We wish to acknowledge the technical assistance of Mr. Elwood Schiffman and Mr. James Bickley.

1. Bothwell. T. H. and Finch, C. A., "Iron Metabolism," pp. 69, 92. Little, Brown, Boston, Massachusetts (1962).
2. Bothwell, T. H., Pirzio-Biroli, G., and Finch, C. A., J. Lab. Clin. Med. 51, 24 (1958).
3. Torrance, J. D., Bothwell, T. H., and Charlton, R. W., S. African J. Med. Sci. 31, 123 (1966).
4. Cortell, S. and Conrad, M. E., Am. J. Physiol. 213, 43 (1967).
5. Forrester, R. H., Conrad, M. E., and Crosby, W. H., Proc. Soc. Exptl. Biol. Med. 111, 115 (1962).
6. Foy, A. L., Williams, H. L., Cortell, S., and Conrad, M. E., Anal. Biochem. 18, 559 (1967).
7. Williams, H. L and Conrad, M. E., J. Lab. Clin. Med. 67, 171 (1966).
8. Goodman, L. S. and Gilman, A., "Pharmacological Basis of Therapeutics," 3d ed., p. 552. MacMillan, New York (1965).

Received Oct. 14, 1968. P.S.E.B.M., 1969, Vol. 130.

Protein Evaluation of Two Species of Cucurbita Seeds* (33650)

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Protein malnutrition is recognized to occur in many areas throughout the world (1, 2). Efforts to develop suitable vegetable protein sources have been investigated as a means of supplementing current dietary protein supplies (3, 4). Surveys have indicated that about two-thirds of the total caloric intakes of the population of underdeveloped countries are derived from cereals and only about 5% from animal protein.

Since information concerning the nutritive value of the protein and the presence of other factors which may affect the protein quality in plants of the Sonora Desert is sparse, we chose to study the nutritive value of native Cucurbita seeds. These data concerning gourd seeds become important since certain species of Cucurbita are xerophytic and can be grown as a food crop in the Sonora Desert region. Two of these species, *C. digitata* gray and *C. foetidissima* HBK, were studied.

Experimental Methods. White mice of weanling age were housed in stainless steel cages with raised wire floors. Twelve mice were used per dietary treatment (equal number of each sex) and were housed two of the same sex per cage. The mice were maintained at 25 ± 1° with feed and water supplied *ad libitum.* The study consisted of seven dietary treatments (Table I). The protein sources employed were dried whole egg, *C. digitata* seeds and *C. foetidissima* seeds. The *C. digi tata* and *C. foetidissima* seeds were dried and prepared in the following manner: (i) ground, (ii) ground and fat extracted, and (iii) autoclaved for 0.5 hr at 120° (15 lb pressure). The *C. digitata* seeds had the following composition: 20% protein and 20.2% fat. The *C. foetidissima* seeds had 32.3% protein and 30.4% fat. All diets were calculated to be isocaloric and each protein source was fed at a level calculated to supply 10% protein.

The mice were weighed and sacrificed at the end of the third week. Livers were extracted from all surviving mice and immediately frozen for glutamic-oxalacetic and glutamic-pyruvic transaminase activity estimations (6, 7). The feed and feces were collected and analyzed for Cr_2O_3 marker, nitrogen, energy (8), and amino acids.

Results and Discussion. The protein sources used in the experiment were analyzed for amino acid composition by a microbiological method (9) (Table II). These results showed the seeds for *C. foetidissima* to be deficient in several amino acids, while the whole egg met the listed NRC amino acid requirements

* Arizona Agricultural Experiment Station Journal Article no. 1282.

TABLE I. Composition of Diets Used.

Dietary ingredients	1 Whole egg (%)	2 *C. digitata* (%)	3 *C. digitata* extracted (%)	4 *C. digitata* extracted, autoclaved (%)	5 *C. foetidissima* (%)	6 *C. foetidissima* extracted (%)	7 *C. foetidissima* extracted, autoclaved (%)
Whole egg	21.93	—	—	—	—	—	—
C. digitata	—	38.46	29.41	29.41	—	—	—
C. foetidissima	—	—	—	—	30.96	21.69	21.69
Corn oil	—	—	3.00	3.00	—	4.00	4.00
Cerelose	74.18	57.83	63.90	63.90	65.35	70.62	70.62
Vitamin mixture	0.14[a]	Same[c]					
Trace mineral mix	0.05[b]						
Calcium carbonate	1.20						
Dicalcium phosphate	1.90						
Salt	0.20						
Choline chloride	0.20						
Cr_2O_3	0.20						
Total	100.00	100.18	100.20	100.20	100.20	100.20	100.20

