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TITLE THE NUTRITIONAL EVALUATION OF CERTAIN NUT PROTEINS

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# THE NUTRITIONAL EVALUATION OF CERTAIN NUT PROTEINS

By

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## INTRODUCTION

Almonds, cashews, English walnuts and pecans have a high content of protein and fat. These concentrated foods have been found to be readily digestible when incorporated in a mixed diet (Jaffa, 1903).

Certain nuts have been studied and have been found to have a variable biological value. Mitchell and Beadles (1937), using the rat as the experimental animal, recorded a biological value of 73 for cashews, which was only slightly less than that of beef protein (76), while pecans, almonds and English walnuts had biological values ranging from 50 to 60. The biological values were determined after expressing some of the oil from the nuts in order to incorporate 14 per cent of protein in the different diets.

Very few studies have been made on the nutritive value of proteins of whole nuts. Also an examination of Block and Bolling's (1951) compilation of the amino acids in common foods includes no data on the amino acid composition of any nuts other than peanuts. Nuts have been widely advised as a means of increasing the nutritive value of a vegetarian diet. Precise knowledge of the composition of this food group is needed. The supplemental value of nuts to a dietary pattern characteristic of a vegetarian population merits investigation.

Rice forms the staple food of a large section of the Indian people. Sample surveys of food intake of the rice eating sections in different parts of India revealed that the pattern of consumption of the rice diet was very similar in all parts of the country (Aykroyd, 1940). In addition to rice, very small amounts of pulses, vegetables, fruit, milk and meat were included in the diets of the low income groups. In a series of experiments by Aykroyd and collaborators (1937-1940), it was demonstrated that milk formed a most effective supplement to this diet. Because of the difficulties of producing milk at a low cost in India, and because of the non-acceptability of proteins of animal origin by sections of the Indian people, the investigators tried a number of vegetable proteins to improve the basic rice diet. It was found that soya beans, pulses, peanut milk in varying amounts did not form a satisfactory supplement to the diet. No work has been performed on the supplementary value of almonds, pecans, cashews and English walnuts to the rice diet although nuts are grown in India and eaten when available.

It was the purpose of this study to investigate the essential amino acids and total protein content of certain nuts and to study the supplementary value of varying amounts of these nuts to a basal diet, the pattern of which was similar in all essentials to the rice diet of low income groups in India.

## REVIEW OF LITERATURE

### Evaluation of the Proteins of Almonds, Cashews, Pecans and English Walnuts

**Digestibility.**

Jaffa (1901, 1903) found the digestibility of nut proteins to be satisfactory. Diet studies were conducted on California fruitarians and nutarians who had lived for many years on diets consisting mainly of nuts and fruits. A few university students, who assumed the nutarian diet during the experimental period also were studied. A careful analysis of the proximate composition of several fruits and nuts was made. Among the nuts studied were almond, pecan, English walnut, Brazil nut, peanut and coconut. A balance period lasted usually for four days. On an exclusively fruitarian diet, which consisted of two types of fruits as sources of carbohydrates and one nut as source of protein and fat, the coefficient of digestibility varied from 70 to 82 per cent. On a mixed diet containing 13 to 29 gm. of protein from nuts per day per person, Jaffa reported a protein utilization of 90 per cent.

Cajori (1918) supported Jaffa's conclusions regarding the utilization of nut proteins, when fed in mixed diets, where almond, pecan, English walnut, lichi or the Brazil nut formed the sole source of protein. Generally the nut pastes (pulverized whole nuts) were better utilized by men than when whole nuts formed part of the diet. Cooking caused no marked change in the digestibility of the nuts. In the case of almonds, the thoroughly roasted nuts showed no advantage over the boiled or the raw product. The utilization of the digestible carbohydrates determined as soluble reducing sugars after hydrolysis with two per cent hydrochloric acid appeared to be excellent in diets containing the various nuts.

Mitchell and Beadles (1937) recorded the digestibility of the cashew nut, almond and English walnut as 96, 94, and 84 per cent respectively.

Studies of growth.

Cajori (1920) tested the value of the proteins of the almond, the English walnut, filbert, pecan and pine nut by the rat growth method. The shelled nuts were passed through a meat grinder and subjected to pressure in a hand press. The process removed considerable quantities of oil from the nut and the nitrogen determination of the residual press cake indicated that the protein content had been sufficiently concentrated to incorporate the press cake in the diet at the desired level of 18 per cent of protein. The experiment was conducted from 10 to 18 weeks and in each group there were 10 to 32 rats. Growth curves were drawn and compared with the rat growth experiment reported by Osborne and Mendel (1915) on a diet containing 18 per cent of casein. Growth was comparable to that on the casein diet in the case of almond, English walnut, filbert and pine nut diets, but the growth rate on the pecan diet was poor.

In this investigation nut diets furnished an opportunity to test the efficiency of the dietary protein for milk production. The mother rats were able to nurse young successfully on diets in which almond, English walnut, pine nut, and the filbert were the sole source of protein.

In 1921 Cajori removed the skins of pecans which contain tannin, and the nuts were dried and pressed through a meat grinder and subjected to pressure to remove enough oil to obtain a press cake of sufficiently high protein content to provide a diet with 18 per cent nut protein. Five young rats grew normally on the diet of treated pecans. Thus the presence of tannin in pecan skin was shown to be the limiting factor for the growth of the rat.

Mignon (1923) demonstrated the adequacy of the walnut globulin as the sole source of dietary protein for white mice, and mixed walnut protein for normal development of both rats and mice. Preliminary experiments indicated that the tannin in the skin of the nut was injurious to the animals. The skin was removed by hand, using hot water as the lye method employed by Cajori (1921) to remove pecan skin was found unsatisfactory. The blanched nuts were ground in a meat grinder, transferred to a muslin bag, and as much oil as possible was pressed out by hand using the ordinary type of hand press. The resulting cake was fat extracted. The fat extracted material contained 45 per cent protein.

Before experimenting with the walnut globulin as the sole source of protein, two rats and three mice were fed on a diet in which walnut press cake was the sole source of protein, incorporated to form 18 per cent protein of a diet adequate in all other respects. The animals consumed the ration well and even when nut protein formed only 12 per cent of the diet, rat growth was entirely satisfactory. On a diet containing walnut globulin alone in an amount providing 18 per cent of protein to the diet, five mice grew normally.

Lane (1931, 1936) reported that almond milk, a vegetable milk prepared to resemble human milk in composition and consisting mostly of protein from almonds, gave satisfactory results in infant feeding. She reared a pair of twins, children of a young woman, whose diet for many years was mainly vegetarian, on almond milk. The twins were started on this milk when they were three months old. During the entire nursing period, there was no sour stomach or regurgitation as is so frequent with cow's milk formulae. The strength and endurance of the children seemed to be above that of the average child. The teeth erupted at the normal time and were healthy. When the children were eight months old, a pediatrician evaluated them as being normally healthy, rickets-free children. When two years old, they weighed 29 and 30 pounds, the height of the boy was 33 5/8 inches and the girl 33 1/2 inches. Their health and development in all its phases was satisfactory. Growth was followed until five years later, when almonds still furnished a good portion of the diet which was entirely vegetarian. Growth was normal. Lane reported that case studies of adults and children receiving almond milk appeared to prove that the human organism can use a generous daily quantity of almonds to advantage for indefinite periods.

Biological value.

Mitchell, Burroughs and Beadle (1936) in their extensive research on the significance and the accuracy of the biological values of protein computed from nitrogen metabolism data, have made an interesting comparison of beef proteins and pecan proteins. They used rats as the experimental animals.

Preliminary digestion experiments indicated a marked difference in the digestibility of the two proteins. Beef protein was completely digested and whole pecan protein was digested to the extent of 70 per cent only. Hence the experimental rations were made up to contain the same percentage of digestible protein, which formed 9.4 per cent of the diet. The investigators found the biological value averaged 75 for beef protein, and 60 for pecan protein.

The above values indicated that 1.33 gm. of digestible nitrogen from beef covered the body's requirement for one gm. of nitrogen, while for the same purpose 1.67 gm. of nitrogen from the pecan were required. Hence, one gm. of absorbed beef nitrogen was the nutritive equivalent of 1.26 gm. of absorbed pecan protein.

When the authors used the paired feeding method for studying growth of rats, eight pairs of rats weighing from 50 to 58 gm. were investigated and the nutritive equivalence of nitrogen of beef round to that of pecan protein was as one is to 1.24, almost the same result as was obtained by the nitrogen balance method.

Mitchell and Beadle (1937) found by using the nitrogen balance technique with rats that the cashews had a biological value of 73 which was higher than that estimated for the almond, filbert, or English walnut. The nuts were analyzed in the routine way. The experimental rations contained approximately 9.2 per cent of protein, 22 per cent of fat, five per cent of Wessen salt mixture as modified by Osborne and Mendel, one per cent of sodium chloride, and 10 per cent of sucrose. The fat was provided by the test sample, butter fat and a lard-cod liver oil mixture in the ratio of four to one to make 22 per cent of total fat. Vitamins B and G were provided by one per cent of dried yeast and Harris vitamin powder given in separate small dishes at the rate of five mg. per gm. of food. Cashew nut, almond and English walnuts were found to have biological values of 73, 51, 56 respectively.

Ujsaghy (1940) found that in healthy infants fed almond milk, the absorption of nitrogen was satisfactory, but the biological value of almond milk while equal to that of cow's milk was slightly inferior to that of human milk.

Campous (1946) reported that the proteins of cashew nuts had high biological value. Roasted cashew nuts forming 40 per cent of the diet produced good growth in rats and corrected the deficiency due to a poor protein derived from dried peas. The biological value of cashew protein was found to be 77.2 per cent.

## Other Nutrients Found in Nuts

Levine (1932) found the pecan nut to be a good source of vitamin A. It was estimated that the pecan nut sample used in rat feeding experiments had a vitamin A potency of approximately 3.6 units per gram of nut, one unit of A according to the definition of Sherman and Smith (1931) being the amount which when fed daily to a standard test animal would suffice to support an average gain in body weight of three grams per week during the test period of four to eight weeks.

De Caro and Franceschini (1939) assayed the vitamin A content of several nuts by biological tests on rats depleted of vitamin A. The vitamin A value expressed in I.U. per gm. of material was, for fresh walnut 1.2, dried walnut 1.3, peanut 3.6, hazel nut 4.4, and almond 5.8.

The vitamin $B_1$ content was estimated by the same authors. The minimum daily dose of test material, required to secure the survival for 60 days of young rats deprived of vitamin $B_1$, was 2.82 micrograms per gm. of walnuts and 2.8, 1.2 and 2.0 micrograms per gram of hazelnuts, almonds and pecans respectively.

Pyke, Melville and Sarsan (1942) found unripe walnuts to be a rich source of vitamin C containing 410-1800 mg. per cent. The dry mature nuts retained only a trace of this element. Most methods of preparing sweet confections from the unripe nut destroyed all the ascorbic acid but if the unripe nut was heated in water and pickled in vinegar or made into jam most of the vitamin C was retained.

Everson, et al. (1948) found the riboflavin in almonds was not as readily available as the riboflavin of ice cream. Eight women subjects consumed a basal diet supplying approximately 2.4 mg. riboflavin per day. Their urinary excretion of riboflavin was surprisingly constant. When the weighed amount of basal diet was supplemented with one mg. of pure riboflavin, an average of 42 per cent of the test dose was recovered in the urine. When a supplement of one mg. of riboflavin was supplied in the form of ice cream, the urinary excretion of riboflavin was comparable to that produced by the pure vitamin. This was taken as indicating that riboflavin of ice cream was as available as that of the pure vitamin. When either frozen peas or almonds furnished one mg. of riboflavin as a supplement, much less riboflavin was excreted in the urine. It was thought that these differences might be due to variations in the absorption of the vitamin.

Jentsch and Morgan (1949) assayed the riboflavin, thiamin, and niacin content of four varieties of walnuts. Thiamin was assayed by the thiochrome method and by rat growth assay. The values obtained from the latter method were 20 to 30 per cent larger than those obtained by the thiochrome procedure. The three varieties had an average of 37 mg. per cent thiamin by the thiochrome method. The riboflavin content of the walnut samples determined by the microbiological method was found to be 20 per cent less than the values obtained by the fluorometric method which gave an average of 0.148 mg. per cent riboflavin.

Niacin was determined microbiologically and gave an average value of 0.71 mg. per cent niacin.

Peterson, Skinner and Strong (1943) reported the percentage of major mineral elements in almonds to be as follows: calcium, 0.228; magnesium, 0.275; potassium, 0.756; sodium 0.024; phosphorus, 0.465; chlorine, 0.037; sulphur, 0.164.

Almonds, cashews, pecans and English walnuts would appear to be valuable sources of vegetable protein. The evidence is that the protein is readily digestible and supports satisfactory growth and nitrogen retention. Few studies have been made with whole nuts. Some form of press cake or protein isolated from nuts has been used in most of the studies recorded.

## Evaluation of Rice Proteins

### <u>Studies of growth</u>.

Kik (1940) compared the growth promoting value of proteins of whole rice and polished rice at 5.5 per cent protein in the diet, using the paired method of feeding rats. The experiment lasted for 130 days. In all the nine pairs of rats fed whole rice, the animals utilized their food better than those fed polished rice. The average gain in weight per gm. of protein intake was 1.8 gm. for the animals fed the whole rice ration and 1.66 gm. for those fed the polished rice ration. The difference between the two weight gains was statistically significant.

The growth promoting value of rice bran and rice polish also were studied at an intake of eight per cent protein. The experiment lasted for 112 days. In all nine pairs of the rats, less nitrogen from rice polish was needed to promote the same gain in weight. The average gain in weight per gm. of protein consumed was 1.79 gm. for the animals fed the rice polish ration and 1.48 gm. for those fed the rice bran ration. The difference of 0.31 gm. was statistically significant.

In further paired feeding experiments, Kik (1940) showed that additions of 25 mg. of cystine, methionine or lysine daily supplemented the proteins of whole rice and polished rice to a slight extent. The addition of tryptophane did not show any beneficial effect.

Sure (1946) found the growth promoting efficiency of rice protein was greater than that of wheat. Thirty rats were given a diet adequate in all respects containing 5.8 per cent of protein from polished rice. The average gain in weight per gm. of protein was 1.86 gm. while on the same plane of protein nutrition the average gain per gram of protein of whole wheat flour was only 0.72 gm.

Substitution of one, three and five per cent of polished rice with equivalent amounts of strain G yeast increased the growth rates of rats by 41 to 69 per cent (Sure, 1947).

Jones, Caldwell and Widness (1948) compared the growth promoting value of the proteins of whole rice, polished rice, yellow corn, hard and soft wheat, pearled barley, rye and rolled oats. The experiments were performed on rats for six weeks. The animals were fed ad libitum. At an intake of 7.5 per cent protein, brown rice gave the best growth in rats. However, at an intake of 4.5 per cent protein, brown rice produced less growth than polished rice. Oats and rye produced slightly greater growth than polished rice.

<u>Biological value.</u>

Rice protein has a satisfactory biological value compared to the proteins of other cereals. Employing the nitrogen balance method on rats, Mitchell (1924) observed that whole rice proteins had an average biological value of 86.1 per cent, on a diet supplying five per cent of protein. At the same protein intake, the biological values of corn and oat proteins were found to be inferior to rice protein, the value for corn being 72.0 and for oats 78.6 per cent.

Basu and Basak (1947) investigated the biological value of the Aman and Aus varieties of Bengal rice and found both varieties had a biological value of 80.0 per cent. Parboiling did not affect the biological value of either variety.

Kik (1939) reported the biological value of whole rice to be 73 per cent on a diet supplying five per cent of protein. This value was lower than that observed by Mitchell (1924) and Basu and Basak (1937) for whole rice. On diets containing five per cent of protein, Kik recorded the biological value for polished rice as 66.6, rice bran as 84.9 and rice polish as 82.9 per cent. At a protein intake of eight per cent rice bran and rice polish had lower biological values. This was thought to be due to the fact that the economy with which amino acids were used decreased as the supply increased.

