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UNIVERSITY OF MISSOURI COLLEGE OF AGRICULTURE
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Research Bulletin 223

GROWTH AND DEVELOPMENT

With Special Reference to Domestic Animals

XXXVI. Endogenous Nitrogen and Basal Energy Relationships During Growth

URAL S. ASHWORTH

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FOREWORD

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

The investigation has been made possible through a grant by the Herman Frasch Foundation, now represented by Dr. F. J. Seviers.

F. B. Mumford,
Director Agricultural Experiment Station

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ABSTRACT

The ratio of endogenous urinary nitrogen to basal metabolism is about 1 mg per Cal. for weanling rats and about 1.5 mg per Cal. for mature rats. The correlation between endogenous nitrogen and body weight is better than between endogenous nitrogen and basal metabolism. Two methods (Time-curve and Composite or Smuts) were used for measuring the minimum endogenous nitrogen excretion of growing rats. These methods gave comparable results when the minimum urinary nitrogen excretion was related to the initial body weight or basal heat production. Variations in the endogenous nitrogen excretion may account for the discrepancies in biological values of proteins as reported in the literature.

Using Smuts' method, in which urine is collected between the 7th and 13th days on a N-poor diet, rats weighing over 100 gms taken from a high (30 per cent) protein diet excreted about 26 per cent more nitrogen per unit body weight than their paired litter mates taken from a low (13 per cent) protein diet. The basal metabolism per unit body weight of the high protein rats was 7.6 per cent lower than that of the low protein rats.

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Ural S. Ashworth

I. INTRODUCTION

The approximately parallel increase of endogenous nitrogen excretion and basal heat production with increasing body weight is apparent from the results of several investigations. A recent analysis of data on *mature* animals ranging in body weight from mice to cattle confirms the above conclusion (Missouri Research Bulletin 220). The basal metabolism increased with the 0.73 power of body weight and the endogenous nitrogen increased with the 0.72 power of body weight. In other words the ratio of endogenous nitrogen to basal metabolism was roughly constant for the entire range of mature animals, namely 2 mg nitrogen per Calorie.

Bulletins 189 and 190 of this series reported data on *growing* rats for the purpose of constructing *age curves* of endogenous nitrogen and basal energy metabolism. The results were not conclusive. The work was therefore continued with the results given in the present bulletin.

Just how accurately can one predict endogenous nitrogen excretion from basal metabolism? Work previously done at this laboratory (Missouri Research Bulletins 189-190) has shown that reliable data on endogenous nitrogen coefficients are difficult to obtain. In the first place the previous dietary protein level exerts a profound influence on the time necessary to reach a minimum level of nitrogen excretion. It was found that the rate of urinary nitrogen excretion in rats on N-free diets may decline for periods as long as 60 days. This is similar to Deuel's results on humans (J. Biol. Chem. *76*, 391). In the second place it is very difficult to keep animals in energy equilibrium during these long periods of specific nitrogen starvation. Undoubtedly there is significant change in the composition of the body during the adjustment to a minimum level of nitrogen excretion. A discussion of the practical and theoretical significance of this subject, together with a review of the literature, is given in Research Bulletin 220.

II. METHODS

This paper reports endogenous nitrogen data obtained by two methods on male rats ranging in size from weanlings to adults.

Paper 91 in the Herman Frasch Foundation Series.

Method 1: Time-Curve Method.—Twenty-two rats were taken from an unusually good stock ration (28 per cent protein) and given a *N-free* (0.5 mg N per gm food) diet. The rats lost weight on this diet at the rate of about 1 per cent per day. The excreta, collected daily, were analyzed in 2-day periods, and the minimum nitrogen excretion was read from the resulting time curve. The *N-free* diet and the method of urine collection were described in Research Bulletin 189. Basal metabolism was measured by the Haldane technique.

Method 2: Composite or Smuts' Method.—To prevent loss of body weight the above N-free diet was supplemented with 5.5 mg N per gm of food in the form of commercial dried egg yolk. The resulting diet was so designed that when the rats ate about 15 per cent more than their basal energy needs, the nitrogen intake was approximately equivalent to their endogenous requirements. It was called the *N-poor* diet, in contrast to the preceding *N-free* diet.

The animals used for this purpose were litter mates previously paired to the same intake of the two protein levels described in Research Bulletin 189 as *low protein* (13 per cent) and *high protein* (30 per cent) diets.

At regular intervals during the growth period each pair of animals in this group was given the N-poor diet for a period of 13 days. Just before putting the rats on this diet, they were starved for 17 hours and a basal metabolism measurement made by the Haldane technique. Six additional days were allowed for the rats to reach a minimum level of nitrogen excretion. The urine and feces were then collected for a period of 6 days. After the urine collection another basal metabolism measurement was made and the rats were returned to their original rations and again paired to the same intake. The above procedure was repeated for other body weights, so that two to three N/Cal. ratios were determined for each rat during the growth period.

