CHEMICAL CONSTITUENTS OF SALIVA AS INDICES OF GLANDULAR ACTIVITY.*

BY J. LUCIEN MORRIS AND VERNON JERSEY.

(From the Biochemistry Laboratory of the School of Medicine, Western Reserve University, Cleveland.)

(Received for publication, February 28, 1923.)

Study of the uric acid content of the secretory products of the digestive glands was made possible when the accurate method of Morris and Macleod1 for the determination of uric acid in small quantities became available about a year and a half ago. Since that time data have been collected, as opportunity offered, and an effort made to interpret the results in relation to the part the glands play in endogenous metabolism. The difficulty of obtaining the secretions from most of the digestive glands has delayed the completion of the work planned. The accessibility of the salivary glands and ease of collecting their secretion made possible the greatest progress. Recent publications of Starr2 on the hydrogen ion concentration of the saliva, Hench and Aldrich,3 and Schmitz4 on the salivary urea content made it seem advisable to publish representative chemical data secured by the authors in their study of the activity of the salivary glands. Data on certain of the other glands will be published later.

The present communication includes extensive data for salivary urea, ammonia, amino-acids, and creatinine in addition to those on uric acid. The former were first required for their value in interpretations in the salivary uric acid content under differing

* A preliminary report of this work was presented before the American Society of Biological Chemists, December 28, 1922 (cf. Morris, J. L., and Jersey, V., J. Biol. Chem., 1923, lv, p. xvi).

conditions. Later, they were secured for the information they contribute in the study of the glandular activity. Finally, all the data bear on the question of the value of saliva analysis as a diagnostic procedure.

Of no small importance in these problems is the method of collecting the saliva specimen. The secretion is much less subject to irregularities in chemical content when collected from the quiescent or resting glands than when obtained from the stimulated glands. In order to increase the quantity of saliva it has been customary in previous investigations for the subject to chew paraffin. Our experience indicates that this method introduces marked variations in volume and chemical content which makes the method quite unsatisfactory where the secretion is to be analyzed quantitatively. Throughout the work here presented the collection is from the resting glands and the output is measured in time intervals. This method, at least to a great extent, avoids the difficulties that result from paraffin chewing.

In Table I is a series of such collections, showing the relative uniformity of volume and chemical content of several consecutive half hour specimens contrasted with a half hour (11.00 to 11.30 a.m.) collection which made use of paraffin chewing. The figures representing the half hour output of the resting glands (first column for each constituent) are more uniform than are the figures (second column in each case) which express the same values in terms of 100 cc. of secretion. The increases due to paraffin chewing differ in amount for the various constituents, the urea plus ammonia nitrogen value paralleling the volume most nearly, the uric acid least. For this reason the figures for urea calculated for 100 cc. are least disturbed by the stimulus of chewing, the corresponding figures for uric acid most. Of the constituents so far studied urea plus ammonia nitrogen values expressed in milligrams per 100 cc. most nearly approach the accuracy of the figures based on the time factor, and may be considered sufficiently accurate for diagnostic purposes; while uric acid certainly cannot be so measured. Amino-acid nitrogen and creatinine lie between these extremes. The extent of these variations cannot be indicated with any definiteness. They differ with the vigor of the chewing. Reference to the uric acid values in Table II makes this apparent. V. J. (adult) chewing paraffin at his usual rate, had
TABLE I.
Secretion from Salivary Glands When Resting and under Stimulus of Paraffin Chewing.

