TABLE I.
Summary of Results Obtained with Rats Fed 3 Starch Preparations Fed at 2 Levels.*

Amount of starch fed	247 mg		317 mg	
Duration of experiment	1 hr mg	2 hr mg	1 hr mg	2 hr mg
Hydrolyzed				
Cereal I	137 ± 7.3†	179 ± 7.7	158 ± 8.5	213 ± 8.3
Cereal II	184 ± 3.8	222 ± 3.1	231 ± 5.2	258 ± 5.8
Corn starch	180 ± 5.8	222 ± 4.3	254 ± 5.2	281 ± 4.8
Emptied				
Cereal I	118 ± 8.8	170 ± 8.3	140 ± 10.0	198 ± 10.4
Cereal II	154 ± 3.8	210 ± 4.6	176 ± 6.2	226 ± 7.2
Corn starch	116 ± 8.2	197 ± 7.6	168 ± 9.6	248 ± 8.3
Absorbed				
Cereal I	117 ± 8.8	170 ± 8.3	130 ± 9.4	195 ± 10.1
Cereal II	148 ± 4.4	210 ± 4.6	158 ± 6.2	220 ± 7.8
Corn starch	115 ± 8.0	197 ± 7.6	165 ± 9.8	248 ± 8.3

* The data presented here were obtained on a total of 240 animals, each group containing from 15 to 30 animals. All values are expressed in terms of starch.
† Standard error

same order of magnitude. Cereal I was hydrolyzed, emptied and absorbed at a consistently slower rate.

The data presented here are in excellent agreement with our previous findings. It is again evident that increasing the size of the meal fed also increases the amount of starch hydrolyzed, emptied and absorbed. The fact that the rates of absorption decreased with time should not be emphasized too strongly since only relatively small amounts of carbohydrate remained unabsorbed during the second hour.

The intimate relationship of the rates of hydrolysis, emptying and absorption and the effect of varying the size of the meal upon these rates support very well our earlier reports in connection with the utilization of dextrose. The results reported here were, however, obtained under conditions which permitted the elimination of forced feeding.

Conclusions. Three starch preparations were fed to a total of 240 animals. The rates of hydrolysis, emptying and absorption are closely related. Increasing the size of the test meal increases these rates. The data were obtained under conditions permitting the elimination of forced feeding.

16372

A Comparison of the Nutritive Value of Egg Proteins and Their Amino Acid Content.

W. C. HESS, E. H. KRAMKE, J. C. FRITZ, AND H. W. HOWARD.
From the Department of Biological Chemistry, Georgetown Medical School, and the Borden Company Nutritional Research Laboratories.

Since the early investigations of Osborne and Mendel[1] the high nutritive value of egg proteins has been recognized. Mitchell and Carman[2] found that whole egg protein fed at an 8% level in the diet had a biological value of 93 as compared with 74 for pork and 67 for wheat. Subsequently the same investigators[3] reported a biological value of 94 for the nitrogen of whole egg and 83 for the nitrogen of egg albumin. Sumner[4] and others reported the biological value of whole egg protein as 94 when fed at a 5% level and 85 when fed at an 8% level and that the biological value was higher for young rats than

for older rats. It was also stated that egg proteins are superior to milk proteins in maintaining nitrogen balance of adult human subjects. Murlin and others[5] gave 97 as the biological value of whole egg protein for human subjects as compared with values of 81, 84, and 83 for soy bean, beefsteak, and peanut proteins, respectively. Hoagland and Snider[6] found that the average growth-promoting value of the proteins in dehydrated pork was 3.75 g gain in body weight per gram of protein consumed in tests with young rats as compared with a gain of 4.28 g for the protein in spray dried eggs. More recently Hoagland, Ellis, Hankins, and Snider[7] stated that the superiority of egg to pork protein was due to the notably more cystine and methionine it contained. Supplementing pork protein with either cystine or methionine gave a growth promoting value equal to that of the protein in eggs.