The technique for urine collection was practically the same as that of method 1. To check the technique, different cage styles were used, and the filter pads were analyzed for nitrogen. Size and form of cage did not affect the results. The feces were preserved in a desiccator; the urine, collected daily, was preserved with sulfuric acid; the filter pads on which the urine and feces were collected, were preserved with an alcoholic solution of benzoic acid.

As additional evidence that the error due to the loss of nitrogen during these collection periods was not large, reference is made to the report of Hogan, Johnson and Ashworth (Proc. Am. Soc. Animal Production, 1934) in which the same technique for urine collection was used. During this investigation complete nitrogen balance experiments were made on young rats. Body analyses of control rats from the same litter

they lost an average of 2.4 gm in body weight per day during the 6 days of urine collection and excreted about 26 mg of body N per gm of body weight lost. There was no doubt that these animals were excreting more than their minimum endogenous level of nitrogen, the N/Cal ratio being above even that given by Smuts for adult rats (2.0 mg N/Cal.). After determining the basal heat production of these large animals a second urine collection of 4 days was marked by a large drop in the N/Cal ratio. This time it averaged 2.27. Then they were given the N-free ration during 4 more days of nitrogen collection. The N/Cal ratio dropped again, this time to an average of 1.87. A second collection period of 6 days on the 8 small rats showed a slightly higher average for the ratio. This time it was 0.98 mg N per Cal. instead of 0.87, but it was still about one-half as large as the corresponding value for the large rats.

3. **Effect of the Preceding Dietary Protein Level.**—Table 3 answers the question of whether rats taken from a 30 per cent dietary protein level excrete more endogenous nitrogen per unit weight when measured by Smuts' method than those taken from a 13 per cent level. These animals are the pairs listed in Table 2, which exceed 100 gms in body weight. The data were analyzed statistically by Student's method modified by Love and Brunson (J. Am. Soc. Agron. *16*, 60). Before a direct comparison could be made by this method the nitrogen excretions had to be corrected for differences in body weight because the high protein animal of each pair was invariably larger (otherwise the animals were comparable since they were litter mates, paired to the same food intake and housed under the same conditions.). This correction was made by first relating urinary nitrogen excretion to body weight for the low protein animals. The equation of this curve, which will be described in more detail later, is $Ur\ N = 0.285\ (Beg.\ Wt.)^{0.90}$. By subtracting the value for urinary nitrogen read from the curve for the low protein animal's body weight from that for the high protein animal's body weight, the correction for body weight (c) in Table 3 was secured. This value was then subtracted from the absolute difference in urinary nitrogen excretion by the two animals to get the net difference or the difference due to the effect of the preceding dietary protein level. When this net difference column is treated by Student's method a very significant difference is noted. The odds are 3000 to 1 that the high protein animals have a higher excretion of nitrogen per unit body weight during the 6th to the 12th days of N-poor feeding. The mean difference was 7.5 mg above the mean excretion of 28.6 mg by the low protein group, or a difference of 26 per cent. Does this actually represent a higher level of endogenous nitrogen excretion by the high protein animals? A preceding publication from this laboratory has shown that only after 28 days on a N-free

diet do the curves for urinary nitrogen per unit body weight for low and high protein rats come together (Fig. 5, Missouri Research Bulletin 190).

By a similar statistical treatment the initial rates of basal energy metabolism of these animals are compared also in Table 3. The equation used to supply the correction factor for the differences in body weight is, Beg. Cal. $=0.267$ (Beg. Wt.)$^{0.84}$. Here again there is a significant difference. The chances are 160 to 1 that the high protein animals have a lower rate of basal metabolism per unit weight. The mean net difference is 1.67 Calories per day. This is 7.6 per cent below the mean of 22 Calories per day produced by the low protein animals. These results confirm earlier work in this respect (Research Bulletin 189). At that time the difference in heat production was attributed to the storage of more adipose tissue by the high protein rats thus lowering the proportion of actively metabolizing tissue. But a recent study of the composition of rats taken from two dietary protein levels showed that the low protein animals contained more fatty tissue than the high protein animals (Hogan, Johnson and Ashworth, Proc. Am. Soc. Animal Production, Dec. 1934). At the present time no satisfactory reason can be advanced for the lower basal metabolism per unit weight shown by the high protein rats.

