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Studies on nitrogen metabolism in rats of different age fed on a protein-free diet.

4. Urinary urea nitrogen excretion in relation to body size and duration on protein-free diet feeding

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ABSTRACT

A study on the excretion dynamics of urea nitrogen (NU) in endogenous urinary nitrogen (EUN) was carried out on 350 male Wistar rats in 14 age groups ($k = 14$), ranging from 23 to 273 days, with an average body mass (SB) from 39 to 446 grams. The rats in each k — group were randomly assigned to one of five subgroups, differing in the duration of the protein - free diet (PFD) feeding, $t = 3, 6, 9, 12$ and 16 days. Urine for EUN and NU determination was collected during the entire experiment.

The age of the rats (k) was found to have a significantly greater influence on the concentration of NU and EUN than the duration of the PFD feeding (t). The interaction of $k \cdot t$ was significant. The proportion of NU in EUN increased distinctly in successive t periods to age of about 80 – 100 days, after which it rose only slightly in older rats. A distinct drop in the proportion of NU in EUN was seen between the period of 3 and 16 days of feeding the PFD.

NU excretion (mg) as a function of urinary EUN excretion (mg) and duration of the PFD feeding described by the linear multiple regression equation after transformation to power form is given below:

$$NU = 0.27 \cdot EUN^{1.187} \cdot t^{-0.185} \quad (1)$$

Daily urea N excretion (NU_d) was highly correlated with body mass (SB_d), increasing with age and decreasing with the duration of feeding the PFD. This relationship describes the multiple regression equation of which exponential transformation is given below:

$$NU_d = 225.4 \cdot SB_d^{1.033} \text{ (in kg)} \cdot d^{-0.242} \quad (2)$$

Equations (1) and (2) make it possible to obtain a satisfactory estimate of the amount of endogenous NU in the urine of rats fed for 16 days on PFD and the daily urea N (NU_d) excretion on days 1 to 16 on a PFD.

KEY WORDS: rat, endogenous nitrogen, urea, protein-free diet

INTRODUCTION

Urea is the main protein metabolite excreted in the urine of ureotelic animals. Ashida and Harper (1961), as well as Schimke (1962a) demonstrated that the amount of urea excreted in the urine of rats was a linear function of the dietary protein level and its biological value. Schimke (1962a) also found that the level of urea cycle enzymes in the liver increased linearly as the amount of dietary protein increased. This fact is reflected in the amount of urea excreted in the urine and its proportion in total-N which, in extreme cases, can even reach 98%. The urea in urine from animals fed protein-containing diets is derived from two sources, urea from deamination of ingested protein and urea from catabolism of endogenous proteins. In nutritional studies, endogenous urea has been determined rarely, usually only once, during the so called period of stabilized urinary nitrogen excretion in humans and animals given a protein-free diet (Smith, 1926; Kiriyama and Ashida, 1964; Berdanier et al., 1967; Calloway and Margen, 1971; Uauy et al., 1982).

Due to the lack of data in the relevant literature on the excretion dynamics of endogenous urea as influenced by either the age of the animal or duration of feeding a protein-free diet, we carried out this study aimed at ascertaining the effects of both of these factors on the quantitative relation between excretion of urea-N and total-N in urine as well as between the excretion of urea-N and body mass of rats in which total urinary N and body mass were a function of age and duration of feeding protein-free diet.

MATERIAL AND METHODS

The experiments were carried out on 350 male Wistar rats assigned to 14 age groups ($k = 14$) from 23 to 275 days, with an average body mass of 39 to 446 g, respectively (Table 1). The rats were fed a protein-free diet to appetite for periods (t) of 3, 6, 9, 12 or 16 days. Details of the experiment are given in previous papers: characteristics of the rats and composition of the protein-free diet (PFD) are given by Bęza (1986); urine collection and total urinary-N content (EUN) by Bęza (1987). Urea and EUN were determined using Brown's method (1959) in urine collected individually from each rat in successive periods of feeding the PFD.

The proportion of urea-N (NU) in urinary EUN of each rat after 3, 6, 9, 12 and 16 days of feeding the PFD was determined statistically using the linear regression model, $\log y = a + b \log x$, and the multiple linear regression model, $\log y = b_0 + b_1 \log x_1 + b_2 \log x_2$ for the relationship NU (EUN, t) and for the relationship NU_d (SB_d, d), i.e. the daily urea-N excretion (NU_d) as a function of body mass (SB_d) and the day of PFD feeding (d).

