Phenolic Compounds in the Treatment of Rheumatic Fever

II. The Metabolism of Gentiisic Acid and the Ethanolamide of Gentiisic Acid

By Norman E. Clarke, M.D., and Robert E. Mosher, Ph.D.

The absorption and excretion of gentisic acid has been studied to help clarify the confusion that exists in the literature. Data are given that support the retarding action of methyl cellulose and Benemid and also the manner in which these compounds are metabolized in the human body. The first metabolic study of the ethanolamide of gentisic acid in human subjects is described. Evidence is presented that the antirheumatic property of certain phenolic compounds may be explained by the phenomena of chelation and the possible existence of a reversible hydroquinone-quinone system in the blood stream.

The published reports on the absorption and excretion of the gentisates in man and in animals are conflicting and incomplete and this confusion has been produced in a large part by the methodology employed for the quantitative estimation of this compound.

As early as 1895, Likhatscheff demonstrated that the oral administration to dogs of gentisic acid and ethyl gentisate, produced an elevation of the urinary ethereal sulfates. He estimated that 18 per cent of the ingested gentisic acid and 46 per cent of the ethyl gentisate was excreted in the urine as ethereal sulfates. Later, Neubauer and Falta, working with normal human subjects concluded that 15 per cent of ingested gentisic acid was excreted in the urine as ethereal sulfates while Meyer and Ragan found that 25 per cent of the ingested gentisic acid was excreted unchanged in the urine and that the blood level of gentisic acid was not detectable in people who had ingested as much as 10 Gm. of the drug within 24 hours. In a recent paper, Consden and Stanier claim that 90 per cent of ingested gentisic acid was recovered unchanged from the urine. Paper chromatograms of urines from patients with rheumatic fever and Still's disease, who were treated with gentisates, showed small amounts of conjugates of gentisic acid as well as phenolic conjugates along with free salicylic acid.

Roseman and Dorfman have reported that 61 to 77 per cent of ingested gentisic acid was excreted in the urine within 24 hours and that hydrolysis of the urine by the addition of sulfuric acid and boiling did not increase the free gentisic acid content, so they concluded that no conjugates were present. In rabbits that were fed sodium gentisate, Benati and his co-workers found that sodium gentisate was excreted by the kidneys and without an increase in urinary ethereal sulfates, glucuronic acid or glycine. Meyer and Ragan have reported no increase in urinary glucuronic acid in patients receiving gentisate therapy while Schaefer found a reducing substance in the urine which “at least in part was a glucuronide.” These conflicting report raise doubts as to the validity of the laboratory procedures used in the quantitative estimation of gentisic acid and its derivatives.

We have studied the metabolism of sodium gentisate and gentisic acid in human subjects in an effort to clarify this problem. The acid and the sodium salt behave alike and since the
sodium salt is more readily available it was used in this study. Our investigation has been extended to consider the metabolism of the ethanolamide of gentisic acid as no report has been published on the fate of this compound in the body.

**METHODS**

The gentisic acid levels were determined quantitatively by the colorimetric procedure of Gerald and Kagan.

This method depends upon the blue color formed by the reaction of gentisic acid with an acid solution of ferric and ferrous chlorides. The method is reproducible and reliable. Urine samples were diluted 50 times with distilled water and the blood sera were measured after deproteinization with a tungstic acid solution. All of the density measurements were made at a wave length of 620 millimicrons with an Evelyn photoelectric colorimeter.

Methods that are based upon an ultraviolet absorption at 320 millimicrons or on the reducing power of gentisates (Folin-Ciocalteu reagent, for example) were avoided since we believe they are not sufficiently specific to differentiate between gentisic acid and its possible conjugates. The colorimetric standards for gentisic acid and sodium gentisate in serum and urine were based upon standard solutions of pure gentisic acid. It was found that the same procedure gave accurate results though a less intense color for the levels of the ethanolamide of gentisic acid. The standards for the determination of the ethanolamide were based upon standard solutions of the pure compound.

