

Effect of Amino Acids on Iron Absorption from a Staple Vegetable Food

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FOOD iron absorption studies in man, using radioiron incorporated biologically into the food, have shown that iron from vegetable foods is poorly absorbed,¹⁻³ especially from staples, such as corn and black beans, which are consumed in large amounts by individuals belonging to the low socioeconomic levels in Latin American countries.

Layrisse, Martínez-Torres and Roche¹ have observed that iron absorption from either corn or black beans is enhanced when these foods are mixed with food of animal origin (veal or fish muscle), or with a mixture of amino acids similar to that present in 100 Gm. of fish. The present contribution provides further information on the effect of fish muscle, or of the amino acids present in fish, on iron absorption from black beans, and the possible effect of the amino acids grouped according to their chemical properties.

MATERIALS AND METHODS

One hundred and seven adult peasants from agricultural areas of Venezuela volunteered for this study; 50 were males and 57 were females. They were in apparent good health with the exception of some with moderate or marked iron deficiency anemia. Hemoglobin,³ packed cell volume, serum iron⁴ and unsaturated iron binding capacity⁵ were performed in each subject.

Absorption Studies

Tagged black beans in which radioiron was incorporated biologically was administered in the morning, after an overnight fast, and no food or drink was allowed for three hours following the test. The next day, black beans tagged with a radioisotope different from that used the preceding day was administered mixed with either fish or with amino acids in number and proportion similar to those present in 100 Gm. of fish,¹ or with amino acids representative of various groups. Blood was drawn 15 days after the feeding test to determine its hematological characteristics and radioactivity. In some experiments, the subjects were fed on days 15 and 16 with the new tagging material and blood was taken again on the 30th day. Duplicate 10-ml. blood samples were prepared for radioactive counting by wet-ashing and electroplating according to the method of Derm and Hart.⁷ Radioactivity was measured in a Tri-Carb Liquid Scintillation Counter (Packard Model 3310). Duplicate standards of the foods administered were counted simultaneously with the blood samples. In the calculation of the per cent of iron absorption, blood volume was estimated from the weight and height of the individuals using the Tulane Table.⁸ No correction was made to determine total iron utilization.

Preparation of the Feeding Material

Black beans (*Phaseolus vulgaris*) were grown in hydroponic culture containing either ⁵⁵Fe or ⁵⁹Fe. The labeled black beans, together with carrier black beans, were boiled and

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This investigation was supported in part by USPHS Grant HE-06507-08.

mashed to make a homogeneous paste. Further details of culture and preparation have been given previously.²

The amino acids administered with black beans were dissolved in 300 ml. of water just before being given to the subjects. When only sulphur amino acids were administered, they were prepared in standard capsules to avoid the unpleasant taste of cysteine. As the gelatine capsules contain a large variety of amino acids,⁹ although in a proportion lesser than the doses administered (0.1 and 1 per cent of the respective doses of cysteine and methionine), a control study was performed comparing iron absorption from black beans given alone, with black beans administered with empty capsules.

The dose of ferrous ascorbate was prepared by mixing tracer doses of about 30–50 μ Gm. of iron as ferric chloride containing about 1–3 μ Ci. ⁵⁹Fe with 3–4 mg. of carrier iron sulphate and adding two moles of ascorbic acid per mole of iron prior to oral administration.

Statistical Analysis

The comparison of the iron absorption from black beans given alone with black beans administered with fish or amino acids was performed by the Student test in paired samples.¹⁰

RESULTS

Characteristics of the Iron Absorption from Black Beans

Figure 1 shows the distribution of the per cent iron absorption from black

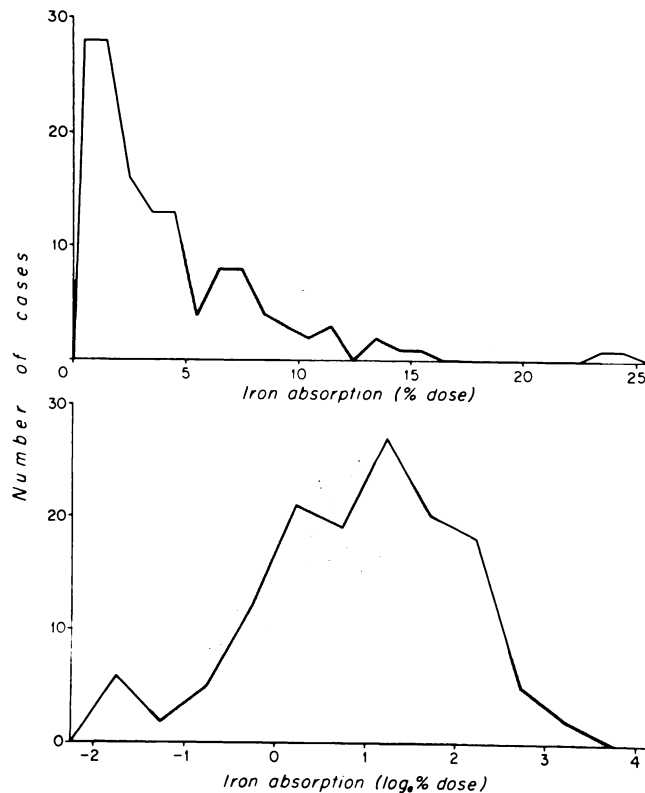


Fig. 1.—Distribution of iron absorption in 137 peasants living in agricultural areas of Venezuela. Doses of 3–4 mg. food iron.

