THE RELATION OF HISTIDINE AND ARGinine TO CREATINE AND PURINE METABOLISM.*

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In a recent paper from this laboratory (Rose and Cox, 1924) it has been shown that histidine is an indispensable component of the diet, and that contrary to the observations of Ackroyd and Hopkins (1916), arginine and histidine are not mutually interchangeable for purposes of growth. In animals deprived of histidine the addition of arginine to the ration is without influence upon the rate of loss in body weight. On the other hand, the inclusion of histidine in the deficient diet occasions an immediate resumption of growth at a normal rate.

In their original communication, Ackroyd and Hopkins concluded that arginine and histidine are interchangeable also as precursors of allantoin. They found that when rats were fed diets devoid of arginine and histidine, but adequate in every other respect to meet the needs of metabolism, the elimination of allantoin decreased 40 to 50 per cent. The addition of either arginine or histidine to the deficient ration was said to result in a much smaller decrease in allantoin excretion than when both of the amino acids were wanting. Tryptophane deficiency was found to produce very little effect upon the allantoin output, although in some cases the losses in body weight were greater than those induced by arginine-histidine deficiency.

* This communication was presented in abstract before the American Society of Biological Chemists at Washington, D. C., December, 1924. See Rose, W. C., and Cook, K. G., J. Biol. Chem., 1925, lxiii, p. xvii.
† The experimental data in this paper are taken from a thesis submitted by K. G. Cook in partial fulfillment of the requirements for the degree of Master of Science in Physiological Chemistry in the Graduate School of the University of Illinois.
Inasmuch as the interchangeability of the two diamino acids as purine precursors seemed scarcely compatible with the results of our growth experiments, it appeared necessary to test the influence of the amino acids in question upon allantoin excretion. We have extended the observations to include uric acid and total creatinine.

EXPERIMENTAL.

Growing rats served as the experimental animals. Each rat was confined in a separate cage, and its food consumption was carefully recorded. The diets employed are indicated in Table I, and are identical with those of like numbers described in the paper of Rose and Cox (1924). The inorganic portion of the food was supplied in the form of Osborne and Mendel's salt mixture (1919). The nitrogenous materials were furnished respectively in the form of purified casein, completely hydrolyzed casein, and hydrolyzed casein from which arginine and histidine had been precipitated by the Kossel-Kutscher procedure. Purified amino acids were added where necessary as indicated in Table I. 25 mg. of Harris yeast vitamin were fed separately to each animal daily. In the experiments involving the feeding of arginine or histidine, the amino acid in question was added to Diet 11. Under such circumstances, 1.40 gm. of arginine nitrate, or 1.19 gm. of histidine monochloride, were employed per 100 gm. of food. Each of these quantities is equivalent to the sum of the arginine and histidine present in casein. The details followed in the preparation of the hydrolyzed casein, and of the hydrolyzed casein from which arginine and histidine were precipitated, have already been described elsewhere (Rose and Cox, 1924).

In the quantitative collection of the urines a procedure was used similar to that recommended by Ackroyd and Hopkins (1916). For this purpose the cages were supported in large funnels over the collecting beakers. In the latter were placed in-

1 These materials were prepared by Mr. G. J. Cox of this laboratory. The experimental animals were also under his immediate care, and were being used in connection with other investigations, the results of which will be published later.
verted 100 cc. round bottom flasks to the necks of which small pedestals had been sealed. The urines flowed down the outer surfaces of the flasks into the beakers, while most of the solid materials were deflected in striking the inverted flasks and did not fall into the urine vessels. The funnels were washed down twice daily with dilute boric acid solution, and the urines and washings were preserved under toluene in an ice chest. Unless distinctly acid, they were rendered so by the addition of a few drops of dilute acetic acid. At the expiration of 8 day intervals,