Mitchell and Block[8] have taken the amino acids of whole egg protein as a standard for comparison of other proteins in an effort to assay biological values on the basis of essential amino acid content. Egg albumin is deficient in both cystine and methionine in comparison with whole egg protein, no data on whole egg white protein or on whole yolk protein were given. Calvery and Titus[9] have studied the sulfur, tryptophane, and cystine content of the whole white and whole yolk proteins and of egg albumin. The whole white protein was higher in all 3 substances than either the albumin or yolk proteins. The whole white protein contained 1.66% sulfur and the yolk protein contained 1.19%. Munk and others[10] found that the total egg protein was composed of 65% white protein and 35% yolk protein. On this basis the sulfur content of whole egg protein should be approximately 1.5%. Patton and Palmer[11] found the sulfur content of the whole egg protein to be 1.35%. Munk[10] found practically the same cystine content in the white as in the yolk protein, 1.9% and 2.2% respectively. Methionine was determined by subtracting the cystine sulfur from the total sulfur and considering the difference as due to methionine. On this basis the whole white protein contained 6.6% and the yolk protein 3.0% methionine. The tryptophane content of the 2 proteins was identical, 1.4%, the phenylalanine content of the whole white protein was 6.2%, approximately 50% greater than the phenylalanine content of the yolk protein. Differences in the other essential amino acids were slight. No feeding studies were reported.

We have prepared whole egg, whole white, and yolk proteins and analyzed them for nitrogen, sulfur, cystine, cysteine, methionine, phenylalanine, tryptophane, threonine, histidine, lysine, and arginine. The same proteins were used in feeding experiments with young rats to determine their protein efficiency values. In addition 3 commercial egg proteins were run similarly.

Experimental. Preparation of proteins. The egg whites and yolks were separated and poured into acetone. After stirring vigorously they were allowed to stand overnight when the acetone was decanted and replaced with fresh acetone. This acetone treatment was repeated 4 times. The whole white protein was prepared similarly. After the final treatment with acetone all the proteins were fairly granular. Following the acetone treatment

1 Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1913, **15**, 311; 1916, **20**, 35.

2 Mitchell, H. H., and Carman, G. G., *J. Biol. Chem.*, 1924, **60**, 613.

3 Mitchell, H. H., and Carman, G. G., *J. Biol. Chem.*, 1926, **68**, 183.

4 Sumner, E. E., Pierce, H. B., and Murlin, J. R., *J. Nut.*, 1938, **16**, 37; Sumner, E. E., *J. Nut.*, 1938, **16**, 129; Sumner, E. E., and Murlin, J. R., *J. Nut.*, 1938, **16**, 141.

5 Murlin, J. R., Edwards, L. E., and Hawley, E. E., *J. Biol. Chem.*, 1944, **156**, 785.

6 Hoagland, R., and Snider, G. G., *Food Research*, 1946, **11**, 494.

7 Hoagland, R., Ellis, N. R., Hankins, O. G., and Snider, G. G., *J. Nut.*, 1947, **34**, 43.

8 Mitchell, H. H., and Block, R. J., *J. Biol. Chem.*, 1946, **163**, 599.

9 Calvery, H. O., and Titus, H. W., *J. Biol. Chem.*, 1934, **105**, 683.

10 Munks, B., Robinson, A., Beach, E. F., and Williams, H. H., *Poultry Sci.*, 1945, **24**, 549.

11 Patton, A. R., and Palmer, L. S., *J. Nut.*, 1936, **11**, 129.

TABLE I.
Amino Acids in Egg Proteins.
All values, except nitrogen, are on the basis of the protein containing 16% nitrogen. Nitrogen values are expressed on the ash and moisture-free original protein.

	Whole egg				Commercial whole egg		
	I	II	Whole white	Whole yolk	I	II	Commercial whole white
Amino acid	%	%	%	%	%	%	%
1. Cystine	2.26	2.25	2.48	1.82	1.33	2.11	2.33
2. Cysteine	0.28	0.10	0.41	0.14	0.00	0.00	0.23
3. Methionine	3.95	3.88	4.15	3.61	4.30	3.65	3.96
Phenylalanine	4.46	4.50	5.42	3.73	4.84	4.87	5.29
Tryptophane	1.29	1.42	1.40	1.16	1.47	1.35	1.56
Sulfur—total	1.57	1.56	1.78	1.30	1.38	1.40	1.62
Sulfur calculated from 1 + 2 + 3	1.53	1.46	1.67	1.30	1.28	1.35	1.54
Nitrogen	13.76	12.62	14.53	12.78	13.34	12.67	12.64
Arginine	6.54	6.46	5.55	6.98	6.40	6.59	6.29
Histidine	1.53	1.43	1.20	1.50	1.38	1.61	1.29
Lysine	5.14	5.05	4.83	5.81	5.09	5.13	5.20
Threonine	3.78	3.88	4.10	3.60	3.94	3.82	4.42

the proteins were treated overnight with ethyl alcohol. The alcohol was removed by filtration and the residues extracted with ethyl ether by allowing them to stand overnight in contact with the ether. The ether extraction was repeated twice. The final products were air dried.

