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9. ABSTRACT

Nitrogen-balance studies in convalescent malnourished infants and children have been used to determine the apparent biological value of the protein in a number of cottonseed flours. A "glandless" CSF proved to approximate most closely the value of milk protein. Cottonseed flour prepared by azeotropic solvent extraction was next in apparent quality and a CSF prepared by heat processing and screw-press extraction gave the poorest results. Cottonseed flour prepared in Peru by combining wet cooking and hexane extraction of a relatively low gossypol variety was no better than this last product. The "glandless" CSF, if used as the main or only source of protein in the diets of infants and children, should provide 8-10% of calories as protein. For the solvent-extracted CSF, a minimum of 10% protein calories seems indicated and for the heat-press extracted CSF, 12% protein calories. These results indicate that both the origin of CSF and the manner of its processing have important effects on the nutritive value of its protein. They suggest that at appropriate levels, a properly processed CSF can be the main or only source of protein in the diets of infants and children. Prolonged feeding trials are necessary to confirm or deny these observations.

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Dietary Protein Quality in Infants and Children

II. Metabolic Studies with Cottonseed Flour[1,2,3]

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A VARIETY of unconventional protein sources is already in use or under consideration for the prevention of malnutrition. Potentially, one of the most abundant and least expensive is cottonseed flour (CSF). Cottonseed meals commonly used in animal feeding have relatively high contents of gossypol, a pigment which chelates the free epsilon-amino groups of lysine, reducing its availability for protein synthesis (1). A variety of methods is used to reduce the "free" gossypol of CSF for human consumption; the Protein Advisory Group of the International Agencies has set up guidelines for its quality and elaboration, specifying the maximum tolerable levels of "free" and "total" gossypol and the minimum level of biologically available lysine (2). Cottonseed flour has been prepared by physical, chemical, or combined processing of "glanded" seeds with an undesirably high gossypol content and, more recently, from "glandless" seed. The present report covers our results with a number of such flours in the diet of convalescent malnourished infants.

In the evaluation of proteins being considered for human consumption, Hansen and Waterlow (3) stress the importance of making comparisons with milk protein at levels that are low enough to accentuate differences in quality, suggesting 1.5 g protein and 100–130 kcal/kg body wt per day. In such diets, protein represents 1.6–6.0% of total calories. In a subsequent document (4), the Pan American Health Organization and the Institute of Nutrition of Central America and Panama suggest testing at two levels of 2.0 and 1.0 g protein/kg per day. At the first level, assuming a calorie intake of 100 kcal/kg per day, protein calories represent 8% of the total, the minimum percentage generally accepted for artificial infant feeding. In human breast milk, protein of the highest quality yields slightly more than 7% of calories. At the lower level suggested, 4% of calories, all proteins are inadequate for the normal growth of human infants.

In 1960, Frenk (5) reported results in a small number of children with a commer-

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cially available CSF. Absorptions and retentions of nitrogen (N), as well as rates of weight gain, were consistently inferior to those from milk. A flour from the same source, identified as *CF-22*, was used in some of our studies.

Scrimshaw et al. (6) made a clinical evaluation in children of their *vegetable mixture* 9, containing 38% CSF. This provided 80% of the protein in the mixture, the rest coming from corn or a mixture of corn and sorghum. At levels of protein intake of 2 or more g/kg per day, no significant differences were observed in the amounts of N retained from milk or from this mixture. At lower levels, retentions from milk were consistently superior. Absorption of N from the vegetable mixture was inferior at all levels. The ages of the subjects and their calorie intakes were not reported, but if these were similar to those of others in their study, they probably were between 1 and 5 years of age and received approximately 100 kcal/kg per day. Thus, on 2 g protein/kg per day, they would have received 8% of their calories as protein.

Snyderman et al. (7) used CSF as the source of protein in the diet of four premature infants. Despite high protein intakes, the rates of weight gain were subnormal. Digestibility, biological value, and net protein utilization were consistently inferior to those of human or cow's milk, although comparable to those of most of the other vegetable proteins tested.

A recent report from India (8) described generally poor results in the initial dietary treatment of patients with kwashiorkor when the main source of protein, at very generous levels, was a CSF with a total gossypol content of 1%. When a solvent-extracted CSF was used, the results were considerably superior.

MATERIALS

The sources of protein in the diets that are the basis of this report are identified as follows:

M: A well-known modified cow's milk infant formula (Similac with iron). Cottonseed oil and cane sugar were added when it was desired to raise the calorie content, maintaining the same proportion of fat to carbohydrate as in the original product.

