ^{14}C-LSD. The penetration rate of LSD through the placental barrier seemed to be great-

er during the first trimester of pregnancy (Fig. 3). The amount of radioactivity measured in mouse embryos during the first trimester of pregnancy, when they are most sensitive to teratogenic agents and LSD (*2–6*), was about 2.3 percent of the initial dose.

The distribution pattern of ^{14}C-LSD in the fetus was very similar to that in the mother (Fig. 3). The highest amounts occurred in the lungs, liver, intestine, brain, and myocardium, in that order. As in the mother, the fetal blood had a very low amount of radioactivity, an indication of a rapid transport of ^{14}C-LSD through cellular membranes into the tissues. The uptake in the placenta was highest 5 minutes after injection, but a moderate concentration remained for 1 hour after injection. The greatest radioactivity concentration in fetal organs was found at 30 minutes, with a significant amount remaining for at least 2 hours. The relatively high affinity of LSD for the maternal organs, causing a rapid decrease in the blood concentration, may diminish the amount available for transfer into the fetus.

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Carbon Monoxide–Induced Arterial Hypoxemia

Abstract. *Inhalation of carbon monoxide produces an increase in the alveolar to arterial oxygen gradient in the presence of veno-arterial shunts or ventilation-perfusion imbalance but has no such effect in normal subjects. The increase in the alveolar to arterial oxygen gradient with rising concentrations of carboxyhemoglobin results from changes induced by carbon monoxide in the shape of the oxyhemoglobin dissociation curve.*

Claude Bernard first pointed out that carbon monoxide (CO) produces hypoxia through its reversible combination with blood to form carboxyhemoglobin (*1*). On combining with hemoglobin, CO causes a functional anemia by decreasing the amount of hemoglobin available for carrying oxygen. In addition, the sigmoid shape of the oxyhemoglobin dissociation curve is changed with increasing carboxyhemoglobin concentrations ([COHb]) toward that of a rectangular hyperbola, with the result that hemoglobin gives up oxygen less readily in the tissues (*2*). Tissue hypoxia produced by these two factors is considered to be the major process involved in clinical CO toxicity (*1*), although it is possible that direct effects of CO on tissue respiration are also important.

There have been few studies of the possible effects of increasing [COHb] on arterial oxygen tension (Pa_{O_2}) and on the alveolar-arterial oxygen gradient ($A\text{-}a\ D_{O_2}$). Until recently it was assumed that Pa_{O_2} was unchanged by inhalation of CO (*1*), but there had been no direct measurements of the effect of CO on Pa_{O_2} until Ayers, Giannelli, and Armstrong (*3*) presented evidence that Pa_{O_2} decreased in a group of patients who breathed concentrations of CO sufficient to produce [COHb] of 5 to 10 percent. These authors later reported, in abstract form, large decreases in Pa_{O_2} and increases in $A\text{-}a\ D_{O_2}$ in dogs given higher concentrations of CO (*4*). As an explanation for their findings they postulated that "carboxyhemoglobin containing red blood cells may impose an abnormal barrier to diffusion of oxygen," or that a "decrease in capacity of the blood to carry oxygen can be shown to magnify the physiological veno-arterial shunting."

In this report we have attempted to determine if arterial hypoxemia may be an additional cause of tissue hypoxia in CO poisoning and to define the possible mechanisms of such hypoxemia.

The effects of the combination of CO with hemoglobin can be compared to the effects of anemia, since in both situations the oxygen-carrying capacity of the blood is decreased. Figure 1 shows the upper portion of oxyhemoglobin dissociation curves calculated for the functional anemia resulting from increasing [COHb] and for the anemia resulting from decreased hemoglobin in the blood. An important difference between the two curves is that the sigmoid shape of the dissociation curve is changed with increasing [COHb] but is not altered with increasing degrees of anemia. It has long been recognized that the shape of

Table 1. Change in $A\text{-}a\ D_{O_2}$ with increased [COHb]. There were no significant changes in oxygen uptake, respiratory quotient, or alveolar ventilation following CO inhalation in any of the subjects. The right to left shunt was 24 percent of cardiac output in patient A.S. and 37 percent cardiac output in patient G.S. The number of normal subjects is given in parentheses; S.D., standard deviation.

Subject	[COHb] (%)		$A\text{-}a\ D_{O_2}$ (mm-Hg)	
	Initial	Final	Initial	Final
Normals (5)	0.9	11.7	12.1	11.6
S.D.	±0.1	±4.7	±4.9	±5.9
P value		< .005		> .5
Shunt				
A.S.	2.1	12.8	36.5	46.9
G.S.*	1.0	12.7	56.7	62.8
$\dot{V}/\dot{Q}$				
J.J.	2.1	12.8	38.0	41.0
R.G.	1.7	11.5	39.8	42.6

* 2, 3-Diphosphoglycerate was elevated in this patient (*8*) which suggests that the oxyhemoglobin dissociation curve was shifted to the right (*9*). This shift might, in part, counteract the effect of [COHb] on $A\text{-}a\ D_{O_2}$.