TABLE 1

The endogenous urea nitrogen (NU) content in endogenous urinary nitrogen (EUN) excreted by male rats of different age fed on a protein-free diet (PFD); n = 25 in each group

(experimental dat)

Age of rats, days (k)	NU mean ± SD in mg and per cent ± SD in EUN after t days on PDF									
	t = 3		t = 6		t = 9		t = 12		t = 16	
	mg	%	mg	%	mg	%	mg	%	mg	%
23	19.2± 2.2	44.1±4.4	30.7± 3.7	36.6± 6.6	41.7± 5.2	36.0± 3.5	55.6± 6.3	36.6±2.4	73.5±12.0	43.7±7.1
26	28.8± 4.1	46.7±3.6	39.6± 4.9	42.4± 6.0	55.0± 9.2	43.7± 3.7	93.9± 13.7	52.5±7.2	89.0±14.8	48.2±5.3
29	29.6± 5.4	53.2±4.4	50.4± 9.6	44.8± 1.7	74.2±14.6	44.9± 5.8	82.3± 14.7	44.0±5.3	90.6±13.7	42.5±3.6
35	91.2±10.2	72.9±1.9	131.4±10.7	64.3± 3.4	181.5±25.6	64.2± 2.6	228.1± 44.6	67.5±5.0	226.5±28.5	54.9±6.4
39	109.2±18.1	65.6±8.5	141.7±16.7	55.9± 7.3	183.3±18.6	56.4± 2.8	162.4± 24.1	51.8±5.4	237.4±31.8	56.4±2.8
42	110.2±16.9	68.7±3.8	145.8±15.6	57.8± 5.4	178.4±24.1	57.6± 5.4	231.8± 33.3	60.4±4.7	214.4±31.3	50.7±2.7
52	127.9± 7.4	68.6±2.4	220.7±43.4	63.9± 3.5	257.6±25.4	61.3± 3.9	366.4± 59.9	60.7±6.2	394.8±62.2	52.2±3.1
62	142.3±12.4	62.8±6.2	249.5±36.3	65.0±12.7	360.8±51.4	68.5±14.7	483.0± 67.8	67.8±6.6	495.7±98.6	57.9±2.8
78	209.0±20.9	61.8±3.6	302.9±28.6	58.6± 7.9	428.3±36.8	62.7± 4.4	525.5± 32.6	65.3±3.4	662.6±64.1	62.8±2.1
98	216.4±23.7	66.1±2.3	321.8±29.5	63.9± 0.9	444.2±54.9	58.9± 3.3	493.6± 35.4	54.8±2.2	722.2±48.7	58.9±4.0
125	163.5±29.0	56.9±4.9	273.8±31.1	51.0± 1.4	343.5±22.5	49.6± 2.2	461.1± 81.1	50.8±2.2	554.4±68.7	50.0±2.4
158	232.5±21.9	65.8±2.7	427.8±39.2	66.0± 5.7	484.0±40.0	60.3± 1.9	612.5± 40.3	58.1±3.4	704.7±62.2	60.2±4.8
264	186.3±15.1	61.9±4.5	365.3±63.0	63.2± 4.2	456.6±43.0	60.9± 5.0	662.1± 59.0	63.5±6.4	811.3±60.4	60.7±3.5
275	234.4±16.4	64.7±3.1	387.2±43.8	60.8± 1.0	593.8±79.6	62.0± 3.1	664.4±112.0	58.6±3.2	834.5±95.7	61.5±3.0

Bifactorial analysis of variance was used to evaluate the significance of the effect of rat age (k) and duration of the PFD feeding (t) as well as the interaction, $k \cdot t$, on the proportion of NU in EUN. Statistical analysis was carried out according to Bliss (1967, 1970).

RESULTS

The NU content (mg) and percentage of NU in urinary EUN, expressed as means SD ± (calculated from 5 rats) during the five periods of feeding the PFD in the 14 age groups of rats, are presented in Table 1.

The coefficients of variation (CV%), calculated for mean NU (mg) did not change with age. The mean CV's% from the 14 age groups were 11.8%, 12.5%, 12.1%, 13.2% and 12.7%, for t = 3, 6, 9, 12 and 16 days, respectively. The mean NU percentage (Table 1) indicates that the proportion of NU in urinary EUN of rats increased more or less regulary in all of the periods of feeding the PFD up to 62 days of age; the mean %NU (for 5 periods) increased from 39.4% in 23-day-old rats to 64.5% in 62-day-old animals. With increasing age in older rats, the %NU decreased in an irregular manner to, on average, a value of 61.5%.