C: A casein product widely used in infant feeding (Casec).

CF-21: Cottonseed flour prepared at the Southern Regional Research Laboratory of the Department of Agriculture by azeotropic solvent extraction (9). Howe et al. (10) and Bressani et al. (11) carried out animal studies with this material, while Barnes et al. (12) also used another batch, *CF-32*, made by the same process. Its characteristics, as well as those of the other CSF's, are given in Table I.

CF-22: A commercially available flour (Proflo) made by heat processing and screw-press extraction.

CF-26: "Glandless" CSF elaborated at the Cottonseed Products Research Laboratory, Texas Engineering Experiment Station, Texas A & M University, by hexane extraction of Cali-

TABLE I

Characteristics of the cottonseed flours used

	Protein content, %	Available lysine, g/16 g N	Gossypol, %		PER (casein 3.0)	References
			Total	Free		
CF-21	59.3	4.38	0.17	0.02	2.36-2.61	9-12
CF-22	58.9	3.69	0.92	0.03	1.64-1.94	10, 12
CF-26	60	4.18	<0.02	<0.01	2.37	
CF-27	60	4.22	0.13	0.02	2.37	
CF-332	49.3		0.84	0.03	2.0-2.22	12, 13

PER = protein efficiency ratio. See text for definition of *CF*'s.

fornia glandless cottonseed and identified as CPRL-27195.

CF-27: A different batch of CSF made by the same acetone-hexane-water extraction as *CF-21*.

CF-332: Cottonseed flour produced at the Agrarian University of Peru by a combination of wet cooking and hexane extraction (13). Barnes et al. (12) found its nutritive value for rats and pigs to be similar to that of *CF-22*

RCF-22: A mixture of rice flour and *CF-22*, with the latter contributing approximately 75% of the protein.

METHODS

The protocol for these studies followed rather closely the recommendations of Hansen and Waterlow (3). The level of protein in the diet, however, was almost invariably higher, as we tried to establish for each child the approximate minimum amount of milk protein which supported "normal" growth, while they suggested a level just above "maintenance" requirements.

Acutely ill, severely malnourished infants suffering from diarrhea and other complications are admitted to the Pediatric Service of the British American Hospital for treatment and study. Once their acute problems are brought under control, they are transferred to the Metabolic Unit to continue their nutritional rehabilitation. In most cases, *M* is the initial source of protein.

If the manifestations of kwashiorkor are present, the total calorie intake is usually kept low (14) until edema is lost and serum albumin approaches our normal level of 4 g/100 ml. At this point, some 30 days after admission, the calorie intake is gradually increased until an accelerated rate of weight gain, usually 3–5 g/kg per day, is established. The protein intake is kept near the minimum that will support this rate of growth and maintain serum albumin above 4 g/100 ml. Occasionally, and particularly when a stubborn or unsuspected infection is present, the rate of initial recovery will be considerably slower, with periodic setbacks. Depending on the age of the patient and the severity of the growth deficit, the total calorie intake required is between 100 and 150 kcal/kg per day, protein intake betwen 2.0 and 2.5 g/kg per day, and the percentage of protein calories between 6.4 and 8.0%, most commonly 6.4 or 6.7%.

Those patients who do not have manifestations of kwashiorkor and are classified as having marasmus are usually younger and present different problems. Initially, and in contrast to those with kwashiorkor, they retain nitrogen (N) poorly, at times for many weeks. Their calorie intake must be made quite high before satisfactory N retentions of approximately 100 mg/kg per day, and weight gain as defined above, are manifest. If calorie intake is increased too rapidly, they develop hypoalbuminemia and sodium retention. Satisfactory "growth" usually requires 125–175 kcal and 2.0–3.0 g of milk protein/kg per day, with 6.4–10.0% protein calories, most commonly 8.0%.