4. **Age Curves by Logarithmic Correlation.**—A series of power equations presented in Table 4, have been fitted by the method of least squares to the data from Tables 1 and 2. In these equations "Beg." designates the beginning body weight or basal heat production of the animals, "End", body weight or basal metabolism at the minimum point of nitrogen excretion, and "Total N", urinary + fecal nitrogen. Equations 21 and 22 represent data selected according to the following criteria: first, the rats were either weanlings or taken from a low protein diet; second, the animals did not lose weight while on the N-poor diet; third, urine collections were made after the effect of the size of the cage and washing technique had been thoroughly checked. These equations then represent the nearest approach to standard conditions for measuring actual age curves of endogenous nitrogen excretion.

Some idea of the significance of these equations can be gained from a study of their standard errors of estimate [68 per cent of the data fall within $\pm$ the standard error of estimate (S_r), and 95 per cent of the data fall within $\pm$ twice the standard error of estimate ($2\ S_r$)]. Sixteen of these equations have been plotted in Fig. 2 for comparative study. If it is assumed that a distance of 1 S_r is slightly significant and a distance of 2 S_r is definitely significant, then the following conclusions follow regarding the first 16 equations listed in Table 4:

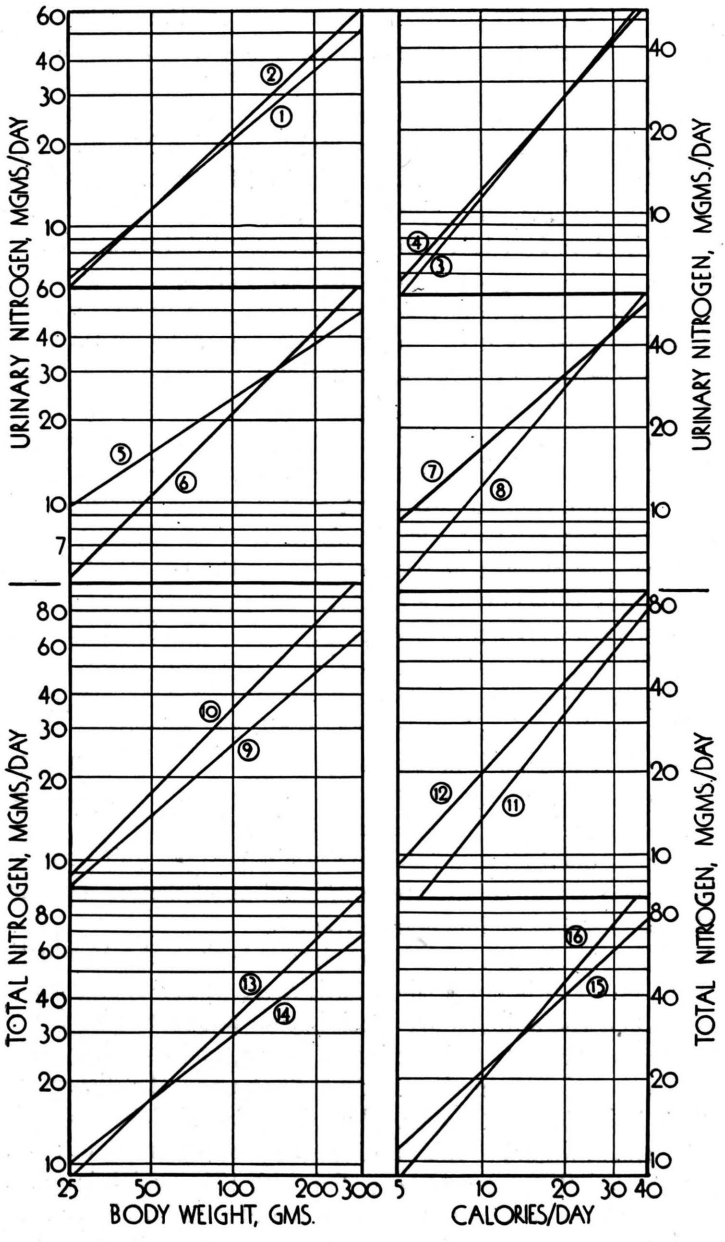

Fig. 2.—A comparison of relationships between nitrogen excretion and body weight or basal metabolism from data by the two methods for determining the minimum nitrogen excretion. The numbers on the curves refer to the equations listed in Table 4.

(1) There is no significant difference between the results secured by the time curve method and by Smuts' method when endogenous *urinary* nitrogen is related to the beginning body weight or the beginning basal heat production (that is, when equation No. 1 is compared to equation No. 2 and equation No. 3 is compared with equation No. 4).