<table>
<thead>
<tr>
<th>Time</th>
<th>Volume of saliva</th>
<th>Urea plus ammonia nitrogen.*</th>
<th>Amino-acid nitrogen.*</th>
<th>Creatinine.*</th>
<th>Uric acid.*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cc.</td>
<td>mg.</td>
<td>mg.</td>
<td>mg.</td>
<td>mg.</td>
</tr>
<tr>
<td>a.m.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.00-7.30</td>
<td>11.0</td>
<td>2.24</td>
<td>10.0</td>
<td>0.10</td>
<td>0.39</td>
</tr>
<tr>
<td>7.30-8.00</td>
<td>15.8</td>
<td>2.18</td>
<td>7.9</td>
<td>0.10</td>
<td>0.57</td>
</tr>
<tr>
<td>8.00-8.30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.30-9.00 Breakfast.</td>
<td>12.5</td>
<td>2.16</td>
<td>17.3</td>
<td>0.10</td>
<td>0.52</td>
</tr>
<tr>
<td>9.00-9.30</td>
<td>19.8</td>
<td>1.92</td>
<td>8.4</td>
<td>0.10</td>
<td>0.69</td>
</tr>
<tr>
<td>9.30-10.00</td>
<td>22.5</td>
<td>2.91</td>
<td>12.9</td>
<td>0.14</td>
<td>0.70</td>
</tr>
<tr>
<td>10.00-10.30</td>
<td>25.0</td>
<td>2.54</td>
<td>12.4</td>
<td>0.13</td>
<td>0.69</td>
</tr>
<tr>
<td>10.30-11.00</td>
<td>22.5</td>
<td>2.91</td>
<td>12.9</td>
<td>0.14</td>
<td>0.64</td>
</tr>
<tr>
<td>11.00-11.30†</td>
<td>19.0</td>
<td>12.52</td>
<td>10.5</td>
<td>0.31</td>
<td>1.14</td>
</tr>
<tr>
<td>11.30-12.00</td>
<td>23.0</td>
<td>2.69</td>
<td>11.7</td>
<td>0.13</td>
<td>0.62</td>
</tr>
<tr>
<td>p.m.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.00-12.30</td>
<td>21.2</td>
<td>2.85</td>
<td>13.4</td>
<td>0.12</td>
<td>0.60</td>
</tr>
</tbody>
</table>

* The figures in the first column for each constituent represent quantities per half hour. The figures in the second column represent quantities calculated for 100 cc. of saliva.
† Paraffin chewed from 11.00 to 11.30 a.m.

TABLE II.
Saliva Collection Methods Compared.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Paraffin chewed</th>
<th>Natural secretion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V. J.</td>
<td>Adult</td>
<td>1.44</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.62</td>
<td>4.2</td>
</tr>
<tr>
<td>J. L. M.</td>
<td>&quot;</td>
<td>2.04</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.48</td>
<td>3.6</td>
</tr>
<tr>
<td>J. S.</td>
<td>8</td>
<td>0.92</td>
<td>2.7</td>
</tr>
<tr>
<td>L. E. M.</td>
<td>7</td>
<td>0.98</td>
<td>3.3</td>
</tr>
<tr>
<td>W. S.</td>
<td>10</td>
<td></td>
<td>3.7</td>
</tr>
</tbody>
</table>

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1.44 and 1.62 mg. of uric acid per 100 cc. Saliva from J. S. (8 year old boy) under similar conditions had 0.92 mg. per 100 cc. In the case of J. L. M. (adult) his usual rate of chewing gave a value of 1.48 mg. per 100 cc. When the last subject chewed about half as rapidly as usual, the uric acid figure was 2.04 mg.; when he chewed about twice as fast as usual, the figure was 0.96 mg. The last figure is as low as the usual value for the 7 and 8 year old boys. Obviously, the vigor of chewing plays a very considerable part in the average figures given in the literature for salivary uric acid. The uric acid values determined for the natural secretion of the resting glands of the same subjects (Table II) are much more uniform. Paraffin collection introduces errors into the analysis of the other salivary chemical constituents also, but they are less exaggerated than in the case of uric acid. Throughout the present study saliva was collected in half hour intervals from the quiescent glands. The following methods of collection, preparation of the specimens, and analysis were used.

Collection of Specimen.—Place a small funnel (1 inch diameter) behind the lips and against the teeth, and hold a graduated cylinder over the stem to catch the secretion. Keep the head bent slightly forward and refrain from chewing or swallowing. Change the cylinder each half hour during the series, thus collecting consecutive specimens (four to twenty-five in the work here presented).

