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Exposure of Aquarium Fish to Dimethyl Sulfoxide (DMSO) with Special Reference to Toxicity and Effects on Uptake of Radioactive Dyes.* (30967)

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It has been reported that dimethyl sulfoxide (DMSO) increases cell membrane permeability and thereby enhances and potentiates the activity of drugs and toxins(1-7). It was felt that this property might prove a valuable adjunct to the use of various fluorescein dyes and radioactive substances currently employed as diagnostic and therapeutic agents.

Aquarium fish were utilized as research models since they could be readily exposed to a steady and constant environment of DMSO. They are also well adapted to study the total body uptake of fluorescent dyes and radioactive materials. The glowlight tetra (*Hemigrammus erythrozonus*-Durbin) was most frequently employed. Other species used in smaller numbers included neon tetras (*Paracheirodon innesi*), platys (*Xiphophorus maculatus*), mollies (*Pescilia latipinna*), guppies (*Poecilia reticulata*), zebras (*Brachydanio rerio*) and catfish (*Corydoras paleatus*). The volume of water per aquarium was about 45 ± 1 ilters. The water employed was distilled and maintained at a temperature of 24-25°C and a pH of 6.8 ± 0.2. Aeration was maintained by means of a pump with a delivery rate of 100 ± 2 ml per minute. The aquaria were algae-free. The fish were fed once daily with 100 mg of a commercially available fish food preparation.†

The work was divided into two phases. 1. *Determination of LD_{50} levels.* Initial studies were performed with the glowlight tetra. Groups of 10 fish were exposed to various concentrations of DMSO. A concentration of 1.9% DMSO killed one-half of fish in the aquarium in 48 hours. Fish surviving a concentration of over 1.9% DMSO for 48 hours appeared to suffer no permanent ill effects when removed at the end of this period and placed in water containing no DMSO. They survived their usual 3 to 4 month life expectancy in the laboratory and reproduced normally.

Toxicity in the glow tetra was first manifested by erratic behavior, such as backward swimming and standing on their tails, suggesting disturbances in neurologic function or abnormalities in vision or maintenance of equilibrium. This was followed by progressive anasarca and death.

Histologically, changes were most prominent in the subcutaneous tissues where cellular enlargement, rupture and an increase in interstitial fluid were noted. These changes resemble those described in the human skin (8).

Neon tetras, platys, mollies and guppies had LD_{50} concentrations very similar to that of the glow tetras (1.9% DMSO) (Table I).

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† Biorell, Manufactured by Long Life Fish Food Products, Harrison, N. J.

TABLE I. Percent of Different Aquarium Fish Lost When Exposed to 1.9% DMSO.

Species*	No. of fish tested	% of fish lost during time period (days) 0–5	6–10	11–15	16–20
Neon tetras	20	50	50	—	—
Glow "	120	100	—	—	—
Black mollies	20	20	60	10	10
Guppies	40	25	25	25	25
Platys	6	16	48	20	16
Crescents	6	0	50	—	—
Zebras	6	0	0	0	0

* Both sexes.

Zebras and catfish had higher LD_{50} concentrations of over 2.5% DMSO. Neither of these species showed evidence of toxicity after prolonged immersion in 2.5% DMSO solution. One group of Zebras has been kept at this concentration for over 3 months without apparent ill effects.

A striking difference in sex sensitivity to DMSO was noted in the sensitive species. In almost every instance all male fish died before any females succumbed (Table II).

2. *Radioactive dye studies.* Diiodofluorescein tagged with ^{131}I was used in the first series of experiments. Six glow tetras were placed in each of 2 aquaria and 10 mg of the radioactive dye containing 100 microcuries were added to each tank. DMSO was added to one aquarium in sufficient quantity to produce a concentration of 1%. At 2 to 4 hour intervals fish were removed from each tank and assayed for total body radioactivity in a crystal well scintillation counter. Considerable overlap in the amount of radioactivity was noted in the 2 groups and no significant difference could be determined.

TABLE II. Survival of Male and Female "Guppies" and "Black Mollies" in 1.9% DMSO.

Species	Days	No. of males lost	No. of females lost	Totals
Guppies (16)	0– 5	4	0	4
	6–10	3	1	4
	11–15	1	3	4
	16–20	0	3	3
	20–25	0	1	1
	Totals	8	8	16
Black mollies (20)	0– 2	4	0	4
	3– 4	4	1	5
	5– 6	2	2	4
	7– 8	0	3	3
	9–10	0	3	3
	11–12	0	1	1
	Totals	10	10	20

Rose aniline labeled with ^{131}I and ^{203}Hg chlormerodrin were used in a similar manner. There were no significant differences in uptake between the control group and those fish exposed to DMSO.