In the initial milk diets and in all subsequent diets we maintain the same proportion of nonprotein calories as in the modified cow's milk or in human breast milk, 53% from fat and 47% from carbohydrates. This is accomplished by the addition of cottonseed oil and cane sugar in appropriate amounts. All children receive the same vitamin and mineral mixture used by Snyderman et al. (15) in their studies. One thousand five hundred units of vitamin A, 400 units of vitamin D, and 25 mg of vitamin C are given daily in one mixture. A separate mixture provides daily the following: thiamine, 0.38 mg; riboflavin, 2.0 mg; nicotinamide, 0.85 mg; calcium pantothenate, 3.5 mg; pyridoxine, 0.67 mg; inositol, 180.0 mg, para-aminobenzoic acid, 0.5 mg; folic acid, 0.05 mg; choline chloride, 147.0 mg; biotin, 0.03 mg; and cyanocobalamin, 0.015 mg. They receive 1 g/kg per day of a mineral mixture with the following composition: NaCl, 17.452%; $MgSO_4 \cdot 7H_2O$, 12.853%; $CaHPO_4$, 23.453%; $KHCO_3$, 40.997%; KCl, 2.659%; ferric citrate, 2.041%; $CuSO_4 \cdot 5H_2O$, 0.347%; $MnSO_4 \cdot H_2O$, 0.156%; KI, 0.014%; and NaF, 0.028%. Appropriate amounts of Na and K were added to make their intakes constant at 2.0 and 3.3 mEq/kg per day. Water intake was also kept constant for each child, from 100 to 125 ml/kg per day.

An appropriate protein and calorie intake from modified milk was established as indicated above before comparisons with one or more of the test proteins were made at isocaloric, isonitrogenous levels. In some children, because of presumed lactose intolerance, it was necessary to make comparisons with a casein-cottonseed oil-sucrose mixture instead of modified cow's milk. By comparing the results with the test

proteins to those with milk protein at levels that were close enough to the minimum requirements for each child, we then hoped to have for each protein a fairly accurate measurement of relative value. If these same comparisons had been made at more generous levels of protein intake, differences in quality would have been obscured. If made at very low levels of protein intake, where milk protein does not support normal indices of protein adequacy, differences in quality would have been considerably exaggerated, making it difficult to determine the level at which the test protein should be used for consistently satisfactory support of growth.

In this type of study, the apparent N retentions are by far the most reliable index of relative protein quality. The nitrogen contents of the total diet, urine, and stool were determined by the micro-Kjeldahl method. Weight gains were reported, but must not be construed to be similarly accurate during such short dietary periods. On many occasions, despite inferior N retention, a child gained as much or more weight on the test protein. This can only mean an unfavorable change in the body composition with excessive fat deposition and little increase in lean body mass, or worse still, retention of sodium and water. When such diets are prolonged and the elevated rate of weight gain continues, seemingly minor declines in serum albumin such as were seen in the present studies assume important proportions and are usually associated with an increase in liver fat. The studies of Thomas and Combs (16) support our contention that serum albumin is an accurate reflection of the body composition in such circumstances. Fortunately, when CSF is the source of dietary protein, and N retention is relatively poor, it is more common for appetite and the rate of weight gain to decrease and for the serum albumin to remain at normal levels. At this point, in our experience with children, lowering the calorie intake while maintaining the same protein intake will usually result in renewed appetite and growth, much as in the experimental animal who reduces his intake of a low protein or imbalanced diet.

RESULTS

Table II and Fig. 1 summarize the results obtained in seven convalescent malnourished infants with two of the "degossypolized" CSF's, *CF-22* and *CF-21*, and modified cow's milk or casein. The CSF's were each given for 6–10 days, almost always for 9, and the milk product given before and after each CSF period, usually for 6 days. This short period was considered adequate to correct any deficits acquired during the limited time of feeding a possibly inadequate protein. Differences in the rates of weight gain often did not reflect the more marked differences in apparent N retention and are of little significance. Little change is to be expected in serum proteins during such studies and, with some exceptions, this was usually the case.

In the table, after the case number and sex of each subject, we have indicated the age in months, the height in centimeters, and the weight in kilograms at the beginning of each study, when all of the children were making a satisfactory recovery on milk diets. In parentheses after the heights and weights are the ages in months to which these measurements correspond on the 50th percentile of the Stuart-Meredith Boston Growth Curves, giving some indication of the degree of undernutrition. After the source of protein on each line, we have listed the protein intake in grams per kilogram body weight per day, the calorie intake in kilocalories per kilogram per day, the percentage of calories as protein, the duration in days of the diet period, the days of that period during which N balances were estimated, the N intake in milligrams per kilogram per day, the apparent N absorption as a percentage of intake (not determined in females whose stool and urine were not separated), the apparent N retention as a percentage of intake and in milligrams per kilogram per day, the change in body weight in grams per kilogram body weight per day, the change in serum albumin in grams per 100 ml, and finally, its level at the end of the diet period.

