Quantitative observations on iliac bone marrow mast cells in chronic renal failure

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SYNOPSIS Mast cells have been counted in sections of iliac bone from 61 control subjects at necropsy. Mast cells were found in all but three, and the range was 0-33.7, median 1.95 per mm² marrow. The majority (82%) had < 4.99 mast cells per mm² marrow; in 37.7% there was < 1 mast cell per mm² marrow.

In a group of 45 patients with chronic renal failure there was a significant increase in the numbers of mast cells ($P < 0.001$) with a range of 0.96-55.63, median 9.55 per mm² marrow. Mast cells were common in the areas of marrow fibrosis associated with osteitis fibrosa but this was not the sole cause of the increase since there was also an excess of mast cells in the non-fibrous parts of the marrow. There was a tendency towards greater numbers of mast cells in those cases with most marked osteitis fibrosa in association with the prominent marrow fibrosis, but there was no significant relationship between mast cell numbers and other features of osteitis fibrosa such as the number of osteoclasts and the amount of woven bone formation. There was no relationship between the numbers of mast cells and the amounts of total bone, osteoid, percentage mineralization of cancellous bone, or the presence of osteomalacia.

Shortly after we began a long-term study of the histopathology of azotaemic renal osteodystrophy some eight years ago one of us observed an apparent increase in the number of marrow mast cells in toluidine blue stained sections of iliac bone from patients with chronic azotaemia. More recently, Neiman *et al* (1972), in a letter to the *Lancet*, referred briefly to their finding of increased numbers of mast cells in the spleen and bone marrow of patients with uraemia.

There is little quantitative information in the literature concerning marrow mast cells in man. In the past, the finding of such cells has been regarded as abnormal (Fadem, 1951) and it is now recognized that mast cells are present in normal bone marrow (Johnstone, 1954; Selye, 1965). The purpose of the present paper is to report quantitative observations on the mast cells in histological sections of iliac bone marrow in 45 patients with chronic renal failure. For comparison samples of iliac bone were obtained at necropsy from 61 cases of sudden death. Numbers of marrow mast cells have been considered in relation to the severity of osteitis fibrosa, the presence of

excess osteoid, and osteomalacia, all of which are features of azotaemic renal osteodystrophy.

Material and Methods

BONE

Samples of iliac bone were examined from 45 patients with chronic renal failure due to a variety of renal disorders. Iliac bone was obtained by transilial trephine bone biopsy in 22 patients and at necropsy in 23. Of the patients coming to necropsy, 11 had terminal peritoneal dialysis, one had a terminal haemodialysis, and two had been treated by peritoneal dialysis for two months. The biopsy patients were near end stage renal failure. Three had been treated by peritoneal dialysis for two to three months, and 12 required dialysis after periods varying from a few days up to three months after bone biopsy.

Control iliac bone samples were obtained at necropsy from 61 previously physically healthy individuals after sudden death due to a variety of causes such as trauma and poisoning.

Blocks of iliac bone were fixed in neutral 10% formalin, decalcified in formic acid, and double embedded using low viscosity nitrocellulose; 5 μ

sections were stained by 0.05% toluidine blue solution at pH 3.8.

MAST CELLS IN MARROW AND FIBROUS TISSUE

We have regarded as marrow tissue mast cells any connective tissue cell possessing cytoplasmic granules that stain metachromatically. Basophils possess similar granules but differ morphologically from mast cells in that they are generally smaller and have a lobulated nucleus. Since human basophilic granules are water soluble, metachromasia is lost after aqueous fixation, and basophils are not liable to be confused with mast cells in histological sections.

Mast cells were counted in the marrow of cancellous bone in a square measuring 0.5×0.5 cm marked out on the cover slip in an area immediately beneath the iliac crest in the case of the necropsy samples and in the middle of the biopsy specimens, using $\times 40$ objective and $\times 10$ eyepiece incorporating a squared graticule. The proportion of total bone within the measured area was determined using a point-counting technique (Ellis and Peart, 1972) and the number of mast cells per mm^2 of marrow was then calculated. In 24 of the patients with renal failure the amount of marrow fibrous tissue resulting from osteitis fibrosa was also measured using a point-counting technique (Ellis and Peart, 1973). In these patients, mast cells were counted separately in fibrous tissue and in mixed fatty and cellular marrow to ascertain whether or not mast cells were concentrated in the fibrous tissue.

