Fate of Gentisic Acid in Man as Influenced by Alkalinization and Acidification. (20121)

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Gentisic acid in the form of its sodium salt has been recently advocated as an anti-rheumatic and analgesic in place of salicylates. It was first pointed out by Neuberg(1) that gentisic acid could be recovered from the urine of subjects receiving salicylates. It was not, however, until 1948 that Ragan and Meyer(2) suggested that gentisic acid might be the active anti-rheumatic factor of salicylates in view of its marked ability to inhibit hyaluronidase. Although the anti-rheumatic activity and analgesic action of gentisic acid has been confirmed(3), there is considerable doubt that gentisic acid *per se* is the sole active component of salicylate metabolism. Since gentisic acid possesses these properties in its own right, it was thought advisable to investigate its fate after oral administration in man in order to determine whether gentisic acid behaves in the body like salicylates. This report will consider primarily the urinary excretion of gentisic acid as compared with salicylates when influenced by acidification and alkalinization.

Method. Groups of subjects without renal or gastrointestinal disease were given 300 mg of sodium gentisate or sodium salicylate orally 3 times daily for 7 days to assure saturation before samples were collected for analysis. The drug was then continued and 24-hour urines were collected for 3 consecutive days. Similar studies were repeated but with the administration of sodium gentisate or sodium salicylate in combination with sodium bicarbonate (0.6 g), dilute hydrochloric acid (1 cc of 0.1 N, U.S.P.), or ammonium chloride (enteric coated 1 g) 3 times daily. The salicylates were then determined by the method of Lester, Lolli, and Greenberg(4). An aliquot of the 24-hour urine was acidified with hydrochloric acid and then hydrolyzed. The hydrolysate was then extracted with ethylene dichloride which was in turn extracted with water. Ferric nitrate was added to the aqueous extract to produce a blue color. The method of determining gentisic acid was a modification of the methods advocated by Lutwak-Mann(5) and Kapp and Coburn(6). The urine was acidified with hydrochloric acid and extracted with ether until the urine failed to give the alkali test (*i.e.*, urine plus sodium hydroxide would give a pink color if any gentisic acid were present). The ether extract was washed with sodium bicarbonate until colorless. The aqueous layer was reextracted with acid and ether. If salicylate was known to be present, chloroform and benzene were used to remove the salicylate. The ether layers were dried, taken up in potassium acid phthalate, and ferric chloride added to produce a blue purple color which could then be measured colorimetrically.

Results. The data on gentisic acid recovery, whether combined or free and the influence of alkalinization or acidification are summarized in Table I. The results of similar studies with salicylates, representing only the total salicylate and its gentisic acid metabolite are indicated in Table II.

We were able to recover approximately 62% of the gentisic acid when the sodium gentisate was administered alone. Of this, approximately 42% existed in the free form. The simultaneous administration of sodium bicarbonate increased the total excretion of gentisic acid by about 35% so that we were able to recover 84% of the gentisic acid administered in the form of its sodium salt. It is of interest to note that although the amount of free gentisic acid recovered was increased by alkalinization, there was no change in the relative proportion of free to the total amount eliminated (Table I).

With acidification produced by administering dilute hydrochloric acid, there was a decrease in the total excretion of gentisic acid at the expense of the combined, the free remaining unaltered. However, when acidifica-

TABLE I. Influence of Alkalinization and Acidification on Recovery of Gentisic Acid. Data present ranges of excretion in patients for 3 consecutive days of urine collection. Data for 3 days averaged. Values represent mean of average excretion for each individual group as indicated.

Group	Patients No.	Total G.A.* recovered, mg		Free G.A.* recovered, mg		% G.A.* recovered		Free/total G.A.* × 100	% change due to alkalinization and acidification	
		Range	Mean	Range	Mean	Total	Free		Free	Total
G.A.*	9	500–551	533	210–241	225	62.4	26.3	42.0	—	—
G.A. + sodium bicarbonate	6	714–726	721	331–346	336	84.4	39.2	46.3	+49.0	+35.2
G.A. + HCl	6	423–471	442	224–246	235	51.7	27.5	53.1	+ 4.5	—17.1
G.A. + NH_4Cl	6	488–509	501	252–257	255	58.7	29.8	50.7	+13.3	— 5.9

* G.A. = Gentisic acid.

TABLE II. Influence of Alkalinization and Acidification on Recovery of Salicylate and Its Gentisic Acid Metabolite. 6 patients in each series. Data present ranges of excretion in individual patients for 3 consecutive days of urine collection. Data for 3 days averaged. Values represent mean of average excretion for each individual group as indicated.

Group	Total S* recovered, mg		% recovered of ingested S*	Total G.A.† recovered, mg		Free G.A.† recovered, mg		% G.A.† recovered of S* ingested		Free/total G.A.† × 100	% change G.A.† recovered due to alkalinization and acidification		% change S* recovered due to alkalinization and acidification
	Range	Mean		Range	Mean	Range	Mean	Total	Free		Free	Total	
S*	594–633	617	68.6	52–58	54.6	4.6–5.1	4.8	6.1	.53	8.8	—	—	—
S + $NaHCO_3$	787–794	790	87.6	75–81	78.2	6.7–7.8	7.2	8.4	.79	9.2	+49.1	+37.7	+27.8
S + HCl	385–496	457	50.7	46–49	48	4.6–5.2	5.0	5.3	.55	10.4	+ 3.7	—13.1	—26.1
S + NH_4Cl	418–440	431	47.8	44–47	46	3.9–4.4	4.1	5.1	.46	9.0	—13.2	—16.4	—30.3

* S = Salicylate.
† G.A. = Gentisic acid.

TABLE III. Statistical Data.