[a] Supplied the following per kg of diet (mg): ascorbic acid, 12.5; thiamine HCl, 12.5; niacin, 100.0; riboflavin, 20.0; pyridoxine HCl, 12.5; *d*-biotin, 1.25; *d*-calcium pantothenate, 75.0; vitamin B_{12} (0.1%), 10.0; folic acid, 4.00; *d*-alpha-tocopheryl-acetate, 200.0; menadione (2-methyl-1,4-naphthoquinone), 1.25; ethoxyquin, 500.0; *i*-inositol, 500.0; para-aminobenzoic acid, 25.0; oxytetracycline, 25.0; (IU): vitamin A, 14,000; vitamin D_2, 1500.

[b] Supplied the following per kg of diet (mg): Mn, 139; Fe, 385; Cu, 55; Zn, 103; Cu, 1.6; Mg, 749; molybdenum, 4.3; and I_2, 4.1 in a cerelose carrier.

[c] Diets 2–7 contained the same amounts of vitamin mixture through Cr_2O_3 as diet 1.

(10). The *C. digitata* seed was low in lysine, threonine, methionine, and extremely low in phenylalanine. The *C. foetidissima* seed was low in lysine, threonine, phenylalanine, and methionine. From these data one would expect growth results in the order of whole egg, *C. foetidissima*, and *C. digitata*.

The third week body weights were found to agree with the amino acid composition data for the dietary ingredients (Table III). The autoclaving of *C. digitata* and *C. foetidissima* seeds produced an improvement in body weight gains. The autoclaved *C foetidissima* diet produced body weights greater than those obtained with *C. digitata*. The autoclaving of *C. digitata* produced only a slight increase in growth which was not equal to *C. foetidissima*. These findings may be attributed to the low phenylalanine and tyrosine contents of the diet. Feed consumption and protein retention data showed a direct relationship to body weights and the availability of dietary amino acids. The analyzed protein values for the feed were 10%. The net protein utilization values indicated

TABLE II. Amino Acid Composition of Dietary Protein Sources.

	(g/16 g of N)		
Amino acids[a]	Whole egg	*C. digitata* seed	*C. foetidissima* seed
Lysine	8.0	4.1	4.0
Histidine	2.6	3.2	2.1
Arginine	1.2	1.2	1.3
Threonine	3.6	1.4	1.5
Valine	8.8	3.4	4.0
Leucine	8.2	4.8	4.6
Phenylalanine	4.4	0.6	3.9
Tyrosine	3.9	5.4	4.9
Methionine	2.9	1.4	1.4
Isoleucine	6.4	3.1	3.2

[a] Determined microbiologically.

TABLE III. Effect of Dietary Protein Source on Growth and Nutrient Utilization on Mice.

Protein source	Third week body wt. (g)[e]	Apparent protein digestion (%)	Av feed consumption/mouse (g)	Protein efficiency ratio[f]	Gross energy (kcal/g)	Digestible energy (kcal/g)
1. Whole egg	18.8[d]	82.23	14.2	6.901	3.97	3.72
2. *C. digitata*	11.8[a]	75.29	11.5	2.435	4.21	3.72
3. *C. digitata*, extracted	11.2[a]	77.40	11.1	1.982	3.81	3.42
4. *C. digitata*, extracted, autoclaved	13.1[b]	71.22	14.6	2.808	3.88	3.36
5. *C. foetidissima*	13.2[b]	72.39	12.2	3.443	3.99	3.54
6. *C. foetidissima*, extracted	13.3[b]	73.45	12.9	3.333	3.83	3.42
7. *C. foetidissima*, extracted, autoclaved	16.7[c]	66.27	16.4	4.695	3.80	3.33

[e] Means having different superscripts are statistically significant at the 0.05 level of probability (Duncan 1955).

[f] PER = gain in body wt./protein intake.

that the whole egg was of the highest protein quality closely followed by the autoclaved *C. foetidissima* (Table III). All diets containing *C. foetidissima* were utilized to a greater extent than the *C. digitata* feeds, apparently as a result of the low phenylalanine and tyrosine contents of the *C. digitata* seeds.

All dietary treatments were calculated to be isocaloric but slightly different values were obtained upon analysis (Table III). The digestible energy figures were virtually the same for all diets except those containing dried whole egg and unextracted *C. digitata*. The whole egg had a 4% greater digestible energy than the other diets. *C. digitata* has been reported to be high in conjugated fatty acids (5) and this may account for the lower energy values.

