EFFECT OF THE RIPENING PROCESS OF CHEESE ON THE NUTRITIVE VALUE OF THE PROTEIN OF MILK CURD[1]

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INTRODUCTION

The ripening of cheese is essentially a hydrolysis of the paracasein in the rennet curd through the agency of proteases (*5*).[2] During this proteolysis the concentration of water-soluble nitrogen, as also of nonprotein and amino nitrogen, in the cheese increases rather evenly with time (*6*). Insofar as these changes predominate in the ripening of cheese, there is no reason to suspect that the nutritive value of the rennet-curd protein would be affected. However, the products of this proteolysis, perhaps largely through the influence of bacteria, undergo certain secondary reactions, involving oxidation, reduction, deamination, and decarboxylation (*2*, *15*). The products of these reactions may differ widely from the products of simple proteolysis with respect to their value in animal nutrition, though their occurrence in cheeses ripened mainly by other agencies than bacteria may be of minor importance. Nelson (*10*) reported the presence of indole and phenols in Limburger cheese, while Camembert cheese contained a trace of indole but no phenols.

No attempt appears to have been made to determine whether and to what extent the nutritive value of the protein of the rennet curd has been affected by the chemical changes incident to ripening. The present and the increasing importance of cheese in the American diet warrants a thorough study of this subject. This paper represents a contribution to it, involving a series of controlled-feeding experiments and metabolism studies on rats.

MATERIAL STUDIED

The cheeses studied were American Cheddar, Roquefort, Swiss, and Limburger. The latter three varieties were furnished by the Kraft-Phenix Cheese Corporation of Chicago. The American Cheddar cheese, and the fresh rennet curd from cow's milk, with which all cheeses were compared, were supplied by the Department of Dairy Husbandry of the University of Illinois.

The fresh cheese was ground, mixed, and sampled for chemical analysis. It was then dried at a low temperature before a blower, ground, and extracted with ether to remove practically all the fat. The dried residue was then analyzed for nitrogen and fat. The ether-soluble material removed from each cheese was also analyzed for nitrogen.

The chemical composition of the fresh cheese is given in table 1. The percentage of nitrogen varied from 9.75 (Roquefort) to 13.25

[1] Received for publication Aug. 7, 1933; issued February 1934.
[2] Reference is made by number (italic) to Literature Cited, p. 18.

(milk curd) in the dried and extracted cheese and in the dried milk curd used in the preparation of the experimental rations. The fat content of these preparations was low (about 0.2 percent). The nitrogen content of the ether-soluble material removed from the cheese was very small, ranging from 0.0037 to 0.031 percent. It is evident that only an insignificant portion of the cheese nitrogen was removed from the experimental samples by ether extraction.

TABLE 1.—*Chemical composition of the fresh cheese*

Variety	Water	Protein (N×6.38)	Fat	Ash	Calcium	Phosphorus	Gross energy per gram
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Calories*
American Cheddar:							
Sample 1	35.33	25.26	28.30	4.04	0.845	0.534	4.46
Sample 2	31.81	24.56	31.17	3.77			4.90
Swiss	35.70	32.03	17.63	4.05	1.05	.728	4.25
Roquefort:							
Sample 1	36.84	22.14	33.35	7.38			
Sample 2	40.33	20.03	32.08	6.89	.550	.424	4.06
Limburger:							
Sample 1	48.00	21.25	26.37	3.68	.656	.418	3.83
Sample 2	57.46	21.56	28.65	3.59	.524	.368	4.17

In a sample of Cheddar cheese and a sample of Limburger cheese the writers attempted, by the use of Richmond's method (*13, p. 231*), to differentiate the primary products of ripening (precipitated as basic copper compounds) and the secondary products of ripening (not so precipitated) in the water-soluble constituents of the cheese. In Cheddar cheese it was found that 29 percent of the total nitrogen was water soluble, and of the water-soluble nitrogen, 62.6 percent was not precipitated by copper sulphate ($CuSO_4$) and sodium hydroxide ($NaOH$). In Limburger cheese, these percentages were, respectively, 34.6 and 56.6.

EXPERIMENTAL PROCEDURE

The first comparisons of cheese protein and milk-curd protein were made by the paired-feeding method, rats being used as subjects. Eight or nine pairs of rats were used in each comparison. Pair mates were always of the same sex, approximately of the same initial weight, and in the large majority of cases they were litter mates. The rats were confined in individual wire cages of the usual design, and the food consumption of pair mates was equalized, so that the only difference in their treatment was the difference in composition of the rations compared. In each comparison of cheese with milk curd, one rat in each pair received the cheese ration and the other the milk-curd ration. These rations contained the same nonprotein ingredients and practically the same percentage of nitrogen, and they had very nearly the same gross energy value as determined by the bomb calorimeter. The nitrogen content of all rations was brought close to 1.28 percent, equivalent to 8 percent of crude protein. This level of protein will permit considerable growth if the protein possesses a high biological value and yet will not promote maximum growth with the best of proteins, a point of great importance in the technic of protein comparison.

All the experimental rations contained 4 or 4.5 percent, either of the original Osborne-Mendel salt mixture (*12*) or, later, of the modified mixture proposed by Wesson (*14*), 8 percent of clarified butterfat, 2 percent of cod-liver oil, 10 percent of sucrose, 1 percent of sodium chloride, and 1 percent of dried yeast. Each ration also contained enough of the dried extracted cheese or the dried rennet curd (approximately 10 percent) to provide 1.28 percent of nitrogen, and enough starch to make up 100 percent. The rations were apparently all deficient in vitamin B_1, since shortly after the start of each experiment it was necessary to give a few drops of tiki-tiki extract to each rat to stimulate a failing appetite. In this, as in every other respect, pair mates were treated exactly alike.

The rats were weighed for 3 consecutive days at the beginning and the end of the feeding period, the average of each group of three weights constituting the initial or the final weight of the rat. Weekly weights were also taken throughout the experiments which continued for 7 or 8 weeks.

In the process of equating the food intakes of pair mates, the amount of food offered to the rats in each pair was increased as long as both rats cleaned up their dishes and was reduced whenever food residues remained in either of the dishes at the end of the day. Notes were kept of the number of times that each rat refused food throughout the experiment. A comparison of these food refusals between pair mates affords information of the comparative avidity with which the experimental rations were being consumed, or possibly of their comparative palatability. Such information is of interest in testing the frequently expressed (or implied) opinion that the better balanced of any two rations will always be consumed in the greater amount in a feeding experiment in which unrestricted access to the feed is permitted.