### TABLE I.

**Composition of the Diets.**

| Composition | Diet No. |  
|-------------|----------|---
| Casein      | 14.70    |  
| Completely hydrolyzed casein | 14.05 |  
| "Deficient digest" (hydrolyzed casein which had been precipitated with Ag$_2$SO$_4$ and Ba(OH)$_2$) | 14.05 |  
| Cystine     | 0.30     |  
| Tyrosine    | 0.45     |  
| Tryptophane | 0.20     |  
| Dextrin     | 40.00    |  
| Sucrose     | 15.00    |  
| Lard        | 19.00    |  
| Cod liver oil | 5.00 |  
| Salt mixture | 4.00 |  
| Agar        | 2.00     |  
| **Total**   | 100.00   | 100.00

the urines of each animal were combined, diluted to a uniform volume of 600 cc., and immediately subjected to analysis. Total nitrogen was determined by the Kjeldahl-Gunning method, total creatinine by the procedure of Folin (1914), uric acid by the method of Benedict and Franke (1922), and allantoin according to the procedure of Wiechowski as modified by Handovsky (1914). In determining total creatinine and uric acid, standards were prepared of such concentrations as to approximate closely the strengths of the unknowns. Where necessary, preliminary analyses of the urines were made, and the standards diluted

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accordingly. In most cases, 10 cc. samples of the urines were found satisfactory. After developing the colors, the solutions were diluted to volumes of 50 or 100 cc. as required. For the allantoin determinations, 400 cc. portions of the diluted urines were taken for the preliminary precipitations, and aliquots of

<table>
<thead>
<tr>
<th>TABLE II. Nitrogen Metabolism on Diets of Whole Casein.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>days</td>
</tr>
<tr>
<td>1-16</td>
</tr>
<tr>
<td>17-32</td>
</tr>
<tr>
<td>33-48</td>
</tr>
<tr>
<td>49-64</td>
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<tr>
<td>65-80</td>
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<tr>
<td>81-96</td>
</tr>
<tr>
<td>97-112</td>
</tr>
<tr>
<td>113-128</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rat 49. ♀. Initial weight, 57 gm. Diet 7 (whole casein) throughout.</th>
</tr>
</thead>
<tbody>
<tr>
<td>days</td>
</tr>
<tr>
<td>1-16</td>
</tr>
<tr>
<td>17-32</td>
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<td>81-96</td>
</tr>
<tr>
<td>97-112</td>
</tr>
<tr>
<td>113-128</td>
</tr>
</tbody>
</table>

225 cc. each of the final filtrates were employed for the titrations with ammonium thiocyanate. Analysis of human and rat urines to which allantoin had been added showed recoveries of 85 to 95 per cent. This is about the usual degree of accuracy observed by others with the Wiechowski-Handovsky method.

The results of the experiments are shown in Tables II to VII inclusive. In most of the experiments the analytical data are
tabulated in periods of 16 days each, although the analyses were made at 8 day intervals. Inasmuch as the quantitative collection of urine in small animals is inherently difficult, we felt that long periods were necessary in order to yield convincing results.