As the duration of feeding the PFD increased, the %NU in EUN dropped in a rather regular manner in the 7 youngest groups (29, 35, 39, 42, 52, 98 and 125-days-old). In the remaining seven age groups, changes in %NU (during feeding the PFD), can be classified as random. A well-defined drop in the %NU occurred in almost all of the age groups between the 3- and 16-day periods of feeding. The difference between the mean %NU's (from 14 age groups) for these extreme periods amounted to about 7%.

Bifactorial analysis of variance, computed on the individual results of the studied rats (n = 350), showed that the age of the rats (k), the duration of the PFD (t) and interaction of $k \cdot t$ had a significant influence ($P < 0.01$) on the %NU in EUN. The F-tests were: for variability k $F_e = 86.33 > 2.28$ at $n_1 = 13$, $n_2 = 280$, for variability t $F_e = 23.27 > 3.41$ at $n_1 = 4$, $n_2 = 280$, and variability $k \cdot t$ $F_e = 3.79 > 1.62$ at $n_1 = 52$, $n_2 = 280$.

The relationship NU (EUN) in the urine of rats of different ages in successive periods of feeding the PFD, describe the regression equations presented in the Table 2. Analysis of variance in regression showed thad the b_t coefficients did not differ significantly among t periods ($P > 0.05$), but that the a_t coefficients did ($P < 0.01$) and decreased with the duration of PFD feeding. The D% coefficients ($r^2 \cdot 100$), which ranged from 95.3 to 97.9%, indicate how strongly the variability of NU was related to the variability of excreted EUN. The calculated slope, common for feeding periods from 3 to 16 days, $b_c = 1.187$ (± 0.012) > 1**

TABLE 2

Relationship between endogenous urea nitrogen (NU) and urinary nitrogen (EUN) excretion by male rats fed on a protein-free diet; $\log y = a + b \log x$; $y = NU$; $x = EUN$ (NU and EUN in mg); $n = 70$ im eael group

Ext. duration in days (t)	$a_t \pm S_a$	$b_t \pm S_b$	t-test	S_E	r_t	A_t	A_t for b_c
3	-0.6656 ± 0.0729	1.1902 ± 0.0321	$b > 1$··	0.0822	0.9762	0.2160	0.2199
6	-0.7637 ± 0.0738	1.2038 ± 0.0295	$b > 1$··	0.0755	0.9802	0.1723	0.1900
9	-0.8399 ± 0.0825	1.2207 ± 0.0314	$b > 1$··	0.0781	0.9782	0.1446	0.1772
12	-0.7438 ± 0.0783	1.1793 ± 0.0289	$b > 1$··	0.0768	0.9802	0.1804	0.1723
16	-0.6775 ± 0.0571	1.1466 ± 0.0204	$b > 1$··	0.0520	0.9894	0.2101	0.1625

Regression significant for $P < 0.05$

Regression lines parallel $F_b = 1.07 < F_{0.05} = 2.39$

Position of regression lines $F_a = 24.02 > F_{0.05} = 2.39$

Common b (b_c) $= 1.1866 \pm 0.0128 > 1^{**}$; $t_b = 14.59 > t_{0.05} = 1.96$

indicates that the rate of NU excretion increased not linearly when the excretion of urinary EUN rose with the age of rats. The amount of excreted NU (mg) decreased significantly ($P < 0.05$), however, when EUN excretion increased during feeding the PFD.

Excretion of NU as a function of two variables, EUN and t, can be equally well described by the multiple regression equation:

$$\log NU = -0.5688 + 1.1866\,(\pm 0.0123)\log EUN - 0.1854\,(\pm 0.0189)\log t, \quad (1)$$

where:
NU and EUN are expressed in mg, t in days; $S_E = 0.0766$; $r_1 = 0.978^{**}$; $r_2 = -0.417^{**}$; $R = 0.983^{**}$; t test $b_1 = 96.47^{**}$; t test $b_2 = 9.81^{**}$; $b_1' = 1.0345$; $b_2' = -0.1136$.

The equation (1) allows to make a close estimation of the amount of excreted NU after a freely chosen time of feeding the PFD, up to 16 days.

The relationship NU (t). Equations describing this relationship were calculated for each of the 14 age groups (k) using logarithms of means (Table 3).