beans in 137 subjects. Observations presented here (Table 1) and those published elsewhere are included.^{1,2} It can be seen that the distribution is asymmetric, as is the case with absorption of inorganic iron,¹¹ but that it changes into a normal distribution when per cent absorption is expressed in logarithms. Accordingly, the mean absorption of each study was calculated from the logarithm of percentage absorption and the results were transformed to antilogarithms to return to the original unit of per cent of iron absorption. A similar procedure was used to calculate the limits of one standard deviation.

Table 1 shows the hematological characteristics of the 107 individuals studied, as well as the per cent absorption from black bean iron given alone and from iron ascorbate. The mean iron absorption from black beans was 2.6 per cent. The mean iron absorption in normal and iron deficient subjects was made according to the per cent transferrin saturation value (normal ≥ 16 per cent). In normal subjects the mean absorption was 2.1 per cent (0.8–5.3) and in iron deficient subjects it was 4.4 per cent (1.2–15.7).

Black bean iron absorption correlates better with inorganic iron absorption than with either plasma iron or per cent transferrin saturation.² However, as the estimation of inorganic iron absorption extends the experiment to 30 days instead of 15, only 27 cases were tested. Accordingly, these cases were pooled with another 25 cases published elsewhere.^{1,2} It was observed that normal subjects who absorbed less than 25 per cent of 1–5 mg. of iron^{12,13} have a geometric mean absorption of 1.3 (0.5–3.3), and iron deficient subjects who absorbed 25 per cent or more of inorganic iron the mean absorption was 2.8 per cent (1.5–5.0).

Intrasubject Variability

Besides variation in iron absorption between subjects, day-to-day intrasubject variation in iron absorption should be taken into consideration when the effect of an agent on the iron absorption from food is evaluated. A control experiment consisting in the administration of black beans alone, followed 24 hours later by the administration of the same food plus empty gelatine capsules was used to observe the day-to-day intrasubject variation in black bean iron absorption. This experiment was planned as a control for subjects fed with black beans together with gelatine capsules containing a certain amount of sulfur aminoacids.

Table 2 shows the two iron absorption tests and the ratio. It is noticed that although variation in absorption on successive days is of the order of –20 per cent to +167 per cent (± 1 SD) of the mean assigned to the value of 100 per cent, the probability that the two absorption tests in this group were similar, is one in 10.

Effect of Fish and Amino Acids on Iron Absorption from Black Beans

Tables 3–5 show the iron absorption when black beans were administered alone and when they were given with fish or amino acids. Due to the small number of individuals in each experiment, normal and iron-deficient subjects were grouped. Iron absorption from black beans increased about twice when the food was administered with either fish or amino acids (Table 3). Summing

