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The Amino Acid Composition of Four Varieties of Rice from India

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THE AMINO ACID COMPOSITION OF FOUR VARIETIES OF RICE FROM INDIA

By

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INTRODUCTION

Rice is believed to be the world's greatest crop. The International Institute of Agriculture estimates a normal annual production of about five hundred billion pounds of rough rice. It is probably the staple food of the greatest number of people.

Rice culture began in an unrecorded past. Rice and food are synonymous in languages so numerous and widespread, especially in India, that we may believe that this was the principal food in the misty dawn of settled life, as long ago as 2,000 B.C. The importance attached to this cereal is seen from the fact that in Sanskrit, the oldest language in India, besides the usual word for rice (Vrihis), the term "dhanya", which signifies the "Sustainer of Human Race" is also used.

India is one of the principal countries where rice is a staple food for the majority of the people. It is one of the most important crops of the populous empire, both in production and in consumption. In eastern Madras, Orissa, Bengal and Assam, for example, more than 75 percent of the total food crops consists of rice.

In India, about 5,000 botanical varieties of rice are grown. However, a great many of these varieties are used only for local consumption. The quality differences in certain varieties are so negligible that for commercial purposes they are classified under the same name.

A more complete knowledge of the constituents of rice and their significance is therefore desirable not only from physiological and biochemical standpoint, but also from that of utility. In recent years, emphasis has been placed on the evaluation of protein quality rather than

the total quantity of protein in foods. This is indeed a timely approach, as utilization of the amino acids, which are constituents of proteins, is dependent on all of the essential amino acids being present simultaneously and in proper proportion.

Wheat is characterized by the high nitrogen and protein content of its kernel. The rice kernel, however, contains less nitrogen or protein, but it is distinguished by its high over-all digestibility.

Because rice is widely used by the people in India and since there is no particularly rich source of protein, other than the pulses, in the diet of common people, more information on the amino acid composition of the foods (especially rice) commonly consumed, is necessary to evaluate rice as a source of protein in the diet. This approach is also of value in the practical nutrition education program in India. Though certain foods may be low in specific amino acids, they may supplement each other when eaten at the same time. Thus knowing the amino acid composition of the rice varieties commonly eaten, a nutritionist may be able to raise the level of health and well being of the population in specific areas, by assisting them in achieving a good state of protein nutrition, without altering the basic dietary pattern.

With the above views in mind, the present study of the amino acids in some varieties of rice eaten in Bombay state of India, was undertaken.

REVIEW OF LITERATURE

A review of rice literature has not been made in recent years. In fact little work has been done on the amino acid composition of the rice grain in either India or United States. The reason for this in India may be due to the lack of trained personnel, modern equipment and lack of funds; while the reason in the United States may be that rice is not a main dietary constituent and is particularly not considered a source of protein, since animal and other rich sources of protein are available. There are a few reviews which cover the literature in India and the United States and these are included in the following discussion along with other historical notes. The following excerpts from the literature are of interest in the development of our present knowledge of protein nutrition.

The following information is taken from a text by Sahyun (25):

> In 1810, the British physician, William Wollashan discovered cystine in certain urinary calculi and named the substance as cystic oxide (25). The same reference states that Proust, working with the flavoring matter of cheese in 1819, isolated a white compound which he called casein oxide. A year later, Barconnot, obtained the same compound from a sulfuric acid digest of muscle fibre and wool and named it leucine. This was the first use of acid hydrolysis for isolation of amino acids and the first demonstration that protein hydrolysis yielded simpler crystalline compounds.
>
> The nutritive value of protein and the dependence of animals on plants for these substances were first pointed out by Mulder around 1840. In 1897, Rubner recognized that proteins of varying origin were not of the same value in nutrition.
>
> The fundamental work on the amino acid composition of proteins continued to gain momentum as the twentieth century opened. In 1910 Kossel, using his own work and that of Fischer and other leaders, presented a summary of the current knowledge regarding the structure of proteins and amino acids. Shortly thereafter, Sir Frederick Howland Hopkins and Sydney Cole isolated the amino acid, tryptophan, which was destined to play a prominent role in

nutrition research.

The amino acid concept led to new concepts of the chemistry of digestion and new understanding of the mode by which proteins from food become available to the organism of the body.

One link was missing in the proof that a mixture containing only amino acids would support life in animals, when used as a sole dietary source of nitrogen. Yandell Henderson and Arthur Dean prepared acid hydrolysates of protein and fed them to dogs. The amino acid mixture was readily absorbed and rapidly converted to urea. Such a mixture exerted a nitrogen sparing effect in animals, but was totally incapable of maintaining nitrogen equilibrium, or promoting the synthesis of flesh. It soon became evident that the failure of acid hydrolysate to replace dietary protein was due to the destruction of an essential amino acid, tryptophan.

Of the literature pertaining to rice the following information is recorded (13):

The analytical results of Wise and Broomell in 1927 showed that Honduras rice (fancy head, uncoated) contains 0.38 percent ash, 0.19 percent ether extract, 0.24 percent crude fiber, 8.74 percent protein, and 2.2 percent pentosans, calculated on moisture-free basis.

Rosenheim and Kajiura state that the protein in table rice makesup about 7 percent of the grain as used for food. They class the proteins as rice globulin, rice albumin and oryzenin. These results were confirmed by Suzuki, Yoshimura and Fuji, who further found that the bran of rice contained 1.165 percent protein nitrogen and 0.035 percent non-protein nitrogen, or a total nitrogen content of 1.20 percent.

Osborne, Van Slyke, Leavenworth and Vinogard have estimated the distribution of nitrogen among the hydrolytic products of oryzenin, the chief protein of the endosperm of rice, and have come to the conclusion that in its general amino acid make-up, it resemblee the protein of animal tissues in a higher degree than do the proteins of wheat and maize. This in their opinion, explains the extensive use of rice as an exclusive diet throughout the Orient in spite of its low protein content.

In 1927, Jodidi worked on the protein nitrogen and non-protein nitrogen of various cereals and his results showed that protein nitrogen constitutes the great bulk of the total nitrogen, and ranges on an average from about 87,88, and 90 percent in rye, wheat, and oats, respectively, to more than 95 percent in corn and rice. Aside from inheritance, the total nitrogen in the kernel of various cereals is attributable more or less to factors such as soil, climate and quality of seed. It does not seem unreasonable to ascribe to these same causes the varying proportion of protein nitrogen found in different samples of cereals.

> There is, however, one factor which may have considerably more influence upon the proportion of protein nitrogen in the cereal kernel than the conditions mentioned above, namely, the degree of ripeness of the seed. From observations of Schulze and other investigators, the conclusion seems to be justified that the proportion of protein and non-protein nitrogen is more or less fluctuating and that it depends on the state of ripeness of the seed. Unripe seed ordinarily contains a higher percent of non-protein nitrogen and the ripe seed ordinarily contains a higher percent of proteins.
>
> The total non-protein nitrogen in the various cereals ranges, in round figures, from about 4 or 5 percent in corn and rice to about 10, 12 and 13 percent in oats, wheat and rye, respectively, calculated on the basis of total nitrogen. Of the small percentage of non-protein nitrogen, however, only part can be extracted with water, which part is distributed among the various nitrogenous compounds such as acid amides, amino acids and polypeptides. Nevertheless their presence in the ungerminated kernel of cereals seems to suggest physiological importance.

