In studies on muscular dystrophy in young individuals investigations of the urine were made along three lines, as follows: (a) the estimation of creatinine and creatine, according to Folin (1) and Benedict (2) respectively; (b) the study as to whether the creatine findings include other substances than creatine; (c) the possibility that in muscular dystrophy guanidine intoxication plays a prominent rôle.

The present paper deals primarily with the guanidine question with some attention to the creatine to creatinine ratio. Unsubstituted guanidine was early shown by Gergens and Baumann (3) and by Putzeys and Swaen (4) to have an injurious action, especially on muscle function, findings later corroborated by Fühner (5); while Kawakita (6) found guanidine toxic for plants, a finding verified by unpublished work from this laboratory.

To facilitate the study of guanidine in relation to health and disease Sullivan (7) devised a new test with a high degree of specificity for unsubstituted guanidine, \( \text{NH:C(NH}_2)2 \). This test is not given by any substituted guanidine. Accordingly, it is not given by methylguanidine which might be formed from creatine and creatinine by oxidation, as early shown by Dessaignes (8) and corroborated by Ewins (9), Baumann and Ingvaldsen (10), and by Greenwald (11).

When the guanidine reaction was applied to urines, little evidence was obtained of the presence of unsubstituted guanidine in normal or pathological urine. A search was then made for simple derivatives comparable to glycoxyamine or glycocyamidine, which
might be converted readily into unsubstituted guanidine in the same manner that Dessaignes (8) and Ewins (9) formed methylguanidine from creatine and creatinine by the use of yellow oxide of mercury or silver oxide respectively.

The mercury oxide, as used by Dessaignes, was first tried but was found to have some disadvantages. In some urines the mercury sulfide was colloidal and hard to get rid of by filtering. The greatest fault is the presence of considerable ammonium salts in the final concentrate, which precipitate on adding picric acid and interfere with the determination of guanidine. Accordingly, most of the search for guanidine in urines was by means of alkaline silver oxide.

Liberation of Guanidine—To 150 cc. of urine, from each patient with muscular dystrophy, a 25 per cent solution of silver nitrate was slowly added, with stirring, as long as a precipitate formed and then in slight excess. Without filtering, the mixture was brought to about pH 8 by means of hot saturated barium hydroxide and set aside for 15 hours. The resulting precipitate, centrifuged and washed with water, was suspended in 100 cc. of water and freed from silver by means of H₂S. The filtrate from Ag₂S was freed from H₂S by a current of air and from barium by H₂SO₄. The resulting filtrate was concentrated on the water bath to 15 to 20 cc. and the concentrate was brought to about pH 7.5 (greenish blue to brom-thymol blue). After filtering, the solution was treated with an equal volume of a saturated solution of picric acid. In urine from patients with muscular dystrophy a precipitate speedily formed. This precipitate gave the guanidine color reaction. Recrystallized, it gave typical crystals of guanidine picrate with a melting point on quick heating of 314° uncorrected, 328° corrected, Anschütz 328–329°.

With this manipulation, guanidine picrate, identified by color reaction, crystalline shape, and melting point of the picrate, was obtained from four patients with progressive muscular dystrophy and in large amounts from four with pseudohypertrophic muscular dystrophy. Two patients with progressive muscular dystrophy, patients Mn and Be, and two with the pseudohypertrophic type, McC and Cy, gave enough picrate to be recrystallized several times for analysis. Recrystallized four times from water and washed with ether the picrates gave practically the theoretical N
for guanidine picrate, patient Mn 28.9, Be 28.9, McC 29.0, and Cy 29.14 (six recrystallizations); theory for guanidine picrate, 29.17 per cent N.

In two cases of progressive muscular dystrophy and one of pseudohypertrophic muscular dystrophy no guanidine could be found. No guanidine could be obtained from the urine of three cases diagnosed as myasthenia gravis, nor from one case of leukemia, one of brain abscess, five cases of cancer, and upwards of ten normal persons. Likewise, no guanidine was found in the urine of a parathyroidectomized dog furnished us by Everette I. Evans of the Bureau of Dairy Industry, United States Department of Agriculture.

The treatment with silver nitrate and barium hydroxide which yielded unsubstituted guanidine in the case of muscular dystrophy did not yield guanidine with creatine, creatinine, or arginine. Glycocoyamine and glycocyamidine on the other hand readily yielded guanidine. Since only a small amount of guanidine was obtained from 100 mg. of guanine, the latter is ruled out as a mother substance of the guanidine found by us.