(2) However, when the *total* (urinary+fecal) nitrogen is related with beginning weight and Calories (i. e., equation No. 9 vs No. 10 and equation No. 11 vs No. 12), there are significant differences which may be attributed to the larger production of fecal nitrogen from the N-poor diet. Both when initial body weight is used as a reference and when initial basal Calories are used, Smuts' method gives high values for total nitrogen. Collecting of feces several times per day as a precaution against contamination with urine did not change the results. Therefore, we attribute the high fecal nitrogen excretion to: first, the nature of the N-poor diet; second, the increased consumption of this diet.

(3) When we compare urinary nitrogen with body weight or basal metabolism at the time of the minimum level of nitrogen excretion, there is a very significant difference between the results for small rats secured by the time-curve technique and those secured by Smuts' technique (equations No. 5 vs No. 6, and No. 7 vs No. 8). This difference is due to the fact that when using the *N-free* diet of the time-curve method the animals lost weight, while on the *N-poor* diet of Smuts' method the smaller rats gained weight. While the animals on the N-free diet (Method 1) had an intake of only about 0.05 mg N daily per gm of body weight, those on the N-poor diet (Method 2) had an intake of 1.5 mg per gm. At the same time the rats having the higher N-intake were excreting about one-half as much urinary nitrogen per unit body weight. This indicates that in small rats additional good dietary protein may lower the excretion of endogenous nitrogen.

These results lead one to speculate whether the production of endogenous nitrogen is not pushed back more nearly to the theoretical minimum when the animals are receiving an adequate diet for normal growth. There probably is built into all cells, and especially the liver, a labile reserve of protein that is dependent upon the dietary protein level. This is gradually depleted while the animal is kept on a diet low in protein, thus producing the "endogenous" nitrogen excretion of Borsook & Keighley (Proc. Natl. Acad. Sci.,-*20*, 179), which is said to account for 60-75 per cent of the nitrogen metabolized under normal conditions of protein supply. The above "endogenous" nitrogen should be distinguished from the nitrogen derived from disintegration of special tissues and fluids such as blood cells, digestive juices and hormones. There is a difficulty in completely depleting the body of reserve or deposit protein, analogous to the difficulty in completely depleting the body

of glycogen. It is conceivable that the enzyme complex involved in protein oxidation changes with advance of time of N-starvation so as to allow more conservative use of protein.

Negative energy balance probably is the cause for most of the individual variations in the endogenous nitrogen level. If protein alone is burnt the mg N/Cal ratio is 40 to 1; if 20 per cent protein and 80 per cent fat is burnt, as during fasting, the mg N/Cal is 8 to 1. It is not surprising then that a minimum N/Cal ratio of 1 to 1, or even 2 to 1, is very difficult to maintain for any length of time. When the mg N/Cal ratio is 1 to 1, only 2.5 per cent of the heat production comes from protein. Moreover, the actual proportion coming from protein oxidation is probably less because under these circumstances the urine contains a larger proportion of less completely oxidized nitrogenous substance such as creatinine and uric acid.

The difference noted in comparing equations No. 5 and 6 may be attributed to the fact that the animals on the ad libitum N-poor diet, were more likely satisfying all their energy requirements than the animals receiving the ad libitum N-free diet. Toward the bottom of Table 2 are recorded data for the 0 series of weanling rats. Those with odd numbers (i.e., 0-1, 0-3, 0-5, 0-7) were given the N-free diet. The remaining members of the 0 series were given the N-poor diet but their intake was paired to that of the N-free animals. Under this limited food supply the N-poor rats excreted as much or more nitrogen than those on the N-free diet. The unusually low nitrogen excretion of these N-free animals may perhaps be attributed to the fact that their activity was confined to the limits of a liter beaker in which the rats were kept for the 6-day period of urine collection. We have no additional data at present to prove this point. But at least these 8 animals offer no evidence that the supplementing of the N-free diet with a small amount of egg yolk lowers the production of endogenous nitrogen.

The standard errors of estimate for the equations listed in Table 4 may be used as indices of the reliability of the equations. The average standard error of estimate for the first 16 equations is 20.4 per cent. In these equations endogenous nitrogen was related to body weight and basal metabolism. Which is the more reliable reference base? If basal metabolism and endogenous nitrogen are the more closely related then, of course, basal metabolism should be considered the better reference base. The average standard error of estimate for the four equations relating *urinary* nitrogen to beginning and ending body weight is 17.6 per cent, while the corresponding value for the equations relating *urinary* nitrogen to basal metabolism is 24.5 per cent. Likewise when *total* endogenous nitrogen is related to body weight and basal metabolism the standard errors of estimate are respectively 16.3 and 23.4 per cent. From this we

might conclude that body weight serves as a better unit of reference for endogenous nitrogen than basal metabolism. Such a conclusion may be criticized because the use of basal metabolism involves another analytical error. A study of the technique used to measure the basal metabolism data, used in the formulation of these equations, leads to the belief that the actual errors of measurement are much lower than the differences noted in the standard errors of estimate.