Table 1.—Iron Absorption from Black Beans

| Identification | Age | Sex | Hb. Gm./100 ml. | Packed Red Cell (per cent) | Serum Iron μGm./100 ml. | Per Cent Transferrin Saturation | Iron Absorption from Black Beans, Three—4 mg. Food Iron (per cent) | Iron Ascorbic Absorption 3 mg. Iron (per cent) |
|----------------|-----|-----|-----------------|-------------------------------|----------------------------|---------------------------------------|---|---|
| 1) 82 JCR | 67 | M | 10.3 | 36.0 | 58 | 13.7 | 0.2 | |
| 2) 58 MCT | 35 | F | 14.4 | 43.0 | 64 | 13.1 | 0.2 | |
| 3) 142 MSG | 29 | F | 12.1 | 41.0 | 123 | 31.8 | 0.2 | |
| 4) 63 MT | 27 | M | 7.2 | 28.5 | 16 | 3.6 | 0.2 | |
| 5) 32 JB | 42 | M | 14.6 | 44.0 | 157 | 29.0 | 0.2 | 22.8 |
| 6) 33 RF | 30 | M | 14.9 | 45.0 | 139 | 43.0 | 0.2 | 13.3 |
| 7) 16 REN | 40 | M | 14.6 | 43.5 | 84 | 23.4 | 0.3 | 12.2 |
| 8) 22 ER | 35 | F | 13.2 | 40.0 | 93 | 23.0 | 0.3 | 7.4 |
| 9) 220 GM | 60 | F | 13.2 | 38.5 | 87 | 28.4 | 0.4 | |
| 10) 35 FV | 36 | F | 13.3 | 41.5 | 104 | 35.5 | 0.4 | 11.1 |
| 11) 18 JAO | 50 | M | 12.2 | 40.0 | 36 | 7.5 | 0.5 | 54.9 |
| 12) 196 CD | 59 | M | 13.5 | 41.0 | 60 | 23.7 | 0.6 | |
| 13) 227 RS | 42 | F | 13.6 | 42.5 | 49 | 18.1 | 0.7 | |
| 14) 223 MN | 53 | F | 13.0 | 41.0 | 90 | 34.4 | 0.7 | |
| 15) 195 RR | 59 | M | 14.6 | 43.0 | 113 | 30.0 | 0.8 | |
| 16) 150 CM | 30 | M | 13.9 | 43.0 | 111 | 28.5 | 0.8 | |
| 17) 214 HS | 28 | F | 12.5 | 39.0 | 78 | 26.5 | 0.8 | |
| 18) 70 MAP | 37 | M | 14.4 | 47.0 | 114 | 29.1 | 0.8 | 22.8 |
| 19) 28 MFT | 19 | F | 12.8 | 41.0 | 114 | 32.5 | 0.8 | |
| 20) 201 PG | 40 | M | 15.6 | 49.0 | 143 | 44.8 | 0.8 | |
| 21) 230 MM | 40 | F | 12.0 | 36.0 | 112 | 33.5 | 0.9 | |
| 22) 144 FR | 50 | F | 14.7 | 41.0 | 122 | 28.1 | 0.9 | |
| 23) 79 JMD | 36 | M | 15.2 | 45.0 | 101 | 35.4 | 1.1 | |
| 24) 30 FB | 50 | F | 14.0 | 41.5 | 89 | 23.2 | 1.1 | 3.7 |
| 25) 240 AS | 40 | M | 14.6 | 44.0 | 137 | 45.2 | 1.2 | |
| 26) 137 IB | 33 | F | 10.8 | 41.0 | 119 | 35.3 | 1.3 | |
| 27) 66 MHM | 20 | F | 11.6 | 37.0 | 89 | 26.8 | 1.3 | |
| 28) 57 MCL | 30 | F | 14.2 | 42.5 | 89 | 27.8 | 1.4 | |
| 29) 211 SS | 60 | F | 12.5 | 40.5 | 41 | 11.6 | 1.4 | |
| 30) 226 EM | 15 | F | 13.9 | 43.5 | 61 | 17.8 | 1.5 | |

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| 31) | 197 | JTU | 50 | M | 9.6 | 32.5 | 37 | 10.2 | 1.5 | | |
| 32) | 146 | MAR | 50 | F | 17.0 | 48.0 | 114 | 29.9 | 1.5 | | |
| 33) | 222 | AJB | 33 | F | 12.2 | 39.0 | 93 | 36.1 | 1.5 | | |
| 34) | 34 | MRB | 33 | M | 16.2 | 48.5 | 154 | 37.3 | 1.6 | 4.3 | |
| 35) | 25 | TGD | 24 | F | 14.0 | 40.0 | 124 | 31.0 | 1.6 | 20.0 | |
| 36) | 228 | JCC | 58 | F | 13.1 | 38.0 | 51 | 18.0 | 1.7 | | |
| 37) | 13 | SV | 60 | M | 14.9 | 45.0 | 89 | 24.7 | 1.7 | 49.0 | |
| 38) | 199 | AM | 17 | M | 17.3 | 49.5 | 176 | 52.5 | 1.7 | | |
| 39) | 36 | MRV | 34 | F | 14.0 | 41.5 | 72 | 21.4 | 1.7 | 11.5 | |
| 40) | 69 | VR | 42 | M | 13.5 | 44.5 | 46 | 14.7 | 1.7 | | |
| 41) | 31 | MC | 20 | M | 14.8 | 48.0 | 119 | 27.3 | 1.7 | 26.7 | |
| 42) | 27 | AT | 15 | M | 13.0 | 42.0 | 89 | 25.0 | 1.8 | 46.2 | |
| 43) | 225 | OM | 33 | F | 14.5 | 47.0 | 80 | 20.7 | 1.9 | | |
| 44) | 77 | RS | 36 | M | 11.0 | 38.0 | 106 | 25.9 | 2.1 | | |
| 45) | 26 | MES | 23 | F | 12.4 | 36.5 | 72 | 24.0 | 2.1 | 33.1 | |
| 46) | 207 | AD | 15 | F | 11.7 | 38.0 | 53 | 14.7 | 2.2 | 63.6 | |
| 47) | 21 | AB | 30 | F | 13.7 | 42.0 | 110 | 34.0 | 2.5 | 6.3 | |
| 48) | 73 | ALA | 27 | F | 12.7 | 40.0 | 93 | 24.2 | 2.5 | | |
| 49) | 224 | GEM | 30 | F | 11.7 | 38.0 | 34 | 12.7 | 2.6 | | |
| 50) | 80 | EC | 25 | M | 12.8 | 42.0 | 71 | 20.2 | 2.7 | | |
| 51) | 147 | CB | 19 | M | 17.8 | 45.0 | 96 | 21.8 | 2.8 | | |
| 52) | 23 | FMC | 46 | F | 12.7 | 40.0 | 76 | 17.2 | 2.8 | 46.5 | |
| 53) | 212 | IV | 45 | F | 12.5 | 41.0 | 62 | 19.4 | 2.9 | | |
| 54) | 213 | EM | 40 | F | 11.6 | 38.5 | 30 | 7.8 | 3.0 | | |
| 55) | 205 | GH | 16 | F | 14.2 | 43.0 | 79 | 23.8 | 3.0 | 15.6 | |
| 56) | 204 | CTA | 18 | F | 13.4 | 40.0 | 97 | 27.5 | 3.0 | 24.8 | |
| 57) | 206 | AR | 29 | F | 11.5 | 36.0 | 52 | 11.8 | 3.0 | 46.7 | |
| 58) | 216 | HS | 24 | F | 13.8 | 43.0 | 111 | 32.8 | 3.1 | | |
| 59) | 17 | SV | 33 | M | 15.8 | 47.0 | 206 | 68.4 | 3.2 | 33.4 | |
| 60) | 61 | IF | 50 | F | 14.5 | 43.0 | 106 | 29.9 | 3.2 | | |
| 61) | 237 | JAD | 30 | M | 16.3 | 47.5 | 111 | 35.2 | 3.3 | | |
| 62) | 84 | AM | 45 | M | 15.1 | 46.5 | 89 | 29.8 | 3.4 | | |
| 63) | 208 | JD | 21 | F | 12.9 | 41.0 | 74 | 21.8 | 3.9 | 64.5 | |
| 64) | 200 | MD | 27 | M | 13.7 | 41.0 | 90 | 32.3 | 3.7 | | |

