THE USE OF COTTONSEED FLOUR IN VEGETABLE PROTEIN MIXTURES FOR HUMAN FEEDING II. CLINICAL TRIALS[1]

by

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An important part of the high mortality in most underdeveloped countries is due directly or indirectly to an inadequate consumption of a sufficient quantity and quality of protein in the diet. While much can be done to increase the availability of animal protein, its cost and

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the pressure of growing populations makes the utilization of suitable vegetable sources of protein important. For tropical and sub-tropical regions cottonseed flour is one of the most promising sources of additional protein for human diets, providing it comes from high quality seed processed in such a way as to be relatively low in gossypol and with protein quality intact. Dr. Bressani has described the basic INCAP experimental work in animals that has led to the development of vegetable mixture formulas using such cottonseed flours for the supplementary and mixed feeding of infants and young children and for improving the protein value of the diets of persons of all ages. Other speakers at this meeting have discussed the specifications for cottonseed flour of suitable quality and described the agricultural and industrial problems involved in its production This presentation will summarize INCAP clinical experience with the various vegetable mixtures containing cottonseed flour. These have not only been shown to be widely acceptable in the diets of persons without obvious malnutrition but also have been found effective in the treatment of severe protein malnutrition in children (kwashiorkor).

Material and Methods

Initial acceptability and metabolic trials were carried out in children 1 to 5 years of age under study in the INCAP metabolic unit and in an advanced stage of recovery from kwashiorkor. As experience was acquired the

Table I
Formulas for INCAP Vegetable Mixtures For Human Feeding

Ingredient	8	8A	9	9A	9B
Lime-treated	50	50	28	----	----
Corn Cooked	----	----	----	29	----
Uncooked	---	----	----	----	29
Sesame flour 33% fat	35	----	----	---	----
18 fat	----	35	---	----	---
Lime-treated	---	---	28	----	--
Sorghum Cooked	----	----	-	29	--
Uncooked	-	---		.	29
Cottonseed flour	9	9	38	38	38
Torula yeast	3	3	3	3	3
Dehydrated leaf meal	3	3	3	---	--
CaCO3	----	----	----	1	1
Vitamin A	---	--	----	4500	4500

formulas were also used in the early and even initial treatment of children with kwashiorkor. The various formulas involved in this program are given in Table I.

Mixture 8A differed from 8 only in the use of a sesame seed flour of lower fat content. Mixture 9′, the uncooked form of Mixture 9, was used only in the animal feeding experiments. From the standpoint of protein quality, there was no difference between mixtures 8 and 8A or between 9, 9A and 9B, although the latter two did not employ lime-treated corn and had the leaf meal as a source of vitamin A activity replaced by the synthetic vitamin.

Mixture 9A, with the corn and sorghum pre-cooked, received 10 minutes of final cooking after blending one part of the dry powder into approximately 10 parts of water; 9B, utilizing raw corn and sorghum, required 15 minutes cooking under the same circumstances. All preparations were served primarily as cereal gruels with added sugar, and flavored with cinnamon, vanilla or chocolate.

The cottonseed flour employed in all of the experimental testing in children was "Pro-flo," produced by the Traders Oil Mill, Fort Worth, Texas. The large-scale commercial trial in Guatemala with INCAP Vegetable Mixture 9B used a cottonseed flour produced by Borgonovo Hermanos, El Salvador, whose characteristics have been described by Dr. Bressani (1).[2] Specifications and sources for other components have been previously described.

