Treatment of Cystinuria with α-Mercaptopropionylglycine: A Preliminary Report with Some Notes on Column Chromatography of Mercaptans* (33461)

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D-Penicillamine[1] has proved useful in the treatment of cystinuria, by virtue of undergoing the disulfide exchange reaction with cystine to form the soluble unsymmetrical mixed disulfide of penicillamine and cysteine [see Ref. (1) and its bibliography]. Cumulative experience now reported in the literature makes it apparent that side-effects are to be expected in as many as 40% of patients so treated. The most serious is a long delayed proteinuria in an as yet incompletely determined proportion of patients (2–4).

A number of other mercaptans have been suggested or tested as a substitute for penicillamine (1, 5). This report describes preliminary clinical results with the most promising one at present, α-mercaptopropionylglycine (MPG),[2] an analog of penicillamine.

This drug has been examined by Japanese workers for its potentialities in other clinical applications. Its acute i.v. LD_{50} for mice is reportedly 2.10 g/kg of body weight (6), as compared to 5.27 for D-penicillamine (7); 30–100 mg/kg doses per os to rats every day for 6 months produced no sign of toxicity (8); no teratogenic effect was found (8). In clinical investigations in Japan involving over 1000 patients (100–1000 mg/day), only 5 cases of side-effects were seen, consisting of itching, temporary nausea, or facial hyperemia (8). Thus there is reason to believe that MPG will be a more desirable, and perhaps less expensive, substitute for penicillamine.

Methods. The MPG was administered in gelatin capsules to 7 cystinurics selected for their intelligent cooperativeness; 5 of them had been on a penicillamine regimen for over 6 months; this drug and the accompanying urine alkalinizers were discontinued some days before beginning the administration of MPG. The 400-mg doses were distributed over the day and careful 24-hr urine specimens collected for analysis.

Twenty-hour cation exchange column chromatographic analyses were carried out as previously described (9, 10). The peaks were measured with an Infotronics CRS-10A integrator. Urine pH was measured electrometrically.

Results. Analytical. The MPG emerges from the column 2 min before taurine. An unidentified 0.8% impurity[3] emerged 5 min earlier. Emergence time and some other data for MPG and some other mercaptans are compared in Table I.

The ninhydrin-MPG reaction products have a 570/440 mμ color yield ratio of 3.7 and color is proportional to concentration up to at least 1 μmole.

Purple color with ninhydrin was unexpected, since most mercaptans that we have examined give a red color, grading off to yellow in lesser concentrations. It cannot be directly attributed to a contribution from the secondary amine, since MPG disulfide gives only a faint yellow color with ninhydrin; the color yield at 570 mμ is less than 1% of that of the standard and consequently MPG disulfide is not seen in the aminogram in the concentrations encountered in urine analyses. (The disulfide was prepared by reaction with Ellman's reagent (12); air oxidation at

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[1] Supplied as Cuprimine capsules by Merck, Sharp and Dohme, West Point, Pa.

[2] Supplied by Santen Pharmaceutical Co., Ltd., Osaka, Japan, through their licensee, Calbiochem, Los Angeles, Cal. (A grade, analyzed).

[3] Expressed in terms of color yield (peak area) compared with that of the standard, cysteic acid (11).

TABLE I. Chromatographic Data on Some Mercaptans.

Compound	Emergence time (min)	570/440 mμ ratio	570 mμ color yield (% of std.)[a]
MPG	52	3.67	28
MPG-MPG disulfide	80		0.9
MPG-cysteine disulfide	140–150		80
β-Mercaptopropionylglycine	55	0.281	0.36
α-Mercaptopropionic acid	67	0.190	0.042
β-Mercaptopropionic acid	85	0.157	0.033
N-Acetylcysteine	37		
Mercaptoacetic acid	35		
Thiomalic acid	54		
Thiolbutyric acid	80		
1:4-Dimercaptobutane-2:3 diol	75		
Diethylaminoethanethiol	1080		
2-Aminoethanethiol	Not eluted		
Thiolacetic acid	62		

[a] Standard was cysteic acid (11).

pH 9 for 6 hr did not produce measurable disulfide.) In test tube experiments it was found that when MPG is in molar excess over ninhydrin in the reaction only a red color is produced, whereas only a purple color is seen when ninhydrin is in excess. An analogous compound, *N*-acetylcysteine, similarly gives only a purple color with ninhydrin excess, as does thiomalic acid in a narrow (2-fold) range of concentration. In contrast, the other compounds in Table I give only the red-yellow color with ninhydrin.

