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Growth in experimental renal failure: Role of calorie and amino acid intake

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Growth in experimental renal failure: Role of calorie and amino acid intake. In order to evaluate the role of calorie and protein intake in growth impairment due to chronic renal failure (CRF), a subtotal nephrectomy was performed in weanling Wistar rats. A two-thirds reduction of renal function was obtained, which induced a marked growth retardation. Growth retardation was identical in nephrectomized and in pair-fed controls, and thus appeared to be entirely due to a deficient food intake. Using low protein diets, administration of a supplement with small amounts of essential amino acids (EAA) resulted in accelerated growth associated with a higher calorie intake, a better utilization of ingested calories for growth and a greater fall of blood urea nitrogen concentration.

Croissance au cours de l'insuffisance rénale chronique expérimentale: Role de l'apport en calories et en acides aminés. L'effet de la ration calorique et protidique sur la croissance au cours de l'insuffisance rénale chronique a été étudiée sur des rats ayant subi une néphrectomie des 7/8. Leur croissance et leur consommation alimentaire ont été comparées à celles de rats témoins nourris ad libitum et à celles de rats témoins "pair-fed" avec les néphrectomisés. On observe un ralentissement de croissance chez les néphrectomisés identique à celui des témoins "pair-fed", donc apparemment dû exclusivement à un défaut de consommation alimentaire. En régime hypoprotidique, un supplément d'acides aminés essentiels en faibles quantités permet une amélioration importante de la croissance, associée à une consommation calorique plus élevée, une meilleure utilisation des calories ingérées pour la croissance, et une urée sanguine plus basse.

Chronic renal failure (CRF) is usually associated with a growth retardation which is rarely improved by extended hemodialysis [1–5], or even by renal transplantation [5,6]. It would be important to prevent growth impairment as soon as possible, but its mechanism and therefore its treatment remain controversial. The role of anorexia has been recently pointed out [7–10]. The present experiment was performed to examine the role of calorie intake, the incidence of low protein content of the diet and the effect of a supplementation of essential amino acids (EAA) on the growth of rats with CRF after subtotal nephrectomy, as compared with growth of control rats pair-fed and control rats fed *ad lib*.

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Methods

Male weanling Wistar rats, aged 25 days with an average weight of 100 g and linear length of 24.8 ± 0.4 mm (mean ± SEM), were used for the experiment. During the 30 days of the study, they were housed in separate cages provided with a grated floor. They lived in an air conditioned room, at 22°C, with a light cycle of 12 hr. All had a free access to tap water.

Two diets (I and II) were used, differing in their protein and EAA content: 7.25 and 8.52% of weight for protein content in diets I and II, respectively. The difference was entirely due to the addition of small amounts of the eight EAA and histidine in diet II. Supplementation provided the minimal requirements for growing rats according to Rama Rao, Chatam Metta and Connor Johnson [11]. The EAA content of the diet is presented in Table 1. Basal protein content (7% of total calories in both diets) was provided by fish flour and had a high biological value. Both diets were dry (less than 1% water content), isocaloric

Table 1. Essential amino acids content of the diets (g/100 g)[a]

	Diet I	Diet II
L = Isoleucine	0.38	0.53
L = Leucine	0.59	0.79
L = Lysine	0.68	0.95
DL = Methionine	0.24	0.39
DL = Phenyalanine	0.32	0.47
L = Threonine	0.34	0.49
L = Tryptophane	0.09	0.14
L = Valine	0.46	0.56
L = Histidine	0.17	0.22
	3.27	4.54

[a] The tyrosine, cystine, and arginine content of both diets was similar, and was, respectively, 0.24, 0.08 and 0.43 g/100 g.

(416 Kcal/100 g) and included a mineral and vitaminic supplement. Vitamin D content of the diet was 350 U/100 g. Calories were provided by fish flour (52 Kcal/100 g), peanut oil (72 Kcal %), saccharose (145 and 140 Kcal % in diets I and II) and cornstarch (147 Kcal %). The food had a pasty consistency and a steady weight in the laboratory atmosphere. It was distributed in cups provided with a holed movable lid, so that spillage was negligible. Food intake was recorded daily and was weighed with an accuracy of 0.01 g.

