UTILIZATION BY HUMAN SUBJECTS OF THE NITROGEN FROM ROUND AND LIVER OF BEEF

by

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INTRODUCTION

The quality of the protein in various food materials has been studied rather extensively during the past decade. Variation in biological value has been found in the proteins of different meats and of different cuts of the same meat, so it was thought profitable to investigate this latter phase further. It was possible to obtain cuts of meat in equal amounts from the same animals which were of known history. In this way variations due to difference in source could be eliminated.

Beef liver is regarded as a good source of iron and the various vitamins. Mitchell (6), working with rats, suggests that it is also a superior source of protein, and to compare it in this respect with beef round, this study was made with human subjects.

WORK IN THE FIELD

It has been shown (7) that with proteins, as with other nutrients, successful nutrition and continued health and physiological efficiency are possible over a wide range of intake. With protein, this may be due to the fact that within 2 to 5 days, the organism adjusts the rate of protein metabolism to the intake (13).

The bulk of the nitrogen passes from the body through the urine and feces. It is commonly stated that ordinarily 2 per cent is lost through the skin, hair, and nails; 8 per cent through the feces, and the remainder through the urine. Since Lusk (5) and also Thomas (15), have suggested that the amount lost through the skin is negligible, particularly on a low protein diet, it is generally ignored in this type of experiment.

Foods of similar chemical composition are found to be utilized differently by the animal organism, being influenced by various factors. Mitchell (6) has indicated the superiority of meat and milk over vegetable proteins from the standpoint of biological value.

He has further shown (8) that this biological value correlates closely with the percentage of nitrogen in the experimental diet, in such a way that within limits, the lower the level of nitrogen feeding, the greater the biological value. He found that when young rats consumed corn, milk, oats, and potatoes at approximately 5 and 10 per cent levels of intake, with all except potatoes, the higher biological values were obtained at the lower level (8). Similar results were obtained with meats (7).

Mitchell also found that rats utilized different meats and certain cuts of the same meat differently. He attributed this mainly to the amount of connective tissue present. With liver, kidney, and heart, he associated a relatively high biological value with a correspondingly low content of collagen and elastin, the main constituents of connective tissue.

A number of observations have been made on the effect of drinking water upon nitrogen metabolism. Orr (11), varying the protein intake from 27 to 319 grams, found that increase in water increased the urinary nitrogen. This was more marked on a low protein diet. There was also a decrease in fecal nitrogen which was interpreted as indicative of a better utilization of food protein.

Sherman, Gillett, and Pope (14) state that the menstrual flow is essentially a blood loss and, as such, is of minor consequence in the nitrogen metabolism but suggest that there is a tendency to retain nitrogen for a day or so at the beginning of the menstrual period. However, in nitrogen balance experiments it is customary to ignore this loss (5).

For some time there has been recognition of a possible effect of crude fiber or "roughage" on the excretion of nitrogen by the body. Mitchell (9), working with rats receiving varying amounts of filter paper in their diet, found that metabolic fecal nitrogen increased as much as 42 per cent under these conditions. Whitacre, Willard, and Blunt (16) comparing results of feeding human subjects two diets, one higher in fiber than the other, obtained noticeably lower coefficients of digestibility for the nitrogen on the diet containing more fiber. They suggest that a large amount of bulk in the diet may interfere with the action of proteolytic enzymes, and so tend to reduce the utilization of nitrogen.

PROCEDURE

Subjects. The subjects for this experiment were three normal young college women, all graduate assistants, with comparable activities.

Diet. The diet used, shown in Table II, was calculated to be sufficient for maintenance of the subjects. It consisted of the meat to be tested, and a basal ration of sweetened orange juice, a practically nitrogen-free bread, and nitrogen-free butterfat. The bread, cracker-like in texture, was made as follows:

	gm.		gm.
Cornstarch....	120	Dextri-Maltose............	15
Lactose.......	40	Baking powder (tartrate)..	3
NaCl..........	3	Butterfat (filtered)......	32
Agar-agar.....	4	Water (distilled).........	80

The dry ingredients were mixed together and the water and melted butterfat added. The resulting paste was spread in a very thin layer on a baking sheet, and baked for 1 hour at 325° F.

