protein. If we pursue this suggestion, the process of virus adaptation to a host which involves an increase of virus virulence and a decrease in its ability to excite the production of interferon might in some way involve some subtle change in the viral nucleic acid by means of which it came to seem less foreign to its new host. This may be the way in which a virus acquires a key to the door of the cell's nucleic-acid-synthesizing chamber. It is our job to learn the combination and to find how to prevent it from being forced.


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# UREA DISTRIBUTION IN THE BODY AFTER HAEMODIALYSIS


BY

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With increasing use of the artificial kidney as a definitive method of treatment in some cases of renal failure, opportunities have arisen to study the effects of haemodialysis on urea distribution in the body. Although it has been generally accepted that urea is freely and rapidly diffusible in the body fluids of normal subjects (McCance and Widdowson, 1951), the validity of this concept in uraemic patients undergoing haemodialysis on an artificial kidney has recently been questioned, and evidence has been adduced to support a contention that a relatively non-freely diffusible intracellular urea exists in such circumstances (Blackmore and Elder, 1961).

In individual uncomplicated cases of acute oliguric renal failure the pre-dialysis daily increment of plasma urea concentration is virtually constant: indeed, practical use is made of this phenomenon to anticipate and plan the day for haemodialysis (Loughridge *et al.*, 1960). After haemodialysis the daily increment of plasma urea concentration is virtually identical except for the first day, when there is almost invariably an accelerated rate of rise (Fig. 1). There seems little reason to believe that this latter phenomenon reflects a temporary increase of metabolic rate in response to rapid removal of urea by haemodialysis, or that it results from an accelerated production of urea due to the trauma of haemodialysis. An alternative explanation—

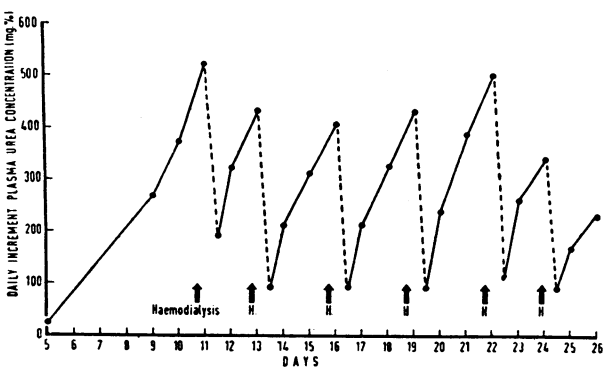


FIG. 1.—Daily increment of plasma urea concentration in a case of acute renal failure treated by haemodialysis on six occasions, showing the accelerated rate of rise during the first day after each haemodialysis.

namely, that the extracellular urea concentration, as measured by the plasma urea, does not reflect, and indeed is less than, the intracellular urea concentration in the immediate post-dialysis period, and that equilibration subsequently takes place in the following day—seems more reasonable. To test the validity of this hypothesis it was decided (1) to determine the intracellular concentration of urea in a series of muscle biopsies taken immediately after haemodialysis and to compare the values with the corresponding plasma values of venous blood samples withdrawn at the time of the biopsies, and (2) to observe post-dialysis hourly rates of rise of the plasma urea concentration and to determine the average time taken for equilibration with the pre-dialysis rates of rise.

## Material and Methods

### Muscle Biopsies

Intracellular urea concentrations (expressed as mg./100 ml. of muscle water) were determined in muscle biopsies obtained from the medial part of the gastrocnemius muscle in 19 unselected patients with acute renal failure immediately after haemodialysis on a rotating coil artificial kidney. Local anaesthesia (2% lignocaine) was used to infiltrate the overlying skin. Care was taken to avoid fat and connective tissue, and 2–4-g. muscle samples were obtained with dry instruments and were placed immediately into dry glass containers. Venous blood samples for urea, electrolyte, and haematocrit determinations were withdrawn at the time the biopsies were taken and all specimens were transferred immediately to the laboratory. There the muscle samples were blotted to remove surface blood, trimmed of any visible fat and connective tissue, and then divided into two approximately equal portions.

