iii) Nitrogen
Chairman: Dr H E de Wardener
Investigations on the Rate of Urea Synthesis and on Nitrogen Balance in Patients with Advanced Chronic Renal Failure

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An increase in endogenous protein breakdown is known to occur in patients with acute renal failure (Borst, 1948) and in patients with chronic renal failure, the same finding has been reported by Herndon et al (1958). These investigators concluded that the minimal protein requirements of patients with chronic renal failure are higher than those of normal subjects. This seems to be in contradiction with satisfactory clinical results obtained in patients with chronic renal failure, who received a selective protein diet containing 23 g of protein per day. Nitrogen balance investigations by Berlyne and Hocken (1968), Boström et al (1968) and Ford et al (1969) have given contradictory results.

In order to investigate protein balance in patients with chronic renal failure we measured the rate of urea synthesis (using $^{14}$C-urea) and carried out nitrogen balances.

The following results are reported:

1. The correlation between the rate of urea synthesis and nitrogen balance.

2. The rate of urea synthesis and nitrogen balance on a conventional low protein diet of 40 g of protein, and on a selective protein diet containing 23 g of protein per day (Kluthe & Quirin, 1966) in patients with advanced chronic renal failure.

The calorie intake was identical in both groups, amounting to 35 Cal/g/day.

The difficulties involved in the measurement of nitrogen balance in patients with chronic renal failure are well known. Wolthuis (1961) has reported extensively on this subject. Changes in the urea nitrogen pool are usually derived from rough estimates of the volume of distribution of urea and the total body water. It is conceivable that the conflicting results already cited could be explained by this fact, since even in normal subjects the total
body water may vary between 40 and 65% of the body weight. To avoid this error we have determined the size of the urea pool with $^{14}C$-urea in each patient and have corrected the nitrogen balance accordingly. The rate of urea synthesis was determined by the method of Walser and Bodenlos (1959). Similar investigations have been reported by Robson et al (1964). This procedure is simpler to perform than nitrogen balance studies. However, it seemed to us questionable whether measurement of the rate of urea synthesis could be substituted for the determination of nitrogen balance. Therefore we have applied both methods simultaneously. The measurements of nitrogen balance and urea synthesis were made over a period of seven days following a period of constant nitrogen and calorie intake lasting two or more weeks. We followed the principles of Reifenstein et al (1945) in carrying out the nitrogen balance. In all patients the nitrogen output in the protein-free urine and in nine patients in the faeces as well was estimated by the Kjeldahl method. The nitrogen intake was measured by analysis of an aliquot of the diet, using the same technique. When the faecal nitrogen was not measured we assumed an average of 1.3 g/day. The rate of urea synthesis was determined by injecting 50 $\mu$Ci $^{14}C$-urea intravenously and by measuring the $^{14}C$ activity and the concentration of blood urea nitrogen after 2 hours, and 1 to 7 days thereafter. Urea synthesis was then calculated from the specific activity and the urea pool. The methods used have been described in full previously, especially the determination of methyl-guanidine (Scholz, 1969).

RESULTS

Figure 1 demonstrates the results of the simultaneous determination of nitrogen balance and the rate of urea synthesis. The difference between the urea nitrogen synthesis and the nitrogen intake is plotted along the abscissa while the nitrogen balance is plotted along the ordinate. Even though identity does not exist in each case there was still a close correlation ($r = 0.883; p<0.001$).

![Figure 1. Correlation between nitrogen balance and the rate of urea synthesis](image-url)
In particular, in those cases in which the rate of urea synthesis leads one to suspect the existence of a hypercatabolic state there was almost always a negative nitrogen balance. Figure 2 shows the results of the nitrogen balance in 21 patients with chronic renal failure who received a conventional protein-restricted diet containing 40 g of protein/day. The abscissa shows the nitrogen intake in grams per day and the ordinate the total nitrogen output. Each circle indicates the nitrogen balance in one patient; 13 of these 21 patients with advanced chronic renal failure had a negative nitrogen balance. The question of whether the negative nitrogen balance in patients with chronic renal insufficiency is due to a reduced protein synthesis or to an increased protein breakdown can be answered by examining the rate of urea synthesis. This is demonstrated in Figure 3 for 28 patients with chronic renal failure. Fifteen patients (characterised by the symbols above the broken line) had a urea nitrogen production greater than could be accounted for by the protein content of the food. The results indicate that in these patients there must have been an increased endogenous protein breakdown. This finding was less frequent in patients on a selective low-protein diet than in patients who received the conventional 40 g of protein.

**DISCUSSION**

The results of the nitrogen balances, as well as the measurements of urea synthesis have shown that frequently there is increased protein catabolism in
patients with advanced chronic renal insufficiency who receive a protein-poor diet of 40 g of protein/day even if the caloric intake is adequate.

There is no indication that this is due to an insufficient protein intake since the patients on a 23 g selective protein diet showed negative protein balances less frequently.

This rules out the possibility of the existence of an increased-protein requirement in chronic renal insufficiency. In addition, previous studies of long term observations of body weight, $^{40}$K determinations in the whole body counter and measurements of the total body water in patients with advanced

Figure 4. Correlation between blood urea nitrogen and nitrogen balance
chronic renal failure have shown that a previously catabolic state may change into an anabolic state with an increase in lean body weight after the institution of regular dialysis treatment (Scholz, 1969). This indicates that the protein catabolism in patients with chronic renal failure is, in all probability, caused by accumulation in the blood of dialysable substances. We have, therefore, correlated nitrogen balance and the concentration of several dialysable substances in the human plasma: there was a positive correlation between nitrogen balance and the blood urea nitrogen \( (r = 0.4; p < 0.01, \text{ Figure 4}) \). On the contrary we could not find a significant correlation between nitrogen balance and the concentration of the plasma standard bicarbonate. The correlation between the nitrogen balance and the blood urea nitrogen levels implies that there is a relationship to protein hypercatabolism.

![Figure 5. Correlation between blood urea nitrogen and methylguanidine concentrations](image)

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![Figure 6. Effect of methylguanidine administration on nitrogen balance in three rabbits. The data are plotted according to the method of Reifenstein et al (1945)](image)

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Furthermore, an increase of urea might be an indication of rising levels of additional protein metabolites which could have even greater significance in this context. In other words they might induce protein breakdown on reaching some critical concentration. We found, for instance, a significant correlation between the blood urea nitrogen and the concentration of methyl-guanidine in the blood \( r = 0.6, \) Figure 5. Methyl-guanidine is known to induce protein catabolism as shown by the findings of Renner and Heintz (1969) in vitro and Giovanetti et al (1968) in vivo. Our group have demonstrated that methyl-guanidine given to rabbits in a dose of \( 5 \times 20 \, \text{g/kg} \) subcutaneously results in a negative nitrogen balance (Figure 6).

CONCLUSIONS

We have found that most of our patients with advanced chronic renal insufficiency developed negative nitrogen balance, and increased endogenous breakdown of protein, when given a conventional protein restricted diet containing 40 g of protein per day. However, this should not lead to the conclusion that the minimal protein requirement of patients with chronic renal failure is greater than normal. Instead if should be concluded that the negative N-balance is due to the retention of protein metabolites other than urea, and among these methyl-guanidine has been shown to cause protein breakdown. It is possible and even highly probable that additional metabolites, as yet unknown, have the same effect.

REFERENCES

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