COMPARISON OF A CHEMICAL AND A BIOCHEMICAL METHOD FOR DETERMINING THE BIOLOGICAL VALUE OF PROTEINS AND AN EVALUATION OF THE ENDOGENOUS NITROGEN [1]

By FLOYD C. OLSON, formerly *research assistant*, and LEROY S. PALMER, *agricultural biochemist, Division of Agricultural Biochemistry, Minnesota Agricultural Experiment Station*

INTRODUCTION

Since the discoveries that animals require specific amino acids and that amino acid distribution in proteins differs with a resultant difference in nutritive quality, a chemical method for determining the nutritive value of proteins has been sought. Almquist, Stokstad, and Halbrook (*1*)[2] proposed a chemical method which differed from any previously used. Analyses of animal protein concentrates were made for intact protein, protein decomposition products, indigestible protein, and hot-water-soluble protein. A formula was then used to give a numerical "protein quality index" (*1, p. 205*).

The purpose of the present study was to compare the method of Almquist et al. with the Mitchell and Carman (*10*) modification of the Thomas-Mitchell (*8*) nitrogen-retention method of determining the biological value of protein. The digestion of the protein-containing materials was compared in vitro and in vivo. In addition, a comparison was made between the endogenous urinary nitrogen output when corrected according to body weight and when corrected according to body surface area (*12*).

PROTEIN-CONTAINING MATERIALS

The protein-containing materials studied are given in table 1. The cereals and cereal protein concentrates were commercial products and were used without purification. Soybean meal 1 was a solvent-extracted meal at 60° C. that had been heated after extraction to 110° for 30 minutes to drive off the residual solvent. Soybean meal 2 was a portion of meal 1 that had been heated at 110° for 15 hours after extraction. It had the characteristic straw color of heated soybean meals. The dry whole-egg and liver meals were commercial preparations that had been soaked in alcohol, extracted with ether for 48 hours and air-dried. The tankage, a commercial meat and bone product having about 50 percent of crude protein, was soaked in 95-percent ethanol, extracted with ethyl ether for 48 hours, and air-dried. The casein was a commercial casein which was purified twice by dissolving in ammonia, running through a supercentrifuge, and reprecipitating with a mixture of acetic and hydrochloric acids; it was then extracted with alcohol for 24 hours, with ether for 48 hours,

[1] Received for publication October 4, 1939. Paper No. 1681, Scientific Journal Series, Minnesota Agricultural Experiment Station. The data in this paper are taken from a thesis presented by Floyd C. Olson in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Minnesota, 1938. The original data are on file in the Division of Biochemistry, University of Minnesota.

[2] Italic numbers in parentheses refer to Literature Cited, p. 341.

and dried at 40° in vacuum. The crude-protein content of the prepared foods is given in table 1.

TABLE 1.—*True digestibility and biological value of proteins tested by the nitrogen-retention method*

Source of protein	Crude protein content as prepared	Proportion in diet	Tests	True digestibility		Biological value	
				Average with standard error	Standard deviation	Average with standard error	Standard deviation
	Percent	*Percent*	*Number*	*Percent*		*Percent*	
Dried whole egg	77.18	8	12	97.9±0.17	0.58	93.9±0.82	2.84
Casein	91.68	8	11	99.7± .35	1.16	62.9±1.83	6.09
Whole wheat	13.40	8	11	91.7± .59	1.97	47.2±1.28	4.24
Wheat gluten	76.38	8	10	99.3± .21	.66	41.9±1.44	4.56
Whole corn	10.06	8	12	91.3± .53	1.83	52.5±1.10	3.80
Corn-gluten meal	42.63	8	10	96.7± .41	1.30	41.8±1.71	5.40
Liver meal	76.25	8	12	88.3± .40	1.38	56.5± .84	2.90
Tankage	56.19	12	14	83.3± .50	1.88	38.1±1.63	6.10
Soybean meal 1	47.70	8	10	83.9±1.10	3.48	61.1±1.16	3.68
Soybean meal 2	46.00	8	10	84.3± .86	2.70	68.5±1.06	3.35

EXPERIMENTAL METHODS

NITROGEN BALANCES

Two different series of nitrogen-balance experiments were run. The first series was performed in the Division of Agricultural Biochemistry, University of Minnesota. The metabolism cages and collection of excreta were essentially as described by Mason and Palmer (*7*). To prevent loss of scattered feed a porcelain feed dish was set into the center of a 1-pound coffee can, the side of which was cut down in such a way that wire netting placed over the top gave an incline of about 40°.