At the end of each experiment, the rats used therein were killed with ether, and their body lengths from mouth to anus were measured. Differences in the stage of growth between pair mates would presumably be the only gross factor determining differences in body length, while other gross factors, such as differences in the deposition of fat, would affect body weight.

EXPERIMENTAL RESULTS

GROWTH-PROMOTING VALUE OF THE PROTEIN OF CHEESE AND OF RENNET MILK CURD

The results of these paired-feeding tests are summarized in tables 2, 3, 4, and 5, while a statistical summary is given in table 6. In all tests but that of the Limburger cheese the results favored the cheese as a source of protein over the milk curd. However, in only one comparison the Swiss cheese with milk curd are the differences between pair mates significant. In this case the rat receiving the cheese ration gained faster than its pair mate in 7 of the 8 pairs, while of the weekly comparisons of gains 39.5 favored the cheese ration and only 16.5 favored the milk-curd ration. When the total gains are analyzed by Student's method (1) the mean difference in gain between pair mates is 7.25 grams (favoring the cheese ration), the standard deviation of differences is 7.15 grams, and the ratio z is 1.01. For $n=8$ and $z=1.01$ the probability that the mean difference was the result of a fortuitous combination of factors common to both groups of rats is only 0.016, and is thus so small (equivalent to 1 of 62 events) that it may be neglected. It may be concluded, therefore, that the differences observed between pair mates in rate of gain in weight was produced by the difference in the character of the dietary proteins. However, no consistent differences between pair mates were noted with respect to body length (4 comparisons favoring one ration and 4 the other), so that there may be a legitimate objection to interpreting the difference in rate of gain on the two rations as a difference in rate of growth.

The Roquefort cheese also promoted greater gains in weight than the milk curd in most of the experimental pairs. Analysis of this experiment, gives $M=+5.75$, $s=10.35$, $z=0.56$, and $P=0.11$. This probability is too large to be neglected, so that the possibility that the outcome was a result of chance cannot be removed.

In all comparisons the cheese rations were considerably less frequently refused; in other words, they were the most palatable, whether or not they promoted the most rapid gains in weight.

TABLE 2.—*Comparison of the growth-promoting value of the protein of Cheddar cheese and of rennet milk curd by the paired-feeding method during a feeding period of 60 days*

Item	Pair 1, males		Pair 2, females		Pair 3, females		Pair 4, females		Pair 5, females	
	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese
Final weight ... grams	151	154	150	145	143	151	127	134	139	142
Initial weight ... do	85	90	91	87	81	86	48	48	49	51
Gain ... do	66	64	59	58	62	65	79	86	90	91
Body length ... centimeters	19.4	19.9	19.0	18.7	18.6	18.9	18.3	18.1	18.3	18.3
Total food ... grams	515	515	512	512	520	520	503	503	521	521

Item	Pair 6, females		Pair 7, males		Pair 8, males		Pair 9, males	
	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese
Final weight ... grams	111	118	118	119	121	120	115	116
Initial weight ... do	35	37	36	37	38	39	37	37
Gain ... do	76	81	82	82	83	81	78	79
Body length ... centimeters	17.4	17.7	18.2	18.6	18.2	18.3	17.7	17.9
Total food ... grams	458	458	448	448	429	429	440	440

TABLE 3.—*Comparison of the growth-promoting value of the protein of Roquefort cheese and of rennet milk curd by the paired-feeding method during a feeding period of 56 days*

Item	Pair 1, males		Pair 2, males		Pair 3, males		Pair 4, females	
	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese
Final weight ... grams	111	128	122	133	97	115	126	112
Initial weight ... do	67	66	71	70	63	63	61	63
Gain ... do	44	62	51	63	34	52	65	49
Body length ... centimeters	17.9	18.2	18.3	18.8	17.7	17.7	17.8	17.6
Total food ... grams	447	447	481	481	379	380	422	425

Item	Pair 5, males		Pair 6, males		Pair 7, females		Pair 8, females	
	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese
Final weight ... grams	128	133	118	126	126	126	133	134
Initial weight ... do	65	66	63	64	61	61	65	63
Gain ... do	63	67	55	62	65	65	68	71
Body length ... centimeters	18.2	18.1	18.0	18.4	18.0	17.8	17.8	18.3
Total food ... grams	408	408	402	402	446	448	451	451

TABLE 4.—*Comparison of the growth-promoting value of the protein of Swiss cheese and of rennet milk curd by the paired-feeding method during a feeding period of 50 days*

Item	Pair 1, males		Pair 2, males		Pair 3, females		Pair 4, males	
	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese
Final weight grams	152	153	129	143	133	133	123	132
Initial weight do	65	68	64	56	66	63	42	39
Total gain do	87	85	65	87	67	70	81	93
Body length centimeters	19.6	19.6	18.8	18.6	19.0	18.8	18.9	18.8
Total food grams	477	477	423	423	430	430	421	421

Item	Pair 5, females		Pair 6, females		Pair 7, males		Pair 8, females	
	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese
Final weight grams	121	124	124	129	123	127	111	122
Initial weight do	38	39	39	39	38	38	38	37
Gain do	83	85	85	90	85	89	73	85
Body length centimeters	17.8	18.0	18.3	18.3	18.7	18.8	17.3	17.6
Total food grams	440	440	450	450	415	415	400	400

TABLE 5.—*Comparison of the growth-promoting value of the protein of Limburger cheese and of rennet milk curd by the paired-feeding method during a feeding period of 50 days*

Item	Pair 1, males		Pair 2, males		Pair 3, females		Pair 4, females	
	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese
Final weight grams	130	138	153	145	136	137	148	145
Initial weight do	49	53	60	57	51	54	56	54
Gain do	81	85	93	88	85	83	92	91
Body length centimeters	19.1	19.0	19.7	19.6	18.9	18.5	19.2	18.8
Total food grams	444	444	457	457	459	459	512	512

Item	Pair 5, males		Pair 6, males		Pair 7, females		Pair 8, males	
	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese
Final weight grams	116	115	121	125	121	119	100	102
Initial weight do	39	41	43	42	42	43	37	36
Gain do	77	74	78	83	79	76	63	66
Body length centimeters	18.3	18.2	18.5	18.4	17.8	18.3	17.2	17.7
Total food grams	373	373	433	433	397	397	350	350

TABLE 6.—*Statistical data on the paired-feeding experiment*

Item	Milk curd	Cheddar cheese	Milk curd	Roquefort cheese	Milk curd	Swiss cheese	Milk curd	Limburger cheese
Number of food refusals	103	33	105	49	29	12	34	4
Comparison of weekly gains of pair mates [1]	33.5	38.5	25	31	16.5	39.5	29.5	26.5
Comparison of total gains of pair mates [1]	3.5	5.5	1.5	6.5	1	7	5	3
Comparison of body length of pair mates [1]	2.5	6.5	3.5	4.5	4	4	6	2

[1] The figures in these comparisons represent the number of comparisons favoring either the rat receiving milk curd or the rat receiving cheese. In the case of equal measurements (gains in weight or body lengths) each pair mate is credited with 0.5.

