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The effect of protein-energy malnutrition on appositional bone growth in the rat[1]

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Summary. During protein-energy malnutrition appositional bone growth in the seventh caudal vertebra of the rat slows and finally ceases. During rehabilitation appositional growth begins agains and attains a rate in excess of that of the controls. This may account for alterations in skeletal proportions resulting from malnutrition.

Protein-energy malnutrition in the weanling rat is characterized by growth failure, with suppression of longitudinal bone growth[3,4]. Studies from this laboratory have demonstrated that longitudinal bone growth slows significantly by the 1st week of severe malnutrition and by the 2nd week has ceased[5]. Others[6] have reported that malnutrition is accompanied by alterations in bone width, relative to length. This may be due to a differential effect of malnutrition on appositional and longitudinal bone growth. The experiments reported here were undertaken to examine the effect of protein-energy deficiency on appositional bone growth in the rat, as assessed by the deposition of fluorescent dyes in the caudal vertebrae[7].

Methods. Male Sprague-Dawley rats (Biobreeding Laboratories, Ottawa, Canada) weighing approximately 150 g each were housed in plastic cages and fed either the 18% lactalbumin (control) or the 0.5% lactalbumin (protein-deficient) diet described by Edozien[8]. After consuming the protein-deficient diet for 12 weeks, some animals were rehabilitated for an additional 4 weeks on the control diet. Controls remained on the control diet throughout the experiment. Food and water were available ad libitum. Food consumption was not recorded.

Control and experimental animals were given tetracycline and DCAF (2,4-bis(N,N′-di′(carboxymethyl)aminomethyl)fluorescein) according to the schedule shown in the table. Both were administered at 50 mg/kg b.wt, by i.p. injection. Animals were sacrificed 6 days after receiving the last injection of dye (groups I and IV at 2 weeks, 6 days; groups II and V at 12 weeks, 6 days; and groups III and VI at 16 weeks, 6 days). The 7th caudal vertebra of each animal was removed, cleaned of adhering tissue, and fixed in buffered formol-saline. The bones were embedded in methylmethacrylate and undecalcified sections were cut transversely with a Gillings-Hance rotary diamond disc. Sections were mounted on glass slides, ground to a thickness of approximately 20 μm, and photographed with fluorescence optics. Tetracycline and DCAF deposition can be distinguished, as the former has a yellow fluorescence, while the latter has a green fluorescence. Photographic prints (50× enlargements) were prepared, and the distance between fluorescent lines adjacent to the periosteal surface was measured at 20 locations on each section, as described by Hammond and Storey[7]. The mean width of each growth band was calculated and appositional bone growth (μm/week) was estimated as the distance between adjacent fluorescent lines, divided by the number of weeks between the administration of the dyes.

Results. Protein-energy malnutrition resulted in an early cessation of weight gain, followed by gradual weight loss; weight gain was rapid during rehabilitation (figure 1). Appositional bone growth of control animals was rapid during the 1st week, but was considerably less when averaged over the first 4 weeks. A further decline is seen between the 1th and 12th weeks and during the periods of 12–14 and 14–16 weeks (figure 2). However, the protein-energy malnourished animals showed a subnormal rate of appositional bone growth as early as the 1st week, and this is further reduced during the first 4 weeks, as well as from the 4th to the 12th weeks. During the first 2 weeks of rehabilitation no appositional bone growth is measurable, but during the next 2 weeks there is a resurgence of appositional growth, much in excess of that of the controls of the same age.

Discussion. It is well recognized that in many animal species, including man, protein and/or energy deficiency is accompanied by a suppression of skeletal growth. Some workers[3,9] have emphasized that even under conditions of severe malnutrition skeletal growth slows, but does not cease. There is some indication that longitudinal bone growth is related to body weight, while bone width is more closely related to age[6], suggesting that longitudinal bone growth may be more severely affected by malnutrition than would be bone width. Thus appositional bone growth may be expected to continue during periods of growth delay, resulting in alterations in the proportions of the long bones.

Schedule of administration of tetracycline (t)* and DCAF (D)* to control, protein-energy deficient, and rehabilitated rats

Group	No.	Time (weeks consuming diet)					
		0	1	4	12	14	16
		Controls					
I	2	T	D				
II	5	T		D	T		
III	5				T	D	T
		Restricted			Rehabilitated		
IV	4	T	D				
V	4	T		D	T		
VI	6				T	D	T

* 50 mg/kg b.wt, i.p.