The biological values of the tankage and soybean meals 1 and 2 were determined in the Department of Agricultural Chemistry at Ohio State University, where a different technique was necessary because of a different type of cage. The technique used here was essentially that of Mitchell (*8*) except that the filter paper containing the absorbed urine was submitted to direct nitrogen determination, blanks being run on the same amount of similar filter paper.

The experimental protein rations furnished 4.5 percent of dry whole-egg protein in the nitrogen-free periods, and 8 percent of protein in the protein-feeding periods, except for the tankage which was fed at a 12-percent level in order to promote growth. To this was added 4.5 percent of Hawk and Oser (*5*) salt mixture, 1 percent of NaCl, 2 percent of cod-liver oil, filtered butterfat to give a total fat content of 10 percent, agar to give a crude-fiber content of about 2 percent, 0.3 percent of vitamin B_1 concentrate,[3] and tapioca dextrin to complete the 100 percent.

In the first series young male rats only were used from the highly inbred strains in the rat colony of the Division of Biochemistry, University of Minnesota. In the second series the rats, including eight females, were purchased from a commercial breeder.

All the experiments involved 4-day transition periods and 7-day collection periods. Two experimental protein-containing rations were

[3] A commercial product, containing 105 International Units of vitamin B_1 per gram and 9.04 percent of nitrogen.

fed in the periods between initial and final nitrogen-free periods. The rats were so divided that one-half the products were tested in the first protein-feeding period and one-half in the second.

The appetite of the rats during the nitrogen-free periods was good, and they all gained slightly in weight. The food intake on the wheat gluten and corn-gluten meal was rather low, but all rats which did not gain weight were discarded.

CHEMICAL ANALYSES

The same protein products used in the feeding experiments were analyzed by the procedure of Almquist and associates (*1*). In this method pepsin HCl alone is the digesting agent. In order to test its completeness, peptic [4] digestion was followed by tryptic,[5] the solution being made alkaline to an alkalinity of 0.5-percent sodium carbonate with solid sodium carbonate, 25 ml. of 1.5-percent trypsin solution was added, and the sample shaken by machine for another 24 hours at 40°C. The insoluble material was analyzed for nitrogen. Blanks for the enzymatic digestion were run in the same manner as when protein was present. The activity of the enzyme preparations is indicated by the fact that they effected nearly 100-percent digestion of casein.

It was noticed in the peptic-tryptic digestion of liver meal and tankage that a copious reddish-brown precipitate formed on neutralizing with sodium carbonate. This was thought to be porphyrin, the hydrochlorides of which are soluble but which are insoluble in neutral or basic solution. A nitrogen analysis of the porphyrin precipitate was made in separate experiments to determine how much of this fraction classed as digestible by the procedure of Almquist et al. (*1*) would be classed as indigestible in the pepsin-trypsin digestion.

EXPERIMENTAL DATA

The endogenous urinary nitrogens in the nitrogen-balance experiments, corrected for body weight, are presented graphically in figure 1. The values used for plotting figures 1 and 2 were taken from table 2 and a third N-free period on 11 rats not shown in this table. The formula for the regression line was first calculated in terms of logarithms and then converted into arithmetical terms. The formula is $N=135.6\ W^{-0.469}]$, where N equals endogenous urinary nitrogen in milligrams and W equals body weight in grams. The negative slope of the curve indicates that the endogenous urinary nitrogen per unit of body weight decreases with an increase in body weight. This formula differs considerably from Ashworth's (*3*) formula, $N=69.3\ W^{-0.274}$ calculated for certain of Mitchell's data on young rats.

Smuts (*12*) reported that the endogeneous urinary nitrogen is more closely related to body surface than to body weight. Figure 2 shows the same data as figure 1 plotted in relation to the body surface, this being calculated by Lee's (*6*) formula, $S=12.54\ W^{0.60}$ where S is the body area in square centimeters and W is body weight in grams. The formula for the regression line in figure 2 is $N=5.61\ A^{0.069}$, where N is the endogenous nitrogen in milligrams and A is body-surface area in square centimeters. When the data are calculated according to Fisher's (*4*) t-value, the exponent is shown to be not significantly different from zero.

[4] Powdered pepsin, U. S. P. X., Merck, was employed. [5] Trypsin, Difco, 1:110, was employed.

