DIETARY PROTEIN AND BLOOD UREA BLOOD UREA LEVELS IN THE RAT AS INFLUENCED BY THE TYPE OF DIETARY PROTEIN AND TIME AFTER A MEAL

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ABSTRACT **Previous work by other investigators indicates a direct relation between the amount of protein in the diet and the level of urea in the blood. However, the present report shows that the type of protein, apart from its level in the ration, also has an effect on blood urea concentrations. When adult male or female rats of two strains were fed a ration in which the only source of protein was wheat flour, the blood urea level was 20 per cent lower than in rats fed an isonitrogenous ration containing casein. This reduction was apparent in the first blood samples taken one week after the start of the wheat ratio. It was most prominen in the blood samples secured 18 hours after removing the feed cups from the cages. The reduction in blood urea was not due to a decreased digestibility of the flour ration nor to a change in the proportion of urinary nitrogen excreted as urea.**

INTRODUCTION Under normal circumstances, the blood urea nitrogen (BUN) level is probably controlled by several factors. The nutritional factor that has received considerable publicity in this respect is the quantity of dietary protein. A relationship between the amount of dietary protein and BUN level in human subjects was pointed out by Addis and Watanabe as early as 1917 (1). These investi-

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gators observed an increase in the BUN levels of their four subjects when the protein intake was raised from 12 g per day to 150 g or more. Thirty years later, the same group observed a doubling in BUN when the protein intake of 10 normal young men was increased from 0.5 g per kg of body weight to 1.5 g (2). A further, but less dramatic, increase in BUN occurred when the protein intake was raised to 2.5 g per kg. MacKay and MacKay (3) stated that the BUN levels of normal medical students increased when their protein intakes were raised from 1.1 g per kg of body weight to 1.7 g. Infants also appear to show an increased BUN whenever the protein ntake is augmented (4). The latter work was done with different groups of infants. This study showed that as the percentage of calories from protein increased from 7 to 20, the BUN increased progressively from 6.0 to 22.6 mg per 100 ml.

The one report contradicting the preceding relationship comes from Chitre, et al (5) who observed no change in the BUN levels of 20 Indian medical students when their protein intakes were increased from 40 to 80 g per day.

Relatively little work on the relation of protein intake to BUN has been done with animals. Osborne and coworkers (6) observed that as the protein in the rations fed to rats was increased from 18 to 80 per cent, the BUN went up from 9.2 to 38 mg per 100 ml.

That the type of dietary protein may influence the BUN is suggested by the work of Puchal, et al (7). When weanling pigs were fed isonitrogenous rations containing different proteins, the urea levels of the blood on the 26th day of the trial were inversely related to the weight gains of the animals. The urea levels ranged from an average of 13.4 mg per 100 ml of Plasma for the pigs fed the dried skim milk ration to 33.1 mg for those fed the meat meal ration. These results suggested to the investigators that an inverse relation existed between the biological value of a protein and the BUN level in the animal consuming that protein.

That the type of dietary protein may influence the BUN levels of human subjects was shown by Bolourchi, Fuerig and Mickelsen (8). They found a marked reduction in the BUN levels of normal men fed a diet which provided 90 to 95 per cent of the protein from wheat. This reduction in BUN levels occurred even though the protein intake was maintained at 70 g per day during both the control and experimental periods.

The present work was undertaken to determine: (1) if a reduction in BUN occurs in rats fed diets in which wheat was the sole source of protein; (2) the effect of time after a meal and source of dietary protein on BUN levels; and (3) whether dif-

cups were removed from the cages at 3 p.m. blood samples were taken by heart puncture the following morning when the animals had been without feed for 18 hours. Immediately thereafter, the fed cups were returned to the cages for three hours; at the end of that time, another blood sample was taken. For the next four hours, the rats were without feed; at the end of that period, the third blood sample was taken. The rats were weighed just prior to each bleeding. The food intake during the three-hour feeding period was recorded. Water was available at all times.

After the preceding study, the grain ration was replaced with the wheat ration (table 1). This was fed for ten days, after which blood samples were secured as previously described.

Both dietary regimens and blood collections were repeated on two separate occasions, with one week intervening between the trials. The animals were fed their allotted rations throughout the study.