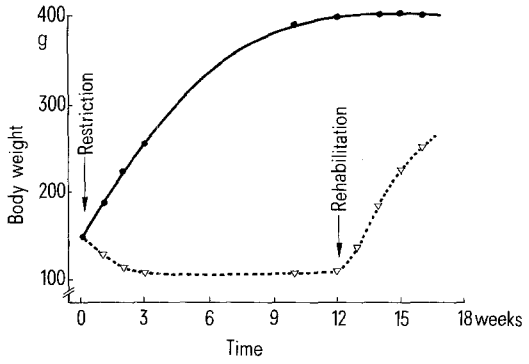

Fig. 1. Body weight of control rats (solid line) and of protein-energy restricted and rehabilitated rats (dotted line).

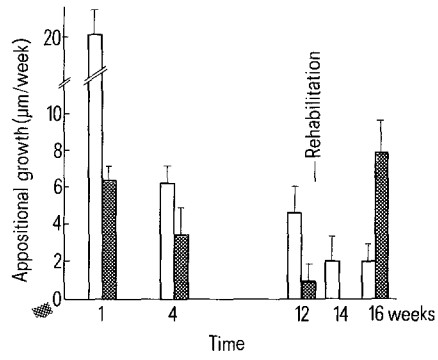

Fig. 2. Appositional bone growth of control rats (white bars) and of protein-energy restricted and rehabilitated rats (cross-hatched bars). Standard deviations are shown.

Studies from this laboratory[5] have demonstrated that, under the same experimental conditions as those described here, longitudinal bone growth slows during the 1st week of malnutrition and then ceases. During rehabilitation longitudinal bone growth resumes (although somewhat later than the resumption of weight gain) and, if rehabilitation continues for a sufficient length of time, bone length approaches that of the controls. The present study demonstrates that a similar pattern is characteristic of appositional bone growth, although the rate of growth suppression is slower. Periosteal growth slows during the 1st week of restriction and finally, by the 14th week, no further growth is observed. The later cessation of appositional growth, relative to longitudinal growth, would account for the changes in proportions of the long bones, as observed by Outhouse and Mendel[6].

Appositional bone growth does not resume immediately upon transfer to the control diet, but is delayed. However, if catch-up growth is defined as 'a growth velocity above the statistical limits of normality for age ... during a defined period of time'[10], then catch-up growth occurs during the early stages of rehabilitation (14–16 weeks). Whether this is temporally correlated with an accelerated rate of longitudinal bone growth remains to be established.

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Lymphatic metastasis of tumour; persistent transport of cells[1]

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Summary. A model of lymphatic metastasis established by injecting Walker rat carcinoma cells into the rat footpad was used to study the output of tumour cells from the footpad. The lymphatic efferent from the footpad was cannulated in a group of rats with advanced neoplasm; it was shown that the output of tumour cells was continuous over periods up to 90 min and ranged from 10^2–10^5 cells/min.

The common human cancers metastasize early by the lymphatics, unlike most experimental animal neoplasms. Experimental models which mimic human lymphatic metastasis are therefore of especial interest[3,4]. A useful model is to inject tumour cells into the footpad and follow their metastasis to the draining popliteal lymph node[5,6]. Among the tumours which successfully metastasize in this manner is the Walker rat carcinoma, but many tumours do not[7]. Detailed studies have not till now been carried out on the lymph directly draining experimental tumours which metastasize by lymphatics. Accordingly, it has not been clear whether tumour cells pass from primary to secondary, singly or in clusters, and discontinuously or continuously. Furthermore, while it has been possible to investigate the entry of tumour cells into the lymphatic vessels in the primary, it has not been possible to pinpoint the time at which this happened by detecting the cells within the lymphatic trunk. This report describes the cannulation of lymphatics efferent from an implanted ('primary') tumour, and the detection and enumeration of tumour cells therein.

A metastasizing model was established by injecting Walker rat carcinoma cells into the left footpad of out bred albino rats. The tumour cells were prepared as described elsewhere[5]. The presence of metastasis was established by histological examination of step paraffin sections of the ipsilateral popliteal lymph node. In newborn male rats the injection of 5 million tumour cells of better than 90% viability adjudged by trypan blue exclusion produced better than 95% successful metastasis in a series of 24 animals. In adult male rats the similar injection of 20 million tumour cells