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# A plasma amino acid method for determining protein quality

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## SUMMARY

Using a method of force-feeding test protein slurries to adult female (200 g) rats, a peak of amino acids was observed at 30 minutes post intubation for every protein tested. The data suggests that after this time the levels of plasma amino acids are controlled by tissue transport, especially into the muscle. Thus, a new steady state concentration of amino acid is set up as the stomach continues to empty and the intestine continues to absorb the diet until six hours post intubation when the stomach is empty. The data also indicate that dietary amino acids are contributing to the increased plasma amino acid levels at 30 minutes.

Plasma amino acid ratios indicate that 30-minute changes in essential plasma amino acids reflect the amino acid composition of the intubated test protein. Plasma amino acid ratios also indicate that later times, such as five hours post intubation do not reflect dietary amino acid composition even though the animal is still absorbing diet at this time.

Thirty-minute blood changes in amino acids were used to compute a new index of protein quality, Plasma Amino Acid Index (PAAI). This index is the geometric mean of the egg protein ratios of the plasma amino acid response at 30 minutes post intubation. It was found that Plasma Amino Acid Index is highly correlated, $r = 0.965$, with Mitchell's Biological Value. The regression equation was $Y = 9.79X + 16$. Using the above relationship, it was shown that values of biological value could be used to predict values for PAA Index computed from blood changes taken from data of Longenecker and Hause, (1959). It is concluded that data which reflect protein quality has existed in the literature but until now the proper interpretation of these values has not been applied.

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### THE AUTHORS

At the time of this study T. R. Whitaker was a Research Assistant in Agricultural Biochemistry; Homer Patrick is Agricultural Biochemist.

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# A Plasma Amino Acid Method For Determining Protein Quality

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The world's supply of protein would be extended if the problems of protein quality were solved. According to the National Research Council's standards for adequate laboratory rat growth, a protein of perfect quality (NPU = 100) is required as 12 per cent of the diet as opposed to a 20 per cent requirement for crude protein (dietary N x 6.25).

The problem of protein quality has been studied for half a century. Many methods for determining the nutritional value of protein have been developed. These have been reviewed by Frost (1959) and Albanese (1959). The **in vitro** methods have recently been reviewed by Sheffner (1967).

Osborne and Mendel (1914) recognized that the nutritional value of a protein could be improved by supplementation with certain amino acids which seemed to be missing in the protein. Later, it became evident that some supplementary patterns had a negative effect on protein quality and the conditions of amino acid imbalance and antagonism became recognized. The effects of amino acid imbalances, toxicities, and antagonisms have been reviewed by Harper (1964).

If the answer to the problem of protein quality is amino acid supplementation, many patterns must be tested until the right ones are found. It is evident that a rapid method of determining protein quality would expedite the work on improving dietary protein.

## LITERATURE REVIEW

In the past, attempts to distinguish differences in protein quality by examining the plasma amino acids after protein feeding have yielded somewhat less than satisfactory results. Comparison of results has been difficult since different species of animals of both sexes at various ages have been studied using various feeding techniques, lengths of fast, dietary pretreatments, levels of protein intake, and times and sites of blood sampling and also methods of amino acid analysis. Several trends do appear in the data. It seems that plasma essential amino acid

levels reflect the availability of amino acids in the dietary protein (Wheeler and Morgan, 1958). An unusually low level of an essential amino acid in the blood after a protein meal indicates a deficiency in the protein's amino acid content. This has been demonstrated with lysine in rats fed bread (McLaughlin **et al.**, 1961), with methionine and lysine in chicks fed peanut meal (Richardson **et al.**, 1962), with methionine in pigs fed soybean oil meal (Puchal **et al.**, 1962), and with tryptophan in dogs fed gelatin (Longenecker and Hause, 1959). Dean and Scott (1965) using a standard reference pattern diet of free amino acids showed that levels of chick plasma essential amino acids reflect the amino acid composition of the diet. Smith and Scott (1965 a,b) demonstrated that this standard reference pattern could be applied to intact proteins. Plasma amino acid ratios as calculated by Longenecker and Hause (1959) detected the most limiting amino acid in wheat gluten, casein, and gelatin when fed to dogs.

Stana **et al.** (1967) observed the effect of fasting as compared to **ad libitum** feeding on the plasma amino acid level of the normal rat (Table 1). Although there is a small decrease in the plasma essential amino acids after a 15-hour fast, the amino acid picture is stable relative to the large increases noted by Hill and Olsen (1963) in the chick in which lysine increased five fold and threonine doubled in a 24-hour fast.

Stana (1966) force-fed (200 gm) male rats a diet containing either wheat gluten, casein-lactalbumin, or a free amino acid

**TABLE 1**

Essential Amino Acids in Fasting and Non-Fasting Plasma of Rats[a]

| Amino Acid | Non-Fasting mg/100 ml | Fasting[b] mg/100 ml |
|---|---|---|
| Arginine | 2.25 | 1.98 |
| Histidine | 1.57 | 1.03 |
| Isoleucine | 1.43 | 1.13 |
| Leucine | 1.94 | 1.83 |
| Lysine | 7.67 | 6.93 |
| Methionine | 1.11 | 0.76 |
| Phenylalanine | 1.20 | 1.18 |
| Threonine | 6.76 | 5.46 |
| Valine | 1.91 | 1.55 |

[a] Stana **et al.** (1967)
[b] Fasted for 15 hours

mixture adjusted to the FAO pattern. She observed a peak concentration of plasma amino acids at 30 minutes post intubation. Guggenheim **et al.** (1960) also observed a response in the plasma amino acids 30 minutes after force-feeding test meals. Stana (1966) did not observe a 30-minute increase in plasma amino acids with a nitrogen free intubation mixture, thus indicating that the 30-minute peak was of dietary protein origin.

## PROCEDURES

### Experimental Protocol

White Wistar rats maintained on the rat breeding ration, Table 2, in the colony of the Agricultural Experiment Station, West Virginia University, were used in this study. When they weighed approximately 200 g, they were fasted for 15 hours prior to treatment. This time represents a compromise between starvation effects and those of dietary pretreatment on plasma amino acids. Water was available during the fast but was removed after the test meal was intubated. Animals were sacrificed at chosen intervals post intubation and samples of systemic blood were taken by heart puncture. Both fasted and sham-intubated groups were sacrificed as controls.