Experiment 3. Nitrogen digestibility and retention, and urea excretion of rats fed grain, wheat or casein rations. Fourteen adult, female Sprague-Dawley rats were kept in individual metabolism cages and fed the grain ration for 9 days (9). The first 3 of the 9 days were considered as the adjustment period. Thereafter, urine and feces were collected for two successive periods of three days each. Feed consumption was measured. As soon as the preceding phase of the study was completed, another 9 day metabolism study was started. For this, 4 rats were maintained on the grain ration; 5 were fed the casein and 5 the wheat ration (table 1). Nitrogen balance studies were carried out for the first three days and the seventh through ninth days of this period.

Nitrogen was determined by the Kjeldahl technique. For this, the feces were homogenized with distilled water. Urinary urea was determined on a 1-250 dilution of the urine by the procedure described for Experiment 1.

RESULTS Experiment 1, First and Second Trial: The BUN levels of the rats fed the wheat ration were significantly lower than those fed the casein ration (table 2). The variability in the values over the eight-week experimental period mas greater for the rats fed the casein ration. In this study, the first blood sample was secured one week after initiating the ration trial. The reduction in BUN levels associated with feeding the wheat ration was not a temporary phenomenon; the lower levels were maintained over the eight weeks of the study. The differences in BUN levels of the casein and wheat-fed rats occurred in both males and females and in rats of two different strains.

The lower BUN levels in the wheat-fed rats was not related to the percentage of nitrogen in the ration nor to the nitrogen intake of the individual animals. The wheat ration by analysis had a slightly higher nitrogen content than the casein ration (2.71% vs. 2.52%). In one pair of female rats, there was no difference in nitrogen intake, while in the other pair, the rat fed the wheat ration consumed about one-third more protein than the casein-fed rat (table 2). In both cases, the wheat-fed rats had lower BUN levels than the casein-fed rats.

The body weight losses experienced by the rats during the early part of the study were partially reversed in some of the animals, toward the end of the period. The slight differences in body weight losses become biologically insignificant when it is realized that these animals ranged in weight from about 200 g for the females to over 600 g for the males.

The reduction in BUN level has been shown by all groups of adult rats fed the wheat ration. In the second trial, which involved 12 animals, there was a consistent difference in BUN levels of the wheat (9.1 mg per 100 ml) and casein (12.0 mg) fed rats (table 3). The BUN levels of both groups of rats during the control period, when they were fed the grain ration, were similar to those of the casein-fed rats (12.8 and 12.0 mg per 100 ml respectively).

Experiment 2. The primary purpose of this study was to evaluate the changes in BUN levels at various intervals following the ingestion of food. The absolute increases in BUN levels immediately following a meal were similar for the wheat- and grain fed rats (9.53 and 10.66 mg per 100 ml respectively— table 4). Since the initial BUN levels of the two groups of rats differed, the relative increases were markedly different (93% and 69% for the wheat and grain groups respectively). After the meal, the in the garin- fed 28% in the BUN levels decreased gradually : wheat - fed - 14% .

Two trials were run in this series with one week intervening between the end of one and the beginning of the other. The results of the two trials were so similar that the values were combined (table 4).

Experiment 3. The digestion coefficients for the rats within each group and for those fed different rations were fairly uniform (table 5). The maximum spread in the values, which was for one of the grain trials, was about 17 per cent; for most of the others, it was closer to 10 per cent.

For all grain trials digestibility ranged from 73 to 80 per cent, whereas the digestibility of the casein and wheat rations

were somewhat greater. The two values for the casein ration agree very well, whereas the first value for the wheat was lower than the second, suggesting that the three days intervening between the first and second trials permitted the rats to adapt to the ration. When that happened, the digestibility of the wheat ration was the same as that of the casein ration. On the basis of this observation, the differences in BUN values of the rats fed the casein and wheat rations cannot be attributed to any difference in nitrogen absorption.

Despite the uniformity of the digestion trials, there was considerable variability in nitrogen retention. The rats fed the casein ration, during the first trial (the first 3 days following the grain ration), retained nitrogen. For some unknown reason, these rats had been in negative nitrogen balance prior to this. During the second trial, the wheat-fed rats were in positive balance while in the first period, two of three rats were in negative balance. A short period of adaptation to that ration probably was essential before the full effects resulting from its feeding could be seen in these adult rats.