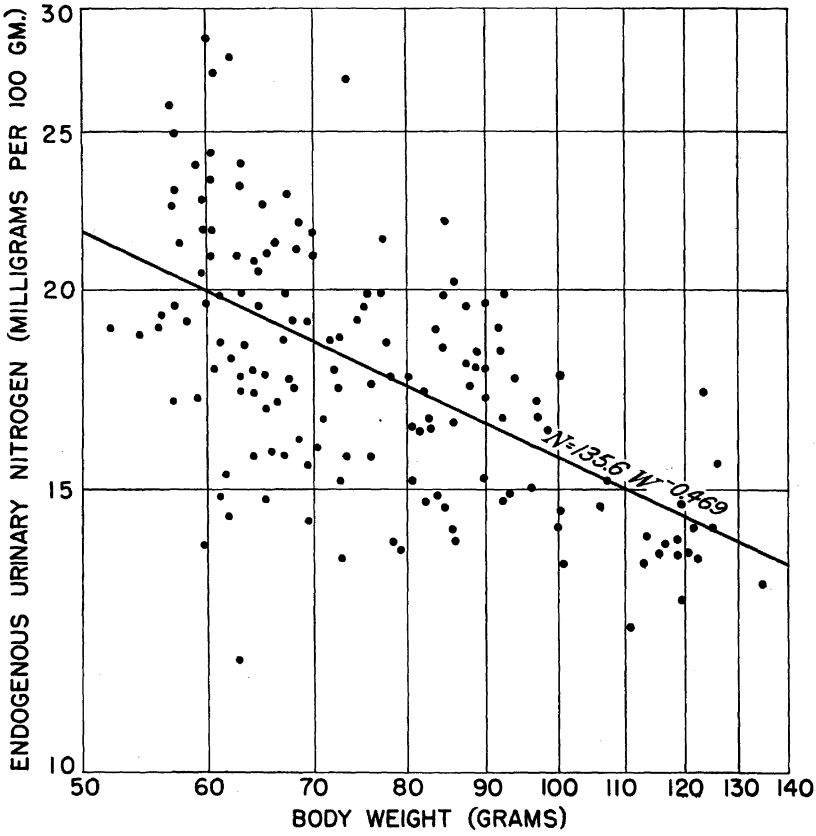

FIGURE 1.—Regression curve of milligrams of endogenous urinary nitrogen per 100 gm. of body weight plotted against body weight.

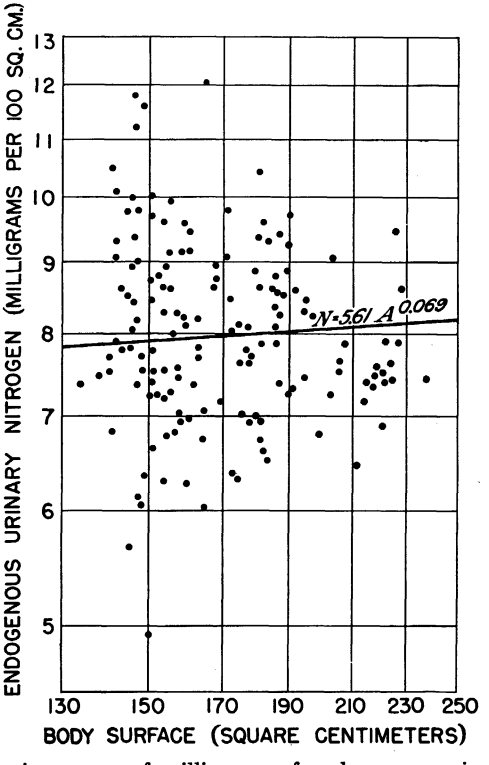

FIGURE 2.—Regression curve of milligrams of endogenous urinary nitrogen per 100 cm.^{2} of body surface plotted against body-surface area.

TABLE 2.—*Summary of endogenous urinary nitrogen data from which figures 1 and 2 were drawn*