COMPARATIVE DIGESTIBILITY OF CHEESE PROTEIN AND MILK-CURD PROTEIN

The comparative digestibility of cheese and milk-curd protein was determined in two of the paired-feeding experiments, those involving Roquefort and Limburger cheese. The collection periods were of 7 days' duration, throughout which the intake of food was kept the same. The coefficients of apparent digestibility for pair mates receiving the same amounts of food are summarized in table 7. For all pairs of rats the milk-curd protein was digested more completely. In one series of pairs the average coefficient for milk-curd protein was 89.65 and that for Roquefort-cheese protein 87.28. In the other series of pairs the average coefficient for the milk-curd protein was 88.78 and that for Limburger-cheese protein 87.22. Furthermore, in a later test (table 9), it was found that Swiss-cheese protein was rather consistently less digestible than milk-curd protein, the average digestion coefficients being, respectively, 89.12 and 90.53.

TABLE 7.—*Coefficients of apparent digestibility of the protein of rennet milk curd and of Roquefort and Limburger cheese for the different pairs of rats*

Pair no.	Milk curd	Roquefort cheese	Milk curd	Limburger cheese	Pair no.	Milk curd	Roquefort cheese	Milk curd	Limburger cheese
1	89.2	86.3	88.4	88.2	6	88.9	86.3	89.0	86.2
2	89.7	87.8	87.4	86.8	7	89.8	86.7	87.8	86.8
3	89.0	87.3	88.6	86.9	8	89.2	88.0	90.5	89.9
4	91.2	86.3	89.6	87.2					
5	90.1	89.5	88.9	85.8	Average	89.65	87.28	88.78	87.22

It thus appears that cheese protein supports a more rapid gain in weight in albino rats than does milk-curd protein, although it is clearly, if only slightly, less digestible. This somewhat anomalous situation is explainable on three possible grounds: (1) The voluntary activity of the rats on the cheese ration was enough less than that of those on the milk-curd ration to reduce the dietary energy available for gains in weight to an extent that compensated (or more than compensated) for the less complete digestibility of the cheese protein; or (2) the biological value of cheese protein is greater than that of milk-curd protein, and enough greater partly to offset the less complete digestibility, and in one case (Swiss cheese) to more than offset it; or (3) the gains in weight produced on the cheese rations contain less protein and more fat than those produced on the milk-curd rations.

Concerning the first possible explanation, the writers have no information to offer at all. Nevertheless, it is certainly well to keep in mind that the results of feeding experiments may be modified, or even determined by a differential effect on the voluntary activity of the rats of the two rations being compared. For example, if one ration, possibly because of a poorer balance of nutrients in it, depresses voluntary muscular activity, it may promote as great a rate of gain in body weight as a better balanced ration, even when consumed in no greater amounts, because a greater proportion of it will be available for increase in weight. The effect of qualitatively different rations upon the activity of experimental animals in nutrition laboratories is practically a virgin field of study, although in the interest of the refinement of the technic of feeding experiments, it is a field that needs careful investigation.

RELATIVE BIOLOGICAL VALUES OF MILK-CURD AND CHEESE PROTEINS

To determine the relative biological values of the proteins of milk curd and of cheese, a study was made of Swiss cheese, which had given clear indications in the paired-feeding experiments of a greater capacity to promote gains in weight than had milk curd. Ten rats were used in these experiments. The plan of the experiment, as well as the technic of the metabolism work, was essentially the same as that developed in this laboratory and described elsewhere (*3*). The 10 rats were used as 2 groups of 5 each, and the plan of feeding was as follows:

	Rats 1 to 5, inclusive	Rats 6 to 10, inclusive
Period 1.	Standardizing ration	Standardizing ration
Period 2.	Milk-curd ration	Swiss-cheese ration
Period 3.	Swiss-cheese ration	Milk-curd ration
Period 4.	Milk-curd ration	Swiss-cheese ration
Period 5.	Standardizing ration	Standardizing ration

The experimental rations had the composition shown in table 8.

TABLE 8.—*Composition of rations used in studying the relative biological values of the proteins of milk curd and of Swiss cheese*

Constituent	Standardizing ration	Milk-curd ration	Swiss-cheese ration
	Percent	*Percent*	*Percent*
Dried, fat-free whole egg	5.47		
Dried rennet-milk curd			10.60
Dried, fat-free Swiss cheese		10.13	
Modified Osborne-Mendel salts	4.5	4.5	4.5
Clarified butterfat	8	8	8
Cod-liver oil	2	2	2
Sucrose	10	10	10
NaCl	1	1	1
Harris yeast vitamin powder	0.3	0.3	0.3
Starch	66.73	62.07	61.6
Pigment	2	2	2
Protein content	4.37	8.39	8.34

In order to improve the consumption of the experimental rations, 3 drops of tiki-tiki extract (containing 9.7 mg of nitrogen) were given to each rat daily when signs of a diminished appetite became apparent.

In these metabolism experiments feces markers were used to differentiate the feces of the experimental periods from those of the preceding and following preliminary (or transition) periods. On the first day of an experimental period the ration contained 2 percent of ferric oxide (Fe_2O_3), which colored the first day's feces a brilliant red; for the remaining 6 days of the period 2 percent of barium sulphate ($BaSO_4$) replaced the ferric oxide, giving to the feces a lighter than normal appearance; then again on the first day following the termination of the experimental period, the ration containing 2 percent of ferric oxide was given.

The purpose of the initial and final standardizing periods was to determine the excretion of body nitrogen in feces (the so-called "metabolic nitrogen") and in urine (the endogenous nitrogen), the former being dependent upon the dry matter consumed and the latter upon the body weight of the rat. The average excretion of metabolic nitrogen in the feces was 1.2 mg and 1.37 mg per gram of food consumed in the two periods. The endogenous nitrogen output in the urine per 100 g body weight averaged 22.0 mg in the first standardizing period and 20.7 mg in the second. These factors differ somewhat in the two periods for each rat, and in computing the body's contribution to urine and feces in the intermediate experimental periods, they are assumed to change in a linear fashion from the initial to the final value determined.

