findings of Shima and Sugahara[7] on Balb/c mice during aging. However in our Balb/c-nu strain, where the recessive nude mutation has been introduced, ploidity classes higher than 4C have not been found. These findings, together with the increase of the mean nuclear volume of hepatocytes during aging, strongly support the hypothesis according to which a strict correlation should exist between the nuclear volume and the degree of polyploidization[3–6]. On the other hand, thymus-grafted old mice display a statistically significant decrease in the percentage of tetraploid nuclei in comparison with the untreated controls (table), although the corrective action of the thymus on the polyploidity level is only partial.

The complex physiological role of the thymus is far from clear. Nevertheless, a hypothesis may be suggested regarding the mechanism that is responsible for the regulatory action of the thymus on the ploidity level of hepatocytes. The thymus is able to modulate either the blood levels of certain hormones, or the adaptive responsiveness of the cells to beta-adrenergic stimulations during aging[9,10]. Since polyploidization is controlled by the endocrine system, as is generally accepted[11,12], it may well be that the thymus acts through the modulation of hormonal levels. Nevertheless, a direct action of the thymus on the hepatocytes cannot be excluded either. The findings revealing a regulation of the physicochemical state of liver cell chromatin by some thymic factors[13–16] are in good agreement with such an assumption.

Our previous experiments showed that athymic young nude mice possessed an increased mean volume of hepatocyte nuclei as compared to their normal littermates, and this alteration was prevented by grafting neonatal thymus into the nudes[17]. Therefore, it seems to be acceptable that thymic regulation may play a role during both the development and aging[17–19]. It should be emphasized, however, that the thymus is able to modify the ploidy level of hepatocytes even in old animals where the general hormonal and metabolic conditions may be considered to be rather unfavorable. This agrees with the general concept regarding the primary role of the thymus in the aging process[18,19].

1 H. Plenck, Arb. zool. Inst. Univ. Wien *19*, 247 (1911).
2 C. Pieri, C. Giuli, M. Del Moro and L. Piantanelli, Mech. Age Dev. *13*, 275 (1980).
3 H. Tauchi, Nagoya J. med. Sci. *24*, 97 (1961).
4 A.O. Pogo, B.G.T. Pogo and J.R.C. Funes, Exp. Cell Res. *20*, 208 (1960).
5 R.C. Guimares, Braz. Resquisas Med. Biol. *4*, 169 (1971).
6 L. Ranek, Acta cytol. *20*, 151 (1976).
7 A. Shima and T. Sugahara, Exp. Geront. *11*, 193 (1976).
8 S. Fujita, J. comp. Neurol. *155*, 195 (1974).
9 L. Piantanelli, A. Basso, M. Muzzioli and N. Fabris, Mech. Age Dev. *7*, 171 (1978).
10 L. Piantanelli, P. Fattoretti and C. Viticchi, Mech. Age Dev. *14*, 155 (1980).
11 F.J. Swartz, Exp. Cell Res. *48*, 557 (1967).
12 C. Nadal and F. Zajdela, Exp. Cell Res. *42*, 117 (1966).
13 D. Amici, G.L. Gianfranceschi, G. Marsili and L. Michetti, Experientia *30*, 633 (1974).
14 G.L. Gianfranceschi, D. Amici and L. Guglielmi, Biochim. biophys. Acta *414*, 9 (1975).
15 G.L. Gianfranceschi, D. Amici and L. Guglielmi, Molec. Biol. Rep. *3*, 55 (1976).
16 G.L. Gianfranceschi, D. Amici and L. Guglielmi, Nature *262*, 622 (1976).
17 C. Giuli, C. Pieri, L. Piantanelli and N. Fabris, Mech. Age Dev. *13*, 265 (1980).
18 L. Piantanelli and N. Fabris, in: Genetic Effects on Aging, p.315. Ed.D.E. Harrison. The National Foundation, 1978.
19 N. Fabris and L. Piantanelli, in: Hormones and Aging. Eds R. Adelman and Roth. CRC Press, Palm Beach 1981; in press.

The roles of phosphorus deficiency and low food intake in the preservation of renal function in uraemic rats[1]

D. Laouari, C. Kleinknecht[2], R. Habib, F. Mounier and M. Broyer

Inserum U. 192, Tour Technique, 6ème étage, Hôpital des Enfants-Malades, 149, rue de Sèvres, F-75643 Paris Cedex 15 (France), 15 June 1981

Summary. While a diet deficient in phosphorus has a protective effect against kidney deterioration in uraemic rats, this effect, in fact, is more closely related to the reduction of food intake than to the phosphorus load itself, even though the latter influenced the pattern of the renal lesions.