Table 1.—(Continued)

| Identification | Age | Sex | Hb. Gm./100 ml. | Packed Red Cell (per cent) | Serum Iron μGm./100 ml. | Per Cent Transferrin Saturation | Iron Absorption from Three-4 mg. Food Iron (per cent) | Iron Ascorbic Absorption 3 mg. Iron (per cent) |
|----------------|-----|-----|-----------------|-------------------------------|----------------------------|---------------------------------------|---|---|
| 65) 55 JBG | 70 | M | 14.2 | 42.0 | 80 | 18.7 | 3.8 | |
| 66) 15 EAO | 15 | M | 12.7 | 41.5 | 61 | 15.0 | 4.0 | 77.6 |
| 67) 210 RM | 28 | F | 10.4 | 34.5 | 48 | 12.2 | 4.1 | |
| 68) 141 CC | 22 | F | 16.1 | 46.0 | 43 | 8.8 | 4.3 | |
| 69) 29 MAT | 36 | F | 11.7 | 39.5 | 120 | 34.0 | 4.4 | 17.5 |
| 70) 24 MRR | 42 | F | 13.1 | 39.0 | 84 | 19.0 | 4.4 | 45.8 |
| 71) 19 MB | 48 | M | 14.6 | 46.0 | 119 | 29.0 | 4.6 | 3.4 |
| 72) 85 RZ | 40 | F | 9.9 | 35.0 | 43 | 8.3 | 4.6 | |
| 73) 62 JM | 16 | M | 12.2 | 37.0 | 128 | 41.6 | 4.7 | |
| 74) 71 JAC | 22 | M | 12.6 | 38.0 | 58 | 10.1 | 5.0 | |
| 75) 74 FA | 38 | M | 15.4 | 47.5 | 245 | 59.6 | 5.5 | |
| 76) 68 JC | 30 | M | 13.3 | 42.0 | 65 | 14.6 | 5.6 | |
| 77) 138 RG | 34 | F | 12.5 | 38.0 | 65 | 20.0 | 6.2 | |
| 78) 59 JS | 50 | M | 13.5 | 42.5 | 62 | 18.2 | 6.3 | |
| 79) 75 IM | 40 | M | 7.4 | 30.0 | 31 | | 6.7 | |
| 80) 60 AL | 16 | F | 13.1 | 42.0 | 50 | 10.5 | 6.7 | |
| 81) 209 NA | 37 | F | 13.6 | 40.0 | 90 | 27.8 | 6.9 | |
| 82) 221 RA | 15 | F | 14.2 | 44.0 | 130 | 37.5 | 6.9 | |
| 83) 151 AQ | 55 | M | 12.9 | 44.0 | 101 | 20.1 | 7.0 | |
| 84) 241 CC | 37 | M | 9.3 | 31.5 | 25 | 6.6 | 7.2 | |
| 85) 203 HM | 16 | M | 13.3 | 42.5 | 144 | 43.0 | 7.3 | |