The mixed disulfide of MPG and cysteine (MPG-Cys) was prepared by bubbling air through a mixture of 5 μmoles of MPG and 1 μmole of cystine /ml at pH 9 for 20 hr. Under these conditions only 20% of the cystine remained unreacted and two adjacent peaks of almost equal height appeared in the aminogram just before the threonine position (9). These were evidently the D,L- and L,L-diastereoisomers, which were not distinguished and will be called I and II, in order of emergence. Th 570/440 mμ absorption ratio for I and II was 2.4 and 2.3, respectively.

Clinical. When urine was collected from a normal individual 2 hr after a 400 mg MPG/hr dose, the subsequent urinary aminogram was normal except for the absence of cystine and the presence of compounds I and II in the ratio of 1.1; no unchanged MPG was seen. After hydrolysis the glycine content of this urine increased from 1.5 to 15.0 μmole/ml; an estimated 80% of the drug was thus unaccounted for.

A cystinuric was given 400 mg of MPG/hr for 5 hr and urine was collected at 1.5 and 5 hr. Some analytical results are given in Table II. Serum amino acids at hour 5 were at normal levels except for a low taurine (22.7 μmoles/liter), a high leucine peak (138 μmoles/liter) and the presence of less than 1 μmole cystine/liter. Hydrolysis and rechromatography of the 5-hr urine showed the expected increase in glycine (equivalent to about 1.0 mg of MPG/mg of creatinine and cysteine, and disappearance of peaks I and II, which supports their identification.

TABLE II. Urinary Excretion of Cystine, Taurine, and Isomers of α-Mercaptopropionylglycine (MPG) by a Cystinuric after an Oral Dose (400 mg of MPG/hr for 5 hr).

Time after first dose (hr)	(mg/g of creatinine)				
	Cyst	Tau	I	II	I/II
0	256	11.3	—	—	—
1.5	153	18.6	379	296	1.28
5.0	58	28.9	156	87	1.79

On a dose of 400 mg of MPG 5 times daily less than 2%, if any, of the drug was excreted in its original form, as demonstrated

TABLE III. Changes in 24-hr Excretion of Some Urinary Amino Acids by Cystinuries during Three Days of Treatment with α-Mercaptopropionylglycine, Compared with the Effect of D-Penicillamine.[a]

