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

by

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INTRODUCTION

It has been suggested that a variation exists in the biological value of the proteins in different cuts of beef. Beef heart, liver, and kidney are thought by Mitchell (8), who worked with rats, to contain proteins of high quality. To continue these investigations, this study was designed to compare the utilization by human subjects of the nitrogen of beef heart with that of beef round. It is a continuation of a series of nitrogen balance experiments which have been conducted at the Kansas State College of Agriculture and Applied Science during the past two years in which different cuts of beef have been studied as to their efficiency in the human body.

REVIEW OF LITERATURE

The protein content of a food is commonly calculated by multiplying the figure obtained from analysis for nitrogen by 6.25. Sherman (14) states that "this involves the two assumptions that the food contains only negligible amounts of nitrogen in forms other than protein, and that the proteins have an average nitrogen content of 16 per cent." Karl Thomas (16) in a discussion of food and tissue proteins says, "the N is the least important of the elements compos-

ing protein. Because of the ubiquity of the Kjeldahl method and the lack of methods to determine the different carbon skeletons, we deal with the N, but we should not do so. Armed in the future with knowledge of the behavior of the carbon skeleton we shall be able to calculate the value of every protein mixture as it occurs in a diet."

Many investigators have attempted to determine the amount of protein required by the body. Sherman (14) in 1920 made a survey of the available literature dealing with nitrogen balance studies on adults in which there was no abnormal condition. He found 109 experiments "in which the diet was sufficiently well adjusted to the probable requirement and the nitrogen balance showed sufficient approach to equilibrium to make it appear that the total output of nitrogen might be taken as an indication of the protein requirement." From the results of these investigations he calculated the average protein requirement for a 70 kilogram man to be 44.4 grams per day.

Rose and McLeod (12) state that "by keeping calories constant and protein at a level which would theoretically just meet requirement, it is possible to compare two kinds of protein as to their biological efficiency." They found that human subjects could maintain nitrogen equilibrium at a level of 85 per cent of their requirement when 97 to 98

per cent of the nitrogen was supplied in the form of milk, lean beef round, or white bread and milk. They also observed that when the subjects changed from the basal diet supplemented with meat to one supplemented with milk and then to one supplemented with bread and milk, there was a storage of 1.4 per cent of the total nitrogen intake on the meat diet, 13.3 per cent on the milk diet, and 9.7 per cent on the bread and milk diet.

Mitchell (8) states that the value of a food depends "upon three determinations, (a) the nitrogen content of the food, (b) the coefficient of digestibility or that percentage of the total nitrogen which is absorbed, and (c) the biological value, or that percentage of the absorbed nitrogen which can be used for maintenance and growth." He used rats to compare the biological value of different meats and found a considerable variation in cuts and kinds. He says, "In all probability the varying content of connective tissue in different cuts of meat modifies the biological value of their nitrogen in such a way that the greater the proportion of the connective tissue the smaller is the biological value." He explains the higher values he found for liver, kidney, and heart in comparison with round by their lower content of connective tissue.

Several investigators have studied factors affecting

nitrogen metabolism in human subjects. Sherman citing Von Noorden (14) observed "that the body tends to adjust its protein metabolism to its protein supply" and "that when the body is accustomed to a certain rate of protein metabolism, it requires an appreciable length of time to adjust itself to a materially higher or lower rate." Von Noorden (14) found that equilibrium could be reached from 3 to 5 days after a change in the level of the protein intake.

Lusk (7), experimenting on himself found, that when he withdrew 350 grams of carbohydrate from a diet on which he had been maintaining nitrogen equilibrium, his protein metabolism was immediately increased. On the second day the nitrogen loss from the body was still greater than it had been on the first day indicating that the store of glycogen had become depleted. In a discussion of the protein-sparing action of carbohydrates, Lusk cites the results of Thomas (7) which are in agreement with his own and those of Tallquist (7) who found that fat also exerted a protein-sparing action but to a lesser degree than carbohydrate. Dakin's theory (14) has been used to explain the protein-sparing action of fats and carbohydrates. In substance he says that pyruvic acid, which may come from the glycerol part of the fat molecule or from glucose, can unite with the ammonia split off in the breaking down of protein and thus build up amino acids again.