Rat No.	First N-free period				Second N-free period			
	Average weight of rat	Food consumed daily	Endogenous urinary N daily	Endogenous urinary N per 100 cm.[2] of body surface	Average weight of rat	Food consumed daily	Endogenous urinary N daily	Endogenous urinary N per 100 cm.[2] of body surface
	Grams	*Grams*	*Milligrams*	*Milligrams*	*Grams*	*Grams*	*Milligrams*	*Milligrams*
1	69	7.6	15.2	9.55	125	10.5	17.9	7.88
2	65	7.4	14.7	9.58	127	10.6	19.8	8.63
3	65	7.1	11.5	7.49	124	10.9	21.4	9.46
4	54.5	5.9	10.3	7.46	106.5	8.1	15.7	7.61
5	67	6.7	12.5	8.00	103.5	6.3	14.7	7.24
6	66.5	6.6	14.2	9.13	106	6.1	15.5	7.53
7	61.5	6.4	9.0	6.06	92.5	5.9	18.5	9.75
8	61	6.3	9.1	6.16	104.5	6.1	18.4	9.02
9	65.5	6.8	9.7	6.29	81	5.1	12.3	7.02
10	71	7.3	13.3	8.22	119.5	8.2	15.3	6.91
12	61.5	6.8	9.5	6.40	108	8.1	16.4	7.88
13	65	6.8	12.7	8.27	86	6.2	12.2	6.72
14	67.5	8.2	13.5	8.60	87.5	8.0	17.1	9.32
15	63	7.2	12.7	8.43	81	7.0	13.3	7.60
16	64.5	7.7	13.5	8.84	83	6.9	13.8	7.77
17	65.5	8.3	13.8	8.95	88.5	8.9	15.4	8.34
18	60.5	7.3	13.2	8.98	72.5	6.8	12.6	7.69
19	58	6.7	12.4	8.65	78.5	8.0	13.8	8.03
20	68	7.4	11.8	7.48	120.5	9.6	16.5	7.42
21	68.5	7.3	11.1	7.01	86	6.0	12.0	6.61
22	62.5	6.7	13.1	8.74	73.5	5.5	11.6	7.02
23	59	5.8	12.4	8.56	70	4.9	11.2	6.98
24	64	7.3	11.0	7.24	79	5.0	11.0	6.38
25	64	7.5	10.1	6.64	111	8.1	13.7	6.47
26	77.5	8.3	15.4	9.03	96.5	5.6	14.5	7.46
27	75	8.0	14.4	8.61	92.5	6.8	13.7	7.22
28	65.5	7.5	11.1	7.20	80	5.0	11.0	6.38
29	69.5	6.7	11.1	6.95	83	5.2	12.3	6.92
30	78.5	8.2	14.6	8.49	98.5	7.6	16.2	8.23
31	60.5	6.1	10.8	7.35	73	4.0	9.9	6.02
32	62	7.4	11.2	7.51	83	8.5	13.7	7.71
33	61.5	6.7	11.4	7.68	75.5	6.2	14.7	8.76
34	58.5	6.0	11.2	7.78	76.5	7.0	12.1	7.15
35	52	6.3	9.9	7.38	67	6.0	10.6	6.78
36	57.5	6.0	11.3	7.92	72	6.3	12.8	7.84
37	57.5	6.2	13.3	9.33	70	6.8	15.2	9.47
38	57.5	6.1	14.4	10.10	69.5	5.7	10.0	6.26
39	71	7.4	11.9	7.35	82.5	5.8	14.3	8.07
40	63	5.9	10.9	7.24	68.5	4.8	14.5	9.15
41	73	7.4	11.1	6.75	81.5	6.1	13.4	7.62
42	66.5	6.3	11.3	7.26	70	4.4	14.7	9.16
43	56.5	6.3	10.9	7.73	69.5	5.3	13.3	8.32
44	63	6.9	11.1	7.37	73.5	6.1	19.9	12.05
45	66	7.4	10.5	6.78	80	6.2	14.1	8.11
46	60	5.9	11.8	8.07	67.5	5.3	15.5	9.87
47	56	6.4	10.6	7.55	68	6.1	11.9	7.55
48	68	6.7	13.0	8.24	78	5.6	16.8	9.81
49	63.5	6.6	11.8	7.80	72	5.5	13.4	8.21
50	63	8.0	(1)	(1)	93	7.7	13.9	7.30
51	60	7.8	8.3	5.67	97	8.0	16.5	8.46
52	61	8.0	12.1	8.19	97	8.0	16.2	8.30
53	61	7.9	14.3	9.68	89	8.0	15.9	8.58
54	63	8.0	7.4	4.91	92	8.0	17.5	9.26
55	57	8.0	9.7	6.84	85	7.4	15.6	8.65
56	63	8.0	(1)	(1)	84	7.9	12.5	6.98
57	59	8.0	11.3	7.80	86	8.0	17.5	9.64
58	60	7.9	12.3	8.41	85	7.9	16.9	9.37
59	62	7.9	17.3	11.60	76	6.8	13.3	7.89
60	59	7.9	(1)	(1)	93	7.9	15.4	8.09
61	60	7.9	13.7	9.36	89	8.0	16.3	8.80
62	60	7.9	13.1	8.95	90	7.9	15.4	8.25
63	65	7.8	13.3	8.66	94	8.0	16.5	8.62
64	64	7.9	11.4	7.50	92	8.0	16.8	8.89
65	63	8.0	14.6	9.69	85	8.0	18.8	10.43
66	63	8.0	15.1	10.02	90	7.9	16.0	8.57
67	60	7.8	14.6	9.98	76	7.5	15.1	8.96
68	62	8.0	(1)	(1)	90	8.0	17.6	9.43
71	60	7.9	17.2	11.76	90	7.7	13.8	7.40
72	59	7.7	14.1	9.74	86	7.7	14.3	7.87
73	60.5	7.9	16.5	11.22	88	7.7	15.8	8.58
74	57	7.7	14.9	10.50	84	7.7	15.9	8.88
75	57	7.9	12.8	9.02	85	7.7	12.5	6.93