### Intubation Procedure

The fasted rats were fed by intubation as reported by Stana (1966). Test meals (Table 3) were mixed in a Waring blender in a 0.3 per cent (w/v) agar solution so that the mixture contained 50 gms of dry diet per 50 mls of agar solution. The intubation apparatus consisted of a 16 gauge, ball-tipped, bent intubation needle and a six ml plastic hypodermic syringe. The rats were fed 2 ml of slurry per 100 gm body weight so as not to overload the stomach.

### Food Passage

The rate of passage of test meal slurries from the stomach to the absorptive areas of the intestine was followed by adding a radioactive marker such as $^{144}Ce$. This radionuclide was assayed by deep-well scintillation. When it became necessary to follow passage of the amino acid of the dietary proteins in the intestinal tract and their absorption into the blood, $^{35}S$ and $^{14}C$-yeast proteins were used. The $^{35}S$-yeast protein was synthesized and isolated by a method of Whitaker (1970) after incorporation of $^{35}SO_4$ by

bakers yeast. The specific activity of the $^{35}S$-yeast was approximately 2.16 x $10^7$ dpm/gm. The $^{14}C$-yeast was purchased from New England Nuclear Company, Boston, Massachusetts, as uniformly labelled $^{14}C$-yeast cells. The $^{35}S$ and $^{14}C$ samples were assayed by liquid scintillation after a method of Bray (1960).

**Amino Acid Analysis**

Plasma amino acids were assayed with a Technicon Autoanalyzer after the procedure of Stana (1966) with modifications described by Whitaker (1970). Protein free filtrates were prepared from fresh plasma with an equal volume of 15 per cent sul-

**TABLE 2**

Composition of the Rat Breeding Ration

| Component | Per Cent |
|---|---|
| Fine Ground Yellow Corn | 52.3 |
| Wheat Middlings | 20.0 |
| Soybean Oil Meal (50% protein) | 15.0 |
| Alfalfa Meal (17% protein) | 5.0 |
| Fish Meal (60% protein) | 5.0 |
| Salts (NaCl + 5% $MnSo_4$) | 0.5 |
| Dicalcium Phosphate | 1.0 |
| Calcium Carbonate | 1.0 |
| Vitamin A (10,000 I.U. per gram) | 0.05 |
| Vitamin $D_3$ (3,000 I.C.U. per gram) | 0.05 |
| Vitamin Premix[a] | 0.1 |
| | 100.00 |

[a] Vitamin premix contained 2 gm riboflavin, 4 gm pantothenic acid, and 8 gm niacin per pound

**TABLE 3**

Composition of Test Meals

| Component | Per Cent |
|---|---|
| Test Protein | 15.0 |
| Corn Starch | 10.0 |
| Glucose Monohydrate (Cerulose) | 70.0 |
| Mineral Premix[a] | 3.0 |
| Vitamin Premix[a] | 2.0 |
| | 100.0 |

[a] N. R. C. requirements after Bernhart and Tomarelli (1966)

fosalicylic acid. Recovery of amino acids from plasma was determined by Whitaker (1970) and were found to be similar to those obtained by Block **et al.** (1966). Protein free filtrates of muscle and liver were assayed for free amino acids from homogenates prepared after Whitaker (1970). The amino acid composition of the test proteins (Table 4) was determined essentially by the method of Blackburn (1966) for acid hydrolysis with modifications described by Whitaker (1970).

## RESULTS AND DISCUSSION

### Purpose of the Study

1. To determine the relationship of the movement of food through the digestive tract to the appearance of plasma amino acid changes at various intervals after force-feeding a protein test meal.
2. To determine if plasma amino acid changes post intubation are due to dietary protein digestion and absorption.
3. To determine if plasma essential amina acids reflect the amino acid composition of the dietary protein.

**TABLE 4**

The Amino Acid Composition of Egg Albumin, Wheat Gluten, and Casein in Grams/100 Grams Protein

| Amino Acid | Egg Albumin | Wheat Gluten | Casein |
|---|---|---|---|
| Asp | 8.1 ± 0.15 | 2.8 ± 0.15 | 6.6 ± 0.03 |
| Thr | 4.2 ± 0.04 | 2.4 ± 0.02 | 4.0 ± 0.10 |
| Ser | 7.9 ± 0.12 | 3.7 ± 0.04 | 5.6 ± 0.04 |
| Glu | 16.2 ± 0.07 | 38.0 ± 1.54 | 22.4 ± 0.31 |
| Gly | 3.1 ± 0.05 | 3.0 ± 0.23 | 2.0 ± 0.04 |
| Ala | 6.9 ± 0.06 | 2.1 ± 0.08 | 3.0 ± 0.01 |
| Val | 6.2 ± 0.05 | 4.2 ± 0.04 | 7.1 ± 0.16 |
| Cys | 2.4 ± 0.15 | 1.9 ± 0.30 | 0.0 ± 0.00 |
| Met | 5.2 ± 0.12 | 1.6 ± 0.02 | 2.9 ± 0.03 |
| Ile | 7.1 ± 0.05 | 4.0 ± 0.02 | 6.5 ± 0.08 |
| Leu | 9.2 ± 0.07 | 6.9 ± 0.04 | 9.8 ± 0.06 |
| Tyr | 3.9 ± 0.06 | 3.1 ± 0.07 | 6.2 ± 0.11 |
| Phe | 6.6 ± 0.13 | 5.0 ± 0.02 | 5.1 ± 0.14 |
| Lys | 6.3 ± 0.01 | 1.6 ± 0.03 | 8.1 ± 0.04 |
| His | 2.1 ± 0.04 | 1.9 ± 0.02 | 3.0 ± 0.02 |
| Arg | 5.6 ± 0.10 | 2.8 ± 0.03 | 4.0 ± 0.08 |

Mean ± standard error represents at least four determinations after the method of Blackburn (1968)

4. To determine if plasma essential amino acid changes post intubation can be used to estimate a new index of protein quality.

**Amino Acid Evaluation of Test Proteins**

The amino acid content of the test proteins as determined from their acid hydrolyzates appear in Table 4. It is clear from this data that casein is lacking in the sulfur amino acids, cystine, and methionine and that a small deficiency in isoleucine exists when compared with the results for egg albumin. Casein is equal or superior to egg in amounts of threonine, leucine, lysine, the aromatic amino acids, and histidine. Casein is also deficient in arginine, but considering the low requirement for arginine in the adult rat (Rose, 1937), this difference may not be important nutritionally. When using the chick this difference in arginine may be very significant as it has a five-fold higher arginine requirement than the rat.