Two experiments (EI and EII), differing by the diet used, were performed (Fig. 1). Each compared the growth rate of three groups of rats. *A*) Groups AI and AII underwent a one-stage seven-eighths nephrectomy (right nephrectomy + excision of the lower and upper poles of the left kidney); they were fed *ad lib* with diets I and II, respectively. *B*) Groups BI and BII were sham-operated and pair-fed with rats of group *A*. *C*) Groups CI and CII were sham-operated and fed *ad lib* with diets I and II.

Weight, total body length and tail length were measured by two observers, to an accuracy of ±1 g and ±1 mm, respectively, under ether anesthesia, at days 1 (before operation) and 30 (before killing). After death, left tibias were removed, fixed in alcohol and radiographied using a tube (Philips PW 1008) and contact films (Kodak). Tibial length was measured on radiographs at magnification ×15. The presence of bone lesions was assessed by radiologic and histologic examination of the tibias.

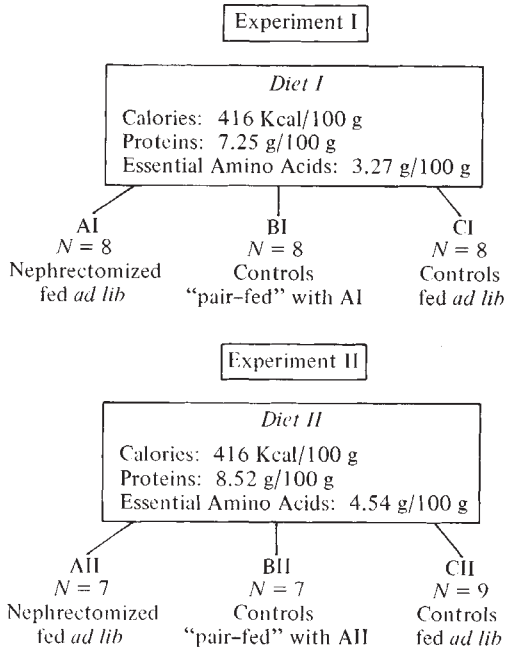

Fig. 1. *Diagram of the experimental protocol.*

In all animals, renal function was evaluated as follows: *1*) plasma creatinine was determined at days 15 and 30 using an autoanalyzer (Technicon); *2*) blood urea nitrogen (BUN) was determined at days 8 and 30. A 24-hr creatinine clearance was performed in 12 animals, 6 nephrectomized and 6 controls. Blood samples were taken from the jugular vein while the animal was under ether anesthesia.

Results

Growth rate. Initial weight and linear length were similar in all the groups. The tibia length of 12 control rats killed at the beginning of the study was 25.0 ± 0.3 mm (mean ± SEM).

After 30 days, marked differences were noted between the groups. Results regarding growth rate are summarized in Table 2.[1] In each experiment, linear growth gain, weight gain and tibia length were significantly lower in nephrectomized rats (AI and AII) than in controls fed *ad lib* CI and CII. Linear growth gain, weight gain and tibial length were identical in groups AI and BI. Comparing groups AII and BII, weight gain and tibial length were identical; linear growth was higher in rats AII, but the difference did not reach significance.

Comparing the results obtained with the two diets, growth rate was higher in animals fed with diet II. The difference for linear growth and weight gain was significant between groups AI and AII ($P < 0.05$), BI and BII ($P < 0.02$) and CI and CII ($P < 0.001$). A parallel difference for tibial length was observed but did not reach a significant level ($0.05 < P < 0.10$).

Food intake. In both experiments, food intake was markedly lower in group A than in group C ($P < 0.01$). Rats of group B, restricted to the intake of the nephrectomized rats, were ravenously hungry, especially rats BI. Calorie intake was higher in rats fed with diet II but the difference was not significant ($P < 0.10$, comparing the groups AI vs. AII, CI vs. CII). The difference in protein intake was highly significant (Table 2).

Relation between growth rate and nutrition. Mean growth gain was approximately the same in rats having the same food intake, nephrectomized or not.

