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GROWTH AND DEVELOPMENT

With Special Reference to Domestic Animals

XXXVII. Interrelations Between Protein Intake, Endogenous Nitrogen Excretion, and Biological Value of Protein

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FOREWORD

The special investigation on growth and development is a cooperative enterprise in which the departments of Animal Husbandry, Dairy Husbandry, Agricultural Chemistry, and Poultry Husbandry have each contributed a substantial part. The plans for the investigation in the beginning were inaugurated by a committee including A. C. Ragsdale, E. A. Trowbridge, H. L. Kempster, A. G. Hogan, F. B. Mumford. Samuel Brody served as Chairman of this committee and has been chiefly responsible for the execution of the plans, interpretation of results and the preparation of the publications resulting from this enterprise.

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F. B. Mumford,
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ABSTRACT

An analysis of the results from 125 determinations of the biological values of proteins taken from the literature indicated that the nature of the protein fed affects the endogenous nitrogen excretion. To support the data from the literature experimental data were secured in our laboratory on 20 pairs of young rats for a comparison of the N excreted on a N-free diet immediately before and after feeding a given protein. No statistically significant effect of the nature of the protein fed (lact-albumin vs corn gluten) on the endogenous N of the final compared with the initial standardization period could be demonstrated, unless the animals were kept on a low protein diet for about 30 days previous to the beginning of the experiment.

ACKNOWLEDGMENT

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XXXVII. INTERRELATIONS BETWEEN PROTEIN INTAKE, ENDOGENOUS NITROGEN EXCRETION AND BIOLOGICAL VALUE OF PROTEIN

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INTRODUCTION

The utilizability of protein, usually called its biological value,* is defined as the percentage of nitrogen retained by the body for maintenance or productive purposes to nitrogen digested or absorbed and is a problem second in importance only to the utilizability of energy. A recent review of the literature on biological values is due to Boas-Fixsen (1935).

The most important factor in the utilization of a given protein is its proportion in the diet. Maximum biological values are reached when the content of protein (N × 6.25) is about 5%. If the protein is complete the urinary N excretion on a 5% protein level is approximately equal to that excreted on a N-free diet. Since the endogenous urinary N excretion represents almost the entire urinary N when complete proteins are fed at 5% levels therefore any variations in the endogenous N excretion reflect as variations in the biological values. Hence the importance for the evaluation of biological values of proteins, of a broad knowledge of the factors influencing endogenous nitrogen excretion.

LITERATURE

Does the nature of the dietary protein intake affect the amount of endogenous N excreted? Since the differentiation between endogenous and exogenous nitrogen is not possible analytically, the above problem can be investigated directly only when the exogenous factor is eliminated by feeding a good source of protein at a very low level (about 5%). In the determination of the biological value of protein by the "balance sheet" method (Boas-Fixsen, 1935) one assumes a constant endogenous N excretion. Mitchell (1924) in proposing this method for determining the biological value of proteins gave two reasons for assuming that the endogenous N excretion remains constant: (1) if the endogenous metabolism were greatly reduced by exogenous protein metabolism then the feeding of a small amount of a protein of high biological value (such as those of egg or milk) would appreciably depress the endogenous urinary

*Biological value $= \frac{\text{N retained}}{\text{N absorbed (or digested)}} \times 100$; N absorbed; = N intake − dietary fecal N; dietary fecal N = total fecal N − metabolic N; N retained = N absorbed − dietary urinary N; dietary urinary N = total urinary N − endogenous N.

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N excretion which, he believed, was not the case; (2) more consistent results for the biological value of the proteins were obtained by assuming a constant endogenous metabolism than by assuming that endogenous metabolism was eliminated when protein was consumed. However, the biological values were not particularly consistent even when a constant endogenous N excretion was assumed.