In six of the seven cases, apparent ab-

TABLE II

Nitrogen balances and changes in body weight and serum albumin in infants receiving cottonseed flour or milk proteins in consecutive diets

Subject	Diet				Nitrogen balances					Changes in wt, g/kg per day	Serum albumin, g/100 ml	
	Protein + g/kg per day		kcal/kg + % protein	No. of days	Study days	mg/kg per day		% of Int				
						Int	Ret	Abs	Ret		Change	Final
043F; *hospital day 279;* age, 16 months; 66.3 cm (7 months); 8.11 kg (8 months)[a]	*M*,	2.0	100, 8.0	45	34–45	320	59		18	3.4	−0.22	3.57[b]
	CF-22,	2.0	100, 8.0	9	1–9	306	53		17	2.5	−0.02	3.55
	M,	2.0	100, 8.0	6	1–6	320	88		27	2.8	0.09	3.64
	CF-21,	2.0	100, 8.0	9	1–9	320	67		21	4.5	0.03	3.67
	M,	2.0	100, 8.0	9	1–6	291	84		29	3.1	0.45	4.12
051M; *hospital day 108;* age, 30 months; 75.5 cm (12 months); 8.74 kg (8 months)	*M*,	2.0	125, 6.4	23	18–22	320	128	69	40	3.0	0.20	4.66
	CF-22,	2.0	125, 6.4	9	1–9	320	41	64	13	3.5	−0.12	4.24
	CF-22,	2.0	100, 8.0	6	1–6	320	35	67	11		−0.19	4.05
	M,	2.0	100, 8.0	6	1–6	320	54	70	17	−0.3	0.10	4.15
	CF-21,	2.0	100, 8.0	9	1–9	320	73	79	23	2.3	0.42	4.57
	M,	2.0	100, 8.0	20	1–9	320	65	70	20	1.3	0.50	5.07
056M; *hospital day 59;* age, 31 months; 75.0 cm (12 months); 8.58 kg (8 months)	*M*,	2.0	125, 6.4	32	24–32	320	59	77	18	3.9	0.19	4.49
	CF-22,	2.0	100, 8.0	9	1–9	320	48	75	15	0.2	0.03	4.52
	M,	2.0	100, 8.0	6	1–6	320	66	88	21	3.7	0.24	4.76
	CF-21,	2.0	100, 8.0	9	1–9	320	34	83	11	1.2	−0.57	4.19
	M,	2.0	100, 8.0	6	1–6	320	60	87	19	1.9	0.68	5.07
058M; *hospital day 46;* age, 12 months; 61.8 cm (4 months); 5.93 kg (3 months)	*M*,	2.5	150, 6.7	9	4–9	400	97	72	24	9.0	0.09	4.42
	CF-22,	2.5	150, 6.7	9	1–9	400	74	67	18	6.9	0.12	4.54
	M,	2.5	150, 6.7	6	1–6	400	120	80	30	6.4	0.25	4.79
	CF-21,	2.5	150, 6.7	9	1–9	400	120	76	30	8.8	−0.32	4.47
	M,	2.5	150, 6.7	7	1–6	400	152	82	38	6.8	−0.10	4.37
061M; *hospital day 16;* age, 5 months; 54.6 cm (1 month); 3.22 kg (newborn)	*M*,	2.5	150, 6.7	34	29–34	400	140	66	35	9.4	0.03	4.29
	CF-21,	2.5	150, 6.7	9	1–9	400	134	77	34	7.2	0.21	4.50
	M,	2.5	150, 6.7	6	1–6	400	147	78	37	5.6	0.30	4.20
	CF-22,	2.5	150, 6.7	10	1–9	400	99	70	25	7.4	−0.10	4.10
	M,	2.5	150, 6.7	12	1–6	400	162	81	40	8.0	−0.15	3.95
063M; *hospital day 30;* age, 35 months; 76.5 cm (13 months); 7.25 kg (6 months)	*M*,	2.0	125, 6.4	12	10–12	320	76	58	24	4.0	0.38	4.24[c]
	CF-21,	2.0	125, 6.4	6	2–6	320	71	76	22	10.8	−0.31	3.93
	C,	2.0	125, 6.4	6	1–6	320	102	83	32	−0.5	0.09	4.02
	CF-22,	2.0	125, 6.4	6	1–6	320	73	71	23	4.0	−0.08	3.94
	C,	2.0	125, 6.4	15	1–6, 10–15	320	90	84	28	3.8	0.29	4.25
	M,	2.0	125, 6.4	6	1–6	320	89	76	28	2.5	0.30	4.55
064M; *hospital day 25;* age, 38 months; 73.0 cm (11 months); 7.08 kg (6 months)	*M*,	2.0	125, 6.4	16	11–16	320	112	67	35	3.0	1.08	4.60
	CF-21,	2.0	125, 6.4	9	1–9	309	54	78	17	3.5	−0.06	4.54
	M,	2.0	125, 6.4	9	1–9	320	85	73	26	4.0	0.25	4.79
	CF-22,	2.0	125, 6.4	9	1–9	320	60	70	18	3.6	−0.43	4.36
	M,	2.0	125, 6.4	16	1–9	320	96	62	29	4.1	0.77	5.13