The effect of giving large amounts of water at meal time on the nitrogen balances of human subjects was studied by Fowler and Hawk (4). They found that when a liter of water was taken at each meal the urinary nitrogen increased and fecal nitrogen decreased. Orr (10) verified their work and added that the excretion of urinary nitrogen was especially marked as a result of copious drinking of water when the diet was low in protein.

Chaney and Blunt (2) observed the effect on nitrogen balances when large quantities of orange juice were added to the diet of two children. They found that with the addition of orange juice, the children retained more nitrogen than during the previous period on the basal diet and that the amount stored was in excess of the weight of nitrogen added by the orange juice. The possible explanations they presented for the increased retention when orange juice was added were, that the vitamins may have promoted the economical use of elements already present in the diet which had not been efficiently used before, and the basic residue may have favored certain activities in the growing body.

Whitacre, Willard, and Blunt (17) conducted nitrogen balance experiments on human subjects to determine the influence of different amounts of fiber in the diet. They found, "that a high proportion of bulk in the diet tends to

reduce the utilization of nitrogen to a lower plane," and they offer these theories to explain their lower coefficients of digestibility for nitrogen, i.e., "The large amount of fiber may interfere with the action of proleolytic enzymes; the diet, as a whole, may occasion an unusually large outgo of metabolic nitrogen." Pittman (11) studied the utilization by human subjects of the nitrogen of the navy bean and found that although its chemical analysis showed it to be a relatively abundant source of nitrogen the coefficients of digestibility were comparatively low. She says, "The lower coefficients obtained in this investigation probably are explained to a large extent by the high crude fiber content of the diet."

The question of loss of nitrogen through the skin was studied by Benedict (1) who found this to be only 0.071 gram daily per person at rest whereas it may be increased during moderate exercise to 0.13 gram per hour. Sherman (14) suggests that in balance experiments this cutaneous loss of nitrogen is usually disregarded since it is so small.

Another possible loss of nitrogen has been investigated by Sherman, Gillett, and Pope (15) who studied the effect of the monthly cycle on the nitrogen metabolism of two healthy women. They found that "there does not appear to be any distinct monthly cycle in the metabolism of either nitrogen,

phosphorus, or calcium (except for the previously known tendency to retain nitrogen for a day or so at the beginning of the menstrual period) nor was the output of any one of these three elements in the menstrual flow large enough to affect materially the estimate of the daily requirement for normal metabolism as averaged for the entire month." They believe that a nitrogen balance study is therefore not affected appreciably by the menstrual period.

PROCEDURE

Subjects. Four healthy young women of approximately the same age served as subjects for this experiment. All were graduate students having similar activities and all ate the same diet in sufficient quantities to meet their energy requirements.

Diet. To compare the biological value of equal amounts of nitrogen from two cuts of beef, a diet was planned in which the chief source of nitrogen was the meat being tested. Sherman (14) calculated the amount of protein required daily by a 70 kilogram man to be 44.4 grams. This figure formed the basis for computing the amount of protein to be used by the subjects according to their fractional weight of 70 kilograms. Since it is desirable that the amount of nitrogen in the diet be low in order that differences in its utiliza-

tion may be observed it was calculated to supply to each individual only 80 per cent of her protein requirement. Rose's (13) figures (1 gram beef heart ⇨ 0.16 gram protein; 1 gram lean round ⇨ 0.213 gram protein) were used in calculating the weights of meat needed to furnish equal amounts of protein.

Meat of known quality was obtained from the Department of Animal Husbandry of this college. The carcasses from which the meat was taken graded prime, choice, and good according to the standards of the U. S. Department of Agriculture. The visible fat was removed from 10 beef hearts after which they were weighed and coarsely ground in order to obtain a uniform sample without greatly breaking down the tissue and thus altering its digestibility. The meat was then weighed as individual portions, each furnishing one-half of the day's protein allowance for a subject. These were wrapped separately in waxed paper, labeled, and held below freezing until the day they were to be used. To eliminate differences due to quality of carcass a weight of beef round to equal the weight of the heart from that animal was treated in the same way. These quantities of meat provided sufficient amounts for the entire study.

