The equivalent of 0.12% lysine enrichment of white wheat flour resulted in significant enhancement of its protein value for the rapidly growing human infant, as demonstrated by increased rates of weight gain and nitrogen retention, stability of serum albumin, and elevation of the molar ratio of plasma lysine when the flour was the only source of protein in the diet. Isoenergetic and isocaloric casein diets were used as controls. Enrichment to the 0.2% level resulted in suggestive further increases in the rates of weight gain and N retention, a further elevation in the molar ratio of plasma lysine and a reduction in that of plasma threonine. Enrichment to the 0.4% level resulted in a suggestive further increase in N retention alone and in further elevation of plasma lysine and reduction of plasma threonine. Enrichment of white wheat flour with lysine to the 0.12% and possibly the 0.2% level is recommended for those areas in which this cereal serves as the main source of protein in the diet, particularly that of infants and children. The improvement in biological value to be expected is enough to make the difference between dietary protein adequacy and deficiency to the many, without adverse effects on the few who might not profit by such enrichment.
Lysine Enrichment of Wheat Flour: Evaluation in Infants

GEORGE G. GRAHAM, M.D.,¹ ROBERT P. PLACKO,¹ GLADYS ACEVEDO, M.D.,⁵ ENRIQUE MORALES, M.D.,⁶ AND ANGEL CORDANO, M.D.⁷

THE UNIVERSAL acceptability of wheat, through the simultaneous consumption of foods rich in this amino acid, particularly fish and legumes. In the diets of infants and young children in the same areas, complementary foods are commonly omitted, making children dependent on the cereal as the main if not only, source of protein. The same is true in many mass feeding programs. It is in these situations that important nutritional gains can be anticipated from the enrichment of wheat flour with appropriate amounts of lysine.

The purpose of these studies is to determine the amount of lysine that should be added to white wheat flour in order to obtain the maximum improvement in its biological value for human beings, without producing adverse effects. Previous investigators (2, 3) have already demonstrated in children the supplementary effect of generous lysine enrichment of wheat. Our own studies suggest that significant improvement in the protein quality of wheat can be demonstrated with the equivalent of as little as 0.12% lysine enrichment of ordinary white wheat flour.

MATERIALS AND METHODS
Six severely malnourished male infants were admitted to the British American Hospital for treatment of infection, rehydration, and correction of acute electrolyte disturbances. Once their acute manifestations were controlled, they were offered a modified cow’s milk preparation and transferred to a metabolic unit for study. After a steady rate of weight gain had been
established, serum proteins returned to normal, and hepatic steatosis corrected, they became the subjects of these studies. For each child, the approximate minimum level of calories and protein that maintained steady growth was determined and the source of protein was then changed to calcium caseinate. Their ages, heights, and weights at that time are given in Table 1. Height ages and weight ages refer to the ages on the 50th percentile of a standard United States growth curve (4), to which these measurements correspond, providing a rough index of their degree of undernutrition.

Cottonseed oil, cane sugar, and starch were added to the casein to achieve the desired caloric intake, maintaining the same proportion of fat and carbohydrate as in the modified cow’s milk and in human breast milk. Appropriate vitamin and mineral supplements (5) were provided and the diet was recalculated daily to yield the predetermined protein and caloric intakes per unit of body weight.

Rates of weight gain were measured and nitrogen (N) balances determined during three consecutive 9-day periods. Apparent absorption was measured by subtracting stool N from ingested N. Apparent retention by subtracting urine N from absorbed N. For the N determination on food aliquots, stool and urine, the standard micro-Kjeldahl technique was used. Serum total proteins by the Biuret method, serum albumin by paper electrophoresis, and fasting plasma amino acids by liquid-column chromatography (6) were determined at the end of this and each subsequent diet period. Following the first 9-day period on casein, the source of protein was changed to white wheat flour or the same flour enriched with lysine at one of three different levels, maintaining the same N and caloric intake per unit of body weight. Each child received all four wheat diets in random sequence, for 15–36 days each, with intervening 9-day periods on casein. Nitrogen balances on each wheat diet were determined for 9–15 days. For each of the casein diets, balances were determined for 6–9 days. With some frequency, because of intercurrent infections, diet periods had to be prolonged. In two children, because of a relatively long hiatus between wheat diets, a lower level of protein and caloric intake was used for the remaining diets but always with a preceding isocaloric and isonitrogenous casein period. In the wheat diets, cottonseed oil and cane sugar were added to make the fat and carbohydrate contents similar to those of milk and of the casein diets.