Orange juice, prepared fresh daily, was strained to remove seeds and coarse pulp. To 1000 grams, one day's portion for each subject, were added 80 grams lactose and 40 grams sucrose.

Subjects A and B, of approximately the same weight, were calculated to have the same energy requirement. Subject C was slightly heavier and more active, so needed more Calories as seen in Table I. These were taken in the form of a fondant candy made from 70 grams sucrose, 4 grams cornstarch, 3 grams butterfat, and 60 grams distilled water.

TABLE I

DAILY CALORIE INTAKE OF SUBJECTS

Subject	Approximate Wt. in Kg.	Calories per Kg.	Total Calories
A	56	39.3	2200
B	56	39.3	2200
C	60	42.0	2520

A preliminary analysis of cornstarch showed it to contain 0.44% protein (N x 6.25). The nitrogen supplied by

TABLE II

DAILY FOOD INTAKE OF SUBJECTS

Food	Beef Round				Beef Liver			
	Subjects A and B[1]		Subject C		Subjects A and B[1]		Subject C	
	Wt.	N[2]	Wt.	N	Wt.	N[2]	Wt.	N
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Bread								
Cornstarch	120	0.084	120	0.084	120	0.084	120	0.084
Lactose	40	-	40	-	40	-	40	-
Dextri-Maltose	15	-	15	-	15	-	15	-
Baking powder	3	-	3	-	3	-	3	-
Salt	3	-	3	-	3	-	3	-
Agar	4	-	4	-	4	-	4	-
Butterfat	32	-	32	-	32	-	32	-
Water (distilled)	80	-	80	-	80	-	80	-
Butterfat	17	-	15	-	20.5	-	18.5	-
Orange juice mixture								
Orange juice	1000	1.881	1000	1.881	1000	1.394	1000	1.394
Lactose	80	-	80	-	80	-	80	-
Sucrose	40	-	40	-	40	-	40	-
Meat	131	4.631	143.2	5.063	136.8	5.012	149.5	5.478
Candy[3]								
Sucrose	0	0	70	0	0	0	70	0
Cornstarch	0	0	4	0.003	0	0	4	0.003
Butterfat	0	0	3	-	0	0	3	-
Water	0	0	60	-	0	0	60	-
Minerals added								
Calcium carbonate	0.55	-	0.67	-	0.54	-	0.64	-
Ferric citrate	0.043	-	0.042	-	0.008	-	0.004	-
Sodium chloride	3	-	3	-	3	-	3	-

1. Subjects A and B, as calculated, had the same food requirements since they had nearly the same weight and activity.
2. Average for experiment.
3. Extra calories from candy were not necessary for subjects A and B.

lactose and maltose was negligible, and the other ingredients of the bread, including the filtered butterfat contained none.

This permitted the nitrogen to be supplied largely by the meat which is desirable when testing a given food. The protein was calculated as 80% of Sherman's (13) average requirement of 44.4 grams per 70 kilograms of body weight. It was believed that if the protein were efficient, this amount would permit the body to be near a state of equilibrium. It was also thought that the degree of utilization would be more apparent.

Two beef livers, of known source, were freed of visible fat, coarsely ground, and mixed. Weighed portions for each individual for each meal were wrapped in oiled paper and kept frozen until used. A corresponding weight of meat from a round from each of the same two animals was prepared in the same manner.

When used, the meat portions were pan-broiled without fat until seared, then allowed to cook slowly in a small amount of distilled water until well done.

The diet as calculated, was low in iron and calcium. It was desired to maintain the standards set by Sherman (13) of 0.015 gram iron daily, and 0.68 gram calcium per 70 kilograms of body weight per day, so iron was added in the form of ferric citrate and calcium as calcium carbonate. No such adjustment was made for phosphorus since it was supplied largely by the meat, and also because the utilization of this element was to be made the basis of another study.

Besides the 3 grams of sodium chloride in the bread, each subject used daily 3 additional grams on her meat and butterfat.