Wheat gluten (Table 4) is lacking in every essential amino acid compared to egg albumin composition except histidine and the aromatic amino acids, tyrosine, and phenylalanine. Glutamic acid represents over one-third of the total amino acid composition of wheat gluten. The low essential N to non-essential N ratio puts wheat gluten in a poor quality protein classification.

**The Relationship Between Food Passage and Appearance of Plasma Amino Acid Changes**

The rate of food passage out of the stomach and into the absorptive areas of the intestine was accomplished by including $^{144}Ce$ in the intubation slurries of wheat gluten and casein. Plasma essential amino acid levels and $^{144}Ce$ content of the intestinal areas were followed until the stomachs had emptied. Although there is no covalent bond between the marker and the dietary protein, the radioactivity emptied from the stomach at rates consistent with those observed for $^{35}S$-yeast protein which was covalently bound (Figure 1). When rats were intubated with the proteins studied, the stomachs emptied at a characteristic reproducible rate (Figure 1). Plasma amino acid data were considered only on the basis that food passage curves were consistent. As might be expected, meal-fed diets empty from the stomach at a slower rate than intubation slurries, since one of the processes occurring in the stomach is hydration of the diet (Figure 1). Gupta **et al.** (1958) determined stomach emptying by measuring

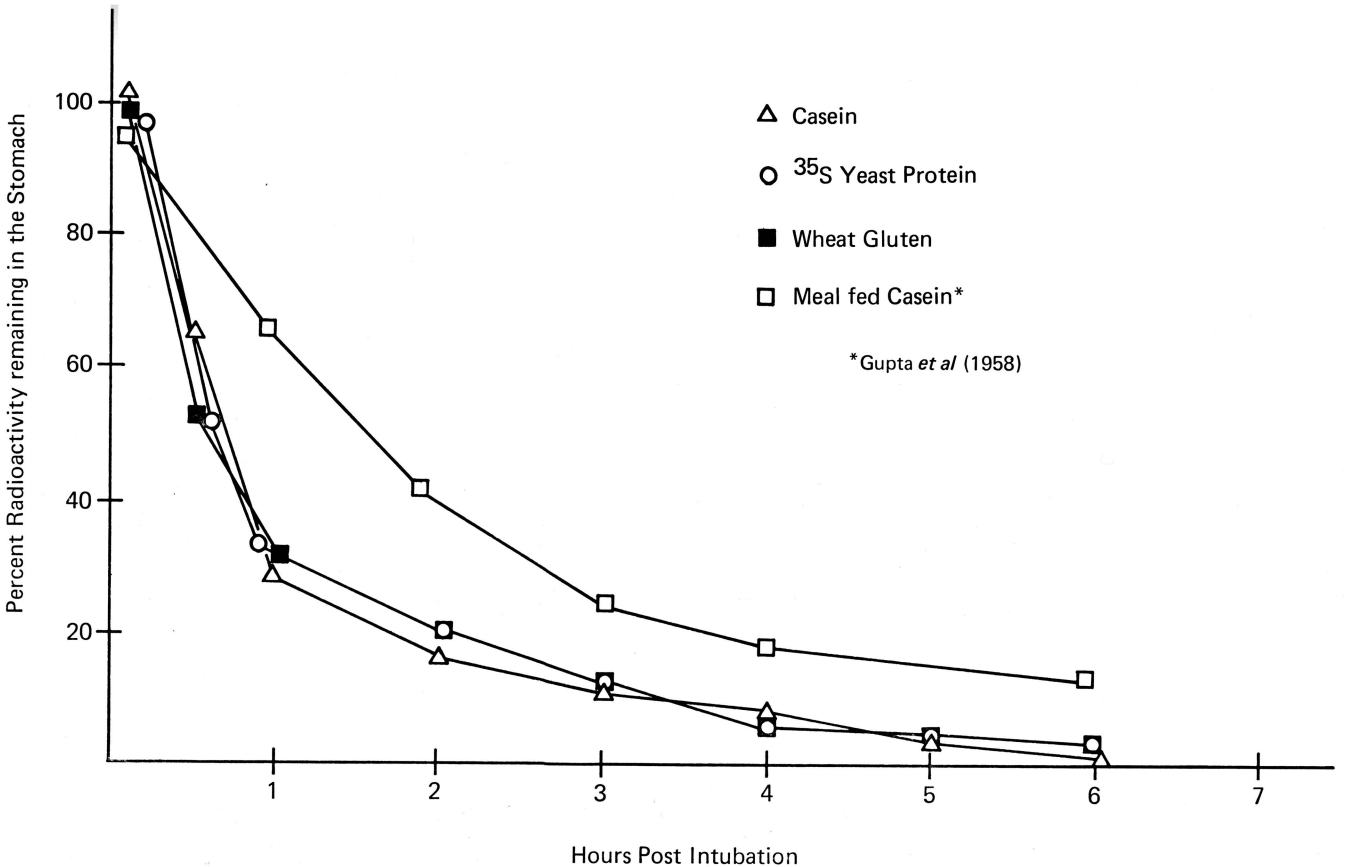



**Figure 1. Rate of Stomach Emptying vs Time.**

nitrogen in the stomach. There was 20 per cent residual nitrogen at six hours post intubation when the stomach was empty of food, indicating the endogenous nitrogen in the stomach. One can see in Figure 1 that if Gupta's curve is corrected for endogenous nitrogen by lowering each value by 20 per cent, it would be more consistent with the intubation curves. This leads us to believe that given the increase in emptying rate due to hydration of the diet, the food passage rates are normal with this feeding method.

The plasma amino acid levels after casein and wheat gluten intubation were followed until all the food had left the stomach (Figure 2). The 30-minute increase described by Guggenheim **et al.** (1960) and Stana (1966) was observed for both proteins. After one hour, the curves are not consistent, but there seems to be a leveling effect. Looking at Figures 1 and 2, the peak in amino acid concentration appears at 30 minutes when 40 per cent of the meal has left the stomach. The same trend is seen when the change in plasma essential amino acids from the fasting control values is considered with respect to time after force-feeding (Figure 3). Casein which is higher in essential N shows a much higher essential amino acid peak at 30 minutes post intuabation than does wheat gluten which is low in essential N (Figure 3), but both peaks are significantly higher than fasting levels (Table 5). The leveling pattern after one hour may represent a new steady state concentration based on the utilization of the amino acids from the diet. It is known that essential amino acids are better utilized when casein is fed, as opposed to wheat gluten. This relationship may be represented in the blood levels between one hour and seven hours post intubation when the blood levels of essential amino acids are higher for wheat gluten than for casein (Figure 3). It is believed that these are new steady state concentrations because most of the diet is absorbed between one and seven hours post intubation (Figure 1), yet there is a stable plasma amino acid picture (Figure 2).