TABLE 3

Relationship between endogenous urea nitrogen (NU) in urine and duration of protein-free diet feeding (t) in male rats of different age, calculated on mean values according to:
$\log y = a + b \log x$; (y = NU in mg; $x = t = 3, 6, 9, 12, 16$ days); n of means = 5 in each group

Age of rats, days (k)	$a_k \pm S_a$	$b_k \pm S_b$	t-test	S_E	r_k
23	0.8790±0.0431	0.8019±0.0461	b < 1*	0.0262	0.9951
26	0.9995±9.1405	0.8324±0.1505	b < 1	0.0853	0.9543
29	1.1952±0.0838	0.6551±0.0897	b < 1*	0.0508	0.9730
35	1.6827±0.0854	0.5877±0.0615	b < 1**	0.0348	0.9840
39	1.8357±0.0851	0.4141±0.0912	b < 1**	0.0517	0.9344
42	1.8005±0.0840	0.4671±0.0900	b < 1**	0.0510	0.9485
52	1.7871±0.0531	0.6871±0.0569	b < 1*	0.0322	0.9899
62	1.7850±0.0618	0.7928±0.0662	b < 1*	0.0375	0.9897
78	1.9703±0.0359	0.6953±0.0385	b < 1**	0.0218	0.9954
98	1.9896±0.0583	0.6888±0.0624	b < 1*	0.0354	0.9879
125	1.8633±0.0265	0.7296±0.0283	b < 1**	0.0161	0.9977
158	2.1023±0.0878	0.6342±0.0940	b < 1*	0.0533	0.9686
264	1.8580±0.0433	0.8753±0.4640	b < 1	0.0263	0.9958
275	2.0058±0.0392	0.7672±0.0420	b < 1*	0.0238	0.9955

Regression highly significant for $P < 0.01$
Regression lines non parallel $F_b = 3.05 > F_{0.05} = 1.99$
Position of regression lines $F_a = 251.72^{**} > F_{0.01} = 1.93$

Variance analysis in regression shows that the coefficients b_k and constants a_k differed ($P < 0.05$) among the age groups. The D% equalled 87.3 to 99.5%. It was found that coefficients b_k were neither linearly nor curvilinearly significantly correlated with either rat age ($r = -0.396$; $R = 0.420$) or initial body mass ($r = -0.307$; $R = 0.473$). However, constants A_k ($A_k = d \log a_k$) were found to be significantly correlated linearly with the age ($r = 0.614^*$) and initial body mass of rats ($r = 0.827^{**}$). Using the derivative of function $\frac{d\,NU}{dt}$ from equation in Table 3, the daily urea excretion $\frac{dt}{(NU_d)}$ on days 1, 3, 6, 9, 12 and 15 of feeding the PFD were calculated.

The obtained amount of excreted NU_d and body mass (SB_d), taken from the work of Bęza (1986a) for the same rats, are presented in Table 4. The relative NU_d excretion, expressed as a percentage of excreted NU_1 and relative EUN_d excretion on the same days of the PFD feeding (taken from the paper of Bęza, 1987), are presented in Figure 1. It can be seen that both curves fall almost in

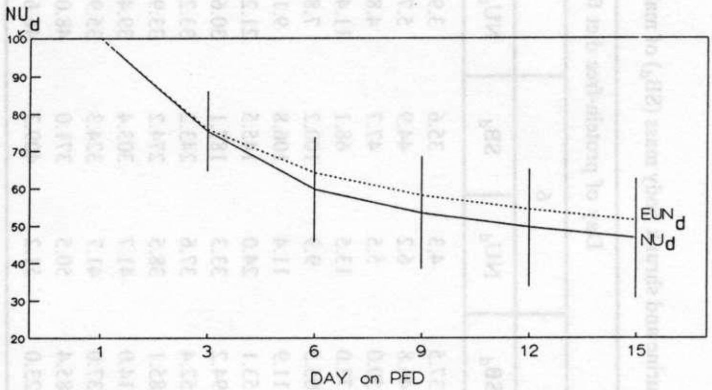

Fig. 1. Daily endogenous urea nitrogen (NU_d) and endogenous urinary nitrogen (EUN_d) as per cent of the excretion on the first day of protein-free feeding of male rats of different age (mean per cent ± SD)

parallel. NU_d excretion decreased mostly to day 6, reaching approximately 60% of NU_1 excretion. On subsequent days of the PFD feeding, NU_d excretion decreased at a much slower rate, equalling about 47% NU_1 on day 15.