**TABLE III.**

*Nitrogen Metabolism on Diets of Completely Hydrolyzed Casein.*

<table>
<thead>
<tr>
<th>Period</th>
<th>Body weight at end of period</th>
<th>Nitrogen intake</th>
<th>Total nitrogen</th>
<th>Total creatinine nitrogen</th>
<th>Uric acid nitrogen</th>
<th>Allantoin nitrogen</th>
<th>Remarks</th>
</tr>
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<tbody>
<tr>
<td>Rat 45</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-16</td>
<td>107</td>
<td>2.43</td>
<td>0.87</td>
<td>22.1</td>
<td>3.2</td>
<td>84.4</td>
<td>Diet 7 (whole casein).</td>
</tr>
<tr>
<td>17-32</td>
<td>117</td>
<td>1.69</td>
<td>0.92</td>
<td>36.2</td>
<td>4.5</td>
<td>87.5</td>
<td></td>
</tr>
<tr>
<td>33-48</td>
<td>123</td>
<td>1.60</td>
<td>1.02</td>
<td>37.1</td>
<td>4.5</td>
<td>95.4</td>
<td></td>
</tr>
<tr>
<td>49-64</td>
<td>136</td>
<td>2.04</td>
<td>1.25</td>
<td>44.7</td>
<td>5.1</td>
<td>94.4</td>
<td>Diet 12 (completely hydrolyzed casein).</td>
</tr>
<tr>
<td>65-80</td>
<td>142</td>
<td>1.74</td>
<td>1.18</td>
<td>50.4</td>
<td>5.1</td>
<td>100.8</td>
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</tr>
<tr>
<td>81-96</td>
<td>153</td>
<td>1.71</td>
<td>1.13</td>
<td>55.9</td>
<td>5.3</td>
<td>93.8</td>
<td></td>
</tr>
<tr>
<td>97-112</td>
<td>158</td>
<td>1.83</td>
<td>1.25</td>
<td>59.7</td>
<td>6.0</td>
<td>95.0</td>
<td></td>
</tr>
<tr>
<td>113-128</td>
<td>166</td>
<td>1.85</td>
<td>1.21</td>
<td>63.2</td>
<td>6.5</td>
<td>100.7</td>
<td></td>
</tr>
</tbody>
</table>

*The first period in this experiment lasted for 8 instead of 16 days. Hence the low analytical figures.*

In Table VII the analytical data are shown in periods of 8 days each. This was done because the two experiments therein recorded were of approximately half the duration of those presented in Tables II to VI inclusive.

Despite the difficulties in collection, and the unsatisfactory
nature of the Wiechowski-Handovsky procedure for allantoin determinations, the results of the experiments are quite consistent. As was to be expected, greater irregularity was observed in the allantoin figures than in the other urinary components. This is undoubtedly to be attributed to errors inherent in the

**TABLE IV.**

**Nitrogen Metabolism on Diets of "Deficient Digest."**

<table>
<thead>
<tr>
<th>Period</th>
<th>Body weight at end of period</th>
<th>Nitrogen intake</th>
<th>Urine.</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total nitrogen</td>
<td>Total creatinine nitrogen</td>
</tr>
<tr>
<td></td>
<td>gM.</td>
<td>gM.</td>
<td>gM.</td>
<td>mg.</td>
</tr>
</tbody>
</table>

**Rat 55. **♂. Initial weight, 85 gm.

<table>
<thead>
<tr>
<th>days</th>
<th>1-16</th>
<th>130</th>
<th>3.02</th>
<th>1.27</th>
<th>29.1</th>
<th>4.0</th>
<th>89.5</th>
<th>Diet 7 (whole casein).</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>17-32</td>
<td>109</td>
<td>1.51</td>
<td>1.31</td>
<td>40.3</td>
<td>3.4</td>
<td>60.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>33-48</td>
<td>107</td>
<td>1.62</td>
<td>1.31</td>
<td>37.4</td>
<td>3.1</td>
<td>63.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>49-64</td>
<td>99</td>
<td>1.62</td>
<td>1.26</td>
<td>31.6</td>
<td>3.3</td>
<td>64.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>65-80</td>
<td>96</td>
<td>1.34</td>
<td>1.07</td>
<td>37.3</td>
<td>3.0</td>
<td>43.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>81-96</td>
<td>92</td>
<td>1.14</td>
<td>0.95</td>
<td>31.3</td>
<td>2.8</td>
<td>44.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>97-112</td>
<td>87</td>
<td>1.12</td>
<td>0.97</td>
<td>30.9</td>
<td>3.3</td>
<td>55.1</td>
<td></td>
</tr>
</tbody>
</table>