In 1941, Kik worked with the proteins of whole rice, rice polishings and rice bran to study quantitatively the amino acids cystine, tryptophan, lysine, arginine and histidine in them (15). All of these amino acids were considered nutritionally essential except cystine, which is only essential when methionine is present at too low a level, and arginine which is not essential for the maintenance of adults. Results showed that whole rice and polished rice are not lacking in cystine, but they do have a low cystine percentage as compared to casein and wheat. Rations containing whole rice or polished rice as the only source of proteins do not furnish enough cystine to support good growth and cystine addition has some supplementary effect. Tryptophan, arginine and histidine content compared favorably with the content of these essential amino acids in wheat and corn. The lysine content of whole rice and polished rice, however, is much lower than that of wheat. Differences were found in the composition of proteins of different varieties. Increases were also

obtained in cystine, tryptophan, lysine, arginine and histidine content of the proteins of rice from plots treated with fertilizers (like super phosphate, ammonium sulfate, sodium nitrate and sulfur) as compared to the amino acid contents of the proteins of rice from untreated plots.

In "Nutritional Reviews" (27) a statement is made that in view of the consideration of the world's food supply, the more economical cereal grains are important as sources of both protein and carbohydrates. Some work was done on the rice samples and it was found that when the animals were fed cereal products as their sole source of protein, rice proteins were found to be superior in supplementing the proteins of corn and wheat. The beneficial effects of rice used in this way, as a supplement to other cereals, were interpreted as due to its content of essential amino acid.

Eighteen free amino acids were identified by paper chromatography from adsorption dialysis extracts of both fresh and oven-aged parboiled rice. Those in greatest initial concentration were alanine, aspartic and glutamic acid; those in intermediate concentration were arginine, asparagine, glycine, leucine, proline, serine and valine; those in lowest concentration were cystine, histidine, methionine, phenylalanine, threonine, tryptophan and tyrosine (12).

Some research work is being done in India on rice samples, but so far more has been done on the vitamin content of rice rather than protein content. There is a plan for doing more work in the near future. In southern India, in the Food Research Laboratory, they studied 35 pure strains of cereals, microbiologically and chemically. Wide variation was found in different varieties of grains and appreciable differences

in various strains of the same grain. They think that heredity and environment influence the amino acid content of cereal grains (8).

Other workers in India took 18 varieties of five cereals (including rice and wheat) and analyzed them for moisture, ash and total nitrogen, as well as amino acids by microbiological methods. Average values of tryptophan, threonine, isoleucine and valine, expressed as a percentages of moisture and ash-free rice were 0.12, 0.76, 0.57, and 0.58, respectively (2).

In the United States Caloro, a short grain variety of the commercially parboiled rice, was analysed by paper chromatography before and after storage for 28 days at 82°C. Eighteen amino acids were detected. It was found that losses during aging were greatest for asparagine (12).

An aqueous extract of brown rice contained 36 percent of the total soluble nitrogen as non-protein-nitrogen, corresponding to 3.5 percent of the total nitrogen in rice. Electrophoresis of an unpurified extract at pH 4.0, 8.4 and 10.3 showed that the proteins were heterogeneous (11).

For further observation on the improvement of polished rice with protein or amino acid supplements, 6 rats were given a diet containing 87 percent rice and also maize oil, salts, choline and vitamins (9). Lysine and threonine, at 0.4 and 0.5 percent, respectively, or a mixture of histidine, tryptophan, methionine and phenylalanine alone or with arginine, valine, leucine or isoleucine, prevented deposition of excess fat in liver, but did not improve rate of growth. When, with these same levels of lysine and threonine, all the essential amino acids were provided, rate of growth was good, but if one acid only was removed

at a time, it was found that isoleucine, leucine, histidine, and to a lesser extent, valine were the limiting growth factors, methionine or tryptophan were intermediate in their action, and arginine or phenylalanine were relatively unimportant. If the amount of leucine, isoleucine, histidine or valine were increased and if tryptophan, methionine and phenylalanine were added, it was still not possible to attain the rate of growth in this work that was obtained with a supplement of good animal protein such as fibrin, pork, casein or kidney meat or in the earlier experiment with a good synthetic diet. But it seems that still more information is required to decide as to which may be the limiting amino acid in such diets, and that a diet, which is mainly of rice, should in practice, be supplemented with foods containing well-balanced protein.

Some experiments indicated that proteins of whole rice and milled rice can be improved by supplementation with lysine, threonine and vitamin B_{12} and those of enriched milled rice by supplementation with lysine and threonine (16). Results also indicated that additions of lysine or threonine resulted in smaller losses of the nitrogen during digestion and metabolism, which resulted in better growth and better utilization of the protein.

MATERIAL AND METHODS

Samples of the following four varieties of rice were received from the Office of Plant-Breeding, Agricultural Research Station, Karjat (Colaba), Bombay State, India. These were designated as follows:

1. K-42; 1957-58
2. Bhadas - 1303; 1957-58
3. 2. Ed. - 68-1; 1957-58
4. Zinya - 31; 1957-58

As used in subsequent analyses, these samples will be referred to as 1, 2, 3, and 4, respectively.

Only brown rice, that is seed rice from which the husk had been removed, was used in the experiments here described.

The rice was dehulled in a barley pearling machine. It was then dried in a vacuum oven at 80°C. for about three hours. The rice was defatted with ether for 6 hours in a Goldfisch fat extraction apparatus (Laboratory Construction Co.) The defatted grain was then ground in a semi-micro Wiley mill using 60 mesh sieves. The resulting powder was used for analysis.

Total nitrogen and water-soluble nitrogen were determined by the Gunning modification of the Kjeldahl method (26). In determining water-soluble nitrogen in terms of water-extractable ninhydrin-positive compounds one gram samples of rice from each variety were transferred in duplicate to test tubes. To the first four samples (one from each variety) was added 4 ml. of distilled water and to the four duplicates was added 4 ml. of 0.1 N HCl. After shaking and mixing them thoroughly,

they were filtered. One milliliter of each filtrate was added to 9 ml. of distilled water. To 0.5 ml. aliquots of these dilutions were added 0.5 ml. of distilled water and 2 ml. of ninhydrin solution (19) and the reaction between the amino acids and ninhydrin was completed in a boiling water bath. The intensity of the blue-colored compound produced was expressed in terms of the amount of leucine that would produce a similar color intensity.

