The Effect of Dialysis and Administration of Essential Amino Acids on Plasma and Muscle Protein Synthesis, Studied with ^{15}N in Uraemic Patients

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It is well known that chronic uraemic patients tend to have a negative nitrogen balance (Herndon et al, 1958). However, it is not clear whether the negative balance is due to a decreased protein synthesis or to an enhanced catabolism of protein.

Presumably, uraemic toxins accumulating in the body fluids could act as inhibitors of the synthesis of individual amino acids or the synthesis of protein from pre-formed amino acids. Thus, there is evidence that the synthesis of L-histidine from non-essential nitrogenous sources is reduced or abolished in uraemia (Bergström et al, 1970; Fürst et al, 1970). An inhibition of transcellular transport of amino acids and other organic acids is another possible factor (Ciccone et al, 1968).

Patients on intermittent dialysis may regain weight and muscular strength. This may be due to the removal of dialysable toxins. Another possibility is that those endocrine mechanisms are stimulated which favour protein synthesis.

In the study reported here we administered ^{15}N-urea intravenously to uraemic patients and determined the labelling of plasma – as well as of muscle – protein and of the protein-free filtrates several times during the course of treatment. The patients were treated with dialysis and with intravenously infused or orally administered essential amino acids.

MATERIAL AND METHODS

Seven patients with chronic uraemia were studied (Table I). ^{15}N-urea (98 excess atom per cent - ONIA, Toulouse, France) was administered to three patients with terminal renal failure who were being treated with intermittent peritoneal dialysis for 24 hours once a week. The isotope solution was infused intravenously during the 4 hours after the completion of a dialysis. One of these patients was also studied in connection with bilateral nephrectomy and a subsequent haemodialysis (Travenol Ultraflo 145, 8 hours).

Six of the uraemic patients were given a standard nitrogen-poor diet (2.7 g of nitrogen per day, Fürst et al, 1969), providing approximately the

Table I. Clinical data, mode of amino acid administration and the early ^{15}N incorporation into muscle and plasma protein

Case	Sex	Age	Body weight kg	Diagnosis	** Amino acids administered g N/day			Route of administration	Day of first muscle biopsy	Per cent of ^{15}N received		$\frac{A}{B}$	^{15}N mg administered
					EAA	Arginine	Histidine			Ⓐ per kg muscle protein	Ⓑ per kg plasma protein		
I-BE	F	50	56	CG	2.2	-	-	i.v.	2	6.2	4.5	1.38	428
NS	M	50	76	CG	2.2	-	-	p.o.	3	2.5	6.7	0.37	615
SW	F	56	67	CP	2.2	-	-	p.o.	3	2.3	7.6	0.30	486
ÅB	M	47	68	CG	2.2	1.15	0.45	i.v.	3	7.5	3.3	2.27	489
GK	F	47	48	CG	-	-	-	-	3	4.0	3.8	1.05	488
RA*	M	44	74	CG	2.2	-	-	i.v.	3	6.6	3.67	1.80	510
DA	F	67	56	CP	2.2	1.15	0.45	i.v.	9	3.36	1.8	1.87	439

* Nitrogen-free diet

** Placed at our disposal by AB Astra, Södertälje, Sweden

CG = Chronic glomerulonephritis

CP = Chronic pyelonephritis

EAA = Essential amino acids

minimum requirement of essential amino acids (Vinnars et al, 1970) according to Rose (1949) and 2400 kcal per day. One patient (RA) was on a nitrogen-free diet consisting of a syrup (Hycal®, Beecham, Bradford, England).

Five of the uraemic patients were treated daily, or every second day, during the study with the essential amino acids, which were either administered by mouth or by a slow intravenous infusion (Josephson et al, 1969). In two of these patients (ÅB, DA) histidine and arginine were also given with the essential amino acids (Josephson et al, 1970). One patient (GC) received only the standard diet, the amino acid treatment being postponed until after the study.

In all the subjects ^{15}N was repeatedly determined in the urine (for dialysis patients in the dialysis fluid as well) and plasma protein and plasma protein-free filtrate. ^{15}N was also determined several times in muscle protein and muscle filtrate. Muscle tissue was obtained from the quadriceps femoris muscle by needle biopsy (Bergström, 1962). In the dialysis patients biopsy was carried out before and after dialysis.

The distribution of non protein nitrogen (NPN) and ^{15}N between extra- and intracellular water in the muscle biopsy material was calculated by the chloride method (Graham et al, 1967) assuming a normal membrane potential of 87 mV.

The technique of preparation of plasma and muscle protein and of protein-free filtrates has been described in a separate paper (Fürst & Jonsson, 1970). The mass spectrometric ^{15}N determinations were carried out in the Laboratory for Mass Spectrometry at Karolinska Institutet. We are grateful to assistant professor R Ryhage for allowing us to use the Atlas CH4 mass spectrometer.

RESULTS

I. Incorporation of ^{15}N into plasma and muscle protein (Table I)

Two to three days (in one case 9 days) after the administration of ^{15}N-urea we found incorporation of ^{15}N into plasma and muscle protein. Oral administration of amino acids resulted in a preferential incorporation of the isotope into plasma protein, whereas intravenous administration favoured the incorporation of this isotope into muscle protein.