In each experiment, linear growth gain was related to calorie intake (r: 0.78 in EI; r: 0.87 in EII). The regression lines in the two experiments were parallel and a higher linear growth was obtained with diet II for the same calorie intake (Fig. 2). No differences were evident when the nephrectomized and control

[1] Tail length was strictly parallel to the total body length and therefore has not been reported.

Table 2. Growth and nutrition in experiments I and II[a]

	Experiment I				Experiment II				Comparison between experiments I and II		
	AI $N = 8$	BI $N = 8$	CI $N = 8$	Comparison between AI-CI[b]	AII $N = 7$	BII $N = 7$	CII $N = 9$	Comparison between AII-CII[b]	AI-AII[b]	BI-BII[b]	CI-CII[b]
Linear growth gain (LGG), *mm/day* ± SEM	1.6 ± 0.1	1.6 ± 0.1	2.2 ± 0.1	<0.001	2.6 ± 0.2	2.2 ± 0.2[c]	3.1 ± 0.1	<0.05	<0.001	<0.02	<0.001
Weight gain (WG), *g/day* ± SEM	1.2 ± 0.4	1.1 ± 0.4	2.4 ± 0.3	<0.05	2.4 ± 0.4	2.4 ± 0.4	4.5 ± 0.4	<0.01	<0.05	<0.01	<0.001
Final tibia length, *mm* ± SEM	29.6 ± 0.4	29.6 ± 0.4	31.5 ± 0.3	<0.001	30.7 ± 0.4	30.7 ± 0.3	32.4 ± 0.4	<0.01	<0.10	<0.10	<0.10
Caloric intake, *Kcal/day* ± SEM	38.5 ± 2	38.5 ± 2	56 ± 3.6	<0.001	46 ± 3.5	46 ± 3.5	65.5 ± 4.0	<0.01	<0.10	<0.10	<0.10
Protein intake, *g/day* ± SEM	0.67 ± 0.03	0.67 ± 0.03	0.97 ± 0.06	<0.001	0.95 ± 0.07	0.95 ± 0.07	1.34 ± 0.08	<0.01	<0.01	<0.01	<0.01
LGG per 100 Kcal ingested, *mm/day*	4.0 ± 0.4	4.0 ± 0.4	4.0 ± 0.4	NS	5.6 ± 0.4	4.8 ± 0.3[c]	4.7 ± 0.1	NS	<0.05	NS	<0.10
WG per 100 Kcal ingested, *g/day*	3.0 ± 0.3	2.9 ± 0.3	4.3 ± 0.4	<0.02	5.0 ± 0.5	5.0 ± 0.5	6.9 ± 0.3	<0.02	<0.01	<0.01	<0.001

[a] For the meaning of A, B and C see Fig. 1.
[b] Student's *t* test.
[c] Comparison between AII and BII, $P > 0.10$.

rats were compared. The slopes of the regression lines were, respectively, 0.03 and 0.04 in experiment I, 0.04 and 0.05 in experiment II ($P > 0.10$). Linear growth was also related to protein intake and was higher with diet II for the same total protein intake (Fig. 3). No significant difference was found between the slopes of the regression lines when the nephrectomized and control rats were compared. The difference was marked only for the lowest protein intakes. A similar relation between growth and calorie or protein intake was observed when growth was assessed by final tibial length.

Linear growth gain per calorie ingested did not differ significantly between groups A, B and C in each experiment (Table 2), though it was slightly higher in group AII than in group BII. Conversely, weight gain per calorie ingested was similar in groups A and B but markedly higher in group C. Rats CII became grossly and obviously obese. At autopsy, the development of adipose tissue was normal in rats CI, and deficient in rats A and B whether or not they received EAA supplements.

Renal function. Plasma creatinine was determined on days 15 and 30 of the study. Mean values were 0.96 ± 0.04 mg/100 ml in the nephrectomized rats and 0.43 ± 0.02 mg/100 ml in controls ($P < 0.001$) (Fig. 4). In rats A plasma creatinine concentration was remarkably homogenous and was steady in each rat. Mean creatinine clearance was 0.37 ± 0.04 ml/min/100 g in six nephrectomized rats, and 1.1 ± 0.08 ml/min/100 g in six controls ($P < 0.001$).

