Comparative Teratogenicity of Isoproterenol and Trypan Blue in the Fetal Hamster* (33745)

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Previous investigation (1) by the author has shown that the psychotomimetic compound, mescaline, was capable of inducing congenital malformations in the hamster. It is postulated that other compounds structurally related to it might also possess teratogenic properties. Whether or not some of the same structural configurations are responsible for both the psychotomimetic and teratogenic properties is also of importance.

Isoproterenol, a sympathomimetic drug used extensively for the treatment of asthma and certain cardiac irregularities, represents a molecule which is structurally related to both the naturally occurring catecholamines and some of the psychotomimetic compounds, *i.e.*, mescaline, psilocybin, 2,-5-dimethoxy-4-methyl-alpha-methyl phenethylamine as well as epinephrine and norepinephrine. However, isoproterenol produces only a limited alteration in higher cerebral activity or behavior (2, 3).

The present study was designed to evaluate the teratogenic potential of isoproterenol, and to compare its activity to that of trypan blue, a proven teratogen (4) with a molecular structure having no resemblance to isoproterenol. In addition, trypan blue possesses no reported behavior-altering properties.

Methods. Pregnant random-bred golden (Lakeview Colony) Syrian hamsters were maintained in individual cages in air-conditioned quarters (75°F) with natural light available through large roof skylights. The females had *ad libitum* access to water, Purina chow, carrots, and lettuce throughout the experimental period. Noise within the animal quarters was kept to a minimum since the noise factor has been demonstrated to exert significant influence on various biochemical, anatomical, and developmental parameters in both the adult and fetal organism (5).

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Both isoproterenol and trypan blue were dissolved in sterile solution 30 min before use. The compounds were administered as a 1-ml volume, single, subcutaneous injection into the pregnant female on the eighth day of gestation since this time has been shown to be an effective period to elicit any teratogenic potential a drug may possess (6). A group of control animals received similar 1-ml injections of saline solution. All groups of animals were returned to their cages and allowed to remain undisturbed until day 12 of pregnancy at which time they were killed in a 500-ml Pyrex beaker containing cotton saturated with ether. When maternal respiration ceased, the uterus was exposed by midline incision and the number and distribution of fetuses was noted. The viability and developmental status of each fetus was determined as it was exposed within the uterine lumen. Each fetus was then removed and placed in 10% formaldehyde for 3 days to allow for tissue hardening in order that a more detailed examination for additional anomalies could be carried out.

Results. The comparative data on isoproterenol and trypan blue are shown in Table I and are contrasted with saline injected control animals. There were no major anomalies found in the control fetuses although a limited number of runts, dead, and reabsorbed fetuses were noted. In general, it was found that a dose–response relationship existed for all parameters measured in the present study, *i.e.*, as the concentration of either isoproterenol or trypan blue was increased, the number of fetuses affected was increased.

The types of anomalies observed with the two compounds were as follows: trypan blue produced exencephaly, meningocele, umbilical hernia, bent tails, and general and local body edema; isoproterenol produced exencephaly, bent tails, umbilical hernia, general and local edema, anophthalmia, myelocele,

TABLE I. Comparison of Fetal Effects of Maternal Administration of Isoproterenol and Trypan Blue.

Dose[a] (mg/kg of body wt.)	No. of females	No. of fetuses (av)	Congenital abnormalities (%)	Runts (%)	Dead fetuses (%)	Resorptions (%)
			Isoproterenol			
0.003	8	12.0	5	4	3	4
0.068	8	11.6	6	6	8	8
0.230	10	10.6	9	7	14	7
5.000	10	9.5	13	8	15	12
18.500	9	9.0	19	8	15	17
34.800	9	8.1	18	10	14	19
			Trypan blue			
0.130	9	10.0	4	3	14	10
0.350	10	9.2	4	4	16	18
1.020	9	8.3	9	4	19	15
2.330	9	5.8	20	7	26	24
			Control			
Saline (0.9%)	25	12.0	0	1	1	2

[a] Dose—all injections 1-ml volume.

microcephalus, hydrocephalus, external heart, and a number of fetuses exhibiting multiple developmental defects.