An hour before meal time the meat was removed from the refrigerator and allowed to stand at room temperature until

TABLE I

CALCULATED DISTRIBUTION OF NUTRIENTS IN THE DIET

Beef Round Experiment

Subject		Food					Bread											
		Meat	Orange Juice	Lactose	Sucrose	Butter Fat	Agar	Baking Powder	Butter Fat	Corn-starch	Dextri-mal-tose	Lactose	NaCl	Distilled Water	NaCl	$CaCO_3$	$FeC_6H_5O_7$	Total
A	Wt. in gms.	113.60	1000.0	80.0	40.0	20.0	5.0	3.0	32.0	120.0	15.00	40.0	3.0	80.0	4.0	0.399	0.0241	
	Pro. gms.	24.20								0.36								24.56
	Fat gms.					20.0			32.0	0.12								52.12
	Carbo. gms.		108.0	80.0	40.0			0.75		104.34	13.95	40.0						387.0
	Ca gms.	0.014	0.290													0.160		0.464
	P gms.	0.261	0.160															0.421
	Fe gms.	0.0027	0.002														0.0055	0.0102
	Calories	132.0	430.0	320.0	160.0	180.0		3.0	288.0	420.0	56.0	160.0						2149.0
	cc. N Acid	15.80																
	cc. N Base		56.1															40.30
B	Wt. in gms.	125.60	1000.0	80.0	40.0	43.2	5.0	3.0	32.0	120.0	15.0	40.0	3.0	80.0	4.0	0.518	0.0276	
	Pro. gms.	26.75								0.36								27.11
	Fat gms.					43.2			32.0	0.12								75.32
	Carbo. gms.		108.0	80.0	40.0			0.75		104.34	13.95	40.0						387.0
	Ca gms.	0.015	0.29													0.207		0.512
	P gms.	0.289	0.160															0.449
	Fe gms.	0.0030	0.002														0.0063	0.0113
	Calories	146.0	430.0	320.0	160.0	389.0		3.0	288.0	420.0	56.0	160.0						2372.0
	cc. N Acid	17.47																
	cc. N Base		56.10															38.63

TABLE I (CONTINUED)

Subject		Food																
			Bread															
		Meat	Orange Juice	Lactose	Sucrose	Butter Fat	Agar	Baking Powder	Butter Fat	Cornstarch	Dextrimaltose	Lactose	NaCl	Distilled Water	NaCl	$CaCO_3$	$FeC_6H_5O_7$	Total
C	Wt. gms.	127.8	1000.0	80.0	40.0	48.0	5.0	3.0	32.0	120.0	15.0	40.0	3.0	80.0	4.0	0.539	0.0281	
	Pro. gm.	27.22								0.36								27.58
	Fat gms.					47.7			32.0	0.12								79.82
	Carbo. gms.		108.0	80.0	40.0			0.75		104.34	13.95	40.0						387.0
	Ca gms.	0.015	0.290													0.216		0.521
	P gms.	0.294	0.160															0.454
	Fe gms.	0.0031	0.002														0.0069	0.0120
	Calories	148.0	430.0	320.0	160.0	429.0		3.0	288.0	420.0	56.0	160.0						2414.0
	cc. N Acid	17.78																
	cc. N Base		56.10															38.32
D	Wt. gms.	131.5	1000.0	80.0	40.0	55.0	5.0	3.0	32.0	120.0	15.0	40.0	3.0	80.0	4.0	0.577	0.0293	
	Pro. gm.	28.01								0.36								28.37
	Fat gms.					55.0			32.0	0.12								87.12
	Carbo. gms.		108.0	80.0	40.0			0.75		104.34	13.95	40.0						387.0
	Ca gms.	0.016	0.290													0.231		0.537
	P gms.	0.303	0.160															0.463
	Fe gms.	0.0032	0.002														0.0068	0.0120
	Calories	153.0	430.0	320.0	160.0	495.0		3.0	288.0	420.0	56.0	160.0						2485.0
	cc. N Acid	18.29																
	cc. N Base		56.10															37.81

