sin[12]. Of course, an indirect way of action via intestinal hormones or nerves must also be considered. At any rate, this observation will stimulate a search for the presence of xenopsin in mammalian tissue to elucidate the possible physiological role of xenopsin in mammals.

1 This work was supported by the Deutsche Forschungsgemeinschaft Fe 127/4.
2 A. Anastasi, V. Erspamer and R. Endean, Archs Biochem. Biophys. *125*, 57 (1968).
3 T. Nakajima, T. Kanimura and J.J. Pisano, Fedn Proc. *29*, 282 (1970).
4 V. Erspamer, G.F. Erspamer, N. Inselvini and L. Negri, Br. J. Pharmac. *45*, 333 (1972).
5 V. Erspamer, Int. Symp. Brain-Gut Axis, Florence 1981; p. 57.
6 K. Araki, S. Tachibana, M. Uchiyama, T. Nakajima and T. Yasuhara, Chem. Pharm. Bull. *23*, 3132 (1975).
7 K. Araki, S. Tachibana, Y. Kato and T. Tajima, Yakugaku Zasshi *99*, 466 (1979).
8 T. Ishida, K. Kawamura, A. Goto, Y. Nishina, J. Takahara, S. Yamamoto, K. Kawanishi and T. Ofuji, Metabolism *25*, 1467 (1976).
9 K. Kawanishi, A. Goto, T. Ishida, K. Kawamura, Y. Nishina, S. Machida, S. Yamamoto and T. Ofuji, Horm. Metab. Res. *10*, 283 (1978).
10 M. Zinner, B. Yaffe, F. Kasher, D. Albert, A. Sank and I. Modlin, Gastroenterology *80*, 1323 (1981).
11 H.T. Debas and M.I. Grossman, Digestion *9*, 469 (1973).
12 I. Baća, G.E. Feurle, A. Schwab, U. Mittmann and W. Knauf, Digestion *23*, 119 (1982).

Thymic control of the polyploidization of hepatocytes during aging

C. Pieri, C. Giuli, C. Bertoni-Freddari, L. Piantanelli and I.Zs. Nagy

F. Verzar International Laboratory for Experimental Gerontology (VILEG), Italian Section, Center of Cytology, and Center of Biochemistry, I.N.R.C.A., Via Birarelli 8, I-60100 Ancona (Italy), and VILEG, Hungarian Section, University Medical School, H-4012 Debrecen (Hungary), 3 June 1981

Summary. Relative DNA-contents of hepatocyte nuclei have been determined in young, old, and thymus-grafted old mice. There is an age-dependent increase in the occurrence of tetraploid nuclei, which is in agreement with previous data regarding the nuclear volume of hepatocytes. The neonatal thymus grafted into old recipients decreased the percentage of tetraploid cells to a statistically significant extent.

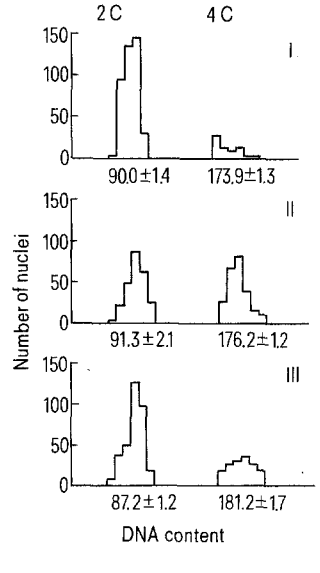
The increase in hepatic cell volume with advancing age is a phenomenon known since the beginning of this century[1]. An age-dependent increase in the mean cellular and nuclear volume was also observed in hepatocytes of haired heterozygous Balb/c-nu mice from our own colony[2]. Furthermore, neonatal thymus grafted into old recipients proved to be able to reverse the age-dependent increase of the nuclear volume to a significant extent[2]. It has been suggested that the increase of the nuclear volume may be the result of the polyploidization of hepatocyte nuclei[3-6]. Experimental evidence has proved the existence of an increased nuclear ploidity level in old animals[7]. The phenomenon of age-dependent polyploidization has been attributed to a hypothesized inability of G_2 phase nuclei to enter the M phase[7]. Therefore, both the age-dependent increase and the thymus-dependent recovery of the mean nuclear volume of hepatocytes[2] may be related to some parallel modifications of the DNA-content of the cell nuclei. The present paper describes an experimental approach to verify this hypothesis.

DNA contents of hepatocyte nuclei were measured by a microfluorimetric method in smears from young (2 months old) and old (24 months old) as well as old, thymus-grafted (24 months old) Balb/c-nu mice. Implantation of the neonatal thymus was performed at an age of 23 months. Smears of the liver were prepared on glass slides by pression, fixed in formaldehyde and stained by the classical Feulgen method. Microfluorimetric measurements of the relative DNA contents were performed according to Fujita[8] by using an OPTON MPM 01 automatic cytofluorimeter connected to a PDP 36/11 computer. Each group consisted of 3 animals, and altogether more than 450 hepatocyte nuclei were measured from each group. The data are presented in a single pool per group in the form of histogram (fig.). Percentual distributions of diploid and tetraploid nuclei are given as averages of the individual values of each group (table). The old animals show a significant increase in the occurrence of tetraploid nuclei as compared to young mice, which is in agreement with the

Percentual distribution of diploid and tetraploid hepatocyte nuclei in young, old, and thymus-grafted old Balb/c-nu mice

Group of animals	2C nuclei (%)	4C nuclei (%)	Significance p<	
Young	83.58±3.42	16.42±3.42	0.01	
Old	49.61±6.28	50.39±6.28	0.02	0.05
Old+thymus	69.06±6.15	30.94±6.15		

Values are means±SD for 3 animals per group. Significance values were calculated by using Student's t-test.



Age- and thymus-dependence of the ploidy levels of hepatocytes in young (I), old (II) and thymus-grafted old (III) Balb/c-nu mice. 2C = diploid cells; 4C = tetraploid cells.

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