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| 86) | 239 | JCD | 28 | M | 16.3 | 46.5 | 64 | 19.2 | 7.4 |
| 87) | 215 | MS | 14 | F | 13.6 | 43.5 | 78 | 20.3 | 7.5 |
| 88) | 202 | CM | 20 | M | 12.4 | 40.0 | 33 | 9.5 | 7.6 |
| 89) | 229 | JT | 20 | F | 8.4 | 30.0 | 28 | 6.6 | 7.7 |
| 90) | 145 | JM | 33 | F | 13.8 | 38.0 | 97 | 28.0 | 8.0 |
| 91) | 72 | MIQ | 26 | F | 13.2 | 41.0 | 46 | 10.2 | 8.6 |
| 92) | 56 | RR | 14 | F | 12.6 | 39.0 | 132 | 25.1 | 8.8 |
| 93) | 198 | MT | 15 | M | 6.4 | 26.0 | 14 | 3.9 | 8.9 |
| 94) | 78 | JMM | 19 | M | 14.9 | 48.0 | 50 | 13.3 | 9.1 |
| 95) | 236 | JAC | 40 | M | 17.5 | 50.0 | 108 | 30.3 | 9.3 |
| 96) | 231 | GS | 15 | F | 11.2 | 36.0 | 42 | 10.2 | 9.6 |
| 97) | 81 | JS | 26 | M | 10.9 | 41.0 | 58 | 13.9 | 10.2 |
| 98) | 86 | MBL | 28 | F | 12.7 | 41.0 | 60 | 21.4 | 10.9 |
| 99) | 149 | PJP | 56 | M | 14.5 | 43.0 | 112 | 23.3 | 11.3 |
| 100) | 65 | PMH | 52 | M | 7.6 | 30.0 | 29 | 5.5 | 11.4 |
| 101) | 143 | SM | 27 | F | 14.5 | 44.0 | 78 | 15.7 | 13.1 |
| 102) | 67 | AMS | 21 | F | 11.9 | 40.0 | 40 | 8.0 | 13.2 |
| 103) | 83 | IR | 48 | M | 9.4 | 35.0 | 46 | 8.0 | 14.9 |
| 104) | 140 | AV | 15 | F | 14.3 | 41.5 | 52 | 11.1 | 15.0 |
| 105) | 64 | JEH | 20 | M | 8.7 | 33.5 | 28 | 4.6 | 15.2 |
| 106) | 76 | JAV | 32 | M | 5.5 | 24.0 | 19 | 3.8 | 24.0 |
| 107) | 218 | VH | 15 | F | 11.8 | 39.0 | 30 | 6.8 | 25.7 |
| Mean | | | | | | | | | 26 |
| Standard Deviation | | | | | | | | | (0.8-8.2) |
| | | | | | | | | | 20.9 |
| | | | | | | | | | (8.4-52.0) |

Table 2.—Intrasubject Variation in Iron Absorption from Black Beans in Successive Days

| Case Number | Per Cent of Iron Absorption from Black Beans (3 mg. Food Iron) | | |
|-------------|---|---|----------------|
| | A Black Beans Alone | B Black Beans + Gelatine Capsules | B / A Ratio |
| 1 | 0.2 | 0.4 | 2.00 |
| 17 | 0.8 | 0.4 | 0.50 |
| 16 | 0.8 | 0.6 | 0.75 |
| 58 | 3.1 | 2.3 | 0.74 |
| 62 | 3.4 | 2.6 | 0.76 |
| 64 | 3.7 | 3.2 | 0.86 |
| 72 | 4.6 | 3.9 | 0.85 |
| 85 | 7.3 | 9.5 | 1.30 |
| 87 | 7.5 | 2.1 | 0.28 |
| 88 | 7.6 | 11.5 | 1.51 |
| 98 | 10.9 | 6.6 | 0.61 |
| 103 | 14.9 | 10.8 | 0.72 |
| Mean | 3.3 | 2.6 | 0.80 |
| S.D. | | | (0.48–1.34) |
| Probability | | | > 0.1 |

up the results of these two series with those recently published by us,¹ the following information is obtained:

In 18 subjects the mean iron absorption from black beans given alone and black beans administered with fish was 1.54 and 2.71 per cent, respectively. The comparison of the two absorptions yield a probability of less than 0.01. In 19 subjects the mean iron absorption from black beans given alone and black beans administered with amino acids was 1.3 and 3.1 per cent, respectively. The comparison of these two absorptions yield a probability of less than 0.01.