Agar was added to the diet in an amount (4 grams) sufficient to produce an approximately normal feces.

The diet was strongly basic. As calculated (1) the average excess base was 37.1 cubic centimeters of normal solution for the beef round series, and 41.2 cubic centimeters for the beef liver series.

Distilled water was used throughout the experiment. An amount for the comfort of each subject was established during the preliminary period, and thereafter kept approximately constant, since this fact is believed to have some effect on nitrogen metabolism.

Meals were eaten regularly in a pleasant environment. Breakfast consisted simply of orange juice, bread and butterfat; while at lunch and dinner, the meat was taken in addition.

Organization. The experiments dealing with each cut of meat were divided into two parts; a preliminary 4 days for adjustment to the diet and routine; and 8 days for collection. The latter was divided into two 4-day periods, during which collections of urine and feces were made. The weighed diet was eaten from the beginning of the preliminary period and continued at the end of the experiment until the marked feces appeared.

Feces were collected quantitatively by marking with carmine at the beginning and end of each 4-day period. They were preserved by drying at approximately 60° F. As a further preservative, acidified alcohol (1 part concentrated HCl to 9 parts C_2H_5OH by volume) was mixed with the drying composite upon each addition of feces. When dry, they were ground, sifted through a 1-mm. sieve, and preserved in glass-stoppered bottles.

Urine was collected quantitatively for each 24-hour day and preserved with toluol and enough concentracted hydrochloric acid (approximately 10 cubic centimeters) to make it definitely acid.

Meat samples were treated in the same manner as feces, except that no added preservative was necessary while drying.

Composite samples of orange juice for each 4-day collection period were preserved by canning by the cold-pack pack method using a water bath.

<u>Methods of Analysis</u>. Quantitative nitrogen determi-

nations were made on food ingested and urine and feces excreted. The Kjeldahl-Gunning method for estimating total nitrogen was used. Accuracy was proved by analyzing a substance of known composition. Determinations were made in triplicate, and accepted standards of sampling were employed.

The content of collagen and elastin of both round and liver was determined, since it is believed to be a factor affecting the utilization of meat cuts. Analyses were made by a revised method for the determination of the collagen and elastin nitrogen in meats, devised and used by the Illinois Agricultural Experiment Station.

DISCUSSION OF RESULTS

Mitchell (6) says, "the nutritive value of a food depends not only upon its nutrient content, but also upon the extent to which the animal organism can use the contained nutrients." He states further, with regard to protein metabolism, "Because of present experimental limitations in the study of protein wastage in digestion, it becomes necessary to speak throughout in terms of nitrogen . . . . . . and the whole problem resolves itself into a study of the utilization of nitrogen in nutrition."

The findings for nitrogen for this study are shown in Tables III and IV.