Despite reduction of renal function, BUN was not elevated as shown in Fig. 5. In all the experimental groups it was lower than in the normal rats fed the usual laboratory 18% protein diet (mean BUN ±

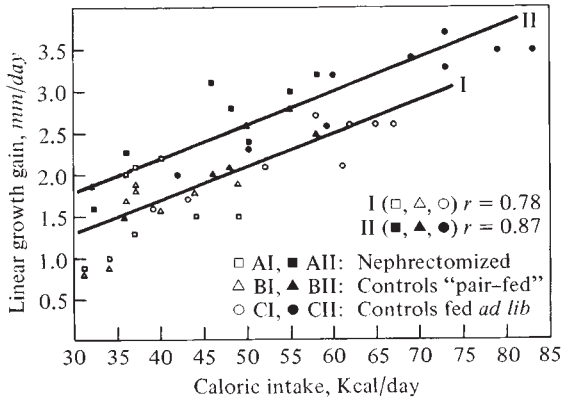

Fig. 2. *Relation between growth and calorie intake.* There is a close correlation between growth and calorie intake. No difference is found between nephrectomized and control rats. For the same calorie intake, growth is higher in animals fed with diet II, containing supplementary essential amino acids.

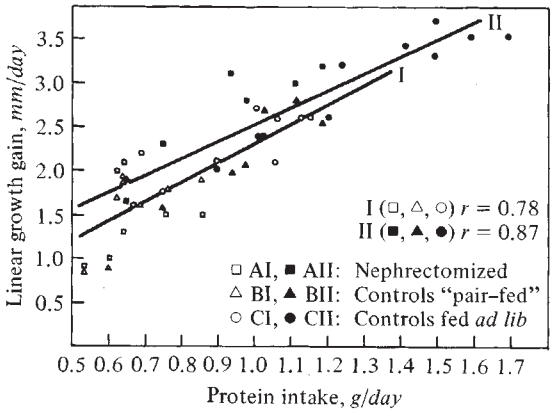

Fig. 3. *Relation between growth and protein intake.* Linear growth gain is highly related to protein intake in each experiment, without any clear difference between nephrectomized and control rats. For the same protein intake, growth is higher in animals fed diet II, containing a higher proportion of essential amino acids.

SEM: 23 ± 1 mg/100 ml). Blood urea nitrogen concentration fell during the course of the study. On day 30, it was below the limits of accurate dosage in most of the control rats. However, BUN was significantly lower when control and nephrectomized were compared (BI + CI vs. AI and BII + CII vs. AII: $P < 0.01$)[2] and when the EAA-supplemented with the non-supplemented groups were compared (AI vs. AII: $P < 0.01$).[2] There was no bone lesion by radiologic or histologic examination of the tibias.

Discussion

Calorie deficiency has been recently incriminated as a major cause of growth impairment in CRF. Simmons et al [7] showed that caloric supplementation of hemodialyzed children resulted in an accelerated growth rate. Holliday [9]; Grushkin, Korsch and Fine [3]; and Betts and Magrath [10] found a good relationship between growth velocity and calorie intake in children with CRF. In children on long-term hemodialysis Broyer et al [4] found that calorie and protein intakes were higher in children with a satisfactory growth rate than in the others, but the difference between the two groups was not significant; osteodystrophy and inadequate dialysis adversely affected growth more evidently than undernutrition. To our knowledge, no clinical study is conclusive. In the report of Simmons et al [7], growth increment has been measured over periods which were too short to be reliable, as was shown by Marshall [12]. In the study of Betts and Magrath [10], only six children showed an impairment of both calorie intake and growth velocity, which was not related to renal function. In addition, this report dealt with a series of nonhemodialyzed children which was not homogeneous for age, causal nephropathy, presence of osteodystrophy and reduction of renal function (creatinine clearance ranged from 2 to 70 ml/min/1.73 m²).

A number of factors are presumably involved in growth retardation and the importance of each cannot be precisely estimated. Moreover, the reliability of the nutritional inquiries is questionable, and the series of children cannot be adequately compared. The difficulties of clinical investigation led Chantler, Lieberman and Holliday to describe an experimental model using nephrectomized rats [13]. This model has been used by Mac Donell, Buzon and Holliday [14] and in the present study.