TABLE 9.—*Nitrogen metabolism of rats on milk-curd and Swiss-cheese rations*

[Results expressed on the daily basis]

RATS 1 TO 5

Ration and rat no.	Initial body weight	Final body weight	Food intake	Nitrogen intake	Nitrogen in feces	Nitrogen in urine
	Grams	*Grams*	*Grams*	*Milligrams*	*Milligrams*	*Milligrams*
Standardizing ration:						
1	48	59	8.1		8.8	11.1
2	47	53	7.3		8.1	10.7
3	50	60	8.3		8.9	12.7
4	45	53	7.2		8.1	11.1
5	45	50	6.8		7.3	11.8
Milk-curd ration:						
1	66	86	10.0	138.3	12.8	45.8
2	60	75	8.5	123.7	11.0	48.5
3	64	81	9.2	129.6	12.6	52.6
4	58	72	7.9	118.3	11.4	45.7
5	58	66	5.8	112.9	8.7	41.6
Swiss-cheese ration:						
1	90	106	10.0	138.1	15.4	55.7
2	78	91	8.7	126.6	12.9	54.7
3	87	97	9.4	128.5	14.8	60.3
4	74	89	8.8	131.3	13.4	50.5
5	64	77	8.2	112.8	15.0	46.5
Milk-curd ration:						
1	117	133	11.5	159.1	15.4	67.1
2	99	112	9.9	136.7	(12.5)	59.7
3	104	122	11.0	149.5	(13.7)	61.9
4	97	108	9.3	131.8	12.4	60.0
5	85	96	8.5	122.2	10.9	47.1
Standardizing ration:						
1	137	150	11.4		15.0	22.6
2	119	123	9.6		12.9	26.7
3	126	140	12.0		14.0	26.1
4	112	122	10.7		13.4	23.4
5	100	110	9.9		12.5	22.7

RATS 6 TO 10

Ration and rat no.	Initial body weight	Final body weight	Food intake	Nitrogen intake	Nitrogen in feces	Nitrogen in urine
Standardizing ration:						
6	50	56	6.7		7.7	10.4
7	46	52	5.8		6.8	10.7
8	49	58	7.3		8.1	11.4
9	49	56	7.1		8.1	12.2
10	47	55	7.1		7.9	11.0
Swiss-cheese ration:						
6	59	68	6.6	90.0	10.1	34.3
7	57	69	6.4	89.9	8.7	30.0
8	65	80	8.9	119.7	11.8	43.6
9	63	77	8.7	118.9	12.2	41.2
10	60	71	8.2	110.1	11.3	45.1
Milk-curd ration:						
6	75	87	8.0	115.7	12.3	49.5
7	74	87	8.1	115.2	10.7	45.6
8	88	102	9.3	131.8	13.2	58.8
9	85	99	9.2	130.8	13.2	55.6
10	76	89	8.7	124.7	12.7	53.6
Swiss-cheese ration:						
6	92	102	8.1	117.9	13.5	55.8
7	93	103	7.2	109.8	12.2	41.9
8	105	117	9.7	133.5	13.5	57.4
9	104	116	9.9	135.3	15.2	64.7
10	95	104	9.3	128.6	14.3	65.6
Standardizing ration:						
6	108	112	8.2		12.6	19.7
7	110	117	8.8		11.7	20.2
8	124	132	10.8		14.4	24.3
9	121	129	11.3		17.1	30.9
10	111	111	7.8		12.6	31.7

All the nitrogen-metabolism data of this experiment are assembled in table 9, while the digestion coefficients and biological values computed from them are summarized in tables 10 and 11. To conserve space in the tables the intermediate calculations are omitted, the method of calculation having been fully explained in earlier publications from this laboratory.

TABLE 10.—*Coefficients of apparent and true digestibility of the protein of Swiss cheese and of rennet milk curd* [1]

Rat no.	Apparent digestibility				True digestibility			
	Milk-curd protein		Swiss-cheese protein		Milk-curd protein		Swiss-cheese protein	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
1	90.7	90.3	88.6	--------	99.0	99.5	97.5	--------
2	91.1	90.9	89.8	--------	99.3	100.0	98.4	--------
3	90.3	--------	88.4	--------	98.2	99.3	96.8	--------
4	90.4	90.6	89.8	--------	98.3	99.3	97.8	--------
5	92.3	91.1	86.7	--------	98.3	99.7	95.3	--------
6	89.3	--------	88.8	88.5	98.8	--------	98.0	98.4
7	90.7	--------	90.3	88.9	99.5	--------	98.9	97.3
8	90.0	--------	90.1	89.8	98.6	--------	98.8	99.0
9	89.9	--------	89.7	88.8	99.3	--------	98.7	99.2
10	89.8	--------	89.7	88.9	99.3	--------	98.9	99.6
Average	90.53		89.12		99.09		98.17	

[1] For rats 1 to 5, 2 digestion trials were run on the milk-curd ration, while for rats 6 to 10, 2 digestion trials were run on the Swiss-cheese ration.

TABLE 11.—*Biological values of the protein of Swiss cheese and of rennet milk curd*

Rat no.	Milk-curd protein		Swiss-cheese protein		Rat no.	Milk-curd protein		Swiss-cheese protein	
	Trial 1	Trial 2	Trial 1	Trial 2		Trial 1	Trial 2	Trial 1	Trial 2
1	77	71	71	--------	7	74	--------	81	78
2	72	73	71	--------	8	70	--------	76	73
3	71	73	67	--------	9	74	--------	79	72
4	73	71	74	--------	10	74	--------	73	70
5	76	78	72	--------					
6	70	--------	75	67	Average	73.1		73.3	

An illustration of the calculation of a biological value for protein is the following: Rat no. 6 in period 2 consumed 6.59 g daily of the Swiss-cheese ration, containing 90 mg of nitrogen. The average daily excretion of nitrogen in the feces was 10.1 mg, of which 8.3 mg (1.26×6.59) was estimated to be of body origin. Hence, the fecal nitrogen of food origin was equal to $10.1 - 8.3 = 1.8$ mg. The absorbed food nitrogen therefore is equal to $90 - 1.8 = 88.2$ mg. The urinary nitrogen for the period averaged 34.3 mg daily, the sum of 12.5 mg of endogenous nitrogen (19.2×65, the average body weight of the rat for the period) and 21.8 mg of food nitrogen unutilized in metabolism. Of the nitrogen absorbed, $88.2 - 21.8 = 66.4$ mg were retained in the body for both maintenance and growth. The biological value is this figure expressed as a percentage of the absorbed nitrogen, i.e. $(66.4 \div 88.2) \times 100 = 75$.