TABLE III

DAILY INTAKE, OUTPUT, AND BALANCES OF NITROGEN FOR THE BEEF ROUND SERIES

Subject	Period	Day	Daily Intake of Nitrogen				Daily Output of Nitrogen			Balance
			Meat	Orange Juice	Cornstarch	Total	Urine	Feces	Total	
			gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
A	I	1	4.631	1.834	0.084	6.549	4.574	1.298	5.872	+ 0.677
		2*	"	"	"	"	-	"	-	-
		3	"	"	"	"	4.056	"	5.354	+ 1.195
		4	"	"	"	"	3.514	"	4.812	+ 1.737
		Av.					4.048		5.346	+ 1.203
	II	1	4.631	1.928	0.084	6.643	4.894	1.425	6.319	+ 0.324
		2	"	"	"	"	4.283	"	5.708	+ 0.935
		3	"	"	"	"	3.638	"	5.063	+ 1.580
		4	"	"	"	"	3.847	"	5.272	+ 1.371
		Av.					4.165		5.590	+ 1.053
	Av.		4.631	1.881	0.084	6.596	4.107	1.362	5.468	+ 1.128
B	I	1	4.631	1.834	0.084	6.549	5.133	1.228	6.361	+ 0.188
		2	"	"	"	"	5.185	"	6.413	+ 0.136
		3	"	"	"	"	4.022	"	5.250	+ 1.299
		4	"	"	"	"	4.116	"	5.344	+ 1.205
		Av.					4.614		5.842	+ 0.707
	II	1	4.631	1.928	0.084	6.643	6.861	0.966	7.827	- 1.184
		2	"	"	"	"	4.162	"	5.128	+ 1.515
		3	"	"	"	"	4.351	"	5.317	+ 1.326
		4	"	"	"	"	4.852	"	5.818	+ 0.825
		Av.					5.057		6.023	+ 0.620
	Av.		4.631	1.881	0.084	6.596	4.836	1.097	5.933	+ 0.664
C	I	1	5.063	1.834	0.087	6.984	6.110	1.187	7.297	- 0.313
		2	"	"	"	"	5.579	"	6.776	+ 0.208
		3	"	"	"	"	5.163	"	6.350	+ 0.634
		4	"	"	"	"	4.771	"	5.958	+ 1.026
		Av.					5.406		6.593	+ 0.391
	II	1	5.063	1.928	0.087	7.078	2.990	0.954	3.944	+ 3.134
		2	"	"	"	"	4.903	"	5.857	+ 1.221
		3	"	"	"	"	4.520	"	5.474	+ 1.604
		4	"	"	"	"	5.519	"	6.473	+ 0.605
		Av.					4.483		5.437	+ 1.641
	Av.		5.063	1.881	0.087	7.031	4.945	1.071	6.015	+ 1.016
	Grand Av.		4.775	1.881	0.085	6.741	4.630	1.177	5.805	+ 0.936

*Sample lost

TABLE IV

DAILY INTAKE, OUTPUT, AND BALANCES OF NITROGEN FOR THE BEEF LIVER SERIES

Subject	Period	Day	Daily Intake of Nitrogen				Daily Output of Nitrogen			Balance
			Meat	Orange Juice	Cornstarch	Total	Urine	Feces	Total	
			gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
A	I	1	5.012	1.516	0.084	6.612	4.278	1.179	5.457	+1.155
		2	"	"	"	"	4.780	"	5.959	+0.653
		3	"	"	"	"	3.184	"	4.363	+2.249
		4	"	"	"	"	4.059	"	5.238	+1.374
		Av.					4.075		5.254	+1.358
	II	1	5.012	1.272	0.084	6.368	4.214	2.194	6.408	-0.040
		2	"	"	"	"	5.319	"	7.513	-1.145
		3	"	"	"	"	3.687	"	5.881	+0.487
		4	"	"	"	"	4.587	"	6.781	-0.413
		Av.					4.452		6.646	-0.278
	Av.		5.012	1.394	0.084	6.490	4.264	1.687	5.950	+0.54
B	I	1	5.012	1.516	0.084	6.612	5.217	1.018	6.235	+0.377
		2	"	"	"	"	5.934	"	6.952	-0.340
		3	"	"	"	"	3.984	"	5.002	+1.610
		4	"	"	"	"	4.197	"	5.215	+1.397
		Av.					4.833		5.851	+0.761
	II	1	5.012	1.272	0.084	6.368	4.233	0.750	4.983	+1.385
		2	"	"	"	"	4.202	"	4.952	+1.416
		3	"	"	"	"	3.786	"	4.536	+1.832
		4	"	"	"	"	4.254	"	5.004	+1.364
		Av.					4.119		4.869	+1.494
	Av.		5.012	1.394	0.084	6.490	4.476	0.884	5.360	+1.130
	I	1	5.478	1.516	0.087	7.081	5.707	1.076	6.783	+0.298
		2	"	"	"	"	5.372	"	6.448	+0.633
		3	"	"	"	"	5.134	"	6.210	+0.871
		4	"	"	"	"	4.208	"	5.284	+1.797
		Av.					5.105		6.181	+0.900
	II	1	5.478	1.272	0.087	6.837	4.620	0.752	5.372	+1.465
		2	"	"	"	"	5.447	"	6.199	+0.638
		3	"	"	"	"	4.886	"	5.638	+1.199
		4	"	"	"	"	4.681	"	5.433	+1.404
		Av.					4.908		5.660	+1.177
	Av.		5.478	1.394	0.087	6.959	5.006	0.914	5.920	+1.039
	Grand Av.		5.167	1.394	0.085	6.646	4.582	1.162	5.743	+0.903

The Calories from total protein (N x 6.25) furnished 7.3 per cent of the total Calories during the beef round series, and 7.2 per cent during the liver series. It was thought that this amount would permit the body to be near a state of nitrogen equilibrium, and that any difference in the utilization of this element would be apparent.