**TABLE 1**

<table>
<thead>
<tr>
<th>Case number</th>
<th>CA, month</th>
<th>Ht, cm</th>
<th>HA, month</th>
<th>Wt, kg</th>
<th>WA, month</th>
</tr>
</thead>
<tbody>
<tr>
<td>117</td>
<td>10</td>
<td>70.5</td>
<td>9</td>
<td>8.15</td>
<td>7</td>
</tr>
<tr>
<td>118</td>
<td>24</td>
<td>78.2</td>
<td>15</td>
<td>8.57</td>
<td>8</td>
</tr>
<tr>
<td>119</td>
<td>11</td>
<td>65.9</td>
<td>6</td>
<td>5.96</td>
<td>3 1/2</td>
</tr>
<tr>
<td>120</td>
<td>11</td>
<td>68.0</td>
<td>7</td>
<td>5.77</td>
<td>3</td>
</tr>
<tr>
<td>121</td>
<td>23</td>
<td>71.1</td>
<td>9</td>
<td>7.09</td>
<td>5 1/2</td>
</tr>
<tr>
<td>122</td>
<td>12</td>
<td>65.3</td>
<td>5 1/2</td>
<td>6.35</td>
<td>4</td>
</tr>
</tbody>
</table>

### Levels of Enrichment

It is possible for adults and older children to consume enough ordinary wheat flour to satisfy their minimum requirements of lysine (7). In preschool children, with higher requirements of N and lysine per unit of body weight, this is not likely, and in the infant it is a practical impossibility. A 1-year-old child weighing 10 kg, with a lysine requirement of 90 mg/kg body wt per day (8) and a caloric requirement of 90 kcal/kg per day, consuming as his only source of protein a wheat flour with 11% protein and 2.5 g of lysine/100 g protein, would have to consume 327 g of flour daily to satisfy his requirement of this amino acid. This amount of flour alone would supply 3.6 g of protein and 118 kcal/kg per day. If he could consume and digest such a diet, which is impossible, he would not only become obese but would probably develop a fatty liver and hypoalbuminemia, the result of an ordinarily high relation of total calories

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*Casein, generously supplied by Mead Johnson International, Evansville, Indiana.

**1-Lysine-HCl, generously supplied by Merck & Co., Rahway, N. J.**
to the first-limiting essential amino acid in the diet.

It is evident that ordinary wheat flour cannot possibly be the exclusive or nearly exclusive source of protein in the diet of infants and young children without the addition of appropriate amounts of lysine. Even so, the proportion of starch in the diet would not allow us to carry out a study in which fat contributed 53% of the diet's nonprotein calories; with the unenriched wheat we would always be providing an excessive caloric intake. We were fortunate in obtaining a white flour processed by air classification to contain approximately 21% protein, making it possible to prepare diets that were very similar in all their components to the control casein diets. The amino acid composition of this flour (Table 11) is quite typical of those in common use throughout the world. Ten “essential” amino acids (the usual eight plus tyrosine and cystine) made up 39.8% of its protein, compared with 48.8% for human breast milk (HBM) protein (Table 11). In the latter, lysine provides 129 mg of every gram of essential amino acids (EAA), while in the flour it supplies only 61% of this amount, 79 mg g EAA. Taking into account the differences in EAA content and assuming equal digestibilities, this wheat flour protein should in theory have a biological value for the human infant of approximately 42% of the value of HBM protein. If a similar calculation is made for the casein used in our studies, its theoretical biological value, relative to that of breast milk, is approximately 76%, and the wheat flour protein should have a biological value approximately 55% of that of casein.