Sure and House (1948) demonstrated that the biological values of milled and whole rice proteins were superior to those of other cereals. At an intake of five per cent of protein, the biological value of milled rice was 75.1, wheat 60.0, rye 63.1 and corn 32. The findings for whole grains were rice 80.0, wheat 76.1, rye 73.2, corn 78.8 and rolled oats 75.6 per cent.

Amino acid content.

Kik (1941) determined the cystine, tryptophane, lysine, arginine and histidine content of whole rice, polished rice, rice bran and rice polish by chemical methods. The amino acid content compared favorably with that for corn and wheat. In comparison with casein, the rice proteins contained less tryptophane, lysine and histidine.

Five varieties of rice, Arkansas 155, Shoemed, Acadia, Zenith and Fortune, were assayed for the above five amino acids. Considerable variation was noted in the amino acid content of these varieties.

Mitchell and Block (1946) calculated the percentage deviations of the content of essential amino acids of white rice protein from the concentration of the corresponding amino acids found in whole egg protein, both standardized to a nitrogen content of 16 per cent. Lysine was the amino acid most deficient in white rice, and probably limited the nutritional value of the rice for maintenance and growth of the laboratory rat.

Lyman and Kuiken (1949) found that unpolished rice had 6.39 per cent crude protein. The percentage of 10 essential amino acids in the sample was as follows: arginine, 0.54; histidine, 0.14; isoleucine, 0.28; leucine, 0.51; lysine, 0.28; methionine, 0.14; phenylalanine, 0.31; threonine, 0.22; tryptophane, 0.10; valine, 0.40. The amino acid composition of polished rice was comparable to that of wheat.

Pecora and Hundley (1951) observed that the addition of two amino acids, lysine and threonine, produced a growth response in white rats three times greater than that obtained with an unsupplemented diet. Neither lysine, nor threonine, nor any other essential amino acid added to rice individually produced better growth than rice alone.

A threefold excess of arginine or methionine when added to rice completely supplemented with all other essential amino acids produced a significant growth depression.

## The Vitamins Found in Rice

Thiamin.

Eijkman (1897) reported that an illness of fowls similar to beriberi in man was produced by feeding fowls with polished rice. This illness was prevented or cured by an extract of rice polishings. The active principle was isolated as vitamin $B_1$ and later characterized as thiamin (Sherman, 1946). Since that time, the antineuritic vitamin content of rice has been of special interest to nutritionists.

Fraser and Stanton (1910) investigated the relationship between the degree of milling to which different kinds of rice had been subjected and the capacity to protect human beings from beriberi and birds from polyneuritis. A convenient way to estimate the vitamin $B_1$ content was to measure the amount of germ and pericarp present. Most of the phosphate present in rice was to be found in the embryo and the integuments. The phosphate content estimated as $P_2O_5$, depended on the degree of milling of rice and was suggested as a useful index of the vitamin $B_1$ value. The ingestion of unmilled rice or parboiled rice, which had a phosphate content of 0.4 per cent was never associated with beriberi in humans. The investigators found that polished rice contained less than 0.4 per cent $P_2O_5$ and such rice was likely to cause beriberi.

McCarrison and Norris (1924) observed that various samples of parboiled rice even when milled to a high degree protected pigeons from polyneuritis when fed to the birds as the sole source of vitamin $B_1$.

Aykroyd (1932) estimated the vitamin $B_1$ potency of a number of rice samples, raw as well as parboiled, by the rat growth method. Groups of rats were given a basal diet deficient in vitamin $B_1$. The rats increased in weight for two to three weeks, after which growth ceased. When the weight was stationary for about one week, the test material was fed either in the form of separate daily additions to the basal diet, or the basal diet was modified to include a definite proportion of the substance under examination. The increase in weight was observed over a further test period of four weeks. The vitamin $B_1$ value of different rice samples was estimated and compared by observing the average weekly gain in weight corresponding to the various daily doses of rice in the basal diet. The vitamin $B_1$ content of unmilled samples of raw and parboiled rice was equal, but when each was milled to an equal degree, the parboiled sample retained the vitamin, while the raw sample did not.

The estimation of the $P_2O_5$ content of rice seemed to serve as an index of the vitamin $B_1$ value of raw rice. This investigation supported the suggestion of Fraser and Stanton (1910) that raw rice, containing less than 0.4 per cent of $P_2O_5$ was apt to be low in vitamin $B_1$ and gave rise to beriberi. The phosphate content of parboiled rice, however, was no guide to the vitamin $B_1$ value, since percentages as low as 0.28 might be present in the samples which contained an abundance of the vitamin.

The investigator was of the opinion that during the parboiling process, when paddy was soaked in water for 24 to 36 hours, subjected to steam at atmospheric pressure for 15 to 20 minutes and then dried and hulled, the antineuritic vitamin was transferred to the inside of the grain and thus not lost to it.

The quantitative estimations of the thiamin content of brown, white and parboiled rice as reported by various investigators have been presented in Table 1. Callieau, Kidder and Morgan (1945) and Miller (1945) determined thiamin in rice by biological assay. All the other investigators assayed thiamin by the thiochrome method.

The distribution of thiamin in the rice was examined by Hinton (1947) by dissecting the various layers and examining them separately. He found 44 to 50 per cent of the total thiamin in the scutellum, 34 to 35 per cent in the pericarp aleurone layers and in the covering of the germ, seven to nine per cent in the endosperm and nine to twelve per cent in the remaining parts of the embryo. He noted that the parboiling process led to the redistribution of thiamin. The endosperm was enriched at the expense of the embryo. There was a marked retention of scutellum in parboiled rice as compared with raw milled rice, due largely to the gelatinization of the endosperm immediately below the germ. This acted as a cementing tissue so that the germ was not so readily knocked out of the grain during subsequent milling.

Simpson (1951) studied the distribution of thiamin and riboflavin in rice by the simple and ingenious photographic method first developed

TABLE 1

Thiamin content of brown, white and parboiled rice

| Investigator | Type of rice | | |
|---|---|---|---|
| | Brown | White | Parboiled |
| | ug/gm | ug/gm | ug/gm |
| Aykroyd, et al. (1940) | 2.90 | 1.00 | 2.40 |
| Swaminathan (1942) | -- | 1.00 | 2.10 |
| Kik (1923) | 3.00 | 0.60 | 1.66 |
| Williams, Knox and Fieger (1943) | 4.20 | 0.80 | -- |
| Calliaeu, Kidder and Morgan (1945) | 3.80 | 0.90 | 2.20 |
| Miller (1945) | 2.10-2.98 | -- | -- |
| Kik and Williams (1945) | 3.55 | 0.84 | 1.35-1.74<br>2.50* |
| Jones, et al. (1946) | 3.71 | -- | 2.00-2.30 |
| Simpson (1951) | 3.84 | 0.60 | 2.03 |

* Converted rice

by Somers, Coolidge and Haworth (1945) for locating these vitamins in wheat kernels. Appropriate filter combinations to record specifically the fluorescence excited by thiochrome and riboflavin, when illuminated by ultra-violet radiation were used in this investigation. In all cases, the maximum amount of thiamin occurred in the scutellum and in the cells of the endosperm adjacent to the embryo. The remainder of the embryo other than the scutellum did not appear to be rich in thiamin, but riboflavin seemed more evenly distributed throughout the tissues of the embryo than in the case of thiamin.

This work brought visual confirmation of the fact that a considerable portion of the thiamin of the scutellum was absorbed into the endosperm during the parboiling process and would remain in the endosperm, even after the parboiled rice was highly milled.

Riboflavin, niacin and other B vitamins.

The riboflavin content of brown, white and parboiled rice as determined by investigators by fluorometric analysis has been presented in Table 2.

The niacin content reported by two groups of investigators has been given in Table 3.

Williams, Knox and Fieger (1943, 1944) determined the pantothenic acid, pyridoxine and biotin content of three typical Louisiana varieties of rice. Pantothenic acid and biotin were assayed microbiologically and pyridoxine by Scudi's (1942) colorimetric method. The average content of these vitamins is presented in Table 4.

## TABLE 2

**Riboflavin content of brown, white and parboiled rice**

| Investigator | Type of rice | | |
|---|---|---|---|
| | Brown | White | Parboiled |
| | ug/gm | ug/gm | ug/gm |
| Kik and Landingham (1943) | 0.53 | 0.26 | 0.38 |
| Williams, and Fieger (1944) | 0.61 | 0.24 | -- |
| Kik and Williams (1945) | 0.60 | 0.27 | 0.37<br>0.25* |

* Converted rice

## TABLE 3

**Niacin content in brown, white and parboiled rice**

| | Type of rice | | | |
|---|---|---|---|---|
| Investigator | Brown | White | Parboiled | Method |
| | ug/gm | ug/gm | ug/gm | |
| Williams, Knox and Feiger (1943) | 47.2 | 12.7 | -- | Microbiological |
| Kik and Williams (1945) | 53.08 | 19.62 | 46.4 | Calorimetric method of |
| | | | 32.5* | Melnick (1942) |

* Converted rice

TABLE 4

The average content of pyridoxine, pantothenic acid and biotin in brown and white rice[1]

| Type of Rice | Pyridoxine | Pantothenic | Biotin |
|---|---|---|---|
| | ug/gm | ug/gm | ug/gm |
| Brown | 17.00 | 10.30 | 0.12 |
| White | 6.40 | 4.50 | 0.04 |

[1] Williams, Knox and Fieger (1943, 1944).

**Fat soluble vitamins, A, E and D.**

Todd et al. (1937) obtained a complex mixture of oily crystalline esters by acetylation of a purified concentrate from the unsaponifiable fraction from the rice germ oil. The crystalline esters on separation and hydrolysis yielded three approximately homogenous crystalline isomeric alcohols, which were biologically inactive. The purified oils remaining after the removal of alcohols from rice germ oil had high vitamin E activity.

Kik (1942) reported that rice and its by products were deficient in vitamins A and D. Polished rice, whole rice, rice polish and rice bran were investigated using young albino rats. Groups of six animals were fed the foodstuffs alone. Addition of one drop of viosterol daily was followed by improvement in growth. After a maintenance curve was reached, a further growth response was obtained after administration of four drops of cod liver oil daily, which furnished vitamin A and additional amounts of D as well. The results showed that rice needed fortification with vitamins A and D to produce good growth.

## Loss of B Vitamins in Washing and Cooking Rice

Aykroyd et al. (1940) reported the average losses of thiamin and niacin during washing and cooking of raw milled rice were from 40 to 50 per cent. Swaminathan (1941, 1942) estimated that raw rice lost 60 per cent of its thiamin and niacin content and parboiled rice eight per cent of the thiamin and 12 per cent of niacin during washing.

An additional 25 per cent of the thiamin in both raw and parboiled rice was dissolved into the cooking water. Cooked parboiled rice even when the cooking water was discarded, contained 1.4 ug. per gram of thiamin.

Miller (1945) determined the loss of thiamin as a result of washing and cooking brown rice and partially polished rice. Unwashed brown rice was cooked for 50 minutes and brown rice was washed five times and cooked in a small amount of water in exactly the same manner as the unwashed sample. Unwashed partially polished rice was cooked in a small amount of water for 40 minutes. A sample of the same rice was washed five times and cooked in the same way as the unwashed partially polished rice. The different types of cooked rice were fed to rats as the sole source of thiamin. As a result of washing and cooking rice, there was an apparent loss of 12 per cent of thiamin. Statistical analysis of the difference between the mean gains of the rats fed washed and unwashed brown rice showed unimportant differences. The differences between the mean gains of the groups of rats fed washed and unwashed partially polished rice appeared to be significant and the apparent loss of thiamin was about 20 per cent. No measure of statistical significance was given by these authors.

Kik and Williams (1945) performed washing experiments on brown, white and converted rice and reported the cooking losses of riboflavin, niacin and thiamin during these processes. One cupful of rice was washed twice in a cupful of water. Brown rice so washed lost 20.1 per cent of its thiamin, 7.7 per cent of riboflavin and 23.0 per cent of niacin. White rice lost 43.0 per cent of thiamin, 25.9 per cent of riboflavin and 23.0 per cent of niacin. Converted rice lost 6.5 per cent of thiamin, 10.5 per cent riboflavin and 16.0 per cent niacin. Converted rice had a lesser loss of vitamins during washing. This confirmed an observation made by Swaminathan (1943).

The investigators cooked rice samples by the double boiler and the open kettle method. One cup of rice and one and one half cups of boiling water were placed in the top of the double boiler and cooked until all the water was absorbed in the cooked rice. In open vessel cooking, one half cup of rice was added to eight cups of boiling water, the rice was cooked, transferred to a colander and drained. The vitamin determination on cooked rice showed that all types of rice lost an average of 4.3 per cent of thiamin in the double boiler method compared to 46.9 per cent when cooked in the open vessel.

The average losses of riboflavin and niacin using the double boiler type of cooking were 6.7 per cent and 3.4 per cent respectively versus 43 per cent for riboflavin and 44.8 per cent for niacin when the open vessel type of cooking was used. The investigators expressed their opinion that all vitamins present in rice before cooking could be saved when the method of cooking used the minimum amount of cooking water and the rice was not rinsed after cooking.

Brown rice contains appreciable quantities of the B vitamins, especially thiamin and niacin. Milling rice reduces its vitamin content while parboiling conserves it. Excessive washing and careless cooking was detrimental to the retention of B vitamins in cooked rice.

## Supplements to Indian Rice Diets

Since McCarrison's (1936) animal experiments revealed the nutritional inadequacy of Indian rice diets, several investigations have been carried on to improve the rice diets of low income groups by appropriate supplements. Aykroyd and Krishnan (1937, 1939, 1940) fed rats with rice diets which closely resembled the diets actually consumed by poor rice eaters in various parts of India. On these diets young rats showed very poor growth, an average increase in weight of three to four gm. per week. The addition of milk greatly improved the nutritive value of the diet for rats. The general condition of the animals improved and the weight gains doubled or trebled on the milk supplemented diet.

Yeast also was an effective supplement to the rice diet. Meat and eggs had some supplementary value but less than that of milk. Soya beans and pulses in varying quantities did not form an effective supplement. The most striking observation of the investigators was that most of the supplemental value of milk could be produced by a calcium salt. The addition of calcium lactate or calcium phosphate to the basal diet improved the growth rate considerably. Parallel experiments with undernourished children corroborated these results. A daily intake of 0.5 to one gm. of calcium lactate or 28 to 48 gm. of skimmed milk powder produced an acceleration of growth in malnourished children. The investigators were of the opinion that the rice diets were not so much deficient in protein, as rice proteins were of high biological value but tended to be very deficient in calcium and vitamin A concentration.

Ranganath and Rau (1938) confirmed Aykroyd and Krishnan's (1937) results concerning the improvement of a rice diet by the addition of calcium salts. They added two per cent calcium carbonate to the rice diet characteristic of the Madras area and observed the apparent improvement of the biological value of the protein of this diet.

Deshikachar (1948) reported milk prepared from ground nuts had no supplemental value to the rice diet. This was surmised to be mainly due to the fact that ground nut milk was deficient in calcium.

Moorjani, et al. (1950) investigated the supplemental effect of ground nut milk fortified with calcium and blended with germinated soya bean to the rice diet. The growth rate of rats was doubled on this supplement.

The nutritive value of the Indian rice diets was improved considerably by the addition of milk or calcium salts to the diets. This improvement was thought to be mainly due to the increase of the ratio of calcium to phosphorus in the diet. The ratio of calcium to phosphorus in rice was approximately as one is to ten, which was thought to be unfavorable for maximum availability and utilization of phosphorus. Supplements in the form of milk or as calcium salts appeared to favor phosphorus assimilation and thus increased the nutritive value of rice diets.