Group*	Total gentisic acid — Mean, mg	Range, mg	SD,† mg	"T"‡
1. G.A.	531	280–805	±124.9	—
G.A. + $NaHCO_3$	721	646–802	±153.9	7.37
G.A. + HCl	431	224–552	± 73.8	3.82
G.A. + NH_4Cl	493	443–631	± 60.2	1.05
2. S	622	538–685	± 48.73	—
S + $NaHCO_3$	787	709–858	± 71.5	11.6
S + HCl	478	344–624	± 74.1	6.3
S + NH_4Cl	435	380–509	± 44.2	13.6
3. G.A. recovered:				
from S	48.8	40.5–59.8	± 5.62	—
from S + $NaHCO_3$	70.9	59.3–86.8	± 7.23	9.1
from S + HCl	43.2	35.0–50.1	± 4.09	3.09
from S + NH_4Cl	41.5	33.7–47.2	± 3.71	4.06

* G.A. = Gentisic acid; S = Salicylate.
† SD = Stand. dev.
‡ "T" = Statistical symbol to indicate significance.

tion was obtained by the use of ammonium chloride, a lesser effect upon combined gentisic acid was demonstrated than with hydrochloric acid.

Approximately 68% of the administered sodium salicylate was recovered in 24 hours from the urine. Approximately 6% of the administered sodium salicylate was recovered as gentisic acid. Upon alkalinization there was a 28% increase in the total salicylate excreted so that we were able to recover approximately 88% of the drug administered (Table II). Alkalinization also affected the excretion of the degradation product, gentisic acid, since its excretion increased to 8.4% which represents an increase of approximately 38%. Although there was an absolute increase in the free gentisic acid, there was no alteration in the proportion of free to combined obtained prior to alkalinization.

Acidification with dilute hydrochloric acid resulted in a decrease of total salicylate so that only 51% of the administered amount was recovered. This decreased excretion was also reflected in the total gentisic acid recovered, but again at the expense of the combined.

In contrast to the effects of ammonium chloride on gentisic acid which were insignificant, the simultaneous administration of ammonium chloride with sodium salicylate resulted in typical changes associated with acidification.

Table III presents a statistical evaluation of the data. The results with gentisic acid are statistically significant for alkalinization with sodium bicarbonate and acidification with hydrochloric acid. The data obtained for salicylate elimination and the elimination of its gentisic acid metabolite are also significant.

Discussion. In the main, gentisic acid followed the same pattern of excretion as salicylate. However, when the gentisic acid produced by salicylate administration was compared to the administration of sodium gentisate alone, there was noted a significant difference in the proportion of free to total gentisic acid recovered. Where the amount of free gentisic acid obtained from salicylate administration was approximately 10% of the total gentisic acid recovered; with the administration of the gentisate alone, free gentisic acid comprised approximately 42% of the total. This proportion of free to total obtained from the salicylate administration remained unaltered under the influence of alkalinization and acidification. We have no explanation as to why the proportion of free gentisic acid to combined is much lower when obtained from salicylate administration than from sodium gentisate alone.

If the anti-rheumatic and analgesic effects of gentisic acid are dependent upon the amount of free gentisic acid present in the body, it is apparent that the amount of the free gentisic acid formed from salicylate would not be of sufficient amount to be of clinical significance. Too small amounts of free gentisic acid are present after salicylate ad-

ministration to account for the latter's pharmacologic effects. The amount of free gentisic acid present in the body cannot be changed by alkalinization or acidification and therefore the effects noted are of pharmacologic but not clinical interest.

Conclusions. 1. The recovery of gentisic acid and salicylate were similar, approximating 62 and 68% respectively of the administered drugs, and 84 and 88% respectively, following alkalinization. 2. Alkalinization increased the combined and free forms of gentisic acid eliminated but did not alter their relative proportions. 3. The amount of gentisic acid recovered from salicylate administration was increased with alkalinization without alteration in the proportion of free to combined gentisic acid obtained with salicylate alone. 4. Whereas the free gentisic acid represented approximately 42% of the total gentisic acid recovered when administered alone, the amount of free gentisic acid obtained from salicylate administration represented approximately 10% of the total gentisic acid. 5. Acidification with dilute hydrochloric acid, but not ammonium chloride decreased the total excretion of gentisic acid at the expense of the combined, the free remaining unaltered. 6. Acidification with both dilute hydrochloric acid and ammonium chloride decreased the total excretion of salicylate and the combined gentisic acid metabolite, the proportion of free to combined gentisic acid remaining unaltered.

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Histochemical Studies of Thermal Injury on Rat Skin.* (20122)

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During the course of an histochemical study of thermally injured rat skin, a unique differential staining property of heat-altered collagen was observed.

Methods. The abdomen of young adult male rats was exposed to various temperatures between 50°C and 80°C for 1-2 minutes in a thermostatically controlled water bath. The area exposed was limited by a shielding device. Immediately following the thermal exposure, skin blocks were excised which included both the exposed and shielded areas. These were immediately fixed in Carnoy's fixative to prevent solution of water soluble substances present as normal constituents of skin or as a result of the heat treatment.

Results. Paraffin sections (4 μ) were stained for 24 hours at room temperature by an aqueous solution of aniline blue (C.I. No. 707) and a mordant, phosphomolybdic acid. Both the normal and the heat-altered collagen of the rat skin stained an intense blue. When the hyperthermic episode ranged between 50 and 60°C, the heat-altered collagen was indistinguishable from that of normal unheated skin. At higher temperatures (70°C and 80°C) the heat-altered collagen showed a variable loss in structure, due to homogenization and swelling of the individual bundles. Orange G (C.I. No. 127) added to the above

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