Lysine, isoleucine, and methionine retentions were examined for all eight diets (Table IV). The results for lysine were approximately the same for all treatments. Whole egg had a 91% retention of isoleucine, while the *C. digitata* and *C. foetidissima* were in the 70% range. Ether extracted *C. digitata* samples showed increased isoleucine retention. The *C. foetidissima* was not affected by fat extraction but did show a reduction in isoleucine retention after autoclaving. Methionine retention values were 91% for whole egg and for the autoclaved *C. digitata* sample; while autoclaving increased methionine retention for *C. digitata* it had little or no effect on the *C. foetidissima* sample.

Additional amino acid retentions were determined using the four diets containing whole egg, and the autoclaved and extracted *C. digitata* and *C. foetidissima* seeds. Whole egg had higher retention values for all the amino acids than did the *C. digitata* or *C. foetidissima* seeds (Table V). *Cucurbita foetidissima* showed lower retention values than *C. digitata* for all amino acids with the exception of phenylalanine. The dietary level

TABLE IV. Amino Acid Retention from Different Protein Sources.

Dietary treatment	Lysine (%)	Isoleucine (%)	Methionine (%)
1. Whole egg	98.55	91.21	91.36
2. *C. digitata*	96.04	71.90	82.86
3. *C. digitata*, extracted	97.00	77.19	81.83
4. *C. digitata*, extracted, autoclaved	95.58	77.25	91.04
5. *C. foetidissima*	96.65	75.83	74.37
6. *C. foetidissima*, extracted	96.85	73.30	76.16
7. *C. foetidissima*, extracted, autoclaved	95.76	67.15	78.15

TABLE V. Percent Amino Acid Retentions from Different Proteins.

Amino acids	No. 1 Whole egg	No. 4 *C. digitata*, extracted, autoclaved	No. 7 *C. foetidissima*, extracted, autoclaved
Leucine	89.84	81.43	70.11
Threonine	82.20	79.10	63.51
Arginine	92.16	87.67	85.25
Histidine	83.02	78.30	72.72
Phenylalanine	85.92	14.10	77.15
Valine	—	70.09	67.54
Tyrosine	79.19	68.01	67.27

of phenylalanine was low for *C. digitata* and in addition a low retention value was obtained (Table V).

TABLE VI. Effect of Different Protein Sources on Liver, Glutamic-Oxalacetic and Glutamic-Pyruvic Transaminase Activities.

Dietary protein source	Liver glutamic-oxalacetic[c] transaminase[d]	Liver glutamic-pyruvic[c] transaminase[d]
1. Whole egg	18.38^{a}	2.67^{a}
2. *C. digitata*	40.38^{b}	6.90^{b}
4. *C. digitata*, extracted, autoclaved	30.91^{ab}	6.54^{ab}
5. *C. foetidissima*	40.08^{b}	7.35^{b}
7. *C. foetidissima*, extracted, autoclaved	31.31^{ab}	6.01^{ab}

[c] Enzyme activity = OD/min/mg of protein.
[d] Means having different superscripts are statistically different at the 0.05 level of probability (Duncan 1955).

A relationship of liver enzyme activity to protein quality and amino acid availability was demonstrated by glutamic-oxalacetic and glutamic-pyruvic transaminase activity measurements (Table VI). The GOT activity was lowest for mice fed the whole egg and was elevated with the feeding of untreated seeds; thus, GOT activities were lowered by the feeding of the autoclaved seeds. The liver activities of GPT showed similar effects to those noted above (Table VI). The higher the quality of the protein, the lower the transaminase activity. These results would indicate that a better balance of amino acids in the protein produced a lowering of transaminase activities.

Summary. An experiment was conducted with weanling mice to evaluate the protein quality of two samples of gourd seeds (*Cucurbita digitata* gray and *C. foetidissima* HBK). These materials were compared with whole egg protein. The gourd seeds were fed after fat extraction and after autoclaving (30 min at 120°) in addition to the feeding of the untreated ground material. Amino acid analyses showed that the *C. digitata* seeds were deficient in phenylalanine plus tyrosine, methionine, and threonine; while, the *C. foetidissima* seeds were deficient in methionine and threonine. Nitrogen retention data, liver transaminase activities and amino acid retention estimates all indicated that *C. foetidissima* seeds were superior to *C. digitata* in protein quality and that *C. foetidissima* seeds could be improved by autoclaving but was still of lower protein quality than whole egg.