Preparation for Analysis.—Note the volume of each half hour quantity (usually 5 to 25 cc.) and transfer to a 100 cc. volumetric flask (200 cc. in those cases where the half hour output, due to some vigorous stimulus, is more than 50 cc.). Add 2.5 cc. of 2 per cent acetic acid and shake. Now add 10 cc. of 10 per cent sodium sulfate and sufficient water to make the total volume about 75 cc. Finally, add 10 cc. of alumina cream and make up to volume. Shake from time to time during 10 minutes and filter.

5 Average figures of 2.1 mg. for men and 1.1 mg. for women were reported by Lowenstein and Gies (Lowenstein, G. A., and Gies, W. J., Proc. Soc. Exp. Biol. and Med., 1918-19, xvi, 53). Similar values (0.6 to 2.9 mg. for men and 0.7 to 2.3 for women) were reported by Lewis and Updegraff (Lewis, H. B., and Updegraff, H., Proc. Soc. Exp. Biol. and Med., 1922-23, xx, 168).
J. L. Morris and V. Jersey

Double the quantities when a 200 cc. flask is required. Filtrates so prepared have been perfectly clear. Any traces of protein remaining did not interfere with any of the determinations made.

Chemical Methods.—Urea plus ammonia nitrogen, ammonia nitrogen, amino-acid nitrogen, and creatinine were determined by the Folin-Wu blood methods, their application to the saliva filtrates proving entirely satisfactory. Uric acid was determined by the Morris and Macleod blood method. (Preliminary work

## TABLE III.

<table>
<thead>
<tr>
<th>Time</th>
<th>Volume of saliva</th>
<th>Urea plus ammonia nitrogen</th>
<th>Ammonia nitrogen</th>
<th>Amino-acid nitrogen</th>
<th>Creatinine</th>
<th>Uric acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>a.m.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.00-8.30</td>
<td>14.0</td>
<td>2.41</td>
<td>17.2</td>
<td>1.71</td>
<td>12.2</td>
<td>1.58</td>
</tr>
<tr>
<td>8.30-9.00</td>
<td>18.2</td>
<td>2.94</td>
<td>16.1</td>
<td>2.03</td>
<td>11.2</td>
<td>1.71</td>
</tr>
<tr>
<td>9.00-9.30</td>
<td>20.4</td>
<td>2.83</td>
<td>13.9</td>
<td>1.38</td>
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<td>1.77</td>
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<td>19.5</td>
<td>2.85</td>
<td>14.6</td>
<td>1.76</td>
<td>9.0</td>
<td>1.63</td>
</tr>
<tr>
<td>10.00-10.30</td>
<td>19.7</td>
<td>3.03</td>
<td>15.4</td>
<td>2.11</td>
<td>10.7</td>
<td>1.71</td>
</tr>
<tr>
<td>10.30-11.00</td>
<td>21.5</td>
<td>3.03</td>
<td>14.1</td>
<td>2.09</td>
<td>9.7</td>
<td>1.65</td>
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<tr>
<td>11.00-11.30</td>
<td>24.4</td>
<td>3.57</td>
<td>14.6</td>
<td>3.00</td>
<td>12.3</td>
<td>1.95</td>
</tr>
<tr>
<td>11.30-12.00</td>
<td>19.8</td>
<td>2.85</td>
<td>14.4</td>
<td>2.39</td>
<td>12.1</td>
<td>1.51</td>
</tr>
<tr>
<td>p.m.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.30-1.00</td>
<td>20.3</td>
<td>3.03</td>
<td>14.9</td>
<td>1.98</td>
<td>9.8</td>
<td>1.49</td>
</tr>
<tr>
<td>1.00-1.30</td>
<td>19.5</td>
<td>3.06</td>
<td>15.7</td>
<td>2.01</td>
<td>10.3</td>
<td>1.41</td>
</tr>
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<td>1.30-2.00</td>
<td>20.2</td>
<td>2.88</td>
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<td>2.04</td>
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<td>1.48</td>
</tr>
<tr>
<td>2.00-2.30</td>
<td>19.0</td>
<td>2.40</td>
<td>12.6</td>
<td>1.92</td>
<td>10.1</td>
<td>1.41</td>
</tr>
<tr>
<td>2.30-3.00</td>
<td>22.5</td>
<td>3.80</td>
<td>16.9</td>
<td>2.66</td>
<td>11.8</td>
<td>1.79</td>
</tr>
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<td>3.00-3.30</td>
<td>20.3</td>
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<td>16.4</td>
<td>2.44</td>
<td>12.0</td>
<td>1.75</td>
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<tr>
<td>3.30-4.00</td>
<td>27.2</td>
<td>3.71</td>
<td>13.6</td>
<td>2.61</td>
<td>9.6</td>
<td>2.03</td>
</tr>
</tbody>
</table>