OSTEITIS FIBROSA, EXCESS OSTEOID, AND OSTEOMALACIA

The severity of osteitis fibrosa was assessed semi-quantitatively on a scale of grades from 0 to 5, and the number of osteoclasts and amount of woven bone were determined as previously described (Ellis and Peart, 1973). The amount of osteoid, expressed as a percentage of the measured area, and the percentage mineralization of cancellous bone were determined by a point-counting technique using undecalcified sections (Ellis and Peart, 1972). Osteomalacia was diagnosed on the basis of the presence of an excess of osteoid with abnormally wide osteoid seams and a reduced or absent calcification front (Ellis and Peart, 1973; Ellis, 1973).

Results

CONTROL SUBJECTS

The arithmetic mean number of marrow mast cells for the 38 males and 23 females, varying in age from 5 to 93 years, was 3.93 per mm^2 with a median of 1.95 per mm^2 . There was no significant difference

($P > 0.10$) between the means for males (4.70 per mm^2) and females (2.65 per mm^2). Plotting the distribution of mast cell values in relation to age revealed no significant relationship in males or females. As the figure shows, there was considerable variation in the number of marrow mast cells between individuals, and the distribution is not normal. Mast cells were found in all but three of the 61 samples examined. The highest value (33.76 per mm^2) was in an 11-year old boy and this in the area immediately contiguous with the zone of endochondral ossification beneath the iliac crest. A second count at a distance of 1.0 cm below this gave a lower value of 4.67 mast cells per mm^2 . In three older individuals aged 21 to 22 years, duplicate counts made at distances of 0.5 cm and 1.5 cm below the crest revealed less marked differences, the paired values being 3.18 and 7.42, 6.32 and 4.80, and 4.7 and 1.68 mast cells per mm^2 of bone marrow, respectively.

The majority (82%) of the control subjects had mast cell values within the range 0 to 4.99 per mm^2 , and in 37.7% of individuals there was < 1 mast cell per mm^2 . The six individuals (10%) with > 10 mast cells per mm^2 were males aged 11 to 47 years. The mode of death was unrelated to the higher values. Thus one died from asphyxia trapped in a disused pit, one from multiple injuries, and the others from various poisons including carbon monoxide, barbiturates.

Figure Distribution of the numbers of iliac bone marrow mast cells in 61 control subjects and in 45 patients with chronic renal failure: $\triangle$ and $\blacktriangle$ males, biopsy and necropsy samples; $\circ$ and $\bullet$ females, biopsy and necropsy samples.

rates, and aspirin. Other subjects dying from these poisons had much lower values. Thus four with carbon monoxide poisoning had 0 to 0.52 mast cells per mm². In addition, in a selected group of previously healthy individuals comprising 25 males and 12 females in whom death had been almost instantaneous and predominantly the result of trauma such as road accidents, the mean values for males and females were 3.81 and 2.21 mast cells per mm², respectively, and these did not differ significantly from those obtained for the control group as a whole.

CHRONIC RENAL FAILURE

Total marrow mast cells

The results of mast cell counts in the iliac bone marrow of the 45 patients with chronic renal failure are summarized in the figure and given in more detail in table I. The values in the figure refer to the numbers of mast cells in the whole marrow, including those in mixed fatty and cellular areas and in any fibrous areas resulting from osteitis fibrosa. Although there is some overlap with values obtained in the control group, in general values were higher and the arithmetic mean was 13.75 per mm² with a median of