A comparison of the endogenous N excretion immediately preceding and following short periods of experimental protein feeding might throw some light on the effect of dietary protein on endogenous N metabolism. Unfortunately, many investigators of the biological values of proteins either use only one standardization period on a N-free diet or the average of the initial and final periods. Since Mitchell published many complete records on endogenous nitrogen excretion preceding and following periods of protein feeding his data offer an opportunity to study this problem. His data were used to construct Table 1. First a line of regression was constructed relating the endogenous N coefficient $\left(\frac{\text{endogenous N}}{\text{body wt.}}\right)$ with body weight:

$$\frac{\text{Ur N}}{\text{body wt.}} \times 100 = 69.3\ (\text{body wt.})^{-0.274}$$

In constructing this curve 50 group averages of 5 rats each were used, 25 for beginning and 25 for ending periods on N-free diets (Fig. 1). Next the deviation from the line of regression for each group average was determined.

The average deviation for all the initial periods was +1.29 mg (the + sign indicating the deviation was above the line of regression) while the average deviation for the final period was −0.124 mg. This mean downward trend in the endogenous N coefficient from initial to final periods is probably due to the fact that the low intake of dietary protein during the intervening periods depleted to a further extent the protein reserves of the body, being analogous to the effect of the long periods of N-free feeding observed by Deuel, et al (1928); by Ashworth & Brody (1933); and by Ashworth (1935). However, only about one-half of the 25 groups showed a downward slope of the line connecting the initial and the final periods of endogenous N determinations. And furthermore, there seems to be a definite correlation between the quality of the protein fed during the intervening periods and the slope of this line. The first 7 groups in Table 1 were given proteins of high quality during the intervening periods. With one exception (group 6) they have a + slope for the curve of endogenous N from the initial to the final period. The reason for the downward slope of group 6 is not apparent. This incon-

TABLE 1.—AN ANALYSIS OF DATA TAKEN FROM THE LITERATURE

Group No.	Diets used during consecutive experimental periods on same rats							Average* end. N. Coef.		Difference final − initial	Ref. Number
	1st	2nd	3rd	4th	5th	6th	7th	initial	final		
1	Initial Standardization Period on a N-free diet	curd	cheese	curd	final**			−1.3	1.2	2.5	1
2		cheese	curd	cheese	final			−2.2	2.7	4.9	1
3		liver, 8%	kidney, 8%	heart, 8%	final			−3.7	−1.2	2.5	2
4		heart, 8%	kidney, 8%	liver, 8%	final			−4.2	4.4	8.6	2
5		heart, 8%	kidney, 8%	liver, 8%	final			0.8	5.1	4.3	2
6		liver, 8%	kidney, 8%	heart, 8%	final			2.7	−2.3	−5.3	2
7		liver, 8%	liver, 16%	final				−5.0	3.5	8.5	2
8		cocoa, 8%	final					4.6	−0.1	−4.7	3
9		cocoa, 8%	final					9.8	−1.2	−11.0	3
10		milk, 8%	cocoa-milk 8%	cocoa, 8%	final			1.3	−5.6	−6.9	3
11		cocoa-milk 8%	final					3.6	−5.4	−9.0	3
12		cocoa-milk 8%	final					−4.9	−7.7	−2.8	3
13		cocoa-milk 8%	final					−5.9	−6.5	−0.6	3
14		egg, 8%	flour-egg 8%	flour 7.5%	flour, 8%	final		5.7	−4.4	−10.1	4
15		flour, 8%	flour-egg 8%	egg, 8%	egg, 8%	final		5.2	2.4	−2.8	4
16		egg, 9%	flour-egg 9%	flour 10%	final			0.6	0.8	0.2	4
17		--------	--------	flour, 10%	flour-egg 9%	egg 9%	final	5.3	6.8	1.5	4
18		--------	egg, 9%	--------	final			−1.6	1.6	3.2	4
19		egg, 9%	--------	--------	final			−1.2	1.9	3.1	4
20		flour, 8%	flour-milk, 8%	--------	--------	egg, 4%	final	5.9	−7.5	−13.4	4
21		milk, 8%	flour-milk 8%	flour, 8%	egg, 4%	final		6.8	−4.2	−11.0	4
22		veal, 8%	flour, 8%	flour-veal 8%	final			−0.7	−5.9	−5.2	4
23		flour, 8%	veal, 8%	flour-veal 8%	final			−1.6	−5.8	−4.2	4
24		beef-flour 9%	flour, 8%	beef, 9%	final			4.7	8.1	3.4	4
25		flour, 8%	beef-flour 9%	beef, 9%	final			7.5	16.2	8.7	4

*Calculated as deviations from average regression curve of $\frac{N}{\text{body wt.}}$ with body weight.