The 30-minute peak was reproducible for all proteins tested for the 200 gm female rat. The males gave an inconsistent response indicating that the peak in plasma amino acids may be occurring earlier than 30 minutes. Young animals (90 gm) did not show a 30-minute peak. Further work is needed to determine if a peak occurs earlier in these animals. Stana (1966) observed the 30-minute peak in anesthetized 200 gm male rats. Growth

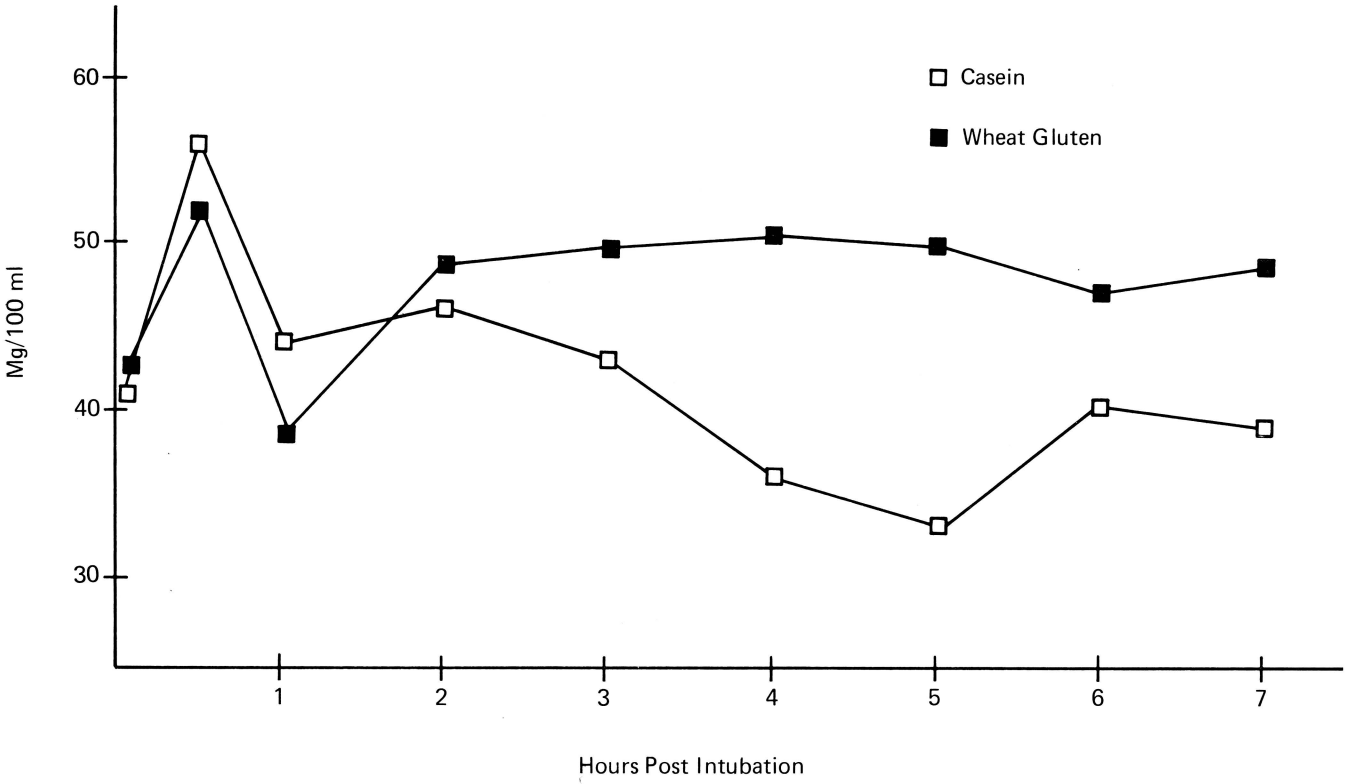


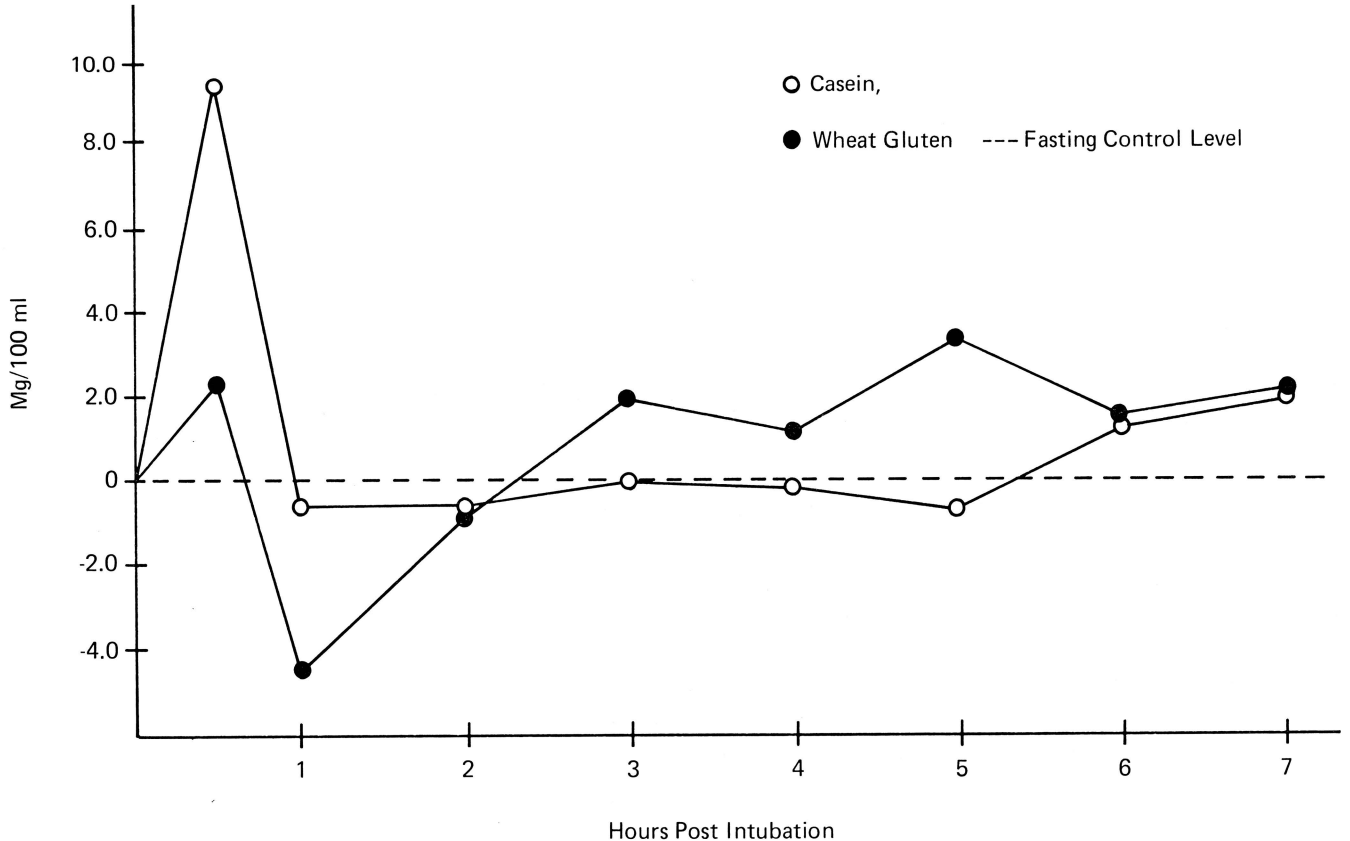

**Figure 2. Total Plasma Amino Acids vs Time.**

## TABLE 5

Mean Total Concentration of Plasma Amino Acids at Selected Intervals Seven Hours Post Intubation of Casein and Wheat Gluten

| Amino Acid | Fasted 15 Hours mg/100 ml | Hours Post Intubation 0.5 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|---|
| **Casein:** | | *** | | | | | | | |
| Essential Amino Acids | 25.21 ±0.37 | 34.12 ±0.14 | 26.29 ±0.47 | 26.40 ±0.89 | 25.46 ±0.26 | 21.93 ±0.51 | 22.58 ±0.73 | 24.74 ±1.25 | 24.74 ±1.49 |
| Total | | *** | * | * | * | * | ** | | |
| Amino Acids | 41.18 ±0.35 | 56.66 ±0.58 | 44.83 ±0.49 | 47.67 ±1.41 | 43.37 ±0.32 | 36.77 ±0.63 | 33.63 ±0.62 | 40.58 ±1.36 | 39.14 ±1.64 |
| **Wheat Gluten:** | | * | ** | | * | | * | * | |
| Essential Amino Acids | 25.91 ±0.31 | 28.22 ±0.35 | 21.41 ±0.52 | 25.18 ±0.66 | 27.73 ±0.30 | 27.13 ±0.67 | 29.31 ±0.69 | 27.29 ±0.10 | 27.97 ±0.50 |
| Total | | ** | | | ** | * | ** | * | * |
| Amino Acids | 42.95 ±0.80 | 52.29 ±0.66 | 39.42 ±0.54 | 48.13 ±1.31 | 50.82 ±0.71 | 51.41 ±1.26 | 50.35 ±0.70 | 47.82 ±0.63 | 48.43 ±0.94 |

Mean ± standard error represents three plasma samples (one sample = plasma of two rats)
Significant difference from fasting level student's t test: *P .05; **P .01; ***P .001




**Figures 3. Change in Plasma Essential Amino Acid Concentration vs. Time.**

hormone and certain androgens are recognized to have strong anabolic effects and to increase amino acid transport into tissues (Christensen, 1964). Estrogens increase transport into female reproductive tissue but not into other tissue (Noal **et al.**, 1957). Thus, if plasma amino acid decreases seen at one hour post intubation are a homeostatic response due to increased tissue transport, the female may have the slower response.