TABLE II

CALCULATED DISTRIBUTION OF NUTRIENTS IN THE DIET

Beef Heart Experiment

Subject		Food					Bread											
		Meat	Orange Juice	Lactose	Sucrose	Butter Fat	Agar	Baking Powder	Butter Fat	Cornstarch	Dextrimaltose	Lactose	NaCl	Distilled Water	NaCl	$CaCO_3$	$FeC_6H_5O_7$	Total
A	Wt. in gms.	151.4	1000.0	80.0	40.0	15.0	5.0	3.0	32.0	120.0	15.00	40.0	3.0	80.0	4.0	0.399	0.0203	
	Pro. gm.	24.21								0.36								24.57
	Fat gms.					15.0			32.0	0.12								47.12
	Carbo. gms.		108.0	80.0	40.0			0.75		104.34	13.95	40.0						387.0
	Ca gms.	0.014	0.290													0.160		0.464
	P gms.	0.261	0.160															0.421
	Fe gms.	0.0036	0.002														0.0046	0.0102
	Calories	176.0	430.0	320.0	160.0	135.0		3.0	288.0	420.0	56.00	160.0						2148.0
	cc. N Acid	21.05																
	cc. N Base		56.10															35.04
B	Wt. in gms.	167.2	1000.0	80.0	40.0	38.0	5.0	3.0	32.0	120.0	15.00	40.0	3.0	80.0	4.0	0.517	0.0233	
	Pro. gm.	26.75								0.36								27.11
	Fat gms.					38.0			32.0	0.12								70.12
	Carbo. gms.		108.0	80.0	40.0			0.75		104.34	13.95	40.0						387.0
	Ca gms.	0.016	0.290													0.207		0.513
	P gms.	0.288	0.160															0.448
	Fe gms.	0.0040	0.002														0.0053	0.0113
	Calories	194.0	430.0	320.0	160.0	342.0		3.0	288.0	420.0	56.00	160.0						2373.0
	cc. N Acid	23.24																
	cc. N Base		56.10															32.85

TABLE II (CONTINUED)

Subject		Food																
							Bread											
		Meat	Orange Juice	Lactose	Sucrose	Butter Fat	Agar	Baking Powder	Butter Fat	Cornstarch	Dextrimaltose	Lactose	NaCl	Distilled Water	NaCl	$CaCO_3$	$FeC_6H_5O_7$	Total
C	Wt. in gms.	170.0	1000.0	80.0	40.0	42.0	5.0	3.0	32.0	120.0	15.0	40.0	3.0	80.0	4.0	0.538	0.0238	
	Pro. gm.	27.21								0.36								27.57
	Fat gms.					42.0			32.0	0.12								74.12
	Carbo. gms.		108.0	80.0	40.0			0.75		104.34	13.95	40.0						387.0
	Ca gms.	0.016	0.290													0.215		0.521
	P gms.	0.293	0.160															0.453
	Fe gms.	0.0041	0.002														0.0054	0.0115
	Calories	197.0	430.0	320.0	160.0	378.0		3.0	288.0	420.0	56.0	160.0						2412.0
	cc. N Acid	23.64																
	cc. N Base		56.10															32.46
D	Wt. in gms.	175.2	1000.0	80.0	40.0	49.3	5.0	3.0	32.0	120.0	15.0	40.0	3.0	80.0	4.0	0.585	0.0248	
	Pro. gm.	28.02								0.36								28.38
	Fat gms.					49.3			32.0	0.12								81.42
	Carbo. gms.		108.0	80.0	40.0			0.75		104.34	13.95	40.0						387.0
	Ca gms.	0.016	0.290													0.230		0.536
	P gms.	0.302	0.160															0.462
	Fe gms.	0.0042	0.002														0.0056	0.0118
	Calories	203.0	430.0	320.0	160.0	444.0		3.0	288.0	420.0	56.0	160.0						2484.0
	cc. N Acid	24.37																
	cc. N Base		56.10															31.73

time of cooking. Each cake was pan-broiled for a few minutes in individual frying pans until it was slightly seared, then distilled water was added to half the depth of the meat after which it was allowed to simmer for approximately 20 minutes. To insure no loss of food material, the liquid remaining in a pan with any necessary rinsings was served as bouillon to that subject.

In addition to the meat, the diet consisted of orange juice, lactose, sucrose, filtered butter fat, a special low-nitrogen bread, and sodium chloride (Tables I and II). The orange juice was expressed the day before it was to be used. It was then strained, weighed, and mixed with 80 grams lactose and 40 grams sucrose. The sugars increased the caloric value without affecting the palatability of the mixture.

The butter fat was prepared by melting butter over hot water, then decanting and filtering through absorbent cotton as quickly as possible in a warm room. It was stored at 50° F. until used on the bread or meat as the individual desired.