The reduction in BUN brought about by feeding the wheat ration was probably due to a reduction in total body load of this compound. It would be almost impossible, under the conditions employed in this study, to detect the increase in urea excretion associated with a reduction in BUN levels. The reason for this is the small amount of urea that has to be excreted to bring this about. Urea is believed to be distributed uniformly in the body water. For the 300 g female rats used in this study, the body water content is approximately 200 ml. A reduction of 4 mg in the BUN levels produced by the wheat ration could be accounted for by 8 mg urea nitrogen. If this were excreted in the 3-day balance period, it would represent a one per cent increase in urinary nitrogen which obviously would be too small to detect.

The urea in the urine represented approximately the same fraction of total nitrogen regardless of the ration fed. The results suggest that the change in BUN level was not associated with any drastic alteration in protein metabolism, at least insofar as this could be detected by its primary degradation product in the urine.

DISCUSSION

The ingestion of a wheat flour ration by adult rats lowers the BUN level as compared to that seen in rats fed in isonitrogenous casein ration. The reduction in BUN brought about by the wheat ration occurred in both male and females of the two strains studied. This reduction occurred regardless of body weight of animals. Other work has shown that weanling rats do not show the same

reduction when fed the wheat ration.

The present study shows that the reduction in BUN cannot be attributed to any difference in nitrogen absorption from the casein or wheat rations. The poorer initial performance of the rats when first fed the wheat ration may be due to the very fine, powdery nature of that ration. Although the flour used in these experiments had a high protein content, approximately twice as high as commercial white patent flour, it was similar to the latter in most other respects. Attempts to overcome the powdery nature of the ration by wetting it only resulted in the formation of a sticky, glutenous mass which could not be managed either by the animals or the investigators. After a day or so, the adult animals started to eat the dry wheat ration. After this initial adjustment period, there were only slight differences in the body weights of the adult rats fed the casein or wheat rations. No relationship existed between body weight changes and BUN levels.

The data in Table 4 indicate that the increase in BUN levels following a meal are proportional to the intake of protein for the grain-fed rats ($r = 0.68$) but no such relationship exists for the wheat-fed animals ($r = 0.16$). These correlatians suggest that a wheat-ration influences BUN levels in a different way than a mixed grain ration. This suggestion is confirmed by the differential changes in BUN levels following the meals of the wheat and grain ration (table 4).

The differences in BUN levels could not be related either to variations in digestibility of the protein or to alterations in urinary urea excretation. By elimination alterations in the kidneys remain as a possible explanation. This would imply that wheat influenced the kidneys so that they excreted more urea for a given BUN level.

A possible explanation for the variation in published reports relating BUN to level of protein intake may come from the observation that the BUN varies with time after a meal, and that if blood samples are taken at certain times, none, or only trivial differences in BUN levels, may be seen despite rather large differences at other times (table 4). Although there were significant differences in BUN levels of the rats fed the grain and wheat rations 18 hours after the removal of feed cups from the animals' cages, these differences were much smaller 4 hours after a meal. Whether similar differences are seen in human subjects remains to be determined.

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TABLE 1

The composition of the casein and wheat diet

	casein diet %	wheat diet %
casein	18.10	—
high protein flour[1]	—	75.00
sucrose	12.75	12.75
dextrose	22.20	—
corn starch	34.70	—
corn oil[2]	6.00	6.00
minerals[2]	4.00	4.00
salt (naCl)	1.00	1.00
vitamins (water soluble)[3]	1.00	1.00
vitamins (fat soluble)[4]	0.25	0.25
% nitrogen (by analysis)	2.52	2.71