**Dietary Protein as a Source of Plasma Amino Acid Increases**

An experiment was designed to determine if dietary amino acids are responsible for the sharp increase in plasma amino acids at 30 minutes post intubation, and if the decrease to fasting level at one hour is the result of an increased tissue uptake. The $^{35}$S-yeast protein was intubated. The results (Table 6) indicate that at least 10 per cent of the protein injected into the stomach had passed the pylorus and was present in the intestinal contents at "zero" time. This may account for the sharp rise to a peak of plasma amino acids at 30 minutes post intubation (Table 7). It is doubtful if this much protein would be evacuated from the stomach with a meal fed or an **ad libitum** fed procedure. Zetzel and Banks (1941) contend that at least for closed loops of human intestine that an amino acid mixture of 10 per cent nitrogen represents the concentration of maximum amino acid absorption. In other words, absorption rates vary with concentration up to 10 per cent nitrogen above which absorption rates are constant and independent of concentration. A major premise of our work is that there exists a concentration of amino acids which will saturate the amino acid transport mechanism such that no further increase in absorption rate is possible. Since 10 per cent of the meal passes into the intestinal tract due to intubation procedure, it is believed that this amount is sufficiently large to

**TABLE 6**

Radioactivity in the Alimentary Tract of Rats at Selected Intervals Post Intubation of $^{35}$S-Yeast Protein

| Sample | 0 Minutes | 30 Minutes | 60 Minutes |
|---|---|---|---|
| | | cpm | |
| Stomach Contents | $2.77 \times 10^{6}$ | $1.43 \times 10^{6}$ | $8.36 \times 10^{5}$ |
| Intestine Contents | $4.31 \times 10^{5}$ | $2.88 \times 10^{5}$ | $2.51 \times 10^{5}$ |
| Total Tract | $3.20 \times 10^{6}$ | $1.72 \times 10^{6}$ | $1.09 \times 10^{6}$ |

Mean represents total activity of 10 rats

achieve maximum absorption. Thus, the magnitude of amino acid increases in the blood would be independent of protein level and would be a function of the relative amounts of these amino acids in the dietary protein.