Int = intake, Ret = retention, Abs = absorption. See text for definition of *CF-21*, *CF-22*, *M*, and *C*.
[a] Case number and sex, day of measurement, age, height, and weight. Values in parentheses are the ages to which these measurements correspond on the 50th percentile of the Stuart-Meredith Boston Growth Curves. [b] Urinary infection under treatment. [c] Intestinal malabsorption.

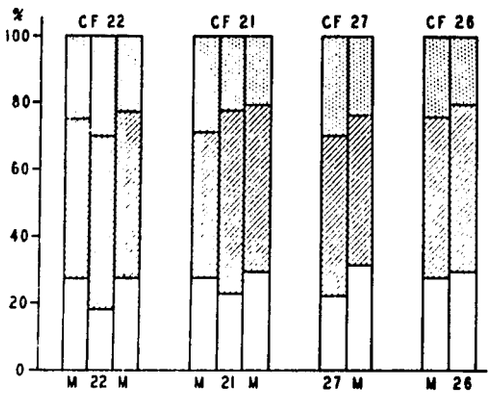

FIG. 1. Graphical summary of N balances with milk protein (*M*) and cottonseed flours (*CF-21*, *CF-22*, *CF-26*, and *CF-27*) as the sole source of dietary protein in convalescent malnourished infants and children. Stippled areas correspond to stool N, cross-hatched areas to urine N, and clear areas to apparent N retentions as a percentage of intake. For *CF-22* and *CF-21*, milk-protein periods preceded and followed the CSF diet periods. For *CF-27*, milk protein followed the CSF, and for *CF-26*, it preceded the CSF.

sorptions of N from *CF-22*, *CF-21*, and milk protein were available for comparison. In all of them, N absorption from *CF-22* was inferior to that from *CF-21* or milk. It averaged 70% of intake with a range of 67–75%. Nitrogen absorption from *CF-21* averaged 78%, with a range of 76–83%. Taking the milk period between the two CFS's, with an average of 79% and a range of 70–88%, the absorption of N, when compared with *CF-21*, was superior in three cases and inferior in three. In this type of study, we occasionally encounter relatively poor absorptions from milk, possibly the result of mild temporary lactose intolerance. In most long-term studies, the absorptions from modified cow's milk are consistently higher, averaging around 85%.

In six of the seven cases, the apparent retention of N from milk was higher than from either CSF. Again, taking the milk period between the two CSF's, the mean percentage of retention from *M* or *C* was 27, with a range of 17–37% of intake. The mean for *CF-22* was 18, with a range of 11–25%, and the mean for *CF-21* was 22.6, with a range of 11–34% of intake.

For statistical analysis, we expressed the N retentions during each CSF period as a percentage of those during the milk protein periods immediately preceding and following it, hoping in this way to eliminate variations which might have been due to changes in physiologic state or advancing state of recovery. Expressed in this fashion, N retention from *CF-22* was 67.8 ± 3.9% and from *CF-21*, 81.5 ± 9.1% of that from milk protein in the same subjects. Using Student's *t* test, the difference between *CF-22* and milk was significant ($P < 0.01$), that between *CF-21* and milk approached significance ($P < 0.1$), and that between *CF-22* and *CF-21* was not significant ($P > 0.1$, < 0.2). The mean rate of weight gain for *M* was 3.1; for *CF-22*, 3.5; and for *CF-21*, 5.5 g/kg per day. Because of the unreliability of this measure in short-term studies, no statistical analysis was done.

When we consider the changes in serum albumin, there was an apparent advantage to *M* over *CF-21* and to this over *CF-22*. For *M*, the mean change was +0.10, for *CF-21* it was −0.06, and for *CF-22*, −0.16 g/100 ml. These lend support to the impression that the superior weight gains on CSF were probably due to a combination of fat deposition and water retention.