**Final Standardization on a N-free diet.

References:

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(2) H. H. Mitchell and Jessie R. Beadles, J. Biol. Chem. *71*, 429 (1927).
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(4) H. H. Mitchell and G. G. Carman, J. Biol. Chem. *68*, 183 (1926).

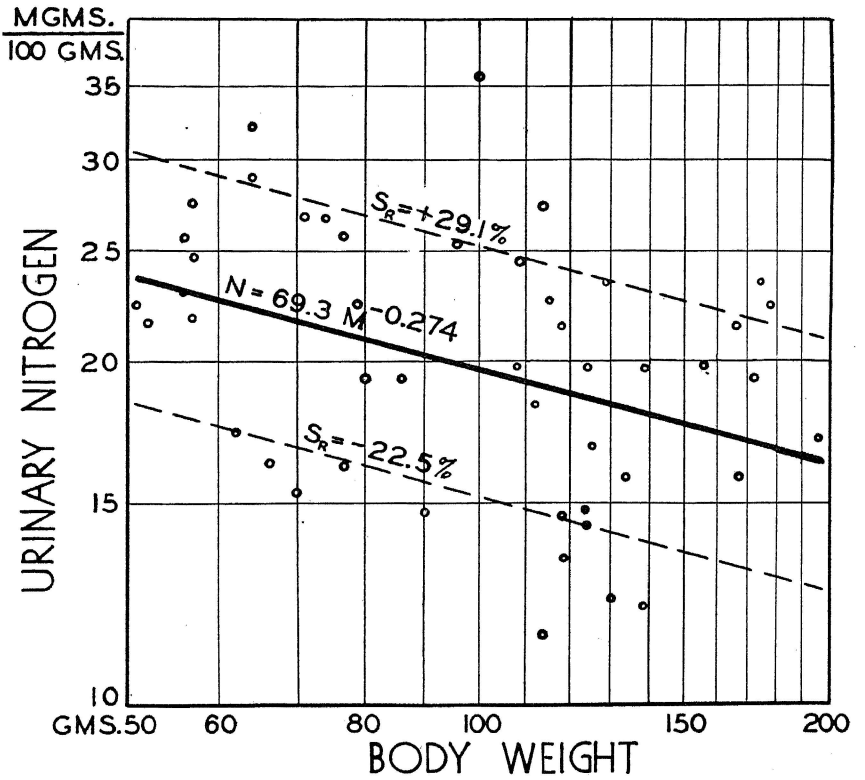

Fig. 1.—Regression curve constructed from data referred to in Table 1.

sistency is not surprising, since other factors influence the endogenous metabolism. Liver protein seems to be particularly potent for building reserve protein when fed immediately preceding the final standardization periods (Groups 4, 5 and 7). Groups 8-13 inclusive were fed cocoa (the protein of which is of low biological value) or a mixture of cocoa and milk. All of these show a negative slope for the curve of endogenous N. Group 14 has a large negative slope. Probably this is due more to the long period of low protein feeding rather than to the biological value of the flour protein. However, the much smaller negative slope of group 15 which differed only in the order of procedure (in which egg protein came last) indicates that egg protein is more efficient in building reserve body protein than flour protein.

The exceptionally large negative slopes of the endogenous N curve for groups 20 and 21 may be attributed to the long experimental periods used and to the fact the final period on a N-free diet was, in each case, immediately preceded by a diet containing only a minimal amount of egg protein. The last 4 groups (22, 23, 24, 25) show that beef at a level of 9.4% protein is a better source of deposit protein than the veal-flour mixture at 8.1% protein level.