Plasma amino acid levels of sham intubation controls were not significantly different from fasting levels (Table 7). No significant difference was observed in blood hematocrits at any time post intubation (Table 8). The results in Table 9 indicate that $^{35}$S-amino acids appear in the plasma at 30 minutes post intubation. The radioactivity appeared in the column effluent at the retention times of cystine and methionine in the 30-minute samples. On the basis of this evidence and the lack of change in hematocrit, it appears as if the 30-minute peak is not due to

**TABLE 7**

Mean Concentration of Essential Plasma Amino Acids at Selected Intervals Post Intubation of $^{35}$S-Yeast Protein

| Amino Acid | Fasted Level 15 Hours | Sham Intubated | 30-Minute Level | 1-Hour Level |
|---|---|---|---|---|
| | | mg/100ml | | |
| Thr | 4.82 ± 0.17 | 4.85 ± 0.17 | 6.02 ± 0.18** | 4.99 ± 0.29 |
| Val | 2.19 ± 0.09 | 2.06 ± 0.07 | 2.98 ± 0.08*** | 2.21 ± 0.07 |
| Met | 0.79 ± 0.01 | 0.82 ± 0.02 | 0.99 ± 0.03*** | 0.89 ± 0.02** |
| Ile | 1.39 ± 0.11 | 1.35 ± 0.04 | 1.74 ± 0.03* | 1.23 ± 0.02 |
| Leu | 2.15 ± 0.05 | 2.08 ± 0.05 | 2.61 ± 0.07** | 1.71 ± 0.04*** |
| Phe | 1.29 ± 0.02 | 1.34 ± 0.05 | 1.68 ± 0.05*** | 1.44 ± 0.04* |
| Lys | 5.16 ± 0.07 | 5.00 ± 0.05 | 6.13 ± 0.10*** | 4.77 ± 0.04** |
| His | 1.10 ± 0.02 | 1.09 ± 0.03 | 1.30 ± 0.02*** | 1.18 ± 0.02 |
| Arg | 1.24 ± 0.06 | 1.20 ± 0.06 | 2.27 ± 0.08*** | 1.70 ± 0.04*** |

Mean ± standard error; each value represents 10 rats
Significant difference from fasting level student's t test: *P .05; **P .01; ***P .001

**TABLE 8**

Blood Hematocrits at Selected Intervals Post Intubation of $^{35}$S-Yeast Protein

| Sample | Blood Hematocrit |
|---|---|
| 15 Hours Fasted | 48.1 ± 0.4 |
| Intubated Zero Time | 49.9 ± 0.8 |
| Intubated 30 Minutes | 49.1 ± 0.6 |
| Intubated 60 Minutes | 48.3 ± 0.7 |

Mean ± standard error; each value represents 10 rats

blood volume changes or stress, and since methionine-$^{35}S$ was detected in the plasma, at least part of the 30-minute increase is due to digestion and absorption of dietary protein.

### Amino Acid Transport as a Control of the Plasma Amino Acid Increases

Although muscle-free amino acid changes after intubation of $^{35}S$-yeast protein (Table 10) are smaller than those for liver, they may be more important in controlling the plasma amino acid levels considering the per cent of the body weight repre-

**TABLE 9**

Radioactivity in the Plasma Amino Acids at Selected Intervals Post Intubation of $^{35}S$-Yeast Protein

| Sample | 30 Minutes | 60 Minutes |
|---|---|---|
| | cpm/ml | |
| Plasma Amino Acids | 5,180 | 7,428 |
| Plasma Methionine | 2,288 | 3,647 |
| Plasma Cystine | 1,431 | 2,919 |

Each mean represents 10 rats

**TABLE 10**

Mean Concentration of Total Essential Free Amino Acids of Liver and Muscle at Selected Intervals Post Intubation of $^{35}S$-Yeast Protein

| | Fasted Level | | |
|---|---|---|---|
| Amino Acids | 15 Hours | 30-Minute Level | 1-Hour Level |
| | u moles/gram | | |
| **Liver:** | | | |
| Total Essential Amino Acids | 6.49 ± 0.08 | 9.49 ± 0.20*** | 9.46 ± 0.37*** |
| Total Amino Acids | 22.56 ± 0.90 | 31.63 ± 0.58*** | 34.11 ± 0.63*** |
| **Muscle:** | | | |
| Total Essential Amino Acids | 6.49 ± 0.08 | 7.88 ± 0.13*** | 8.83 ± 0.17***,# |
| Total Amino Acids | 12.85 ± 0.23 | 15.12 ± 0.23*** | 16.61 ± 0.31***,# |

Mean ± standard error represents four rats
Significant difference from fasting level student's t test: ***P .001
Significant difference from 30-minute level: #P .05

sented by muscle as compared to that of liver. One hour levels of muscle free amino acids are significantly higher than 30-minute levels. This may explain the drop in plasma amino acid levels between 30 minutes and one hour post intubation. The homeostatic control of a plasma amino acid load by tissue transport was first demonstrated by Van Slyke and Meyer (1913) in one of the first **in vivo** experiments with amino acids.

This experiment was repeated using uniformly labeled $^{14}C$-yeast cells. The results reported by Whitaker (1970) confirm the data presented for $^{35}S$-yeast protein. Since the $^{14}C$-label was distributed through all the amino acids and all the $^{35}S$-amino acid effects were reproduced, it was concluded that the 30-minute plasma amino acid peaks are due to dietary amino acids and not due to specific reactions of sulfur amino acid metabolism.

**The Plasma Amino Acids Reflect the Amino Acid Composition of Dietary Protein**

Wheat gluten, casein-lactalbumin, and egg albumin were intubated to determine if plasma essential amino acids after force-feeding reflect the amino acid composition of dietary protein. The results of plasma essential and non-essential amino acids of the above protein at 30 minutes and five hours post intubation and fasting levels are reported by Whitaker (1970). When plasma amino acid ratios (Longenecker and Hause, 1959) are calculated for 30-minute and five-hour plasma essential amino acid changes from fasting level, lysine was detected as the first limiting amino acid in wheat gluten using 30-minute changes (Table 11) but methionine was first limiting using five-hour changes. When plasma amino acid ratios were calculated from plasma amino acid changes after intubation of casein-lactalbumin, methionine was first limiting for 30-minute changes, but the first limiting amino acid using five-hour changes was histidine (Table 12). Therefore, PPA ratios for 30-minute plasma essential amino acid changes produce limiting amino acids orders which are consistent at least for the first limiting amino acid with chemical score orders while ratios for five-hour changes show no agreement with chemical score (Tables 11 and 12). This is further evidence that the peak at 30 minutes represents absorption of amino acids released by digestion of dietary protein, and that plasma amino acid values between one and seven hours after force-feeding represent a new steady state and no longer reflect the amino acid composition of the dietary protein.

**Plasma Amino Acid Index (PAAI)**

Egg albumin, wheat gluten, yeast, and casein-lactalbumin were intubated, and 30-minute plasma essential amino acid changes from fasting level were computed (Table 13). A new index of protein quality using egg albumin values as a standard was computed for wheat gluten (Table 14), yeast (Table 15), and casein-lactalbumin (Table 16). This index is an adaptation of the method of Oser (1951) for Essential Amino Acid Index (EAAI) to plasma amino acids. Plasma Amino Acid Index (PAA Index) is the geometric mean of the egg ratios of 30-minute plasma amino acid changes.