The digestion coefficients given in table 10 again reveal a lower digestibility of cheese nitrogen than of milk-curd nitrogen. The average coefficients of apparent digestibility are 89.12 and 90.53, respectively. If the fecal nitrogen is reduced by the amount of metabolic nitrogen estimated to be present in the feces, it is possible to compute coefficients of true digestibility, which average 98.17 and 99.09, respectively, the advantage again resting with the milk-curd protein.

However, the biological values computed for the two protein sources were very nearly the same, averaging 73.1 for milk-curd protein and 73.3 for Swiss-cheese protein (table 11).

These determinations of the biological values of the protein of milk curd and of Swiss cheese indicate that the good showing of the cheese proteins in the paired-feeding experiments, in spite of a distinctly lower digestibility, cannot be explained on the basis of a higher utilization of their absorbed nitrogen in the anabolic chemical reactions of maintenance and growth. The remaining possibility is that the explanation is to be found in the composition of the gains in weight produced on the two types of rations, one containing milk curd as its main source of protein and one containing cheese as its predominant source of protein.

NITROGEN AND ENERGY CONTENT OF PAIRED RATS ON MILK-CURD AND LIMBURGER-CHEESE RATIONS

In order to investigate this possibility, the 16 rats used in the paired-feeding comparison of milk curd and Limburger cheese (table 5) were analyzed after removal of the gastrointestinal tract and contents. The feeding experiment had shown no advantage for either sort of protein in the promotion of gains in weight but did show a clear advantage of the milk-curd protein over the cheese protein in digestibility (table 7). The nitrogen contents of the rat carcasses were determined by the Kjeldahl method, and their gross energy values (heats of combustion) by the Parr oxgyen bomb calorimeter. The results of these determinations are given in table 12.

TABLE 12.—*Nitrogen and energy contents of paired rats on milk-curd and Limburger-cheese rations*

Pair no.	Protein in ration	Body weight of rat minus alimentary tract	Nitrogen content		Energy content	
			Percent	Grams	Per gram	Total
		Grams			*Calories*	*Calories*
1	Milk curd	123	2.57	3.16	2.24	276
	Cheese	130	2.32	3.02	2.43	315
2	Milk curd	144	2.69	3.87	2.53	365
	Cheese	135	2.74	3.70	2.34	316
3	Milk curd	129	2.70	3.48	2.26	292
	Cheese	129	2.55	3.29	2.33	301
4	Milk curd	140	2.68	3.75	2.22	311
	Cheese	137	2.52	3.45	2.42	332
5	Milk curd	109	2.69	2.93	1.89	206
	Cheese	109	2.68	2.92	2.21	241
6	Milk curd	113	2.82	3.19	1.84	208
	Cheese	117	2.36	2.76	2.11	247
7	Milk curd	114	2.65	3.02	2.00	228
	Cheese	112	2.39	2.68	2.51	281
8	Milk curd	94	2.90	2.73	2.06	194
	Cheese	95	2.67	2.54	2.09	199

An analysis according to the Student method of the differences between pair mates with respect to their nitrogen and energy contents is given in table 13.

TABLE 13.—*Analysis according to the Student method of the differences between pair mates with respect to nitrogen and energy contents*

Item	Nitrogen content		Energy content	
	Percent	Grams	Per gram	Total
			Calories	*Calories*
Mean of differences, M [1]	+0.182	+0.221	−0.175	−19.0
Standard deviation, s	.148	.122	.196	29.9
Ratio $z=M\div s$	1.23	1.81	.89	.64
Probability, P	.0071	.001	.025	.068

[1] The differences between pair mates were given a plus sign when the value for the rat on the milk-curd ration was the larger, and a negative sign when it was the smaller.

In every pair of rats but one, the percentage of nitrogen was higher for the rat on the milk-curd diet, and in all eight pairs the weight of nitrogen was higher. The probabilities that these paired differences were determined only by chance are so low (i.e., 0.0071 and 0.001, respectively) that they may be neglected. It seems certain, therefore, that the difference in the rations produced the difference in nitrogen content of the rats, the milk-curd ration promoting, to a greater extent, the deposition of nitrogen during growth.

The values for the energy contents of the rats presents a different picture, though not so convincing. In every pair but one the energy content of the rat receiving the Limburger-cheese ration, whether expressed in calories per gram of rat or in total calories, was greater than that of the rat raised on the milk-curd ration. The probabilities that chance alone could have produced these results may not be small enough to disregard entirely, although with respect to the gross energy per gram chance would be expected to produce such a one-sided outcome once in about 40 times. In this case at least it may be said with practical certainty that the difference in ration was responsible, the Limburger-cheese ration producing a carcass with the higher gross-energy content presumably because of a greater content of fat.

TABLE 14.—*Estimated gains in nitrogen and gross energy of paired rats on milk-curd and Limburger-cheese rations*

Pair no.	Protein of ration	Body weights minus intestinal tract		Estimated initial content of rats in—		Observed final content of rats in—		Gains in—	
		Initial [1]	Final	Nitrogen	Energy	Nitrogen	Energy	Nitrogen	Energy
		Grams	*Grams*	*Grams*	*Calories*	*Grams*	*Calories*	*Grams*	*Calories*
1	Milk curd	42	123	1.14	63	3.16	276	2.02	213
	Cheese	46	130	1.25	69	3.02	315	1.77	246
2	Milk curd	53	144	1.44	80	3.87	365	2.43	285
	Cheese	50	135	1.36	75	3.70	316	2.34	241
3	Milk curd	44	129	1.20	66	3.48	292	2.28	226
	Cheese	47	129	1.28	71	3.29	301	2.01	230
4	Milk curd	49	140	1.33	74	3.75	311	2.42	237
	Cheese	47	137	1.28	71	3.45	332	2.17	261
5	Milk curd	33	109	.90	50	2.93	206	2.03	156
	Cheese	35	109	.95	53	2.92	241	1.97	188
6	Milk curd	37	113	1.01	56	3.19	208	2.18	152
	Cheese	36	117	.98	54	2.76	247	1.78	193
7	Milk curd	36	114	.98	54	3.02	228	2.04	174
	Cheese	37	112	1.01	56	2.68	281	1.67	225
8	Milk curd	31	94	.84	47	2.73	194	1.89	147
	Cheese	30	95	.82	45	2.54	199	1.72	154

[1] Estimated.