* The figures in the first column for each constituent represent quantities per half hour. The figures in the second column represent quantities calculated for 100 cc. of saliva.

on many saliva specimens gave results by the Folin-Wu and Morris-Macleod methods which agreed, warranting the conclusion that uric acid alone was represented.)

Attempts to use the Folin-Wu method for the determination of glucose resulted uniformly in the absence of more color than a

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Saliva Constituents and Gland Activity

Blank gives. No reducing sugar is present in saliva either from the resting glands or as a result of any of the stimuli here used.

Table III presents the values for volume and chemical constituents obtained in an 8 hour series of half hour specimens.

Table IV.
Uric Acid in Saliva during Day on Non-Purine Diet.

<table>
<thead>
<tr>
<th>Time</th>
<th>Volume of saliva</th>
<th>Uric acid per half hr</th>
<th>Uric acid per 100 cc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>a.m.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.00-7.30</td>
<td>2.8</td>
<td>0.15</td>
<td>5.36</td>
</tr>
<tr>
<td>7.30-8.00</td>
<td>5.7</td>
<td>0.42</td>
<td>7.37</td>
</tr>
<tr>
<td>8.00-8.30</td>
<td>4.5</td>
<td>0.21</td>
<td>4.67</td>
</tr>
<tr>
<td>8.30-9.00</td>
<td>Breakfast</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.00-9.30</td>
<td>3.5</td>
<td>0.20</td>
<td>5.65</td>
</tr>
<tr>
<td>9.30-10.00</td>
<td>6.8</td>
<td>0.39</td>
<td>5.74</td>
</tr>
<tr>
<td>10.00-10.30</td>
<td>6.3</td>
<td>0.29</td>
<td>5.41</td>
</tr>
<tr>
<td>10.30-11.00</td>
<td>6.7</td>
<td>0.37</td>
<td>5.59</td>
</tr>
<tr>
<td>11.00-11.30</td>
<td>5.2</td>
<td>0.26</td>
<td>5.00</td>
</tr>
<tr>
<td>11.30-12.00</td>
<td>7.6</td>
<td>0.35</td>
<td>4.71</td>
</tr>
<tr>
<td>p.m.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.00-12.30</td>
<td>12.1</td>
<td>0.44</td>
<td>3.66</td>
</tr>
<tr>
<td>12.30-1.00</td>
<td>11.2</td>
<td>0.40</td>
<td>3.00</td>
</tr>
<tr>
<td>1.00-1.30</td>
<td>Lunch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.30-2.00</td>
<td>10.0</td>
<td>0.30</td>
<td>3.00</td>
</tr>
<tr>
<td>2.00-2.30</td>
<td>11.3</td>
<td>0.31</td>
<td>2.81</td>
</tr>
<tr>
<td>2.30-3.00</td>
<td>7.4</td>
<td>0.22</td>
<td>2.97</td>
</tr>
<tr>
<td>3.00-3.30</td>
<td>8.2</td>
<td>0.26</td>
<td>3.22</td>
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<td>3.30-4.00</td>
<td>14.0</td>
<td>0.46</td>
<td>3.34</td>
</tr>
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<td>4.00-4.30</td>
<td>11.8</td>
<td>0.37</td>
<td>3.16</td>
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<td>4.30-5.00</td>
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<td>0.42</td>
<td>2.98</td>
</tr>
<tr>
<td>5.00-5.30</td>
<td>14.2</td>
<td>0.44</td>
<td>3.12</td>
</tr>
<tr>
<td>5.30-6.00</td>
<td>Dinner</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.00-6.30</td>
<td>10.6</td>
<td>0.38</td>
<td>3.61</td>
</tr>
<tr>
<td>6.30-7.00</td>
<td>17.6</td>
<td>0.55</td>
<td>3.18</td>
</tr>
<tr>
<td>7.00-7.30</td>
<td>18.2</td>
<td>0.49</td>
<td>2.70</td>
</tr>
<tr>
<td>7.30-8.00</td>
<td>15.5</td>
<td>0.40</td>
<td>2.58</td>
</tr>
<tr>
<td>8.00-8.30</td>
<td>11.3</td>
<td>0.37</td>
<td>3.22</td>
</tr>
<tr>
<td>8.30-9.00</td>
<td>16.0</td>
<td>0.41</td>
<td>2.64</td>
</tr>
</tbody>
</table>