[1] First endogenous urinary N lost. Results based on second period.

When the data from Mitchell's laboratory, as used by Ashworth (*3*) are plotted in figure 3 on the body-surface-area basis, the regression formula $N=3.44\ A^{0.200}$ is obtained. This formula shows a larger increase in endogenous urinary nitrogen per unit of body surface with an increase in body surface than does the regression line in figure 2. An inspection of figure 2 shows a fair grouping of the values around the regression line. However, the values from Mitchell's laboratory show no grouping around the regression line but a wide scattering over the range of 5 to 17 mgm. per 100 cm.^{2} of body surface. This wide scattering of the points appears from the original data to be owing more to a variation between groups on different rations than to differences between individual animals. This would indicate that the variation was due to lack of complete control of the experimental procedure. For example, in some cases, even with whole egg in the ration, the animals lost weight, indicating that the food intake was not sufficient for the energy requirements. This would tend to increase the endogenous urinary nitrogen. Ashworth (*2*) reported that a 4-day preliminary period on a nitrogen-free ration is not sufficient for attainment of a low food intake. Mitchell and Beadles (*9*) reported a case in which the true endogenous level was not reached with a 4-day preliminary period.

However, it is to be noted that some of the values from Mitchell's data are for rats larger than those plotted in figure 2. Other determinations of endogenous urinary nitrogen on adult rats (not reported here) have given values considerably higher than 8 mgm. per 100 cm.^{2} of body surface even though a longer depletion period on a nitrogen-free ration was used. Smuts (*12*) found that rats weighing 150 gm. or more give an average endogenous urinary nitrogen of 15.40 mg. daily per 100 cm.^{2} of body surface. Calculations made from results obtained by Mason and Palmer (*7*) and by Lohn [6] from this laboratory on adult rats show values almost as high as those obtained by Smuts. Thus, it would seem that, whereas young rats of 50 to 100 gm. body weight show an endogenous urinary nitrogen output of 6 to 10 mg. per 100 cm.^{2} of body surface, older rats attain a considerably greater average output when calculated per unit body surface.

Table 1 gives the biological value and true digestibility with the standard errors and standard deviations for the test proteins. For the biological value calculations, the endogenous urinary nitrogen was corrected for changes in body surface area, and the metabolic fecal nitrogen was corrected for each gram of dry food intake.

Table 3 shows the significance of the differences between certain means, by using formulas given by Treloar [7] and referring to Sheppard's (*11*) tables for the relative deviate, x', in the determination of the probability for comparisons of biological value and digestibility. For comparisons of endogenous urinary nitrogen where correlation is expected, Fisher's (*4*) formula was used.

[6] LOHN, C. A STUDY OF THE EFFICIENCY OF FOOD METABOLISM FOR THE MAINTENANCE OF INBRED ANIMALS DIFFERING IN THEIR EFFICIENCY OF FOOD UTILIZATION DURING GROWTH. Thesis, Ph. D., Univ. Minn.

[7] TRELOAR, ALAN E. AN OUTLINE OF BIOMETRIC ANALYSIS. 3 v. in 1. Burgess Publishing Co., Minneapolis. 1935. [Mimeographed.] See pp. 28–29, and 56.

