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NITROGEN UTILIZATION DURING CALORIC RESTRICTION

II. THE EFFECT OF VARIATION IN NITROGEN INTAKE [1]

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ONE FIGURE

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Numerous investigations have shown that nitrogen utilization is a function of both caloric and protein intake. Benditt et al. ('48) found increased nitrogen utilization by the protein-depleted rat up to intakes of 6 gm of protein per kilogram per day and up to 1240 Cal./m^2/day when the factors were varied independently. However, it was demonstrated by Allison and co-workers ('46) that the nitrogen balance index in the normal dog was unaltered provided that 50% or more of the caloric requirement was met; the index was markedly reduced at the 25% level of energy intake. In studies employing protein-depleted rats, supplementation with egg protein or methionine was effective in sparing body protein only when 50% or more of the energy requirement was supplied (Stevenson et al., '46). Rosenthal ('52) reported a normal index with a low fat, low-casein (0.15 gm N/kg) diet fed at 25% caloric requirement, and positive nitrogen balance with a reduction in index when the casein nitrogen was in-

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creased to 0.30 gm/kg. Because of the techniques involved in these studies all of the animals employed were restricted in protein for variable lengths of time.

In the experiments presented here the relationship between nitrogen intake and utilization was examined in normal adult rats restricted to half the caloric requirement.

PROCEDURE

Male albino rats, Sprague-Dawley strain, were standardized for two weeks as described in the preceding paper (Calloway and Spector, '55). Three series were conducted: in series I, commercial stock diet was fed during the standardization period; in series II, a complete 18% casein diet; and in series III, commercial stock diet for two days (until the shipping weight loss was recovered) and an 18% casein diet for the remaining 12 days. In each series body weight was maintained at 300 gm.

After standardization, 5 or 6 control animals from each series were sacrificed for tissue analysis. During the next 4-day period an egg albumin diet was fed to supply half the previous caloric intake and graded levels of nitrogen as shown in table 1. The nitrogen content of the diet was increased at the expense of sucrose; fat, salts and vitamins [2] were held constant. In series I and II the diets contained 30% fat by weight and in series III, 5%. The amounts of nitrogen fed in series I and II were at or below the normal requirement, 160 mg per day; in series III the nitrogen consumptions were increased from 160 to 600 mg. In addition, fasted and protein-free fed controls were employed. Six animals comprised each group in series I and III; 5 animals per group were used in series II.

[2] Vitamins were given by dropper daily to supply the following amounts in micrograms:
Thiamine 25; riboflavin 50; pyridoxine 25; nicotinamide 200; calcium pantothenate 200; para-amino benzoic acid 500; biotin 1; folic acid 5; vitamin B_{12} 1; vitamin K 10; and inositol 1 mg; choline chloride 10 mg; α-tocopherol 1 mg; vitamin A 1,200 I.U.; vitamin D 20 I.U.

TABLE 1

Diet plan

	SERIES I	SERIES II	SERIES III
Standardization diet	Commercial stock [1]	18% casein	Commercial stock, 2 days; 18% casein, 12 days
Caloric intake, Cal./day	46	47	52
Nitrogen intake, mg/day	460	270	315
Restriction			
Egg albumin, % of diet	0–28.2	0–28.2	0–90
Fat,[2] % of diet	30	30	5
Caloric intake, Cal./day	23	23	25
Nitrogen intake, mg/day			
a	0	0	0
b	69	81	162
c	98	119	347
d	126	160	505
e	156		604

[1] Purina Laboratory Chow.
[2] Hydrogenated vegetable fat.

TABLE 2

Body weight loss after 4 days of caloric restriction

SERIES	NITROGEN INTAKE	WEIGHT LOSS
	mg/day	*%*
	At 50% caloric requirement	
I	0	11.8 ± 0.3 [1]
	69	11.2 ± 0.4
	98	11.1 ± 0.8
	126	10.6 ± 0.6
	156	10.5 ± 0.5
II	0	8.0 ± 0.2
	81	7.3 ± 0.3
	119	8.0 ± 0.4
	160	6.9 ± 0.6
III	0	7.2 ± 0.4
	162	6.3 ± 0.2
	347	7.5 ± 0.4
	505	7.4 ± 0.2
	604	5.6 ± 0.2
	Fasting	
I		17.0 ± 0.2
II		14.4 ± 0.3
III		15.1 ± 0.6

[1] Standard error.

Urine and feces were collected and analyzed in the manner described in the preceding paper (Calloway and Spector, '55). Livers were analyzed as before and total serum protein as determined by the copper sulfate specific gravity method (Phillips et al., '50) was measured in series II.

RESULTS

Body weight changes

Body weight losses were independent of the level of nitrogen fed during caloric restriction. Average losses of the groups were 10.5 to 11.8% in series I, 6.9 to 8.0% in series II, and 5.6 to 7.2% in series III (table 2). Losses in fasted animals were greater. These variations in weight loss among series are attributable to the diet fed during standardization (Calloway and Spector, '53).