Ammonia nitrogen values are noticeably less regular than the other substances. This irregularity is characteristic of the ammonia in all series and indicates that it is not a direct product of the glands, but is formed after the secretion leaves the glands,
probably through hydrolysis of urea as suggested by Hench and Aldrich. The other four substances follow the volume of the saliva (from the resting gland) with their usual degree of uniformity. A general rise is noted in all values as the series continues. All the following series show this same tendency, with the fact emphasized, of course, in the longer series. It is especially apparent in Table IV which presents the half hour volumes and uric acid contents of 14 consecutive hours of quiescent secretion.

**Chart 1.**
Paraffin chewed 11.00–11.30 a.m.

Resulting increases:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Percentage Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>429</td>
</tr>
<tr>
<td>$\text{NH}_2\text{N}$</td>
<td>722</td>
</tr>
<tr>
<td>Urea + $\text{NH}_3\text{N}$</td>
<td>374</td>
</tr>
<tr>
<td>Creatinine</td>
<td>118</td>
</tr>
<tr>
<td>Uric acid</td>
<td>63</td>
</tr>
</tbody>
</table>

(with three breaks of half an hour each for the meals of a non-purine diet). Rising above the local irregularities and the more general constancy of the volume and uric acid figures, there is very definite evidence that the activity of the quiescent glands increased as the day advanced. Reference to graphic records of the other
experimental series presented in this paper proves the general nature of the phenomenon. Apparently, the increase is an index of the physiological activity involved.

On the basis of the demonstrated character of the secretion of the salivary glands in the normal resting state, the effects of various stimuli were studied. Paraffin chewing is a mechanical stimulus. Its effect is expressed graphically in Chart 1. A volume increase of 429 per cent was accompanied by increases in the constituents analyzed as follows: amino-acid nitrogen, 722 per cent; urea plus ammonia nitrogen, 374 per cent; creatinine, 118 per cent; and uric acid, 63 per cent. In the series represented in Chart 2, the stimulation was due to the presence of acetic acid in the mouth (1 cc. of 2 per cent acid dropped into the mouth every 6 minutes). The volume increased 560 per cent; amino-acid nitrogen, 302 per cent; urea plus ammonia nitrogen, 200 per cent; creatinine, 124 per cent; and uric acid, 71 per...
cent. Though the general effect resembles that of paraffin chewing, certain points of difference were observed. The volume increase was much greater than before, amino-acid nitrogen and urea plus ammonia nitrogen increases were about half the former amount while creatinine and uric acid were increased only slightly more than with paraffin chewing. Evidently, the acid brings about the pouring out of a greater volume of secretion, but the constituents are washed out with it to a less extent than in paraffin chewing. Also, it is the group which ordinarily follows the volume more closely (i.e. urea plus ammonia and amino-acids), which lagged markedly in this case. Taken together with the fact that uric acid and creatinine increased more than in the paraffin series, this might suggest that the two stimuli act upon different parts of the secretory mechanism. Possibly acetic acid acts more
locally upon the glands while chewing exerts a general accelerating effect upon the entire process of filtration from the blood.