The bread for each subject for one day was made according to the following recipe:

Ingredients	grams
Cornstarch	120
Dextri-maltose	15
Lactose	40
Salt	3
Baking powder (tartrate)	3
Agar	5
Butter fat	32
Water, distilled	80

The dry ingredients were well mixed, and combined with the softened butter fat and water. The resulting dough, which was worked until of uniform consistency, was then spread on an oiled baking sheet in a layer about one-eighth inch thick and baked for one hour at 325° F. The bread had a hard, cracker-like appearance and texture and tended to crumble unless it was loosened from the sheet by means of a long spatula a few minutes before it was taken from the oven. After cooling the bread was placed in an individual bread box until eaten in the course of the day.

Each subject consumed daily four grams of sodium chloride in addition to that in the bread. This was served individually and eaten on the meat and butter fat.

Throughout the experiment distilled water was used. In a preliminary adjustment period each subject determined the amount she desired and continued to use this amount through-

out the study.

According to accepted standards of 0.68 gram calcium, 1.32 grams phosphorus and 0.015 gram iron per day for a 70 kilogram man, the diet was not adequate in these minerals. Additions of calcium carbonate ($CaCO_3$) and ferric citrate ($FeC_6H_5O_7$) were made in quantities sufficient to supply the deficit of these two elements. No phosphorus salt was added because a phosphorus balance study was being carried on simultaneously as another phase in the comparison of the two cuts of beef.

Plan of Experiment. The experiment was divided into two parts of 12 days each. In the first part most of the protein was furnished by beef round and in the second it was supplied by beef heart. The first four days were used as a preliminary period during which the subjects ate the weighed diet which enabled them to orient themselves to the conditions and to adjust their caloric intake to maintain a constant weight. It was at this time also that each subject determined the volume of water she would take daily. The eight days following the preliminary period were divided into four-day periods during which samples of food were taken and quantitative collections of urine and feces were made.

To mark the feces, size 00 gelatin capsules containing carmine were taken one-half hour before breakfast at the

beginning and end of each period. The diet was continued at the close of the experiment until the marker appeared.

Care of Samples.

Food. Samples of meat to which two drops of formaldehyde had been added were preserved by drying in a gas oven at 55° C.-60° C. When they reached approximately constant weight they were ground fine, sifted through a one-millimeter sieve, and stored in glass-stoppered bottles.

Aliquot portions of each day's orange juice for the given periods were made into composites. These were canned in half-pint jars in a water bath by the cold-pack method and later analyzed for nitrogen. Agar and psyllium seed were also sampled, as they were used for laxatives in the experiment. Previous analysis of the butter fat showed it to be nitrogen-free. The nitrogen in the cornstarch was not determined but calculated according to the report of the Committee on Foods of the American Medical Association (3). The other ingredients of the diet were of C. P. quality and were regarded as 100 per cent carbohydrate.

Urine. Urine was collected under toluol for daily determinations of nitrogen. The 24-hour collection was measured, well mixed, and a 250 cc. portion reserved for analysis. This was further preserved by acidifying with three cc. of C. P. hydrochloric acid.

Feces. The first collection of feces from a subject was preserved by mixing with acidified alcohol (9 parts 95 per cent ethyl alcohol and 1 part C. P. hydrochloric acid) and then dried in an oven. Each additional collection was acidified in the same manner and added to the fecal material previously obtained. In this way the feces collected by a subject during one period were combined into one composite. After drying the composite was treated in the same manner described above for meat.

Methods of Analysis.

The residual moisture from the aid-dried samples was removed by heating them in a Freas oven at 60° C. for three hours. The Gunning-Arnold modification of the Kjeldahl procedure was followed in analyzing all samples. To test the technique and reagents used, the nitrogen in a known sample was recovered. Determinations were made in triplicate and repeated if there was a deviation of more than two per cent between the lowest and highest results. Collagen and elastin were determined by Mitchell's modified procedure (9).

RESULTS AND DISCUSSION

The diet was easily digested by these young women, and with the exception of subject B, who was ill for one day and unable to take food, they were in good health throughout the experiment. During the 3-day period following the day sub-

ject B was incapacitated, she showed a marked retention of nitrogen (Table III). This bears out the observations of Sherman and Von Noorden (14) that a period of starvation during which nitrogen continued to be excreted by the body is followed by a period of nitrogen retention.