We think that this is due to the difference in initial distribution of the amino acids supplied. When given by mouth, the amino acids will be distributed to the liver by the portal circulation. Intravenously administered amino acids, on the other hand, will be directly distributed to all tissues, including the muscle compartment (Fürst et al, 1970).

II. The ^{15}N labelling of plasma and muscle as influenced by dialysis treatment

Patient NS (Figure 1) received essential amino acids orally during the study.

The level of total NPN was about 10 times higher in intracellular water than in plasma. During the days before dialysis there was a decline of ^{15}N-excess in plasma and muscle protein. A decrease in ^{15}N excess was also found in plasma NPN and in intracellular NPN in muscle tissue. When the patient was dialysed, an increase of ^{15}N-incorporation into plasma and muscle protein was observed. A more rapid disappearance of ^{15}N excess in extra- and intracellular NPN was also found. A reduction of both extra- and intracellular NPN and of plasma urea-N was achieved by dialysis. The pattern of ^{15}N incorporation observed in the first dialysis was repeated in the second dialysis. Over the whole study the ^{15}N excess in plasma NPN decreased more rapidly than the ^{15}N excess in intracellular water, indicating a redistribution of ^{15}N.

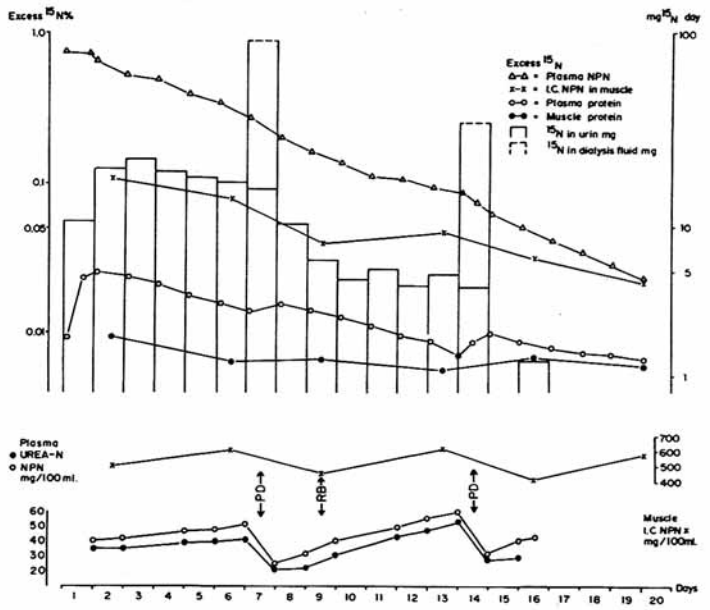

Figure 1. Heavy nitrogen labelling of muscle and plasma protein and body fluids, also concentrations of urea and total NPN in plasma and muscle cell (IC NPN) are shown for regular dialysis patient NS (Table I).
PD = peritoneal dialysis. RB = open renal biopsy

Patient SW (Figure 2) was also given essential amino acids orally and was studied over two consecutive peritoneal dialyses. The pattern of ^{15}N incorporation was essentially similar to that in case NS, although the ^{15}N incorporation in muscle protein brought about by dialysis was less pronounced.

Patient IBE (Figure 3) received daily intravenous infusions of essential

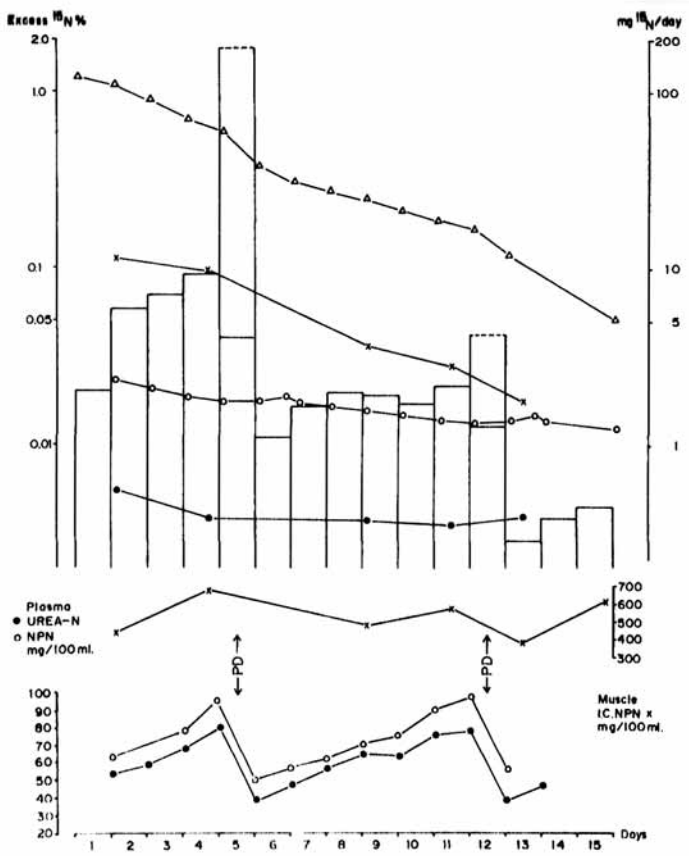

Figure 2. Heavy nitrogen labelling of muscle and plasma protein and body fluids, also concentrations of urea and total NPN in plasma and muscle cell (IC NPN) are shown for regular dialysis patient SW (Table I) PD = peritoneal dialysis. RB = open renal biopsy