Further experiments were carried out by comparing iron absorption of black beans given alone, with the absorption of the food mixed with either basic amino acids (Histidine, Arginine and Lysine) or aromatic amino acids (Phenylalanine, Tyrosine and Tryptophan) (Table 4). Significant differences were not found. Similar findings were obtained when the food was combined with various amino acids grouped under the heading of "aliphatic amino acids" (Glycine, Alanine, Leucine, Isoleucine, Serine, Threonine, Aspartic acid and Glutamic acid). In each experiment, the amount of amino acids administered was the same as present in 100 Gm. of fish.

Two sulfur amino acids (Cysteine and Methionine) were used to determine their effect on the absorption of black bean iron (Table 5). In the group of subjects fed with cysteine plus methionine, the absorption of black bean iron was significantly increased as compared with the iron absorption from the food given alone. One dose or three doses of methionine, i.e., the amount of methionine present in 100 and 300 Gm. of fish, respectively, did not increase the absorption of black bean iron, but there was a significant increase when one or three doses of cysteine was mixed with the food.

Table 3.—Effect of Fish Muscle and Amino Acids on Iron Absorption from Black Beans

| Case No. | Per Cent of Iron Absorption from Black Beans | | | B/A or C/A Ratio |
|-----------------------|--|--|----------------------------------|------------------|
| | A. Black Beans Alone 2-3 mg. Fe | B. Black Beans + Fish (0.5 mg. Fe) | C. Black Beans + Amino Acids* | |
| Effect of Fish | | | | |
| 11 | 0.5 | 5.9 | — | 11.80 |
| 19 | 0.8 | 5.2 | — | 6.50 |
| 42 | 1.8 | 7.4 | — | 4.11 |
| 46 | 2.2 | 6.3 | — | 2.86 |
| 57 | 3.0 | 2.7 | — | 0.90 |
| 55 | 3.0 | 3.9 | — | 1.30 |
| 56 | 3.0 | 3.6 | — | 1.20 |
| 63 | 3.9 | 4.0 | — | 1.03 |
| 69 | 4.4 | 6.5 | — | 1.48 |
| 71 | 4.6 | 3.7 | — | 0.80 |
| Mean | 2.2 | 4.7 | | 2.08 |
| S. D. | | | | (0.83-5.23) |
| Probability | | | | < 0.05 |
| Effect of Amino Acids | | | | |
| 7 | 0.3 | — | 0.8 | 2.67 |
| 8 | 0.3 | — | 0.9 | 3.00 |
| 24 | 1.1 | — | 4.1 | 3.73 |
| 35 | 1.6 | — | 4.7 | 2.94 |
| 37 | 1.7 | — | 5.4 | 3.18 |
| 45 | 2.1 | — | 2.4 | 1.14 |
| 47 | 2.5 | — | 2.4 | 0.96 |
| 52 | 2.8 | — | 7.7 | 2.75 |
| 59 | 3.2 | — | 1.3 | 0.41 |
| 66 | 4.0 | — | 5.5 | 1.38 |
| 70 | 4.4 | — | 6.3 | 1.43 |
| Mean | 1.6 | | 2.9 | 1.81 |
| S. D. | | | | (0.92-3.57) |
| Probability | | | | < 0.02 |

* Amino acids in the same number and proportion present in 100 Gm. of fish.

DISCUSSION

The study of iron absorption from black beans in 137 subjects, including individuals with normal iron stores and with various degrees of iron depletion, provides a spectrum of the iron absorption of this food in an agricultural rural community of Venezuela. As mentioned before, the mean absorption is 2.6 per cent and the theoretical variation in absorption in 67 per cent of the individuals tested was from 1.0-7.6 per cent.

The great individual variability of iron absorption in consecutive days, when either inorganic or food iron was used, limits to some extent the interpretation of the effect of any given substance on iron absorption from food. It was found that suitable information on the effect of food or amino acids on the iron absorption from black beans is obtained when eight to 10 individuals are