PAA Index was calculated from plasma amino acid data for each rat intubated. When results (Table 17) were compared to the corresponding biological values, a marked similarity was noted. Unfortunately, only a mean yeast biological value was re-

**TABLE 11**

Comparison of PAA Ratios[a] Calculated from 30-Minute and 5-Hour Changes in Plasma Amino Acid Levels Post Intubation of Wheat Gluten

| Amino Acid | PAA Ratio | Limiting Order | Chemical Score[b] Order |
|---|---|---|---|
| **30-Minute Change:** | | | |
| Thr | 17.2 | 7 | 3 |
| Val | 19.1 | 8 | 4 |
| Met | 3.6 | 2 | 2 |
| Ile | 7.2 | 4 | 5 |
| Leu | 7.5 | 5 | 7 |
| Phe | 6.8 | 3 | 6 |
| Lys | −11.6 | 1 | 1 |
| His | 11.5 | 6 | 8 |
| **5-Hour Change:** | | | |
| Thr | 54.4 | 8 | 3 |
| Val | 28.8 | 6 | 4 |
| Met | 5.3 | 1 | 2 |
| Ile | 22.8 | 5 | 5 |
| Leu | 22.0 | 4 | 7 |
| Phe | 12.2 | 3 | 6 |
| Lys | 30.2 | 7 | 1 |
| His | 8.5 | 2 | 8 |

PAA ratios calculated from data Table

[a] Longenecker and Hause (1959)

[b] Block and Mitchell (1946)

ported. A regression analysis indicates a close linear relationship between PAA Index and Biological Value (Figure 4). The regression equation was $Y = 0.79X + 16$. The correlation coefficient, $r = 0.965$ was significant at the P 0.001 level.

Since biological value does not take into account amino acid availability and PAAI does, such a close relationship might not be expected. Biological value should slightly overestimate protein quality. This has been compensated for by the fact that egg albumin is slightly lower in biological value (97) than whole egg (100). This becomes clear when we substitute 97 into the regression equation for biological value and obtain a PAA Index value of 100. Since egg albumin was not the protein of highest biological value, PAA Index slightly overestimates protein quality. It should be noted that the indispensible amino acid tryptophan was not included in the PAA Index since it was not quantitatively recovered from the ion exchange column. Tryptophan should be

**TABLE 12**

Comparison of PAA Ratios[a] Calculated from 30-Minute and 5-Hour Changes in Plasma Amino Acid Levels Post Intubation of Casein-Lactalbumin

| Amino Acid | PAA Ratio | Limiting Order | Chemical Score[b] Order |
|---|---|---|---|
| **30-Minute Change:** | | | |
| Thr | 60.8 | 8 | 3 |
| Val | 32.8 | 6 | 5 |
| Met | 7.6 | 1 | 1 |
| Ile | 18.8 | 4 | 2 |
| Leu | 19.2 | 5 | 7 |
| Phe | 11.7 | 2 | 4 |
| Lys | 35.6 | 7 | 6 |
| His | 14.5 | 3 | 8 |
| **5-Hour Change:** | | | |
| Thr | 17.2 | 7 | 3 |
| Val | 26.0 | 8 | 5 |
| Met | 3.6 | 2 | 1 |
| Ile | 17.6 | 6 | 2 |
| Leu | 15.5 | 5 | 7 |
| Phe | 8.5 | 3 | 4 |
| Lys | 13.8 | 4 | 6 |
| His | −0.5 | 1 | 8 |

PAA ratios calculated from data Table

[a] Longenecker and Hause (1959)

[b] Block and Mitchell (1946)

assayed by other methods and included in future determinations of PAA Index.

When the PAA Index was calculated using one-hour essential

**TABLE 13**

Mean 30-Minute Change in Plasma Amino Acids After Intubation of Wheat Gluten, Yeast, Casein-Lactalbumin, and Egg Albumin

| Amino Acid | Egg Albumin | Wheat Gluten | Yeast Protein | Casein-Lactalbumin |
|---|---|---|---|---|
| | | mg/100 ml | | |
| Thr | 2.54 | 0.43 | 1.20 | 1.52 |
| Val | 1.15 | 0.67 | 0.79 | 1.15 |
| Met | 0.47 | 0.11 | 0.20 | 0.23 |
| Cys | 1.05 | 1.32 | ..... | 0.71 |
| Met + Cys | 1.52 | 1.43 | | 0.93 |
| Ile | 0.55 | 0.18 | 0.35 | 0.47 |
| Leu | 0.54 | 0.30 | 0.46 | 0.77 |
| Tyr | 0.49 | 0.50 | 0.76 | 0.60 |
| Phe | 0.20 | 0.24 | 0.39 | 0.41 |
| Phe + Tyr | 0.69 | 0.74 | 1.15 | 1.01 |
| Lys | 1.76 | −0.58 | 0.97 | 1.78 |
| His | 0.25 | 0.23 | 0.20 | 0.29 |

Each mean represents four rats

**TABLE 14**

Calculations of Plasma Amino Acid Index Using Egg Ratios of 30-Minute Changes in Plasma Amino Acids After Intubation of Wheat Gluten

| Amino Acid | Egg Albumin (mg/100 ml) | Wheat Gluten (mg/100 ml) | Ratio (%) | Log Ratio |
|---|---|---|---|---|
| Thr | 2.54 | 0.43 | 17 | 1.2304 |
| Val | 1.15 | 0.67 | 58 | 1.7634 |
| Met + Cys | 1.52 | 1.43 | 94 | 1.9731 |
| Ile | 0.55 | 0.18 | 33 | 1.5185 |
| Leu | 0.54 | 0.30 | 56 | 1.7482 |
| Phe + Tyr | 0.69 | 0.74 | 100 | 2.0000 |
| Lys | 1.76 | −0.58 | 0 | 0.0000 |
| His | 0.25 | 0.23 | 92 | 1.9638 |
| | | | Total | 12.1374 |
| | | | Ave. Log | 1.5171 |

Plasma Amino Acid Index is the Geometric Mean: Antilog 1.5171 = 32.9

plasma amino acid changes for dog No. 365 meal fed casein (Longenecker and Hause, 1959), a PAA Index of 70.2 was obtained (Whitaker, 1970). A value of 30 was calculated for dog No. 364 meal fed wheat gluten. These are in line with the pre-