The urinary glucuronie acid levels were determined by the Tollens' or naphthoresorcinol colorimetric method as modified by Fishman and his co-workers. While it would appear that all of the current methods for the determination of glucuronic acid are in considerable error, we believe this procedure to be the most reliable.

The urinary ethereal sulfate levels were determined by the procedure of Hawk, Oser, and Summers. The sulfate in 1 aliquot of urine is precipitated by barium chloride and is then filtered, washed, dried and weighed. Another aliquot is then acidified with hydrochloric acid and boiled to hydrolyze sulfate esters. The difference between these two values is then converted to millimoles of sulfate per 24 hours and is termed "ethereal sulfate." All pH determinations were made with a Beckman line-operated pH-meter (Model H-2) using glass and calomel electrodes. The absorption spectra were determined with a Beckman Model DU Quartz Spectrophotometer using 1 cm. fused silica cells. All of the values listed for the concentrations of these components in the urine are given in millimoles which makes it possible to compare directly the drug, glucuronic acid and ethereal sulfate levels. Standard deviations are given rather than average deviations.

**RESULTS**

The Absorption of Gentiates

The rapidity of absorption of a substance from the gastrointestinal tract may be determined by measuring the time before it appears and also reaches its peak level in the blood stream. We found that a measurable amount of gentisate appears in the blood stream within 15 minutes after its oral administration. This observation is of qualitative value only since the exact magnitude of the level reached in this period will depend, among other things, upon the form in which the compound is administered. Our tests made with gentisic acid ethanolamide and sodium gentisate with and without Benemid, and with tablets compounded of sodium gentisate and methyl cellulose confirm this statement.

In most patients the blood serum level reached its peak within one hour, while a few patients required twice as long. Figure 1 shows the blood serum gentisate levels after the ingestion of 33 mg. of sodium gentisate per kilogram of body weight. All data presented in this figure were obtained on the same individual. Combinations of sodium gentisate with Ben-
emid and with methyl cellulose were tested to note any retarding influence on the blood levels. The methyl cellulose was incorporated directly with sodium gentisate in compressed tablets for the purpose of delaying its absorption and thereby obtaining a more constant blood level. The patient was “conditioned” with 2 Gm. of Benemid every 24 hour period for several days before the tests were performed. The maximum blood concentration occurred one hour after ingestion of the drug and then dropped off rapidly. The lower blood serum levels obtained with the tablets containing methyl cellulose indicate a retarded absorption from the gastrointestinal tract. It appears evident that Benemid helps to maintain a higher blood level and for a longer time. Although the blood serum levels are higher after any given interval of elapsed time when Benemid is used, a more constant level is maintained by the combination with methyl cellulose. There is a linear relationship between the dose of gentisate administered and the blood serum level attained under any given set of circumstances.

In figure 2 is presented data from a similar study in which the ethanalamide of gentisic acid was administered alone and in combination with Benemid. It is significant that Benemid causes a displacement but no elevation of the gentisate blood level curve. Presumably the action of Benemid may be associated with the presence of a free carboxy group in the drug.

The Excretion of Gentisates

The gentisates are eliminated rapidly from the body. A measurable amount of gentisate can be detected in the urine within 30 minutes after its oral administration. Table 1 presents the 24 hour excretion data from a number of rheumatic fever patients who were treated with various gentisates alone and also in combination with Benemid or methyl cellulose.