Another indication of an influence of dietary protein on endogenous N metabolism is the statement made by Mitchell and Carman (1926) that when the protein of whole egg was fed the estimated quantity of body N excreted in the urine was greater than the actual amount excreted. Consequently they gave a biological value of 100% to the whole egg protein at a 7.9% level.

Still another indication of the influence of dietary protein on the endogenous N excretion is the recent report of Mason and Palmer (1935). Unfortunately, the original data are not given, although, the 7th, 8th and 9th factors correlated in their Table 4 indicates a significant influence of the dietary protein used on the final level of endogenous N reached.

The object of our work was to determine whether lactalbumin produces more reserve protein (thus increasing the endogenous N excretion) than corn gluten when both proteins are fed at the same low dietary level.

EXPERIMENTAL TECHNIQUE

Pairs of rats were selected from the same litter, of the same sex, and approximately the same weight. Soon after weaning they were given the milk-yeast diet of Table 2 containing about 8% protein, in order to standardize them all to a low level of reserve (or deposit) protein. The length of time they were given this diet varied with the different series in the following manner:

Series Number	No. of pairs	Length of time on milk-yeast
1	5	10 days
2	6	26 days
3	5	2 days
4	4	7 days

After this they were all given the N-poor diet of Table 1 for 4 days. Then the N-free diet was given for 1 day before the start of the excreta collection period. These final 5 days on a practically N-free diet are considered by many as sufficient to deplete the body of all reserve protein. Food consumption records were kept for all diets.

TABLE 2.—COMPOSITION OF THE DIETS

Food Constitutents	Milk yeast	N-poor	N-free	Experimental Rations					
				Series 1		Series 2		Series 3 and 4	
				A	B	A	B	A	B
Dried yeast %	5	----	----	----	----	----	----	----	----
Dried milk %	21	----	----	----	----	----	----	----	----
Dried egg yolk %	----	10	----	----	----	----	----	----	----
Lactalbumin %	----	----	----	7	----	8	----	16	----
Corn gluten %	----	----	----	----	10	----	12	----	24
Corn starch %	56	65	71	65	62	64	60	53	45
Sucrose %	----	10	10	10	10	10	10	10	10
Salts 14A %	4	4	4	4	4	4	4	4	4
Starch—cellophane %	4	4	2	4	4	4	4	4	4
Butter %	5	----	5	5	5	5	5	5	5
Tikitiki (70% dry mat.) %	3	3	3	3	3	3	3	3	3
Liver ext. (50% dry mat.) %	----	3	3	----	----	----	----	3	3
Cod liver oil %	2	1	2	2	2	2	2	2	2
N-content mg./gm.	12.8	6.0	1.6	7.8	3.8	9.7	10.1	21.0	20.0

During the collection period of 5 days the rats were housed in 3-liter beakers. Separation of urine and feces was accomplished as shown in Fig. 2. A false bottom of ½ inch mesh hardware cloth is supported on a frame about 10 cm. above the beaker floor. Under the false bottom a copper wire gauze is set at an angle so the feces caught on it will roll down to one side and be caught in a trough while the urine runs through the gauze. The sheet metal shed is used to protect the feces in the trough from contamination with urine. A film of benzoic acid put on in alcoholic solution was used to preserve the excreta. Each collection period was of 5 days duration, although daily collections were made of urine and feces.

After the first collection period the rats were transferred back to round wire cages and given the experimental diets A and B (Table 2) for a preliminary period which varied with the series, being 2 days for the first, 3 days for the second, and 5 days for the third and fourth series. Liver extract was not included in any of the diets for the first series and only in the final N-free diet for the second series. The reason was to minimize the N content of these rations. However, since a few of the rats in the second series started losing their coat of hair it was believed advisable to include this supplement. The third and fourth series were given liver extract in the N-poor and all succeeding diets.