Before hydrolyzing the proteins in rice one gram of each sample of powdered rice was weighed out and a digestion of starch with amyloytic enzymes was performed (5). Each sample was pasted with 8 to 9 volumes of hot water until no lumps remained. Then dilute acetic acid was added to bring the pH to 4.5 and the suspension was heated for one hour in a boiling water bath, after the initial temperature reached 90°C. The suspension was diluted with 3 volumes of water and the pH adjusted to 7.0 with NaOH. After cooling the suspension to 37°C., an excess of fresh, centrifuged saliva was added. Starch digestion was allowed to proceed for 7 days at 37°C. Toluene and chloroform were added as preservatives. At the end of the digestion period the chloroform and toluene were removed by dissolving them in a small amount of ether, drawing off the ether layer, and repeating the extraction with several small portions of ether. The remaining traces of ether were removed by passing a current of air through the solution.

The suspension was quantitatively transferred to a centrifuge tube and centrifuged. The supernatant liquid was decanted and the centrifugate was re-suspended in distilled water and re-centrifuged. After

decanting the supernatant liquid, 25 ml. of 6 N HCl was added to the centrifugate. An acid hydrolysis was then performed in an oil bath at 115°C for 24 hours under a reflux condenser. During this acid hydrolysis, humin formed in the solution. The humin was filtered out and a determination of the nitrogen in it was separately carried out by the Gunning modification of Kjeldahl method.

The filtrate from the hydrolysis, containing the free amino acids, was combined with the water rinses from the humin filtration and reduced to dryness under reduced pressure (about 25 mm mercury), using heat as necessary (temperature did not exceed 50°C.). In order to more completely remove the hydrochloric acid, 10 ml. of distilled water were added to the residue and it was again reduced to dryness.

To this dry material one milliliter of citrate buffer (pH 3.42) was added and the solution was transferred to a 10 ml. volumetric flask. After adjusting the solution to pH 2.5-3.0 by adding a few drops of 0.5 N NaOH, the solution was made up to volume by adding more of the pH 3.42 buffer. One ml. of solution was placed on a 100 cm. Dowex 50 resin column, prepared as directed by Stein and Moore (21). The resin column technique was chosen instead of the starch column technique, since resin columns possess higher resolving power than starch columns and the performance of ion-exchange columns is not adversely affected by the presence of inorganic salts in the material being chromatogrammed.

Using the 100 cm. resin column, it is possible to quantitatively recover all of the amino acids with the exception of the basic amino acids, histidine, lysine and arginine. For better recovery of the basic

amino acids, a shorter Dowex-50 column of 15 cm. length was employed. One ml. of the solution was placed on the 15 cm. column and the chromatogram made in the sodium cycle, using the citrate buffers recommended by Stein and Moore (21).

Low recoveries could be expected of cystine and methionine. The low recovery of methionine could result from the failure of thiodiglycol in the buffer to completely protect sulfur from oxidation, and for both cystine and methionine it could be due to hydrolytic destruction. Separate estimations of cystine and methionine were made in an attempt to obtain better values. The gravimetric method of Evans, as outlined by Block and Bolling (4) was used. This is based on the principle that cystine, but not methionine, is oxidized to sulfate with hot concentrated HNO_3. Two grams of rice powder were heated in a 500 ml. Kjeldahl flask with 35 ml. of concentrated HNO_3 on a steam bath for 24 hours. The solution was evaporated to dryness in a 250 ml. beaker containing 500 mg. of KNO_3, then 10 ml. of concentrated HCl were added and the solution again evaporated to dryness. The residue was taken up in 200 ml. of hot water containing one ml. of dilute HCl.

The solution was then heated to boiling and 25 ml. of 5 percent $BaCl_2$ solution were added dropwise from a pipette, while stirring the solution constantly. After allowing the precipitate to settle overnight, it was transferred to filter paper and ashed in a furnace at 900°C. for a few hours, and sulfur determined as sulfate.

Total sulfur was determined by Parr Bomb ignition followed by precipitation of sulfate by barium chloride as outlined.

Calculations: Cystine = 3.747 x (sulfur after HNO_3 oxidation-inorganic sulfur)

Methionine = 4.651 x (total sulfur-sulfate sulfur).

One of the serious unsolved problems in the analytical chemistry of proteins concerns the extent of destruction of amino acids during the hydrolytic procedure. The rates of destruction depend upon the presence or absence of other constituents such as carbohydrates, either in the protein or in the hydrolysing mixture. It seems that the basic amino acids are quite stable under the conditions necessary for complete hydrolysis of proteins by acids, although slight but detectable destruction of histidine has been shown to occur in one instance. On the other hand tryptophan, serine, threonine, cysteine, tyrosine and phenylalanine are subject to considerable destruction under these conditions. In order to have some estimate of the tryptophan content in these rice samples, an alkaline hydrolysis procedure was used.

For the estimation of tryptophan 0.5 gram of powdered rice from each sample was mixed with 20 gm. of anhydrous barium hydroxide and to this was added 20 ml. of distilled water (18). This mixture was hydrolysed by autoclaving at 15 lb. pressure for about 8 hours. To this hydrolysate enough of 10 N H_2SO_4 was added dropwise (until pH 6 to 6.8 was reached) to precipitate barium as barium sulfate. The contents were filtered, the precipitate washed with warm water a few times to extract all the tryptophan and the washings were combined with the original filtrate. In almost all the cases the filtrate was slightly cloudy and was therefore kept on the steam bath overnight and then filtered in order to obtain a clear filtrate.

The clear filtrate was evaporated to dryness under reduced pressure. 1.5 milliliters of 0.1 N HCl were added to the dry mass and 0.5 ml of this solution was placed on a starch column, which had previously been prepared with an eluting solution prepared by mixing equal portions of n-propanol and 0.5 N HCl (20). Elution was done with 0.1 N HCl, collecting 0.5 ml. fractions. Tryptophan was then estimated by spectrophotometric measurement of the color produced by its reaction with ninhydrin solution. (19)

EXPERIMENTAL RESULTS

To furnish supplementary information on the composition of the four rice varieties prior to analyzing them for their amino acid content, the rice samples were examined for differences in weight and in water-extractable nitrogen.

A comparison was made of the weight of the seeds of each variety with and without husk. Data shown in Table I represent the weight per

Table I. The Weight of the Four Varieties of Rice Samples in Grams, Per 100 Kernels.

Rice Sample	Wt. of 100 Kernels With Husk (Grams)	Wt. of 100 Kernels Without Husk (Grams)
1	1.3364	1.0576
2	2.1300	1.6788
3	2.5488	1.8396
4	1.2464	0.9152

100 kernels. Considerable variation in average weights of the kernels of the four varieties is evident.

Total nitrogen was determined on all four varieties of dehulled, defatted and moisture-free ground rice by the Kjeldahl method. Table II shows the total nitrogen in milligrams per gram of moisture-free samples. There seems to be no correlation between the total nitrogen values and kernel weight values.

Table II. Total Nitrogen Values for the Four Varieties of Rice Samples.

Rice Sample	Milligrams Nitrogen Per Gram of Rice (Moisture-Free)
1	12.00
2	7.71
3	11.18
4	9.11

Column 2 in Table III shows the water-extractable nitrogen in micrograms per gram of rice sample as determined by the Kjeldahl method. The figures in columns 3 and 4 represent the nitrogen extractable in cold

Table III. Water-Extractable Nitrogen in Four Varieties of Rice Samples.