## EXPERIMENTAL PROCEDURE

### Proximate Analysis of Nuts and Rice

The proximate analysis of nuts and rice was made as a knowledge of the major food constituents was necessary in order to prepare the test diets for biological assay.

<u>Moisture determination.</u>

Moisture was determined by drying the foods at 70° C. with the aid of a Brabender moisture tester. The nuts were coarsely powdered in a Waring blender and rice was ground with a pestle and mortar. Triplicate samples of 10 gm. were weighed into the pans of the instrument. Percentage moisture was read directly at intervals of one half an hour. When the difference between two readings was within 0.05 per cent, the final reading was taken as the percentage moisture of the sample.

<u>Ether extract.</u>

The moisture free samples were fat extracted in a Soxhlet extractor using ethyl ether. Fat extracting thimbles were first extracted for one half an hour and after all trace of ether had evaporated, were placed in a desiccator. Triplicate samples of 9.5 to 10 gm. of moisture free samples were weighed into the dry fat extracted thimbles and fat extracted until the samples reached constant weight.

Crude protein.

Nitrogen was assayed on duplicate samples of one gm. of the fat extracted materials by the Kjeldahl method. The quantity of nitrogen was multiplied by the factor 6.25 to express the crude protein concentration of the samples.

Ash determination.

Ash was determined on powdered whole nut and rice samples. Duplicate samples of 0.5 to one gm. were weighed into ashing dishes and placed in a muffle furnace. The temperature of the furnace was raised gradually to 525° C. in order to prevent rapid burning of the materials. The samples were left in the furnace at 525° C. until a white ash of constant weight was obtained.

Carbohydrate determination.

Carbohydrate content was obtained by difference. The percentage of water, ether extract, crude protein and ash were added and the difference of this sum from 100, was taken as the carbohydrate content of the samples. Carbohydrate by difference included available and non-available carbohydrates and any organic acids present.

Gross energy.

The gross energy value of nuts and rice was determined by the aid of a bomb calorimeter.

## Microbiological Assay of Ten Essential Amino Acids in Almonds, Cashews, Pecans and English Walnuts

It was planned to study the essential amino acid content of the nuts as this would give an indication of the nutritional value of nut protein. However, it was necessary first to determine the effect of the high percentage of fat in the nuts on the growth of the micro-organism. Therefore, a preliminary experiment was carried out in which the methionine content of fat extracted and non-fat extracted nuts was determined. The results of this experiment have been presented in Table 5. Slightly higher values were consistently found in fat extracted samples. Therefore, the fat extracted nuts were used for the analysis of amino acids.

Preparation of hydrolysates for amino acid assays.

Acid hydrolysates for the assay of arginine, histidine, methionine, threonine, lysine, leucine, isoleucine, valine and phenylalanine were made by adding 100 ml. of 2N hydrochloric acid to 1.5 gm. of each fat extracted sample of nuts in an Erlenmeyer flask.[1] The recovery of added amino acids was studied by treating a sample of defatted cashew nuts as follows. A hydrolysate was prepared by adding to the above mixture of nut and acid, two ml. of DL-phenylalanine, two ml. L-histidine hydrochloride solutions and four ml. each of solutions of DL-Valine, L-Leucine, DL-isoleucine, DL-threonine, DL-methionine, L-arginine hydrochloride and L-lysine hydrochloride.

TABLE 5

Methionine content of fat extracted and whole nuts

| Method | Type of nut | | | |
|---|---|---|---|---|
| | Walnut | Almond | Cashew | Pecan |
| | mg/gm | mg/gm | mg/gm | mg/gm |
| Fat extracted samples | 1.719 | 1.900 | 3.176 | 1.536 |
| Whole nut sample | 1.679 | 1.632 | 2.716 | 1.453 |

The solution of amino acids used each contained 10 to 40 mg. of the material or its hydrochloride. The flasks were plugged with glass wool and autoclaved for five hours at fifteen pounds pressure (Schweigert, et al. 1946). The hydrolysates were cooled to room temperature, filtered, made to volume with distilled water and stored in screw-top bottles in the refrigerator.

Alkaline hydrolysis was employed for the assay of tryptophane, using cysteine as a stabilizing agent (Kuiken, Lyman and Hale, 1947). Two hundred mg. portions of L-Cysteine were weighed into five alkaline resistant Erlenmeyer flasks. Into each flask, 32 ml. of 4N sodium hydroxide were added. The flasks were stoppered with cotton and autoclaved at 15 pounds pressure for one hour. While the solutions were still hot, another 200 mg. portion of L-Cysteine and 0.4 gm. of the sample were added to each flask. Duplicate samples of almonds were prepared and to one sample of almond 2.5 ml. of tryptophane solution containing 12 to 15 mg. of DL-tryptophane were added for the purpose of determining the percentage recovery of the amino acid. The mixtures in the five flasks were autoclaved for fourteen hours at fifteen pounds pressure. Each sample was transferred to a 100 ml. volumetric flask, while still hot, using warm rinse water. When cool the samples were diluted to volume and stored in screw top bottles in the refrigerator.

Organisms used.

*Leuconostoc mesenteroides* P-60 was used for lysine assay.

*Lactobacillus arabinosus* 17-5 was utilized in the assay of isoleucine, leucine, phenylalanine and valine.

*Streptococcus faecalis* R was used for the assay of arginine, histidine, methionine, threonine and tryptophane.

Basal medium.

For the assay for all amino acids except methionine, the medium of Schweigert, et al. reported in 1949 was employed. For the determination of methionine, a medium was used which was a combination of that of Schweigert, et al. (1949) and Lyman, et al. (1946), in which the amino acids were replaced by hydrogen peroxide treated peptone, cystine, tyrosine and tryptophane. Mills (1951) reported the composition of the medium for methionine as employed in this laboratory.

Assay method.

The method was essentially that of Sauberlich and Bauman (1946) with slight modifications as routinely followed in this laboratory (Ingalls, et al. 1950).

The organisms were maintained on stab cultures of yeast extract-dextrose-agar. Transfers were made weekly and incubated at 37° C. for 48 hours. Inoculums for the assay tubes were made by transferring cells of the organism from stab cultures to tubes containing five ml.

of the complete basal media plus five ml. of water. This liquid culture was incubated for 24 hours at 37°C. and then stored in the refrigerator. Inoculum, which was over five days old was discarded. At the time of inoculation, the inoculum was centrifuged and the supernatant liquid discarded. The cells were suspended in 20 ml. of 0.9 sterile saline solution. One drop of this suspension was used to inoculate each assay tube.

Assays were carried out in pyrex culture tubes and in a total volume of two ml. Graded amounts of the amino acid to be assayed were added to a series of tubes in order to establish a standard curve. The pH of the standard solutions of amino acids from which the dilutions were obtained was adjusted to suit the needs of the assay organism. For streptococcus faecalis R the pH was adjusted to 8.4 to 8.6 with thymol blue as indicator and for Lactobacillus arabinosus 17-5 and Leuconostoc mesenteroides P-60 the pH was adjusted to 6.6 to 6.8 with brom thymol blue as indicator.

Twenty-four tubes were used to establish the standard curve; triplicate tubes were employed for the first six dilutions and duplicate tubes for the three highest dilutions. Water was added to each tube to bring the volume to one ml.

Aliquots of the sample hydrolysates were taken and further diluted to a suitable volume after adjusting to the desired pH. Duplicate tubes at dilutions of 0.3, 0.6 and one ml. respectively of the sample were prepared. Water was added to bring the total volume to one ml. in each tube.

The basal medium to be used was mixed omitting the amino acid to be assayed. The pH of the medium was suitably adjusted before diluting the solution to 100 ml. volume. One ml. of the medium was added to all tubes to bring the total volume to two ml.

The racks of tubes were covered with heavy towelling and autoclaved for eight minutes at 20 pounds pressure. The cooled tubes were inoculated with one drop of inoculum, suspended in sterile saline solution and incubated in a water bath for 72 hours at 37° C.

At the end of the incubation period, the growth response of the test organisms was measured by titrating the lactic acid produced against 0.05 N sodium hydroxide.[1] Brom thymol blue was used as indicator for tests using Lactobacillus arabinosus 17-5 and Leuconostoc mesenteroides P-60 as the test organisms, and thymol blue for those using Streptococcus faecalis R. During titration, air was blown into the tubes to provide complete mixing.

Standard curves were drawn by plotting the milliters of 0.05 N sodium hydroxide used in titration against the micrograms of standard amino acid. The amount of amino acid in the samples was obtained by interpolation from this standard curve. Typical standard curves obtained for the ten essential amino acids have been shown in Figures 1 to 10 in the Appendix.

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[1] The 0.05 N sodium hydroxide was standardized against a standard solution of potassium acid phthelate.

The recoveries of the added amino acids were usually within $\pm$ 10 per cent, Table i, Appendix. However in the case of tryptophane the recoveries were consistently low. Possibly there was a slight destruction of tryptophane during the preparation of the alkaline hydrolysate, even though cysteine was used as a stabilizer, which according to Kuiken, Lyman and Hale (1947) should completely prevent destruction of tryptophane under these conditions. Recoveries in the case of threonine were found to be consistently high. The reason for this is not clear.

## Growth Experiment on Rats

A growth study was conducted for a four week period on young female albino rats, weighing 55 to 75 gm. to test the growth promoting value of nut supplements to a rice diet. The rice diet, which was used as a negative control diet was similar to Aykroyd's (1940) "Poor rice eater's diet," in that it contained little else than rice. Calcium hydroxide was added because of the evidence that calcium greatly increased the growth promoting value of the rice diet and because many Indians obtain significant amounts of added calcium from the practice of chewing betel nuts. Irradiated ergosterol was added because the rice consuming section of Indian people live in a climate full of sunshine and do not lack vitamin D. Vegetable fat was added to the diet, as Indian people do not commonly use animal fat in their diets. The composition of this diet is given in Table 6.

## TABLE 6

### The composition of the rice control diet

| Food[1] | Weight | Calories* | Protein | Fat |
|---|---|---|---|---|
| | gm. | | gm. | gm. |
| Rice | 100.0 | 380.2 | 7 | 1 |
| $Ca(OH)_2$ | 1.0 | --- | --- | --- |
| Ergosterol | --- | --- | --- | --- |
| Vegetable fat | 13.9 | 131.4 | --- | 13.9 |
| Total | 114.8 | 511.6 | 7.0 | 14.0 |

* Gross energy value.

1 About 6 gm. of greens were fed to animals every alternate day during the experimental period.

2 [illegible]

Two nut supplemented rice diets were prepared from each of the four experimental nuts by displacing five per cent and 25 per cent of rice protein in the rice control diet by proteins of the intact nuts. The object of this procedure was to find out if the supplementation of rice protein with nut proteins improved the growth promoting value of the rice protein alone. All of the eight nut supplemented diets were isocaloric with the energy value of the rice control diet and, like it, contained 511.6 calories, seven gm. of protein, 14 gm. of vegetable fat, one gm. of calcium hydroxide and 1600 I.U. of ergosterol. A positive control diet was prepared from fat extracted dried defatted whole egg to match the rice control. The protein in this diet was derived solely from the dried whole egg. The composition of the various nut supplemented rice diets and the whole egg control diet have been given in Tables ii and iii in the Appendix.

In the preparation of the rice diets, converted rice was washed, cooked and dried. Two pound lots were washed with water once and cooked in an open pan for 15 minutes in a large amount of water. The cooking water was discarded, the cooked rice was dried and ground to a fine powder.

Pecans and English walnuts were prepared with the skins. Almonds were blanched and the coats of the cashew nuts were peeled as the nuts were purchased for the experiment.

Incorporation of the nuts into the diets presented a problem as it was difficult to grind the nuts into small pieces without loss of oil. Therefore, all the dry ingredients of each diet were weighed into a large pan, mixed and ground in a Waring blender and passed through a sieve and then the requisite amount of vegetable oil was added to each diet and mixed thoroughly. Two ml. of ether solution of viosterol containing 1600 I.U. of vitamin D were sprinkled on each diet. After all the ether had evaporated, the diet was sieved three times and mixed well.

Groups of five rats were fed each experimental diet. The rats in each group were of approximately the same weight. The animals were placed singly in cages with large mesh screen floors and given diets and distilled water, ad libitum. A record of the food intake of each animal was kept. The animals were weighed on alternate days. Every other day each animal was fed about six gm. of green cabbage which supplied a source of vitamin A.

At the end of a week, the animals in all the groups showed very poor growth. The consumption of food was low and the physical condition unsatisfactory. Food scattering by some animals in each diet was noticeable. The egg control animals were in a slightly better condition than the other animals. During the first part of the second week of the experiment, 11 animals discharged orange-red colored urine. On the 10th day of the experiment, one rice control animal died and on the 12th day, one animal on rice-cashew diet 1 died. The condition of the animals deteriorated noticeably. A few lost weight. The weights of the majority reached a plateau. The coats of the animals on the rice control and rice and nut diets became rough. The coat of one of the animals on rice-cashew diet 2 was distinctly oily. Except for the changes noted above, the animals showed no specific vitamin deficiency.

The diets contained 6.1 to 6.6 per cent protein which should have been adequate for slow growth in the rat. There was sufficient calcium in the diet to supplement the minerals of rice to give satisfactory growth (Kik, 1942). The test diet, with the exception of the egg control diet consisted of converted rice or largely of converted rice. The rice was cooked as was characteristic in parts of India by the open kettle method in a large amount of water and the cooking water was discarded, and this cooking method leads to large losses of thiamin, riboflavin and niacin (Kik and Williams, 1945).

It was surmised that poor appetite, failure of growth and the low condition of animals might be due to lack of some of the water soluble vitamins. Therefore it was decided to supplement the diets with water soluble vitamins.

A vitamin mixture[1] of the composition below was prepared.

| | |
|---|---|
| Calcium Pantothenate | 0.280 gm. |
| Niacin | 0.100 gm. |
| Riboflavin | 0.050 gm. |

---

[1] By courtesy of Mrs. Annanell Jubb. This mixture was routinely used in this laboratory.

| | |
|---|---|
| Thiamin | 0.040 gm. |
| Pyridoxine | 0.050 gm. |
| Inositol | 2.000 gm. |
| Ascorbic Acid | 2.000 gm. |
| p-amino Benzoic Acid | 2.000 gm. |
| Choline H | 5.000 gm. |

The vitamins were weighed separately into a small beaker and made up to 20 gm. with cornstarch and mixed well. Two gm. of the mixture were added to each kilogram of ration. About six gm. of spinach were fed to each animal instead of cabbage to increase the vitamin A content of the food.

By the end of the third week, the animals on the whole improved in physical condition and showed weight gains and the urine became normal in appearance in all animals. The muscle tone of the animals was much better. By the end of the fourth week, most of the animals showed substantial weight gains.

## Nitrogen Balance Experiment

During the last three days of the 28 day growth period a balance period for determining nitrogen retention was done. Urinary and fecal collections were made. Urine was absorbed on filter paper, following Mitchell's (1924) procedure. The feces for all the animals in each group were pooled into a single sample. Twenty per cent hydrochloric acid was used as a preservative. The same procedure was followed for the urine collection. A careful record of the food intake of each group of animals was made. Weights of animals at the beginning and the close of the balance period were taken.

The urinary and fecal samples were digested on a hot plate until a brown digest uniform in color and consistency was obtained. The brown digests were passed through sieves into volumetric flasks and made to volume. Thoroughly mixed samples were stored in screw topped bottles. The nitrogen analysis of diet samples and urinary and fecal samples were made by the Kjeldahl method.

After the balance period, all the animals were sacrificed and autopsied. About 55 per cent of the animals were mildly infested with *Taenia taeniaformin* cysts.

## RESULTS AND DISCUSSION

### Chemical Analysis

The results of the chemical analyses of the experimental nuts and converted rice with the gross energy value of these foods are presented in Table 7. The moisture, crude protein, total ash of nuts and the ether extract values for almond and walnut were within the range of values reported by Mitchell and Beadles (1937), Peterson, Skinner and Strong (1943), Winton (1945), Campous (1946), Watt and Merrill (1950) for the above nuts. The ether extract values for pecans and cashews were two and five per cent lower.