Case No. Age and Sex	Mast Cells per mm ²		Mineralization Data			Osteitis Fibrosa Data			
	All Marrow	Non-Fibrous Marrow	O	M	OM	OCL	WB	MF	OFG
1 15M	9.52	3.01	2.57	90.5	+	0.22	—	8.6	3
2 16F	44.74	2.63	0.72	98.4	—	5.63	—	14.1	4
3 24F	31.89	29.52	0.09	99.6	—	0.71	—	1.4	2
4 25M	33.29	32.91	0.15	99.3	—	0.24	—	0.5	1.5
5 27F	14.23	11.69	0.39	97.5	—	0.56	—	2.5	2
6 29M	2.89	2.62	0.28	98.9	—	0.79	—	0.9	2
7 31M	22.37	8.21	4.02	88.5	+	1.55	—	6.5	3
8 32M (2)	1.18	0.93	0.57	98.0	—	0.38	6.8	0.4	1.5
9 33F	19.98	18.06	6.44	80.8	+	1.49	—	3.3	3
10 40M (17)	26.37	26.36	0.31	98.7	—	0.96	5.3	0.7	2
11 42F	1.86	1.46	0.04	99.9	—	0.27	—	0.5	2
12 45F (6)	7.87	6.03	3.94	82.2	+	7.70	50.8	8.0	4
13 45M	26.02	7.21	0.49	98.1	—	2.67	—	13.1	4
14 45M	1.32	1.32	0.18	99.3	—	0.20	—	0	1
15 47F	44.06	10.95	1.05	95.6	—	1.31	—	4.8	3.5
16 48F	3.25	2.82	0.11	99.3	—	0.28	—	1.0	1
17 49M (18)	25.03	21.70	1.00	95.0	—	5.00	61.6	5.7	3.5
18 50M	15.51	13.74	1.51	93.9	—	3.15	—	11.5	4
19 52M	55.63	43.75	1.81	92.4	—	9.46	—	10.4	4
20 53F	7.85	2.61	1.84	91.8	—	1.58	—	3.6	2.5
21 54M	12.10	8.44	0.75	96.2	—	0.33	—	0.6	2
22 58F	13.01	5.75	0.48	98.2	—	2.16	—	1.5	3
23 23F (27)	1.21	—	0.10	99.5	—	0.02	9.6	0.1	1
24 27F (28)	0.96	—	5.35	85.6	+	2.49	23.3	2.0	2.5
25 29M (57)	11.26	11.09	0.15	99.6	—	0.56	10.1	0.9	2
26 35F (15)	2.62	—	2.89	92.7	—	1.62	36.9	1.6	3
27 37M (24)	10.77	—	0.08	99.6	—	0.30	0	0.2	2
28 40M (3)	20.19	—	0.04	99.8	—	0.74	7.4	0.7	2
29 42F (31)	10.49	5.47	4.65	86.6	+	2.64	29.9	2.1	3
30 42M (39)	8.88	—	0.03	99.9	—	0.79	5.1	0.4	2
31 43M (48)	2.39	—	0.01	100.0	—	0.59	3.0	1.0	2
32 45F (44)	9.77	—	0.02	99.9	—	0.36	4.3	0.3	2
33 45F (5)	17.54	—	6.22	79.7	+	0.67	33.6	0.6	2.5
34 50M (45)	1.62	—	0.03	99.9	—	0.06	2.6	0.1	1
35 50M (32)	5.89	—	0.02	99.9	—	0.48	21.2	1.1	2.5
36 51F (40)	6.05	—	0.04	99.4	—	0.27	8.7	0.3	2
37 53M (41)	7.62	—	0.59	98.2	—	0.36	9.6	0.7	2
38 54F (42)	25.05	—	0.18	99.1	—	0.96	43.7	2.6	3
39 55F (19)	4.04	—	0.12	99.2	—	0.06	6.6	0	0
40 58F (10)	29.51	—	8.08	63.6	+	0.96	16.6	1.4	2
41 59F (43b)	1.57	—	0.36	98.4	—	0.59	23.1	1.0	2
42 60F (11)	9.55	—	0.04	99.6	—	0.66	8.3	0.4	2
43 62F (21b)	7.98	—	2.64	73.9	+	4.40	43.8	13.8	4.5
44 68M (47)	1.39	—	17.16	61.0	+	0.35	18.3	1.6	2
45 70F (37)	2.56	—	0.01	100.0	—	0.11	0	0.1	1