Nutritional factors have been thought to be implicated in the rapidity of the kidney deterioration subsequent to subtotal nephrectomy. Two main factors have been studied; the protein[3,4] and the phosphorus (P)[5,6] content of the diets. In the former studies, however, proteins were supplied by fish flour and had a high P content. In the paper of Ibels et al.[5] demonstrating the preservation of renal function in rats fed a diet deficient in P, the total amount of food ingested was not measured so that any change of appetite resulting from the various P diets was not taken into consideration.

The present experiment was aimed at separating the role of P per se from the role of total food ingested on the survival of uraemic rats.

Material and methods. Male Sprague-Dawley rats (150 g b.wt) underwent a subtotal nephrectomy and were distributed into 5 groups (A-E). They were matched according to their serum creatinine, so that the mean initial serum creatinine concentration was 152 ± 12 µmoles/l in all groups. 3 diets were used. They were dry, isocaloric, had the same protein (18 g/100 g bovine fibrin), vitamin and mineral content except for phosphorus which was 0.03%, 0.2% and 0.5 g%. Phosphorus was supplied by calcium phosphate and calcium was maintained constant (0.5 g%) by the addition of calcium carbonate. 3 groups of rats (A, B, C) were fed ad libitum the 0.03%, 0.2% and 0.5 g% phosphorus diets respectively. The D and E groups were fed the 0.2% and 0.5 g% phosphorus diets, but each rat was matched with one of the A group and was pair-fed with his A counterpart (table). Hence group A, D and E had the same nutritional intake as far as calories, proteins, sodium and calcium was considered, and they differed exclusively in their phosphorus intake.

The experiment lasted for 36 weeks. Food consumed was weighed daily and serum creatinine, calcium and phosphorus concentrations were determined monthly. At time of death the remnants of the kidneys were taken for histologic examination.

Results. Main results are summarized in the table. As expected, food consumption rapidly decreased in the P-deficient group A rats and was significantly lower than

Rats	A n=10	B n=10	C n=10	D n=10	E n=9
Phosphorus diet (g/100 g)	0.03	0.2	0.5	0.2	0.5
Feeding	ad libitum	ad libitum	ad libitum	pair-fed with A	pair-fed with A
Food intake (g/day)	13.1±0.9	19.6±9.8[a]	18.2±0.9[a]	13.1±0.9	13.1±0.9
Phosphorus intake (mmoles/day)	0.131±0.08	1.16±0.04[a]	2.89±0.09[a]	0.90±0.06[a,b]	2.25±0.15[a,b,c]
Plasma phosphorus concentration (mmoles/l)	0.70±0.02	1.31±0.02[a]	2.00±0.10[a]	1.31±0.06[a,b]	1.74±0.02[a,b]
Surviving rats at week 36	8	1[d]	1[d]	7	5

Main characteristics of the 5 experimental rat groups. A vs B, C, D, E: [a] $p<0.001$; B vs D and C vs E: [b] $p<0.01$; B vs E: [c] $p<0.001$ (Student's t-test). A vs B, C: [d] $p<0.01$ (χ^2-test); A vs D, E: $p>0.05$ (χ^2-test). The 5 groups differed either in the P content of the diet or in the daily allotments (ad libitum or restricted to the quantity of food consumed by A rats). The mean values ± SEM were calculated during the first 14 weeks of the experiment, before a significant number of animals developed terminal renal failure. The number of surviving rats in that of the end of the experiment, 36 weeks after its beginning.

that of the 2 other groups fed ad libitum, which had a similar food intake. Group A rats gained no weight during the experiment. Pair-fed group D and E rats gained significantly more weight despite an identical amount of food ingested, and gained less weight than group B and C rats fed the same diets without restriction.

Plasma phosphorus concentration showed an important decrease in P-deficient A rats, was moderately lower than normal in B and D fed the 0.2% P diet and was normal in C and E rats fed the 0.5% P diet, at least until week 18. At that time, it markedly increased in most of the C rats fed the 0.5% P diet ad libitum, and was associated with deterioration of renal function. Plasma calcium remained within the normal range in C and E rats, was slightly elevated in B and D rats, and was significantly high (3.50±0.08 mmoles/l) in A rats.