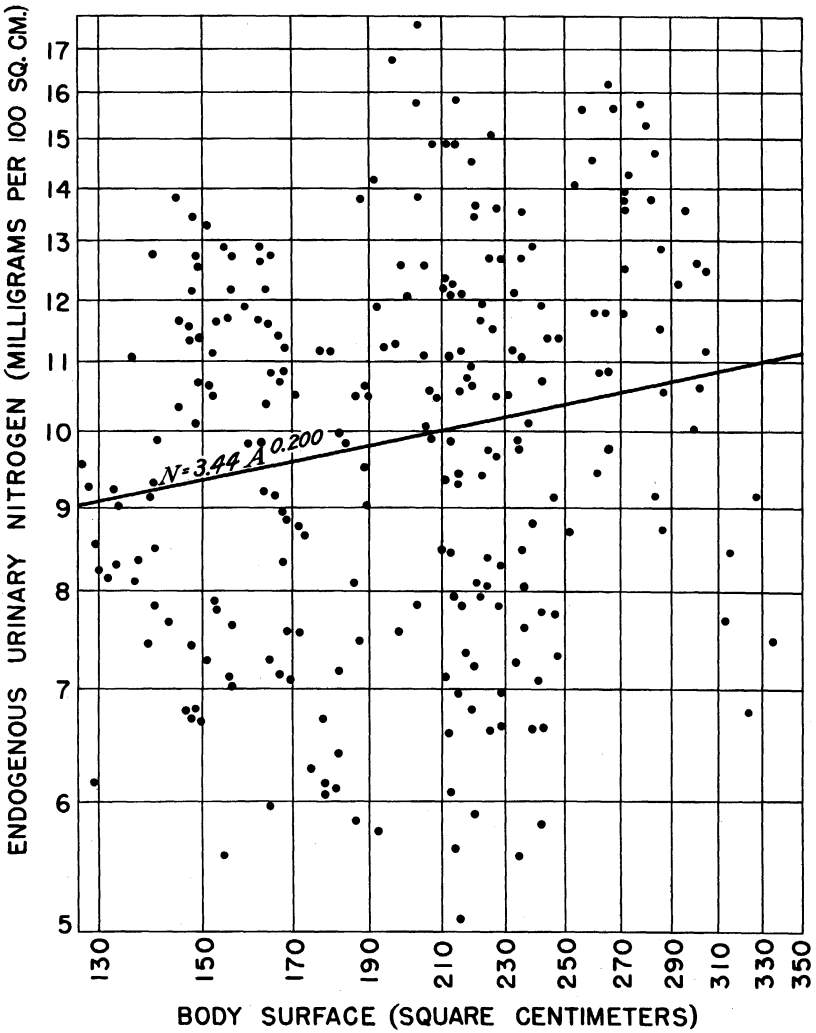

FIGURE 3.—Regression curve of milligrams of endogenous urinary nitrogen per 100 cm.^{2} of body surface plotted against body-surface area. Mitchell's data as cited by Ashworth (*3*).

TABLE 3.—*Determination of significance of differences in means for endogenous urinary-nitrogen and other data*

Item	Period or value	Mean	Standard deviation	Probability
		Milligrams		
Endogenous urinary nitrogen per 100 gm. of body weight.	First N-free period	19.6	3.4	0.001
	Second N-free period	17.1	2.6	
Endogenous urinary nitrogen per 100 cm.² body surface.	First N-free period	8.2	1.4	.674
	Second N-free period	8.1	1.1	
Endogenous urinary nitrogen per 100 gm. of body weight, Mitchell's data (*3*).	First N-free period	22.1	5.0	.001
	Second N-free period	18.8	6.1	
Endogenous urinary nitrogen per 100 cm.² of body surface, Mitchell's data (*3*).	First N-free period	10.2	2.2	.92
	Second N-free period	10.3	3.3	
		Percent		
Whole wheat	Biological value	47.2	4.2	.006
Wheat gluten	do	41.9	4.6	
Whole corn	do	52.5	3.8	.001
Corn-gluten meal	do	41.8	5.4	
Soybean meal 1	do	61.1	3.7	.001
Soybean meal 2	do	68.5	3.3	
Soybean meal 1	Digestibility	83.9	3.5	.83
Soybean meal 2	do	84.3	2.7	

Table 3 shows that when the endogenous urinary nitrogen is corrected for body weight, a significant fall is noted between the first and second periods, but not when the correction is made for body surface. This is also shown to be true for the data from Mitchell's laboratory (*3*). A significant difference is shown between the mean biological value of whole-wheat and wheat-gluten proteins, between whole-corn and corn-gluten proteins, and between raw and heated soybean-meal proteins. However, no significant difference is shown in the digestibility of the two soybean meals.