Table III and Fig. 1 summarize the results in four children receiving *CF-27* and *M* as the sources of protein. The circumstances, however, were quite different. All four had been receiving *CF-27* as the sole source of dietary protein for periods of from 71 to 178 days, when this was changed to milk. The last three had been receiving the CSF from the time of admission. Assuming, as is undoubtedly true, that CSF protein is inferior in quality, a change to milk protein would be expected to result in a temporarily exaggerated rate

TABLE III

Nitrogen balances and changes in body weight and serum albumin in infants receiving "degossypolized" cottonseed flour and modified cow's milk as the source of dietary protein in consecutive periods

Diet				Nitrogen balances					Changes in wt, g/kg per day	Serum albumin, g/100 ml	
Protein + g/kg per day	kcal/kg + % protein		No. of days	Study days	mg/kg/day		% of Int				
					Int	Ret	Abs	Ret		Change	Final
095F; *hospital day 229;* age, 14 months; 68.5 cm (8 months); 10.08 kg (13 months)[a]											
CF-27 2.0	100,	8.0	6	1–6	302	71		23	3.4	0.10	4.96
M 2.0	100,	8.0	18	1–6	289	137		47	1.3	−0.10	4.86
110M; *hospital day 29;* age, 18 months; 66.6 cm (6 months); 5.42 kg (3 months)											
CF-27 3.0	150,	8.0	43	1–21	480	88	70	18			
				35–43	480	71	67	15	2.7	−0.19	3.75
M 3.0	150,	8.0	14	1–8	472	149	72	32	5.3	0.38	4.13
120M; *hospital day 86;* age, 21 months; 72.9 cm (11 months); 8.29 kg (8 months)											
CF-27 2.0	125,	6.4	9	1–9	312	75	72	24	0.2	0.15	4.41
M 2.0	125,	6.4	41	1–5, 18–20	320	108	74	34	3.0	0.45	4.86
126M; *hospital day 133;* age, 24 months; 70.0 cm (8 months); 7.75 kg (6 months)											
CF-27 3.0	100,	12.0	46	38–46	480	132	71	27	0.4	1.01	4.87
M 3.0	100,	12.0	16	3–11	480	128	82	26	1.6	−0.53	4.34

Int = intake, Ret = retention, Abs = absorption. See text for definition of *CF-27* and *M*.

[a] Case number and sex, day of measurement, age, height, and weight. Values in parentheses are the ages to which these measurements correspond on the 50th percentile of the Stuart-Meredith Boston Growth Curves. [b] Persistent respiratory infection.

of N retention (17) and this was the case in all but one child, *126,* who had been receiving 12% of his calorie intake from *CF-27* protein at the time the comparisons were made. In the other three, retentions from *M* exceeded those from *CF-27* by a good deal more than would have been expected on the basis of the previous comparisons between *M* and *CF-21,* which is essentially the same product as *CF-27.* In three of the four cases, N absorptions from *CF-27* and *M* were measured. Those from *CF-27* were slightly inferior to those from *CF-21.* The differences were not impressive, however, and in two of the three subjects, the absorptions were almost identical to those from milk, suggesting that the variations were due to the physiologic state of the individual.

The mean percentage of N retention from *CF-27* in the four subjects was 22.8, with a range of 17–27%, while the mean for *M* was 34.8, with a range of 26–47%.

A limited evaluation was made of a CSF (*CF-332*) produced in Peru from a low gossypol variety of cottonseed by combined cooking and hexane extraction. Results are summarized in Table IV.

In *case 079,* the absorption of N from this product was inferior to that from milk; the retention was notably inferior. Rates of weight gain were of a similar magnitude, but as indicated previously, they cannot be interpreted as synonymous with growth.

In *case 084,* absorption and retention of N from *CF-332* were again inferior to those from milk and the rates of weight gain were again similar. In this infant, an addi-

TABLE IV

Nitrogen balances and changes in body weight and serum albumin in infants receiving a locally produced cottonseed flour and modified cow's milk or a rice-cottonseed flour mixture as the source of dietary protein

Diet			Nitrogen balances					Changes in wt, g/kg per day	Serum albumin, g/100 ml	
Protein + g/kg per day	kcal/kg + % protein	No. of days	Study days	mg/kg/day		% of Int				
				Int	Ret	Abs	Ret		Change	Final
079M; *hospital day 27;* age, 13 months; 69.7 cm (8 months); 6.59 kg (5 months)[a]										
M 2.0	125, 6.4	89	84–89	320	115	84	36	3.9	0.59	3.94
CF-332 2.0	125, 6.4	38	1–20	320	62	72	19	3.9	−0.01	3.93
variable intake		21						−2.4	0.03	3.96[b]
M 2.0	100, 8.0	19	11–19	320	75	84	23	2.1	0.70	4.66
084M; *hospital day 47;* age, 7 months; 63.7 cm (4 months); 5.75 kg (3 months)										
M 2.5	150, 6.7	9	1–9	399	129	75	32	5.3	0.25	4.04
CF-332 2.5	150, 6.7	19	2–19	400	68	64	17	6.1	−0.10	3.94
RCF-22 2.5	150, 6.7	15	1–3	400	121	74	30	3.2	0.34	4.28