Serum creatinine concentration rose in some but not in all rats. A marked increase often occurred suddenly just before death. Samples taken at that time showed creatinine levels compatible with those of terminal renal failure (300–600 µmoles/l) in 2 P-deficient group A rats, 3 and 4 rats of the pair-fed groups D and E, and in all but one of both groups B and C fed ad libitum the 0.2% and 0.5% P diet (A vs B or C: $p<0.05$. χ^2-test).

The survival curve differed markedly from one group to another. The earliest deaths occurred in group C rats fed the highest P diet ad libitum; their mortality rate reached 50% at week 20, but group B rats who received the 0.2% P diet experienced a similar high mortality a few weeks later. At the end of the study, 2,3 and 4 rats were dead with terminal renal failure in groups A, D and E respectively; these rats had the same food consumption with different P intakes: these differences were not significant. Mortality was 90% among group B and C rats (B and C vs A and D: $p<0.01$; vs E: $p<0.05$, χ^2-test).

The kidneys of rats that died from renal failure could be examined in 1,9,9,2 and 3 rats of groups A, B, C, D and E respectively. Of these, most rats of groups A, B and D given the deficient or low P diets showed prominent glomerular lesions and dilatation of tubules without calcium deposits. In contrast, tubular dilatation was the major finding in group C and E rats, fed the normal P diet, and they were associated with diffuse calcium deposits impregnating all tubular basement membranes in group C rats. The lesions were less impressive in group E rats. Calcium deposits, however, were absent or few in the single group C and in the 5 group E rats killed at the end of the experiment when their plasma creatinine concentrations ranged from 110 to 210 µmoles/l.

Discussion. The present experiment demonstrates that the preservation of renal function in uraemic rats given a phosphorus-deficient diet, as described by Ibels et al.[5], was related much more to the reduction of food ingested than to the amount of phosphorus ingested; survival was significantly higher in group E than in group B rats, although there was a higher daily phosphorus intake in the former group. From our previous results[3,4], it is likely that the amount of protein ingested played the major role. It cannot be asserted, however, that phosphorus intake had no influence. The mortality was somewhat higher in group E than in group A and D rats, and deaths occurred earlier in group C than in group B rats. These differences, although not significant, cannot be neglected. Several mechanisms may be suggested; a) the enhanced weight gain increasing the amount of metabolic waste products to be cleared by the kidney; b) the enhanced hyperparathyroidism; c) the direct toxicity of phosphorus load per nephron as suggested by Haut et al.[6]. The differences in histological lesions strongly favour the last 2 hypotheses. In the experiment by Haut et al.[6], however, as in those showing the occurrence of renal lesions in rats fed a diet with excessive phosphorus, the phosphorus/calcium ratio of the diet was far above the normal value, a well-known cause of severe hyperparathyroidism resulting in renal lesions, even in the presence of normal serum phosphorus levels and normal renal function. It has been shown that such lesions do not occur in parathyroidectomized animals[7]. In a previous study[4] rats given the highest protein and phosphorus (1.6%) diets had no calcium deposits, despite a phosphorus intake 3 times as high as that of group C rats, associated with terminal renal failure. The phosphorus/calcium ratio of the diet was normal (≠1) in our previous study as well as in the 0.5% phosphorus diet of the present experiment. The parathormone levels under these conditions would be of significance in the understanding of the formation of lesions.

In conclusion, the variations of phosphorus intake play but a minor rôle in comparison with that of total food ingested, particularly protein, in the renal deterioration of rats with reduced kidney mass. The rôle of calcium deposits and the toxicity of phosphorus itself, its relation to the calcium intake and absorption, to the parathyroid response, and to other factors involved in the progression of renal failure, are not yet clearly delineated.

1 Acknowledgment. This work was in part supported by a grant from the Association pour l'Utilisation du Rein Artificiel (AURA, Paris).

2 Author to whom correspondence and reprint requests should be addressed.

3 C. Kleinknecht, I. Salusky, M. Broyer and M.C. Gubler, Kidney int. *15*, 534 (1979).
4 I. Salusky, C. Kleinknecht, M. Broyer and M.C. Gubler, J. Lab. clin. Med. *97*, 21 (1981).
5 L.S. Ibels, A.C. Alfrey, L. Haut and W.E. Huffer, N. Engl. J. Med. *298*, 122 (1978).
6 L.L. Haut, A.C. Alfrey, S. Guggenheim, B. Buddington and N. Schrier, Kidney int. *17*, 722 (1980).
7 I.K. Clark and F. Rivera-Cordero, Proc. Soc. exp. Biol. Med. *139*, 803 (1972).