The gross energy value of nuts per 100 gm., determined with the aid of a bomb calorimeter, was 705 calories for almonds,659, 808, and 773 calories for cashews, pecans and English walnuts, respectively. Mitchell and Beadles (1937) reported the gross energy value of nuts in calories per 100 gm. as follows: almonds, 676; cashews, 648; pecans 769; and English walnuts 732. These values were lower than the values obtained in this study in comparison with the percentage composition of nuts in the two studies. Gross energy values were computed using as average heats of combustion 4.1 calories per gm. for carbohydrates, 9.45 calories per gm. for fat, and 5.65 calories per gm. for protein (Sherman, 1946). The calculated values, expressed in calories per 100 gm. for nuts used in this study were: almonds, 702; cashew, 639; pecans 772; and English walnuts 750. The

Table 7

The proximate composition of nuts and rice

| Type of food | Moisture | Ether extract | Crude protein* | Total ash | Carbohydrate by difference | Gross energy value/100 gm.[1] |
|---|---|---|---|---|---|---|
| | per cent | per cent | per cent | per cent | per cent | calories |
| Nuts | | | | | | |
| Almond | 4.18 | 53.34 | 23.49 | 2.99 | 16.00 | 704 |
| Cashew | 4.85 | 42.75 | 19.35 | 2.58 | 30.47 | 659 |
| Walnut | 3.80 | 63.47 | 15.85 | 1.88 | 15.00 | 773 |
| Pecan | 4.02 | 60.06 | 9.87 | 1.33 | 15.72 | 808 |
| Rice, cooked and dried | 10.6 | 0.10 | 7.04 | 0.39 | 81.87 | 380 |

* N x 6.25

[1] Acknowledgement is made to Mrs. Dorothy Dunsing for determination of gross energy values.

differences between the computed caloric values and the actual determinations were within five per cent and are believed to be more consistent with the proximate composition than the data reported by Mitchell and Beadles (1937).

The proximate analysis of converted rice, cooked by the open kettle method and dried after discarding the cooking water has not been reported in the literature.

### Essential Amino Acid Composition of Almonds, Cashews, Pecans and English Walnuts

The amino acid content of four nuts, as obtained in this study has been given in Table 8. Kuiken and Lyman's (1949) analysis of whole egg and unpolished rice and amino acid composition of white rice calculated from compiled data (Block and Bolling, 1951) also are included in this table for comparison.

There were no studies found in the literature on the essential amino acids of total proteins of the nuts studied in this investigation. However Cajori (1921) partitioned the nitrogen present in globulin by the method of Van Slyke. Following the same method, Mignon (1923) partitioned the nitrogen of English walnut globulin, and Damodaran and Sivaswamy (1936) partitioned the nitrogen of cashew nut globulin. Table 9 summarizes the data of their investigations. These data show that the extracted proteins of cashews, pecans and walnuts have a high content of arginine and fair amounts of histidine and tryptophane.

Table 8

Essential amino acids of four nuts, brown rice, white rice and whole egg

| Amino acid | Walnut | Almond | Cashew | Pecan | Brown rice[1] | White rice[2] | Whole egg[1] |
|---|---|---|---|---|---|---|---|
| | per cent | per cent | per cent | per cent | per cent | per cent | per cent |
| Arginine | 3.32 | 4.42 | 4.92 | 2.01 | 0.54 | 0.54 | 0.85 |
| Histidine | 0.47 | 0.70 | 0.70 | 0.36 | 0.14 | 0.11 | 0.33 |
| Isoleucine | 0.70 | 1.12 | 1.07 | 0.49 | 0.28 | 0.38 | 0.80 |
| Leucine | 1.08 | 1.54 | 1.55 | 0.65 | 0.51 | 0.59 | 1.23 |
| Lysine | 0.45 | 0.70 | 0.97 | 0.30 | 0.28 | 0.24 | 1.03 |
| Methionine | 0.17 | 0.19 | 0.32 | 0.15 | 0.14 | 0.26 | 0.45 |
| Phenylalanine | 0.71 | 1.16 | 1.14 | 0.48 | 0.31 | 0.48 | 0.66 |
| Threonine | 0.67 | 0.82 | 0.91 | 0.37 | 0.22 | 0.30 | 0.64 |
| Tryptophane | 0.10 | 0.11 | 0.13 | 0.07 | 0.10 | 0.10 | 0.21 |
| Valine | 0.73 | 1.10 | 1.23 | 0.52 | 0.40 | 0.47 | 0.93 |

1 Lyman and Kuiken's (1949) analysis.

2 Calculated from compiled data, Block and Bolling (1952).

## Table 9

Partition of nitrogen from cashew, pecan and walnuts globulins reported in the literature

| Source of N | Cashew[1] | Pecan[2] | Walnut[3] |
|---|---|---|---|
| | per cent | per cent | per cent |
| Amide N | 11.91 | 9.8 | 9.99 |
| Humin N | 1.40 | 3.6 | 1.84 |
| Arginine N | 21.57 | 22.9 | 39.13 |
| Histidine N | 4.78 | 3.7 | 5.74 |
| Lysine N | 1.54 | 6.2 | 10.61 |
| Mono-amino N | 29.5 | 51.7 | 30.36 |
| Non-amino N | 10.09 | 0.8 | 0.88 |

1 Damodaran and Sivaswamy (1936).

2 Cajori (1921).

3 Mignon (1936).

This result was in agreement with the findings of the present study on total protein. However, the lysine content of total proteins of pecans and walnuts was found to be much lower than that of nut globulins alone.

The nuts on the whole were richer in lysine and lower in methionine than white rice. In comparison with whole egg all the nuts were low in methionine as well as lysine. Fang and Butts (1949) reported an analysis of amino acids in filbert globulins, which suggested that the globulins were deficient in lysine and methionine. In the case of lysine this finding confirms the earlier studies. Biological assay (Fang and Butts, 1949) showed that lysine and methionine were the growth limiting amino acids in filbert globulins for rats. Whether this was true of the proteins of almonds, cashews, pecans and English walnuts was not known, as the proteins of the test diets was not derived solely from the above nuts.

The arginine content of cashews was more than four times greater than that of whole egg. Also histidine, isoleucine, leucine, phenylalanine and threonine occurred in greater concentration in cashews than in whole egg, while lysine, methionine and tryptophane were found to be in lower amounts. The pecans have a lower content of all essential amino acids compared to whole egg, with the exception of arginine. In walnuts and almonds, arginine and histidine occurred in greater concentration than in whole egg; lysine, tryptophane and methionine were present in lesser amounts. Isoleucine, leucine, phenylalanine, threonine and valine occurred in greater amounts in almonds than in whole egg, while these amino acids were found in comparable amounts in walnut and whole egg.

Efficiency of Rice and Nut Protein Mixtures for the Growth of Rats

The weight gains of animals in relation to the protein of the diet are given in Table 10. The individual differences in growth were great even though the protein intake was approximately the same. The animals infested with Taenia taeniaformin seemed to have grown as well as the healthy animals, under the conditions of this experiment (Table 10). This also was true of a preliminary experiment not reported here.

The growth promoting efficiency of the protein of whole eggs was superior to that of rice. Rice protein promoted slightly better growth than most of the rice and nut protein mixtures. The order of the protein efficiency computed as gm. gain per gm. protein ingested, for the different diets was as follows:

| | |
|---|---|
| whole egg control | 2.19 |
| rice control | 1.50 |
| walnut-rice diet 2 | 1.36 |
| cashew-rice diet 1 | 1.13 |
| cashew-rice diet 2 | 1.01 |
| walnut-rice diet 1 | 0.91 |
| pecan-rice diet 1 | 0.77 |
| almond-rice diet 2 | 0.64 |
| almond-rice diet 1 | 0.50 |
| pecan-rice diet 2 | 0.28 |

The differences between the protein efficiency of the rice control and the egg control diets and rice control and each of the following diets: pecan-rice 1, pecan-rice 2, almond-rice 1, almond-rice 2, cashew-rice 1, cashew-rice 2, were statistically significant. The differences between the rice control and each of the walnut-rice diets werenot statistically significant (t-test, Fischer, 1938). The "t" values obtained in the statistical analysis are presented in Table 11.

The average protein efficiency of whole egg on a diet containing 6.8 per cent of protein was found in earlier work on rats in this laboratory[1] to be 3.0 gm. gain in weight per gm. of protein ingested. Ruegmer, Poling and Lockhart (1950) and Arnrich, Hunt, Axelrod, and Morgan (1951) report a protein efficiency of 15 gm. gain per gm. of nitrogen ingested, or 2.4 gm. per gm. of protein ingested, on diets containing 9.5 to 10 per cent protein derived from powdered whole egg.

The efficiency of proteins of the rice control diet in promoting growth was also comparable to studies reported in the literature. On a diet containing 5.5 per cent protein, the protein being derived solely from rice, Kik (1940) reported a protein efficiency of 1.80 gm. Sure (1946) reported the protein efficiency of polished rice at 5.8 per cent protein intake as 1.86 gm. The protein efficiency of rice in this study was found to be 1.50 gm. However, in making these comparisons, it was noted that the studies quoted above were on diets complete with respect to all dietary essentials, while in the present investigation the diet was that characteristic of a human population but which had never been demonstrated to give maximum growth in the rat.

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[1] Unpublished data, by courtesy of Miss Louise Kelley.

Table 10

Growth in weight in relation to protein intake of rats on experimental diets

| Type of diet | Animal number | Total Weight gain | Total food intake | Protein in diet | Total protein intake | Gain per gram of protein intake |
|---|---|---|---|---|---|---|
| | | gm. | gm. | per cent | gm. | gm. |
| Rice Control | 1 | 17 | 171 | 6.2 | 10.60 | 1.60 |
| | 2[2] | | | | | |
| | 3[1] | 21 | 190 | | 11.78 | 1.78 |
| | 4 | 14 | 189 | | 11.72 | 1.19 |
| | 5[1,2] | 16 | 183 | | 11.35 | 1.41 |
| Average | | 17.0 | 183 | | 11.36 | 1.50 |
| Egg Control | 1[1] | 29 | 159 | 6.6 | 10.49 | 2.76 |
| | 2[1] | 26 | 162 | | 10.69 | 2.43 |
| | 3[2] | 11 | 148 | | 9.78 | 1.12 |
| | 4 | 25 | 160 | | 10.56 | 2.37 |
| | 5[2] | 22 | 148 | | 9.77 | 2.25 |
| Average | | 22.6 | 158 | | 10.26 | 2.19 |

Table 10 (contd)

| Type of diet | Animal number | Total Weight gain | Total food intake | Protein in diet | Total protein intake | Gain per gram of protein intake |
|---|---|---|---|---|---|---|
| | | gm. | gm. | per cent | gm. | gm. |
| Pecan Rice Diet 1 | 1[1] | 3 | 160 | 6.5 | 10.40 | 0.29 |
| | 2[1] | 16 | 171 | | 11.12 | 1.44 |
| | 3 | 1 | 139 | | 9.04 | 0.11 |
| | 4 | 11 | 169 | | 10.99 | 1.00 |
| | 5[1,2] | 10 | 153 | | 9.95 | 1.01 |
| Average | | 8.2 | 158 | | 10.30 | 0.77 |
| Pecan Rice Diet 2 | 1 | -2 | 170 | 6.4 | 10.88 | -1.84 |
| | 2[1] | 10 | 178 | | 11.39 | 0.88 |
| | 3[1] | 11 | 205 | | 13.12 | 0.84 |
| | 4[1] | 8 | 183 | | 11.71 | 0.68 |
| | 5[2] | 10 | 184 | | 11.78 | 0.85 |
| Average | | 7.4 | 184 | | 11.78 | 0.28 |

Table 10 (contd)

| Type of diet | Animal number | Total weight gain | Total food intake | protein in diet | Total protein intake | Gain per gram of protein intake |
|---|---|---|---|---|---|---|
| | | gm. | gm. | per cent | gm. | gm. |
| Walnut Rice Diet 1 | 1[1] | 1 | 172 | 6.6 | 11.35 | 0.08 |
| | 2 | 4 | 172 | | 11.35 | 0.35 |
| | 3 | 16 | 183 | | 12.08 | 1.32 |
| | 4 | 19 | 172 | | 11.35 | 1.67 |
| | 5 | 12 | 159 | | 10.49 | 1.14 |
| Average | | 10.4 | 172 | | 11.32 | 0.91 |
| Walnut Rice Diet 2 | 1[1] | 15 | 180 | 6.1 | 10.98 | 1.37 |
| | 2 | 18 | 191 | | 11.65 | 1.55 |
| | 3[2] | 19 | 184 | | 11.22 | 1.69 |
| | 4[1] | 13 | 199 | | 12.14 | 1.07 |
| | 5[2] | 12 | 175 | | 10.68 | 1.12 |
| Average | | 15.4 | 185 | | 11.33 | 1.36 |

Table 10 (contd)

| Type of diet | Animal number | Total weight gain | Total food intake | Protein in diet | Total protein intake | Gain per gram of protein intake |
|---|---|---|---|---|---|---|
| | | gm. | gm. | per cent | gm. | gm. |
| Almond Rice Diet 1 | 1[1] | 2 | 170 | 6.1 | 10.37 | 0.19 |
| | 2[1] | 3 | 152 | | 9.27 | 0.32 |
| | 3[1] | 13 | 169 | | 10.31 | 1.26 |
| | 4[1] | 3 | 150 | | 9.15 | 0.33 |
| | 5[1] | 4 | 171 | | 10.43 | 0.38 |
| Average | | 5.0 | 162 | | 9.91 | 0.50 |
| Almond Rice Diet 2 | 1[1] | 14 | 192 | 6.1 | 11.71 | 1.20 |
| | 2 | -1 | 165 | | 10.07 | -0.10 |
| | 3 | 0 | 158 | | 9.64 | 0.00 |
| | 4[1] | 9 | 172 | | 10.49 | 0.86 |
| | 5[1] | 14 | 186 | | 11.35 | 1.23 |
| Average | | 7.2 | 175 | | 10.65 | 0.64 |

Table 10 (contd)

| Type of diet | Animal number | Total weight gain | Total food intake | Protein in diet | Total protein intake | Gain per gram of protein intake |
|---|---|---|---|---|---|---|
| | | gm. | gm. | per cent | gm. | gm. |
| Cashew Rice Diet 1 | 1[2] | | | 6.5 | | |
| | 2 | 18 | 208 | | 13.52 | 1.33 |
| | 3 | 14 | 176 | | 11.44 | 1.22 |
| | 4 | 13 | 184 | | 11.96 | 1.09 |
| | 5 | 9 | 155 | | 10.27 | 0.88 |
| Average | | 13.5 | 182 | | 11.80 | 1.13 |
| Cashew Rice Diet 2 | 1[1] | 19 | 188 | 6.2 | 11.66 | 1.63 |
| | 2[1,2] | 10 | 171 | | 10.60 | 0.94 |
| | 3[1] | 14 | 181 | | 11.22 | 1.25 |
| | 4[1] | 6 | 163 | | 10.11 | 0.59 |
| | 5[1] | 8 | 200 | | 12.40 | 0.65 |
| Average | | 11.4 | 181 | | 11.20 | 1.01 |

[1] Animals mildly infested with _Taenia taeniaformin_.

[2] Animals with orange-red urine, cause undiagnosed..