Three measures were used to assess linear growth: total body length, tail length and tibial length, the latter being the most accurate and objective measure; all three indexes were highly related. On the contrary, linear and ponderal growth showed no regular parallelism, particularly in animals with high calorie intakes who developed obesity. Compared to normal rats of the same age fed the usual laboratory diet (18% protein), rats CII showed a greater weight gain (4.5 vs. 3.25 g/day) associated with a lower length gain (3.1 vs. 3.9 mm/day). Therefore, weight is not a satisfactory index to assess growth in rats as it is not in children.

The procedure used to assess daily food intake was satisfactory, spillage being rare and minimal. Each animal had a steady food intake except for the nephrectomized rats during the two days following surgery. However, the reliability of the pair-feeding procedure to provide a similar nutrition may be questioned, even in the absence of any spillage. *1*) The physical activity may be different. In the present experiment, pair-fed controls (especially rats BI) were

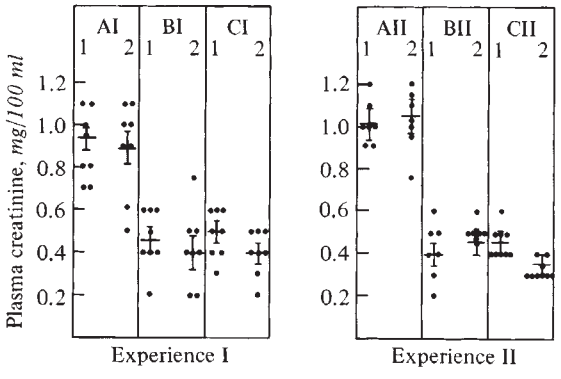

Fig. 4. *Plasma creatinine concentration on days 15 (1) and 30 (2) of the study.* For the meaning of A, B and C see Fig. 1.

[2] Mann and Whitney test.

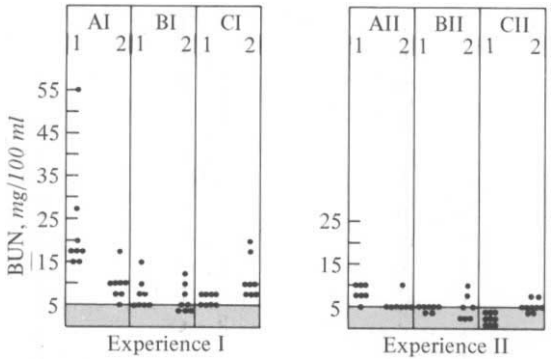

Fig. 5. *Blood urea nitrogen values on days 8 (1) and 30 (2) of the study.* For the meaning of A, B and C see Fig. 1.

persistently hungry, aggressive and hyperactive. They clearly expended more calories for activity than the nephrectomized rats. *2*) The normal timing of eating may be altered in control animals. Rats A and C consumed food at frequent intervals throughout the 24-hr period, while rats B consumed their whole daily allowance within a few hours. Pocknee and Heaton [15] demonstrated that normal rats fed on these two patterns showed the same total body weight gain. The consequences of various feeding patterns in pathologic states have not yet been evaluated.

Subtotal nephrectomy led to a moderate renal failure with a two-thirds reduction of glomerular filtration rate (GFR). In most of the reports dealing with the same experimental procedure, BUN is used as the only index of renal failure [13, 14, 16]. When GFR is measured, it is reduced to approximately one-third the control values [17]. In the presence of such a moderate renal failure, BUN is highly dependent on protein intake and is elevated when high protein diets are used [13, 14, 16, 17], as we confirmed in other experiments. Attempts to further reduce GFR by using radiotherapy on the remaining renal tissue led to an increased mortality without changes in mean GFR in the surviving rats (personal data). Weanling rats appeared most sensitive to renal failure, suggesting that the experimental model used would be inadequate for studying severe renal failure.