Table I Details of the numbers of marrow mast cells, mineralization of bone, and severity of osteitis fibrosa in 45 patients with chronic renal failure

O, % measured area comprising osteoid; M, % mineralization of cancellous bone; OM, osteomalacia + or -; OCL, number of osteoclasts per mm² of cancellous bone and marrow; WB, woven bone % of cancellous bone trabeculae; MF, % measured area comprising fibrous tissue; OFG, osteitis fibrosa grade
Figures in brackets refer to the case numbers in a previous paper (Ellis and Peart, 1973). Present cases 1 to 22 biopsy bone samples; cases 23 to 45 necropsy bone samples.

9.55 per mm². Thus in only one individual (2.2%) was there < 1.0 mast cell per mm² compared with a corresponding value of 37.7% in the control group. In contrast, the number of mast cells exceeded 5 per mm² in 68.9% of patients with chronic renal failure compared with 18.0% of controls. Comparison of the control and renal groups by the Mann-Whitney test revealed a significantly higher mean number of marrow mast cells in patients with chronic renal failure (table II). This was true for renal patients whether the bone sample had been obtained by transilial biopsy or at necropsy. The biopsy series had significantly more marrow mast cells than did the necropsy series ($P < 0.02$).

Mast cells and marrow fibrosis

Mast cells in the marrow of patients with chronic renal failure occurred both in the mixed fatty and cellular areas and in the zones of marrow fibrosis constituting part of the repair reaction to osteitis fibrosa. Mast cells were commonly conspicuous within the cellular fibrous tissue occupying resorption lacunae and lying alongside cancellous trabeculae. In 24 patients with renal failure (22 bone biopsy and 2 necropsy samples) differential counts were made of the number of mast cells in the various components of the marrow. Very high values were found in the fibrous areas within the range 0 to 536.4 mast cells per mm², arithmetic mean 148.8 and median 98.27 per mm². When the number of total marrow mast cells was plotted against the percentage of marrow occupied by fibrous tissue there was a significant linear relationship ($P < 0.001$) with a correlation coefficient (r) = 0.474, although there

was a fairly wide scatter of individual values about the calculated regression line.

The large number of mast cells within the fibrous parts of the marrow did not alone account for the increase in total marrow mast cells in the patients with chronic renal failure. Thus the differential counts in 24 cases also revealed an increased number of mast cells in the mixed fatty and cellular component of the marrow with from 0.93 to 43.75 mast cells per mm² (arithmetic mean 11.60 and median 7.71 per mm²), which was significantly higher than the number of mast cells found in the control marrows ($P < 0.001$).

Number of mast cells and severity of osteitis fibrosa

That mast cells were common within the fibrous component of the marrow suggested the possibility that the increased number of mast cells in azotaemia might be the result of the osteitis fibrosa due to secondary hyperparathyroidism. Since the osteitis fibrosa grades are non-parametric it was not possible to calculate any possible linear relationship between the number of marrow mast cells and the severity of the osteitis fibrosa. There was a wide range of mast cell numbers within each osteitis fibrosa grade, with a tendency towards most mast cells in the bones showing the more severe grades of osteitis fibrosa. Comparison of the mean numbers of mast cells in bone with osteitis fibrosa grades 0 to 2 and grades 2.5 to 5 showed more mast cells in the group with severe osteitis fibrosa (table III) when biopsy and necropsy specimens were taken together ($P < 0.05$). There were similar numbers of mast cells in the non-fibrous parts of the marrow in the two osteitis

Group	Number	Mast Cells per mm ² Marrow			Comparisons with Controls (Mann-Whitney Test)
		Range	Mean	Median	
Control	61	0-33.76	3.93	1.95	—
Renal (necropsies)	23	0.96-29.51	8.65	7.62	$P < 0.001$
Renal (biopsies)	22	1.18-55.63	19.09	14.87	$P < 0.001$
All renal	45	0.96-55.63	13.75	9.55	$P < 0.001$