Table 11

**Statistical comparison between protein efficiency on the various diets**

| Comparison of diets | "t" values Obtained | Required for significance Probability < .01 | Required for significance Probability < .05 |
|---|---|---|---|
| Rice and egg | 2.464 | 3.499 | 2.365 |
| Rice and pecan-rice 1 | 2.920 | 3.499 | 2.365 |
| Rice and pecan-rice 2 | 2.346 | 3.499 | 2.365 |
| Rice and walnut-rice 1 | 1.967 | 3.499 | 2.365 |
| Rice and walnut-rice 2 | 1.167 | 3.499 | 2.365 |
| Rice and almond-rice 1 | 5.250 | 3.499 | 2.365 |
| Rice and almond-rice 2 | 2.965 | 3.499 | 2.365 |
| Rice and cashew-rice 1 | 4.111 | 3.707 | 2.447 |
| Rice and cashew-rice 2 | 2.578 | 3.499 | 2.365 |

The growth-promoting value of the protein of the nut supplemented rice diets was in no case equal to that of rice. It was apparent that the proteins of nuts do not form an effective supplement to the rice diet as measured by growth per gm. of protein fed.

Nitrogen Intake Excretion and Retention by Rats on Test Diets

The nitrogen intake, excretion and retention by young rats on the test diets is given in Table 12. The data show that the fecal losses of nitrogen of rats on rice and nut supplemented rice diets were much greater than the fecal nitrogen loss on whole egg protein, indicating that the vegetable proteins were not as digestible as the whole egg or that the other constituents of the vegetable food interfered with the digestion of protein. Fecal losses were greater on walnut-rice diets than on the rice diet, while this loss on the other nut supplemented rice diets was comparable to that on rice. True digestibility of proteins of test diets was not obtained since endogenous excretion levels were not tested. The apparent digestibility[1] of the whole egg protein surpassed that of the other diets. The digestibility of this protein was found to be 85 per cent which was comparable to Arnrich's (1950) figure of 89 per cent on the apparent digestibility of whole egg. The apparent digestibilities of rice and nut supplement rice diets ranged from 70 to 78 per cent. None of the apparent

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[1] Formulae for computing apparent digestibility: $\frac{\text{N intake-FecalN}}{\text{N intake}} \times 10($

Table 12

Nitrogen intake, excretion, and retention per day per animal on the proteins of the experimental diets

| Diet | Ave. initial wt. | Food intake | N intake | Fecal N | Urine N | N absorbed | N retained | Apparent digestibility | N retained/gm. absorbed | N retained /gm. N.intake |
|---|---|---|---|---|---|---|---|---|---|---|
| | gm. | gm. | gm. | gm. | gm. | gm. | gm. | per cent | gm. | gm. |
| ice control | 73 | 7.2 | 0.0704 | 0.0159 | 0.0318 | 0.0545 | 0.0227 | 77 | 0.4165 | 0.3224 |
| gg control | 77 | 6.0 | 0.0630 | 0.0095 | 0.0191 | 0.0535 | 0.0344 | 85 | 0.6430 | 0.5460 |
| ecan rice 1 | 77 | 5.6 | 0.0577 | 0.0165 | 0.0269 | 0.0412 | 0.0143 | 71 | 0.3471 | 0.2478 |
| ecan rice 2 | 77 | 6.4 | 0.0650 | 0.0154 | 0.0310 | 0.0496 | 0.0186 | 76 | 0.3750 | 0.2862 |
| alnut rice 1 | 78 | 6.3 | 0.0662 | 0.0197 | 0.0322 | 0.0465 | 0.0143 | 70 | 0.3075 | 0.2160 |
| alnut rice 2 | 80 | 6.7 | 0.0664 | 0.0180 | 0.0267 | 0.0484 | 0.0217 | 73 | 0.4483 | 0.3268 |
| lmond rice 1 | 67 | 5.8 | 0.0566 | 0.0146 | 0.0305 | 0.0420 | 0.0115 | 74 | 0.2719 | 0.2032 |
| lmond rice 2 | 70 | 6.3 | 0.0615 | 0.0150 | 0.0305 | 0.0465 | 0.0160 | 76 | 0.3441 | 0.2602 |
| ashew rice 1 | 75 | 6.2 | 0.0635 | 0.0141 | 0.0286 | 0.0494 | 0.0208 | 78 | 0.4211 | 0.3276 |
| ashew rice 2 | 68 | 6.4 | 0.0638 | 0.0141 | 0.0323 | 0.0497 | 0.0174 | 78 | 0.3501 | 0.2727 |

digestibilities of the rice supplemented diets can be said to be better than that of rice.

Nitrogen retained per gm. nitrogen intake again showed that whole egg protein promoted greater retention of nitrogen than either rice proteins or proteins of nut supplemented rice diets. Whole egg protein showed a nitrogen retention of 0.5460 gm. per gm. nitrogen intake.

The rice diet and the walnut and rice diet 2 and cashew and rice diet 1 have the same order of nitrogen retentions per gm. of nitrogen intake, namely, 0.3224; 0.3268; and 0.3276 gm. in comparison with 0.5460 for the egg diet. The remaining diets resulted in lower retention values in all cases. From the point of nitrogen retention it can be generalized that none of the nut supplemented diets provided for better protein nutrition than the rice diet alone, since the retention of nitrogen on these diets ranged from 0.2034 to 0.3276 gm.

Endogenous nitrogen excretion was not determined on this series of rats. However, in a recent study in the department endogenous nitrogen excretion was determined on a series of animals of comparable size and under comparable conditions of management. By using the average endogenous nitrogen[1] excretion from this study, the true digestibility and biological values were calculated and these are presented in Table 13. The egg control diet was superior to all other diets in both estimated true digestibility and biological value (Table 13.)

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[1] Unpublished data by courtesy of Miss Louise Kelley.

Table 13

Estimated true digestibilities and biological values of the proteins of experimental diets

| Diet | N intake | Fecal N | Metabolic Fecal N[1] | Fecal N -Metabolic N | Urine N | Endogenous[2] N | Urine N -endogenous N | N absorbed | N retained | True digestibility | Biological value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | gm. | gm. | gm. | gm. | gm. | gm. | gm. | gm. | gm. | per cent | per cent |
| ce control | 0.0704 | 0.0159 | 0.0089 | 0.0070 | 0.0318 | 0.0126 | 0.0192 | 0.0634 | 0.0442 | 90 | 70 |
| g control | 0.0630 | 0.0095 | | 0.0006 | 0.0191 | | 0.0065 | 0.0624 | 0.0559 | 99 | 90 |
| can rice 1 | 0.0577 | 0.0165 | | 0.0076 | 0.0269 | | 0.0143 | 0.0501 | 0.0358 | 87 | 71 |
| can rice 2 | 0.0650 | 0.0154 | | 0.0065 | 0.0310 | | 0.0184 | 0.0585 | 0.0401 | 90 | 69 |
| lnut rice 1 | 0.0662 | 0.0197 | | 0.0108 | 0.0322 | | 0.0196 | 0.0554 | 0.0358 | 84 | 65 |
| lnut rice 2 | 0.0664 | 0.0180 | | 0.0091 | 0.267 | | 0.0141 | 0.0573 | 0.0432 | 86 | 75 |
| lmond rice 1 | 0.0566 | 0.0146 | | 0.0057 | 0.0305 | | 0.0179 | 0.0509 | 0.0330 | 90 | 65 |
| lmond rice 2 | 0.0615 | 0.0150 | | 0.0061 | 0.0305 | | 0.0179 | 0.0554 | 0.0375 | 90 | 68 |
| shew rice 1 | 0.0635 | 0.0141 | | 0.0052 | 0.0286 | | 0.0160 | 0.0583 | 0.0423 | 92 | 74 |
| shew rice 2 | 0.0638 | 0.0141 | | 0.0052 | 0.0323 | | 0.0197 | 0.0586 | 0.0389 | 91 | 66 |

Average metabolic nitrogen per rat per day, Kelley (1952).

Average endogenous nitrogen per rat per day, Kelley (1952).

Kelly (1952) found the average true digestibility and biological values to be 96 per cent and 89 per cent respectively on a diet containing 6.8 per cent protein derived from defatted whole egg. These values are in accord with the findings of this experiment.

The true digestibility of protein which was found for rice (90 per cent) was slightly lower than 97 per cent reported by Kik (1943) for brown rice. True digestibilities of rice diets supplemented with nuts ranged from 84 to 92 per cent and were in agreement with Jaffa's report (1903) and Cajori's (1921) observation that the nuts in a mixed diet gave a 90 per cent protein digestibility.

The calculated biological value observed for the rice diet was 70 per cent. Kik (1943) reported a biological value of 73 per cent for brown rice, but Mitchell (1924) and Basu and Basak (1937) found 80 to 86 per cent of the proteins of brown rice used by the animal body. The biological values of the proteins of cashew-rice diet 1 and walnut-rice diet 2 and pecan-rice diet 1 ranged from 71 to 75 per cent, the proteins of other test diets had a lower biological value than rice alone. The biological values of protein calculated as outlined above showed close agreement with protein efficiency. Whole egg protein had the highest biological value and the best protein efficiency. The rice control diet, walnut-rice 2, cashew-rice 1 had about the same biological value and about the same protein efficiency. The pecan-rice diet 1 had a biological value approximately the same as that of rice alone but the protein efficiency of this diet was poor. Almonds had low protein efficiency as well as low biological value. The other nut supplemented rice diets had lower efficiency and lower biological values than the rice diet. It was evident that the addition of nuts to a rice diet did not improve the digestibility or the biological value of the proteins over that of rice under the conditions of this experiment. None of the diets were comparable to dried egg in the efficiency of the protein supplied.

## Nutritive Value of the Rice Diets Supplemented with Nuts

The supplementation of rice diets with nuts did not improve the nutritive value of the experimental diets over that of the rice control. Cashews contain more of all the essential amino acids than white rice. Almonds, pecans, and English walnuts have a higher concentrate of amino acids, with exception of methionine, than white rice (Table 8). It was expected that the amino acids in the nuts would supplement the amino acids of rice, and this would result in good growth and better utilization of nitrogen of the nut mixed rice diets. The results of this experiment did not support this hypothesis; possibly the decrease of the nutritive value of the nuts and rice mixed diets may be the result of amino acid imbalances due to addition of nut protein to rice protein. Arginine imbalance could be a factor in decreasing the nutritive value of nut mixed rice diets. Pecora and Hundley (1951) reported a three fold excess of arginine in the rat diet produced by adding arginine to polished rice caused significant growth depression in rats. Rose (1937) estimated the growth requirement of rat for arginine as 0.2 per cent of the diet. Polished rice contained 0.54 per cent of arginine. The nuts also have a high percentage of arginine (Table 8). Addition of nuts to rice increased the amount of arginine in the nut-rice mixtures. In the rice control diet, the arginine content was 0.47 per cent. In the various rice diets supplemented with nuts the arginine content ranged from 0.50 to 0.71 per cent, and the deviation of arginine from the growth requirement of the rat ranged from + 150 to + 255 per cent in the diets. This excess of arginine in diets could be harmful to the growing animal. Lyman and Elvehjem (1951) also have reported that amino acid imbalances resulted on the addition of excessive amino acids to a casein-gelatin diet, as measured by the growth depression of weanling rats.

It may be possible that there were interfering factors in the nuts which decreased the nutritive value of the nut supplemented rice diets, and prevented proper utilization of the protein of the diet. Cajori (1921) found tannin in the skins of pecans toxic to the rat. Mignon (1923) reported the tannin in the skin of English walnuts as harmful to the rats. Fecal nitrogens were found to be higher on all the vegetable diets than on that of the egg control diet, indicating a lower digestibility for the vegetable diets than that of the egg control diet. Urinary nitrogens also were higher for the vegetable diets than the egg control indicating that the amino acidsabsorbed were less available to the animal body.

The rice control diet and the nut supplemented rice diets were protected by addition of calcium. This, according to Akyroyd (1940) and Ranganath and Rau (1937) should stimulate the growth efficiency of the diets. The diets also contained greens, ergosterol, and the water soluble vitamins which should have made the diet effective, but in spite of these additions, the growth was slow on all the experimental diets except the rice control. It averaged from five to 15.4 gm. for four weeks per animal. Three extra animals from the same consignment, one of them infested with Taenia taeniaformin, were kept on the stock diet routinely used in this laboratory. The growth of the animals during the four week period was 67, 75 and 86 gm. It is of interest that the infected animal made the greatest growth. It was clear that the experimental animals did not attain maximum potentiality for growth on a diet simulating an Indian rice diet, or on the experimental rice diets supplemented with nuts. Evidence from this experiment supports the observations concerning the slow growth of human beings whether in India or elsewhere in Asia on a rice diet.

The improvement of the rice diet for India and other Asian countries has been the concern of nutritionists. Milk, eggs, or meat have been found to supplement the rice diet very efficiently.(Aykroyd and Collaborators ,1937-1940). Owing to the difficulties of the production of milk and non-acceptability of other animal proteins by some section of the Indian people, other sources of protein to supplement the basic Indian rice diet need to be explored. From this experiment it was found that nuts do not form an effective supplement to the rice diet. A consistent testing program is necessary for other forms of vegetable protein of high biological value which would both be acceptable to the Indian population and which might improve the quality of the rice diet. Cereal germs and brewer's yeast are possible products for this purpose, both of which could be produced economically.

## SUMMARY AND CONCLUSIONS

The nutritive value of four nuts, almonds, cashews, pecans and walnuts was investigated for proximate composition and percentage of 10 essential acids. The effectiveness of the supplementation of the basic rice diet of low income groups in India with nuts also was studied.

Weanling rats were maintained on the experimental diets for four weeks. The protein efficiency was measured as gain in weight per gm. of protein ingested. The efficiency of the different diets was as follows: defatted whole egg 2.19 gm., rice 1.50 gm., and from 0.28 to 1.36 gm. for the eight nut supplemented rice diets.

The apparent digestibility of the proteins and nitrogen retention per gm. of nitrogen intake was investigated for each diet use of the balance experiment. The apparent digestibility of proteins of test diets was found to be: whole egg 85 per cent, rice 77 per cent, and nut supplemented diets from 70 to 78 per cent.

The nitrogen retained per gram intake was for whole egg 0.5460 gm., rice 0.3224 gm. The range for nut supplemented rice diets was from 0.2032 to 0.3276 gm.