**Rat 56. **♀. Initial weight, 90 gm.

<table>
<thead>
<tr>
<th>days</th>
<th>1-16</th>
<th>131</th>
<th>3.02</th>
<th>1.19</th>
<th>32.6</th>
<th>4.3</th>
<th>93.0</th>
<th>Diet 7 (whole casein).</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>17-32</td>
<td>111</td>
<td>2.01</td>
<td>1.23</td>
<td>40.3</td>
<td>3.6</td>
<td>70.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>33-48</td>
<td>108</td>
<td>1.89</td>
<td>1.44</td>
<td>41.4</td>
<td>3.8</td>
<td>74.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>49-64</td>
<td>104</td>
<td>1.67</td>
<td>1.37</td>
<td>37.4</td>
<td>3.8</td>
<td>62.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>65-80</td>
<td>99</td>
<td>1.39</td>
<td>1.20</td>
<td>40.2</td>
<td>3.8</td>
<td>57.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>81-96</td>
<td>94</td>
<td>1.12</td>
<td>1.04</td>
<td>34.6</td>
<td>3.5</td>
<td>46.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>97-112</td>
<td>91</td>
<td>1.19</td>
<td>1.11</td>
<td>33.7</td>
<td>3.5</td>
<td>53.2</td>
<td></td>
</tr>
</tbody>
</table>

allantoin method. On the diet of whole casein (Table II) the animals gained in weight at normal rates, and the output of all urinary components steadily rose. The figures for total creatinine and uric acid show increases during the course of the experiments of approximately 300 per cent. The allantoin elimination, though irregular, increased 30 to 50 per cent. Similar results were secured with the diet of completely hydrolyzed
casein (Table III), but inasmuch as the animals upon this diet did not grow quite as rapidly as those on the whole casein ration, the increments in urinary components were not quite so large.

The results of the experiments with the "deficient digest" (Table IV) are in striking contrast to those with whole or hydro-

**TABLE V.**

_Nitrogen Metabolism on Diets of "Deficient Digest" Plus Arginine._

<table>
<thead>
<tr>
<th>Period</th>
<th>Body weight at end of period</th>
<th>Nitrogen intake</th>
<th>Total nitrogen</th>
<th>Total creatinine nitrogen</th>
<th>Uric acid nitrogen</th>
<th>Allantoin nitrogen</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>gms</td>
<td>gms</td>
<td>mg.</td>
<td>mg.</td>
<td>mg.</td>
<td></td>
</tr>
<tr>
<td>1-16</td>
<td></td>
<td>104</td>
<td>2.36</td>
<td>0.78</td>
<td>19.0</td>
<td>2.2</td>
<td>74.9</td>
</tr>
<tr>
<td>17-32</td>
<td></td>
<td>90</td>
<td>1.88</td>
<td>1.28</td>
<td>41.0</td>
<td>2.8</td>
<td>59.5</td>
</tr>
<tr>
<td>33-48</td>
<td></td>
<td>87</td>
<td>1.63</td>
<td>1.25</td>
<td>35.5</td>
<td>2.5</td>
<td>52.2</td>
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<tr>
<td>49-64</td>
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<td>82</td>
<td>1.65</td>
<td>1.36</td>
<td>33.3</td>
<td>3.2</td>
<td>55.4</td>
</tr>
<tr>
<td>65-80</td>
<td></td>
<td>81</td>
<td>1.48</td>
<td>1.35</td>
<td>30.4</td>
<td>2.7</td>
<td>56.3</td>
</tr>
<tr>
<td>81-96</td>
<td></td>
<td>77</td>
<td>0.92</td>
<td>0.90</td>
<td>28.4</td>
<td>2.3</td>
<td>40.0</td>
</tr>
<tr>
<td>97-112</td>
<td></td>
<td>74</td>
<td>1.03</td>
<td>1.05</td>
<td>27.6</td>
<td>2.4</td>
<td>56.2</td>
</tr>
<tr>
<td>113-128</td>
<td></td>
<td>70</td>
<td>0.88</td>
<td>0.85</td>
<td>25.0</td>
<td>2.4</td>
<td>53.9</td>
</tr>
</tbody>
</table>

Rat 44. ♂. Initial weight, 71 gms. 

Diet 7 (whole casein).

Diet 11 ("deficient digest") plus arginine.

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Rat 51. ♂. Initial weight, 69 gms. 

Diet 7 (whole casein).