The relationship NU_d (SB_d) was calculated on the basis of the values presented in Table 4 after logarithmic transformation. The regression equations calculated for days 1, 3, 6, 9, 12 and 15 of the PFD feeding along are presented in Table 5. Statistical comparison of the slope ratios shows that the b_d coefficients did not differ significantly, and the common coefficient b_c for d from 1 to 15 equalled 1.0328 (±0.0421) did not differ significantly from 1. This permits the

TABLE 4

Daily endogenous urea nitrogen (NU_d) excretion in urine and shrunk body mass (SB_d) of male rats of different age fed on a protein-free diet (NU_d, mg; SB_d, g)

Age of rats, days (k)	Day of protein-free diet feeding (d)											
	1		3		6		9		12		15	
	NU_d	SB_d	NU_d	SB_d	NU_d	SB_d	NU_d	SB_d	NU_d	SB_d	NU_d	SB_d
23	6.1	39.4	4.9	37.5	4.3	35.6	3.9	34.9	3.7	34.3	3.5	33.9
26	8.3	49.8	6.9	46.8	6.2	44.9	5.7	43.9	5.5	43.2	5.3	42.6
29	10.3	53.7	7.0	50.0	5.5	47.7	4.8	46.5	4.4	45.6	4.0	44.9
35	28.3	78.9	18.0	72.0	13.5	68.1	11.4	65.8	10.2	64.2	9.3	63.1
39	28.4	113.5	14.9	105.5	9.9	100.2	7.8	97.5	6.6	95.5	5.8	94.2
42	29.5	120.8	16.4	111.9	11.4	106.8	9.1	103.8	7.8	101.8	7.0	100.2
52	42.1	165.6	29.8	153.1	24.0	145.5	21.2	141.6	19.3	138.7	18.0	136.5
62	48.3	206.5	38.5	194.2	33.3	187.1	30.6	182.8	28.9	179.9	27.6	177.4
78	64.9	267.3	46.5	252.4	37.6	243.2	33.2	238.2	30.4	234.4	28.4	231.7
98	67.2	304.1	47.8	285.1	38.5	274.2	33.9	268.0	31.0	263.6	28.9	260.0
125	53.3	331.1	45.8	314.0	41.7	303.4	39.4	297.2	37.9	293.1	36.8	290.0
158	80.3	357.3	53.7	337.0	41.7	324.3	35.9	317.0	32.3	312.6	29.8	307.6
264	63.1	403.2	55.0	385.4	50.5	371.0	48.0	363.1	46.3	358.1	45.0	354.8
275	77.7	445.7	60.2	423.0	51.2	409.3	46.6	402.0	43.6	396.3	41.4	392.0

TABLE 5

Relationship between daily endogenous urea nitrogen (NU_d) and shrunk body mass (SB_d) of male rats of different age during protein-free diet feeding; $\log y = a + b \log x$; where: $y = NU_d$; in mg, $x = SB_d$ in kg; n of means = 14 ineach group

$NU_{d/(NUd/day)}$ day	$a_d \pm S_a$	$b_d \pm S_b$	t-test	S_E	r	A_d	A_d for b_c
1	2.3161 ± 0.0729	0.9891 ± 0.0836	$b<1$	0.1088	0.9597	207.1	224.4
3	2.2211 ± 0.0552	1.0153 ± 0.0614	$b>1$	0.0806	0.9788	166.4	172.0
6	2.1636 ± 0.0597	1.0336 ± 0.0652	$b>1$	0.0860	0.9769	145.7	145.5
9	2.1307 ± 0.0681	1.0447 ± 0.0735	$b>1$	0.0973	0.9716	135.1	132.0
12	2.1076 ± 0.0758	1.0529 ± 0.0812	$b>1$	0.1077	0.9661	128.1	123.1
15	2.0899 ± 0.0826	1.0593 ± 0.0880	$b>1$	0.1169	0.9610	123.0	116.7

Regression significant for $P < 0.01$

Regression lines parallel $F_b = 0.12 < F_{0.05} = 2.35$

Position of regression lines $F_a = 16.98 > F_{0.01} = 3.25$

Common b (b_c) $= 1.0328 \pm 0.0421 > 1$; $t_b = 0.78 < t_{0.05} = 2.00$

conclusion that the rate of NU_d excretion in relation to body mass, which increases with age, was constant within the limits of error, regardless of the duration of the PFD feeding. The amount of excreted NU_d, however, decreased from 224.4 mg $\cdot SB_{(kg)}^{1.033}$ on the first day to 1167 mg $\times SB_{(kg)}^{1.033}$ on day 15 of the PFD feeding, i.e. it decreased to about 52% of NU_1. However, the greatest drop in NU_d excretion occurred by day 3 to 77% and by day 6 to 65% of NU_1.