The subjects maintained their body weights throughout the experiment on an intake of 45 Calories per kilogram which indicated that the diet was adequate for energy. From 83.9 to 86.1 per cent of the total Calories was furnished by carbohydrates. This is thought to be desirable when different sources of nitrogen are to be compared as the experiments of Lusk (7) and others have shown.

The average coefficients of digestibility of the nitrogen were calculated by the following formula:

$$\frac{\text{(grams N ingested - grams N in feces)100}}{\text{grams N ingested}} = \text{per cent N absorbed}$$

For all subjects the average figure so obtained was 86.0 per cent for the beef round series and 86.3 per cent for the beef heart. This difference may be regarded as negligible. Subjects B and D had higher coefficients of digestibility for heart than for round while the opposite was true for subjects A and C (Table IV). Subject D had the lowest coefficient for the beef round series and the next to the lowest for the beef heart series. Previous workers have found that the utilization of nitrogen is decreased by a large content

TABLE III

DAILY URINARY OUTPUT

Period	Day	Subject A		Subject B		Subject C		Subject D	
		Volume	Nitrogen	Volume	Nitrogen	Volume	Nitrogen	Volume	Nitrogen
		cc.	gm.	cc.	gm.	cc.	gm.	cc.	gm.
Beef Round I	1	1670	5.32	1580	7.56	1120	5.06	1480	4.67
	2	1570	5.11	985	5.04	1400	4.41	1320	4.89
	3	1080	4.69	1610	5.33	1780	5.09	1600	4.82
	4	1320	4.22	1480	4.78	1520	4.64	1420	3.73
	Average		4.83		5.60		4.80		4.53
	Average*		4.74		5.52		4.73 ⁂		4.40⁂
Beef Round II	1	1660	4.68	980	3.46	1330	5.37	1140	4.02
	2	980	4.12	1500	5.82	1340	5.18	1300	3.86
	3	980	2.48	1190	3.22	1130	4.82	740	2.51
	4	1570	6.47	1020	5.61	1290	4.90	1080	4.12
	Average		4.43		4.52		5.06		3.83
	Average*		4.43		4.45		4.95		3.75
Beef Heart III	1	1740	4.59	**	**	1320	5.37	1490	5.12
	2	1470	5.15	525	2.76	1950	3.01	1510	5.40
	3	910	3.90	1180	4.95	2820‡	10.93‡	1300	4.97
	4	830	4.23	1280	4.25			1200	4.93
	Average		4.46		3.74		5.32		5.11
	Average*		4.45		3.72		5.26		5.10
Beef Heart IV	1	1660	4.17	940	4.69	760	4.28	1300	4.35
	2	870	3.68	1310	5.18	1335	5.05	1180	4.44
	3	1200	3.96	1260	5.04	1070	4.44	1480	5.13
	4	1330	3.57	1310	5.18	1740	4.79	1350	4.86
	Average		3.84		5.02		4.64		4.69
	Average*		3.75		5.11		4.73		4.73

* Average obtained from composite.
⁂ Average obtained from composite of last 3 days.
** No collections made.
‡ Two-day collection combined.

TABLE IV

AVERAGE COEFFICIENTS
OF DIGESTIBILITY FOR NITROGEN

Period	Subject	Coefficient of Digestibility per cent
Beef Round	A	87.51
	B	86.38
	C	88.96
	D	81.00
	Average	86.00
Beef Heart	A	83.44
	B	90.38
	C	86.49
	D	84.50
	Average	86.30

of fiber in the diet which may explain why subject D had these low values as she took 18 grams of psyllium seed daily for a laxative.

In accordance with the idea that cellulose is a factor in the utilization of nitrogen, agar was used in the bread in the smallest quantity necessary to produce normal feces, and to further reduce the fiber in the diet, the orange juice was strained before it was weighed.

Under the conditions of this experiment both heart and round of beef were well utilized by the subjects. The average daily nitrogen balance as may be seen from Table V for the beef round series was -0.05 gram which may be regarded as equilibrium, and that for the beef heart series (Table VI) was +0.49 gram. The difference is small and several factors may have influenced these results. The level of nitrogen intake was not the same for both cuts of meat as analysis showed that the round contained only 19.1 per cent of protein instead of the 21.3 per cent suggested by Rose (13) and used in calculating the diet. On the other hand, the percentage of protein in the heart was found to be 16.4 which was slightly higher than the expected 16.0 per cent (13).