**TABLE 15**

Calculation of Plasma Amino Acid Index Using Egg Ratios of 30-Minute Plasma Amino Acid Changes after Intubation of Yeast Protein

| Amino Acid | Egg Albumin (mg/100 ml) | Yeast Protein (mg/100 ml) | Ratio (%) | Log Ratio |
|---|---|---|---|---|
| Thr | 2.54 | 1.20 | 47 | 1.6721 |
| Val | 1.15 | 0.79 | 69 | 1.8388 |
| Met | 0.48 | 0.20 | 42 | 1.6232 |
| Ile | 0.55 | 0.35 | 64 | 1.8062 |
| Leu | 0.54 | 0.46 | 85 | 1.9294 |
| Phe + Tyr | 0.69 | 1.15 | 100 | 2.0000 |
| Lys | 1.76 | 0.97 | 55 | 1.7404 |
| His | 0.25 | 0.20 | 80 | 1.9031 |
| | | | Total | 14.5132 |
| | | | Ave. Log | 1.8141 |

Plasma Amino Acid Index is the Geometric Mean: Antilog 1.8141 = 65.2

**TABLE 16**

Calculation of Plasma Amino Acid Index Using Egg Ratios of 30-Minute Plasma Amino Acid Changes after Intubation of Casein-Lactalbumin

| Amino Acid | Egg Albumin (mg/100 ml) | Casein-Lactalbumin (mg/100 ml) | Ratio (%) | Log Ratio |
|---|---|---|---|---|
| Thr | 2.54 | 1.52 | 60 | 1.7782 |
| Val | 1.15 | 1.15 | 100 | 2.0000 |
| Met + Cys | 1.52 | 0.94 | 61 | 1.7853 |
| Ile | 0.55 | 0.47 | 85 | 1.9294 |
| Leu | 0.54 | 0.77 | 100 | 2.0000 |
| Phe + Tyr | 0.69 | 1.01 | 100 | 2.0000 |
| Lys | 1.76 | 1.78 | 100 | 2.0000 |
| His | 0.25 | 0.29 | 100 | 2.0000 |
| | | | Total | 15.4929 |
| | | | Ave. Log | 1.9366 |

Plasma Amino Acid Index is the Geometric Mean: Antilog 1.9366 = 86.4

dicted values obtained by substituting the biological values of Mitchell (1948) into the regression equation (Whitaker, 1970). Thus further evidence has been obtained that early essential plasma amino acids changes after protein feeding reflect dietary protein amino acid composition such that they may be used as an index of protein quality.

**The Advantages of Using PAA Index to Determine Protein Quality**

1. The principal advantage of the PAA Index is the ease and speed with which the data is obtained. The animals are fed only once and samples are secured in less than two hours. By comparison, data must be collected for two weeks to obtain reliable results with methods based on weight gain or nitrogen balance.

2. Since the PAA Index is an integrated method, the results

**TABLE 17**

Comparison of PAA Index Values of Test Proteins with Their Biological Values

| Test Proteins | PAA Index | Biological Value |
|---|---|---|
| Casein-Lactalbumin | 87 | 86[a] |
| | 84 | 85 |
| | 83 | 84 |
| | 80 | 83 |
| | 83.5 | 84.5 |
| Yeast Protein | 72 | 63[b] |
| | 64 | 63 |
| | 61 | 63 |
| | 58 | 63 |
| | 63.8 | 63.0 |
| Wheat Gluten | 36 | 46[c] |
| | 32 | 42 |
| | 31 | 40 |
| | 28 | 38 |
| | 31.8 | 41.8 |

[a] Mitchell (1924)
[b] Mitchell (1948)
[c] Mitchell and Beadles (1950)

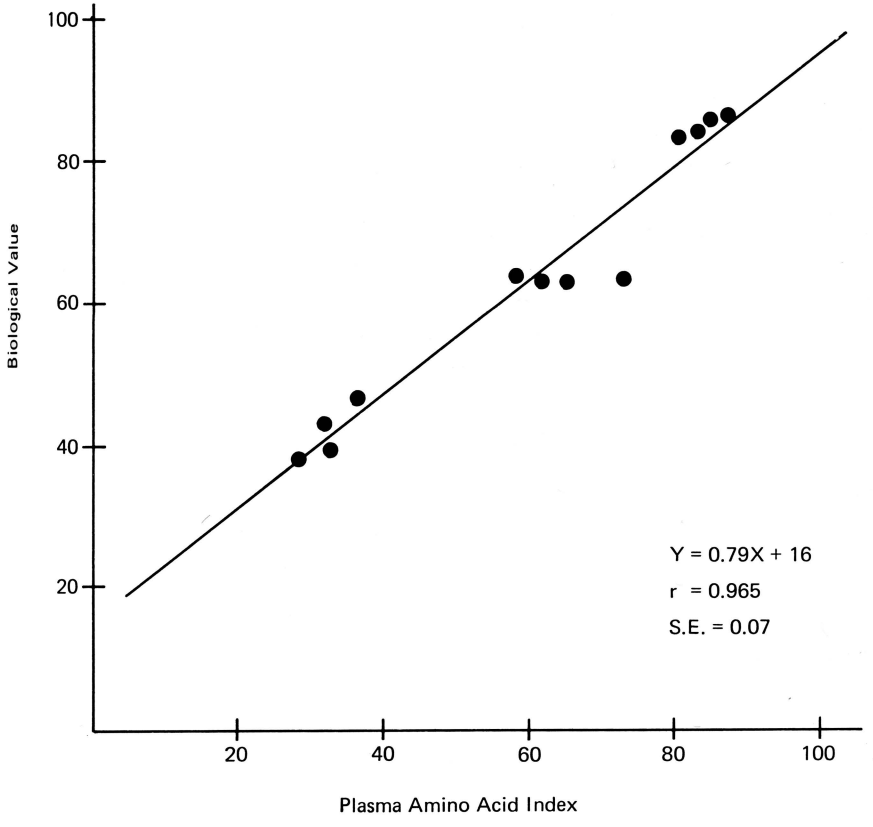



**Figure 4. Comparison of Biological Values of Test Proteins with Their Respective PAA Index.**

explain not only the relative nutritional value of the dietary protein, but also the specific essential amino acid deficiencies and their relative magnitudes. Only supplementation with specific amino acids involving additional time and animals could produce the same information using nitrogen balance or weight gain methods.

3. Since the PAA Index is based on amino acids which have been absorbed into the blood from the digestive tract, differences in amino acid availability in the dietary protein should be detected. This would be an advantage over **in vitro** methods such as Chemical Score and Essential Amino Acid Index which may provide the same information but do not take into account digestibility.

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