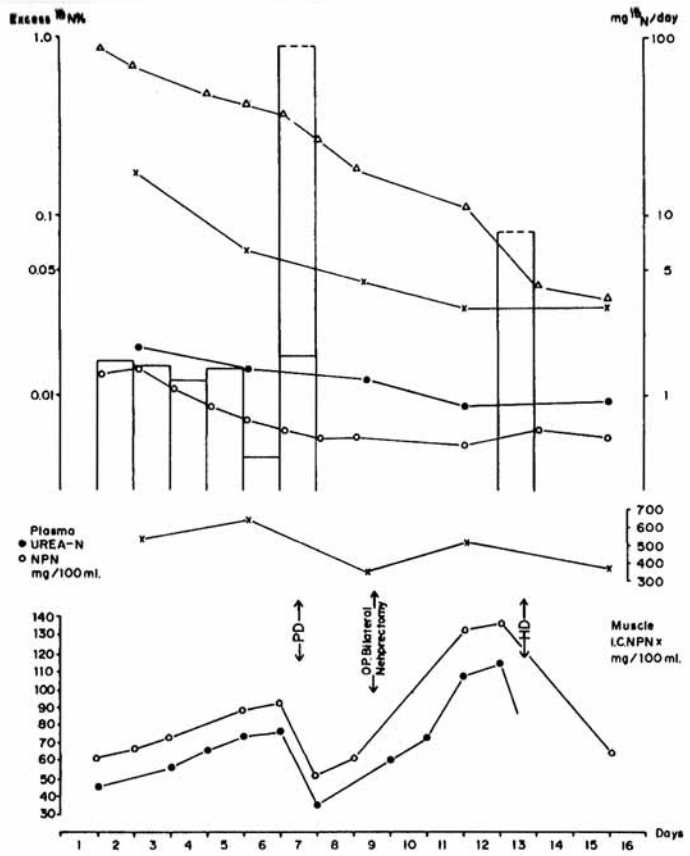

Figure 3. Heavy nitrogen labelling of muscle and plasma protein and body fluids, also concentrations of urea and total NPN in plasma and muscle cell (IC NPN) are shown for patient IBE in whom haemodialysis (HD) was performed 4 days after bilateral nephrectomy. PD = peritoneal dialysis. RB = open renal biopsy

amino acids. The ^{15}N excess in plasma protein was increased by peritoneal dialysis and the decrease of plasma NPN was accelerated. However, no clearcut effect was observed on the ^{15}N excess in muscle protein and in intracellular NPN. Two days after the dialysis bilateral nephrectomy was performed because of rapidly progressing glomerulonephritis with severe arterial hypertension. After the operation the disappearance of ^{15}N excess in plasma and muscle protein was more rapid. This trend was reversed when the patient was haemodialysed for 8 hours. The incorporation of ^{15}N increased in plasma and muscle protein. There was a pronounced fall in ^{15}N excess in plasma NPN, whereas ^{15}N excess in muscle NPN was unchanged.

COMMENT

The present results indicate that dialysis influences nitrogen metabolism, not only by withdrawing NPN products from the body fluids, but also by bringing about an increased incorporation of ^{15}N from labelled nitrogenous precursors into plasma and muscle protein. This occurs despite the fact that the level of total NPN in the body fluids decreases due to the dialysis.

A decrease or an increase of ^{15}N labelling in a heterogenous structure such as the proteins of plasma and muscle is difficult to interpret in terms of protein catabolism or anabolism. Any interpretation would require a more detailed knowledge of the ^{15}N-containing protein precursors as well as of the protein into which these are preferentially incorporated, and of its degradation products.

Lacking this information, we conclude that the increased ^{15}N labelling of protein after dialysis demonstrates an enhancement of the protein synthesis in the uraemic patient, even if other explanations cannot be excluded. The changes in ^{15}N labelling in plasma and muscle tissue brought about by dialysis are similar to the changes produced when a uraemic patient on a protein-poor diet receives essential amino acids intravenously, turning the previously negative nitrogen balance positive (Fürst et al, 1970). This further indicates that these changes in ^{15}N labelling are signs of an increased protein synthesis.

The underlying mechanisms for the increase in protein synthesis remain obscure.

The removal of uraemic toxins which inhibit transport mechanisms or synthetic enzymes is one possible explanation (Bergström & Bittar, 1969).

The effect of insulin, stimulated by the glucose taken up from the dialysate, may be another factor which could enhance the synthesis of protein (Manchester, 1961).

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