Diet 11 ("deficient digest") plus arginine.

lyzed casein. As will be observed, the values for allantoin, instead of increasing, promptly diminished 40 to 50 per cent following the change from the casein to the deficient ration. In each experiment the most pronounced decrease was observed in the first period following the dietary alteration, but further though somewhat variable decreases occurred in subsequent
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periods. The uric acid elimination also diminished, but the variations from the normal values are not quite so striking as in the case of allantoin. Total creatinine manifested a moderate increase followed by a decline, but at no time did the elimination on the inadequate diet fall below the output on the adequate ration.

**TABLE VI.**

*Nitrogen Metabolism on Diets of "Deficient Digest" Plus Histidine.*

<table>
<thead>
<tr>
<th>Period</th>
<th>Body weight at end of period</th>
<th>Nitrogen intake</th>
<th>Total nitrogen</th>
<th>Total creatinine nitrogen</th>
<th>Uric acid nitrogen</th>
<th>Allantoin nitrogen</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat 48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>days</td>
<td>gm.</td>
<td>gm.</td>
<td>gm.</td>
<td>mg.</td>
<td>mg.</td>
<td>mg.</td>
<td></td>
</tr>
<tr>
<td>1-16</td>
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<td>2.47</td>
<td>0.90</td>
<td>20.0</td>
<td>2.2</td>
<td>79.1</td>
<td>Diet 7 (whole casein).</td>
</tr>
<tr>
<td>17-32</td>
<td>118</td>
<td>2.51</td>
<td>1.15</td>
<td>29.5</td>
<td>3.1</td>
<td>78.9</td>
<td></td>
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<td>33-48</td>
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<td>95.3</td>
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</tr>
<tr>
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<td>2.83</td>
<td>1.53</td>
<td>40.4</td>
<td>4.0</td>
<td>90.3</td>
<td></td>
</tr>
<tr>
<td>65-80</td>
<td>151</td>
<td>2.51</td>
<td>1.60</td>
<td>46.4</td>
<td>4.6</td>
<td>90.4</td>
<td>Diet 11 (&quot;deficient digest&quot;) plus histidine.</td>
</tr>
<tr>
<td>81-96</td>
<td>163</td>
<td>2.35</td>
<td>1.31</td>
<td>62.3</td>
<td>5.3</td>
<td>94.2</td>
<td></td>
</tr>
<tr>
<td>97-112</td>
<td>172</td>
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<td>1.66</td>
<td>61.2</td>
<td>5.3</td>
<td>93.1</td>
<td></td>
</tr>
<tr>
<td>113-128</td>
<td>179</td>
<td>2.69</td>
<td>1.66</td>
<td>60.2</td>
<td>6.4</td>
<td>105.6</td>
<td></td>
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<tr>
<td>Rat 52</td>
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</tr>
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<td>1-16</td>
<td>78</td>
<td>1.84</td>
<td>0.54</td>
<td>15.5</td>
<td>2.0</td>
<td>63.0</td>
<td>Diet 7 (whole casein).</td>
</tr>
<tr>
<td>17-32</td>
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<td>1.79</td>
<td>0.84</td>
<td>22.7</td>
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<td>1.81</td>
<td>0.97</td>
<td>27.7</td>
<td>2.9</td>
<td>70.1</td>
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<td>49-64</td>
<td>126</td>
<td>2.27</td>
<td>1.27</td>
<td>31.2</td>
<td>4.1</td>
<td>87.3</td>
<td>Diet 11 (&quot;deficient digest&quot;) plus histidine.</td>
</tr>
<tr>
<td>65-80</td>
<td>138</td>
<td>1.97</td>
<td>1.19</td>
<td>42.5</td>
<td>4.5</td>
<td>89.6</td>
<td></td>
</tr>
<tr>
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<td>1.08</td>
<td>56.0</td>
<td>4.7</td>
<td>80.2</td>
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</tr>
<tr>
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<td>1.22</td>
<td>55.1</td>
<td>4.0</td>
<td>88.7</td>
<td></td>
</tr>
<tr>
<td>113-128</td>
<td>154</td>
<td>1.75</td>
<td>1.28</td>
<td>55.1</td>
<td>5.2</td>
<td>93.8</td>
<td></td>
</tr>
</tbody>
</table>

As is shown in Table V, the addition of arginine to the deficient diet failed entirely to induce growth, or to influence the output of any of the urinary components. Indeed, the experimental data obtained from the two arginine-fed rats are in every respect similar to those secured with the "deficient digest" animals.
It appears that the addition of arginine, under the conditions of our experiments, exerted no influence upon either purine or creatine-creatinine metabolism.