The first level of supplementation with lysine was calculated to make it approximately as limiting as threonine, the presumed next-limiting amino acid in wheat. Again referring to Table 11, we see that

\[ \text{Lysine Enrichment of Wheat Flour} \]

<table>
<thead>
<tr>
<th>HBM protein</th>
<th>Wheat protein</th>
<th>EAA</th>
<th>mg</th>
<th>g</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>Pro</td>
<td>%</td>
<td>mg</td>
<td>g</td>
<td></td>
</tr>
<tr>
<td>Iso</td>
<td>4.4</td>
<td>1.11</td>
<td>4.22</td>
<td>125</td>
<td>95</td>
</tr>
<tr>
<td>Leu</td>
<td>9.9</td>
<td>1.83</td>
<td>6.51</td>
<td>195</td>
<td>105</td>
</tr>
<tr>
<td>Lys</td>
<td>6.3</td>
<td>1.29</td>
<td>2.67</td>
<td>79</td>
<td>64</td>
</tr>
<tr>
<td>Phe + Tyr</td>
<td>10.1</td>
<td>2.07</td>
<td>8.25</td>
<td>244</td>
<td>110</td>
</tr>
<tr>
<td>Cys + Met</td>
<td>4.3</td>
<td>0.88</td>
<td>3.62</td>
<td>107</td>
<td>121</td>
</tr>
<tr>
<td>Thr</td>
<td>4.6</td>
<td>0.94</td>
<td>2.88</td>
<td>83</td>
<td>88</td>
</tr>
<tr>
<td>Try</td>
<td>1.6</td>
<td>0.33</td>
<td>1.2</td>
<td>36</td>
<td>109</td>
</tr>
<tr>
<td>Val</td>
<td>6.6</td>
<td>1.35</td>
<td>4.5</td>
<td>133</td>
<td>98</td>
</tr>
</tbody>
</table>

| Total       | 40.8 | 1,000 | 11.77 | 1,000 |

- The % EAA for each amino acid in wheat as a percentage of that in HBM.

in the wheat flour there are 83 mg of threonine/g EAA, or 88% of the proportion in HBM. By adding 215 mg of lysine (as l-lysine-HCl) to every 100 g of the high protein wheat flour, its lysine content becomes approximately 3.75 g 100 g of protein or 108 mg g EAA. At this point, the lysine and threonine levels, in mg g EAA, are both approximately 85% of those for HBM and the wheat flour protein should have 60% of the biological value of HBM protein and 80% of the biological value of casein. An infant of 10 kg, receiving 2 g protein kg body wt per day from this source, would receive 75 mg lysine/kg per day, considerably more than the 53 he would get from an equal amount of unenriched wheat protein but still below the estimated requirement of 90 mg kg per day (8). Further supplementation with lysine should in theory produce no further enhancement of biological value, as beyond this point threonine becomes the first-limiting amino acid. This first level of supplementation is the equivalent of 0.12% enrichment of an ordinary white

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*Beem, generously supplied by Pillsbury Co., Minneapolis, Minn.
flour with 11% protein. In this report the unenriched flour is identified as (IV), while the flour enriched to this first level is identified as (IVL-1).

The second level of lysine supplementation (IVL-2) was calculated to make lysine supply the same proportion of the EAA's as in HBM protein, 129 mg/g EAA, and was achieved by adding 359 mg lysine/100 g of flour, the equivalent of 0.2% enrichment of ordinary white flour. At this point threonine should be clearly first-limiting and an infant receiving 2 g protein/kg per day from this source would have a lysine intake of 89 mg/kg per day, the approximate average requirement. The rate of growth, however, should be dependent on the threonine intake.

The third level (IVL-3) was chosen so that the lysine content of the wheat flour protein would be the same as that of HBM protein, 6.3%, and was achieved by adding 725 mg lysine/100 g wheat flour, the equivalent of 0.4% enrichment of ordinary white flour. This should represent an excess of lysine, and an infant ingesting 2 g protein/kg per day would receive 125 mg/kg per day.