Table II Marrow mast cell values in 61 controls and 45 patients with chronic renal failure

Group	Osteitis Fibrosa Grade 0-2			Osteitis Fibrosa Grade 2.5-5		
	Number	Mast Cells per mm ²		Number	Mast Cells per mm ²	
		Mean	Median		Mean	Median
Biopsies	10	12.84	7.68	12	24.30 ^a	21.18
Necropsies	16	8.02	6.84	7	10.08 ^a	7.98
Combined	26	9.88	6.83	19	19.06 ^a	15.51

Table III Relationship between number of marrow mast cells and severity of osteitis fibrosa

^aSignificant difference between groups with grade 0-2 and 2.5-5 osteitis fibrosa, $P < 0.05$

^bNo significant difference between groups grade 0-2 and 2.5-5 osteitis fibrosa, $P > 0.10$

fibrosa groups in the 24 cases in which differential counts were made. Thus the groups osteitis fibrosa grades 0-2 and 2.5-5 had respective means of 11.74 and 11.47 mast cells per mm² of mixed fatty and cellular marrow.

Plotting the numbers of osteoclasts per square millimetre of bone against the corresponding numbers of total marrow mast cells or non-fibrous marrow mast cells revealed a random scatter of points with no suggestion of any significant relationship. The number of marrow mast cells was not related to the amount of woven bone formation.

Number of mast cells and state of bone mineralization

The presence of osteomalacia was not related to the number of marrow mast cells. The values for the range, mean, and median for 10 cases with osteomalacia were 0.96-29.51, 12.76, and 10.01 mast cells per mm², respectively. The corresponding values for the 35 cases without osteomalacia were, respectively, 1.18-55.63, 14.04, and 9.55 mast cells per mm².

Attempts to relate the amounts of osteoid, expressed as a percentage of a measured area of bone and marrow, to the numbers of marrow mast cells were unsuccessful in osteomalacic and non-osteomalacic samples. Similarly, there was no significant relationship between the percentage mineralization of cancellous bone and the number of marrow mast cells.

Discussion

The present observations indicate that mast cells are to be found in histological sections of iliac bone marrow in the majority of individuals. We have regarded as tissue mast cells all those mononucleated cells in the marrow with intracytoplasmic metachromatic granules. It seems to be generally accepted that the solubility characteristics of basophilic leucocytes make them unrecognizable in tissue when an aqueous or alcoholic fixative has been used (Johnstone, 1954), whereas human mast cell granules are relatively insoluble in watery fixatives (Johnstone, 1954; Kramer and Windrum, 1955; Sagher and Even-Paz, 1967). The solubility of mast cell granules varies between different animal species. Mouse, rat, and human mast cell granules are well preserved after formalin fixation but non-aqueous fixatives are necessary in the case of lower vertebrates and in the guinea-pig and dog (Benditt and Dunn, 1963). The shape of mast cells was noted to vary, most being ovoid or rounded but some were elongated around the surface of fat cells or were distinctly spindle shaped. Granules were frequently sufficiently dense and numerous to obscure nuclear detail. Individual mast cells were scattered throughout the marrow

with a tendency to lie alongside cancellous trabeculae, around blood vessels or arranged in groups with wide intervening areas devoid of mast cells. This type of distribution inevitably limits the precise value of mast cell counts made in small areas which may not necessarily be representative. Any changes observed in serial bone biopsies from individual patients must therefore be interpreted with caution. In the present study we are concerned mainly with a comparison of two different groups of subjects so that any differences encountered between the groups as a whole may be regarded as meaningful.

In the control necropsy series comprising 61 individuals, the distribution of values for the numbers of marrow mast cells was not a normal one and we have therefore not quoted the mean and standard error. The control values were range 0.33-76, arithmetic mean 3.93, and median 1.95 mast cells per mm² marrow. We have no explanation for the fact that 10% of individuals had mast cell values in excess of 10 per mm² marrow, and these values were apparently unrelated to the mode of death.