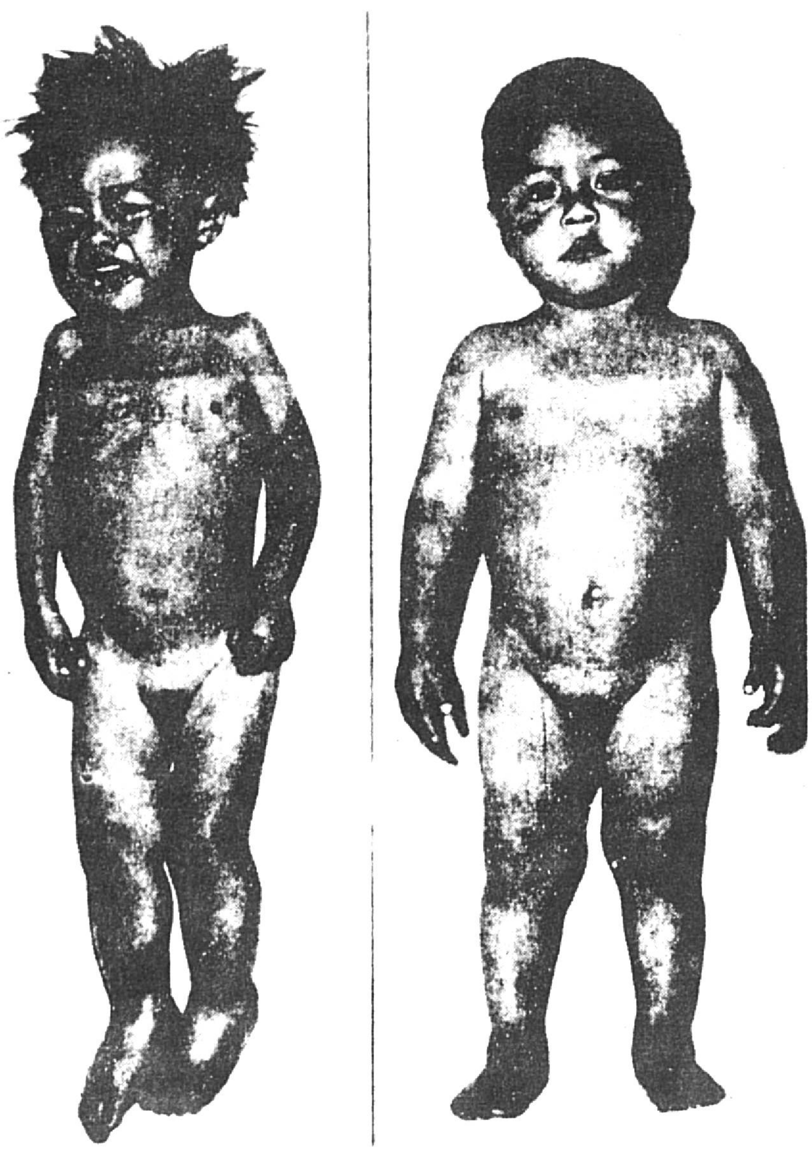
Mixture 8 contained approximately 25.1% protein and Mixture 9, 27.5%. As described in detail elsewhere (2,3,4), both mixtures contained a balanced complement of other essential nutrients except ascorbic acid. The chemical analysis and biological trials cited by Dr. Bressani (1, 5) indicated that, as a protein source, each should give results in children similar to those obtainable with skim milk.

Therapeutic and Metabolic Studies

After preliminary tests of palatability and initial tolerance, Mixture 8 with its content of 9% cottonseed flour was given as the sole source of protein to seven children who had recovered from the acute phase of kwashiorkor but who were not yet ready for discharge from the hospital (2,4,6). The longest periods for

[2] Figures in parenthesis refer to References at end of this article.

which Vegetable Mixture 8 was fed as the sole source were 59, 50 and 33 days. In every case the clinical response was excellent and the mixture resulted in firm and regular stools. Following this favorable experience, 6 children with full-blown kwashiorkor were treated from the time of admission with Mixture 8 as the sole protein source with a clinical response indistinguishable from that usually achieved with milk (2,4). The recovery of one of these children is illustrated by the first child in Figure 1.



In eight of these children, 5 day nitrogen balance periods with the vegetable mixture as the protein source were compared with similar periods when milk was given isonitrogenously. Table II, taken from a recent publication (4) shows that at levels of intake between 2.00 and 2.8 gm., there was no difference between the average retention of nitrogen expressed as percent of intake when the protein was derived from Vegetable Mixture 8 and that when it was supplied by cows milk. Mixture 8A gave equally satisfactory results when used in balance experiments but was less well tolerated for the initial therapy of kwashiorkor due to unknown differences in the sesame flour which were unrelated to the value of cottonseed flour in the formula.

Tolerance of patients to Mixture 9 was established during nitrogen balance studies in 3 children and treatment of 2 children partially recovered from kwashiorkor. Table III, from a recent INCAP publication (4), summarized the results reported to date from 72 five-day balance periods with one or another of the Mixture 9 formulas and a similar number of control periods in which milk was the protein source. At levels of protein intake of 2.0 gm. and above, the retentions obtained with the Vegetable Mixtures 9A and 9B were fully comparable to those obtained with milk, al-

Fig. 1. Child PC-88 in the two photographs was a boy 2 years, 8 months, weighing 18 lbs., 7 oz. on admission to the hospital and treated with INCAPARINA as a sole source of protein. He is shown on the right after 4½ months of this treatment at which time he weighed 22 pounds.