In Table VI are presented the results of two experiments upon the effects of including histidine in the ration. As will be seen,

### TABLE VII

<table>
<thead>
<tr>
<th>Period</th>
<th>Body weight at end of period</th>
<th>Nitrogen intake</th>
<th>Urine.</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total nitrogen</td>
<td>Total creatine nitrogen</td>
<td>Uric acid nitrogen</td>
</tr>
<tr>
<td>days</td>
<td>gm.</td>
<td>gm.</td>
<td>gm.</td>
<td>mg.</td>
</tr>
<tr>
<td>1-8</td>
<td>88</td>
<td>0.93</td>
<td>0.45</td>
<td>9.8</td>
</tr>
<tr>
<td>9-16</td>
<td>81</td>
<td>0.61</td>
<td>0.36</td>
<td>12.3</td>
</tr>
<tr>
<td>17-24</td>
<td>78</td>
<td>0.45</td>
<td>0.42</td>
<td>16.2</td>
</tr>
<tr>
<td>25-32</td>
<td>76</td>
<td>0.52</td>
<td>0.44</td>
<td>17.9</td>
</tr>
<tr>
<td>33-40</td>
<td>76</td>
<td>0.44</td>
<td>0.26</td>
<td>14.8</td>
</tr>
<tr>
<td>41-48</td>
<td>73</td>
<td>0.42</td>
<td>0.34</td>
<td>14.3</td>
</tr>
<tr>
<td>49-56</td>
<td>73</td>
<td>0.42</td>
<td>0.35</td>
<td>12.7</td>
</tr>
</tbody>
</table>

- Rat 123. ♀. Initial weight, 72 gm.
- Diet 7 (whole casein).
- Diet 12 minus tryptophane.

<table>
<thead>
<tr>
<th>Period</th>
<th>Body weight at end of period</th>
<th>Nitrogen intake</th>
<th>Urine.</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total nitrogen</td>
<td>Total creatine nitrogen</td>
<td>Uric acid nitrogen</td>
</tr>
<tr>
<td>days</td>
<td>gm.</td>
<td>gm.</td>
<td>gm.</td>
<td>mg.</td>
</tr>
<tr>
<td>1-8</td>
<td>131</td>
<td>1.43</td>
<td>0.64</td>
<td>16.1</td>
</tr>
<tr>
<td>9-16</td>
<td>121</td>
<td>0.80</td>
<td>0.59</td>
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</tr>
<tr>
<td>17-24</td>
<td>116</td>
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<td>0.50</td>
<td>19.2</td>
</tr>
<tr>
<td>25-32</td>
<td>114</td>
<td>0.82</td>
<td>0.58</td>
<td>21.1</td>
</tr>
<tr>
<td>33-40</td>
<td>114</td>
<td>0.68</td>
<td>0.57</td>
<td>19.4</td>
</tr>
<tr>
<td>41-48</td>
<td>112</td>
<td>0.63</td>
<td>0.55</td>
<td>19.5</td>
</tr>
<tr>
<td>49-56</td>
<td>110</td>
<td>0.61</td>
<td>0.52</td>
<td>17.6</td>
</tr>
</tbody>
</table>

- Rat 124. ♀. Initial weight, 109 gm.
- Diet 7 (whole casein).
- Diet 12 minus tryptophane.

the addition of this amino acid to Diet 11 not only permitted growth, but led to regular increases in the excretion of total creatinine, uric acid, and allantoin. Furthermore, the increments in output of the urinary components are of the same order as in animals upon the whole casein diet. Obviously, histidine and arginine bear quite different relations to purine metabolism.