Rice Sample	Water-Extractable Nitrogen (mcg. N/g. of sample)	Water-Extractable Nitrogen (mcg.leucine equivalent/g. of sample)	Nitrogen Extractable in 0.1 N HCl (mcg. leucine equivalent/g. of sample)
1	140	204	595
2	350	320	435
3	190	240	555
4	300	270	445

water and the nitrogen, extractable in 0.1 N HCl, respectively; since

ninhydrin reagent was used and concentrations were determined with a spectrophotometer, these values are expressed as milligrams of leucine equivalent per gram of rice.

From columns 3 and 4 of Table III it can be seen that more ninhydrin-positive material can be extracted using 0.1 N HCl than with water alone. The figures in columns 3 and 4 cannot be compared directly with those of column 2, because column 2 represents total water-soluble nitrogen, whereas in columns 3 and 4, it represents only the nitrogen compounds that give ninhydrin color reaction. The intensities of color produced by this mixture of compounds was arbitrarily equated to the color intensity produced by known amounts of leucine.

During hydrolysis of the rice proteins, in 6N HCl at temperature of 110°-120°C. for 24 hours, a brown to black colored substance called humin was formed. The insoluble humin was filtered and the nitrogen in it determined by the Kjeldahl method. A negligible amount of nitrogen (less than 0.1 mg. nitrogen/gram of rice) was detected in the humin formed during the hydrolysis of the proteins of each of the four varieties of rice.

Table IV shows the net amount of nitrogen available for hydrolysis. It was determined by substracting water-soluble nitrogen from total nitrogen. Column 5 of Table IV shows the protein equivalent hydrolyzed. These figures were obtained by multiplying the amount of nitrogen hydrolyzed by the protein factor 5.95 for rice (14).

Table IV. Net Amount of Nitrogen or Protein Available for Hydrolysis Per Gram of Rice.

Rice Sample	Milligrams of Total Nitrogen	Milligrams of Water-Soluble Nitrogen	Milligrams of Nitrogen Hydrolyzed	Milligrams of Protein Hydrolyzed
1	12.00	0.14	11.86	70.6
2	7.71	0.35	7.36	43.8
3	11.18	0.19	10.79	65.5
4	9.11	0.30	8.81	52.5

The protein hydrolysates, after humin filtration, were dried under partial vacuum and reduced temperature as described in the Material and Methods section. The amino acids were quantitatively separated by chromatography on Dowex 50 resin columns operated in the sodium cycle. Table V shows the data for the separated amino acids in micrograms per gram of moisture-free rice for the four rice varieties studied.

Table V. The Amino Acid Content of the Protein From Four Rice Samples Expressed as Micrograms of Amino Acid Per Gram of Moisture-Free Rice.

Amino Acid	Rice Sample			
	1	2	3	4
Threonine	2,330	1,780	1,740	1,420
Valine	2,600	2,120	2,810	1,830
Methionine	970	505	758	524
Iso-leucine	2,190	1,340	1,780	1,214
Leucine	5,740	3,460	4,770	3,260
Phenylalanine	3,060	2,330	2,640	2,460
Tryptophan	1,040	841	832	642
Lysine	3,010	1,990	1,800	1,870
Serine	3,660	2,510	3,140	2,270
Glutamic Acid	11,200	6,800	9,540	6,330
Glycine	2,770	2,020	2,900	1,890
Alanine	3,120	2,060	3,290	2,150
Cystine	556	1,005	862	785
Tyrosine	3,620	2,140	2,850	2,410
Histidine	1,640	905	1,310	950
Arginine	4,170	2,680	3,140	3,670
Ammonia	1,020	520	830	680
Aspartic Acid	2,800	1,590	1,660	1,070
Proline	3,090	1,950	2,340	2,210
Unidentified "Ninhydrin-Positive" Compounds	6,220	5,320	5,690	4,380

Tryptophan is destroyed during acid hydrolysis, hence an alkaline hydrolysis was used for the determination of tryptophan in rice samples. Subsequently, tryptophan was separated by starch column chromatography using 0.1 N HCl as the eluent. The amount of tryptophan recovered from different varieties is shown in Table VI.

Table VI. The Amount of Tryptophan Recovered from Four Varieties of Rice.

Rice Sample	Micrograms of Tryptophan/gm. of Rice
1	1,040
2	841
3	832
4	642

Low recoveries of cystine and methionine can be expected when a long period of hydrolysis (24 hours) is used to obtain a maximum amount of amino acids from a protein. Cystine is destroyed to a great extent (3) during acid hydrolysis and in presence of carbohydrate. The methionine present in protein is also affected during hydrolysis, but to a lesser extent. Low recoveries of methionine may also result from the failure of thiodyglycol in buffer to protect sulfur completely from oxidation.

Because of low recoveries for these two amino acids, for the reasons given above, separate estimation of cystine and methionine was

attempted gravimetrically. The results shown in Table VII are expressed as milligrams of methionine and cystine per gram of moisture-free sample.

These data do not compare favorably with the results for methionine and cystine given in other literature on rice. The methionine and cystine values shown in Table VII are two to three times higher than those previously reported. Hence, these results are not considered to

Table VII. Methionine and Cystine Values For Four Rice Samples, as Determined Gravimetrically

Rice Sample	Milligrams of Methionine/gm. Rice	Milligrams of Cystine/gm. Rice
1	2.17	2.73
2	2.77	1.83
3	2.76	2.69
4	3.78	1.57

be accurate. This large error is probably due to the small amounts of sulfur (cystine and methionine) present in the rice. Two grams of rice flour were used, whereas the method recommends 2 grams of a protein preparation. Relatively small differences in weights of the barium sulfate precipitates, would result in sizeable errors when calculating methionine and cystine values.

DISCUSSION

Frequently, amino acid data is recorded on the basis of grams of amino acid per 100 gram of protein. The data for the amino acid content of the four rice varieties as presented in Table VII has been recalculated on this basis and is reported in Table VIII. On comparing the results obtained with those reported for some rice samples in Calcutta, India, (7) in Table IX, it has been found that there is some variation in the results. The average amino acid values as reported in Table VIII, for threonine, leucine, phenylalanine, tryptophan, lysine and histidine are larger than reported in the Calcutta data, while the values for valine, methionine, isoleucine and arginine are lower. The average values reported here agree quite well with the data for brown rice (see Table IX) with regard to phenylalanine, lysine, serine, cystine and tyrosine content; however, they were lower for threonine, valine, methionine, isoleucine, proline, glycine and aspartic acid and were higher for tryptophan, glutamic acid, histidine and arginine. (22)

Other data on rice, presented in Table IX seem to differ from ours considerably with regard to the amino acids, valine, methionine, isoleucine and arginine.