In the present experiment, low protein diets were used. In previous reports comparing growth in controls and in uremic rats, high protein diets were used: 37% of total calories for Chantler et al [13] and Mac Donell et al [14], 37% and 15% for Holliday [9], and 23% for Kaufman et al [17]. Such diets differ widely from those commonly used in children with CRF. Our children treated by extended hemodialysis consume 7 to 8% of calories as proteins with a low calorie intake. A similar protein content has been arbitrarily chosen for experimental use although the protein minimal requirement is probably higher in growing rats than in children. Obviously the 7% protein diet was insufficient to promote a normal growth in normal rats, and protein deficiency was a factor for stunting. In rats CII the high calorie intake and the presence of obesity favors the role of protein deficiency as explanation of the moderate impairment of growth observed. However, growth impairment was observed by Chantler et al [13] and Mac Donell et al [14] despite the high protein content of the diet used (43% of weight, 37% of calories).

In the present study and in previous reports [13, 14, 16] nephrectomized rats evidenced growth impairment. Pair-fed controls showed a similar impairment, as has been previously shown by Kaufman et al [17] and Mac Donell et al [14]. Kaufman et al considered surgery to be the cause of anorexia, but Chantler et al [13] showed that the discrepancy in weight between nephrectomized rats and controls increased with time. In the present experiment, food intake remained low in nephrectomized rats until the end of the study, and this could reasonably be attributed to renal failure.

At possible variance with Holliday [9] and Russel and Avioli [18], we found neither histologic nor radiologic evidence of osteodystrophy.

The similar growth in nephrectomized rats and pair-fed controls suggests that growth failure in the former group is entirely due to food deficiency, without any change in utilization of calories for growth. Chantler et al [13] pointed out that weight gain per calorie ingested was lower in uremic rats than in controls, and suggested that calorie cost for growth was higher in renal failure. The data shown in Table 2 confirm these results but not their hypothesis: weight gain per calorie ingested was higher in controls fed *ad lib* than in nephrectomized rats, as it was in the study of Chantler et al, but it was similar in groups having the same food intake whether nephrectomized or not; and linear growth gain per calorie ingested was similar in the three groups of each experiment. Irrespective of CRF, rats having the highest calorie intake used a greater percentage of calories for weight gain but it was not so for linear gain.

Anorexia may be considered, therefore, to be a major cause of growth impairment in moderate renal failure, but the factors involved have not been clearly defined. The role of urea per se may be eliminated from the present study, but not the role of other waste products or "toxins", or the responsibility of other consequences of nephrectomy such as hypertension, acidosis or hyposthenuria. Adelman and Holliday promoted appetite stimulation and accelerated growth by administration of a saccharin supplement

in uremic rats [19] while gavage led to a decreased spontaneous food intake and failed to achieve normal nutrition [19]. In our clinical practice, long-term calorie supplementation proved unable to achieve a sufficient calorie intake in dialyzed children.

Low protein diets are always used in patients with severe renal failure, as high protein diets lead to the accumulation of metabolic toxins. The protein diets usually prescribed in children with CRF (1 to 2 g/kg/day according to age) are considered to be sufficient when compared to the recommended dietary allowances. But these are much lower than the actual protein intakes of normal children. Moreover, there is some protein loss during dialysis. It is uncertain whether these protein diets are able to promote normal growth, especially when calorie intake is low as it is in CRF. Thus, the optimum protein regimen, taking into account the level of renal failure and the expected growth rate, remains to be defined.

Because of this dilemma, we chose to study the effects of adding small amounts of EAA to low protein diets in rats. Results were dramatic, with improvement of both calorie intake and utilization for growth of the calories ingested. Despite a greater amount of nitrogen ingested, rats fed with the supplemented diet showed a greater and earlier drop of BUN. This result suggests a better re-utilization of urea nitrogen and is consistent with the finding of Giordano [20]. The benefit of a low protein diet supplied with EAA in the management of adults with CRF has long been shown, particularly by Giovanetti and Maggiore [21] and Lee et al [22]. They demonstrated an improvement in clinical condition and in nitrogen balance using EAA-supplemented diets.

Our findings suggest that an EAA supplement could be used in the long-term management of children with CRF, in order to promote satisfactory growth by improving both nitrogen balance and calorie intake.

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