**Table III**

<table>
<thead>
<tr>
<th>Case number</th>
<th>Diet kg per day</th>
<th>kcal</th>
<th>Pro</th>
<th>g kg</th>
<th>Δ wt</th>
<th>g kg</th>
<th>N ret.</th>
<th>mg kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>107</td>
<td>2.0</td>
<td>110</td>
<td>7.3</td>
<td>3.5</td>
<td>87</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>108</td>
<td>2.0</td>
<td>100</td>
<td>8.0</td>
<td>2.6</td>
<td>83</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>188</td>
<td>2.0</td>
<td>125</td>
<td>6.4</td>
<td>4.1</td>
<td>115</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>192</td>
<td>2.0</td>
<td>125</td>
<td>6.4</td>
<td>5.5</td>
<td>106</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>203</td>
<td>2.0</td>
<td>125</td>
<td>6.4</td>
<td>4.7</td>
<td>99</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>204</td>
<td>2.0</td>
<td>90</td>
<td>6.7</td>
<td>2.7</td>
<td>58</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>205</td>
<td>1.65</td>
<td>100</td>
<td>6.6</td>
<td>2.8</td>
<td>89</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Pro = protein, ret = retention.

In theory, the wheat flour protein enriched to the second and third levels (IVL-2 and IVL-3) should have a biological value no higher than that of IVL-1, that is, 60% of that of HBM and 80% of that of casein, as this will then be determined by the availability of threonine, if this is its second-limiting essential amino acid.

**RESULTS**

All diets were well accepted and tolerated. The percentage absorption of nitrogen was in each instance as high or even higher than that for the preceding and following casein diets. All values were between 80 and 91%, with most of them between 84 and 90% of intake. This is considerably higher than that of other cereal diets we have studied and moderately higher than the usual values for modified cow's milk in our unit. Because of the consistently good results, no attempt will be made to further analyze these.

The rates of weight gain and the apparent N retention from each diet in each child have been expressed as a percentage of the same during the preceding and following casein diets. In Table III, along with the protein and calorie intakes for each child, we have listed the rates of weight gain in grams per kilogram body weight per day and the apparent nitrogen retention in milligrams per kilogram body weight per day during the casein periods.

In Table IV we have tabulated the rates of weight gain on each diet as previously defined. It must be borne in mind that rates of weight gain during relatively short periods of time are not always an accurate reflection of the adequacy of protein in the diet. Thus, one may see accelerated weight gain during periods of poor N retention and very little or no weight gain during periods of good retention, probably reflecting retentions and losses of sodium and water. Nevertheless, in this study the diet periods were of suf-
significant duration to make changes in weight more meaningful. The difference in rates of weight gain between W and WL-1 approached significance (P < 0.1) by the Student's t test. The differences between W and WL-2 and W and WL-3 were statistically significant (P < 0.05). None of the differences between the various supplemented diets were significant.

Table v summarizes the apparent N retention for each child on each wheat diet as a percentage of the same during the casein periods immediately preceding and following it. The differences between unenriched wheat and all three supplemented diets were significant (P < 0.01). The differences between WL-1 and WL-2 and between WL-2 and WL-3 were not statistically significant, but that between WL-1 and WL-3 was (P < 0.02).

Table vi compares the mean changes in serum albumin and the mean level at the end of each diet. The changes were generally not striking, nor are they very likely to be during such relatively short periods. With each of the wheat diets there was occasionally a more impressive fall in serum albumin, usually following an infection. With these exceptions, the final levels were very satisfactory, above 4.0 g/100 ml. Changes in serum albumin must be interpreted in relation to rates of weight gain; if the levels remain normal or improve during periods of little or no weight gain they cannot be considered indicators of adequate protein value of the diet (9). If, as in most cases in this study, they remain in the normal range during periods of accelerated weight gain, they are suggestive of a satisfactory protein intake.