After the above preliminary period the excreta were collected during which time the daily food intake was kept nearly constant. Next the rats were put back into the wire cages and fed the N-free diet for another adjustment period of the same length for each series, respectively, as that immediately preceding the collection period on the experimental diets. Following that was the third collection period.

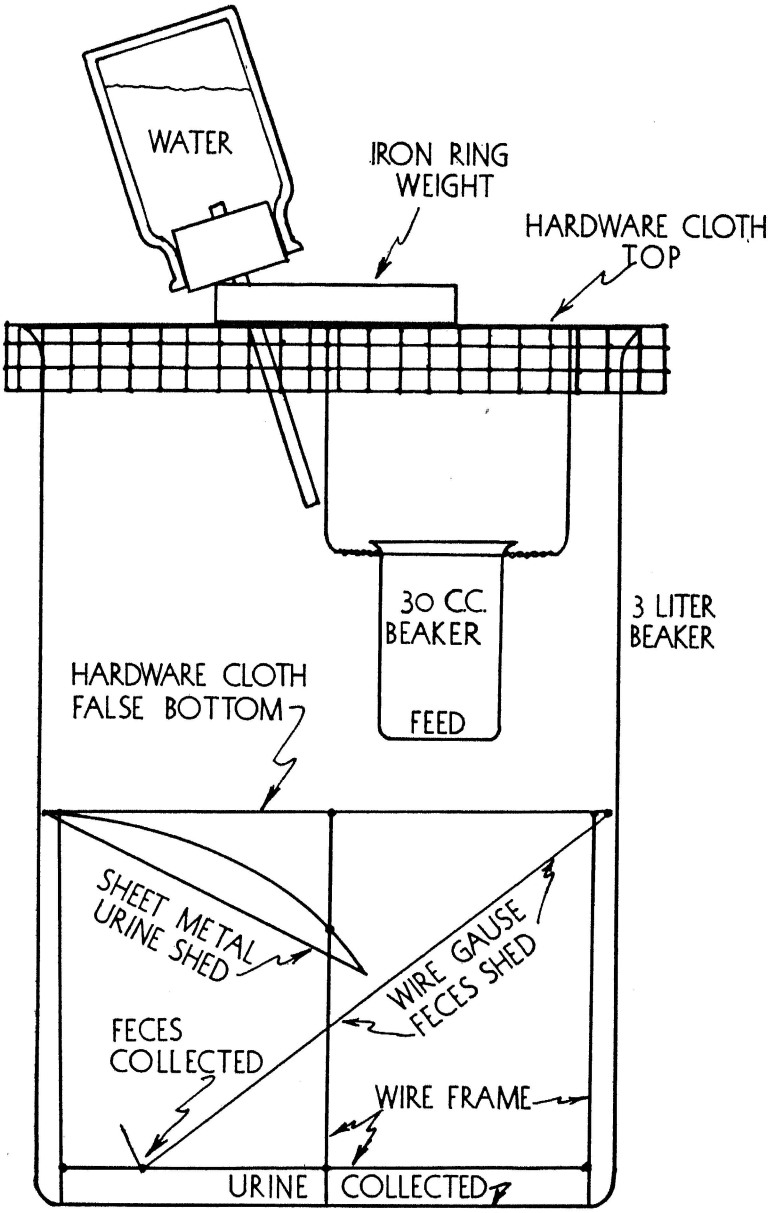

Fig. 2.—Diagram of excreta collection chamber.

EXPERIMENTAL RESULTS

The original data for each rat are shown in Table 3. No attempt was made to strictly pair the animals to the same food intake except in series 3. It was very difficult to get a sufficient intake of the corn gluten ration. Even the nitrogen-free ration seemed to be more palatable. The calculated biological values using both the initial and final standardization periods of Table 3 are shown in Table 4. Very often the rats