The paired portions were transferred to two dry tared weighing-bottles and accurately weighed (wet weight). One of the paired portions was shredded with fine dry scissors and then crushed with a glass rod. Then 1.5–2 ml. of distilled water was added to the crushed specimen in the weighing-bottle, washing off the glass rod, and the whole was accurately weighed and then put into a refrigerator at 4° C. for 24 hours to allow for equilibration. At the end of this time 0.2 ml. of the

fluid in the weighing-bottle was pipetted out and its urea concentration determined by a modification of the method of Skeggs (1957), using phosphoric–nitric acid and potassium persulphate. The remaining fluid was evaporated off by subjecting the weighing-bottle and its contents to a temperature of 100–110° C. for 48 hours and the dry weight determined by weighing. The difference between the previously observed wet weight and the subsequent dry weight was taken to represent the weight of the intracellular and extracellular muscle water and is expressed as ml./kg. of wet muscle.

The extracellular component was determined from the chloride space in the second of the paired portions after fat extraction had been effected (Hastings and Eichelberger, 1937; Talso *et al.*, 1953). The specimen was first dried at 100–110° C. for 48 hours, and, after reweighing, approximately 2.5 ml. of ethyl ether was added to the dried muscle in its weighing-bottle. The muscle was ground with a glass rod and a further 2.5 ml. of ethyl ether was added. After 10 minutes the ether was removed by centrifugation, aspiration, and evaporation. A further single extraction with ethyl ether and then two more with petroleum ether were completed before reweighing. The weight loss was taken to represent the weight of the neutral fat in the muscle biopsy. After fat extraction, approximately 2 ml. of distilled water, accurately weighed, was added to the dry fat-free specimen in its weighing-bottle. The bottle was capped and agitated before it was left to stand at room temperature for two hours, during which time it was agitated repeatedly. The chloride content of the fluid was then determined by a modification of the method of Van Slyke and Hiller (1947): the nomogram for temperature correction (Van Slyke, 1947) was applied. After correcting for the chloride content of red blood cells contained in the biopsy (Barnes *et al.*, 1957) and applying the Donnan factor (0.96), the chloride space was calculated from the tissue and plasma chloride (Flear *et al.*, 1960) and expressed as ml./kg. of fat-free wet muscle.

Plasma urea concentrations were determined by the modified method of Skeggs (1957), and plasma chloride by the method of Van Slyke and Hiller (1947); the intracellular urea concentration was deduced from the equation:

$$Iu = \frac{(Tu \times Tw) - (Eu \times Ev)}{(Tw - Ev)}$$

Where Iu = intracellular urea concentration (mg./100 ml.), Tu = total urea (mg./100 ml. muscle water), Tw = total water (ml./kg.), Eu = extracellular (plasma) urea concentration (mg./100 ml.), and Ev = extracellular volume (ml./kg.).

#### Post-dialysis Hourly Increments of Plasma Urea

In 14 unselected patients with acute tubular necrosis post-dialysis venous blood samples were withdrawn repeatedly at intervals of one to two hours so that post-dialysis plasma urea concentrations (Fig. 2) could be determined in each sample by an automatic analyser using the modified method of Skeggs (1957). The rate of rise of plasma urea in each case was plotted against time, and the regression curve so obtained could be seen gradually to approximate the average pre-dialysis rate of rise (determined by dividing each average daily rise by 24), so making it possible to deduce the time required for equilibration in each case.

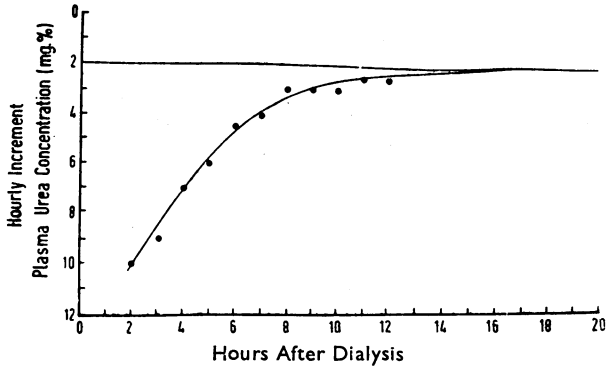


FIG. 2.—Post-dialysis hourly increment of plasma urea concentration. The horizontal line represents the pre-dialysis hourly increment (2 mg./100 ml.) obtained by dividing the average daily rise by 24. The point of intersection of the horizontal line by the regression curve of the post-dialysis hourly increment is taken as the equilibration time, which is 18 hours in this case.

## Results

*Muscle Biopsies* (Table I).—In the muscle biopsies taken immediately after haemodialysis in 19 unselected patients the average total water content was 78% of wet muscle (range 754 to 825 ml./kg.), and the average extracellular (chloride) component was 35% of fat-free wet muscle (range 214 to 476 ml./kg.). In 14 cases the post-dialysis intracellular urea concentration was calculated to be greater than the observed extracellular (plasma) urea concentration: it was less in 5. For the 19 cases the average difference between the intracellular and extracellular urea concentrations was 26.1 mg./100 ml., which is statistically significant at the 5% level (t = 2.41 with 18 degrees of freedom).