The ratio of essential to total amino acids in plasma gives some indication of the adequacy of dietary protein, as to quantity if not as to quality (10). In our estimation of these ratios, the figure for the total essential amino acids was derived from the sum of the fasting levels in plasma, in micromoles per milliliter, of isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tyrosine, and

### Table iv

Rates of weight gain during the four wheat diets as a percentage of the rates during the casein periods

<table>
<thead>
<tr>
<th>Case number</th>
<th>W, %</th>
<th>WL-1, %</th>
<th>WL-2, %</th>
<th>WL-3, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>107</td>
<td>63</td>
<td>94</td>
<td>105</td>
<td>66</td>
</tr>
<tr>
<td>102</td>
<td>61</td>
<td>69</td>
<td>100</td>
<td>85</td>
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<tr>
<td>101</td>
<td>57</td>
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<td>102</td>
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<td>203</td>
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<tr>
<td>204</td>
<td>86</td>
<td>100</td>
<td>86</td>
<td>99</td>
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<tr>
<td>Mean</td>
<td>67</td>
<td>83</td>
<td>97</td>
<td>91</td>
</tr>
<tr>
<td>SD</td>
<td>11.6</td>
<td>14.2</td>
<td>22.7</td>
<td>13.0</td>
</tr>
</tbody>
</table>

### Table v

Apparent nitrogen retentions during the four wheat diets as a percentage of those during the casein periods immediately preceding and following each diet

<table>
<thead>
<tr>
<th>Case number</th>
<th>W, %</th>
<th>WL-1, %</th>
<th>WL-2, %</th>
<th>WL-3, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>107</td>
<td>64</td>
<td>77</td>
<td>116</td>
<td>111</td>
</tr>
<tr>
<td>102</td>
<td>62</td>
<td>101</td>
<td>94</td>
<td>105</td>
</tr>
<tr>
<td>101</td>
<td>78</td>
<td>93</td>
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<td>103</td>
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<td>102</td>
<td>43</td>
<td>80</td>
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</tr>
<tr>
<td>203</td>
<td>71</td>
<td>91</td>
<td>103</td>
<td>96</td>
</tr>
<tr>
<td>204</td>
<td>39</td>
<td>79</td>
<td>94</td>
<td>123</td>
</tr>
<tr>
<td>Mean</td>
<td>63</td>
<td>87</td>
<td>98</td>
<td>106</td>
</tr>
<tr>
<td>SD</td>
<td>11.9</td>
<td>9.6</td>
<td>11.0</td>
<td>9.6</td>
</tr>
</tbody>
</table>

### Table vi

Mean change (Δ) and final serum albumin (SA) after each of the four wheat diets and the casein periods

<table>
<thead>
<tr>
<th></th>
<th>Δ SA, g/100 ml, mean and range</th>
<th>Final SA, g/100 ml, mean and range</th>
</tr>
</thead>
<tbody>
<tr>
<td>W</td>
<td>-0.00 (-0.27, 0.18)</td>
<td>4.02 (4.04, 4.22)</td>
</tr>
<tr>
<td>WL-1</td>
<td>-0.20 (-1.09, 0.28)</td>
<td>4.12 (4.45, 4.60)</td>
</tr>
<tr>
<td>WL-2</td>
<td>-0.99 (-0.47, 0.84)</td>
<td>4.32 (4.09, 4.45)</td>
</tr>
<tr>
<td>WL-3</td>
<td>0.08 (-0.76, 0.55)</td>
<td>4.14 (4.50, 4.70)</td>
</tr>
<tr>
<td>Casein</td>
<td>0.05 (-0.10, 0.36)</td>
<td>4.23 (3.90, 4.38)</td>
</tr>
</tbody>
</table>
valine. Cystine and tryptophan, both present in relatively low concentration, were excluded for technical reasons. The figure for total amino acids includes the above eight essentials plus the nonessentials: alanine, arginine, asparagine, glutamine, glycine, histidine, ornithine, proline, and serine. Aspartic and glutamic acids, as well as taurine, were also excluded for technical reasons.

Table vii summarizes the mean values for each wheat diet and the respective ratios, comparing them with the same values and ratios for a large number of casein diets of similar protein and caloric value. The differences between the various wheat diets were not significant but the differences between these and the casein diets was significant ($P < 0.05$). Whether this difference reflects the difference in EAA content of the respective proteins or actual differences in protein adequacy is not clear.