1. Pillsbury Co., Minneapolis, donated this special high protein flour which contained 21.5% protein (NX5.75).
2. Mineral mixture composition (%): KCl. 11.59; KI. 0.002; FeSO4, 0.80; CuSO4, 0.11; MnSO4, 0.11; ZnSO4, 0.46; MgCO3, 2.30; NaHCO3, 28.74; CaHPO4, 41.39; CaCO3, 14.37; CaCl2, 0.11.
3. Vitamin (water-soluble) mixture contained (mg/500 g): Thiamin HCl, 150; Riboflavin, 300; Nicotanic Acid, 2000; Ca-pan tothenate, 1500; Pyridoxine, 100; P-aminobenzoic acid, 650; Ascorbic acid, 4000; Inositol, 6,500; Choline chloride, 65,000; Folic acid, 13; Biotin, 2.5; B12, 5; made to 500 g with dextrose.
4. Vitamins (fat-soluble) mixture contained / 125 g: tocopherol acetole 500 mg; vitamin A, 214,300 I.U.; 2-methyl, 1, 4-naphthoquinone, 2 mg; calciferol, 0.6 mg; made to 125 g with sucrose.

Table 2

Effect of wheat flour and casein rations on blood urea levels and body weights.
Blood was secured 4 hours after removing feedcups from the cages.
All rats were fed their respective rations for 8 weeks.

BUN (mg%)				N intake (mg/day)		Initil Body weights (g)		Average body weight change g/week	
Casein		Wheat		Casein	Wheat	Casein	Wheat	Casein	Wheat
M[1]	11.1 ± 0.82[2]	M[1]	8.0 ± 0.40[2]	475	414	558	624	- 3.5	- 7.5
F	12.9 ± 1.30[3]	F	10.1 ± 0.66[3]	368	363	398	406	3.5	2.2
F	12.1 ± 1.13[4]	F	10.5 ± 0.40[4]	238	326	196	196	- 4.5	-- 3.9
Av.	12.0		9.8	360	368	384	408	- 1.5	- 3.1
L.S.D.P. = 0.05	1.4				145				3.1

1 The letters M and F refer to the sexes of the individual rats.

2 The mean and standard error of 8 weekly determinations.

3 The mean and standard error of 7 weekly determinations.

4 The mean and standard error of 6 weekly determinations.

The standard errors were calculated by the method of N. Mantel, Am. Statistician S : 26 (Oct. 1951).

Table 3

Blood urea nitrogen, feed consumption and body weight changes of adult male Osborne-Mendel rats. The 6 animals in each group were fed a grain ration for the first 2 weeks and then fed either the wheat or casein ration for the next 2 weeks. Blood samples were taken each week.

	BUN (mg/100 ml)		Feed intake (g/day)		Body weight (g) [1]	
Group	Control	Experimental [2]	Control	Experimental	Control	Experimental
I	Grain 12.8 ± 1.3 [3] (1)	Wheat 9.1 ± 1.0 [3] (2)	15.8 ± 2.3 [3]	13.9 ± 1.9 [3]	-11.5	-1
II	Grain 13.0 ± 1.7 (3)	Casein 12.0 ± 1.0 (4)	15.1 ± 3.1	18.6 ± 2.9	-7.2	+7

1 Body weight changes represent the differences between the initial and final weights for each 2 week period.

2 During the experimental period, Group I rats were fed the wheat and Group II the casein ration.

3 Means and standard deviations.
Significance of the differences:

(1) vs (2) p 0.001
(1) vs (4) p 0.1
(2) vs (3) p 0.001
(2) vs (4) p 0.001

Table 4

Blood urea levels (gm per 100 ml), feed intake and body weight gains (g per rat) at different intervals after eating a meal of the wheat or grain diets. The result of two trials with the some 12 rats are given. The experiment was repeated for each ration on two separate occasions.

	Grain diet	Wheat diet
BUN after 18 hours of festing	15.37 ± 0.92 *	10.16 ± 0.79
BUN immediately after feeding for 3 hours	26.03 ± 1.43	19.69 ± 1.00
BUN 4 hours after removing feed cups	18.87 ± 1.18	16.93 ± 1.53
Food intake during the 3 hours (g/rat)	5.8 ± 0.72	3.9 ± 0.39
Body weight gain during the 3 hours (g/rat)..	10.6 ± 1.00	0.5 ± 1.00
Body weight gain measured 4 hours after removing feed cups (g/rat)..	6.0 ± 1.14	5.6 ± 0.67

* Averages ± Standard Error of two trials with 12 rats in each trial.

** Body weight gain measured from termination of fast.

Table 5

Digestibility and retention of nitrogen and urea excretion of adult female rats fed the grain ration followed by either the wheat or casein ration for an additional 9 day period.