Displacement of five and twenty-five per cent of the protein of the basic rice diet by the proteins of four whole nuts did not improve the growth promoting efficiency, the digestibility, or nitrogen retention of the proteins of nut supplemented rice diets over that of rice alone. Therefore, it was concluded that the nuts did not form an efficient

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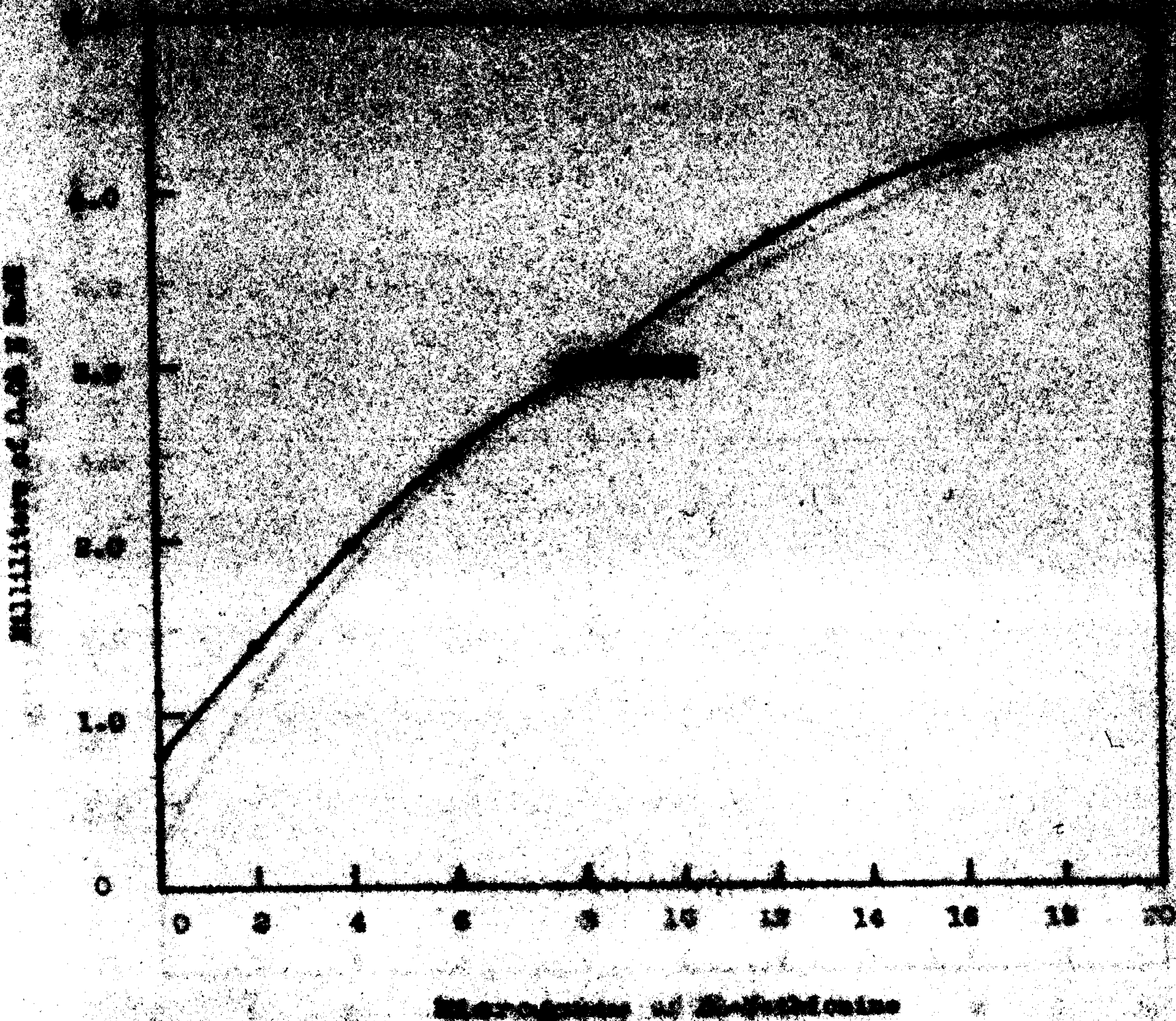
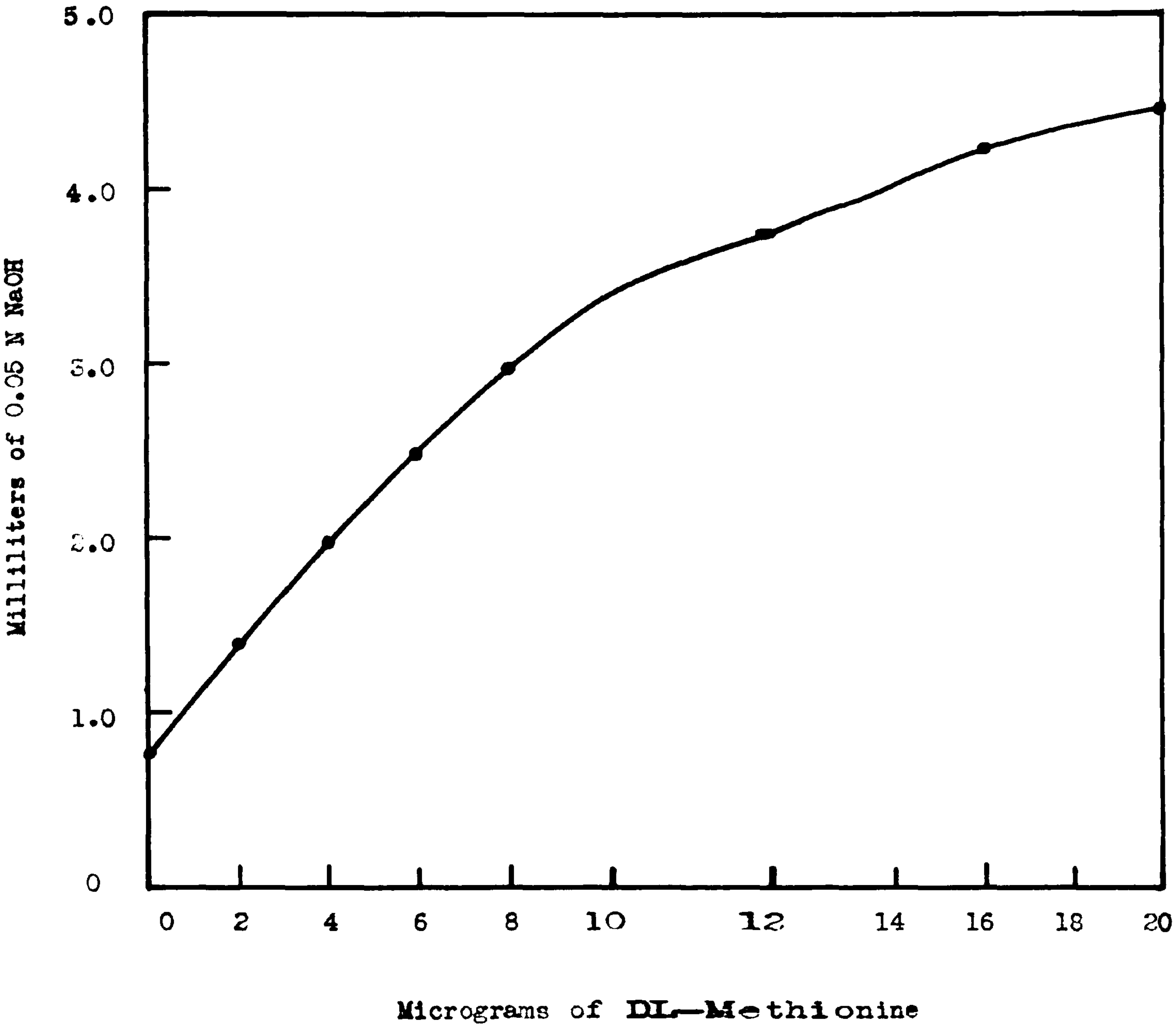