Table 1.—Twenty-four Hour Excretion of Gentisates

<table>
<thead>
<tr>
<th>Drug</th>
<th>No. of Patients</th>
<th>No. of Samples</th>
<th>Ave. % Excreted</th>
<th>Std. Dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium gentisate</td>
<td>8</td>
<td>28</td>
<td>58.3</td>
<td>±14.8</td>
</tr>
<tr>
<td>Genticic acid</td>
<td>4</td>
<td>16</td>
<td>60.9</td>
<td>±18.1</td>
</tr>
<tr>
<td>Ethanalamade (adults)</td>
<td>3</td>
<td>19</td>
<td>25.8</td>
<td>±6.3</td>
</tr>
<tr>
<td>Ethanalamade (children)</td>
<td>3</td>
<td>29</td>
<td>44.1</td>
<td>±11.1</td>
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<tr>
<td>Ethanalamide and Benemid (adults)</td>
<td>2</td>
<td>12</td>
<td>35.5</td>
<td>±5.9</td>
</tr>
<tr>
<td>Ethanalamide and Benemid (children)</td>
<td>5</td>
<td>37</td>
<td>23.5</td>
<td>±9.5</td>
</tr>
<tr>
<td>Sodium gentisate and methyl cellulose</td>
<td>4</td>
<td>30</td>
<td>61.7</td>
<td>±13.9</td>
</tr>
</tbody>
</table>

* Free Drug.

Table 2.—The Excretion of Total and Free Gentisate

<table>
<thead>
<tr>
<th>Patient</th>
<th>Drug</th>
<th>No. of Samples</th>
<th>Ave. Total Drug (Free Drug)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. L.</td>
<td>Sodium gentisate</td>
<td>10</td>
<td>1.11 ± 0.03</td>
</tr>
<tr>
<td>R. S.</td>
<td>Ethanalamide of gentisic acid</td>
<td>7</td>
<td>2.09 ± 0.24</td>
</tr>
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</table>

The excretion, although high, is never entirely complete for reasons as yet unexplainable. Less than 1 per cent of the drug is excreted through the bowel. The authors believe that occasionally this missing fraction has resulted from drug build-up in body fluids or tissues since in some patients with pleural effusion, the pleural fluid contained from 5 to 6 mg. of drug per 100 ml. The excretion of the ethanalamide of gentisic acid is much lower than that of sodium gentisate. There is no correlation between the urine volume and pH, and the drug output for a 24-hour period.
Evidence that conjugates of gentisic acid occur as metabolic end-products is presented in Table 2 which lists the ratios of total to free gentisate found in the 24-hour urines of rheumatic fever patients who were treated with sodium gentisate and with the ethanolamide of gentisic acid. The total drug levels were determined by applying the colorimetric method for gentisates to aliquots of urine which had been hydrolyzed by boiling with dilute hydrochloric acid.

It appears from these data that a certain amount of both drugs is “bound” in the urine and can be liberated only by an acid hydrolysis. Although the bound portion is low with sodium gentisate, it appears in significant amounts with the ethanolamide. The nature of this conjugate fraction has not been established with certainty but it may conceivably exist in one or all of three different forms; namely, as ethereal sulfates, glucuronides or as the glycine derivative.

Table 3 presents data on the daily excretion of glucuronic acid and ethereal sulfate by rheumatic fever patients who were treated with the ethanolamide of gentisic acid, sodium gentisate and of both compounds in combination with Benemid. The Benemid was given for a few days before the gentisate compound was administered.

There is a distinct increase in the urinary excretion of ethereal sulfate and glucuronic acid after the ingestion of the ethanolamide of gentisic acid but these urinary metabolic products are not increased by the ingestion of Benemid alone. In the rheumatic fever patient treated with sodium gentisate there is a correlation between the millimoles of total drug, ethereal sulfate and glucuronic acid excreted in the urine. However, there is no direct relationship between the total drug and the conjugates excreted in the urine when the patient received both sodium gentisate and Benemid although the levels of the conjugates are higher but without a definite ratio pattern.

Table 4 presents data from a patient with rheumatic fever who was treated with the ethereal sulfate and glucuronic acid of Benemid...
fall of the total drug excreted during this same period. This is suggested by the curves for the urinary excretion of the ethereal sulfate, glucuronic acid and for the total drug, which is additional evidence that conjugates of gentisates are formed.