The commercial proteins studied included a dried egg white and 2 dehydrated, defatted whole egg preparations, both made by the same manufacturer.

Analysis. Total nitrogen was determined on all samples by the macro Kjeldahl procedure. Total sulfur was determined by the method of Pollack and Partansky.[12] Phenylalanine, methionine, and tryptophane were determined, following alkaline hydrolysis, as described by Hess and Sullivan.[13] Cystine and cysteine were determined by the method of Sullivan, Hess, and Howard[14] as used by Hess and Sullivan.[15] Threonine was determined by the method of Shinn and Nicolet.[16] Arginine, histidine, and lysine were determined by the methods described by Block and Bolling.[17] The values given in Table I are expressed on the basis of the proteins containing 16% nitrogen since this method was used in determining the protein content of the diet. The nitrogen values in the table, however, are on the original sample corrected for moisture and ash.

Feeding experiments. Albino rats of the Sprague-Dawley strain were used. The diets were made up with the egg proteins to contribute 10% protein to the diet. The nitrogen of

TABLE II.
Composition of the Diet.

Constituent	%
U.S.P. No. 2 salt mixture	4
Wilson's 1:20 liver concentrate	1
Crisco	5
Corn oil	5
Protein—N × 6.25 to contribute	10
Cerelose and corn starch to make up	100%.

Synthetic vitamins were added in the following quantities per 100 g of diet: alpha tocopherol 4.0 mg, 2-methyl-1-4-napthoquinone 1.0 mg, thiamine hydrochloride 0.8 mg, riboflavin 1.6 mg, pyridoxine hydrochloride 0.8 mg, niacin 4.0 mg, calcium pantothenate 4.4 mg, para-aminobenzoic acid 4.0 mg, choline chloride 200.0 mg, and inositol 21.6 mg. Vitamins A and D were fed separately, 3 drops of reference cod liver oil weekly.

In the diet containing 0.25% cystine an equal weight of corn starch was omitted.

[12] Pollack, R. N., and Partansky, R. M., *Ind. and Eng. Chem., Anal. Ed.*, 1934, **6**, 330.

[13] Hess, W. C., and Sullivan, M. X., *Ind. and Eng. Chem., Anal. Ed.*, 1945, **17**, 717.

[14] Sullivan, M. X., Hess, W. C., and Howard, H. W., *J. Biol. Chem.*, 1942, **145**, 621.

[15] Hess, W. C., and Sullivan, M. X., *J. Biol. Chem.*, 1943, **151**, 625.

[16] Shinn, L. A., and Nicolet, B. H., *J. Biol. Chem.*, 1941, **138**, 91.

[17] Bosshardt, D. K., Ydse, L. C., Ayres, M. M., and Barnes, R. H., *J. Nut.*, 1946, **31**. 23.

TABLE III.
Growth of Weanling Rats on Egg Protein Diets.

Protein	No. of rats	Initial wt, g	Final wt, g	Gain, g	Food intake, g	PE ratio
Whole egg I	5	47.0*	97.0	50.0	151.4	3.30
		45.0	100.0	55.0	141.0	3.90
		55.0	108.0	53.0	177.0	2.99
Egg white	4	49.0	105.8	56.8	161.5	3.51
		54.0	120.0	66.0	177.0	3.73
		48.0	104.0	56.0	169.0	3.31
Yolk	4	46.8	96.0	48.8	151.0	3.23
		47.0	97.0	50.0	137.0	3.65
		49.0	106.0	57.0	191.0	2.98
Commercial egg white	8	49.8	104.1	54.4	161.1	3.37
		55.0	117.0	62.0	159.0	3.90
		45.0	87.0	42.0	168.0	2.50
Commercial whole egg I	9	49.4	90.9	41.4	148.4	2.80
		52.0	100.0	48.0	151.0	3.18
		44.0	83.0	39.0	159.0	2.45

* First value in each case is the average, followed by the values for the rats having the highest and lowest PE ratios in the group.

TABLE IV.
Growth of Weanling Rats on Egg Protein Diets.