Although the mean radioactive uptake appeared to be higher in the fish exposed to DMSO for 8-24 hours, the standard deviation was high and the figures are statistically unreliable.

TABLE III. "Uptake" of Radioactive Dyes by "Glow Tetras" With and Without 1% DMSO.

Time (hr)	Dye	"Uptake" by groups of (6) fish in counts/min With 1% DMSO	Control
0– 2	Diiodofluorescein	174	160
2– 8	^{131}I	258	236
8–24		600	300
	Difference in avg count increase DMSO *vs* control 300 ($P = .20$)		
0– 2	Rose aniline ^{131}I	25	20
2– 8		67	47
8–12		120	50
	Difference in avg count increase DMSO *vs* control 70 ($P < .10$)		
0– 2	Chlormerodin ^{203}Hg	894	705
2– 8		780	780
8–24		3010	2320
	Difference in avg count increase DMSO *vs* control 690 ($P > .50$)		

Summary. The LD_{50} concentration of DMSO was found to be 1.9% in the case of glow tetras, neon tetras, guppies, mollies and tetras. Zebras and catfish appear to tolerate higher concentration of DMSO. In addition to the species differences, a striking increase in sensitivity to DMSO was noted in males. In almost every instance, all male fish died before the first female succumbed. No consistent difference in uptake of radioactive labeled dyes was noted in a group of fish exposed to DMSO when compared with a control group of fish not exposed to DMSO.

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Effects of a Hepatocarcinogenic Diet on Adrenal Glands and Liver Of Rats. (30968)

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There is an increasing interest in the hormonal influences in liver carcinogenesis and the role adrenal glands play in it(1-7). The adrenal glands of rats bearing primary hepatomas induced by azo-dye, show marked histological changes including heavy deposit of lipoid droplets which stain deep red with scharlach R(4). These observations suggested further study of the chemistry of these lipoids of the adrenal glands of rats during azo-dye carcinogenesis.

This paper reports the effect of a low-protein, low-riboflavin diet alone and with an azo-dye carcinogen on the chemistry of the adrenal glands and liver of rats during the induction period of the primary hepatoma and after.

Materials and methods. Rats of the NIH Osborne-Mendel strain were used in all experiments. They were about 3 months old at the start of experiments and males weighed 150-175 g and females 100-125 g. One group of rats was used to study the effects of the hepatocarcinogenic diet and a second group to study the effects of hormonal changes on the chemistry of the adrenal glands and liver.

The diets used in these experiments were: 1) Purina Laboratory Chow Pellets—standard control diet; 2) Semi-Synthetic Diet—low protein, low riboflavin diet as described before(8); and 3) Hepatocarcinogenic Diet—semi-synthetic diet in which 0.06% N,N-dimethyl-*p*-(m-tolylazo) aniline (3'-Me-DAB) was incorporated.

The early effects of hepatocarcinogenic diet on the chemistry of the adrenal glands and liver were studied by feeding hepatocarcinogenic diet to male rats for 2, 5, 7 and 17 weeks. Rats fed on Purina Laboratory Chow pellets and those fed on semi-synthetic diet were used as controls.

The effects of hormonal changes were studied on male and female rats, intact or gonadectomized. A month after the surgical procedures, the rats were fed the hepatocarcinogenic diet for 17 weeks and the experiment terminated 4 weeks later. During the experimental period some animals received implants of hormone pellets. In all there were 6 subgroups according to treatment as listed in Table 2. Hormone pellets were prepared in melted cholesterol and a weighed pellet containing 50 mg of hormone was implanted under the skin at the start of the carcinogenic diet and another 8 weeks later. Diethylstilbesterol and testosterone pellets were implanted in gonadectomized males and females, respectively.

There were 10 rats in each sub-group and all animals were housed singly in partitioned cages, with individual feeding jars and water bottles. The animals were examined every day except Saturday and Sunday and their food intake checked. Rats were killed at the end of the experimental period and each rat examined for macroscopic lesions, and tissues were taken for microscopic and chemical study.

The adrenal glands and livers were analyzed for total fat, "steroids,"* and ascorbic acid. Some samples of adrenal glands were

* This includes cholesterol, corticosteroids and the intermediates found in the adrenal gland and reacting with ferric chloride (11).