Table II
Comparison of Vegetable Mixture 8 and Milk in Young Children

				MILK				VEGETABLE MIXTURE 8			
Children	Age	Weight	Cal/kg	Days	Intake g/kg	Absorption % of intake	Retention % of intake	Days	Intake g/kg	Absorption % of intake	Retention % of intake
48	1 yr. 9 mos.	7.5	101	5	2.5	87	0	5	2.3	73	11.3
23A	3 yrs.	11.2	106	5	2.6	74	23.2	5	2.6	78	23.2
48	1 yr. 9 mos.	9.9	110	5	3.8	90	14.8	5	3.8	71	12.0
56	3 yrs. 6 mos.	9.5	101	5	2.8	74	11.0	5	3.0	71	13.5
57	4 yrs. 3 mos.	11.6	108	5	2.9	80	33.6	5	2.8	68	22.5
73	1 yr. 1 mo.	7.4	100	5	3.0	83	17.6	10	3.5	78	31.3
75	1 yr. 3 mos.	7.5	95	10	2.6	83	38.2	5	2.9	70	23.8
88	3 yrs.	10.7	108	5	2.0	78	25.9	10	3.3	67	15.2
Average			104		2.8	81	20.5		3.0	72	19.1

though once again, the absorption, expressed as percent of intake, was slightly higher with milk. At levels below 2.0, inadequate for children of the age studied, the retentions with Vegetable Mixture 9 were still strongly positive although somewhat higher levels were attained with milk.

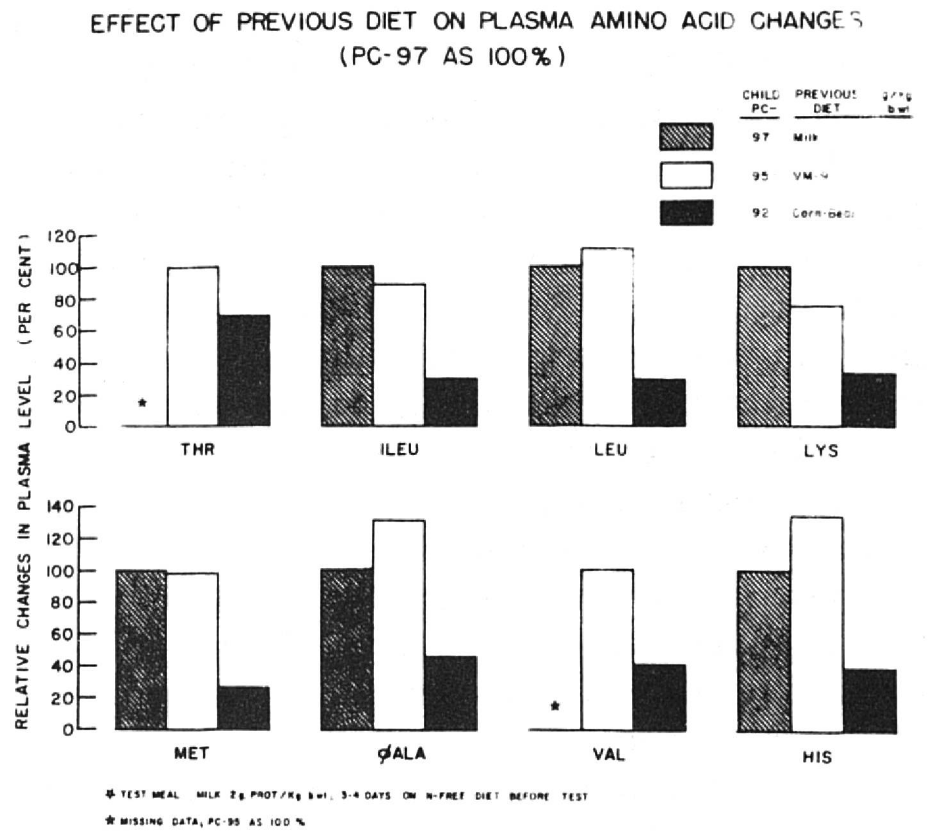
Five children with kwashiorkor admitted to the hospital in this period were treated with Vegetable Mixture 9 with results not distinguishable from those previously observed with milk and with Mixture 8. One of these is illustrated in Figure 1.