Following the general procedure of Ackroyd and Hopkins we
have performed two experiments involving tryptophane deficiency. The experiments were begun after the others were well under way, and are of shorter duration than those which involved histidine deficiency. The results are shown in Table VII. Although the percentage losses in weight were almost as great, for equal periods of time, as in the rats deprived of histidine, the values for allantoin and uric acid excretion remained quite constant throughout.

DISCUSSION.

The data outlined above are of interest from several points of view. First, they appear to indicate that histidine is the precursor of tissue purines. In this respect our results are in accord with those of Ackroyd and Hopkins. We believe that this deduction is justified despite the irregularities in the figures for allantoin excretion. The unsatisfactory nature of the Wiechowski-Handovsky method is recognized by all who have employed it, and the undesirable features are emphasized when the method is applied to dilute urines, such as are obtained in metabolism studies upon rats. That the fluctuations in some of our allantoin data are to be attributed to errors inherent in the method of analysis, and not to incompleteness in urine collection, is indicated by the values for total creatinine and uric acid. The latter are quite regular in all of the experiments. Where the diet permitted growth, the increases in output of total creatinine are roughly proportional to the increments in body weight of the animals. We do not attach much significance to the absolute quantities of allantoin obtained either in our or in any other experiments upon rats hitherto reported in the literature, but we believe that the relative increases observed uniformly when histidine is present in the diet, and the relative decreases seen invariably when the amino acid is excluded from the ration, justify the conclusion that histidine and allantoin are closely related to each other in metabolism.

Furthermore, we have reached this conclusion somewhat contrary to our earlier ideas. During the progress of the experiments, it seemed to us not unlikely that the differences in the effects of histidine and arginine upon purine metabolism might
be due solely to the fact that the addition of one amino acid permitted growth, while the inclusion of the other did not. In other words, it seemed probable to us that the alterations in urine composition were the result of, and secondary to, the ability or inability of the rats to increase in body weight, and did not indicate necessarily a precursor relationship between histidine and body purines. We were inclined to the opinion that with loss in weight, and the resulting decrease in mass of active protoplasm, there might occur a diminution in the output of all endogenous products. In order to test this possibility, it was evidently necessary to determine the effects upon urinary composition of another type of dietary deficiency. Such an experiment appeared to be capable of serving as a crucial test for a possible relationship between histidine and purines. With these considerations in mind, we conducted the experiments upon the influence of excluding tryptophane from the diet. The results were striking. Instead of obtaining falls in allantoin and uric acid as we had anticipated, the values for both of these substances remained quite constant throughout the experiments. On the other hand, the total creatinine behaved just as it did during histidine deficiency, showing a tendency to increase at first, followed later by a decrease, but at no time falling below the original level observed on the adequate diet. It is true that the experiments upon tryptophane deficiency were only half as long as those which involved a deficiency of histidine; but in the latter, very pronounced decreases in allantoin excretion occurred during the first periods following the change to the inadequate ration. The conclusion appears to be warranted, therefore, that under ordinary conditions of diet, histidine is a mother-substance of allantoin. If this conclusion is correct, it is evident that one reason why histidine is an indispensable component of the diet is that it is required for nuclear synthesis.

In this connection it should be noted that our results are not necessarily in conflict with those of Abderhalden and Einbeck (1909), and Abderhalden, Einbeck, and Schmid (1910). These authors report that the feeding of histidine to fasting dogs, or the addition of histidine to the diet of a well fed animal, fails entirely to influence the allantoin output. We have discussed this question, and the literature bearing upon it, in a former
communication (Rose, 1921). Ackroyd and Hopkins (1916) also properly raise the question as to whether an abnormal condition like fasting affords the best opportunity for investigating the fate of an amino acid. In regard to the addition of excess histidine to an already adequate diet the same authors make the following interesting comment; "When an animal is in a state of full nutrition it does not follow that such a process as the synthesis of the purine ring would necessarily be much accelerated or increased by mere increase in the supply of its raw material."