In the group of 45 patients with chronic renal failure taken as a whole there were significantly more marrow mast cells (arithmetic mean 13.75 and median 9.55 per mm²) than in the control group ($P < 0.001$). Again the distribution of values was not a normal one and comparisons between the various groups throughout this study have been made using the appropriate Mann-Whitney test (Snedecor and Cochran, 1971). Within the renal group there was a significantly higher range of mast cell values for those samples obtained by iliac crest biopsy as compared to those obtained at necropsy. This raises the possibility that better preservation of mast cells and hence easier recognition result from rapid fixation of the biopsy samples, whereas in the case of the necropsy specimens there would inevitably be a delay between death and the subsequent fixation of tissues at the time of the necropsy. We cannot exclude this possibility, but it does not alter the significance of the increased numbers of marrow mast cells observed in renal disease since even in the necropsy specimens significantly more mast cells occurred in the renal failure patients than in the controls ($P < 0.001$). Furthermore, we have observed in serial bone biopsies from patients treated by renal transplantation, where conditions of fixation of specimens were identical, a return of marrow mast cells to the control range.

The difference between renal biopsy and necropsy cases may also be attributable in part to the fact that there were more severe examples of osteitis fibrosa in the biopsy series with more marrow fibrous tissue which included frequent mast cells. In the bone of patients with chronic renal failure there was com-

monly evidence of osteitis fibrosa due to secondary hyperparathyroidism. Mast cells were often conspicuous within the zones of marrow fibrosis in such cases, and we were able to demonstrate a significant relationship between the total number of marrow mast cells and the amount of marrow fibrosis. We have also observed mast cells in the fibrous tissue of organizing iliac bone biopsy sites, and it is well recognized that mast cells may occur in many sites of repair where there is connective tissue proliferation such as fracture callus (Selye, 1965; Lindholm *et al*, 1969; Lindholm and Lindholm, 1970). From our observations we are not certain that all the cells counted as mast cells in the marrow fibrous tissue are true mast cells. Some of the cells may have been fibroblasts which had taken up metachromatic granules from adjacent disrupted mast cells (Higginbotham *et al*, 1956).

That the mast cells in marrow fibrous tissue did not alone account for the increased numbers of marrow mast cells in renal failure was shown by the finding of increased numbers of mast cells in the mixed fatty and haemopoietic marrow of these patients. This suggests that there is some other factor in renal failure which is responsible, at least in part, for the increase in marrow mast cells. This concept is supported by the report that mast cells are increased in tissues other than the bone marrow in chronic renal failure, such as the spleen (Neiman *et al*, 1972). These authors put forward the hypothesis that hyperparathyroidism, which is almost invariably present in chronic renal failure (Gilmour, 1947; Ellis and Peart, 1973), may account for tissue mast cell proliferation since there is evidence to suggest in experimental animals that parathormone stimulates the accumulation of mast cells in bone (Rockoff and Armstrong, 1970; Rasmussen, 1972). This could be the explanation for the excess of mast cells in the fibrous tissue component of the osteitis fibrosa and in the remaining non-fibrous marrow. To test this hypothesis we have tried to relate the severity of the osteitis fibrosa, representing the degree of hyperparathyroidism, to the number of mast cells both in the marrow as a whole and in the non-fibrous marrow. The results showed a tendency towards greater numbers of mast cells in those bone samples with the more severe grades of osteitis fibrosa. When the combined renal biopsy and necropsy samples were considered, there was a low order of significant difference ($P < 0.05$) between the number of total marrow mast cells in the group with osteitis fibrosa grades 0 to 2 and that with grades 2.5 to 5. This difference was almost certainly associated with the greater amounts of fibrous tissue with mast cells in the marrow of the samples with more severe grades of osteitis fibrosa.