TABLE 3.—ORIGINAL DATA

Rat Number	Body wt., aver. gms.			Food Intake, gms./day			Urine N, mg./day			Fecal N, mg./day		
	1st*	2nd*	3rd*	1st*	2nd*	3rd*	1st*	2nd*	3rd*	1st*	2nd*	3rd*
				1st Series 5 Pairs of Animals								
1A	80	81	81	4.7	6.0	4.7	12.27	10.06	11.19	7.12	8.48	3.88
1B	89	84	81	5.3	3.0	4.6	13.28	16.50	10.89	6.40	2.88	7.41
2A	93	90	90	4.3	5.0	5.5	13.32	10.90	11.41	5.62	7.54	6.05
2B	103	94	90	4.4	2.7	4.6	16.42	20.09	13.18	5.26	3.12	6.16
3A	67	69	68	4.4	4.8	3.5	13.32	11.98	13.55	5.09	6.92	3.28
3B	71	66	62	3.6	1.9	4.0	12.98	14.92	9.94	4.15	1.94	5.70
5A	64	67	67	3.7	5.5	4.1	13.03	8.29	10.34	5.71	8.83	5.49
5B	63	61	57	5.2	2.9	3.7	12.55	13.56	10.14	7.55	6.14	8.61
6A	55	59	59	3.4	4.7	5.0	13.48	8.66	10.58	5.62	6.35	5.45
6B	59	56	54	5.2	2.9	3.5	12.10	14.59	9.05	5.57	5.21	4.81
				2nd Series 6 Pairs of Animals								
7A	46.5	50.5	50.0	2.5	3.3	2.5	9.88	8.20	8.06	3.41	5.35	3.66
7B	49.5	49.5	46.0	2.6	3.3	2.2	10.01	27.71	8.96	3.51	4.84	3.09
8A	49.5	54.0	55.0	3.1	3.7	3.0	9.30	10.81	8.41	4.01	5.83	3.30
8B	51.0	51.5	49.0	3.3	3.5	2.5	9.24	25.64	9.33	3.85	5.43	3.13
9A	58.5	65.5	64.0	3.2	4.7	3.0	10.99	10.13	10.31	3.56	7.77	2.75
9B	58.5	58.0	56.0	3.4	3.4	2.6	10.11	26.79	10.85	4.31	5.98	4.25
10A	68.0	77.5	76.0	3.8	6.0	4.3	11.18	10.87	12.00	4.76	7.17	5.38
10B	72.5	70.0	67.0	4.6	4.2	3.1	11.32	31.65	10.72	4.84	7.27	3.17
11A	69.0	75.0	77.0	3.8	5.4	4.6	11.41	11.11	11.71	3.86	7.57	5.27
11B	60.5	56.0	55.0	2.5	2.8	2.6	10.24	26.51	11.28	3.91	4.63	2.49
12A	77.0	82.0	82.0	4.1	6.0	4.8	12.91	11.06	13.49	7.95	12.11	8.91
12B	75.0	74.5	73.0	5.1	4.5	4.3	10.07	33.26	12.00	4.91	5.58	5.15
				3rd Series 5 Pairs of Animals								
13A	58.5	67.0	66.5	4.6	4.1	4.3	12.47	14.74	14.46	6.26	6.73	3.72
13B	58.5	58.5	55.0	3.8	3.3	2.6	14.54	50.32	10.40	4.44	9.14	5.97
14A	60.0	58.0	59.0	4.5	1.9	3.3	13.15	13.69	10.52	5.13	2.22	4.65
14B	62.5	57.5	55.0	5.4	2.1	2.7	11.65	28.10	9.50	6.67	4.48	4.23
15A	57.0	60.0	60.5	4.9	2.4	4.1	13.94	19.94	12.31	6.07	4.44	6.10
15B	61.0	58.0	54.0	4.9	2.4	2.7	14.23	34.39	9.58	5.19	4.31	3.03
16A	61.5	65.5	66.0	4.6	2.6	4.3	16.03	18.88	13.69	6.08	3.31	5.50
16B	63.0	60.0	53.5	4.1	2.6	2.9	17.98	42.85	12.34	6.75	10.82	4.77
17A	62.5	67.5	69.0	4.7	3.0	4.3	12.77	13.80	12.75	6.16	5.22	3.83
17B	63.0	61.0	59.0	4.9	3.1	2.8	13.10	46.19	9.40	6.28	8.04	3.59
				4th Series 4 Pairs of Animals								
18A	55.5	63.5	65.5	5.7	4.0	4.5	15.72	17.07	14.24	5.35	9.41	6.43
18B	55.0	51.5	48.5	3.4	1.9	2.5	14.08	33.26	9.76	6.31	5.30	2.23
19A	60.0	68.0	69.5	3.5	4.0	4.4	11.33	10.85	13.48	7.13	8.38	5.66
19B	60.0	55.0	54.5	4.3	2.2	3.1	11.05	37.62	9.77	4.89	6.76	4.00
20A	65.0	72.0	74.0	4.5	4.0	4.5	13.22	13.02	15.46	5.51	7.77	6.78
20B	64.5	63.0	60.0	3.9	2.9	3.2	12.66	46.85	10.35	4.41	6.26	2.98
21A	59.5	67.5	70.0	3.0	4.0	4.5	13.29	13.61	14.50	6.30	7.97	5.68
21B	64.5	61.5	58.0	4.6	2.6	3.5	13.19	41.97	10.84	5.42	10.02	5.45