					(mg/24 hr)										
Subject	Dose (g/day)	Duration	No pen (days)	Urine pH	Tau	Gly	Cyst	Cys–Homo	Orn	Lys	Arg	I/II	I + II	Dis–Cys	Total Cyst.
1	0		Pre-pen	—	9	160	752	110	271	1960	437	—	—	52	804
	2	4 days		—	152	—	222	—	—	—	—	—	(1201)	543	765
	0		28	6.7	27	125	866	88	304	1608	270	—	—	42	908
	2			6.5	11	200	383	26	304	938	390	0.15	1400	596	979
	1.6			6.5	11	229	495	36	334	1012	432	0.14	1291	549	932
	1.2			8.7	9	149	307	25	323	1565	346	1.17	533	227	534
2	0		Pre-pen	6.8	27	166	839	103	477	2255	1328	—	—	49	888
	0		3	6.6	120	281	1200	29	557	2380	1780	—	—	14	1214
	2			6.8	127	257	417	—	455	1500	1348	(not measurable)			—
	2			6.5	124	230	247	—	547	1925	1495	2.08	1285	548	795
	2[b]			5.8	89	225	572	5	698	2690	1245	1.64	1491	634	1206
3	0		Pre-pen	—	10	107	820	102	318	1020	44	—	—	49	869
	2	3 days		7.0	143	137	337	—	610	1315	630	—	(886)	396	733
	4	7 mo.		—	175	—	211	—	—	—	—	—	—	—	—
	4	14 mo.		—	281	—	204	—	369	1000	326	—	(720)	323	527
	0		10	6.8	168	115	1020	54	472	1880	558	—	—	26	1046
	2			6.1	277	58	696	62	450	2141	482	1.32	1228	522	1218
	2			6.5	184	77	541	67	436	2020	501	—[c]	1265	537	1078
	2			5.7	214	69	465	34	502	1540	269	(I only)	1540	655	1220
4	0		Pre-pen	—	21	—	483	82	232	1470	849	—	—	39	522
	2	3 days		—	92	112	328	11	469	—	—	—	(482)	216	549
	2	3 mo.		—	43	—	104	—	—	—	—	—	—	—	—
	2		4	6.7	15	112	232	—	261	970	682	3.4	584	248	480
	2			6.5	23	103	203	—	265	1170	635	—[c]	971	414	617
	2			6.8	35	111	226	—	250	1105	836	1.8	812	347	573
5	0			6.6	47	82	491	10	379	1110	755				
	1.6			6.6	21	76	261	—	420	1118	—				
	1.6			6.0	10	67	284	13	180	620	466				
	1.6			6.1	12	50	204	26	166	550	500				

TABLE III
(continued)

Subject	Dose (g/day)	Duration	No pen (days)	Urine pH	(mg/24 hr) Tau	Gly	Cyst	Cys–Homo	Orn	Lys	Arg	I/II	I + II	Dis–Cys	Total Cyst.
6	1.6	45 days													
	0			8.0	68	54	607	—	168	643	—				
	0			7.5	59	76	587	—	290	1110	286				
		Day 10		7.0	205	101	585	—	—	—	—	1.11	859	365	950
		17		6.9	217	116	456	—	585	1205	992	1.40	656	279	735
		31		6.5	133	168	240	—	697	1312	944	One peak	1212	515	755
		45		6.2	55	75	300	—	581	1290	692	One peak	882	374	674

[a] This table includes, for comparison, control data obtained before patients went on penicillamine (Pre-pen) and after having been on it for the indicated dosages and times [see also Ref. (10)]. In the "I + II" column, the values in parentheses refer only to pen–cys mixed disulfide. Symbols: pen, penicillamine; Tau, taurine; Gly, glycine; Cyst, cystine; Cys–Homo, cysteine–homocysteine mixed disulfide (not always measurable, after treatment, at sample concentrations analyzed); Orn, ornithine; Lys, lysine (does not include 1-methylhistidine peak, which was separated here); Arg, arginine; I/II, ratio of two isomers; I + II, mg of both isomers; Dis–Cys, cysteine present as cysteine–MPG disulfide (and Cys–Homo); Total Cys, cystine plus Dis–Cys.

[b] Patient collected only first 8-hr urine on this day; these figures extrapolated to 24 hr.

[c] Peaks not separated sufficiently.

both by chromatography and sulfhydryl determination (12).

Results of MPG administration to 6 other cystinurics are given in Table III, which illustrates the necessity for individual adjustment of dosage, the salutary effect of increasing urinary pH (the pH of these urines was high despite no alkalinizing drug intake), and the degree of variability in cystine and other characteristic cystinuric amino acid output in the same patient from time to time. A rise in pH from 5.7 to 6.7 apparently increases the MPG-bound disulfide cysteine from 53 to 60% of the total. Some data from these patients while on penicillamine are included for comparison. The glycine measurements reveal no significant gastrointestinal hydrolysis of MPG.

Patient 6 was the only one receiving MPG for more than 3 days. Although the analytical results indicated he was taking the drug, his urinary cystine did not fall appreciably until day 17, for unknown reasons. A stone in one kidney, later analyzed as pure cystine, was also unaffected. Blood counts, serum nonprotein nitrogen and hemoglobin remained normal and no protein or glucose was detected in these or any of the other patients' urines.

No side effects, except for one instance of mild and transient nausea, were reported.