The difficulties involved in securing reliable values for the endogenous nitrogen excretion, no doubt, explains many of the discrepancies in the biological values of proteins as given in the literature. Maximum biological values must necessarily be measured at very low dietary concentrations of protein, otherwise part of the protein will be wasted. The endogenous nitrogen excretion, as measured by the nitrogen excretion on a N-free diet, accounts for a large proportion of the total urinary nitrogen when these low protein diets are fed. What might seem to the investigator to be a minor detail in the experimental technique may involve a 20 per cent difference in the endogenous nitrogen excretion and a consequent 20 per cent difference in the biological value secured.

CONCLUSIONS

1. Weanling rats excrete less than 1 mg endogenous urinary nitrogen per Calorie of basal metabolism. Under the same conditions adult rats excrete about 1.5 mg endogenous urinary nitrogen per Calorie. In other words, the N/Cal ratio ~~declines~~ rises with increasing age during growth.

2. Using Smuts' technique, whereby the endogenous nitrogen is taken as the average excretion between the 7th and 13th days of N-poor feeding, rats weighing over 100 gms taken from a high protein diet (30 per cent) were found to excrete an average of 26 per cent more nitrogen per unit body weight than their litter mates paired to the same intake of a low protein diet (13 per cent). The high protein animals produced 7.6 per cent less basal heat per unit body weight than their low protein pair mates.

3. Endogenous nitrogen follows more closely a logarithmic function of body weight than it does a logarithmic function of basal heat production.

4. More consistent results can be secured by relating the minimum endogenous nitrogen excretion to the initial body weight than by relating it to the body weight at the time the minimum level of nitrogen excretion is reached.

TABLE 1.—DATA SECURED BY TIME-CURVE METHOD

Rat Number	Energy Metabolism: Beginning Body Wt. gms.	Beginning Cal. per day	at endogenous level Body Wt. gms.	Cal. per day	Days on N-free	Food Intake* gms. per day	Nitrogen excretion mg per day: Urinary	Fecal	Ratios: Ur. N (mg) Beg. Wt. (kg)	Ur. N (mg) Beg. Cal
9-P	51	11.5	44	7.3	10	2.0	12.	3.9	235.	1.04
9-N	71	13.	53	11.	15	3.3	18.5	3.2	260.	1.42
10-P	56	11.5	42	6.4	18	2.3	11.5	2.4	205.	1.00
11-P	64	14.	53	8.9	14	3.8	15.	3.8	234.	1.07
11-N	86	18.	84	17.	3	6.0	23.	7.1	267.	1.28
6-P	65	12.	51	7.6	17	3.1	13.	2.8	200.	1.08
6-N	135	23.	109	18.	14	4.9	19.	4.6	141.	0.83
5-P	72	13.	54	7.8	19	2.6	15.	2.0	208.	1.15
7-P	96	15.	88	12.5	5	5.9	23.5	8.7	245.	1.57
7-N	113	18.	92	14.5	16	3.0	24.0	6.6	212.	1.33
8-P	115	21.	99	13.0	10	4.0	22.5	4.8	196.	1.07
8-N	135	22.	120	16.	6	4.7	32.	8.4	237.	1.86
16-N	120	17.	107	17.5	17	4.4	23.	5.5	191.	1.35
15-N	130	19.	115	18.	14	4.5	23.	6.9	177.	1.21
17-N	140	21.	142	22.	4	8.8	29.	7.8	207.	1.38
1-P	210	31.	170	15.	25	3.1	36.	6.4	171.	1.16
1-N	183	20.	160	20.5	17	6.8	31.	8.1	169.	1.55
13-N	230	27.	220	27.5	10	10.8	44.	10.5	191.	1.63
14-N	240	27.5	203	22.5	16	5.7	45.	9.4	188.	1.64
3-P	322	36.	320	32.	4	10.6	52.	15.7	161.	1.44
3-N	245	24.	250	21.	4	6.3	43.	15.0	176.	1.79
4-P	250	40.	240	32.	3	10.5	55.	15.0	220.	1.38

*Food intake at endogenous level.