Table 4.—Effect of Nonsulfur Amino Acids on Iron Absorption from Black Beans

| Case Number | Per Cent of Iron Absorption from Black Beans (3 mg. of Food Iron) | | |
|--|--|---------------------------------------|--------------|
| | A. Black Beans Alone | B. Black Beans + Amino Acids | B/A Ratio |
| Effect of Aromatic Amino Acid Mixture (Phenylalanine, Tyrosine and Tryptophan) | | | |
| 20 | 0.8 | 0.8 | 1.00 |
| 23 | 1.1 | 1.0 | 0.91 |
| 32 | 1.5 | 1.0 | 0.61 |
| 47 | 2.5 | 0.6 | 0.24 |
| 52 | 2.8 | 3.4 | 1.21 |
| 51 | 2.8 | 0.8 | 0.29 |
| 90 | 8.0 | 6.3 | 0.79 |
| 94 | 9.1 | 12.9 | 1.42 |
| Mean | 2.6 | 1.8 | 0.70 |
| Limits of 1 S.D. | | | (0.36–1.34) |
| Probability | | | > 0.1 |
| Effect of Basic Amino Acid Mixture (Histidine, Arginine, and Lysine) | | | |
| 35 | 1.6 | 0.8 | 0.50 |
| 37 | 1.7 | 3.5 | 2.06 |
| 50 | 2.7 | 4.4 | 1.63 |
| 59 | 3.2 | 2.1 | 0.66 |
| 66 | 4.0 | 5.0 | 1.25 |
| 83 | 7.0 | 9.8 | 1.40 |
| 97 | 10.2 | 13.0 | 1.27 |
| 99 | 11.3 | 5.2 | 0.46 |
| Mean | 4.0 | 4.1 | 1.02 |
| Limits of 1 S.D. | | | (0.58–1.79) |
| Probability | | | > 0.9 |
| Effect of Aliphatic Amino Acid Mixture (Glycine, Alanine, Valine, Leucine, Isoleucine, Serine, Threonine, Aspartic Acid and Glutamic Acid) | | | |
| 9 | 0.4 | 1.2 | 3.00 |
| 22 | 0.9 | 1.6 | 1.78 |
| 28 | 1.4 | 2.3 | 1.64 |
| 65 | 3.8 | 4.4 | 1.16 |
| 78 | 6.3 | 8.9 | 1.41 |
| 80 | 6.7 | 4.3 | 0.64 |
| 92 | 8.8 | 6.8 | 0.77 |
| 101 | 13.1 | 2.6 | 0.20 |
| 107 | 25.7 | 26.1 | 1.02 |
| Mean | 4.0 | 4.1 | 1.04 |
| Limits of 1 S.D. | | | (0.48–2.26) |
| Probability | | | > 0.8 |

* The dose of each amino acid is the same as that present in 100 Gm. of fish muscle.

tested and the results are statistically compared by the Student test in paired samples. Nevertheless, in some instances, it would be necessary to increase the number of subjects tested in order to define the difference between the two parameters. The group of 10 subjects (Table 3) in which the effect of fish on black bean iron absorption was tested could be used as an example. Al-

Table 5.—Effect of Sulfur Amino Acids on Iron Absorption from Black Beans

| Identification | Per Cent of Iron Absorption from Black Beans (3 mg. of Food Iron) | | |
|---|--|---------------------------------------|--------------|
| | A. Black Beans Alone | B. Black Beans + Amino Acids | B/A Ratio |
| Effect of One Dose of Methionine + One Dose of Cysteine | | | |
| 14 | 0.7 | 2.3 | 3.29 |
| 24 | 1.1 | 2.9 | 2.64 |
| 45 | 2.1 | 23.3 | 10.62 |
| 44 | 2.1 | 3.6 | 1.71 |
| 48 | 2.5 | 7.1 | 2.84 |
| 49 | 2.6 | 12.9 | 4.96 |
| 70 | 4.4 | 11.5 | 2.61 |
| 75 | 5.5 | 12.4 | 2.25 |
| 79 | 6.7 | 7.2 | 1.07 |
| 106 | 24.0 | 29.1 | 1.21 |
| Mean | 3.1 | 8.2 | 2.65 |
| Standard deviation | | | (1.36–5.17) |
| Probability | | | < 0.01 |
| Effect of One Dose of Cysteine ° | | | |
| 13 | 0.7 | 1.6 | 2.29 |
| 27 | 1.3 | 4.5 | 3.46 |
| 30 | 1.5 | 4.3 | 2.87 |
| 43 | 1.9 | 5.7 | 3.00 |
| 60 | 3.2 | 7.0 | 2.19 |
| 68 | 4.3 | 7.1 | 1.65 |
| 73 | 4.7 | 4.2 | 0.89 |
| 100 | 11.4 | 18.3 | 1.61 |
| 104 | 15.0 | 20.2 | 1.35 |
| 105 | 15.2 | 19.5 | 1.28 |
| Mean | 3.7 | 6.9 | 1.90 |
| Standard deviation | | | (1.23–3.03) |
| Probability | | | < 0.01 |
| Effect of Three Doses of Cysteine | | | |
| 21 | 0.9 | 4.3 | 4.77 |
| 25 | 1.2 | 1.7 | 1.42 |
| 36 | 1.7 | 3.1 | 1.82 |
| 61 | 3.3 | 8.2 | 2.48 |
| 84 | 7.2 | 14.1 | 1.96 |
| 86 | 7.4 | 17.0 | 2.30 |
| 89 | 7.7 | 23.4 | 3.04 |
| 95 | 9.3 | 24.3 | 2.61 |
| 96 | 9.6 | 28.0 | 2.92 |
| Mean | 5.4 | 13.8 | 2.45 |
| Standard deviation | | | (1.7–3.5) |
| Probability | | | < 0.01 |