The nitrogen intake was somewhat higher than was calculated, due to the high nitrogen content of orange juice. The latter ranged from 20.0 to 28.5 per cent of the total nitrogen of the diet, and averaged 27.9 per cent for the beef round series and 21.1 per cent for the liver series.

The cornstarch supplied an average of 1.3 per cent of the total dietary nitrogen.

Beef round furnished, for the three subjects, an average of 70.8 per cent of the total nitrogen of the diet, while liver supplied 77.8 per cent. The meats for the two series were originally designed to furnish equal amounts of nitrogen, but upon analysis it was found that variation in the individual foods was sufficient to make this difference.

It is interesting to note that the cuts of meat used in this experiment were slightly higher in protein (N x 6.25) than suggested by Rose (12). Her figures are 21.3 and 20.4 per cent protein for beef round and liver respectively, while for this experiment, they were found to be 22.1 and

22.9 per cent. However, this amount of variation is to be expected in different samples as has been shown by numerous workers with a variety of foods.

The nitrogen output was determined for only the feces and urine since it was assumed that the elimination through the skin would be negligible and that the loss during the menstrual period could be ignored (14).

The daily variation in nitrogen output through the urine for each subject was often greater than the average variation between subjects. The per cent of total nitrogen eliminated through the urine ranged from 74.5 to 83.9 per cent for all subjects in the beef round series, averaging 79.6 per cent. For the liver series, the range was from 67.0 to 86.7 per cent, averaging 80.1 per cent. This is somewhat lower than 90 per cent suggested by Chittenden (3), but there was some nitrogen retention by the subjects for which he made no allowance.

In comparing the two periods for the same subject in either series, where the fecal nitrogen was higher, the urinary nitrogen was also higher, in every case except one. This suggests that with poorer absorption, as indicated by a lower coefficient of digestibility (higher fecal nitrogen), there may also have been poorer usage of the absorbed nitrogen, since slightly more was excreted by the kidneys at this time.

The nitrogen balances show that the average nitrogen retention for all subjects in the beef round series was + 0.94 gram nitrogen daily, while in the liver series it was + 0.90 gram. The difference may be regarded as negligible.

The subjects showed wide individual differences. Subject A utilized beef round more efficiently, subject B showed better results with liver, while subject C used both cuts about equally well.

The coefficients of digestibility, as shown in Table V, varied from 78.5 to 86.5 per cent with an average for all subjects of 82.5 per cent for beef round, while for liver, the range was from 76.5 to 89.0 per cent with an average of 82.4 per cent. Both the highest and lowest values for nitrogen balances and coefficients of digestibility were obtained in the liver series.

TABLE V

COEFFICIENTS OF DIGESTIBILITY FOR NITROGEN

Subject	Period	Beef Round	Beef Liver
		per cent	per cent
A	I	80.2	82.2
	II	78.5	65.5
	Av.	79.4	73.9
B	I	81.3	84.6
	II	85.5	88.2
	Av.	83.4	86.4
C	I	83.0	84.8
	II	86.5	89.0
	Av.	84.8	86.9
	Grand Av.	82.5	82.4

The average coefficients of digestibility for the subjects in both the round and liver series are seen to be in the proportion of $A<B<C$. There is fairly close agreement of the coefficients with round, but the figure for subject A on liver is so much lower than the others that it seems questionable whether, in general, the coefficient of digestibility for beef liver is as low as the average indicates.