In order to eliminate in these comparisons the results of variable initial contents of nitrogen and energy among the paired rats, the gains in nitrogen and in energy were computed from the observed final contents and from estimates of the initial contents based on the initial weights. The initial weights of the rats were corrected to the weights minus the intestinal tract by the use of Donaldson's tables (*4*). The remainder of the carcass was then assumed to contain 2.72 percent of nitrogen and 1.505 calories of gross energy per gram on the basis of the results of Mitchell and Carman (*9*). The computation of the gains of nitrogen and energy is given in table 14.

The differences between pair mates in the storage of nitrogen averaged 232 mg in favor, in each pair, of the milk-curd ration. The standard deviation of differences was 113 mg, and the probability that this outcome is a result of chance is only 0.0005, clearly negligible. The differences between pair mates in energy storage averaged 18.5 calories, and in all pairs but one favored the Limburger-cheese ration. However, the standard deviation of differences is 27.9 calories and the desired probability is 0.063. These results are therefore merely highly suggestive of a ration effect.

It appears that the less digestible Limburger-cheese nitrogen promoted a smaller storage of nitrogen in the paired-feeding experiment than the same intake of milk-curd nitrogen. Nevertheless, the gains in weight were just as large, due in all probability to a greater deposition of fat in the rats on the cheese ration.

TABLE 15.—*Estimation of the biological values of the protein of milk curd and Limburger cheese when fed to paired rats*

Pair no.	Protein of ration	Average body weight	Total intake of digestible nitrogen	Total endogenous nitrogen	Nitrogen stored in tissues	Total nitrogen utilized	Biological value of nitrogen
		Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*
1	Milk curd	90.4	5.25	0.99	2.02	3.01	57
	Cheese	96.5	5.24	1.06	1.77	2.83	54
2	Milk curd	106.0	5.34	1.17	2.43	3.60	67
	Cheese	100.6	5.30	1.11	2.34	3.45	65
3	Milk curd	95.7	5.44	1.05	2.28	3.33	61
	Cheese	96.0	5.34	1.06	2.01	3.07	57
4	Milk curd	104.1	6.13	1.15	2.42	3.57	58
	Cheese	100.5	5.96	1.11	2.17	3.28	55
5	Milk curd	79.5	4.45	.87	2.03	2.90	65
	Cheese	78.5	4.29	.86	1.97	2.83	66
6	Milk curd	84.5	5.16	.93	2.18	3.11	60
	Cheese	84.0	5.00	.92	1.78	2.70	54
7	Milk curd	81.9	4.67	.90	2.04	2.94	63
	Cheese	81.0	4.62	.89	1.67	2.56	55
8	Milk curd	67.7	4.25	.75	1.89	2.64	62
	Cheese	68.5	4.23	.75	1.72	2.47	58

It is possible from the data at hand to determine more or less certainly whether the smaller storage of nitrogen on the cheese ration was entirely accounted for by the smaller digestibility of the cheese nitrogen. In table 15 will be found computations similar in import to those leading to the determination of biological values from the data of metabolism experiments. The only explanation necessary relates to the computation of the endogenous nitrogen. The values given for this item cover the entire period of the feeding experiment. They are based upon the average weight of the rat for the feeding period and an average value, taken from metabolism experiments, of 22 mg of endogenous nitrogen per day per 100 g of body weight. It should be said of the biological values given in the last column that they may be less than those obtained in a short metabolism experiment if, during the prolonged feeding period, the rat has shed appreciable amounts of hair and scurf. In such case the nitrogen stored in the tissues is really greater than the difference between the initial and final nitrogen content of the rat.

From the table it is evident that in all pairs but one the computed biological value of the dietary protein is smaller for the Limburger cheese than for the milk curd, the averages being, respectively, 58 and 62. The average difference is 3.63 and the standard deviation of differences 2.49. The probability that chance alone would account for the paired differences is only 0.0031. It seems, therefore, that the biological value of Limburger-cheese protein is somewhat less (about 6 percent) than that of its mother substance, the milk-curd protein. Such a difference was not detected in metabolism experiments affording a comparison of the protein of Swiss cheese and of milk curd (table 11), though this fact cannot be considered inconsistent with the finding for Limburger cheese because of the more extensive ripening to which the latter cheese was evidently subjected.

If the protein of milk curd, casein, has suffered a deterioration in nutritive value during the conversion of the curd into Limburger cheese, then it may be expected that the amino acid deficiency of the casein would be altered. It has been demonstrated that casein is deficient in the sulphur-containing amino acid, cystine (*11*). If, during the ripening process, cystine is destroyed more rapidly than other amino acids indispensable in nutrition, then the cystine deficiency of the cheese protein will be greater than the cystine deficiency of casein, so that a greater improvement in its biological value will result when it is supplemented with an excess of cystine. On the other hand, if some other indispensable amino acid is destroyed more rapidly than the cystine, then the resulting cheese protein will possess a smaller deficiency of cystine, or even no cystine deficiency at all.

CYSTINE DEFICIENCIES OF MILK-CURD AND LIMBURGER-CHEESE PROTEINS

In measuring the cystine deficiencies of the protein of milk curd and the protein of Limburger cheese, the paired-feeding technic was modified. The ordinary technic as used in the preceding experiments is for some purposes open to the criticism that the better of two rations being compared is in a sense being penalized, because the rats receiving it are growing at the faster rate and are correspondingly increasing their maintenance requirements over those of their pair mates. Hence, of the equal intakes of food, a smaller proportion of the better ration will be available for growth over and above the maintenance requirements. Osborne and Mendel (*11, p. 9*) have discussed the same situation and have concluded: "The only strict basis for comparison is afforded by experiments in which the animals receive the same amount of food during the same period of time and make the same gain in weight."

This principle was incorporated into the paired-feeding technic, as illustrated by the milk-curd experiment. Several kilograms of a milk-curd ration containing 8 percent of protein, and otherwise similar in make-up to the rations used in the preceding experiments, were prepared, thoroughly mixed in an electric mixer, and divided into two equal portions. One portion was used unchanged, while to the other portion was added 0.3 percent of cystine, equivalent to 3.75 percent of the protein. This proportion of cystine is more than enough to supplement the casein. The two rations were then fed to a series of pairs of rats in the ordinary way until differences in weight developed between pair mates. Then the rat in each pair that received the cystine diet was fed a combination of this diet and of a low-nitrogen diet similar to it except that it contained no cystine and that starch was substituted for the dried milk curd. The total amount of the two rations fed to the rats receiving cystine was kept the same as the amount of food consumed by their pair mates on the unsupplemented milk-curd ration, while the proportion between the amounts of the two combined rations was so adjusted that the gains in weight of pair mates were equalized as closely as possible. Thus, as the rats on the cystine ration increased in weight beyond their pair mates, they were given a greater proportion of the low-nitrogen diet, and vice versa. From 3 to 5 drops of tiki-tiki extract were given daily to each rat in order to maintain the appetite.