The results obtained by the chemical method of Almquist et al. (*1*) are given in table 4. The digestibility determinations of the proteins in vitro do not agree very closely with the corrected digestibility obtained from the rat data. The corn proteins show a higher digestibility in vivo while whole-wheat, soybean, and tankage proteins tend to give better digestion in vitro. The differences between digestion of tankages and liver meal by pepsin HCl and on peptic-tryptic digestion show that a considerable amount of porphyrin-like material is present, and therefore there is a distinct error in the method employed by Almquist and associates for determining digestibility. The digestibility used in calculating the protein quality index was the value obtained in vitro which most nearly approached the digestibility in vivo. The explanation of this is given in the last column of table 4. The data indicate that no single method of enzymatic digestion is comparable with animal digestion.

TABLE 4.—*Results obtained for digestibility of proteins from various sources, expressed as percentage of total nitrogen, chemical (in vitro) means of digestion and determination being used*

Source of protein	Copper precipitable (A) [1]	Phosphotungstic acid precipitable (D) [1]	Undigested with pepsin (B) [1]	Undigested with pepsin neutralized after digestion	Undigested with pepsin and trypsin	Undigested residue as determined from rat data	Hot-water soluble (C)	Protein quality index	Method of digestion used for calculation
Dried whole egg	100.0	0.0	4.2	----------	0.0	2.2	4.1	93.3	Pepsin.
Casein	95.7	.5	4.7	----------	.1	.3	3.8	93.5	Trypsin.
Whole wheat	81.3	4.2	5.8	----------	3.5	8.3	13.4	69.2	Pepsin.
Wheat gluten	84.0	8.3	.6	----------	.2	.7	16.0	77.1	Do.
Whole corn	85.4	8.7	41.0	----------	17.4	8.7	2.0	70.3	Trypsin.
Corn-gluten meal	94.0	2.3	10.8	----------	5.9	3.3	1.8	87.9	Do.
Liver meal	86.9	5.9	11.0	15.3	11.6	11.7	11.5	73.1	Do.
Tankage	78.9	11.7	11.1	13.9	9.0	16.7	16.2	60.0	Pepsin, neutralized.
Soybean meal 1	95.4	.3	9.9	----------	3.5	16.1	9.0	80.1	Pepsin.
Soybean meal 2	96.2	.4	11.3	----------	3.7	15.7	6.5	81.2	Do.

[1] Protein quality index $=A-(B+0.6\ C)+0.4\ D$.

The data of tables 1 and 4 are compared in figure 4. There seems to be little correlation between the two methods. However, it will be noted that the natural foods, to which no protein material had been added or from which none had been subtracted, lie on a fairly straight line. Casein, corn gluten, and wheat gluten, which are more or less isolated proteins, give quite different results by the chemical method as compared with the nitrogen-retention method.

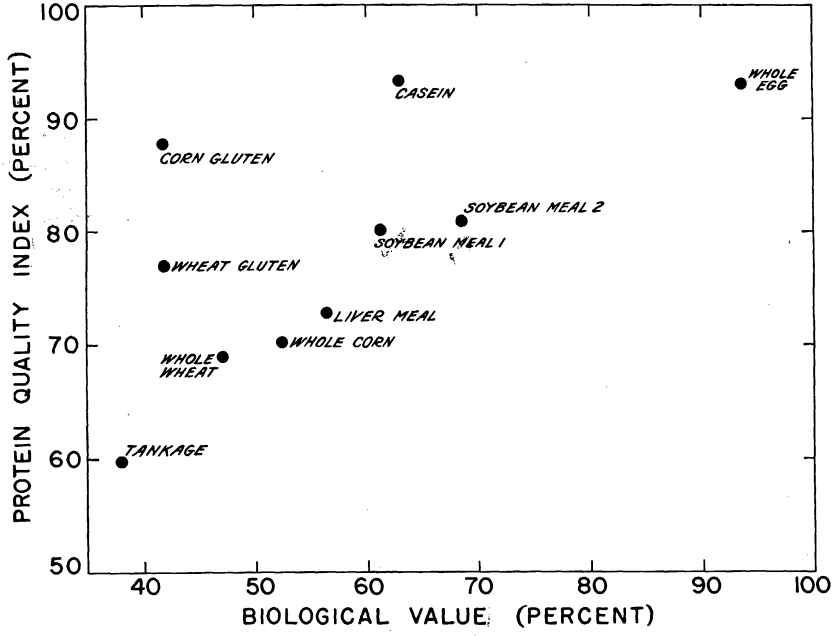

FIGURE 4.—Comparison of the protein quality index with the biological value for proteins from different sources. Data from tables 1 and 4.