On comparing the results of the present research with the results shown in literature, there seems to be no set pattern of variation. Comparison of the average amino acid values for the four rice varieties seem to be higher in some instances and lower in others. In all comparisons these data were lower in valine, methionine and isoleucine.

These variations may be due in part to the different assaying

Table VIII. The Amino Acid Composition of Four Varieties of Rice Expressed as Grams of Amino Acid Per 100 Grams of Protein

	Rice Variety				
Amino Acid	1	2	3	4	Average
Threonine	3.30	4.06	2.66	2.70	3.18
Valine	3.68	4.84	4.29	3.49	4.07
Methionine	1.37	1.15	1.16	1.00	1.17
Isoleucine	3.10	3.06	2.72	2.31	2.80
Leucine	8.13	7.90	7.28	6.21	7.38
Phenylalanine	4.33	5.32	4.03	4.69	4.59
Tryptophan	1.47	1.92	1.27	1.22	1.47
Lysine	4.26	4.54	2.75	3.56	3.78
Serine	5.18	5.73	4.79	4.32	5.00
Glutamic Acid	15.86	15.52	14.57	12.06	14.50
Glycine	3.92	4.61	4.43	3.60	4.14
Alanine	4.42	4.70	5.02	4.09	4.56
Cystine	0.79	2.29	1.32	1.49	1.47
Tyrosine	5.12	4.88	4.35	4.59	4.73
Histidine	2.32	2.07	2.00	1.81	2.05
Arginine	5.90	6.12	4.79	6.99	5.95
Ammonia	1.44	1.19	1.27	1.30	1.30
Aspartic Acid	3.96	3.63	2.53	2.04	3.04
Proline	4.38	4.45	3.57	4.21	4.15
"Ninhydrin-Unknowns"	8.81	12.16	8.68	8.34	9.50

Table IX. The Values for Amino Acid Content of Rice From Different Literature, Expressed as Grams of Amino Acid/16 Grams of Nitrogen (or 100 Grams of protein)

Amino Acid	Calcutta Rice 1/	Texas Patna Rice 2/	Texas Patna Polished Rice 2/	Converted Rice 3/	Rice 4/	Average Values in Five Studies	Brown Rice 5/
Threonine	2.86	3.57	3.43	3.90	3.80	3.51	3.73
Valine	4.71	6.38	6.34	5.70	6.2	5.87	6.06
Methionine	1.32	2.30	2.54	2.09	3.0	2.25	1.71
Isoleucine	3.08	4.59	4.44	4.03	5.2	4.27	4.46
Leucine	5.91	7.91	7.99	7.23	8.20	7.45	8.21
Phenylalanine	3.00	4.87	4.57	4.31	5.0	4.35	4.78
Tryptophan	0.61	1.59	1.59	1.11	1.30	1.24	1.02
Lysine	2.51	3.96	3.30	3.62	3.20	3.32	3.76
Serine							4.83
Glutamic Acid							13.04
Glycine							6.51
Alanine							
Cystine				1.81	1.30	1.56	1.30
Tyrosine				2.64	5.70	4.17	4.35
Histidine	1.22	2.30	2.41	1.95	1.70	1.92	1.60
Arginine	6.14	8.29	8.25	5.70	7.20	7.12	5.49
Ammonia							
Aspartic Acid							4.50
Proline							4.61

References (foot-notes) for Table IX

[1] Chatterjee, K. P. Essential amino acid composition of pulses and rice. Food research 21: 569-570. Sept.-Oct. 1956. (7).

[2] Lyman, C. M., Knuiken, K. A., Hale, Fred. Essential Amino Acid Content of Farm Feeds. J. Agr. Food Chem. 4: 1008-1013. Dec. 1956. (17).

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techniques employed. In nearly all of the other cases individual amino acids were determined by microbiological assay. A summing up of the 18 amino acids and liberated ammonium ion determined in this study shows that approximately 83, 88, 75 and 72 percent of the calculated amount of protein hydrolyzed has been recovered from the hydrolysates of rice samples 1, 2, 3 and 4, respectively. Calculated on the basis of percent of total nitrogen, the nitrogen recovery represents 73, 76, 65 and 66 percent for the four samples, respectively. This reflects on the completeness of protein hydrolysis with the conditions employed and may reflect, to some extent, the degree of amino acid decomposition occurring with these same conditions. With regard to measurements of amino acids as affected by incomplete hydrolysis, peptides were not determined by the resin column technique used; however, some of the amino acids which they contained may be available in microbiological assays. This could contribute to the higher values recorded for certain of the amino acids in previously published reports.

The recoveries of amino acids and "ninhydrin-unknowns" were very good for samples 1 and 2, that is 91.7 and 100.1 percent, respectively. With samples 3 and 4, the recoveries were only 83.5 and 80.0 percent, respectively. The compounds designated as "ninhydrin-unknowns" emerged from the resin column as several discrete peaks preceeding aspartic acid. One of these contained hydroxyproline and a sufficient amount of another ninhydrin-positive compound to make quantitative estimation of the amount of hydroxyproline impracticable. The compounds reacting with ninhydrin, being unidentified, were recorded in terms of their leucine equivalent,

that is the amount of leucine which would yield a total color intensity, on reacting with ninhydrin, equivalent to the total color intensity of the unknown compounds. Many ninhydrin reacting compounds yield similar color intensities on a mole basis. However, if many of the compounds now grouped in the class of "ninhydrin-unknowns" had molecular weights greatly exceeding that of leucine, it would be quite inaccurate to add their equivalent weights in terms of leucine to the total amount of amino acids recovered in determining the completeness of recovery. Thus the recoveries, of amino acids, for example - 100 percent for rice sample 2, are not as precise as presented here for the reason just noted and, also, the fact that no account has been made of the total weight of water reacting with the protein during hydrolysis.

Since the total recovery of amino acids from sample to sample was not approximately the same, the amino acid data were recalculated on the basis of grams of amino acid per 100 grams of total amino acids emerging. These data are presented in Table X. It was thought that this basis would permit comparison of amino acid content among the four rice varieties studied. As recorded in Table X, the amount of individual amino acids still differs from variety to variety. Only in methionine, isoleucine, tryptophan, serine and histidine content is there fair agreement. With regard to the other amino acids, each variety has certain points of agreement with one or two of the other varieties.