Discussion. Recent studies demonstrated that the administration of isoproterenol to adult rats resulted in a significant increase in the size of the salivary glands due to hyperplasia and hypertrophy. In addition, polyploid nuclei were common in the enlarged cells of the glands (7–9). These effects of isoproterenol were considered to be of a direct action on the tissues by the drug since it was demonstrated (10) that superior cervical ganglionectomy did not prevent or block the salivary gland enlargement or increased DNA synthesis. However, pretreatment with propranolol, a beta adrenergic receptor blocking agent, prevented isoproterenol-induced cardiomegaly and salivary gland enlargement (11).

In the present study it is suggested that isoproterenol may have produced the disruptions of normal fetal development by altering the pathways of DNA synthesis within the cells of certain organs and tissues during critical differentiation phases. Coupled to this reaction, hyperplasia and hypertrophy may have played a role dependent upon the extent of disruption of the normal cellular metabolic pathways.

While still controversal, trypan blue has been implicated in the production of an abnormal type of serum protein (12), an alteration in the relative percentages of the alpha and beta serum globulins (13), and has been postulated to inhibit thyroxine release from the thyroid gland by inhibition of cellular cathepsin (13). These several factors may have contributed to the mechanism whereby trypan blue produced its effects on the fetus since the above mentioned abnormal serum protein percentages have been associated with stillbirths, miscarriages, and congenital malformations (14); and decreased thyroxine release could lead to a decrease in oxidative metabolism in the pregnant rat which in turn could result in a decreased supply of oxygen and essential nutrients to the fetus (13).

Summary. Isoproterenol, administered subcutaneously in a single dose on the eighth day of gestation to pregnant hamsters produced a variety of gross malformations of the brain, eyes, spinal cord, heart, liver, and skeleton. General and localized body edema was found alone or in conjunction with other anomalies. In addition, the percentages of dead, resorbed, or runt fetuses were increased over those found in control litters. The qual-

itative and quantitative parameters of the teratogenicity of isoproterenol were compared to those of trypan blue.

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The Metabolism of Glyceryl Thioethers* (33746)

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Alkyl O-ethers of glycerol are readily cleaved by a pteridine-requiring enzyme found in mammalian livers (1, 2). The activity of the cleavage enzyme is lower in other tissues and varies among species (2); neoplasms that contain high levels of ether-linked lipids (3) appear to lack the cleavage enzyme (4). Although the general reaction sequence for the cleavage has been postulated (1), experimental evidence on the actual mechanism is lacking.

We believed that the glyceryl thioethers (S-ethers) would be valuable model compounds to shed light on the reaction steps involved in the biocleavage of the *O*-alkylglycerols, since we could isotopically label the thioethers at the three critical positions of the glyceryl ether molecule (the ether linkage with ^{35}S, the alkyl chain with ^{3}H or ^{14}C, and the glycerol moiety with ^{3}H or ^{14}C). Glyceryl thioethers have not yet been isolated in nature nor is anything known about their behavior in biological systems although their chemical synthesis (5) and their physical properties and quantitative analysis (6) have been investigated. Therefore, our initial feeding experiments with rats were directed toward comparing the metabolism of alkyl O- and S-ethers of glycerol having identical 1-^{14}C-alkyl moieties.

Methods. The synthesis and radiopurity of the 1-^{14}C-hexadecyl-O-glycerols (6.35 μCi/mg) used in this study were previously described in detail (7). The 1-^{14}C-hexadecyl-S-glycerol (2.01 μCi/mg) and 1-^{14}C-octadecyl-S-glycerol (1.95 μCi/mg) were synthesized (5) from 1-thioglycerol and 1-^{14}C-hexadecyl bromide or 1-^{14}C-octadecyl bromide, respectively, under conditions similar to those described by Lawson *et al.* (8). The 1-isomers were racemic mixtures. Radiopurities of all compounds used in our study were >98% as determined by zonal profile scans (9, 10) prepared after thin-layer chromatography on Silica Gel G in a solvent system of hexane:diethyl ether:methanol:acetic acid (80:20:10:1, v/v). The chemical purities of the O-ethers

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