A further phenomenon, worthy of mention, is the frequent occurrence of dark urines from patients being treated with the gentisates. Usually, this color formed in the urine after it had been exposed to the air for some time, but occasionally the urine was dark when voided. Similar results have been reported in human subjects and animals following the ingestion of salicylates or gentisates. In many respects these urines resemble alkaptonuric urines in that they darken from the top down, and only in the presence of atmospheric oxygen. The darkening process may be inhibited by the addition of ascorbic acid. As pointed out by Abbott and Salmon and by Blum, the distinctive color cannot be due to homogentisic acid. The most reasonable explanation is that this color change is due to the presence of the quinone of gentisic acid. The light absorption curve of the dark gentisate urines is essentially the same as that of the air-oxidized gentisic acid solution as is demonstrated in figure 5. It was found that the gentisate-treated patients' urines darkened more rapidly upon exposure to air than did the solutions of gentisic acid. The gentisic acid solutions at a pH of 6.6 could be exposed to air for as long as six weeks without appreciable darkening, but many urines from patients on gentisate therapy started to oxidize or darken within two to three days. It seems probable that the urine contains a catalyst for this oxidation process and that it must also contain an antioxidant since not all urines of patients on gentisate therapy darkened immediately. Figure 6 demonstrates the change in density of various dilutions of urine from an untreated normal male adult to which had been added the same amounts of

<table>
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<tr>
<th>M. moles</th>
<th>24 Hour Urinary Excretion in Millimoles</th>
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<tr>
<td>54.1</td>
<td>33.9</td>
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<td>54.1</td>
<td>37.0</td>
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<tr>
<td>54.1</td>
<td>24.8</td>
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<td>32.4</td>
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<tr>
<td>54.1</td>
<td>37.7</td>
</tr>
<tr>
<td>54.1</td>
<td>20.8</td>
</tr>
<tr>
<td>54.1</td>
<td>12.6</td>
</tr>
</tbody>
</table>

![Fig. 3. The eight-hour excretion of sodium gentisate, glucuronic acid and ethereal sulfate.](image-url)
this alkalinity the oxidation-reduction potentials of gentisic acid or the natural anti-oxidants of urine or of both are so modified that they fail to protect gentisic acid from assuming the

in the urine. Those urines that are deficient in this agent will be dark when excreted or will start to darken shortly after they are excreted.

**Discussion and Conclusions**

Since gentisic acid or carboxy-p-benzoquinone is structurally related to phenol, hydroquinone, salicylic acid and benzoic acid, it is reasonable to expect that the mechanisms utilized by the body in detoxifying these latter compounds may be involved in the elimination of gentisic acid and the conjugates should be similar, at least qualitatively. Benzoic acid, when administered in small doses, is excreted entirely as hippuric acid, but when given in larger amounts there is some benzoylglucuronic acid as well as a small amount of free acid excreted in the urine. There is evidence that hydroquinone is excreted both as the ethereal sulfate and as a glucuronide, while phenol is excreted as the free compound, the ethereal sulfate, the glucuronide or other conjugates.

According to Kapp and Coburn, 80 per cent of ingested sodium salicylate is excreted in forms containing intact salicylic acid, the most important being glucuronic acid derivatives, free salicylic acid, the glycine derivative and gentisates. Apparently ethereal sulfates are not formed in appreciable quantities.

By comparing these compounds we find that salicylic acid does not form ethereal sulfates as do other phenols and that the amount converted to the glycine derivative is lower than for benzoic acid. This action is apparently due to the chelation or hydrogen-bonding which oc-
curs between the two groups situated in adjacent positions on the aromatic ring. It is known that the increased acid strength of salicylic acid over benzoic acid is due to this chelation. Since the glucuronide of salicylic acid does occur it is possible that this means of detoxication may occur despite the chelation. With gentisic acid the ortho placement of groups occurs also and the conjugation products should be similar with respect to this grouping, although some modification may be expected since there is a free hydroxy group in the 3- position which should confer further phenolic properties to gentisic acid. The anticipated metabolic products of gentisic acid are free gentisic acid or gentisates, conjugated gentisic acid or ethereal sulfate, glucuronides, glycinic derivatives, and possible oxidation products and their conjugates. The gentisates are among the main oxidation products of salicylic acid in the human body. Schayer working with salicylic acid which was labeled with carbon-14 has shown that salicylic acid and its metabolic products are completely eliminated from the body with no tissue retention. With only an insignificant amount released as carbon dioxide it would appear likely that the above tabulation covers the major possible metabolic by-products of gentisic acid and the conjugated free form co-exist in the urine. The presence of glycine derivatives is doubtful. Since an occasional patient will void a dark urine, it is reasonable to include carboxy-p-benzoquinone, in the list of metabolites. This last component does not occur ordinarily in the urine although the work of Heubner on the reversible oxidation and reduction of hydroquinones in blood strongly suggests that the quinone of gentisic acid does exist in the body. Whether this has any bearing on the antirheumatic action of the gentisates is at present a moot question.