The daily excretion of urea-N, as a function of two variables SB_d and d, are equally well described by the multiple linear regression equation:

$$\log NU_d = 2.353 + 1.033\ (\pm 0.029) \log SB_d - 0.242\ (\pm 0.027) \log d \quad (2)$$

where: NU_d is expressed in mg; SB_d in kg; d — day 1 to 15 on the PFD; $S_E = 0.0952$; $r_1 = 0.940^{**}$; $r_2 = -0.308^{**}$; $R = 0.972^{**}$; t test for $b_1 = 35.13^{***}$; t test for $b_2 = 9.33^{***}$; $b_1' = 0.924$; $b_2' = -0.095$.

Both this equation (2) and the equations given in Table 5 show that, in the presented experiments, the principle factor affecting decreased NU_d excretion/kg $SB_d^{1.033}$ was the duration of feeding the PFD.

DISCUSSION

In studies on the metabolism of endogenous protein in humans and animals fed on protein-free diet, total urinary-N per unit of total or metabolic body mass was predominantly determined. However, the excretion of nitrogenous compounds, i.e. protein metabolites, in urine has not been examined in detail.

The results of the experiment presented in this study deal with the excretion of urea-N (NU), the main component of endogenous urinary nitrogen (EUN). They showed that NU excretion was highly correlated with EUN excretion, rat body mass (SB) and duration of feeding PFD for a period of up to 16 days. From the equations describing relationship NU (EUN, t) (Table 2) and from equation (1) it can be concluded that the rate of NU excretion increased significantly in a nonlinear manner ($b_c - 1.187 > 1.187 > 1^{**}$), when EUN excretion increased with the age of the rats, in all of the periods of feeding the PFD.

The equations describing the relationship NU (SB_d, d) (Table 5) and equation (2) show that the rate of urea-N excretion (NU_d), regardless of the day of the PFD feeding, was constant within limits of error ($b_c = 1.033 > 1$, non significant), when SB_t increased with the age of the rats. In addition, equations (1) and (2) show that the rate of NU and NU_d excretion decreased significantly during feeding the PFD, according to $b_2 = -0.185$ and $b_2 = -0.242$, respectively.

The nonlinear course of the relationship NU (EUN, t) suggests a nonlinear increase the NU concentration in EUN with age and a nonlinear decrease of %NU during feeding the PFD; %NU, computed using equation (1) increased over all of the periods of feeding the PFD, from an average 44% in 23-day-old rats to about 62% in 98-day-old rats. The %NU in the older animals increased slightly, reaching 64% in 275-day-old rats. Both, the mean percentages of NU, calculated using equation (1) and those obtained directly from experimental values, in spite of some differences between them, unequivocally indicate that the concentration of NU in EUN increased significantly with age only in young rats.

Significantly smaller changes in %NU in EUN occurred during feeding the PFD. In spite of the fact that in equation (1) $b_2 = -0.1854$ indicates that the ratio of NU to EUN steadily decreases with PFD feeding duration a distinct difference in %NU occurred only between the 3- and 16-day periods of feeding the PFD. A similar direction of change is indicated by the changes in mean %NU in Table (1) These results suggest that in the shorter periods of feeding the PFD, changes in the %NU in EUN can be unnoticeable. The results obtained by Kiriyama and Ashida (1964) also indicate a tendency towards increasing the %NU in EUN with age from 21 to 158 days in rats fed a protein-free diet.

The increased concentration of NU in EUN with age suggests that the rate of endogenous urea synthesis increases with age also. However, when NU_d and EUN_d excretion were related to SB_d, i.e. to body mass, that also increases with age, showed that the excretion rate of NU/kg SB was relatively stable ($b_c = 1.033 > 1$ insignificant) while the rate of EUN excretion, EUN/kg SB, in the same rats decreased in accordance with $b_c = 0.872 < 1^{**}$ (Bęza, 1987). This indicates that the increasing with age the excretion of NU per unit of excreted EUN, thus the increased concentration of NU in EUN with age, was caused by the decreasing excretion of EUN with age, and was not related to increased urea synthesis with age.