Table 5.—Continued

| Identification | Per Cent of Iron Absorption from Black Beans (3 mg. of Food Iron) | | B/A Ratio |
|--------------------|--|---------------------------------------|--------------|
| | A. Black Beans Alone | B. Black Beans + Amino Acids | |
| | Effect of One Dose of Methionine † | | |
| 18 | 0.8 | 1.5 | 1.88 |
| 26 | 1.3 | 1.7 | 1.31 |
| 33 | 1.5 | 1.6 | 1.07 |
| 40 | 1.7 | 3.6 | 2.12 |
| 74 | 5.0 | 3.5 | 0.70 |
| 76 | 5.6 | 8.7 | 1.55 |
| 77 | 6.2 | 9.7 | 1.56 |
| 82 | 6.9 | 3.3 | 0.48 |
| 91 | 8.6 | 10.4 | 1.21 |
| 102 | 13.2 | 14.8 | 1.12 |
| Mean | 3.6 | 4.3 | 1.20 |
| Standard deviation | | | (0.77–1.88) |
| Probability | | | > 0.20 |
| | Effect of Three Doses of Methionine | | |
| 29 | 1.4 | 1.9 | 1.36 |
| 31 | 1.5 | 1.0 | 0.67 |
| 38 | 1.7 | 1.2 | 0.71 |
| 53 | 2.9 | 4.9 | 1.69 |
| 54 | 3.0 | 1.9 | 0.63 |
| 67 | 4.1 | 4.2 | 1.02 |
| 81 | 6.9 | 6.6 | 0.96 |
| 93 | 8.9 | 12.4 | 1.39 |
| Mean | 3.1 | 3.0 | 0.99 |
| Standard deviation | | | (0.63–1.33) |
| Probability | | | > 0.5 |

* One dose of cysteine = 0.21 Gm.

† One dose of methionine = 0.53 Gm.

though the mean iron absorption from black beans administered with fish was twice that obtained when black beans were given alone, the probability that the two results were different, was between 0.02 and 0.05. However, this difference became more evident ($p < 0.01$) when 18 subjects were analyzed.

The studies presented here show that fish muscle enhances iron absorption from black beans. This observation agrees to some extent with experimental studies on the effect of protein in the diet on inorganic iron absorption. Klavins et al.^{14,15} observed that a low-protein diet is associated with low iron absorption and that a certain amount of protein is necessary for adequate iron absorption. These authors suggested that amino acids derived from dietary protein probably affect iron absorption.

Of the amino acids tested, only cysteine proved to enhance iron absorption from black beans. As far as is known, there is no reference in the literature reporting the effect of cysteine or other amino acids on human iron absorption from food, but there are some experimental studies in rats on the effect of

amino acids, including cysteine, on the absorption of inorganic iron. Green et al.¹⁶ observed enhanced absorption of iron when cysteine was added. Kroe et al.¹⁷ found that any of the following amino acids: glutamic acid, serine, phenylalanine, proline and methionine increase inorganic iron absorption.

The way in which cysteine enhances absorption from inorganic iron is not known. Other agents, such as fructose¹⁸ and ascorbic acid,^{19,20} which increase inorganic iron absorption, form soluble chelates with iron. This iron compound prevents the formation of insoluble polymers and precipitates in the lumen of the gut.²¹⁻²³ It has also been demonstrated that amino acids, in general, are effective chelating agents,²⁴ and studies of Brown and Rother²⁵ suggest that iron-binding amino acids occur in the lumen of the gut before entry into the mucosal cells. It might be possible, therefore, that in the lumen of the gut, cysteine forms with iron from black beans a soluble chelate which facilitates iron absorption. Experimental studies are in process to test this working hypothesis.

SUMMARY

Black beans, a staple food consumed in large amounts in Central and South America, were used as a model for the studies of the effect of fish or amino acids present in fish on iron absorption from vegetable food.

The iron absorption rate in 137 subjects showed an asymmetric distribution. The mean iron absorption was 2.6 per cent and the limit of one standard deviation was from 0.8-8.2 per cent.

Iron absorption from black beans increased about twice when the food was administered with either fish or with amino acids in number and proportion as present in 100 Gm. of fish. Further experiments showed that the absorption is not enhanced if the black beans were mixed with either basic amino acids (Histidine, Arginine and Lysine), aromatic amino acids (phenylalanine, Tyrosine and Tryptophan) or other amino acids grouped as "aliphatics" (Glycine, Alanine, Leucine, Isoleucine, Serine, Threonine, Aspartic acid and Glutamic acid). However, either cysteine plus methionine, or cysteine alone enhances iron absorption from black beans in similar proportion as observed when this food was mixed with fish or total amino acids present in fish.

ACKNOWLEDGMENT

The authors wish to thank Sr. Guillermo López Calzón for his technical assistance.

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