Titration [illegible] [illegible]ained from

Str[illegible] [illegible]lis [illegible] for

Known Concentrations of DL-Methionine

FIGURE 1

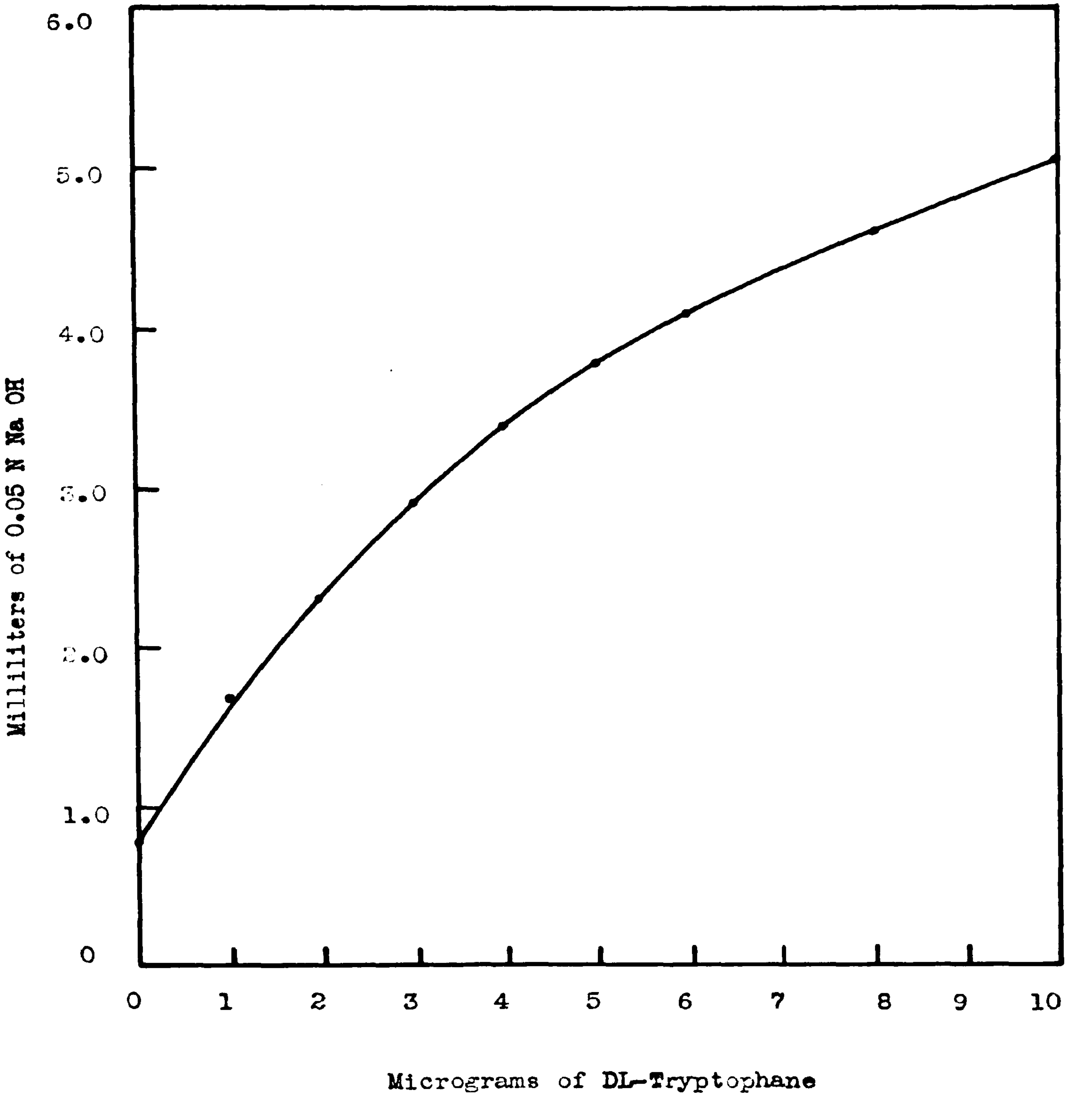



Titration Values Obtained from

<u>Streptococcus</u> <u>faecalis</u> <u>R</u> for

Known Concentrations of DL-Methionine

FIGURE 2

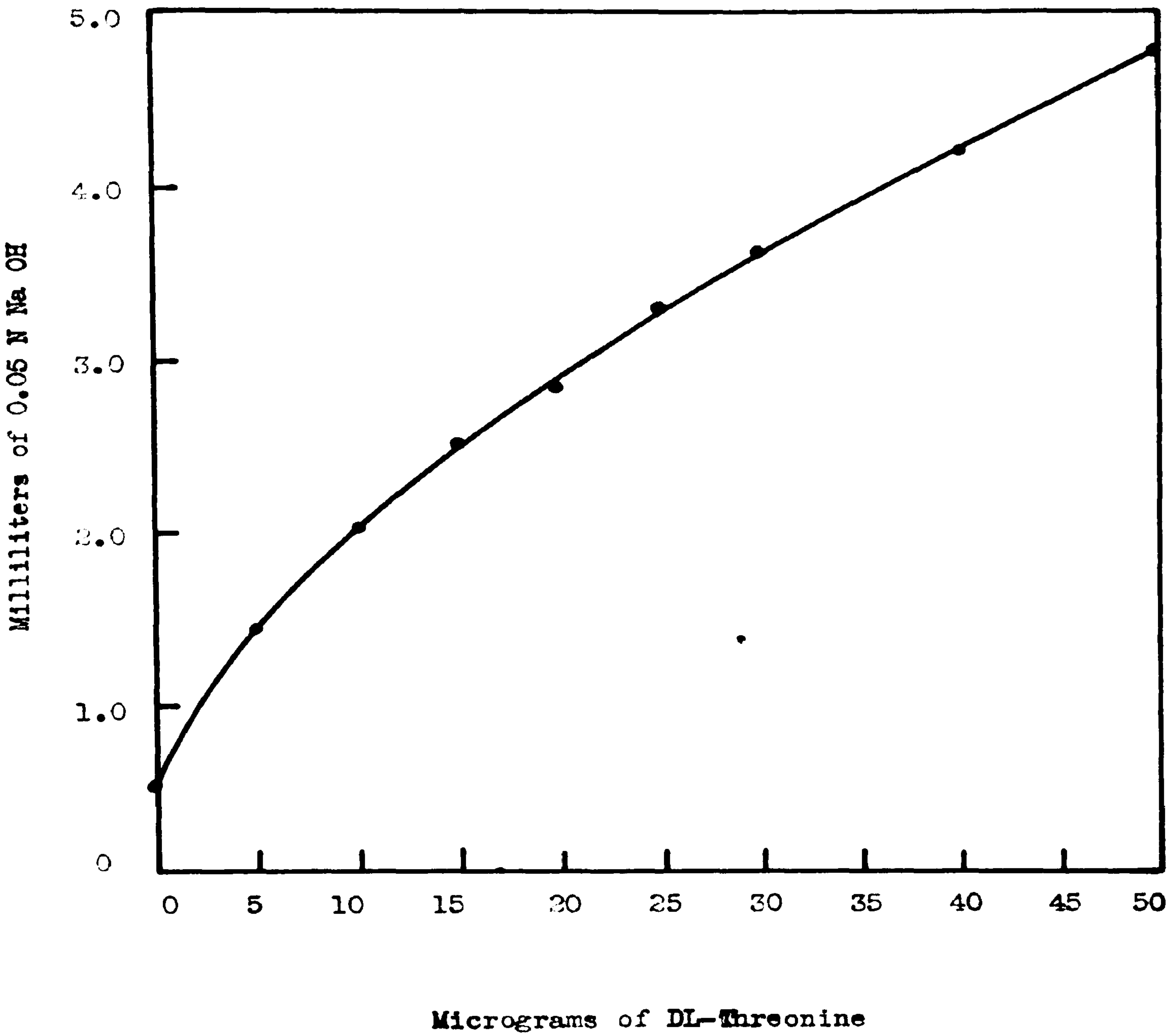



Titration Values Obtained from

<u>Streptococcus</u> <u>faecalis</u> <u>R</u> for

Known Concentrations of DL-Tryptophane

FIGURE 3

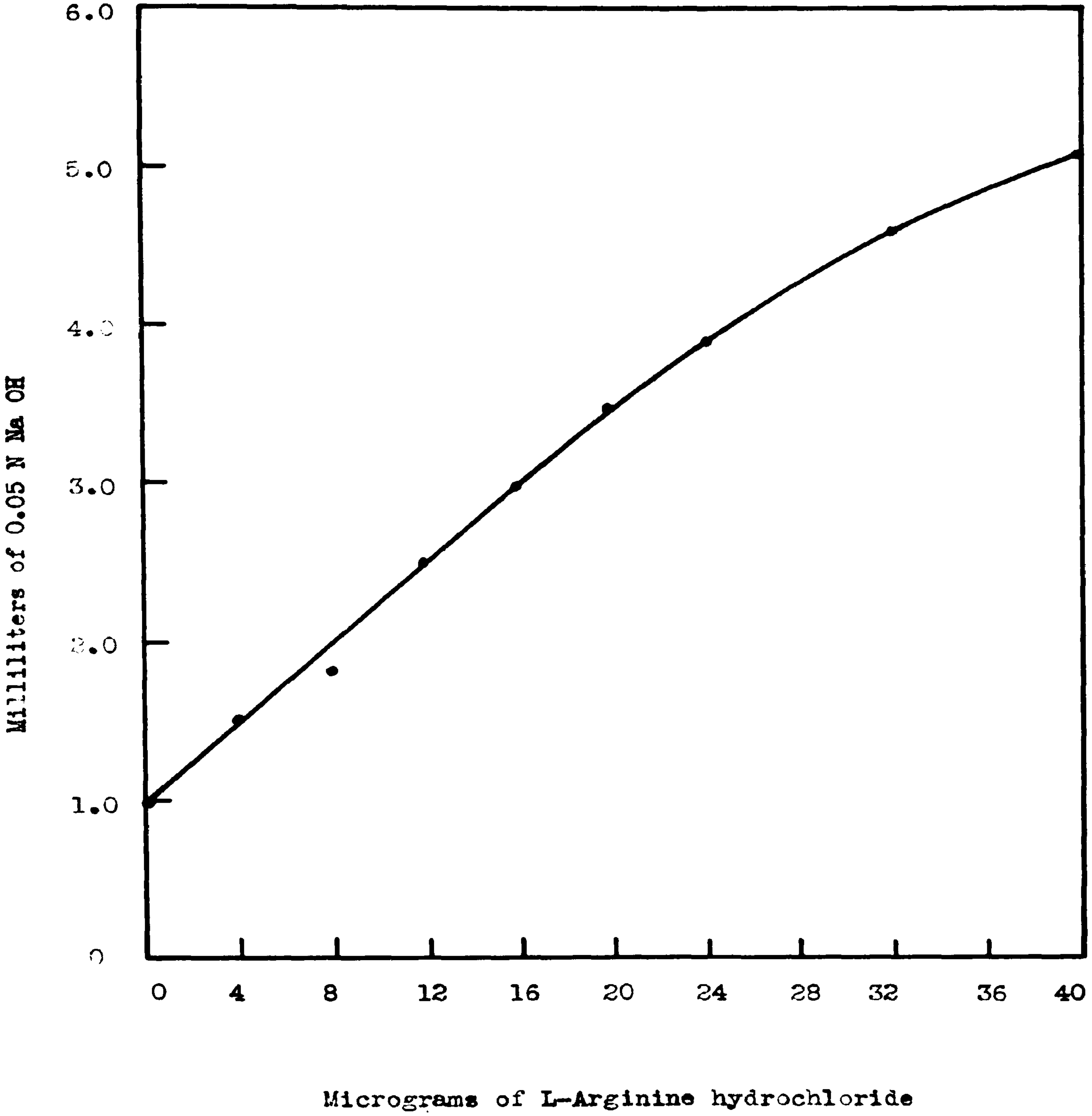



Titration Values Obtained from

<u>Streptococcus</u> <u>faecalis</u> <u>R</u> for

Known Concentrations of DL-Threonine

FIGURE 4

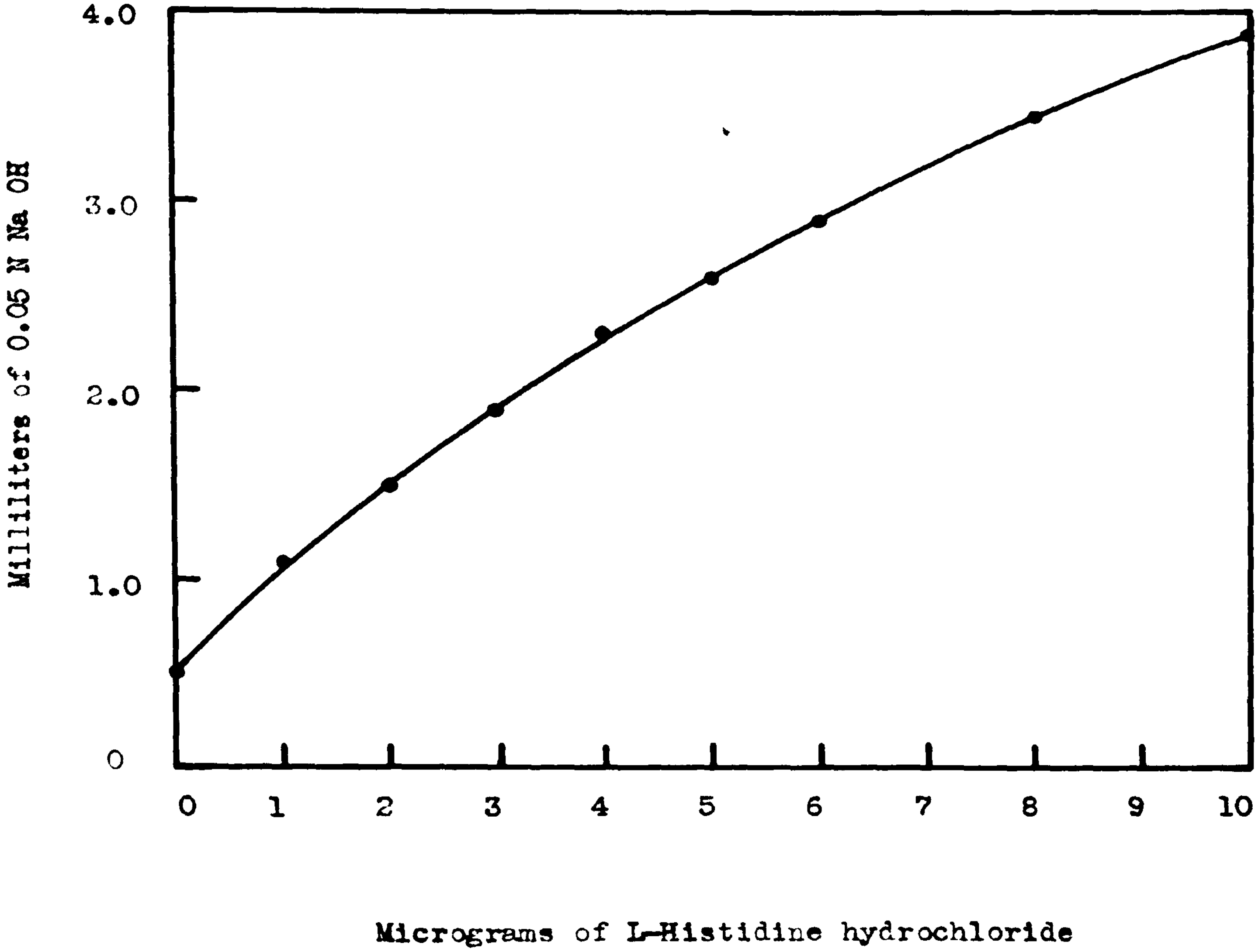



Titration Values Obtained from

*Streptococcus faecalis R* for

Known Concentrations of L-Arginine hydrochloride

FIGURE 5

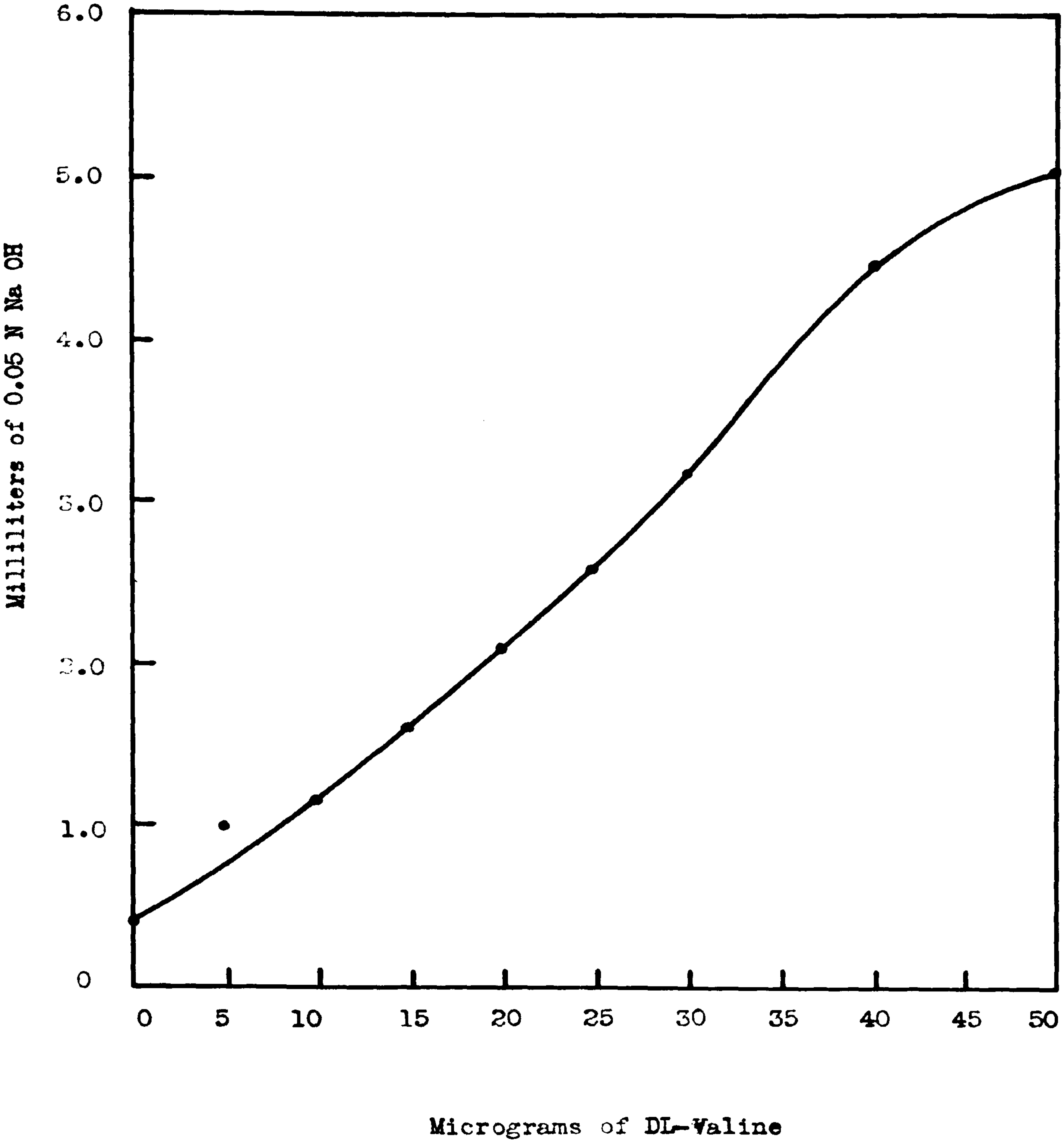



Titration Values Obtained from

Streptococcus faecalis R for

Known Concentrations of L-Histidine hydrochloride

FIGURE 6

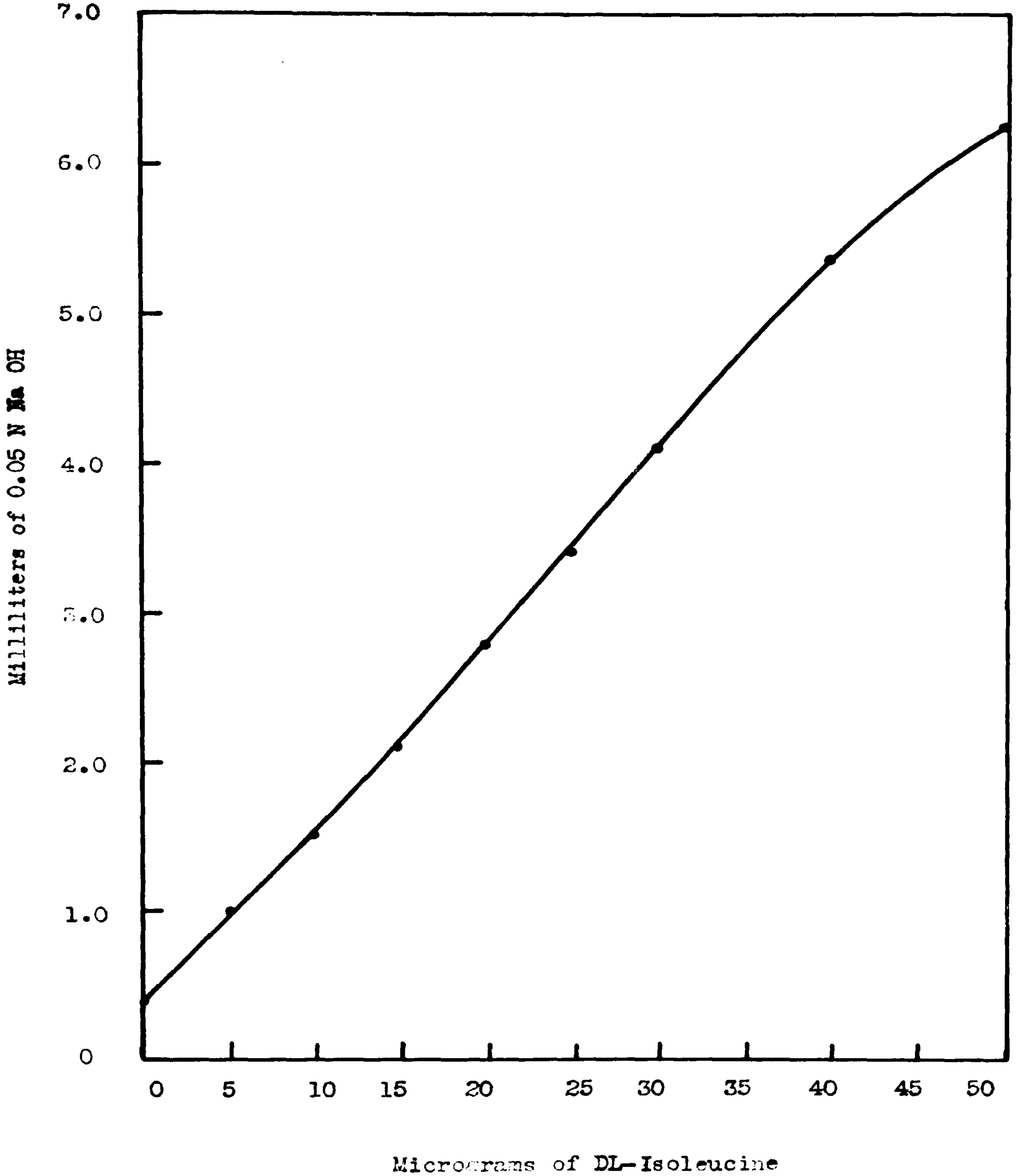



Titration Values Obtained from

Lactobacillus arabinosus P-60 for

Known Concentrations of DL-Valine

FIGURE 7




Titration Values Obtained from

Lactobacillus arabinosus 17-5 for

Known Concentrations of DL-Isoleucine

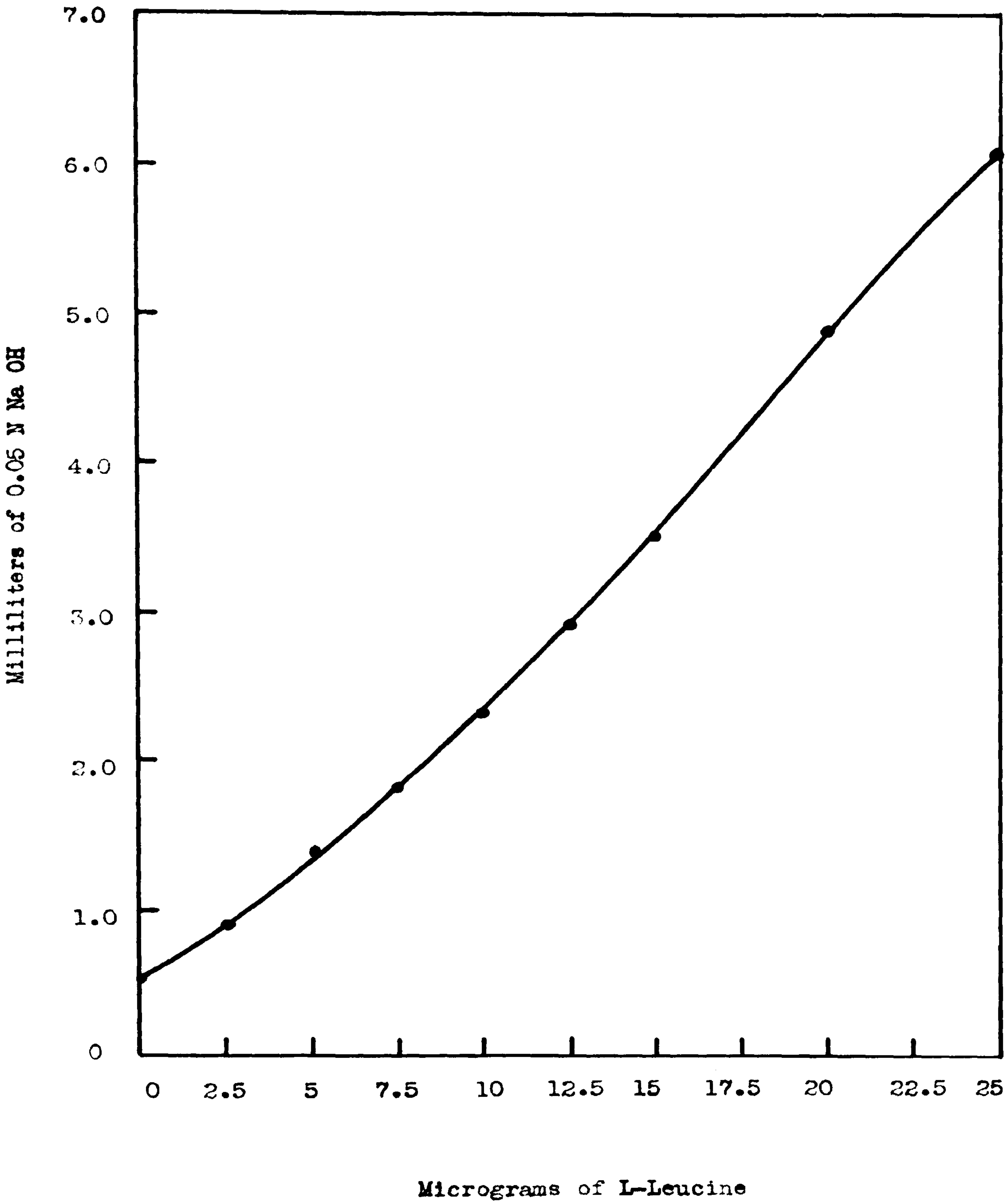
FIGURE 8


Titration Values Obtained from

<u>Lactobacillus</u> <u>arabinosus</u> <u>17-5</u> for

Known Concentrations of DL-Phenylalanine

FIGURE 9

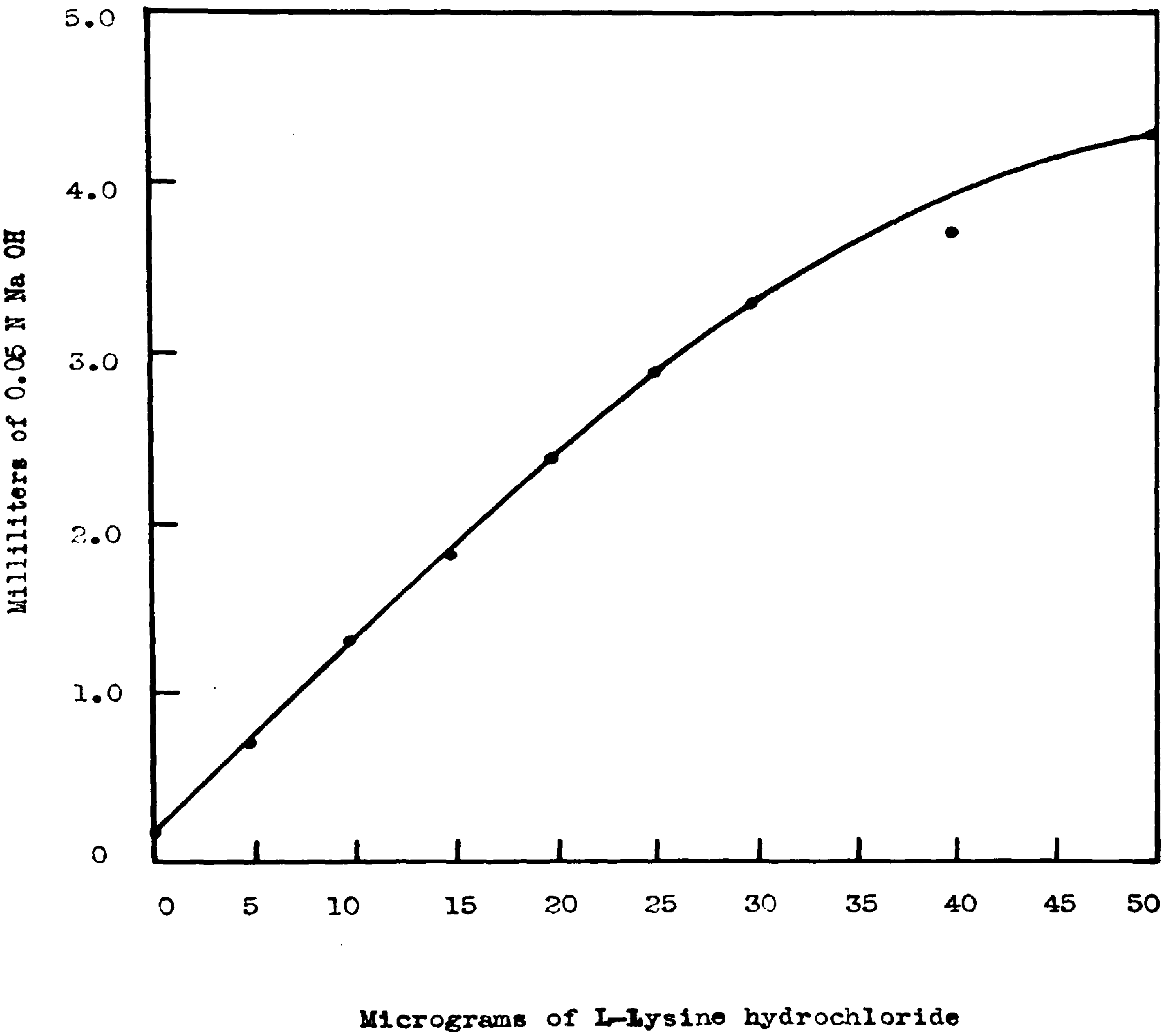



Titration Values Obtained from

Lactobacillus arabinosus 17-5 for

Known Concentrations of L-Leucine

FIGURE 10




Titration Values Obtained from

Leuconostoc mesenteroides P-60 for

Known Concentrations of L-Lysine hydrochloride

**Table 1**

**Essential amino acids in four nuts and recoveries of added amino acids**

| Test Number | Walnuts | Almonds | Cashews | Pecans | Recovery |
|---|---|---|---|---|---|
| | mg/gm | mg/gm | mg/gm | mg/gm | per cent |
| | | | **Threonine** | | |
| 1. | 6.633 | 7.522 | 8.488 | 3.651 | 116.9 |
| 2. | 6.713 | 8.357 | 8.879 | 3.912 | 116.1 |
| 3. | 6.641 | 8.857 | 9.852 | 3.660 | 109.6 |
| Average | 6.662 | 8.246 | 9.073 | 3.741 | |
| | | | **Valine** | | |
| 1. | 7.350 | 10.962 | 13.021 | 5.585 | 94.3 |
| 2. | 7.102 | 11.234 | 12.128 | 5.177 | 95.4 |
| 3. | 7.467 | 10.796 | 11.826 | 4.793 | 100.5 |
| Average | 7.306 | 10.997 | 12.325 | 5.185 | |
| | | | **Methionine** | | |
| 1. | 1.705 | 1.833 | 2.797 | 1.479 | 101.6 |
| 2. | 1.718 | 1.862 | 3.026 | – | 94.2 |
| 3. | 1.779 | 1.909 | 3.909 | 1.545 | 103.0 |
| 4. | 1.673 | 1.996 | 2.971 | 1.569 | 96.0 |
| Average | 1.719 | 1.900 | 3.176 | 1.531 | |

Table i (contd)

| Test Number | Walnuts mg/gm | Almonds mg/gm | Cashews mg/gm | Pecans mg/gm | Recovery per cent |
|---|---|---|---|---|---|
| | | | **Tryptophane** | | |
| 1. | 1.162 | 1.217 | 1.558 | 0.784 | 71.5 |
| 2. | 0.876 | 0.946 | 0.835 | 0.574 | 73.2 |
| 3. | 0.944 | 0.997 | 1.426 | 0.620 | 75.0 |
| 4. | 1.066 | 0.925 | 1.003 | 0.804 | 79.3 |
| 5. | 1.074 | 1.624 | 1.805 | 0.842 | 81.7 |
| Average | 1.024 | 1.142 | 1.325 | 0.723 | |
| | | | **Histidine** | | |
| 1. | 4.311 | 7.946 | 7.462 | 4.006 | 111.7 |
| 2. | 4.808 | 6.659 | 7.386 | 4.267 | 103.1 |
| 3. | 5.084 | 6.475 | 6.675 | 3.058 | - |
| 4. | 4.682 | 6.847 | 6.450 | 3.138 | 104.9 |
| Average | 4.721 | 6.982 | 6.993 | 3.617 | |
| | | | **Arginine** | | |
| 1. | 35.892 | 46.846 | 50.000 | 19.982 | 98.3 |
| 2. | 30.424 | 47.966 | 55.556 | 21.906 | 91.2 |
| 3. | 33.227 | 37.801 | 42.010 | 18.490 | 107.5 |
| Average | 33.181 | 44.204 | 49.189 | 20.126 | |

Table i (contd)

| Test Number | Walnuts | Almonds | Cashews | Pecans | Recovery |
|---|---|---|---|---|---|
| | mg/gm | mg/gm | mg/gm | mg/gm | per cent |
| | | | Isoleucine | | |
| 1. | 7.000 | 10.939 | 10.707 | 6.142 | -- |
| 2. | 8.253 | 11.326 | 9.947 | 4.298 | 90.8 |
| 3. | 7.623 | 11.232 | 11.182 | 4.669 | 97.9 |
| 4. | 7.480 | 11.326 | 10.873 | 4.375 | 97.2 |