From the relationship NU_d (SB_d, d) it can also be concluded that the rate of endogenous NU excretion is determined for the most part by body mass, while the amount of NU excreted per unit SB was determined, in these experiment, by the duration of the PFD feeding; excretion of NU_d decreased from 224.4 mg/kg $SB_d^{1.033}$ on day 1 to 116.7 mg/kg $SB_d^{1.033}$ on day 15 of the PFD feeding. In a study by Kendall et al. (1982) carried out on adult dogs fed a protein-free diet, whose body mass increased form about 3 to 51 kg, the relationship between NU and EUN was found to be linear, since the daily EUN/kg $W^{0.75}$ excretion was constant.

Both Schimke (1962b) and Nakano et al. (1972) found that the decrease in urea excretion in the urine of rats fed a protein-free diet was caused by the decrease in the amount and activity of urea cycle enzymes in the liver, as well as by the decreased activity of enzymes degrading amino acids due to a fall in the hepatic c-AMP level.

The steepest decline in urea excretion in the urine of rats, as compared with day 0, when a diet containing 15% casein was provided, was found by Schimke

(1962b) on the fourth day of feeding a PFD (75%, while on day 7 it rose to only 79%).

In our experiment, urinary NU excretion in rats of different ages fell, in comparison with the first day of feeding the PFD, by an average of 25%, 40% and 53% on days 3, 6 and 15, respectively. Such significant differences in the reduction of NU excretion between our and Schimke experiments are the effects of the high level of urea derived from the catabolism of casein provided on day 0 in Schimke's experiment. The sharp decrease in urea excretion on day 4 of feeding the PFD indicates, according to Schimke, the rapid adaptation of urea cycle enzymes to altered nutritional conditions. The decrease in NU excretion on the first day of feeding the PFD in our study can be attributed to the depletion of the more labile proteins, whose catabolic rate, as estimated by Yamaguchi and Kandatsu (1968, 1973), was above one hundred times higher than of less labile proteins. It was calculated (Bęza, 1992) that the more labile proteins were almost completely catabolized within the first 3 days of feeding the PFD. This suggests that the NU_d calculated from equation (2) and, on day 4, equal to 160.4 mg/kg $SB_d^{1.033}$, represents urea-N derived only from less labile proteins.

In summary, it can be said that endogenous NU_d excretion can be best and with a high degree of accuracy estimated from equation (2) which describes NU_d as a function of (SB_d and d), because determining SB_d is easier than determining EUN_d in urine. Once the value of NU_d, excreted on a specified day between days 1 and 15 on PFD, is known, the amount of NU_d derived from deamination of dietary protein, provided at maintenance or higher levels, can be calculated from the difference:

$$NU_d \text{ deamination} = \text{total } NU_d - \text{endogenous } NU_d$$

for rats of the same SB_d at which the endogenous NU_d was determined. The ratio of NU_d from deamination to endogenous NU_d can be an indicator also used as an index of the biological value of provided dietary protein.

REFERENCES

Ashida K, Harper A.E., 1961. Metabolic adaptations in higher animals. VI. Liver arginase activity during adaptation to high protein diet. Proc. Soc. Exp. Biol. Med. 107, 151–156

Berdanier C.D., Brush M.K., Fisher H., 1967. Significance of endogenous nitrogen excretion in protein evaluation studies with rats. J. Nutr. 93, 411–417

Bęza R., 1986a. Studies on nitrogen metabolism in rats of different age fed on a protein-free diet. 1. Changes of body mass. Arch. Tierernähr. 36, 341–350

Bęza R., 1986b. Studies on nitrogen metabolism in rats of different age fed an a protein-free diet. 2. The feed intake. Arch. Anim. Nutr. Berlin 36, 571–583

Bęza R., 1987. Studies on nitrogen metabolism in rats of different age fed on a protein-free diet. 3. Urinary endogenous nitrogen excretion in relation to body-size and protein-free feeding duration. Arch. Anim. Nutr. Berlin 37, 1101–1114

Bęza R., 1992. Biological evaluation of a protein-free diet, based on the body chemical composition in rats. Warsaw Agric Univ. Press, 1992, p. 61