TABLE 2.—DATA SECURED BY SMUTS' METHOD

Rat Number	Diet Taken from	Basal energy metabolism				Food Intake* gm/day	Nitrogen excretion mg per day		Ratios	
		Beginning		Ending					Ur. N (mg)	Ur. N. (mg)
		Body Wt. gms.	Cal. per day	Body Wt. gms.	Cal. per day		Urinary	Fecal	Beg. Wt. (kg)	Beg. Cal
A-1	Mother	32.5	6.89	38.8	6.24	5.1	7.68	6.93	236	1.11
A-10	Mother	36.6	8.55	37.7	7.07	3.7	7.25	6.23	198	0.85
B-1	Mother	30.5	6.01	35.5	7.41	4.6	7.60	6.60	249	1.26
B-10	Mother	28.9	7.25	37.5	6.84	5.4	9.98	7.50	345	1.38
C-1	Mother	27.4	5.60	36.5	6.29	5.0	9.68	6.77	353	1.73
C-10	Mother	29.0	5.46	37.5	5.96	4.8	9.14	6.70	315	1.67
D-1	Mother	24.2	4.95	33.2	6.19	4.7	8.59	4.67	355	1.74
D-10	Mother	25.3	5.15	34.9	6.21	5.2	9.66	6.86	382	1.88
A-2	high†	55.9	10.10	59.	9.50	5.1	20.3	6.93	363	2.01
A-20	low†	44.6	9.44	57.	8.46	7.5	14.3	8.77	321	1.51
A-3	high	71.0	13.5	75.	10.50	6.4	22.0	7.73	310	1.63
A-30	low	60.0	11.6	63.	9.24	4.8	14.0	5.55	233	1.21
C-3	high	78.0	14.6	87.	12.30	8.8	22.3	11.0	286	1.53
C-30	low	67.0	12.7	76.	10.80	7.6	17.1	10.0	255	1.35
A-1	high	104.	18.9	104.	14.40	7.5	21.5	12.3	207	1.14
A-10	low	76.	15.8	87.	15.10	8.2	16.9	11.0	222	1.07
B-1	high	96.	17.6	102.	17.10	9.6	21.4	12.5	223	1.22
B-10	low	75.	14.9	83.	17.50	8.5	20.4	10.7	272	1.37
C-1	high	108.	19.4	103.	15.20	8.7	22.9	14.3	212	1.18
C-10	low	74.	12.4	77.	11.00	6.5	16.6	10.1	224	1.34
B-3	high	84.	15.1	88.	14.90	6.5	18.3	8.9	218	1.21
B-30	low	58.	11.5	59.	11.60	3.8	14.8	6.6	255	1.29
A-3	high	160.	24.1	147.	20.40	8.0	47.0	16.8	294	1.95
A-30	low	109.	19.4	106.	14.60	6.9	31.5	11.3	289	1.62
A-2	high	133.	22.2	140.	21.00	12.2	25.2	19.2	189	1.14
A-20	low	91.	17.3	98.	16.80	9.0	23.5	16.7	258	1.36
D-1	high	107.	20.2	117.	19.90	9.5	23.0	14.7	215	1.14

*during urine collection.

†High-protein and low-protein diets.

TABLE 2 (CONTINUED).—DATA SECURED BY SMUTS' METHOD

Rat Number	Diet Taken from	Basal energy metabolism				Food Intake* gm/day	Nitrogen excretion mg per day		Ratios	
		Beginning		Ending					Ur. N (mg)	Ur. N. (mg)
		Body Wt. gms.	Cal. per day	Body Wt. gms.	Cal. per day		Urinary	Fecal	Beg. Wt. (kg)	Beg. Cal
D-10	low	74	15.3	74.	9.0	4.5	16.6	8.27	224	1.08
C-3	high	150	24.2	165	23.80	16.3	39.2	22.8	261	1.62
C-30	low	109	20.8	120	16.90	10.2	23.7	15.6	217	1.14
B-2	high	126	21.4	131	21.30	9.6	30.9	15.2	245	1.44
B-20	low	89	16.5	95	18.50	7.5	19.0	10.2	213	1.15
C-2	high	130	22.9	126	22.40	9.5	40.3	14.8	310	1.76
C-20	low	86	17.8	98	20.30	10.6	24.8	13.5	288	1.39
E-1	Mother	30.0	6.35	37	8.74	6.5	7.11	6.87	237	1.12
E-2	Mother	24.0	3.44	26.6	6.52	7.7	6.06	5.40	253	1.76
G-1	Mother	29.4	6.60	34.0	8.76	3.2	10.63	5.86	362	1.61
G-2	Mother	22.2	3.96	24.0	6.26	2.5	5.80	3.79	261	1.46
My-1	Milk	56.6	13.5	58.0	8.76	5.1	11.85	7.14	209	0.88
My-2	Milk	55.5	15.0	60.0	10.80	5.0	11.81	6.68	213	0.79
My-3	Milk	64.0	14.9	63.0	11.1	4.8	13.02	6.30	203	0.87
My-4	Milk	59.6	14.9	62.0	11.7	4.8	10.76	7.27	181	0.72
B-2	high	202	29.0	196	24.4	13.3	47.01	22.26	233	1.62
B-20	low	141	21.1	149	20.9	11.4	23.3	17.12	165	1.10
A-1	high	163	22.7	179	24.3	14.9	40.3	26.5	247	1.78
A-10	low	128	18.5	149	21.4	14.2	30.7	14.3	240	1.66
B-1	high	170	26.4	178	26.6	15.5	38.2	24.9	225	1.45
B-10	low	123	21.8	137	22.2	15.3	24.4	23.2	198	1.12
C-1	high	163	26.0	181	28.4	17.5	45.9	25.9	282	1.77
C-10	low	126	23.5	130	21.0	11.0	34.0	15.0	270	1.45
D-1	high	165	25.1	175	28.9	16.9	41.8	29.8	253	1.67
D-10	low	120	20.4	123	19.7	12.5	29.0	23.4	242	1.42
A-2	high	200	24.4	206	29.6	15.3	41.9	23.6	210	1.72
A-20	low	146	23.4	159	27.0	14.3	28.3	24.5	194	1.21