Mitchell (8), working with rats, attributed the relatively high biological values he obtained for heart, liver,

TABLE V

AVERAGE DAILY NITROGEN FINDINGS FOR THE BEEF ROUND PERIODS.

Period	Subject	INTAKE					OUTPUT			
		Corn-starch	Orange juice	Meat	Psyllium seed	Total	Urine	Feces	Total	Balance
		gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
I	A	0.06	1.53	3.47		5.06	4.83	0.63	5.46	-0.40
	B	0.06	1.53	3.84		5.42	5.60	0.83	6.43	-1.01
	C	0.06	1.53	3.91		5.49	4.80	0.61	5.41	+0.08
	D	0.06	1.53	4.02	0.349	5.96	4.53	1.26	5.79	+0.17
II	A	0.06	1.50	3.47		5.03	4.43	0.63	5.06	-0.03
	B	0.06	1.50	3.84		5.40	4.52	0.64	5.16	+0.24
	C	0.06	1.50	3.91		5.47	5.06	0.60	5.66	-0.20
	D	0.06	1.50	4.02	0.349	5.93	3.83	1.03	4.86	+1.07

Average -0.05

TABLE VI

AVERAGE DAILY NITROGEN FINDINGS FOR THE BEEF HEART PERIODS

Period	Subject	INTAKE					OUTPUT			
		Corn-starch	Orange juice	Meat	Psyllium seed	Total	Urine	Feces	Total	Balance
		gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
III	A	0.06	1.70	3.98		5.74	4.46	0.94	5.40	+0.34
	B	0.06	1.70	4.40		6.15	3.74	0.48	4.22	+1.93
	C	0.06	1.70	4.48		6.23	5.32	0.90	6.22	+0.00
	D	0.06	1.70	4.61	0.349	6.71	5.11	1.01	6.12	+0.60
IV	A	0.06	1.50	3.98		5.54	3.84	0.93	4.77	+0.77
	B	0.06	1.50	4.40		5.96	5.02	0.68	5.70	+0.26
	C	0.06	1.50	4.48		6.04	4.64	0.75	5.39	+0.64
	D	0.06	1.50	4.61	0.349	6.53	4.69	1.05	5.74	+0.77
									Average	+0.49

and kidney to their correspondingly low content of connective tissue. In the present experiment, results of analyses for collagen and elastin, the two principal kinds of connective tissue in meat, showed that the collagen content of beef heart was 0.497 per cent which is considerably higher than the 0.265 per cent obtained for round. However, the difference in the collagen content should not have affected the utilization of the nitrogen since collagen is thought to be converted by the body into a digestible protein. Elastin, on the other hand, is not considered digestible but since beef round contained 0.0111 per cent and beef heart 0.0102 per cent the difference between the elastin content of the two meats was so slight it could not have influenced the results.

Another possible factor influencing the nitrogen balances in this study was that each subject's intake of protein may have been lower than she was receiving previous to this study. Although it was expected that all the subjects would have adjusted their protein metabolism to the protein supply in the four-day preliminary period, the results given in Table III indicate that subject B may not have reached equilibrium. She excreted 7.56 grams of urinary nitrogen on the first day and then dropped to 5.04 grams on the second day. The average amount excreted through the kidneys for the last

three days of the period was 4.95 grams. The large output of nitrogen on the first day of period I caused her to be in negative balance for the first period during which beef round was tested.

The actual average daily nitrogen intake for each subject with her requirement, and standard appear in Table VII. It shows that although the subjects received enough protein to meet their requirement, (0.6 gram per kilogram of body weight) they were supplied only 64 to 76 per cent of their standard (1 gram of protein per kilogram of body weight).

The meat was expected to furnish most of the nitrogen of the diet but analysis of the orange juice showed that the latter supplied from 25 to 30 per cent of the nitrogen consumed in the beef round experiment, and from 23 to 29 per cent when beef heart was used. The cornstarch was calculated to yield from 0.9 to 1.1 per cent of the nitrogen, and the psyllium seed used by subject D furnished her from 5.22 to 5.88 per cent. It was assumed, however, that the latter was not available for use in her body. The meat, therefore, furnished only from 68 to 74 per cent of the total nitrogen.