*Collection periods.

TABLE 4.—CALCULATED BIOLGICAL VALUES

	Rat Pair	Lactalbumin		Corn Gluten	
		Initial	Final	Initial	Final
Series 1	1	105.9	102.7	78.4	64.8
	2	106.5	101.3	70.4	48.0
	3	103.7	104.6	79.4	54.6
	5	111.8	105.1	91.9	74.6
	6	113.3	105.3	78.1	47.8
Series 2	7	105.9	99.5	44.9	40.9
	8	95.6	92.8	51.6	50.7
	9	102.1	100.4	49.2	51.3
	10	100.6	102.0	49.4	45.6
	11	100.6	101.4	41.2	42.0
	12	103.4	104.4	48.4	53.0
Series 3	13	97.3	98.9	41.6	20.5
	14	98.7	92.5	60.3	55.4
	15	88.5	85.4	68.8	46.9
	16	95.0	90.9	48.1	33.6
	17	98.4	98.3	45.1	36.1
Series 4	18	98.3	96.5	50.8	32.7
	19	100.6	103.2	36.9	32.5
	20	100.2	102.9	39.1	33.3
	21	99.6	101.1	39.3	34.4

receiving lactalbumin excreted less urinary nitrogen during the intermediary period than during the initial or final periods. This situation made the biological value greater than 100% which is an argument in favor of the existence of an effect of the protein fed on the endogenous nitrogen excretion. The calculated biological values of corn gluten show much irregularity, being similar to the observed variations in the biological values of zein and gelatin as reported by Mason and Palmer (1935).

A statistical analysis of the data is summarized in Table 5. The difference between the endogenous urinary nitrogen excreted per 100gms body weight during the first and final standardization periods for each rat was treated by the method of Love and Brunson (1925) for the probability of significance. The relative difference between pairs of animals was determined by subtracting the difference found between the urinary N excretion of initial and final standardization periods for the lactalbumin rats from that found for the corn gluten rats. This relative difference was then tested for significance by the same statistical method. A significant relative difference was taken to indicate a specific effect of the nature of the protein fed during the intermediary period.

The differences and relative differences in the biological values shown in Table 4 were tested for significance in the same manner as the endogenous nitrogen excretion. These results are also summarized in Table 5.