These results were highly gratifying but did not in themselves assure that any of these mixtures could be successfully introduced for the local use of persons with growing children and grossly inadequate economic resources. This could be determined only by field acceptability and marketing trials.

Special Biochemical Findings

A new approach for evaluating the quality of dietary protein in human subjects is under investigation in INCAP by Arroyave (7). It involves the determination of the plasma amino acid pattern by ion exchange column chromatography before and after a small test feeding of milk. Observations in dogs and children indicate that the plasma amino acids increases, after a standard protein feeding, are influenced by the nutritional state of the subject as determined by the previous diet. A decreased response seems to occur if a protein of poor nutritional value has been fed for some time before the test.

In two separate 4-week experiments each of three children was fed either a diet containing Vegetable Mixture 9B as the sole protein source, milk or a mixture of corn and beans in which each furnished half of the protein. After a fasting blood sample, a test meal of skim milk providing 2 gm. of protein/k. of body weight was administered and a second blood sample taken 2-½ hours later. The shift in plasma amino acid for the first of these trials is given in Figure 2, taken from a recent publication, and the results with the second were almost identical.



They show that the changes in the child fed the vegetable mixture were essentially the same as those of the child receiving milk and very much higher than those of the child who had been subsisting on the corn and beans diet. The results tend to confirm the impressions of the metabolic and therapeutic trials that the

Table III
Comparison of Vegetable Mixture 9A or 9B and milk in Young Children

g prot/kg	No. Children	No. balance Periods	MILK Intake g/kg	MILK Absorption % of Intake	MILK Retention % of Intake	VEGETABLE MIXTURE 9A Or 9B Intake g/kg	VEGETABLE MIXTURE 9A Or 9B Absorption % of Intake	VEGETABLE MIXTURE 9A Or 9B Retention % of Intake
4.0	1	2	4.0	84.4	22.0	4	66.1	24.1
3.0-3.9	4	11	3.0	84.9	17.1	3.0	70.2	16.8
2.0-2.9	9	48	2.3	82.6	16.3	2.3	68.9	17.8
1.0-1.9	4	13	1.2	78.1	24.9	1.2	66.2	15.5
1	2	3	0.5	67.2	8.1	0.5	59.1	4.5

[1] Scrimshaw et al. (ref. 4)

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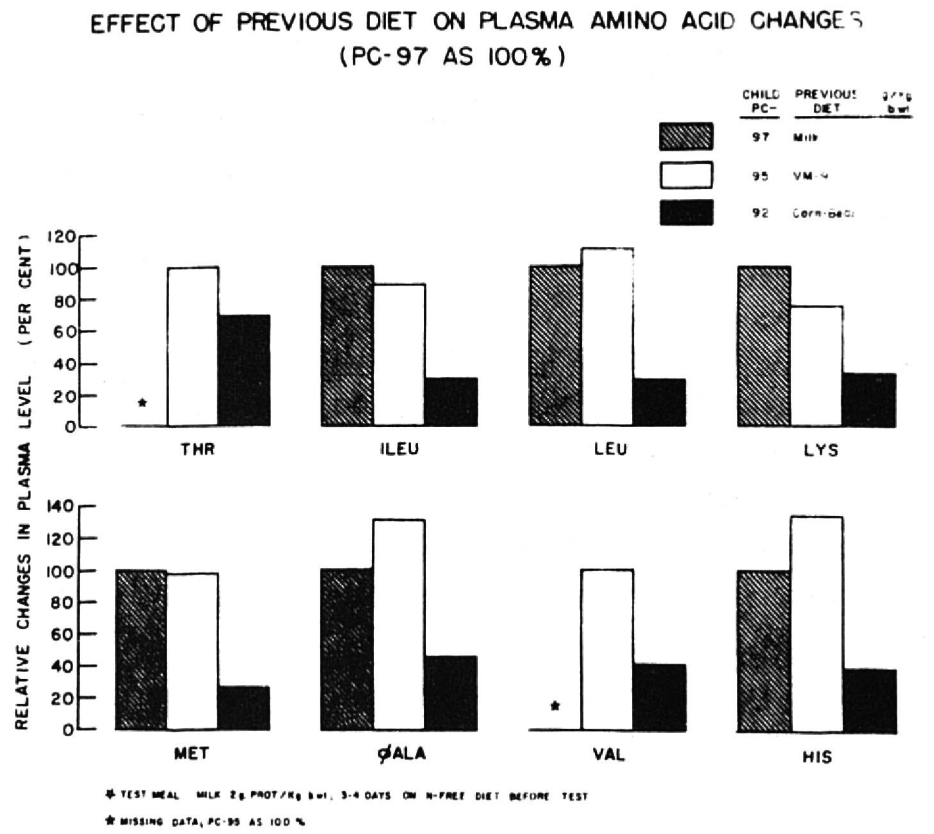