| Average | 7.589 | 11.206 | 10.677 | 4.871 | |
| | | | Leucine | | |
| 1. | 11.314 | 15.138 | 15.847 | 6.901 | 110.0 |
| 2. | 10.962 | 16.470 | 16.168 | 6.430 | 100.9 |
| 3. | 10.209 | 14.738 | 14.351 | 6.103 | 102.7 |
| Average | 10.828 | 15.449 | 15.455 | 6.478 | |
| | | | Lysine | | |
| 1. | 4.766 | 6.910 | 9.344 | 3.034 | 92.5 |
| 2. | 3.955 | 7.768 | 10.470 | 3.221 | 104.4 |
| 3. | 4.476 | 6.376 | 9.230 | 2.963 | 98.0 |
| 4. | 4.746 | 6.959 | 9.572 | 2.964 | 99.4 |
| Average | 4.486 | 7.003 | 9.654 | 3.046 | |

Table i (contd)

| Test Number | Walnuts | Almonds | Cashews | Pecans | Recovery |
|---|---|---|---|---|---|
| | mg/gm | mg/gm | mg/gm | mg/gm | per cent |
| | | | **Phenylalanine** | | |
| 1. | 6.601 | 11.326 | 11.214 | 4.712 | 94.7 |
| 2. | 7.128 | 11.834 | 10.090 | 4.829 | 86.5 |
| 3. | 7.537 | 11.590 | 13.010 | 4.931 | 84.8 |
| Average | 7.089 | 11.583 | 11.438 | 4.824 | |

Table ii

The composition of rice diets supplemented with nuts

| Food[1] | Weight | Calories | Protein | Fat |
|---|---|---|---|---|
| | gm. | | gm. | gm. |
| | | **Almond-Rice Diet 1** | | |
| Rice | 75.00 | 285.15 | 5.25 | 0.08 |
| Almonds | 7.45 | 52.49 | 1.75 | 3.98 |
| Vegetable fat | 9.94 | 93.93 | --- | 9.94 |
| Cornstarch | 19.52 | 80.03 | --- | --- |
| Total | 112.91 | 511.60 | 7.00 | 14.00 |
| | | **Almond-Rice Diet 2** | | |
| Rice | 95.00 | 361.19 | 6.65 | 0.10 |
| Almonds | 1.49 | 10.45 | 0.35 | 0.80 |
| Vegetable Fat | 13.1 | 123.8 | --- | 13.10 |
| Cornstarch | 3.94 | 16.16 | --- | --- |
| Total | 114.53 | 511.6 | 7.00 | 14.00 |
| | | **Pecan-Rice Diet 1** | | |
| Rice | 75.00 | 285.15 | 5.25 | 0.08 |
| Pecan | 17.73 | 143.33 | 1.75 | 12.25 |
| Vegetable fat | 1.67 | 15.78 | --- | 1.60 |
| Cornstarch | 16.42 | 67.34 | --- | --- |
| Total | 111.82 | 511.60 | 7.0 | 14.0 |

Table ii (contd)

| Food[1] | Weight | Calories | Protein | Fat |
|---|---|---|---|---|
| | gm. | | gm. | gm. |
| | | **Pecan-Rice Diet 2** | | |
| Rice | 95.00 | 361.19 | 6.65 | 0.10 |
| Pecan | 3.55 | 28.66 | 0.35 | 2.45 |
| Vegetable fat | 11.45 | 108.20 | --- | 11.45 |
| Cornstarch | 3.30 | 13.55 | --- | --- |
| Total | 114.30 | 511.60 | 7.00 | 14.00 |
| | | **Walnut-Rice Diet 1** | | |
| Rice | 75.00 | 285.15 | 5.25 | 0.08 |
| Walnut | 11.04 | 85.39 | 1.75 | 7.01 |
| Vegetable fat | 6.91 | 65.30 | --- | 6.91 |
| Cornstarch | 18.48 | 75.76 | --- | --- |
| Total | 112.43 | 511.60 | 7.00 | 14.00 |
| | | **Walnut-Rice Diet 2** | | |
| Rice | 95.00 | 361.19 | 6.65 | 0.10 |
| Walnut | 2.21 | 17.08 | 0.35 | 1.40 |
| Vegetable fat | 12.50 | 118.13 | --- | 12.50 |
| Cornstarch | 3.71 | 15.20 | --- | --- |
| Total | 114.42 | 511.60 | 7.00 | 14.00 |

Table ii (contd)

| Food[1] | Weight gm. | Calories | Protein gm. | Fat gm. |
|---|---|---|---|---|
| | | Cashew-Rice Diet 1 | | |
| Rice | 75.00 | 285.15 | 5.25 | 0.08 |
| Cashews | 9.09 | 59.60 | 1.75 | 3.87 |
| Vegetable fat | 10.05 | 94.97 | --- | 10.05 |
| Cornstarch | 17.53 | 71.88 | --- | --- |
| Total | 112.77 | 511.60 | 7.00 | 14.00 |
| | | Cashew-Rice Diet 2 | | |
| Rice | 95.00 | 361.19 | 6.65 | 0.10 |
| Cashews | 1.81 | 11.92 | 0.35 | 0.78 |
| Vegetable fat | 13.12 | 123.98 | --- | 13.12 |
| Cornstarch | 3.54 | 14.51 | --- | --- |
| Total | 114.47 | 511.60 | 7.00 | 14.00 |

[1] One gm. of $Ca(OH)_2$ and 1600 International units of irradiated ergosterol were added to each test diet. Animals on the diet were fed six gm. of greens every alternate day during the experimental period.

Table iii

The composition of whole egg control diet

| Food[1] | Weight gm. | Calories | Protein gm. | Fat gm. |
|---|---|---|---|---|
| Dried whole egg | 10.00 | 60.35 | 7.00 | 2.00 |
| Fat | 12.00 | 113.40 | ---- | 12.00 |
| Cornstarch | 82.40 | 337.70 | ---- | ---- |
| Total | 105.40 | 511.60 | 7.00 | 14.00 |

[1] About six gm. of greens were fed to animals on the whole egg control diet during the experimental period. 1600 International units of irradiated ergosterol were added to the diet.