TABLE 16.—*Supplementing effect of cystine on the protein of rennet milk curd during a feeding period of 49 days when fed to paired rats*

Item	Pair 1, females		Pair 2, females		Pair 3, females		Pair 4, females	
	No cystine	Cystine	No cystine	Cystine	No cystine	Cystine	No cystine	Cystine
Final weight grams	122	121	129	128	115	121	128	125
Initial weight do	46	44	50	50	49	49	50	50
Gain do	76	77	79	78	66	72	78	75
Body length centimeters	18.4	18.3	18.4	18.4	18.3	18.6	18.2	18.1
Food intake grams	443	443	466	466	428	428	447	447
Nitrogen intake do	5.71	4.41	6.01	4.23	5.53	3.86	5.77	4.34
Ratio of nitrogen intakes	1.29	1	1.42	1	1.43	1	1.33	1

Item	Pair 5, females		Pair 6, females		Pair 7, males		Pair 8, females	
	No cystine	Cystine	No cystine	Cystine	No cystine	Cystine	No cystine	Cystine
Final weight grams	138	133	126	120	153	152	138	142
Initial weight do	48	46	49	45	57	55	53	56
Gain do	90	87	77	75	96	97	85	86
Body length centimeters	18.8	18.9	18.0	18.2	19.5	19.4	18.9	19.1
Food intake grams	447	447	451	451	500	500	488	488
Nitrogen intake do	5.77	5.15	5.82	4.50	6.45	4.96	6.30	5.22
Ratio of nitrogen intakes	1.12	1	1.29	1	1.30	1	1.21	1

The results of this type of an experiment on milk-curd protein are given in table 16. On the same intakes of food the gains in weight of pair mates were kept very nearly the same (except in one pair in which the difference amounted to 6 g) simply by varying the nitrogen intake. A considerably smaller amount of nitrogen from the mixture of casein and cystine promoted the same gains in weight. From the ratios between the nitrogen intakes of pair mates, given in the last line of the table, it appears that 1 g of nitrogen from the casein-cystine mixture produced the same nutritive effect as 1.12 to 1.43 g of nitrogen (averaging 1.30 g) from the unsupplemented casein. Taking the biological value of casein as 73, that of the supplemented casein would be $73 \times 1.3 = 95$,[3] on the reasonable assumption that cystine did not affect the digestibility of the casein. Mitchell (*8*) has reported metabolism data on a rat, indicating a biological value of 75 for casein, and a value of 92 for casein fully supplemented with cystine.

Three pairs of rats in this experiment, nos. 1, 7, and 8, were analyzed at the end of the feeding period to determine whether the composition of the carcasses had been affected by the difference in dietary treatment. Only the nitrogen and energy contents of the empty carcasses were determined. The results are given in table 17.

TABLE 17.—*Nitrogen and energy contents of empty carcasses of three pairs of rats used in experiments for which data are recorded in table 16*

Pair no.	Dietary protein	Nitrogen content	Energy content per gram
		Percent	*Calories*
1	Milk curd	3.34	2.07
	Milk curd+cystine	3.30	2.18
7	Milk curd	3.03	2.30
	Milk curd+cystine	3.02	2.64
8	Milk curd	3.18	2.31
	Milk curd+cystine	3.28	2.22

The differences between pair mates were slight and did not show any consistency, so that it may be concluded that the similar gains of pair mates signified similar nitrogen retention. For these six rats computations of the biological value of the dietary protein were made, according to the method used in a preceding experiment (tables 14 and 15), with the results given in table 18. The unsupplemented milk-curd protein exhibited in this test biological values of 73, 73, and 70, which are of the same magnitude as those determined by the nitrogen-balance studies (table 11). The supplemented casein gave biological values of 93, 95, and 88, averaging 92. The results of the feeding experiment led to an estimate of 95.

TABLE 18.—*Estimation of the biological values of the protein of milk curd with and without a cystine supplement when fed to paired rats*

Pair no.	Supplement	Total intake of digestible nitrogen	Total endogenous nitrogen	Total nitrogen stored in tissues	Total nitrogen utilized	Biological value
		Grams	*Grams*	*Grams*	*Grams*	*Percent*
1	None	5.15	0.95	2.83	3.78	73
	Cystine	3.98	.95	2.75	3.70	93
7	None	5.80	1.12	3.09	4.21	73
	Cystine	4.43	1.12	3.09	4.21	95
8	None	5.66	1.06	2.91	3.97	70
	Cystine	4.71	1.08	3.06	4.14	88

[3] It must be admitted that part of this increase in the biological value of casein accompanying the addition of cystine may be due to the fact that the supplemented casein was fed at a lower percentage of the diet than the unsupplemented. This in itself would tend to increase the biological value, although all proteins do not show this effect (*7*).

The Limburger cheese was tested by the same methods applied to a study of the cystine deficiency of milk-cured protein. Eight pairs of rats were used in a preliminary test and were carried for 2 weeks on equalized intakes of the supplemented and unsupplemented rations containing equal percentages of nitrogen. The purpose of this test was to determine whether the protein of Limburger cheese was deficient in cystine. At the end of 2 weeks of feeding such a deficiency was clearly established. In seven pairs, the greater gains were made by the rat receiving the cystine-supplemented diet, while in one pair the gains were equal. The average difference in gain between pair mates was 3.50 g, and the standard deviation of differences was 2.46 g. The probability that the average difference was the result merely of a fortuitous combination of factors common to both rats of a pair is only 0.0036, certainly small enough to be negligible.