On comparing the average figures shown in Table X for four varieties of rice with the other literature values, it can be seen that, 1) on comparison with the results obtained in Calcutta research, the

Table X. The Amino Acid Composition of the Four Varieties of Rice Expressed as Grams of Amino Acid Per 100 Grams of Amino Acids Emerging From Column

	Rice Variety				
Amino Acid	1	2	3	4	Average
Threonine	3.98	4.62	3.55	3.77	3.95
Valine	4.44	5.50	5.73	4.86	5.13
Methionine	1.65	1.31	1.55	1.39	1.47
Isoleucine	3.74	3.47	3.63	3.22	3.51
Leucine	9.80	8.97	9.73	8.66	9.29
Phenylalanine	5.22	6.04	5.39	6.54	5.80
Tryptophan	1.77	2.18	1.70	1.70	1.84
Lysine	5.14	5.16	3.67	4.97	4.73
Serine	6.25	6.51	6.41	6.03	6.30
Glutamic Acid	19.12	17.64	19.47	16.82	18.26
Glycine	4.73	5.24	5.92	5.02	5.23
Alanine	5.32	5.34	6.71	5.71	5.77
Cystine	0.95	2.61	1.76	2.08	1.85
Tyrosine	6.18	5.55	5.82	6.40	5.99
Histidine	2.80	2.35	2.67	2.52	2.58
Arginine	7.12	6.95	6.41	9.75	7.56
Ammonia	1.74	1.35	1.69	1.81	1.65
Aspartic Acid	4.78	4.12	3.39	2.84	3.78
Proline	5.27	5.06	4.77	5.87	5.24

figures obtained in the current study are higher in case of almost every amino acid analyzed, 2) on comparing these results with those reported by Block and Bolling or the values reported for brown rice, the results of the current research are higher for most of the amino acids except for valine, methionine and isoleucine.

The comparisons made in Table X provide a more consistent pattern for the amino acid composition of the four rice varieties than found in Table VIII. Any comparisons based on Table X, however, should be qualified by considerations of variation in rates of cleavage of amino acids from protein and in rates of amino acid decomposition. The literature provides sufficient evidence of lack of uniformity for either the process of amino acid liberation or decomposition in the hydrolytic medium. Also, hydroxyproline and the "ninhydrin-unknown" compounds liberated during protein hydrolysis were not considered in the calculations entering into Table X.

On comparing the amino acid makeup of the protein fraction of rice (using either Table VIII or X) as determined in this study with that of the protein from egg, which is considered a complete protein (see Table XI), it has been found that the content of certain essential amino acids is higher in case of egg. The figures for threonine, valine, methionine, isoleucine and lysine are particularly low in rice.(22)

On comparing the averaged amino acid values of the four varieties of rice (see Table X) with the amino acid composition of wheat grain (22) (see Table XI), it can be seen that wheat grain is lower in almost all essential amino acids than is rice grain, an exception being isoleucine.

Table XI. Amino Acid Composition of Egg and Whole Wheat Expressed as Grams, of Amino Acid Per 100 Grams of Protein

Amino Acid	Egg [1]	Whole Wheat [1]
Threonine	4.98	2.69
Valine	7.42	4.32
Methionine	3.14	1.42
Isoleucine	6.64	4.05
Leucine	8.80	6.26
Phenylalanine	5.78	4.61
Tryptophan	1.65	1.15
Lysine	6.40	2.56
Serine	8.40	4.30
Glutamic Acid	12.37	29.15
Glycine	3.54	5.34
Alanine	---	3.26
Cystine	2.34	2.05
Tyrosine	4.30	3.49
Histidine	2.40	1.90
Arginine	6.56	4.46
Ammonia	---	---
Aspartic Acid	7.01	5.09
Proline	4.24	9.74

[1] "Amino Acid Content of Foods" -- Home Economics Research Report No. 4, United States Department of Agriculture, p. 24.

A similar comparison based on Table VIII shows rice to be slightly lower than wheat in valine, methionine and phenylalanine content, and considerably lower in isoleucine.

Various other experiments on rice by other research workers (2) have shown that lysine content is predominantly low for rice protein, ranging from 2.2 to 4.4 percent, while the value for a quality protein, such as egg, is 7 percent; also, the protein efficiency ratio (P.E.R.) of rice and other cereals in general is considerably lower than that of skim milk and of the whole egg protein.

It is possible that P.E.R. of rice protein is influenced by lysine and methionine as limiting amino acids. An enrichment of rice with these two amino acids would be one way of improving the P.E.R. of rice, but this suggestion is not capable of implementation on as large a scale as would be required for human nutrition in India. It is more practicable at present to attempt to improve the nutritive value of cereal protein by incorporation in the diet of other foodstuffs which will supply the deficient amino acids. In fact Patwardhan has shown that in Indian dietaries a combination of pulses and cereals provides sufficient lysine for the daily adult requirement, and improves its biological value in human subjects (23).

In a similar way, investigation was also made by Phansalkar, Ramachandran and Patwardhan to find if similar methods would succeed in providing dietary protein of vegetable origin which would have a protein efficiency ratio comparable to that of skim milk or that of whole egg. It was concluded from the above experiment that it is practicable to

obtain, through a proper combination of vegetable foodstuffs, protein mixtures of high biological value, as measured by protein efficiency ratios and the maintenance of normal levels of hemoglobin and plasma protein in albino rats (23).

Basu and Basak have found that the average biological value of the mixed proteins of a diet composed of rice, pulse and vegetable was 75 as compared to 100 for diets based on balanced proteins (23).

Rice is the main source of protein in the poor rice eater's diet. While an increase in protein content of typical rice diets is desirable, there is not much evidence that protein deficiency is among the more serious faults of such diets. No specific pathological conditions in rice eaters, ascribable to protein deficiency have been reported. Experiments have shown that deficiency of calcium is one of the major faults of the diet of rice eaters (1).

Diet surveys carried out in India between 1935 to 1948 have shown that the average consumption of cereals per adult man is 16 ounces per day. On this basis, the quantity of essential amino acids, except for methionine supplied by rice could meet the minimum requirements prescribed for adults. The authors of the experiment (24) suggest that even though the rice protein is known to be deficient in lysine, the adult human requirements can be met from 16 ounces of rice (see Table XII).

From the results in the various tables referred to above it can be seen that there is considerable difference in the amounts of specific amino acids in the four different varieties of rice samples. Samples 1 and 3 are higher in total protein than samples 2 and 4. If the protein

and amino acid values for each sample were to correctly reflect the

Table XII. Minimum Daily Requirement of Amino Acids in Grams, and the Amount of Amino Acids in Grams Received from 16 Ounces of Rice, as Determined in Diet Surveys in India During 1935-1948 (24)

Amino Acid	Quantity of Amino Acid 16 oz. Rice (in Grams)	Minimum Daily Requirement (in Grams)
Threonine	1.45	0.50
Valine	0.85	0.80
Methionine	0.95	1.10
Isoleucine	2.58	0.74
Leucine	3.46	1.10
Phenylalanine	1.97	1.10
Tryptophan	0.53	0.25
Lysine	1.68	0.8

average composition for this particular variety instead of for the one sample available for analysis, it may be possible that a plant breeding program could be used to improve the quality of the essential amino acid makeup of rice. Since samples 1 and 3 came from the rice breeding station in the Bombay State of India, it is apparent that rice breeding programs are capable of increasing the protein content of the experimental varieties as compared to the present protein level of some of the varieties commonly sold in markets in that area, for example varieties 2 and 4.

In India, the main problem in rice breeding is to increase the level of production per unit area and to increase the protein content of rice. Ever since systematic rice breeding was undertaken in the country, this problem has received constant attention and has been a major objective in all the breeding projects undertaken (24).