SUMMARY

Nitrogen-balance studies were made with young rats, 10 food proteins being used. The values obtained (rounded) were: Whole egg 94, casein 63, whole wheat 47, wheat gluten 42, whole corn 52, corn-gluten meal 42, liver meal 56, meat and bone tankage 38, solvent-extracted soybean meal 61, and heated solvent-extracted soybean meal 68.

The same foods were used for the determination of the protein quality index by the Almquist chemical method.

The digestibility of the proteins was calculated from data obtained by the nitrogen-retention method. An attempt was made to compare these values with the digestibility determined by means of enzymes. The digestibility in vitro showed a poor comparison with the digestibility in vivo.

The protein quality index showed no comparison with the biological value by the nitrogen-retention method when used on isolated proteins. For natural foods the comparison is fair if the enzymic digestibility most similar to that in vivo is used for the calculation of the protein quality index.

Heated soybean meal was found to have a higher biological value than raw, but no difference was found in the digestibility of the raw and heated meals.

Correction of the endogenous urinary nitrogen according to body surface was found to give less variation than correction according to body weight. When the body surface correction was made, no difference was shown in endogenous urinary nitrogen between the first and second nitrogen-free periods.

The regression lines were plotted for the endogenous urinary nitrogen when calculated according to body weight and body surface area. The formula for the regression line for the body weight graph is $N=135.6W^{-0.469}$, and for the body surface graph, $N=5.61A^{0.069}$.

LITERATURE CITED

(1) Almquist, H. J., Stokstad, E. L. R., and Halbrook, E. R.
1935. Supplementary values of animal protein concentrates in chick rations. Jour. Nutr. 10: 193–211, illus.

(2) Ashworth, Ural S.
1935. Growth and development with special reference to domestic animals. XXXVI. Endogenous nitrogen and basal energy relationships during growth. Mo. Agr. Expt. Sta. Res. Bul. 223, 20 pp., illus.

(3) ———
1935. Growth and development with special reference to domestic animals. XXXVII. Interrelations between protein intake, endogenous nitrogen excretion, and biological value of protein. Mo. Agr. Expt. Sta. Res. Bul. 228, 14 pp., illus.

(4) Fisher, R. A.
1928. Statistical methods for research workers. Ed. 2, rev. and enl., 269 pp., illus. Edinburgh and London.

(5) Hawk, Philip B., and Oser, Bernard L.
1931. A modification of the Osborne and Mendel salt mixture. Science 74: 369.

(6) Lee, Milton O., and Clark, Elizabeth.
1929. Determination of the surface area of the white rat with its application to the expression of metabolic results. Amer. Jour. Physiol. 89: 24–33, illus.

(7) MASON, INEZ D., and PALMER, LEROY S.
1935. UTILIZATION OF GELATIN, CASEIN, AND ZEIN, BY ADULT RATS. Jour. Nutr. 9: 489–505.
(8) MITCHELL, H. H.
1924. A METHOD OF DETERMINING THE BIOLOGICAL VALUE OF PROTEIN. Jour. Biol. Chem. 58: 873–903.
(9) ——— and BEADLES, JESSIE R.
1937. THE NUTRITIVE VALUE OF THE PROTEINS OF NUTS IN COMPARISON WITH THE NUTRITIVE VALUE OF BEEF PROTEINS. Jour. Nutr. 14: 597–608.
(10) ——— and CARMAN, G. G.
1926. THE BIOLOGICAL VALUE OF THE NITROGEN OF MIXTURES OF PATENT WHITE FLOUR AND ANIMAL FOODS. Jour. Biol. Chem. 68: 183–215.
(11) SHEPPARD, W. F.
1902. NEW TABLES OF THE PROBABILITY INTEGRAL. Biometrika 2: [174]–190.
(12) SMUTS, D. B.
1935. THE RELATIONSHIP BETWEEN THE BASAL METABOLISM AND THE ENDOGENOUS NITROGEN METABOLISM, WITH PARTICULAR REFERENCE TO THE ESTIMATION OF THE MAINTENANCE REQUIREMENT OF PROTEIN. Jour. Nutr. 9: 403–433.