Int = intake, Ret = retention, Abs = absorption. See text for definition of *M*, *CF-332*, and *RCF-22*.
[a] Case number and sex, day of measurement, age, height, and weight. Values in parentheses are the ages to which these measurements correspond on the 50th percentile of the Stuart-Meredith Boston Growth Curves. [b] Infection present.

tional comparison was made with a mixture of rice flour and *CF-22*. The results with this last mixture were similar to those with milk, but distinctly superior to those with *CF-332*. This is in contrast with the poor results reported by Frenk (5) for a similar combination of rice and CSF. We often encounter marked variations in the digestion and utilization of rice by infants. Even when prolonged cooking of rice is employed, many of them digest it poorly and retain inadequate amounts of N.

The limited results with *CF-332* were not unlike those obtained with *CF-22*, confirming the similarities found by Barnes et al. (12).

Table v and Fig. 1 summarize the results in five children who received *M* and *CF-26*, the flour from "glandless" cottonseed, as the source of protein. Again the circumstances were different from those under which *CF-21* and *CF-22* were evaluated, this time favoring the CSF, as it invariably followed the milk-protein period. In such comparisons, a "carry-over" effect and the assumed good physiologic state of the subjects might give deceivingly good results for the test-protein period. This is not necessarily so, as shown by the relatively poor results with *CF-332*, even when it followed milk protein.

Apparent absorption of N from *CF-26* in these five children averaged 80% of intake, as against 74% from milk protein. The relatively poor value from milk was possibly the result of lactose intolerance in at least two of the children, who were subsequently shown to absorb N much more effectively from a casein-cottonseed oil-sucrose diet. Nevertheless, the values for *CF-26* are very satisfactory, being the same or a little better than those from *CF-21* and superior to those from *CF-22*, *CF-27*, and *CF-332*.

The apparent retention of N from *CF-26* in these five children averaged 29.4% of intake as against 27.4% from *M*. Considering the fact that three of the comparisons were made at levels of 6.4% or 6.7% pro-

TABLE V

Nitrogen balances and changes in body weight and serum albumin in infants receiving modified cow's milk and "glandless" cottonseed flour as the source of dietary protein in consecutive periods

Diet			Nitrogen balances								
Protein + g/kg per day	kcal/kg + % protein	No. of days	Study days	mg/kg per day		% of Int		Changes in wt, g/kg per day	Serum albumin, g/100 ml	
				Int	Ret	Abs	Ret		Changes	Final
120M; *hospital day 95;* age, 21 months; 72.9 cm (11 months); 8.31 kg (8 months)[a]										
M, 2.0	125, 6.4	41	36–41	296	87	81	29	3.0	0.45	4.86
CF-26, 2.0	125, 6.4	14	2–13	309	112	82	36	1.9	−0.11	4.75
123M; *hospital day 21;* age, 4 months; 60.9 cm (3 months); 4.80 kg (2 months)										
M, 2.5	150, 6.7	6	1–6	399	121	68	30	8.6	−0.89	4.06
CF-26, 2.5	150, 6.7	40	1–9	399	94	73	23	5.0	0.06	4.12[b]
125M; *hospital day 102;* age, 7 months; 56.0 cm (1 month); 4.58 kg (1 month)										
M, 3.0	150, 8.0	13	8–13	480	180	86	37	9.2	0.21	4.32
CF-26, 3.0	150, 8.0	21	1–2, 13–18	467	152	82	32	8.8	−0.49	3.83
130M; *hospital day 127;* age, 26 months; 73.0 cm (11 months); 8.38 kg (8 months)										
M, 2.0	100, 8.0	49	44–49	320	54	74	17	2.2	−0.52	4.28
CF-26, 2.0	100, 8.0	46	1–9	320	90	86	28			
			38–46	320	70	75	22	2.8	−0.71	3.57
M, 2.0	100, 8.0	16	1–6	320	68	79	21	2.9	0.73	4.30
135M; *hospital day 135;* age, 32 months; 78.8 cm (15 months); 9.80 kg (11 months)										
M, 2.0	125, 6.4	16	4–16	320	77	60	24	4.8	−0.09	4.08
CF-26, 2.0	125, 6.4	22	1–9	320	101	78	31	3.5	−0.02	4.06[b]

Int = intake, Ret = retention, Abs = absorption. See text for definition of *M* and *CF-26*. [a] Case number and sex, day of measurement, age, height, and weight. Values in parentheses are the ages to which these measurements correspond on the 50th percentile of the Stuart-Meredith Boston Growth Curves. [b] Pellagra-like dermatitis.

tein calories, they do suggest a relatively high biological value, at least in terms of measurable N retention. In one case, *130*, a further comparison with *M* was made at the end of the 46-day *CF-26* period. Whereas in the initial comparison, absorption and retention from *CF-26* were higher than from *M*, in the final comparison there was little or no difference.