SUMMARY

Rice is a staple food for the majority of the people in India and many other countries. This study was undertaken to provide a more complete knowledge of the amino acid constituents of rice.

Four varieties of rice received from the Office of the Plant-Breeding, Agricultural Research Station, Karjat, Bombay State, India, were used in this study. The rice was dehulled, dried, defatted, ground to pass a 60 mesh sieve and treated with salivary amylase. Total nitrogen, water-soluble nitrogen and the amino acid values for 18 amino acids were recorded. The amino acids were separated on Dowex-50 resin column eluted with sodium citrate buffers; amino acid concentration in the effluent fractions was determined spectrophotometrically, after reaction with ninhydrin reagent. Tryptophan was separately determined following alkaline hydrolysis and separation by means of starch column chromatography. Only a very small portion of the total nitrogen of rice was found to be water-soluble.

Experimental varieties (1 and 3)were found to be higher in protein content than the two market varieties of rice (2 and 4).

Since complete hydrolysis was not obtained, the amino acid data have been presented on two bases: 1) grams of amino acid per 100 grams of protein, and 2) grams of amino acid per 100 grams of amino acids emerging from the resin column. Data on both bases have been used in comparing the values obtained in this study with those previously reported for rice in the literature. On either of the bases of comparison mentioned, the figures obtained in this study yield lower values for the

valine, methionine and isoleucine content of rice, as compared to the literature values.

The average values for essential amino acids in the four rice varieties have been discussed from the standpoint of their significance in nutrition in India. Comparisons were made with whole egg protein and protein of wheat, with regard to protein efficiency ratio. At the present level of rice consumption by adults in India (16 ounces daily), rice appears to supply a sufficient amount of all essential amino acids.

Plant breeding programs for rice varieties seem to show promise of improving this dietary cereal, principally by increasing the protein content of the kernel and thus, indirectly the amount of essential amino acids provided by it.

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APPENDIX

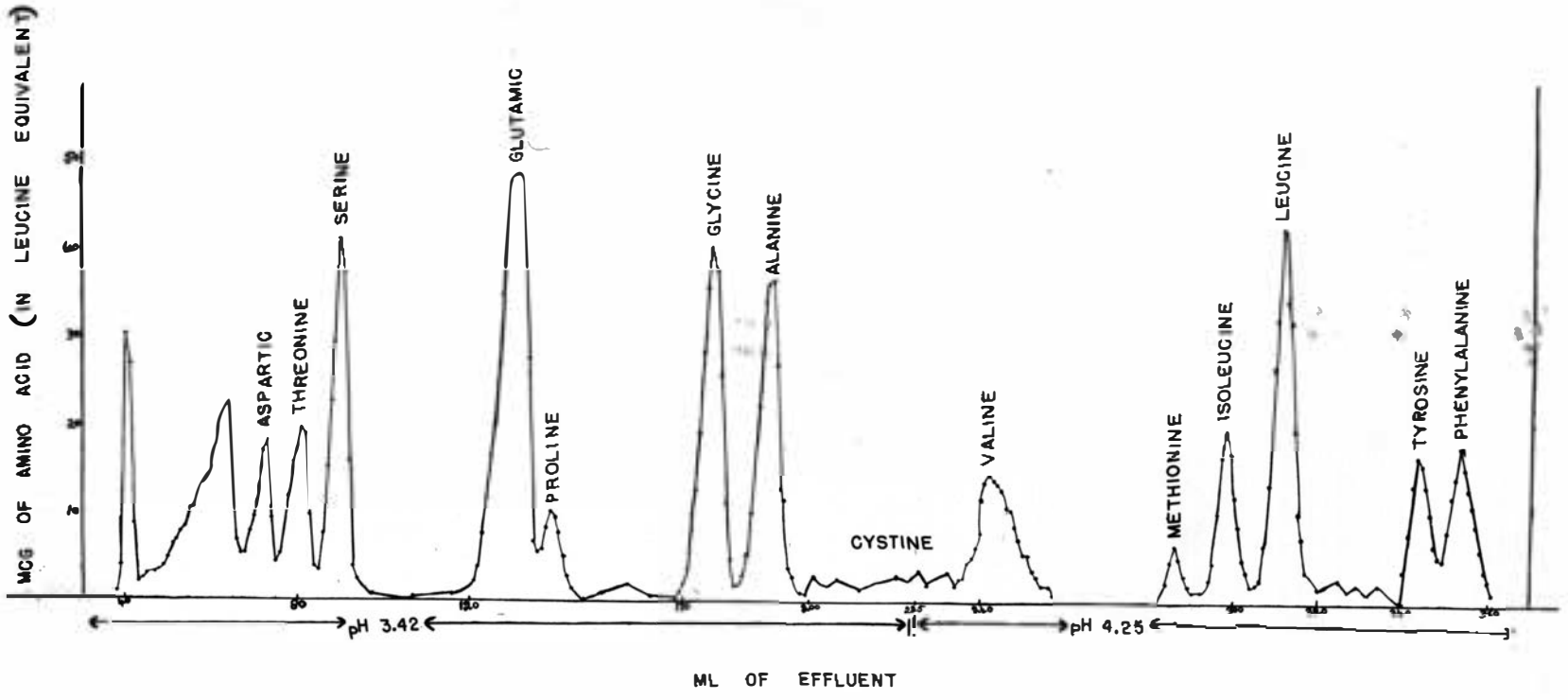

Figure I. Separation of amino acids from the acid hydrolysate of rice sample 2. The column of Dowex-50, 0.9 x 100 cm., was operated in the sodium form with buffers of the pH indicated as eluants. Elution with pH 3.42 buffer was performed at 37°C., while elution with pH 4.25 buffer was performed at 50°C. initially, and later at 75°C. following the emergence of leucine.