Ration	group 1 I				group 2 I				group 3 I			
	grain[2]	grain	grain	grain	grain[2]	grain	casein	casein	grian[2]	grain	wheat	wheat
Nitrogen intake [3]	1495	2018	2227	1797	1424	1446	1710	1246	1305	1606	1158	1104
Nitrogen in feces[3]	290	400	448	396	368	390	177	139	309	444	192	118
Nitrogen in urine [3]	945	1119	1312	1261	1171	1154	1164	1005	1346	1140	989	873
Urea-N in urine [3]	798	828	1179	1143	1054	1011	1067	841	1179	1032	869	742
% Urea-N from Urine-N	84.4	74.0	89.9	90.6	90.0	90.0	91.7	83.7	87.6	90.5	87.9	85.0
% N digestible-Mean	79.6	79.9	80.0	79.5	74.2	72.6	89.4	88.8	76.0	72.4	82.6	89.3
-Low	74.9	76.4	77.9	78.1	69.2	66.7	87.5	87.2	72.6	68.8	75.5	88.9
-High	83.2	81.7	81.8	82.2	79.7	79.5	92.4	91.4	79.3	74.8	87.8	90.1
% N retention-Mean	13.7	17.1	21.1	29.3	-9.4	-7.2	21.3	27.9	-8.6	-1.3	3.1	-9.9
-Low	-3.1	-1.6	15.2	5.7	-22.3	-11.9	14.6	3.1	-14.9	-3.5	-9.5	2.3
-High	25.5	28.9	25.8	15.9	3.3	-2.1	26.7	17.5	1.4	7.5	7.5	16.3

1 Averages of 4 rats in group 1 and 5 rats in groups 2 and 3.

2 The first 2 columns in each group represent the two consecutive 3-day balance trials The last two columns represent the balance trials on the first and last 3 days of the 9-day period which immediately followed the two consecutive trials.

3 mg/rat/3 days collection period.

ference in BUN levels can be accounted for by differences in nitrogen digestibility or retention.

EXPERIMENTAL

Experiment 1. Effect of wheat flour and casein on blood urea levels of rats.

First trial: For 8 weeks, three adult rats(one male and one female Osborne-Mendel and one female MSU grey) were fed a wheat ration. Another 3 rats matching these in strain, sex, age and body weight were fed a casein ration (table 1) Feed and water were available to the animals at all times.

Blood samples were secured under light ether anesthesia from all animals each week by heart puncture. The blood samples were taken 4 to 5 hours after the feed cups were removed from the cages. This time interval was chosen since Winsten (9) indicated that for human subjects this was sufficient for the re-establishment of basal blood urea levels. The serum was analyzed for urea by the following procedure: 0.1 ml serum and 0.2 ml urease solution (5 mg crystalline urease in 4 ml water) were incubated at 37°C for 30 minutes after which 0.1 ml 10% sodium tungstate solution, 0.1 ml 2/3 N sulfuric acid and 1.0 ml distilled water were added. After centrifugation, 1.0 ml of the supernatant was added to 0.2 ml Nessler's reagent. The absorption at 470 mu was determined with a Beckman DU spectrophotometer and compared with standard solutions of ammonium sulfate. These were carried through the same procedure. For each sample, a blank was also put through the procedure except that distilled water was used in place of the urease solution.

Second Trial: This was essentially a check on the preceding study. For it, 16 adult male Osborne-Mendel rats were fed a grain ration (10). Feed consumption and body weights were measured weekly. Late in the afternoon of the last day of each week, the feed cups were removed from the cages. The next morning, after a 16 hour fast, blood for BUN determinations was secured by heart puncture. For this, the animals were anesthetized.

At the end of the second week, the 12 rats whose BUN levels were most uniform during the two week period were chosen for the study. They were divided into two equal groups on the basis of body weights, feed intakes and BUN levels. One group was fed the wheat and the other the casein ration (table 1). Body weights and blood samples were secured at the end of each week. The rats were fed the casein or wheat rations for two weeks.

Experiment 2. Changes in the blood urea levels with time after a meal. For a period of 10 days, twelve adult Sprague—Dawley rats were fed a grain ration (10). On the tenth day, feed