TABLE I.—*Post-dialysis Muscle Biopsies in 19 Patients*

| Case No. | Total Water (ml./kg.) | Chloride Space (ml. kg.) | Intracellular Urea (mg./100 ml.) | Extracellular Urea (mg./100 ml.) |
|---|---|---|---|---|
| 1 | 786 | 380 | 94 | 43 |
| 2 | 772 | 272 | 148 | 108 |
| 3 | 778 | 220 | 126 | 84 |
| 4 | 775 | 263 | 298 | 275 |
| 5 | 764 | 267 | 140 | 80 |
| 6 | 814 | 392 | 189 | 145 |
| 7 | 766 | 391 | 152 | 140 |
| 8 | 777 | 341 | 218 | 150 |
| 9 | 754 | 311 | 235 | 155 |
| 10 | 825 | 417 | 269 | 126 |
| 11 | 800 | 407 | 134 | 92 |
| 12 | 782 | 417 | 106 | 83 |
| 13 | 766 | 396 | 98 | 94 |
| 14 | 789 | 406 | 126 | 116 |
| 15 | 784 | 320 | 63 | 65 |
| 16 | 807 | 421 | 85 | 87 |
| 17 | 806 | 389 | 124 | 168 |
| 18 | 777 | 476 | 83 | 134 |
| 19 | 767 | 214 | 92 | 140 |
| Average | 784 | 353 | | |

The average total water content is 78% of wet muscle and the average extracellular (chloride space) component is 35% of fat-free wet muscle. The intracellular urea concentration exceeds the extracellular urea concentration in Cases 1–14.

*Post-dialysis Hourly Increments of Plasma Urea* (Table II, Fig. 3).—Compared with the pre-dialysis hourly increments of plasma urea concentration, which ranged from 1.2 to 3.5 mg./100 ml. (average 2.2) in 14 patients, the post-dialysis increments were significantly increased for varying periods of time: two hours after completion of haemodialysis the increments ranged from 5 to 18 mg./100 ml. (average 9.8), but these values gradually decreased until they approximated the pre-dialysis values at times ranging from 7 to 20 hours (average 15).

## Discussion

Since the molecular size of urea is only 3.0 Ångstroms, it would be reasonable to believe that it passes freely across cell membranes and becomes rapidly diffused throughout all the body fluids: indeed, this concept was imputed when it was suggested by McCance and Widdowson (1951) that orally ingested urea might be used to measure total body water in normal subjects. Yet it had been suggested previously that urea is not equally distributed between red-cell and plasma water (Ralls, 1943), and McCance and Widdowson (1951) themselves stated that the cerebrospinal fluid, some fluid in the kidneys, and the renal veins might be exceptions. More recently, urea concentrations in red-cell water and plasma water in human blood samples have been compared *in vitro* by Murdaugh and Doyle (1961): they found the concentration of urea in red-blood-cell water to be consistently higher than in plasma water (the mean ratio was 1.19, range 1.07 to 1.38), and they concluded that the difference was due to binding of the urea by the haemoglobin in the red blood cells. The retrospective deductions of Blackmore and Elder (1961), based on urea clearances in uraemic patients during haemodialysis on a twin-coil artificial kidney (Aoyama and Kolff, 1957), also raise doubt about the free diffusibility of urea in such circumstances: their observations, in fact, led them to suggest that urea might exist as diffusible and non-diffusible moieties, that there is a greater ratio of the non-diffusible form in the cells of such patients, and that it is difficult to accept urea as a freely diffusible and rapidly equilibrating substance. Our studies tend to support such a concept.

