Influence of Antihistamines on Mast Cell Disruption Following X-Irradiation.* (23905)

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It has been shown that tissue mast cells contain histamine(1), and that they undergo widespread disruption in animals exposed to x-irradiation(2-3). More recently, the claim has been made(4) that antihistamine, pyrilamine maleate, prevents destruction of mast cells seen in the x-irradiated hamster. The present paper is concerned with experiments in which antihistamine was administered to the irradiated rat and irradiated hamster. The results suggest that the protective effect is species specific and confined to the region of application of the antihistamine.

Methods. At various times after treatment with x-rays and antihistamines, male, 200-g Sprague-Dawley rats and 100-g Syrian hamsters were sacrificed with ether anesthesia. Samples of mesentery, skin and cheek pouch were removed, prepared as whole mounts(2), stained with toluidine blue(2) and examined under microscope at magnifications up to 1000 ×. To evaluate damage from irradiation and protection by antihistamine treatment, separate counts were made of typical mast cells and of all other cells containing metachromatic inclusions. Of the latter a few are abnormal mast cells(2), while most are fibroblasts and macrophages which contain metachromatic material phagocytized from disrupted mast cells. Thus this count is an indication of amount of damage and disruption of mast cells. Irradiations consisted of single, whole-body exposures to x-rays (250 KV, 15 ma, 0.5 mm Cu and 3.0 mm Bakelite filters, 26.7 cm target distance, 1.5 mm Cu half-value layer, and 215-225 r/min). The antihistamines, pyrilamine maleate† (Neo-Antergan, Merck) and promethazine hydrochloride (Phenergan, Wyeth) were dissolved in Tyrode's solution and administered intraperitoneally each day beginning on day of irradiation.

Results. Both in rat and hamster, x-irradiation was followed by significant damage and disruption of mast cells, as indicated by increases in number of abnormal mast cells and of macrophages and fibroblasts containing metachromatic material (Table I). Administration of pyrilamine maleate after exposure of hamster to 900 r greatly reduced the signs of mast cell damage in the mesentery but not in the cheek pouch or skin (Table I). In similar experiments, not tabulated here, similar results attended treatment with pyrilamine maleate after exposure of hamster to 600 r or 1200 r. In the irradiated rat pyrilamine maleate was without influence on mast cell damage (Table I).

Antihistamine, promethazine hydrochloride, in dosage of 5.0 mg/kg/day was without influence on disruption of mast cells in the mesentery, skin and cheek pouch of hamsters exposed to 900 r or 1200 r. In these experiments groups of 3 animals were sacrificed on days 1, 2, 3, 5 and 7 after irradiation and/or treatment with promethazine hydrochloride. Administration of 25 mg/kg/day of this compound to irradiated hamsters was attended by greater evidence of mast cell disruption in the mesentery than that caused by x-radiation alone (Table I). In nonirradiated hamsters this dosage of promethazine hydrochloride over 6 days caused complete disappearance of mesenteric mast cells (Table I) but had no effect on mast cells of the cheek pouch.

Discussion. The present evidence for damage to mast cells as a result of x-irradiation agrees with our previous findings(2,3,5). The present data clearly show that in the x-irradiated hamster, pyrilamine maleate prevents most or all damage to mast cells of the mesentery but does not affect such damage in skin and cheek pouch. The experiments do not yield information either on the mechanism of protection or on the basis of restriction of protection to the region of application of the anti-

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TABLE I. Influence of Antihistamines on Damage to Mast Cells in the X-irradiated Animal.

Treatment	Days after irrad.	Cell counts*: Mesentery Typical mast cells	Mesentery Phagocytes†	Cheek pouch Typical mast cells	Cheek pouch Phagocytes	Skin Typical mast cells	Skin Phagocytes
I. Hamster—Pyrilamine maleate							
900 r	1	225	20	412	1	652	0
	2	206	85	748	3	726	3
	3	186	63	452	6	463	16
	5	143	94	643	33	408	8
	7	100	133	349	52	372	47
900 r + pyrilamine maleate, 5.0 mg/kg/day intraper.	1	253	2	138	2	539	2
	2	237	2	524	4	547	3
	3	298	5	373	16	553	27
	5	168	39	345	78	145	52
	7	236	20	264	113	421	16
Pyrilamine maleate, 5.0 mg/kg/day intraper.		257‡	3	407	1	512	0
Untreated controls		245	2	603	0	603	0
II. Rat—Pyrilamine maleate							
800 r	2	128	45			374	173
	5	72	29			251	71
	7	102	7			264	3
800 r + pyrilamine maleate, 5.0 mg/kg/day intraper.	1	171	70			476	109
	2	194	34			328	73
	3	156	27			331	215
	5	154	9			251	29
	6	81	18			283	9
	7	114	7			327	0
Pyrilamine maleate, 5.0 mg/kg/day intraper.		192‡	1			583	1
Untreated controls		201	2			623	4
III. Hamster—Promethazine hydrochloride							
900 r + promethazine hydrochloride, 25 mg/kg/day intraper.	1	94	335	376	7		
	2	71	61	365	6		
	3	158	128	431	38		
	5	0	0	550	88		
	7	0	0	265	79		
Promethazine hydrochloride, 25 mg/kg/day intraper.	1	182	0	639	2		
	2	70	159	262	3		
	3	159	0	1201	12		
	5	0	0	499	0		
	6	0	0	365	0		
	7	15	18	661	5		