The rates of weight gain on the *M* diets were generally superior to those on *CF-26*, and in two of the children, *125* and *130*, serum albumins fell below 4.0 g/100 ml, to 3.83 and 3.57 g/100 ml, after 21 and 46 days on *CF-26*, respectively. Two of the others, *123* and *135*, developed skin changes suggestive of mild pellagra on the *CF-26* diet. In the first one, it was discovered that a vitamin mixture lacking niacin and pyridoxine had been inadvertently substituted, but in the second one, the usual mixture had been given all along. This does have a low niacin content, but subsequent experience has shown that the etiology of these skin lesions is complex and that they cannot be prevented by administering 10 mg of niacin daily. They develop almost as readily on casein diets when the protein-to-calorie ratio is as low as it was in these *CF-26* diets.

DISCUSSION

Metabolic studies in convalescent malnourished infants and children have been used to evaluate a number of cottonseed flours as the only source of dietary protein. These studies were carried out at levels of total calorie and protein intake that were close to the minimum necessary for accelerated weight gain and maintenance of normal serum albumin in each child when cow's milk was the source of protein. Rates of weight gain proved to be an unreliable indicator of dietary protein quality. Changes in serum albumin, however, were generally indicative of the rates of N retention, which corresponded fairly well to the PER determinations in rats.

Azeotropic solvent-extracted CSF (*CF-21* and *CF-27*) proved to be distinctly superior to a commercial heat and screw-press extracted CSF (*CF-22*). At 6.4–8% protein calories, *CF-22* was inferior to milk protein in almost all children, while *CF-21* and *CF-27* more closely approximated its apparent biological value. Because there were differences, however, it seems advisable that if these two CSF's are to be used in the diets of infants and children, the diet should supply 10% or more of calories as protein. In the case of *CF-22*, a level of 12% is probably advisable.

The CSF (*CF-332*) produced in Peru, with free and total gossypol contents similar to those of *CF-22*, had an apparent biological value in children which was as low or lower than that of the *CF-22*, again confirming the protein efficiency ratio determination in rats.

Although comparisons were not made in the same subjects, "glandless" cottonseed flour, *CF-26*, had an apparent biological value in children, as measured by the N-balance technique, which more nearly approximated that of milk protein than that of any of the other CFS's tested. This difference had not been predicted by the animal studies. At critical levels of intake (6.4–6.7%), rate of N absorption and retention as well as rates of weight gain were practically indistinguishable from those for modified cow's milk in the same children. Nevertheless, a slight but definite decline of serum albumin levels in two of five children would argue for the use of this source of protein in the diets of infants and small children at levels of at least 8 and preferably 10% of calories as protein.

Although metabolic studies of this type serve as a fairly reliable indicator of relative protein quality, they must be complemented by prolonged feeding trials before categorical recommendations can be made for mass feeding of children. These later studies can bring out signs of toxicity or intolerance as well as effects of amino acid imbalance that are not apparent during shorter periods of observation.

SUMMARY

Nitrogen-balance studies in convalescent malnourished infants and children have been used to determine the apparent biological value of the protein in a number of cottonseed flours. A "glandless" CSF proved to approximate most closely the value of milk protein. Cottonseed flour prepared by azeotropic solvent extraction was next in apparent quality and a CSF prepared by heat processing and screw-press extraction gave the poorest results. Cottonseed flour prepared in Peru by combining wet cooking and hexane extraction of a relatively low gossypol variety was no better than this last product. The "glandless" CSF, if used as the main or only source of protein in the diets of infants and children, should provide 8–10% of calories as protein. For the solvent-extracted CSF, a minimum of 10% protein calories seems indicated and for the heat-press extracted CSF, 12% protein calories. These results indicate that both the origin of CSF and the manner of its processing have important effects on the nutritive value of its protein. They suggest that at appropriate levels, a properly processed

CSF can be the main or only source of protein in the diets of infants and children. Prolonged feeding trials are necessary to confirm or deny these observations.

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