As we have stated on several occasions elsewhere (Rose, 1921, 1923), it does not seem unreasonable to suppose that the synthesis of a tissue component may be limited quantitatively to the anabolic needs of the organism for that particular ingredient. If this conception is correct, the addition of histidine to an already adequate diet should not necessarily lead to an increase in allantoin excretion.

In contrast to the observations of Ackroyd and Hopkins our experiments show quite clearly that arginine cannot replace histidine in purine synthesis. This result serves to emphasize and reinforce the conclusion of Rose and Cox (1924), that in the absence of histidine from the diet its functions cannot be assumed vicariously by arginine. It appears evident that neither in growth nor in purine metabolism can the two diamino acids be regarded as mutually interchangeable.

Under the conditions which pertained in our experiments, no relationship was observed between the arginine content of the diet and the total creatinine elimination in the urine. The information in the literature concerning the relationship of arginine to creatine-creatinine metabolism is quite conflicting. Thompson (1917), and Gross and Steenbock (1921) expressed the opinion that increased arginine consumption leads to an exaggerated creatine production. Somewhat similar conclusions had been reached at an earlier date by McCollum and Steenbock (1912–13), and others. On the contrary, Myers and Fine (1915), Baumann and Marker (1915), and Baumann and Hines (1918) were unable to observe any relationship between the arginine content of the food and the creatine or creatinine output in the

\[ \text{For a review of the literature, see the paper of Hunter (1922).} \]
excreta. As we have stated elsewhere (Rose and Cox, 1924), it is quite likely that the Kossel-Kutscher method of precipitating the diamino acids does not completely remove arginine. Assuming, therefore, that this amino acid is the precursor of creatine, it is possible that even on the deficient diets our animals may have received adequate amounts for creatine anabolism, and that quantities furnished in excess of the anabolic needs were catabolized by more direct methods. In order to answer definitely the question of a possible relationship between arginine and creatine-creatinine metabolism, it would appear necessary to employ a diet known to be absolutely devoid of the amino acid in question. We are endeavoring to prepare such a material at the present time.

SUMMARY.

1. Studies have been made of creatine and purine metabolism in growing rats upon diets in which the nitrogen was supplied, respectively, in the form of casein, completely hydrolyzed casein, and hydrolyzed casein from which histidine and arginine had been precipitated by the Kossel-Kutscher procedure. The diets of whole casein and of completely hydrolyzed casein led to the excretion of progressively increasing quantities of total creatinine (creatine plus creatinine), allantoin, and uric acid, which were roughly proportional to the increments in body weight of the animals. On diets of hydrolyzed casein from which histidine and arginine had been precipitated, the output of allantoin decreased 40 to 50 per cent. The uric acid excretion also decreased, but the variations from the normal values were not quite so striking as in the case of allantoin. Total creatinine manifested an increase followed by a decline, but at no time did the output fall below the level seen on the adequate ration.

2. The effects upon purine metabolism are not to be attributed solely to the losses in weight of the rats on the deficient diets as is indicated by the results of experiments in which a deficiency of tryptophane was induced. When the ration was adequate in every respect except as regards its tryptophane content, the animals steadily declined in weight, but the output of allantoin and uric acid remained quite constant.
3. The addition of histidine to diets in which the component hydrolyzed casein had previously been subjected to silver precipitation, led to increases in the excretion of total creatinine, uric acid, and allantoin, until the quantities eliminated were of the same order as in animals upon whole casein. On the contrary, the addition of arginine to the deficient rations failed entirely to affect the output of any of the urinary components. It is evident from these investigations that arginine and histidine are not interchangeable in purine metabolism. Our data also appear to indicate that histidine is one of the precursors of purines.

4. Under the conditions which pertained in our experiments, no relationship was observed between the arginine content of the diet and the total creatinine elimination in the urine. This may have been due to the fact that none of our diets were completely devoid of arginine.

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