In the experimental animal under special conditions (11) and in the human infant, when an essential amino acid is lacking in the diet or present in marked excess (12), the plasma amino acid levels will be clearly indicative of the situation. To our knowledge, the plasma "aminogram" has not been shown to indicate the first-limiting amino acid in human diets as consumed in actual practice. If such were possible, the aminograms of our subjects consuming the unenriched wheat diet would have been expected to reveal a lower level of lysine, relative to the other essentials, than in the supplemental diets. Similarly, the diets with a calculated excess of lysine, WL-2 and particularly WL-3, should have resulted in a relatively elevated level of lysine in the aminogram and a relatively lower level of threonine, if this were indeed the second-limiting amino acid in white wheat flour for the human infant, as is the case for the laboratory rat (1). Although such trends were apparent in individual cases, there were enough exceptions to suggest that this determination is not a reliable indicator of the first-limiting amino acid in the diet of individual infants and children. As has already been documented (10, 12), the levels of certain essential amino acids, particularly tyrosine, isoleucine, and valine, tend to fall whenever the diet is low in total protein or in any essential amino acid. The same trend was usually present in our studies.

The level of plasma lysine was expressed as its fraction of the total EAA (the eight previously mentioned). In four of the six children there was a distinct upward trend as the level of dietary lysine increased (Table viii). The differences between the means for $W$ and $W1-1$ approached significance ($P < 0.1$), while the difference between $W$ and $W1-2$ ($P < 0.02$) and $W$ and $W1-3$ ($P < 0.01$) were significant. None of the other differences were of significance. In a group of 35 infants receiving modified cow's milk or casein diets with 6.4–8.0% of calories as protein, the mean lysine–EAA ratio was 0.157 (so 0.041), very similar to that of the $W1-1$ diets.

The level of plasma threonine was expressed in a like manner (Table ix). As dietary lysine was increased, there was a downward trend in threonine values in the same four infants in which the up-

---

**Table vii**

Mean fasting plasma amino acids of the six infants at the end of each wheat diet

<table>
<thead>
<tr>
<th>EAA, Amino Acid</th>
<th>W</th>
<th>W1-1</th>
<th>W1-2</th>
<th>W1-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu M$</td>
<td>5.02</td>
<td>5.57</td>
<td>5.15</td>
<td>5.57</td>
</tr>
<tr>
<td>$\mu g/100 g$</td>
<td>0.56</td>
<td>0.17</td>
<td>0.17</td>
<td>0.17</td>
</tr>
<tr>
<td>$\mu M$</td>
<td>2.23</td>
<td>2.33</td>
<td>2.23</td>
<td>2.33</td>
</tr>
<tr>
<td>$\mu g/100 g$</td>
<td>0.41</td>
<td>0.31</td>
<td>0.41</td>
<td>0.31</td>
</tr>
<tr>
<td>$\mu M$</td>
<td>0.26</td>
<td>0.39</td>
<td>0.26</td>
<td>0.39</td>
</tr>
<tr>
<td>$\mu g/100 g$</td>
<td>0.02</td>
<td>0.01</td>
<td>0.02</td>
<td>0.01</td>
</tr>
</tbody>
</table>

EAA = total essential amino acids. AA = total amino acids. * Values after casein diets with 6.4 to 6.7% of calories as protein in 13 infants are included for comparison.
ward trend in lysine values was evident. These were the same four infants in whom the effect of lysine supplementation on rates of weight gain was most evident. The difference between these ratios (Thr/EAA) for W and WL-1 was not significant, but the differences between W and WL-2 (P < 0.05) and between W and WL-3 (P < 0.02) were significant. The difference between WL-1 and WL-3 approached significance (P < 0.1), the other differences did not. For the modified milk or casein diets, this ratio was 0.165 (SD 0.038), again very similar to that of the WL-1 diets.

**DISCUSSION**

Apparent nitrogen retentions from iso-caloric and isonitrogenous diets at critical levels of intake are considered the single most reliable measure of relative biological value of dietary proteins for human infants (13). By this criterion we have been able to demonstrate a significant improvement in the biological value of white wheat flour protein when enough lysine was added to the diet to make lysine no more limiting than threonine, the second-limiting amino acid in wheat. By this same criterion there was an apparent further enhancement when a moderate excess of lysine was added, apparently equating the biological value of casein, which was not to be expected.