Further evidence in favor of an interpretation of this kind appears in the data of a pilocarpin series, graphically represented in Chart 3. After two normal half hour specimens had been collected, 1/2 grain of pilocarpin was taken by mouth. The volume rose to its maximum (84.4 cc.) an hour later, with an increase of 502 per cent. Urea plus ammonia nitrogen reached its maximum with an increase of 445 per cent. Amino-acid nitrogen increased 249 per cent. Creatinine increased 171 per cent, somewhat more than in the other series. Uric acid increased 183 per cent. The direct action of pilocarpin upon the glands differs not only from the mechanical stimulation of chewing paraffin, but also from the indirect action of an acid solution in the mouth. Amino-acids are influenced less by pilocarpin than by either of the other two stimuli, while uric acid and creatinine respond to a greater extent. Urea in this as in the paraffin series increases with the volume more closely than under the stimulation of acetic acid.

The pilocarpin-stimulating effect, though diminishing, continued for some hours. Reference to the curves (Chart 3) shows that amino-acids returned to the normal level more rapidly even than the volume. This fact and a relatively smaller increase with pilocarpin are in marked contrast with the response to paraffin chewing and acetic acid stimulation. The other constituents decreased less rapidly than the volume. This delayed return or sustained elevation is most evident in the case of uric acid. Another series of uric acid values under stimulation of pilocarpin was obtained (first set of curves in Chart 4). The same behavior of volume and uric acid values is observed with the uric acid elevation even more prolonged as the volume decreased. Without offering an explanation for this, the facts seem to warrant the statement that the larger quantity of uric acid results from some stimulating effect of the pilocarpin upon the glandular mechanism which differs from its action upon the volume of liquid produced.

Ingestion of atropine affects the volume and uric acid to a very similar degree. In the experiment, represented in the second set of curves of Chart 4, 1/8 grain of atropine was taken by mouth. The resultant paralysis of the glands decreased the volume from 20 to 2.5 cc. (88 per cent fall) and the uric acid from 0.5 to 0.17 mg. (66 per cent decrease). The return to normal values of uric acid paralleled the gradual increase of volume.
Extremes in diet evidently bring no definite changes in volume or uric acid content. Data were collected from two subjects when fasting and when eating non-purine and purinediets. The results are shown in Charts 5 and 6. The usual increase in volume and uric acid as the day advanced, took place in all six series. When non-purine food was eaten, the increases of the day were slightly less than on the fasting days. When purine food was eaten, the increases were slightly greater. Certainly there is little evidence that presence of food in the stomach has any effect in increasing the secretory activity of the salivary glands. It is also obvious that the conditions of increased uric acid elimination through the kidneys (after ingestion of large quantities of purine-rich food) are not duplicated to any noticeable degree in the filtration through the salivary glands.

Evidently, the variations observed in saliva secretion as a result of stimuli of different kinds bear definite relations to the nature of the activating forces. Final interpretations are not yet warranted, but the selective effects of the stimuli indicate that several factors are involved in the elaboration of the secretion and that these are stressed in different proportions under various stimuli. The factors concerned may be roughly grouped as relating more directly to filtration or metabolism of the glandular tissue. Work, planned to give further evidence leading to interpretation of the problems connected with the elaboration of saliva and the conditions controlling the chemical content, is being carried out by this laboratory on normal and pathological
The data in the present communication seem to justify the working hypothesis that uric acid, more than any other constituent, represents the actual cellular activity and might well serve as an index of the gland metabolism.
CHEMICAL CONSTITUENTS OF SALIVA AS INDICES OF GLANDULAR ACTIVITY
J. Lucien Morris and Vernon Jersey

J. Biol. Chem. 1923, 56:31-42.

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