A significant decrease generally was found when the endogenous nitrogen excretion of the initial period was compared with that of the final period. This was to be expected since the protein intake of the intermediary period was low, especially in the first two series. Even after the very long (31 days) preliminary period of low protein feeding used in series 2, the odds are 51:1 that less urinary nitrogen was excreted

TABLE 5.—STATISTICAL ANALYSIS OF DATA

Series Number	1	2	3	4
Pairs of rats in series	5	6	5	4
Protein content of experimental diet (%)	6	6	13	13
Length of preliminary period (days)	15	31	7	12
Mean endogenous N mg/100 gm body wt.				
(A) Lactalbumin, initial	18.9	18.1	22.8	22.5
Lactalbumin, final	15.9	15.9	19.8	20.7
Probability of significant diff.	23:1	51:1	44:1	4:1
(B) Corn gluten, initial	18.0	16.9	23.2	21.0
Corn gluten, final	15.7	18.5	18.6	18.5
Probability of significant diff.	302:1	29:1	356:1	17:1
Relative decrease, mg (B − A)	−1.5	−3.8	+1.66	+0.75
Probability of significance	9:1	832:1	4:1	3:1
Mean biological values				
(A) Lactalbumin, initial	108.2	101.4	95.6	99.7
Lactalbumin, final	103.8	100.1	93.2	100.9
Probability of significant diff.	32:1	6:1	9:1	4:1
(B) Corn gluten, initial	79.6	47.5	52.8	41.5
Corn gluten, final	58.0	47.3	38.5	33.2
Probability of significant diff.	454:1	1:1	44:1	9:1
Relative decrease (B − A)	19.3	−1.6	9.9	12.1
Probability of significance	212:1	9:1	14:1	16:1

during the final than during the initial standardization period for the lactalbumin rats. In this same series the corn gluten animals showed a significant increase in endogenous nitrogen excretion by odds of 29:1. This increase is no doubt due to an acceleration of their endogenous nitrogen excretion which was not prevented by the corn gluten diet.

The decrease in the endogenous excretion of the corn gluten rats with respect to that of the lactalbumin rats was in all series except No. 2 not significant because of the parallel decrease of the nitrogen excretion of the rats from both diets. Series 2 is an exception in that the corn gluten rats showed a significant increase in nitrogen excretion while the lactalbumin rats showed a significant decrease, thus making the relative difference significant. These data show then that until the body's supply of reserve protein is reduced to a low level, as in series 2, the specific effect of the nature of the protein fed on the endogenous nitrogen excretion is not apparent.

In most cases, after the biological values of the proteins were calculated using the above endogenous N figures it made little difference whether the initial or final standardization period was used. Series 1 seems to be the exception. There is an unmistakable decrease in the biological value of both lactalbumin and corn gluten when the final standardization period was used. The greater decrease of the biological value of the corn gluten with respect to that of the lactalbumin was significant. Unfortunately the nitrogen levels of the corn gluten and lactalbumin diets were not the same. By mistake the nitrogen content of the corn gluten fed was only about one-half as great as that of the lactalbumin diet (Table 2). With the smaller amount of nitrogen absorbed by the corn gluten animals the same difference in correction for maintenance (endogenous nitrogen) made a larger difference in the retained nitrogen than it did for the lact-

albumin rats. This unintentional difference in the nitrogen levels of the lactalbumin and corn gluten probably accounts for the differences in the first series. These data show then that for the calculation of biological values a correction factor, due to the nature of the protein fed previously to the standardization period, need not be applied to the endogenous nitrogen excretion.

SUMMARY AND CONCLUSIONS

The nature of the protein fed affected but slightly the endogenous nitrogen excretion when short experimental periods were used. However, when the reserve protein supply of the body was reduced to a low level by long periods on N-free diets an effect of the nature of the protein fed on endogenous nitrogen excretion did appear. In most of the work reported by Mitchell and used in the construction of Table 1, a relatively long period of a low protein diet preceded the final standardization period. This long period may account for the observed effect of the nature of the protein fed on the final endogenous nitrogen excreted relative to that excreted at the beginning of the experiment. The results of the present paper suggest the use of short experimental periods to avoid the effect of the nature of the protein fed on the endogenous N excretion; but short experimental periods increases the variability of: the amount of reserve protein present in the body, the endogenous N excretion and therefore the biological values of the protein. The use of short experimental periods makes the method of doubtful value in determining small differences in the biological value of different proteins or protein mixtures. Before these small differences can be determined with reliability a method of securing more consistent-values for the endogenous nitrogen excretion must be devised.

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