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3.0-3.9	4	11	3.0	84.9	17.1	3.0	70.2	16.8
2.0-2.9	9	48	2.3	82.6	16.3	2.3	68.9	17.8
1.0-1.9	4	13	1.2	78.1	24.9	1.2	66.2	15.5
1	2	3	0.5	67.2	8.1	0.5	59.1	4.5

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quality of the protein in Vegetable Mixture 9 is indistinguishable by these means from that of milk, at least at normal and therapeutic levels of protein intake.

Field Studies

Field acceptability trials were carried out only with formulas 9A and 9B used interchangeably in a total of 129 children in four Guatemalan communities for periods of 15 to 19 weeks (4, 8). The mixtures were supplied to the mother in plastic bags containing 75 gm., each sufficient for the preparation of the three daily glasses of INCAPARINA which were recommended. An average of 78% of all children in the study consumed 2 or more glasses daily. Ninety-seven percent of the glasses offered were accepted, and only 3% rejected; no instance of intolerance to the mixture was encountered. Subsequently, similar results were obtained in El Salvador where 53 children were given the mixture for 4 weeks, and in preliminary acceptability trials in Honduras and Nicaragua.

These studies established the acceptability of the product when offered without cost but did not measure the willingness of the parents to purchase the preparation for their child or for the entire family. Arrangements were then made by INCAP and the government of Guatemala for a market trial in the predominantly Indian village of Palin with an estimated population of 4,000. The mixture was marketed under the generic name of INCAPARINA using the same type of plastic bag containing 75 gm. of powder which had been employed in the acceptability trials. Within 3 weeks sales stabilized at approximately 1,200 bags per week and remained at this average level for the 5-month trial period. No commercial advertising was used, but the product was recommended by personnel of the health center and by the local school teachers.

The name INCAPARINA has now been adapted as a generic name to refer to any vegetable mixture developed by INCAP suitable for feeding to young children and containing at least 25% protein of a quality comparable to that of milk and other products of animal origin.

Similar arrangements were made for larger place marketing trials to involve 43 communities with health centers or units and limited commercial advertising. This trial began March 22, 1960, and was terminated September 30, 1960, at which time over 840,000 of 75 gm. portions had been sold. Initial demand for the product was unexpectedly high and the full production was absorbed by the two principal cities and nearby towns. For this reason, the original plan could not be carried out because adequate production and packaging facilities were lacking. For the same reason, almost no commercial advertising was employed. The response was so encouraging, however, that negotiations are in progress for large-scale production and distribution of INCAPARINA based on formula 9B, in Guatemala, El Salvador and Nicaragua. It is hoped that arrangements will subsequently be made for production in a number or the other countries in which protein malnutrition in children is an acute problem and which possess or can develop suitable local sources of cottonseed flour.

Discussion

The basic objective of INCAP's work with protein-rich vegetable mixtures for human consumption has been to develop a low-cost protein food suitable for feeding to young children which could be produced in technically underdeveloped areas at a price which families of limited economic resources could afford. There are many potential formulas, but for use in Central America, milk was both expensive and in short supply, soybeans were not grown in quantity and the prospect of producing a suitable quality of fish flour very doubtful. For a time sesame flour seemed promising but soon proved to be too expensive.

Cottonseed flour was employed cautiously at first in Vegetable Mixture 8 because of uncertainty about specifications for protein quality and possible adverse effects of its gossypol content. The favorable biological and clinical results with this formula encouraged INCAP to replace sesame flour entirely with the less expensive cottonseed flour. Sorghum was also substituted for part of the corn to further reduce the price and increase commercial flexibility.