There may be a correlation between the chemical structure of these compounds and their excretion as ethereal sulfates. Salicylic acid, gentisic acid or sodium gentisate and the ethanolamide of gentisic acid differ a great deal in the extent to which they are excreted as sulfates and in the following order: salicylates < sodium gentisate < ethanolamide of gentisic acid. This may be due to the number and the placement of the hydroxy groups. Salicylic acid is reported as not forming ethereal sulfates in human subjects. In this compound the carboxy and hydroxy groups exist in adjacent or ortho positions on the ring, and the hydroxy group is bound so tightly by chelation that it is not available for reaction or coupling with sulfate. With gentisic acid, however, we have an additional hydroxy group in the 5- position which is available and as we have shown this compound does produce an elevation of the urinary ethereal sulfate excretion. With gentisic acid ethanolamide the carboxy group is tied up by a nonionic linkage and although some chelation could occur it is relatively weak when compared with that in the free acid. The net result is that a statistically larger number of hydroxy groups is available for conjugation and the amount of conjugates excreted is increased. As reported by Williams this is true of other amides of phenolic aromatic acids. Von Oettingen explains this phenomenon by saying that the “basic” —CONH₂ grouping attracts the “highly acid” —OSO₃H group thus favoring an increase of ethereal sulfates. If this were true, one would expect the —OSO₃H to attach itself to the amide group rather than to a nearby hydroxy group. Furthermore, the —OSO₃H group can hardly be expected to exist as such in the body while sulfate ion itself must be considered to be a base rather than an acid since it is an electron donor rather than an acceptor. It was found by Lihkatscheff that ethyl gentisate caused a greater excretion of ethereal sulfates than did gentisic acid. This is in accord with the above explanation since esters of gentisic acid would exhibit weaker chelate bonds than the acid.

Although our evidence for the existence of conjugates of gentisic acid is suggestive rather than conclusive, we know that the actual isolation and positive identification of each conjugate will be a difficult task. Their labile nature poses great difficulties and a definite answer is not expected in the near future.

**Summary**

The absorption and excretion of gentisates in the human body has been discussed, with
demonstration of the influence of Benemid and methyl cellulose on the gentisate blood serum levels.

Gentisic acid and its ionizable salts are to a small extent excreted as ethereal sulfates and glucuronides as well as the free drug. In some patients the oxidized or quinone form has been present.

A larger portion of the ethanolamide of gentisic acid is excreted as ethereal sulfates and glucuronide conjugates than with gentisic acid itself. This behavior is explained on the basis of differences in molecular structure or hydrogen bonding.

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Sumario Español

La absorción y la excreción del ácido gentísico ha sido estudiada para ayudar a aclarar la confusión que existe en la literatura. Datos se presentan para sostener la acción retardante de la metilcelulosa y Benemid y también la manera en que estos compuestos son metabolizados por el organismo humano. El primer estudio metabólico de la etanolamida de ácido gentísico en sujetos humanos se describe. Evidencia se presenta en favor de que las propiedades antirreumáticas de ciertos compuestos fenólicos puede explicar por el fenómeno de chelación y la posible existencia de un sistema reversible de hidroquinona - quinona en la sangre.

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