*during urine collection.

TABLE 2 (CONTINUED).—DATA SECURED BY SMUTS' METHOD

Rat Number	Diet Taken from	Basal energy metabolism				Food Intake* gm/day	Nitrogen excretion mg per day		Ratios	
		Beginning		Ending					Ur. N (mg)	Ur. N. (mg)
		Body Wt. gms.	Cal. per day	Body Wt. gms.	Cal. per day		Urinary	Fecal	Beg. Wt. (kg)	Beg. Cal
C-2	high	192	27.1	175	22.2	9.3	49.8	19.8	259	1.84
C-20	low	148	25.3	156	27.4	10.3	27.3	21.8	184	1.08
A-3	high	246	30.0	233	30.2	11.1	58.3	21.0	237	1.94
A-30	low	189	27.6	175	20.2	10.5	38.0	18.8	201	1.38
B-3	high	167	22.6	173	26.2	11.2	33.7	29.5	202	1.49
B-30	low	138	19.9	149	19.4	10.1	23.9	18.1	173	1.20
C-3	high	234	30.0	260	31.8	18.3	35.8	32.5	153	1.19
C-30	low	178	22.0	204	29.9	15.2	28.5	23.4	160	1.30
F-1	high	89	16.6	113	19.6	11.1	17.1	16.6	192	1.03
F-2	high	106	16.7	129	21.3	11.8	18.4	18.5	174	1.10
F-3	low	106	19.2	129	20.7	13.1	18.2	18.0	172	0.95
My-1	high	112	19.0	115	13.7	6.6	23.3	13.3	208	1.23
My-2	low	109	26.8	116	16.1	8.6	21.8	14.9	200	0.81
My-3	low	111	21.4	109	15.4	7.1	19.4	11.2	175	0.91
My-4	high	114	20.2	125.0	14.9	8.5	21.6	15.5	189	1.07
0-1	Mother	42	8.40	37.4	9.44	2.7	6.20	2.29	148	0.74
0-2	Mother	42	7.93	39.6	5.70	2.7	7.58	2.15	180	0.96
0-3	Mother	36	8.30	34.2	7.26	2.7	5.97	3.04	166	0.72
0-4	Mother	37	8.49	36.7	8.46	2.7	7.21	3.82	195	0.85
0-5	Mother	42	7.27	40	9.36	3.2	6.51	2.58	155	0.90
0-6	Mother	41	7.61	42	8.70	3.2	6.93	4.31	169	0.91
0-7	Mother	43	7.55	39	7.56	3.2	6.89	3.57	160	0.91
0-8	Mother	42	8.12	40	8.74	3.2	7.51	2.95	179	0.92
106	Milk	406	43.2	364	34.1	10.3	113.9	20.3	281	2.64
308	Milk	403	42.0	369	28.8	10.2	92.4	29.4	229	2.20
404	Milk	363	32.3	330	27.5	8.2	69.7	20.9	192	2.09
405	Milk	382	39.9	340	38.2	8.5	102.1	21.3	267	2.56

*during urine collection.