Discussion. The color produced with ninhydrin by mercaptans is probably explained by a mechanism analogous to that which has been indicated for chromogen production with amines (13), the purple color reflecting the relative amount of bis-1,3-diketoindanyl present. The separation on the chromatographic column of the diastereoisomers has a precedent (14). The appearance of erratic proportions of peaks I and II is not understood, but suggests that the two isomers react with cystine to different extents or rates under subtly different conditions, which diastereoisomers can commonly do. Rapid action of the drug is to be expected, since it is rapidly excreted and disulfide exchanges take place quickly (15).

Taurine excretion is usually quite low in cystinuric stone-formers (1) and is promptly restored to normal by treatment with penicillamine. On going off penicillamine it is evident (Table III) that several days are required for taurine excretion to fall to low levels again and that MPG does not normalize it quickly. The corresponding changes in cystine excretion are much more rapid.

As is the case with penicillamine (16), the nitroprusside reaction can be useful in conveniently following the control of cystinuria with MPG. MPG gives the reaction, but its disulfide and mixed disulfide give a much weaker test. Since essentially no MPG as such appears in the urine, a positive nitroprusside test indicates the necessity for an increased dosage.

Summary. In acute trials α-mercaptopropionylglycine (MPG) appears to be approximately as effective as D-penicillamine in lowering to safe levels the urinary cystine excretion by cystinurics. Analytical results show the mechanism of action to be the same. No side effects were seen here. Some new analytical information on other mercaptans is reported, for comparison.

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Catheterization of Renal Artery in Rats* (33462)

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This paper describes a modification, for use in rats, of the method of Rudolph *et al.* (1) for chronic catheterization of the renal artery in dogs.

Design of catheter. The renal artery catheter is made by stretching a heated polyethylene tubing (PE10) to an outside diameter of about 0.009 in. A length of 3–4 mm of the thinned part forms the tip of the catheter. Three small ridges are made on the unstretched part of the tubing, one at the beginning of the taper to give it a pear shape and others at different distances from it; these are used to anchor the catheter (Fig. 1). The total length of the catheter is 25 cm. The PE10 tubing is heated by holding it inside a nichrome wire loop the temperature of which is adjusted by a powerstat. When the part of the tubing inside the loop starts softening it can be stretched or compressed to make the taper or the ridges, respectively, while the powerstat is momentarily turned off.

Catheterization of the renal artery. Female rats of the Holtzman strain (204–290 g) were operated on under ether anesthesia and aseptic conditions. The animal is laid on its right flank. The skin is incised about 5 mm lateral and parallel to the left spinal muscle mass, and the abdominal aorta and the origin of the left renal artery are exposed retroperitoneally. A "O" thread is passed loosely around the aorta between the origins of the two renal arteries (Fig. 2). A stitch (6 "O") is then tied into the adventitia of the aorta about 1 mm medial to the origin of the left renal artery with ends left long to be used to secure the catheter in place. The adventitia of the renal artery is grasped and held still, and the artery wall is pierced close to its origin with a 27-gauge needle while the blood flow in the aorta is arrested by pulling the "O" thread. The needle is withdrawn, and traction on the "O" thread is slowly reduced until a drop of blood appears at the puncture site. The tip of the catheter (filled with heparin in saline and connected at the distal end by a needle to a stopcock and syringe) is inserted into the artery and the grasp on the artery released. Blood flows into the catheter upon further release of traction on the aorta; the blood is reinjected and the stopcock is closed. The catheter is secured by tying the 6 "O" thread around it above the pear shaped tip. The stopcock is then opened and blood permitted to flow out to make sure small clots are not obstructing the catheter. The catheter is then refilled with fresh heparin solution and sealed by tying off its distal end.

The catheter is secured further by sutures in the abdominal muscle fascia tied around its other ridges; then it is brought under the skin to a stab wound in the back of the neck where it is coiled under the skin and the sealed end tied to the wound. The abdominal wound is closed.

The aortic blood flow should not be arrested longer than 3–4 min at a time. If the catheter cannot be introduced at the first attempt, the aortic flow is released slowly

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