1. Anderson, W., French, D., Scrimshaw, N. S., and McNaughton, J. W., Am. J. Pub. Health 49, 1964 (1959).

2. Behar, M., Bressani, R., and Scrimshaw, N. S., *in* "World Review of Nutrition and Dietetics," Vol. 1, p. 75. Pitman, New York (1959).

3. Scrimshaw, N. S., Squibb, R. L., Bressani, R., Behar, M., Viteri, F., and Arroyave, G., *in* "Amino Acid Malnutrition" (W. H. Cole, ed.), p. 28. Rutgers Univ. Press, New Brunswick, New Jersey (1957).

4. Squibb, R. L., Wyld, M. K., Scrimshaw, N. S., and Bressani, R. J., Nutr. 69, 343 (1959).

5. Bemis, W. P., Berry, J. W., Kennedy, M. J., Woods, D., Moran, M., and Deutschman, A. J., Jr., J. Am. Oil Chem. Soc. 44, 429 (1967).

6. Karmen, A., J. Clin. Invest. 34, 131 (1955).

7. Wroblewski, F. and LaDue, J. S., Proc. Soc. Exptl. Biol. Med. **91**, 569 (1956).
8. Weber, C. W. and Reid, B. L., Poultry Sci. **64**, 1190 (1967).
9. Association of Official Agricultural Chemists, Methods of Analysis, 1965.
10. Natl. Res. Council, Natl. Acad. Sci. #10 (U. S.) Publ. **990**, (1962).

Received Oct. 14, 1968. P.S.E.B.M., 1969, Vol. 130.

Immunogenicity of Electrophoretically Purified Guinea Pig Transplantation Antigen (33651)

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Transplantation antigens are substances which induce specific accelerated (second-set) rejection of donor tissue grafts (1). Billingham *et al.* (1) first demonstrated hastened graft rejection following the administration of subcellular extracts. It is now generally accepted that subcellular membrane (2–5) and nonsedimentable (4, 6, 7) fractions can induce specific allograft immunity.

Recently there has been interest in solubilized transplantation antigens, i.e., in substances released from their usual location on the surface membrane of cells (8). Sonic energy was first employed to liberate transplantation antigen from spleen cells as a water-soluble material which was immunogenic when administered subcutaneously (6, 7). Recently, water-soluble transplantation antigen has been isolated from the spleen and lungs of inbred, histoincompatible guinea pigs and purified 10 thousandfold by discontinuous electrophoresis on polyacrylamide gels (9). Only one electrophoretic component (component 15, R_f 0.73–0.74) possessed antigenic activity as determined by its ability to elicit specific, delayed type hypersensitivity reactions in presensitized, allogeneic guinea pigs. This assay system was exceedingly rapid and sensitive, since 0.1 μg of antigen could be detected. Once the antigenic activity had been localized by this method, the immunogenicity tests described herein were performed to establish this material as a transplantation antigen. Only component 15 among the 17 electrophoretic fractions resolved on acrylamide gel was able to specifically sensitize allogeneic hosts against donor strain skin grafts.

Methods. Preparation of the antigen. Spleen and lungs obtained from 240 inbred, histocompatible strain 2 guinea pigs were minced in Tris–sucrose buffer (0.05 *M* Tris, 0.008 *M* magnesium chloride, 0.0025 *M* potassium chloride, 0.15 *M* sucrose, adjusted to pH 7.45, at 25°). Single cell suspensions were prepared by consecutive passes through nylon screening of 50 and 200 mesh. The suspension was centrifuged at 950*g* and the cell pellet obtained was suspended in Tris–sucrose buffer containing 3% (w/v) of acetic acid to lyse the erythrocytes. The residual cells were washed twice with Tris–sucrose buffer and then passed through a fine nylon mesh sieve. The cell concentration was adjusted to 30–40 $\times 10^6$ cells/ml as determined on a model D Coulter counter at a threshold of 45/∞. Fifty-ml aliquots of the cell suspension were exposed to a 3-min burst of 15.5 W/cm^2 sonic energy generated over a diaphragm of 4.7 cm in a Raytheon model DF 101 magnostrictive oscillator. The sonication chamber was maintained at less than 5° by perfusion with alcohol at −10°. The sonicated suspension was first centrifuged at 3500 rpm, and then at 105,000–140,000*g* to remove the cellular membranes. The ultracentrifugal supernate was concentrated against Aquacide I, C Grade (Calbiochem) and then was passed in ascending fashion at a flow rate of 3–6 ml/hr through a column of Sephadex G-200 (2.5 $\times$ 100 cm) which had been equilibrated with Tris–glycine buffer,