Nitrogen utilization

The degree of negative nitrogen balance was inversely proportional to the nitrogen intake (fig. 1, table 3). Positive nitrogen balance was not attained although it was approached in the group fed 604 mg of nitrogen daily, approximately 2 gm per kilogram of body weight. The slope of the line in figure 1 indicates that positive balance might be reached at higher intakes.[3] A difference in response among the three series is evident, the series II groups being in less negative balance than those of series I. At the 160 mg intake, the balances were significantly different ($p < .001$) although the balances were essentially the same when no nitrogen was fed.

The percentage of nitrogen utilization, shown in table 3, was calculated by the formula:

Per cent utilization $= \frac{\text{Absorbed N} - (\text{Urinary N} - \text{Urinary N on protein-free regime})}{\text{Absorbed N}} \times 100$. This formula is the same as that derived by Allison

[3] An attempt was made to feed pure egg albumin but the data are omitted because the animals had to be force-fed and diarrhea was marked. However, under these conditions positive balance was obtained at an intake of 750 mg of nitrogen per day.

and Anderson ('45) for obtaining nitrogen balance index but in this case expresses nitrogen utilization as percentage of that absorbed; since the term "nitrogen balance index" has a specific derivation its use has been avoided here. The application of the formula in this instance differs from that of the originators in that a group of animals fed a protein-

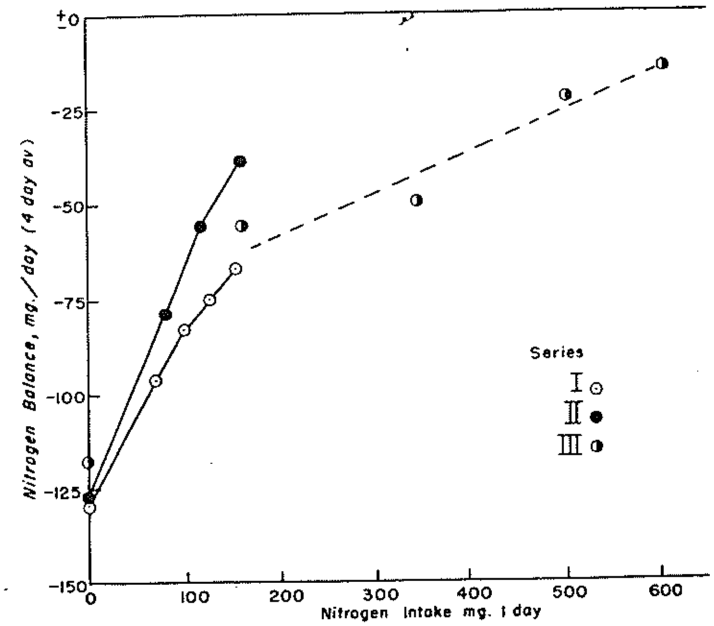

Fig. 1 Nitrogen balance during 4 days of 50% caloric restriction.

free diet provided the base line rather than each animal serving as its own control. This technique was selected in order to obviate the possible complication of even a short period of protein restriction. In each series percentage utilization diminished as the dietary level of nitrogen increased, representing progressively larger fractions of dietary protein expended for energy. In no case, however, was there

TABLE 3

Nitrogen utilization during 4 days of caloric restriction

SERIES	NITROGEN INTAKE	ABSORBED NITROGEN[1]	URINARY NITROGEN	NITROGEN BALANCE	NITROGEN UTILIZATION[2]	REDUCTION OF NITROGEN DEFICIT COMPARED WITH Fasting	REDUCTION OF NITROGEN DEFICIT COMPARED WITH Equal non-protein calories
	mg/day	*mg/4 days*	*mg/4 days*	*mg/4 days*	%	%	%
				At 50% caloric requirement			
I	0	0	488	— 518 ± 8[3]		38	
	69	269	617	— 386 ± 32	52		25
	98	387	681	— 333 ± 21	50		36
	126	495	762	— 302 ± 17	45		42
	156	617	849	— 269 ± 29	41		48
II	0	0	469	— 508 ± 10		32	
	81	316	593	— 315 ± 15	61		38
	119	471	653	— 222 ± 29	61		56
	160	628	749	— 160 ± 29	55		68
III	0	0	432	— 470 ± 29		39	
	162	642	829	— 225 ± 26	38		52
	347	1374	1536	— 200 ± 19	20		57
	505	1982	2041	— 90 ± 24	19		81
	604	2383	2415	— 60 ± 38	17		87
				Fasting			
I			801	— 834 ± 36			
II			714	— 744 ± 29			
III			741	— 772 ± 25			

[1] Absorbed nitrogen = nitrogen intake — (fecal nitrogen — fecal nitrogen, protein-free).

[2] Nitrogen utilization, $\% = \frac{AN - (UN \cdot UN \text{ pro-free})}{AN} \times 100$.