As the nitrogen in the diet was well used, the results of this experiment agree with those of Rose and McLeod who studied the maintenance values for the proteins of lean beef round freed from visible fat and connective tissue. They

TABLE VII

AVERAGE NITROGEN INTAKE
COMPARED WITH ACCEPTED STANDARDS

Period	Subject	Body Wt. kg.	N Requirement gms.*	N Standard gms.*	Fraction of Standard Received per cent
I	A	47.72	4.84	7.63	66.31
	B	52.72	5.35	8.43	64.29
	C	53.63	5.44	8.57	64.06
	D	55.22	5.60	8.83	67.49**
II	A	47.72	4.84	7.63	65.90
	B	52.72	5.35	8.43	64.06
	C	53.63	5.44	8.57	63.82
	D	55.22	5.60	8.83	67.15**
III	A	47.72	4.84	7.63	75.23
	B	52.72	5.35	8.43	72.95
	C	53.63	5.44	8.57	72.70
	D	55.22	5.60	8.83	75.99**
IV	A	47.72	4.84	7.63	72.61
	B	52.72	5.35	8.43	70.69
	C	53.63	5.44	8.57	70.48
	D	55.22	5.60	8.83	73.84**

* Sherman.

** Includes psyllium seed.

found that their subjects reached equilibrium when they received from 97 to 98 per cent of their protein from the meat even though the level of intake was approximately 15 per cent below each subject's protein requirement. Long (6) compared the utilization of beef round with that of beef liver and found that when her subjects received 70.8 per cent of their protein from the round at a level of 78.1 per cent of their standard they were also in equilibrium.

Since the protein of the diet in the present study was supplied at a low level and was furnished for the most part by beef heart or beef round, both cuts of meat must have supplied protein of high quality or some other constituent in the diet must have exerted a favorable influence in the utilization of the nitrogen. It is possible that both were factors. If the work of Chaney and Blunt (2) concerning the effect of orange juice on nitrogen retention in children is applicable to adults, the large amount of orange juice used in this study may have increased the utilization of the nitrogen from the two meats. Because of the orange juice, the diet was decidedly alkaline being equivalent to 38.8 cc. of normal alkali (5) in the beef round series and 33.1 cc. in the beef heart series (Tables I and II). It is possible that the reaction of the diet may have favored the economical usage of the nitrogen.

The volume and the nitrogen content of the urine fluctuated from day to day for each subject (Table III), and there was apparently no correlation between the volume of urine and the grams of nitrogen it contained. This variation, commonly found in balance experiments, is difficult to explain since the food and water intake were constant and the activity and temperature were similar each day. Loss of nitrogen and moisture through perspiration would be an improbable explanation since under conditions of moderate activity, such loss of nitrogen is so small that it is commonly disregarded in balance experiments of this type.

A study of a number of nitrogen balance experiments reported in the literature indicated that it is customary to make daily nitrogen analyses of the urine of each subject. From the results so obtained the average daily output of nitrogen over a given period is determined. In contrast to this method for nitrogen, the mineral content of urine is most frequently reported as obtained from analysis of a composite sample which is made by mixing aliquot portions of the daily collections for the period. The analytical work involved to obtain the figures for the average output of the mineral in this way requires less time than if it were reached through averaging the output of the separate days, and if the two averages agree it would appear to be a time-

saving procedure. This question was investigated in connection with the present study.

Composite samples consisting of a tenth of each day's volume of urine for a given subject were therefore analyzed in the same manner as described previously for determining the nitrogen in a daily urine collection, and it was found that the sum of the daily outputs of nitrogen for any given four-day period for any subject agreed closely with results (Table III) obtained from analysis of the composite samples for that period. The deviations ranged from a minimum of 0.039 per cent to a maximum of 2.05 per cent which are within the limits of experimental error. If in addition to the average amount of nitrogen excreted in the urine, the daily fluctuation is to be studied, daily analyses are necessary as there was more variation in the daily output of one subject than there was between different subjects.

CONCLUSIONS

The average daily output of urinary nitrogen may be obtained either by analysis of composite or daily samples. The two methods agree within the limits of experimental error.

Under the conditions of this experiment the nitrogen from beef round and the nitrogen from beef heart were well

utilized by four human subjects. It is impossible to state that one cut was better used than the other as several factors may have influenced the balances obtained.

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