* Cells per 2.028 mm². Each value represents avg of 3-6 animals.

† Comprised of fibroblasts and macrophages containing metachromatic material and a few abnormal mast cells.

‡ Avg of 18 animals—3 animals sacrificed on each of days 1, 2, 3, 5, 7, and 9. Values are lumped since there were no significant differences from day to day.

histamine. It is of interest to note, however, that pyrilamine maleate has been reported(1) to prevent the disruption of mast cells caused by injection of stilbamidine, d-tubocurarine and agar serotoxin.

Our finding that pyrilamine maleate failed to reduce disruption of mast cells in the irradiated rat demonstrates a distinct species specificity for the effectiveness of this compound. The absence of protection in hamsters treated with promethazine hydrochloride shows that not all antihistamines are effective.

The mechanism of damage to mast cells by higher dosages of promethazine hydrochloride is not shown by present data. It should be noted, however, that this destructive effect was restricted to the region of application and did not involve mast cells in distant tissues.

Summary. X-irradiation was followed by evidence of marked damage to mast cells in mesentery, skin and cheek pouch of rat and hamster. Daily intraperitoneal injection of pyrilamine maleate largely prevented damage to mast cells in the mesentery but not to those in skin and cheek pouch of the irradiated hamster. Pyrilamine maleate offered no protection against damage to mast cells in tissues of the irradiated rat. Intraperitoneal injections of promethazine hydrochloride in low dosage failed to protect mast cells against radiation damage in any tissue of the hamster. In high dosages this compound destroyed mast cells of mesentery of the nonirradiated hamster, but did not affect those of skin and cheek pouch.

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Effect of Tetracyclines on Formation of Amines by Bacteria.* (23906)

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Many intestinal bacteria are known to produce amines from amino acids(1). Melnykowicz and Johansson(2) found that rats fed small amounts of tetracyclines contained in their gastrointestinal tract less amines than control animals. They found that tetracyclines interfered with formation of some amines by the intestinal flora.

The effect of tetracyclines on formation of amines by single strains of bacteria isolated from human feces was therefore studied. Formation of isoamyl amine and isobutyl amine by *Proteus vulgaris* and of tyramine and phenyethyl amine by *Streptococcus fecalis* is the subject of the present communication.

Methods. Test organism and antibacterial assay. Str. fecalis and *Proteus vulgaris*, isolated from feces of patients suffering from gastro-enteritis were used. Sensitivity towards antibiotics was determined by 2-fold dilutions in nutrient broth (Difco). *Str. fecalis* was inhibited by 2 μg/ml oxytetracycline hydrochloride, tetracycline hydrochloride or chlortetracycline hydrochloride after 24 hours of incubation. *Proteus vulgaris* was inhibited by 50 μg/ml tetracycline hydrochloride or by 20 μg/ml chlortetracycline hydrochloride after 24 hours of incubation. *Culture media.* For amine production a casein hydrolysate medium of the following composition was used:

Casein hydrolysate vit. free (NBC) 10%	3	ml
Yeast extract (Difco)	.5	g
Glucose	.1	g
Sodium chloride	.5	g
Tryptophan	.002	g
Salt solution	1	ml
Distilled water to	100	ml

The salt solution was composed of: $MgSO_4 \cdot 7H_2O$ – 22.5 g, $CuSO_4 \cdot 5H_2O$ – 0.5 g, $ZnSO_4 \cdot 7H_2O$ – 0.4 g, $MnCl_2 \cdot 4H_2O$ – 0.15 g in distilled water to 500 ml pH adjusted to 5.5 and the medium autoclaved 20 minutes. For manometric experiments the strains were grown on brain heart infusion medium (Difco) with 1.5% agar. After incubation at 37°C for 14-18 hours the cells were harvested, washed 3 times with 0.85% sodium chloride solution and kept at –5°C until used. *Analytical methods. Determination of volatile amines by DNFB method.* Volatile amines were quantitatively determined by colorimetric method based on dinitro fluorobenzene (DNFB) reaction(3): 1 ml of a sample was alkanized by addition of 2 ml 40% potassium hydroxide solution and steam distilled

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