The elastin and collagen content (N x 6.25) of beef round was 0.063 and 1.37 per cent respectively, while for liver it was 0.045 and 0.69 per cent. Equal amounts of meat were taken from each of two carcasses, one of low choice, the other of high good grade, and mixed together. The results for collagen and elastin nitrogen in the beef round of this experiment compared favorably with those obtained by averaging Mitchell's (10) results for inner and outer round on a good grade carcass. He found wide variation in connective tissue content of cuts from the same grade carcass, and stated, "there appears to be no relation between the grading of a carcass and the connective tissue content of its lean." He finds, however, that the collagen and elastin content of the inner round, generally recognized as the more tender portion of the cut, tends to be lower than that of the outer round. In this experiment liver, the more tender cut, contained less than half as much collagen, and considerably less elastin than round, yet in spite of the difference in the amount of connective tissue, the utilization of these two cuts seems to have been about equally good. However, both cuts were comparatively low in connective tissue, and had the amounts been higher differences might have been evident.

On an ordinary mixed diet, various authorities claim that the daily excretion of feces by an adult male will average 110 to 170 grams, with a solid content ranging between 25 and 45 grams (4). The average wet weight of feces in this experiment ranged from 47.7 to 197.9 grams daily. The average for all days for all subjects on both series was 107.3 grams. This indicates that even on this simple diet, with the help of the agar, a nearly normal bulk of feces was obtained.

TABLE VI

DAILY FECES ELIMINATION (DRY WEIGHT)

Subject	Period	Beef Round	Beef Liver
		gm.	gm.
A	I	26.7	22.3
	II	29.4	36.6
	Av.	28.1	29.5
B	I	21.7	17.6
	II	18.0	12.9
	Av.	19.9	15.3
C	I	23.6	19.1
	II	18.6	13.9
	Av.	21.1	16.5
	Grand Av.	23.0	20.5

The dry weights of feces for all subjects for the beef round series, as presented in Table VI, averaged 23.3 grams; for the liver series, 20.4 grams.

The losses of nitrogen in digestion were noticeably higher for subject A, who consistently excreted a much larger bulk of feces.

It is surprising that adult subjects showed such a tendency to store nitrogen. It was expected that they would have reached equilibrium at an early period in the experiment. No explanation presents itself, unless it may be that the large quantity of orange juice resulted in better storage as suggested by Chaney and Blunt (2) in their work with children. Orange juice is potent in vitamins A, B (complex), and C, and these, or some other factors, may have been the cause of the consistent nitrogen retention.

This suggests a possibility that the ordinary diets of these subjects, which were doubtless adequate, were brought closer to the optimal in the experiment.

SUMMARY

Three normal young women served as subjects for a balance study comparing the utilization of nitrogen in beef round and beef liver.

The diet consisted of the cut of meat to be tested, and a basal ration of sweetened orange juice, a practically nitrogen-free bread, and butterfat. It was believed to be adequate in all respects for each subject except for phosphorus and possibly vitamin D.

Beef round and liver, in equal amounts from the same two animals, supplied 70.8 and 77.8 per cent respectively of the total dietary nitrogen. These proportions are sufficiently high to warrant interpreting the results as being due mainly to the meat. Calories from total protein (N x 6.25) furnished an average of 7.25 per cent of the total Calories of the diet. It was thought that this level would permit the body to be near a state of equilibrium, and that any difference in the utilization of nitrogen would be apparent.

Nitrogen determinations were made on food and excreta.

There was no significant difference in nitrogen utilization of the beef round and liver as indicated by the average balances of +0.94 and +0.90 gram respectively. It was suggested that, as in similar experiments with children, this rather high retention by adults may in some way be associated with the large amount of orange juice used.

Differences in coefficients of digestibility for the two cuts of beef were not significant, the average for both being about 82 per cent. Both coefficients of digestibility and nitrogen balances showed marked individual variations.

Collagen and elastin content, which is a recognized factor inhibiting utilization of meat, showed round to contain considerably more of these proteins than liver, but the utilization of the two cuts of beef seems about equally good in this experiment on human subjects.

CONCLUSION

Under the conditions of this experiment, the nitrogen of round and liver of beef appears to be utilized about equally well by human subjects.

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