The resulting formula for Mixture 9 represents a product in which the three principal factors of cost, nutritive value and acceptability were watched equally closely. The high nutritive value has been confirmed by very extensive animal and clinical trials. Because it is also highly acceptable and cheap, INCAP Vegetable Mixture 9, depending on cottonseed flour for the quantity and quality of its protein, seems likely to have a considerable competitive advantage in comparison with comparable mixtures based on more costly protein concentrates.

The most serious limiting factor, the present lack of mills producing a sufficiently good grade of cottonseed flour, can be overcome through the application of existing technology. The INCAP results to date indicate that even when fed as 38% of a mixture furnishing the sole protein source of the diet, cottonseed flours containing levels of total and free gossypol in the range of 1.0, 0.04 and 0.06%, respectively, can have good nutritive values and produce no detectable adverse biological results, at least when the seed is processed in such a way that the quality of the protein is not damaged by heat.

While it is evident from the discussions of this meeting that more data are needed on the nature of the so-called "gossypol toxicity" in sensitive animals fed flours of poor quality, the results help to confirm the safety and effectiveness of cottonseed flours of high protein quality in which the initial seed does not contain excessive gossypol and is processed so that most of the pigment present is separated with the oil. Since cotton grows well in the tropical and subtropical regions where protein malnutrition is most common, the initial success of a vegetable mixture for human consumption containing 38% cottonseed flour, points to an important role for cottonseed protein in helping to meet the critical protein shortages of such areas.

Summary

INCAP Vegetable Mixture 8 containing 9% cottonseed flour and Mixture 9B containing 38% cottonseed flour, 58% whole ground corn and sorghum, 3% Torula yeast, 1% $CaCO_3$, and added vitamin A. have been found highly acceptable when prepared in the form of flavored and sweetened cereal gruels. Mixture 8 proved highly satisfactory in 15 children recovering from kwashiorkor and 5 with acute kwashiorkor, even as the sole source of protein. In 8 children fed from 2.3 to 3.5 gm. of protein per k. body weight nitrogen retention with milk averaged 19.1% of intake compared with 20.5% for Mixture 8 fed isoproteically.

Initial tolerance to Mixture 9B was established in children partially recovered from kwashiorkor; it was then used successfully in the treatment of 5 children with acute kwashiorkor. Plasma amino acids changes following a protein test-meal were studied in 6 children given either milk, Mixture 9B or a corn-bean diet for several weeks. The increase in plasma amino acid levels was essentially the same when milk or Mixture 9B constituted the protein source of the previous diet and much higher than that following the corn-bean feeding. Since this response bears a direct relationship to the protein value of the previous diet, the results are further confirmation of the similarity of Mixture 9B and milk in this respect.

In 9 children, 48 balance periods each on milk and Mixture 9B at a protein intake between 2.0 and 3.0 gm./k. day gave an average retention of 16.3% for milk and 17.8% for Mixture 9B. In 13 balance periods each at higher levels of protein intake, nitrogen retentions were still essentially the same for milk and Mixture 9B, although in 16 periods each at levels of 1.0 gm./k./day and below, retentions with milk were somewhat higher. No effect of fat on nitrogen retention was detected when it was added to either of the mixtures.

In field trials with Mixture 9B involving 115 children in Guatemala for 15-19 weeks and 53 in El Salvador for 4 weeks, approximately 95% liked the mixture and consumed it regularly. Preliminary results of acceptability trials in Honduras and Nicaragua are similar. In a marketing trial in Guatemala from March 22 to September 30, 1960, over 840,000 packages containing 75 gm. each of Mixture 9B at 3 cents each or their bulk equivalent were consumed with very favorable acceptability and no reports of intolerance.

The cottonseed flours employed were specially prepared for human consumption and contained over 50% protein of a relatively high biological value. They had approximately 1% total and 0.04 to 0.06% free gossypol. At no time was an intolerance or other adverse effect due to the cottonseed flour encountered even though it furnished nearly 80% of the dietary protein for periods ranging from a few weeks to 3 months.

Mixtures 8 and 9, and their several variations, have been given the generic name "INCAPARINA." On the basis of the extensive and favorable biological and clinical findings, they are recommended for the supplementary and mixed feeding of young children, and as low-cost protein-rich foods of good quality for persons of all ages in any area where the basic ingredients are available and where protein of animal origin is expensive or in short supply.

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