Protein	No. of rats	Initial wt, g	Final wt, g	Gain, g	Food intake, g	PE ratio
Whole egg II	9	49.6*	82.1	32.6	104.4	3.12
		61.0	107.0	46.0	141.0	3.26
		60.0	94.0	34.0	124.0	2.74
Commercial whole egg II	9	48.8	73.2	24.6	100.9	2.33
		45.0	74.0	29.0	101.0	2.90
		50.0	68.0	18.0	103.0	1.75
Commercial whole egg II + 0.25% cystine	9	50.2	75.8	25.6	107.2	2.38
		44.0	70.0	26.0	89.0	2.92
		48.0	77.0	29.0	131.0	2.21

* First value in each case is the average, followed by the values for the rats having the highest and lowest PE ratios in the group.

the sample times 6.25 gave the protein content and the amounts of food consumed by the rats were carefully determined and recorded. Two series of feeding experiments were run. The average food intake and weight gains for a period of 2 weeks of each group of rats are given in Tables III and IV. Bosshardt and others[18] found that the protein efficiency ratio* reached a maximum in approximately 10 days and state that this period is sufficiently long to permit reliable calculations. If the experiments are run for a long period of time the PR ratio decreases.

Discussion. The first series of experiments, Table III, indicated that the whole white protein has a higher PE ratio than either the whole egg or yolk proteins. The commercial whole white protein has a PE ratio that is only slightly less than that of the laboratory whole white protein while commercial whole egg protein 1 has a markedly lower PE ratio than the laboratory prepared sample. In comparing the amino acid content of the laboratory prepared whole egg protein with that of commercial sample 1 it was noted that the sulfur amino acid content of the commercial sample was markedly lower. No

* The protein efficiency ratio (PE ratio) is calculated by dividing the weight gained by the grams of protein ingested.

marked differences were noted in the content of the other essential amino acids.

In an effort to determine whether the variation in total sulfur amino acid content, particularly cystine, would explain the difference in the PE ratio another series of feeding experiments were run. A new preparation of whole egg protein and another sample of commercial whole egg protein from the same producer were fed at the same level as previously. At the same time 0.25% cystine was added to the commercial whole egg protein diet and fed to another set of rats. These results are given in Table IV. The PE ratio of whole egg protein II was 3.13 as compared with the 3.3 in the first series while commercial whole egg protein II was 2.33 compared with 2.8 in the first series. The commercial whole egg protein II plus cystine had a PE ratio of 2.38, practically identical with that of the diet without the added cystine. It will be noted from Table I that whole egg protein II has an amino acid content the same as that of Sample I within the limits of experimental error. Commercial whole egg protein II contains slightly more cystine than commercial Sample I and slightly less methionine, there is little variation in all the samples of whole egg proteins in the other essential amino acids tested. It is apparent that the addition of cystine has not improved the PE ratio of the commercial whole egg protein. The only essential amino acids which were not determined in the proteins are leucine, isoleucine, and valine. These, according to Mitchell and Block,[8] are present in such large amounts in whole egg protein that it is difficult to imagine any lack of these amino acids in any of the samples. These results emphasize the difficulty of assaying the nutritional values of proteins by analyzing for the essential amino acids. In spite of the similarity of the amino acid content of the commercial whole egg and laboratory whole egg proteins, except for cystine, they are biologically different, even when the commercial sample is reenforced with cystine.

Summary. The proteins of whole egg, whole egg white, and yolk, were prepared and analyzed for cystine, methionine, phenylalanine, tryptophane, threonine, arginine, histidine, lysine, sulfur, and nitrogen. These proteins were incorporated into diets at a 10% level and fed to young rats. The protein efficiency ratio (gain in weight per gram of protein ingested) was determined after a 2-week feeding period. Commercial preparations of whole egg and whole white protein were similarly analyzed and their PE ratio determined. The PE ratio of the whole white protein was higher than that of either the whole egg or the yolk proteins. The PE ratio of the commercial whole white protein was almost as high as that of the laboratory prepared sample. The PE ratio of commercial whole egg protein was markedly lower than that of the laboratory prepared sample. The amino acid content of all the whole egg proteins were quite similar with the exception of the cystine content of the commercial samples which was lower than that of the laboratory samples. When the commercial whole egg protein II was reenforced with 0.25% cystine the PE ratio was not improved. Other factors in addition to the amino acid content apparently play a role in the biological value of proteins.