TABLE 3.—COMPARISON OF RATS PAIRED TO LOW AND HIGH PROTEIN LEVELS

Pair No.	Initial body wt.		Urinary-N mg per day		(a-b) (d)	Correction for body wt. (c)	Net difference (d-c)	Initial basal Cal. per day		(e-f) (h)	Correction for body wt. (g)	Net difference (h-g)
	High Protein (gm)	Low Protein (gm)	High Protein (a)	Low Protein (b)				High Protein (e)	Low Protein (f)			
A-3 & 30	160	109	47.0	31.5	15.5	8.0	7.5	24.1	19.4	4.7	6.4	−1.7
C-3 & 30	150	109	39.2	23.7	15.5	6.6	8.9	24.2	20.8	3.4	5.1	−1.7
B-2 & 20	202	141	47.0	23.3	23.7	9.5	14.2	29.0	21.1	7.9	7.2	+0.7
A-1 & 10	163	128	40.3	30.7	9.6	5.5	4.1	22.7	18.5	4.2	4.2	0.0
B-1 & 10	170	123	38.2	24.4	13.8	7.6	6.2	26.4	21.8	4.6	5.8	−1.2
C-1 & 10	163	126	45.9	34.0	11.9	5.9	6.0	26.0	23.5	2.5	4.5	−2.0
D-1 & 10	165	120	41.8	29.0	12.8	7.0	5.8	25.1	20.4	4.7	5.6	−0.9
A-2 & 20	200	146	41.9	28.3	13.6	8.4	5.2	24.4	23.4	1.0	6.4	−5.4
C-2 & 20	192	148	49.8	27.3	22.5	6.5	16.0	27.1	25.3	1.8	5.1	−3.3
A-3 & 30	246	189	58.3	38.0	20.3	8.6	11.7	30.0	27.6	2.4	6.5	−4.1
B-3 & 30	167	138	33.7	23.9	9.8	4.4	5.4	22.6	19.9	2.7	4.7	−2.0
C-3 & 30	234	178	35.8	28.5	7.3	8.9	−1.6	30.0	22.0	8.0	6.4	+1.6
Mean				28.6			7.5		22.0			−1.67

$$Z = \frac{\text{mean net difference}}{\text{standard deviation}}$$

for endogenous nitrogen $Z = \frac{7.5}{4.5} = 1.7$, odds = 3332 to 1

for basal metabolism $Z = \frac{1.67}{1.88} = 0.89$, odds = 160 to 1.

TABLE 4.—EQUATIONS RELATING ENDOGENOUS NITROGEN EXCRETION TO BODY WEIGHT AND BASAL METABOLISM. S_r = STANDARD ERROR OF ESTIMATE WITHIN WHICH ⅔ OF THE DATA ARE INCLUDED.

Time Curve Method	*Smuts' Method*
1. Ur N = $0.444 (\text{Beg. Wt.})^{0.84}$ S_r = +14.1 and −12.4%	2. Ur N = $0.298 (\text{Beg. Wt.})^{0.94}$ S_r = +25.5 and −20.3%
3. Ur N = $0.674 (\text{Beg. Cal})^{1.22}$ S_r = +19.4 and −16.3%	4. Ur N = $0.904 (\text{Beg. Cal})^{1.13}$ S_r = +33.0 and −24.8%
5. Ur N = $1.18 (\text{End Wt.})^{0.65}$ S_r = +13.9 and −12.2%	6. Ur N = $0.215 (\text{End Wt.})^{1.00}$ S_r = +23.3 and −18.9%
7. Ur N = $2.15 (\text{End Cal.})^{0.90}$ S_r = +21.3 and −17.6%	8. Ur N = $0.752 (\text{End Cal})^{1.22}$ S_r = +36.8 and −26.9%
9. Total N = $0.500 (\text{Beg. Wt.})^{0.86}$ S_r = +15.8 and −13.7%	10. Total N = $0.322 (\text{Beg. Wt.})^{1.02}$ S_r = +22.7 and −18.5%
11. Total N = $0.766 (\text{Beg. Cal.})^{1.25}$ S_r = +21.1 and −17.4%	12. Total N = $1.62 (\text{Beg. Cal})^{1.09}$ S_r = +31.4 and −23.9%
13. Total N = $0.818 (\text{End Wt.})^{0.68}$ S_r = +13.2 and −11.7%	14. Total N = $0.387 (\text{End Wt.})^{0.97}$ S_r = +18.9 and −15.9%
15. Total N = $2.48 (\text{End Cal.})^{0.93}$ S_r = +22.2 and −18.2%	16. Total N = $1.25 (\text{End Cal.})^{1.20}$ S_r = +30.2 and −23.2%
17. Beg. Cal = $0.943 (\text{Beg. Wt.})^{0.63}$ S_r = +13.1 and −11.6%	18. Beg. Cal = $0.472 (\text{Beg. Wt.})^{0.78}$ S_r = +15.3 and −13.2%
19. End Cal. = $.474 (\text{End Wt.})^{0.74}$ S_r = +19.4 and −16.2%	20. End Cal. = $0.516 (\text{End Wt.})^{0.74}$ S_r = +16.2 and −13.9%

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21. Ur N = $0.285 (\text{Beg. Wt.})^{0.90}$ S_r = +20.2 and −16.9%	22. Beg. Cal. = $0.267 (\text{Beg. Wt.})^{0.84}$ S_r = 19.0 and −15.9%