The liberation of guanidine from the simple guanidine complex in the urine is probably not quantitative, since glycocyamine put through the silver nitrate-barium hydroxide treatment rarely gives quantitative results. Our findings vary from 40 to 100 per cent of the theoretical guanidine and generally are about 60 per cent of the theoretical.

Despite the incompleteness of the conversion of glycocyamine to guanidine and in an analogous way the incompleteness of the conversion of the simple guanidine derivative in the urine to unsubstituted guanidine, we obtained guanidine from eight of the eleven patients with muscular dystrophy studied in this laboratory. From Cy 700 mg. of purified guanidine picrate were obtained from 1346 cc. of urine, the 24 hour amount. It would seem then as suggested by us earlier (12, 13) that the urine in muscular dystrophy contains a simple guanidine derivative or mother substance comparable to glycocyamine which yields guanidine on oxidation with silver nitrate and barium hydroxide. Weber (14) more recently has reported that glycocyamine is present in the urine in cases of muscular dystrophy.

Of the negative cases, patient Lock had been helpless for a
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number of years and recently died from pneumonia. Another patient (Fr) with the typical high creatine is a spinal type and may have a spinal muscular atrophy distinct from muscular dystrophy. The third negative case with no guanidine in the urine has been diagnosed as pseudohypertrophic muscular dystrophy. This patient, a boy 17 years old, had been under treatment with ephedrine and glycine for some time before coming to us for study. It can be said, however, that by the procedure given in this paper we have isolated guanidine as a picrate from the urine of several individuals given 10 to 15 gm. of glycine per day for many months,

**Table I**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Diagnosis, muscular dystrophy</th>
<th>Volume</th>
<th>Creatinine</th>
<th>Creatine</th>
<th>Guanidine obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn</td>
<td>7</td>
<td>Progressive</td>
<td>340</td>
<td>316</td>
<td>305</td>
<td>1.4</td>
</tr>
<tr>
<td>Be</td>
<td>7</td>
<td>&quot;</td>
<td>360</td>
<td>171</td>
<td>358</td>
<td>2.1</td>
</tr>
<tr>
<td>Bt</td>
<td>12</td>
<td>Pseudohypertrophic</td>
<td>585</td>
<td>129</td>
<td>174</td>
<td>1.35</td>
</tr>
<tr>
<td>Cy*</td>
<td>13</td>
<td>&quot;</td>
<td>620</td>
<td>135</td>
<td>643</td>
<td>4.8</td>
</tr>
<tr>
<td>Fr</td>
<td>13</td>
<td>Progressive (spinal atrophy?)</td>
<td>720</td>
<td>466</td>
<td>762</td>
<td>1.64</td>
</tr>
<tr>
<td>McCoy</td>
<td>12</td>
<td>Pseudohypertrophic</td>
<td>1285</td>
<td>754</td>
<td>1014</td>
<td>1.34</td>
</tr>
<tr>
<td>Lock*</td>
<td>10</td>
<td>Progressive</td>
<td>435</td>
<td>318</td>
<td>923</td>
<td>2.9</td>
</tr>
<tr>
<td>Ser</td>
<td>14</td>
<td>&quot;</td>
<td>510</td>
<td>153</td>
<td>366</td>
<td>2.4</td>
</tr>
<tr>
<td>Kr</td>
<td>7</td>
<td>&quot;</td>
<td>960</td>
<td>288</td>
<td>674</td>
<td>2.34</td>
</tr>
<tr>
<td>Alr</td>
<td>10</td>
<td>Pseudohypertrophic</td>
<td>860</td>
<td>363</td>
<td>690</td>
<td>1.90</td>
</tr>
<tr>
<td>Pas</td>
<td>17</td>
<td>&quot;</td>
<td>1310</td>
<td>947</td>
<td>1201</td>
<td>1.27</td>
</tr>
</tbody>
</table>

*Died.

so that, if medication has altered the guanidine picture, it must be due to the ephedrine or other factors.

The chemical data covering our first analysis in each patient are given in Table I. 70 per cent of our patients diagnosed by the medical profession as cases of muscular dystrophy have given guanidine by our procedure. No evidence for the presence of simple non-methylated guanidine derivatives was obtained in three cases of myasthenia gravis. Normal urines have been invariably negative. It may be said further that our work leaves the question of the presence of methylated guanidines untouched.
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STUDIES IN MUSCULAR DYSTROPHIES: THE PRESENCE OF SIMPLE GUANIDINE DERIVATIVES IN THE URINE

M. X. Sullivan, W. C. Hess and Filadelfo Irreverre


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