[3] Standard error.

quantitative excretion of dietary protein, i.e., zero utilization. In the groups of series II, utilization was superior to that obtained in I and III; when the intake was 160 mg of nitrogen daily the values were 55% as compared with 41 and 38%.

Analysis of these data in terms of what they represent in body economy shows that feeding half the caloric requirement reduced body losses by 32 to 39% as compared with fasted animals. When the normal nitrogen requirement was met in addition, further savings of 48 to 68% were induced. At the highest level shown, 604 mg daily nitrogen intake, the reduction of nitrogen deficient was 87% above that due to calories alone. In order to increase the net protein saving from 52 to 87%, however, the intake had to be raised 377% and the concomitant increase in urinary nitrogen excretion was 291%.

The nitrogen retention reflected in the percentage utilization recorded here is markedly less than that reflected in the nitrogen balance indexes of egg albumin as reported: 0.91 in man, 1.14 in the dog and 0.94 in the rat under conditions of full feeding (Rutgers, '46–'50). The reason for the divergence of these data is not clear. Allison ('51) has stated that ". . . indexes are essentially constant and independent of the magnitude of the protein stores . . ." and are unaffected by 50% caloric deficit. Other factors to be considered as sources of variance are: (1) the variation in the base line of protein-free feeding and (2) the preceding diet of the animals. These factors will be elaborated in a subsequent paper.

Tissue composition

From table 4 it may be observed that there were distinct differences among the livers of control groups of the three series. These differences were based largely upon the size of the organs, since there was no significant difference in percentage content of moisture or of nitrogen. In the first series the livers weighed an average of 10.3 gm, 3.42 gm per

100 gm of body weight; in series II, these values were 12.0 and 4.03 gm; and in series III, 14.0 and 4.62 gm. Because of the similarity in percentage nitrogen content, this variation in organ size resulted in significantly different total amounts of liver nitrogen ($p < 0.05$).

TABLE 4

Liver composition of rats fed varying amounts of nitrogen and calories

SERIES	NITROGEN INTAKE	LIVER WET WEIGHT	MOISTURE	NITROGEN (dry basis)	NITROGEN Total
	mg/day	*gm*	*%*	*%*	*mg*
		Control			
I	460	10.3	70.2	12.0	369 ± 8 [1]
II	270	12.0	72.3	12.2	404 ± 14
III	315	14.0	72.3	12.8	496 ± 17
		After 4 days of 50% caloric restriction			
I	0	10.2	72.4	12.6	354 ± 9
	69	10.6	72.6	12.5	364 ± 8
	98	10.3	72.3	12.5	358 ± 10
	126	10.3	72.3	12.6	356 ± 14
	156	9.8	72.5	12.8	350 ± 10
II	0	8.5	68.8	11.5	304 ± 7
	81	8.9	67.6	11.1	318 ± 4
	119	9.2	67.0	11.1	337 ± 15
	160	9.7	67.7	11.0	346 ± 12
III	0	9.9	72.8	12.4	335 ± 17
	162	10.6	72.6	12.4	362 ± 10
	347	10.5	71.9	12.2	360 ± 17
	505	11.4	72.4	12.2	386 ± 7
	604	11.3	72.0	12.1	383 ± 12
		After 4 days of fasting			
II		7.4	67.0	11.5	280 ± 15
III		8.9	72.3	12.7	312 ± 6

[1] Standard error.

It is readily apparent that after restriction there were no significant changes from control liver nitrogen in the first series where that control value was low; in the second and third series where measurable losses were encountered, those

losses tended to diminish with increasing nitrogen intake, but the response was not uniform. The process of restriction served to eliminate the aberrant character of the series; that is, among the groups given 160 mg of nitrogen there were no significant differences and among the protein-free groups only the extremes (I vs. II, $p = 0.003$) were different. There was no correlation between nitrogen utilization as measured by nitrogen balance and by the changes in liver nitrogen content.

Neither kidney weight nor total serum protein showed significant changes as a result of either caloric restriction or alteration in dietary nitrogen. Kidney weight averaged 2.1 gm in controls and 2.0 gm after fasting; serum protein was 7.0 gm % initially and 6.9 gm % after fasting.

SUMMARY

Diets supplying graded amounts of egg albumin nitrogen, zero to 604 mg per day, were fed to normal adult rats limited to 50% of caloric requirement for 4 days. Nitrogen utilization was measured by nitrogen balance and by alteration in liver nitrogen content.

The degree of negative nitrogen balance was inversely proportional to nitrogen intake but positive balance was not attained. Nitrogen utilization diminished as the level of dietary nitrogen increased from 52 to 61% at 70 to 80 mg daily to 17% at 604 mg, indicating increased use of dietary protein for energy. Supplying half the caloric requirement reduced the nitrogen deficit 32 to 39% as compared with fasting and the addition of dietary nitrogen produced up to 87% sparing beyond that of the calories alone.

There was no significant correlation between liver nitrogen loss and nitrogen balance.

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