TABLE 19.—*Supplementing effect of cystine on the protein of Limburger cheese during a feeding period of 45 days when fed to paired rats*

Item	Pair 1, females		Pair 2, males		Pair 3, females		Pair 4, males	
	No cystine	Cystine	No cystine	Cystine	No cystine	Cystine	No cystine	Cystine
Final weight……grams	85	86	98	97	95	98	110	108
Initial weight……do	42	42	40	39	44	47	47	45
Gain……do	43	44	58	58	51	51	63	63
Body length……centimeters	16.5	16.8	17.0	17.2	16.9	17.0	17.4	17.6
Food intake……grams	305	305	290	290	292	292	282	282
Nitrogen intake……do	4.14	3.61	3.93	3.47	3.97	3.67	3.83	3.20
Ratio of nitrogen intakes	1.15	1	1.13	1	1.08	1	1.20	1

Item	Pair 5, males		Pair 6, males		Pair 7, females		Pair 8, females		Pair 9, females	
	No cystine	Cystine	No cystine	Cystine	No cystine	Cystine	No cystine	Cystine	No cystine	Cystine
Final weight……grams	87	87	100	100	112	112	100	99	102	101
Initial weight……do	38	39	43	42	48	48	46	43	32	32
Gain……do	49	48	57	58	64	64	54	56	70	69
Body length……centimeters	17.8	17.8	17.3	17.7	17.8	18.3	17.3	17.4	17.4	17.5
Food intake……grams	255	255	296	296	375	375	319	319	328	328
Nitrogen intake……do	3.46	3.33	4.02	3.57	5.08	4.51	4.33	3.75	4.45	3.95
Ratio of nitrogen intakes	1.04	1	1.13	1	1.13	1	1.15	1	1.13	1

A cystine deficiency in the protein of Limburger cheese having been established, the extent of this deficiency was quantitatively assessed by the modified paired-feeding technic. Nine pairs of rats were used in the experiment. The results obtained are summarized in table 19. A very close equalization of the gains of pair mates was secured in this test, but a relatively greater proportion of the cheese-cystine nitrogen was required than of the casein-cystine nitrogen in the preceding test. The ratios of nitrogen intakes from the unsupplemented and the supplemented cheese rations ranged from 1.04 to 1.20 to 1, and averaged 1.13 to 1. If the biological value of the Limburger-cheese protein may be taken as 6 percent less than that of casein (Table 11), namely 69, then the addition of cystine increased it only 13 percent, giving a value of 78. This is appreciably less than the value of 92 to 95 given by milk-curd protein plus cystine. It may be concluded, therefore, that the ripening of the curd in the manufacture of Limburger cheese lowered the biological value of the protein by reactions involving the destruction of cystine to some extent, but to a greater extent the destruction of some one or more of the other amino acids indispensable in animal nutrition. We may infer that cystine has been involved to some extent in these reactions both because it is still the limiting deficiency of the cheese protein, and because the biological value of the cheese protein appears to be somewhat less than that of the curd protein. We may infer that other indispensable amino acids are also involved, because the improvement in biological value induced by the addition of cystine is considerably less than the improvement noted in the case of casein. In other words, a second limiting amino acid deficiency is manifest in the supplemented cheese protein at a lower biological value than in the supplemented curd protein.

DISCUSSION

The most general and clear-cut finding of the feeding and metabolism experiments reported in this paper on four varieties of cheese (Cheddar, Roquefort, Swiss, and Limburger) was that in the ripening process to which milk curd is subjected in the manufacture of cheese, the digestibility of the protein of the curd is depressed 1 or 2 percent. However, the capacity of the cheese protein to promote gains in weight was not lowered. In fact, in the case of Swiss cheese, its gain-promoting capacity was definitely, though slightly, increased over that of milk curd. In metabolism experiments on 10 rats no differences in the biological values of rennet curd and of Swiss cheese could be distinguished. Hence, the depressed digestibility of the protein of this cheese could be reconciled with its increased capacity to promote gains in weight in young rats, most probably on the assumption that the chemical composition of the weight increases on cheese portein showed less protein and more fat than those produced by milk-curd protein.

This assumption was tested for Limburger cheese, the protein of which was found to be as efficient as milk-curd protein in promoting gains in weight, within the limits of accuracy of the paired-feeding method, although it also was distinctly less digestible. By analyzing the carcasses of the rats at the conclusion of the paired-feeding tests, and from average analyses of rats at the initial weight, computing the nitrogen and gross energy content of the gains in weight produced, it was clearly shown that the assumption was correct. It was also shown that in the case of Limburger cheese the biological value of the protein was slightly depressed, about 6 percent, below that of the casein of milk curd. The contrast in this respect between this protein and that of Swiss cheese is probably related to the more extensive ripening which the Limburger cheese undergoes.

The fact that equal gains in weight put on in the same periods on the same amounts of two rations may differ appreciably in composition, indicates that a gram gain in weight in experimental rats possesses a variable nutritive significance, although in the current interpretation of feeding experiments on rats, less well controlled than were these, the nutritive significance of gains in weight are assumed to vary only with their size. This experiment on Limburger cheese confirms the conclusion of Mitchell and Carman (*9, p. 410*), expressed 8 years ago:

> Therefore, the gain in weight of an animal, no matter how carefully experimental conditions may be prescribed, is not a definite measure of the nutritive effect of the ration fed, but is subject to considerable variation due to the operation of unknown factors.

It proves beyond doubt that the exact interpretation of feeding experiments, no matter how well controlled they may be, requires an investigation of the chemical composition of the carcasses produced by the experimental feeding.

The analyses of the carcasses of experimental rats in these studies have permitted the computation of the biological value of dietary protein by another method than that based on the metabolism data secured over a short period. The former biological values may be less than the latter on account of the loss of hair and scurf during a long feeding period, but in one case, involving a study of milk-curd protein, the two computations led to practically identical results.

The nutritive value of Limburger-cheese protein, and presumably of that of the proteins of other cheeses in which the chemical changes of ripening are no more extensive, is limited by a deficiency of cystine, in a similar manner, though not to so great an extent, as the paracasein of milk curd. In studying the quantitative aspects of the cystine deficiency of cheese and milk curd, a modification of the paired-feeding method was used, involving a principle enunciated by Osborne and Mendel in one of their earlier series of feeding experiments, namely, that in the comparison of the growth-promoting values of proteins the experimental animals should receive "the same amount of food during the same period of time and make the same gain in weight", the only variable being the intake of protein.

CONCLUSIONS

The digestibility of the protein of Roquefort, Swiss, and Limburger cheese is from 1 to 2 percent less than that of rennet milk curd.

The biological value of the protein of Swiss cheese is practically the same as that of rennet curd, i.e., 73, while the biological value of the protein of Limburger cheese is about 6 percent less.

In Limburger cheese, and presumably in all cheeses in which ripening has proceeded no further, cystine is the amino acid limiting its biological utilization, as is true of casein itself. However, the biological value of the protein of Limburger cheese is not increased as much by cystine supplementation as is the biological value of casein.

Cystine destruction is involved in the ripening reactions in the preparation of Limburger cheese, as is also one other, at least, of the amino acids indispensable in animal nutrition.

The accurate interpretation of the gains in weight of animals secured in feeding experiments, no matter how well controlled, requires an investigation of their nutrient content.

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