When the rates of weight gain were used as the measure of biological value, the further advantage of additional supplementation beyond the level of the next-limiting amino acid was not nearly as apparent. This might be explained on the basis of equal caloric intakes, as these set the upper limit for the rate of weight gain (14). If this apparent situation is true, nearly equal rates of weight gain despite inferior nitrogen retentions, it should be accompanied by differences in body composition: lower protein content, higher fat, and possibly higher sodium and water contents. Changes in the serum total protein levels, and particularly in serum albumin, represent a close approximation of such changes in the experimental animal (15) and do occur when infants and children continue to gain weight despite inadequate protein intakes and poor nitrogen retentions (9).

In the present study, the changes in serum albumin concentration and the final levels reached on each of the four wheat diets and the casein diets were not significantly different, but this may be a

**TABLE VIII**

Fasting plasma lysine of the six infants at the end of each wheat diet as a fraction of the total essential amino acids (Lys/EAA) in µmoles ml

<table>
<thead>
<tr>
<th>Case number</th>
<th>W</th>
<th>WL-1</th>
<th>WL-2</th>
<th>WL-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>107</td>
<td>0.209</td>
<td>0.226</td>
<td>0.261</td>
<td></td>
</tr>
<tr>
<td>102</td>
<td>0.130</td>
<td>0.198</td>
<td>0.183</td>
<td></td>
</tr>
<tr>
<td>103</td>
<td>0.154</td>
<td>0.163</td>
<td>0.179</td>
<td></td>
</tr>
<tr>
<td>104</td>
<td>0.120</td>
<td>0.167</td>
<td>0.174</td>
<td></td>
</tr>
<tr>
<td>105</td>
<td>0.080</td>
<td>0.149</td>
<td>0.174</td>
<td></td>
</tr>
<tr>
<td>106</td>
<td>0.136</td>
<td>0.162</td>
<td>0.174</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.191</td>
<td>0.178</td>
<td>0.191</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>0.046</td>
<td>0.039</td>
<td>0.028</td>
<td>0.015</td>
</tr>
</tbody>
</table>

**TABLE IX**

Fasting plasma threonine of the six infants at the end of each wheat diet as a fraction of the total essential amino acids (Thr/EAA) in µmoles ml

<table>
<thead>
<tr>
<th>Case number</th>
<th>W</th>
<th>WL-1</th>
<th>WL-2</th>
<th>WL-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>107</td>
<td>0.103</td>
<td>0.110</td>
<td>0.110</td>
<td></td>
</tr>
<tr>
<td>102</td>
<td>0.148</td>
<td>0.170</td>
<td>0.170</td>
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<tr>
<td>103</td>
<td>0.110</td>
<td>0.114</td>
<td>0.114</td>
<td></td>
</tr>
<tr>
<td>104</td>
<td>0.165</td>
<td>0.174</td>
<td>0.174</td>
<td></td>
</tr>
<tr>
<td>105</td>
<td>0.165</td>
<td>0.174</td>
<td>0.174</td>
<td></td>
</tr>
<tr>
<td>106</td>
<td>0.190</td>
<td>0.190</td>
<td>0.190</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.147</td>
<td>0.142</td>
<td>0.142</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>0.041</td>
<td>0.039</td>
<td>0.027</td>
<td>0.039</td>
</tr>
</tbody>
</table>
duration of the relatively short duration of each diet period. Prolonged feeding trials now under way with each of the enriched wheat diets may provide answers to this question. Studies of body composition on two of these infants (body water compartments, creatinine excretions and biopsies of muscle and fat) suggest that the chemical nature of their growth during the duration of these studies was not different from that of similar children receiving exclusive milk or casein diets during recovery.

There was no difference between the molar content of total fasting plasma amino acids after the wheat diets and those after isonitrogenous, isocaloric casein diets. The molar content of eight essential amino acids was moderately higher after casein diets and the molar ratio of essential-total amino acids was significantly lower after the wheat diets. There were no differences between the various wheat diets. These differences probably represent the lower level of dietary essential amino acids and of the first-limiting amino acid. They could possibly represent a lesser adequacy of the dietary protein for these children.