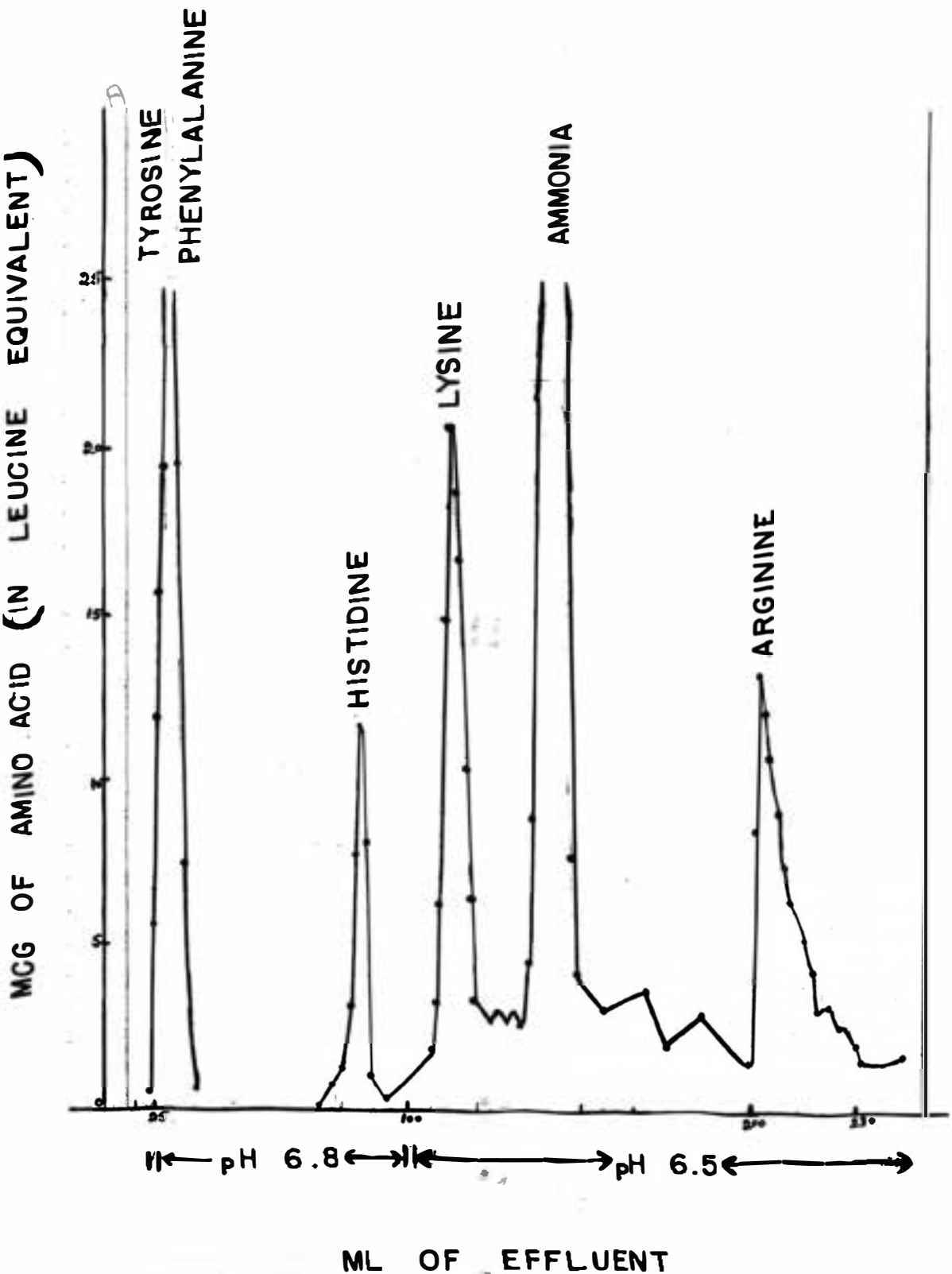

Figure II. Separation of basic amino acids from the acid hydrolysate of rice sample 2, on a column of Dowex 50, 0.9 x 15 cm. The column was operated in the sodium form at room temperature, with the buffers indicated. The large peak, A, comprises all the amino acids emerging before tyrosine in Figure 1.

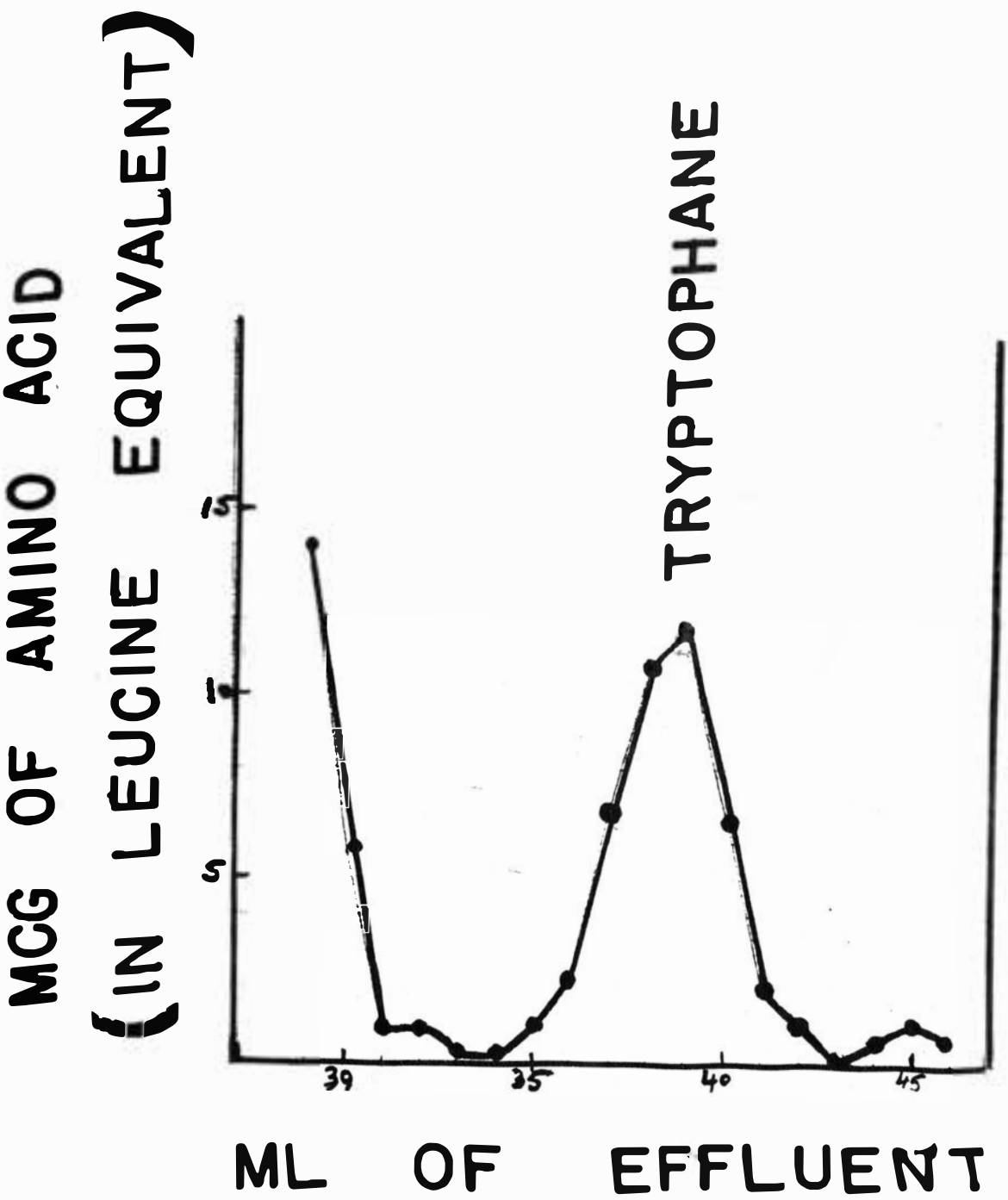

Figure III. Separation of tryptophan from an alkaline hydrolysate of rice sample 2, on starch column, 0.9 x 30 cm. Solvents used were 1:1 n-propanol: 0.5 N HCl followed by 0.1 N HCl