TABLE II.—*Pre- and Post-dialysis Hourly Increments of Plasma Urea and Equilibration Times in* 14 *Unselected Cases*

| Case No. | Pre-dialysis Hourly Rise of Plasma Urea (mg./100 ml.) | 2-Hour Post-dialysis Hourly Rise of Plasma Urea (mg./100 ml.) | Equilibration Time (Hours) |
|---|---|---|---|
| 1 | 2·5 | 12 | 15 |
| 2 | 2·2 | 10 | 7 |
| 3 | 2·2 | 10 | 20 |
| 4 | 2·0 | 5·5 | 16 |
| 5 | 1·25 | 13 | 11 |
| 6 | 1·5 | 8 | 16 |
| 7 | 1·5 | 18 | 13 |
| 8 | 2·0 | 12 | 14 |
| 9 | 2·25 | 5 | 14 |
| 10 | 3·5 | 6 | 19 |
| 11 | 2·5 | 9 | 11 |
| 12 | 1·2 | 10 | 20 |
| 13 | 3·5 | 9·5 | 16 |
| 14 | 1·5 | 9 | 16 |
| Average | 2·2 | 9·8 | 15 |

The pre-dialysis increments (mg./100 ml.) represent the average daily values divided by 24. Two hours after dialysis the average hourly increment is more than four times the average pre-dialysis value. The average equilibration time is 15 hours.

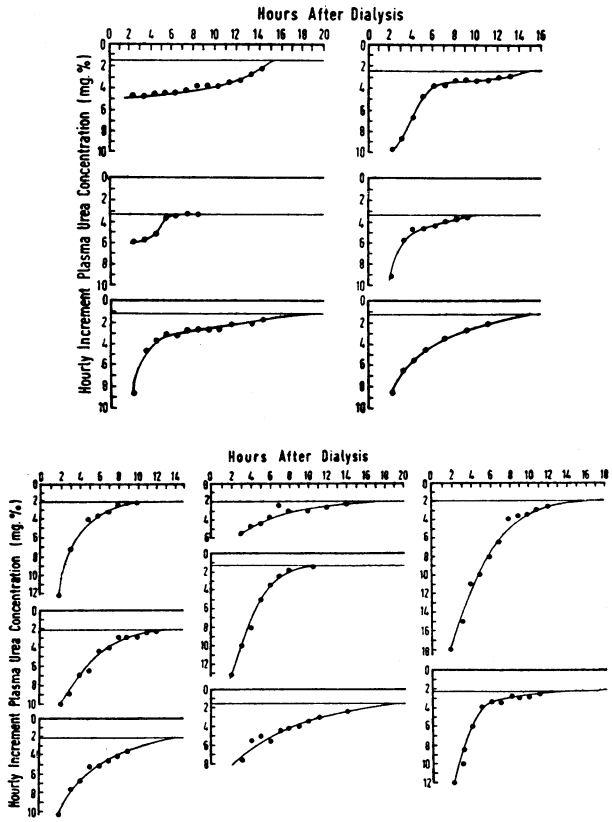


FIG. 3.—Post-dialysis hourly increments of plasma urea in 14 unselected cases. The horizontal lines represent the average pre-dialysis hourly increments of plasma urea (obtained by dividing the average daily rise by 24). The point of intersection of the horizontal line by the regression curve is taken as the equilibration time in hours.

In the muscle biopsies taken on completion of haemodialysis the intracellular urea concentration was found to exceed the extracellular urea concentration in 14 out of 19 instances. Since such observations depended on concomitant determinations of the total and the extracellular water in the muscle samples, it is pertinent to point out that, although the average value (78%) for the total water content of wet muscle in the present studies compares closely with values found in non-uraemic subjects by other authors (Flear *et al.*, 1960), the average value (35%) for the extracellular (chloride) space was significantly greater. While post-dialysis expansion of the extracellular space could result from transference of water from the artificial kidney into patients during haemodialysis, this is unlikely to be the whole explanation in view of the fact that the pre-dialysis average value for the extracellular space, determined in the same patients from muscle biopsies taken from contralateral legs immediately before haemodialysis, was found to be 31.5%: this would suggest, therefore, that an expanded extracellular space is present in some patients with acute oliguric renal failure even before haemodialysis, although it is possible that excessive intracellular chloride, due to abnormal cell permeability of disease states (Barnes *et al.*, 1957), could explain the observation.

The temporary accelerated rate of rise of plasma urea concentration following haemodialysis in uncomplicated cases of acute oliguric renal failure could be rationally explained on the basis of the present observations that the concentration of the intracellular urea exceeds the extracellular concentration at the end of haemodialysis and that there is then a subsequent and gradual equilibration of the relatively greater intracellular urea concentration with the relatively lower extracellular urea concentration. The average time taken for the post-dialysis hourly urea increments to equilibrate with the respective pre-dialysis values was found to be 15 hours, and this accords with the recognized clinical observation that the accelerated rate of rise of plasma urea concentration in cases of uncomplicated acute oliguric renal failure after haemodialysis is only temporary and occurs during the first post-dialysis day.