When the results for the six children were combined there was a progressive rise in the molar ratio of plasma lysine and a fall in that of plasma threonine, suggesting that for a group of individuals consuming nearly identical diets it might be possible to identify the first-limiting amino acid through its molar plasma ratio. The individual trends were not consistent enough to suggest that under conditions similar to those of this study, such identification could be made reliably in an individual. Further experience with these diets and others limiting in different amino acids is being accumulated.

In attempting to explain the apparent benefit of lysine supplementation beyond the level of the next-limiting amino acid that is suggested by the nitrogen retention, various possibilities may be considered:

1) The availability of lysine in wheat flour and in the cooked diets may be less than indicated by the composition or, more likely, the more rapid absorption of the supplemental lysine might result in wastage.

2) Human breast milk may contain a relative excess of threonine, and, consequently, the first level of supplementation did not in fact reach the next-limiting factor.

3) A relative threonine deficiency does not manifest itself as a reduced rate of weight gain but could be apparent later as a fall in serum albumin and an increase in liver fat. We suspect this to be the case when methionine is the first-limiting amino acid (9).

4) An excess of lysine might produce a transitory increase in nitrogen retention by an unexplained mechanism, possibly growth hormone stimulation.

We hope that prolonged feeding trials will help explain this interrogant. Various trends might be apparent:

1) The differences in the rates of weight gain between WL-1 and WL-2 and WL-3 might become more apparent, suggesting that one of the first two alternatives is true and that there would be a real advantage to enrichment beyond the first level.

2) Similar rates of weight gain might continue to be supported but a more striking fall in serum albumin and an increase in liver fat might become apparent on diet WL-1, again supporting one of the first two alternatives and further enrichment with lysine.

3) Similar rates of weight gain might continue, but a greater fall in serum albumin and an increase in liver fat become evident with WL-2 or WL-3, or both, suggesting that the third alternative is true and that there is no virtue to enrichment.
Lysine Enrichment of Wheat Flour

at the level or levels that produce these changes.

4) No differences in the rates of growth and in serum proteins or liver fat might become apparent, suggesting that the fourth alternative is true and that there is no virtue to enrichment beyond the first level.

Our prolonged feeding trials are thus far incomplete and the results inconclusive but do suggest that there is further advantage to be gained by enrichment to the second level.

These studies indicate that there are very real gains in protein value from enrichment of white wheat flour with 0.12% lysine and a probable further advantage to enrichment at the 0.2% level. The possibility of significantly improving the nutritional value of this important cereal grain by such a simple expedient and without in any way altering its very favorable characteristics has enormous practical implications for programs designed to prevent malnutrition in deprived populations anywhere in the world. They do not in any way deny the possibility of even greater benefits to be derived from enrichment with proteins rich in lysine, such as those of fish and soy, at least until problems of technology and acceptability are overcome, or wherever they cannot be overcome. The constantly falling cost of this amino acid has already made this an economically practical solution to the appalling problem of protein deficiency, at least in those areas of the world in which significant consumption of wheat by infants and children is already an established habit.

SUMMARY

The equivalent of 0.12% lysine enrichment of white wheat flour resulted in significant enhancement of its protein value for the rapidly growing human infant, as demonstrated by increased rates of weight gain and nitrogen retention, stability of serum albumin, and elevation of the molar ratio of plasma lysine when the flour was the only source of protein in the diet.

Lysine enrichment of casein diets used as controls. Enrichment to the 0.2% level resulted in further increases in the rates of weight gain and N retention, a further elevation in the molar ratio of plasma lysine and a reduction in that of plasma threonine. Enrichment to the 0.4% level resulted in a further increase in N retention alone and in further elevation of plasma lysine and reduction of plasma threonine.

Enrichment of white wheat flour with lysine to the 0.12% and possibly the 0.2% level is recommended for those areas in which this cereal serves as the main source of protein in the diet, particularly that of infants and children. The improvement in biological value to be expected is enough to make the difference between dietary protein adequacy and deficiency to the many, without adverse effects to the few who might not profit by such enrichment.

REFERENCES