Bliss C.I., 1967, 1970. Statistics in Biology. McGraw-Hill Book Company, New York, Vol. I, II
Brown H.H., 1959. Determination of blood urea with p-dimethylaminobenoaldehyde. Anal. Chem. 31, 1844-1846
Calloway D., Margen S., 1971. Variation in endogenous nitrogen excretion and dietary nitrogen utilization as determinants of human protein requirement. J. Nutr. 101, 205-216
Kendall P.T., Blaza S.E., Holme D.W., 1982. Assessment of endogenous nitrogen output in adult dogs of contrasting size using a protein-free diet. J. Nutr. 112, 1281-1286
Kiriyama S., Ashida K., 1964. Effect of the quality of dietary protein on nitrogen compouds in the urine of rats. J. Nutr. 82, 127-134
Nakano K., Katsuzaki M., Ashida K., 1972. Further studies on the effect of dietary carbohydrate and fat on protein metabolism in rats. J. Nutr. 102, 283-290
Nakano K., Ashida K., 1975. Possible intervention of insulin, cyclic AMP glucocorticoids in protein-sparing action of dietary carbohydrate in rats. J. Nutr. 105, 906-913
Schimke R.T., 1962a. Adaptive characteristics of urea cycle enzymes in the rat. J. Biol. Chem. 237, 459-468
Schimke R.T., 1962b. Differential effects of fasting and protein-free diets on levels of urea cycle enzymes in rat liver. J. Biol. Chem. 237, 1921-1924
Smith M., 1926. The minimum endogenous nitrogen metabolism. J. Biol. Chem. 68, 15-31
Uauy R., Yánez E., Ballester D., Barrera G., Guzmán E., Saitúa M.T., Zacarias I., 1982. Obligatory urinary and faecal nitrogen losses in young Chilean men given two levels of dietary energy intake. Br. J. Nutr. 47, 11-20
Yamaguchi M., Kandatsu M., 1968. Catabolic rate of body protein in rat during the earlier stages of protein depletion. Nature 217, 668-669
Yamaguchi M., Kandatsu M., 1973. Catabolic rate of body protein in adult rat over an entire period of protein depletion. Agric Biol. Chem. 37, 579-586

STRESZCZENIE

Metabolizm azotu u szczurów w różnym wieku, żywionych dietą bezbiałkową. 4. Wydalanie azotu mocznikowego w moczu w zależności od masy ciała i czasu żywienia dietą bezbiałkową

Badania nad dynamiką wydalania N mocznikowego (NU) w N endogennym moczu (EUN) przeprowadzono na 350 samcach szczurów typu Wistar w 14 grupach wiekowych (k = 14), od 23 do 275 dni, o średniej masie ciała (SB) od 23 do 446 g. W każdej k-grupie szczury podzielono losowo na 5 podgrup, odpowiadających okresom żywienia dietą bezbiałkową (PFD) t = 3, 6, 9, 12 i 16 dni. Zawartość NU i EUN oznaczono w moczu zebranym oddzielnie od każdego szczura w ciągu przyjętych okresów żywienia PFD.

Wykazano, że na koncentrację NU w EUN istotny i znacznie większy wpływ miał wiek szczurów (k) niż czas żywienia PFD (t), przy istotnej interakcji k · t. Procent NU w EUN zwiększał się w czasie żywienia PFD do około 80 lub 100 dnia życia szczurów, a u starszych zwierząt zwiększał się z wiekiem już nieznacznie. W czasie żywienia PFD wyraźne obniżenie udziału NU w EUN wystąpiło między okresem 3 a 16-dniowym.

Wydalanie NU w zależności od wydalania EUN (mg) w moczu i t-okresu żywienia PFD najlepiej opisywało równanie liniowej regresji wielokrotnej, które po zdelogarytmowaniu ma postać:

$$NU = 0{,}27\ EUN^{1{,}187}\ t^{-0{,}185} \quad (1)$$

Dobowe wydalanie N mocznikowego (NU_d) było wysoce istotnie skorelowane z masą ciała (SB_d), zwiększającą się z wiekiem szczurów, a obniżającą się w czasie żywienia PFD. Zależność

tę najlepiej opisywało równanie liniowej regresji wielokrotnej, które po zdelogarytmowaniu ma postać:

$$NU_d = 226{,}4 \cdot SB_d^{1,033} \text{ (w kg)} \cdot d^{-0,242} \quad (2)$$

Na podstawie równania (1) i (2) można z dużą dokładnością oszacować ilość NU pochodzenia endogennego w EUN w dowolnym okresie żywienia PFD między 3 a 16 dniem oraz dobowe wydalanie N mocznikowego (NU_d) w dowolnym dniu między 1 a 15 dniem żywienia PFD. Otrzymaną z równania (2) ilość NU_d-endogennego można wykorzystać do oszacowania ilości NU_d pochodzącego w dezaminacji białka diety, skarmianego na poziomie bytowym lub powyżej bytowego. Stosunek między obu zawartościami NU_d może być wskaźnikiem wartości biologicznej skarmianego białka.