When haemodialysis effectively removes urea from the body and the clearance from the extracellular compartment exceeds that from the intracellular compartment, the process could result in an osmotic gradient tending

to produce intracellular oedema: this should be minimal when a twin-coil ultrafiltration artificial kidney (Aoyama and Kolff, 1957) is used, because this machine can effectively remove water at a rate of 800 ml./hour. Animal experiments (Alexander *et al.*, 1961) have shown that intravenous infusions of urea, similar to those used to decrease brain bulk at craniotomy (Stubbs and Pennybacker, 1960), produce a significant transitory increase of plasma volume which is believed to reflect withdrawal of fluid from body tissues as a result of a generalized blood-tissue osmotic gradient. To postulate that reversal of such a process in uraemic patients treated by haemodialysis, especially when a high rate of urea clearance is achieved and the plasma protein concentration is low, could produce a reversed osmotic gradient should therefore cause little surprise. Indeed, the development of cerebral oedema, which has been observed on occasion during haemodialysis, together with the gradual restitution of the neurological state which begins shortly after haemodialysis is stopped, can be reasonably attributed to urea-concentration differences in the intracellular and extracellular compartments and their subsequent spontaneous equilibration. The cause of the delayed urea diffusion remains obscure: it is possible that a specific non-diffusible urea moiety exists in such circumstances (Blackmore and Elder, 1961), but expansion of the extracellular compartment physically affecting urea transfer might also be significant.


### Summary

Intracellular concentrations of urea were determined in muscle biopsies taken from 19 unselected patients with acute renal failure immediately after haemodialysis on a rotating-coil artificial kidney.

The values were compared with corresponding plasma urea values of venous blood samples withdrawn at the time of the biopsies and were found to be significantly greater in 14 instances and less in 5.

Post-dialysis hourly rates of rise of plasma urea concentration were determined in 14 unselected patients and the average time taken for equilibration with the respective pre-dialysis hourly rates of rise was found to be 15 hours.

The observations suggest that haemodialysis on a rotating-coil artificial kidney may sometimes, as a result of delayed diffusion of urea across cell membranes, be followed by an intracellular/extracellular urea concentration ratio greater than unity.

A raised intracellular/extracellular urea concentration ratio, by producing an osmotic gradient, could rationally explain the development of cerebral oedema during haemodialysis, its gradual spontaneous regression when haemodialysis is stopped, and the well-recognized temporary accelerated rate of rise of the plasma urea concentration during the first post-dialysis day.


We are indebted to Dr. Ruth Haslam and Dr. I. D. P. Wootton, of the Department of Biochemistry at the Postgraduate Medical School, for their technical assistance and critical interest.

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# ENDOCRINE CONTROL OF SKELETAL DEVELOPMENT IN MAN*


BY

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Exhaustive studies at the Institute of Experimental Biology, University of California, have shown that skeletal development in the rat entails two distinct processes each of which is under separate endocrine control. The first process, usually termed "bone growth," consists in the progressive increase in the dimensions of a bone. The dimension usually measured is length because of its simplicity. The second process is called "bone maturation," and this consists in the gradual acquisition of the adult shape of the particular bone. Maturation is assessed by radiological methods in clinical medicine. Apart from this experimental support there is also clinical evidence in favour of dissociated control of bone growth.

Evidence is produced here to show that in man at least three separate factors operate in skeletal development. Bone maturation as defined above is divisible into two separate elements, and these, together with the other element of bone growth, are each under different endocrine control.

*Evidence from Experimental Animals.*—The University of California workers (Becks *et al.*, 1946; Simpson *et al.*, 1950) demonstrated by their carefully controlled experiments on the rat that growth hormone chiefly increases the size of the skeleton and that thyroxine is mainly concerned with maturation, although it potentiates the action of growth hormone when used in combination. Alone, thyroxine has no effect on bone growth. Other hormones were found to have relatively little effect, although it was noted that oestrogens inhibit growth by antagonizing growth hormone, while androgens stimulate growth in the presence of the pituitary.

*Clinical Evidence.*—Pituitary disorders in the growing period of life are instructive. In gigantism the overacting hypophysis causes a marked acceleration of skeletal growth which does not stop at the usual time of puberty, since the epiphyses remain open. In infantilism the epiphyses again remain open but there is an almost complete absence of growth in length. It follows that the agent promoting growth does not also control epiphysial closure. The well-known acceleration

*Based on a paper read to the combined Sections of Orthopaedics and Radiology at the Annual Meeting of the British Medical Association, Auckland, New Zealand, 1961.