Thus there were similar numbers of mast cells in the non-fibrous parts of the marrow in the two osteitis fibrosa groupings in 24 individuals where differential counts of mast cells in fibrous and non-fibrous marrow were made. As another measure of hyperparathyroidism (Ellis and Peart, 1973) we considered the number of osteoclasts per square millimetre of bone in relation to the number of mast cells but there was no significant relationship. We therefore have no definite evidence to indicate a relationship between the degree of hyperparathyroidism and the number of marrow mast cells. Indeed, what evidence exists suggests that there may not be any direct relationship. Preliminary observations we have made in serial bone biopsies from patients with chronic renal failure before and after treatment with calcium carbonate and aluminium hydroxide or by parathyroidectomy, and in whom there has been a fall in serum parathormone and a reduction in the severity of osteitis fibrosa histologically, have shown no corresponding reduction in the excess of mast cells. This is against the hypothesis that there is a relationship between hyperparathyroidism and numbers of marrow mast cells. A further difficulty in any attempt to relate the number of marrow mast cells to the degree of osteitis fibrosa is the duration of the changes. The number of mast cells may be a function of the stage of development of the osteitis fibrosa and the age of the marrow fibrous tissue component. By analogy with the changing numbers of mast cells in the processes of organization and repair elsewhere, such as in the healing of a wound (Wichmann, 1955), one would expect most mast cells to be present during the active phase of marrow fibrosis and subsequently to be reduced.

Johnstone (1954) observed that the presence and number of mast cells in the marrow were not related to the iron content of the marrow or to the degree of erythropoiesis and that anaemia was the only factor common to all the cases in which mast cells were prominent. The majority of our patients with chronic renal failure had some degree of anaemia but there was no relationship between the number of marrow mast cells and the haemoglobin concentration of the blood within the group of renal patients.

There are no data in the literature strictly comparable with that presented here but Rebel *et al* (1974) have reported marrow mast cell counts in 21 patients with renal failure treated by haemodialysis in the range 0 to 60.0 per mm², which is within the range we have found in renal failure. These authors do not report any control values however. In 11 other patients with, for example, osteomalacia or osteoporosis, the range was 0.1 to 23.0 mast cells per mm² of marrow. Highest marrow mast cell values were

observed in patients with osteomalacia whether this was the result of renal failure or other causes, and the authors suggested that osteomalacia alone is sufficient to bring about an increase in the number of marrow mast cells. We were unable to confirm this relationship between osteomalacia and numbers of marrow mast cells in our patients with chronic renal failure, and there was no significant relationship between numbers of mast cells and the amount of osteoid per cent of the measured bone area, or the percentage mineralization of cancellous bone. The nature of the bone changes in chronic azotaemia depends upon the severity and duration of the renal disease. Hyperparathyroidism occurs at an early stage, so that in an unselected series of patients with chronic renal failure one can expect about 90% to show histological evidence of osteitis fibrosa whereas osteomalacia, which develops later, occurs in only about one-third (Ellis and Peart, 1973). In any unselected series of patients with chronic renal failure the duration and severity of osteomalacia will vary from case to case. The rate of formation of osteoid also seems to vary so that some patients show relatively little increase in osteoid in serial biopsies whereas in others it is more rapid, and large amounts of osteoid accumulate and make a significant contribution to osteosclerosis. If there is a relationship between the numbers of marrow mast cells and osteomalacia then it might be that mast cells are associated with some particular stage in the development or persistence of osteomalacia. The incidence of osteomalacia in haemodialysed patients increases with time on dialysis (Ellis and Peart, 1971a) so that in a series of dialysed patients, such as that reported by Rebel *et al* (1974), some patients would have recent and active development of osteomalacia. If this early stage of osteomalacia is associated with increased marrow mast cells, then this could explain the discrepancy between the results of these authors in haemodialysed patients and in our own non-dialysed patients. We are currently accumulating data on patients treated by long-term haemodialysis who have developed osteomalacia and we hope to be able to elucidate this aspect of the problem in the future. Increased numbers of mast cells alongside bone cancellous trabeculae have been described in rats on a low-calcium and vitamin D deficient diet (Shipley *et al*, 1922; Urist and McLean, 1957; Cass *et al*, 1958) and the mast cells were reduced in number after treatment with vitamin D.

Increased numbers of marrow mast cells appear to be associated with experimental resorption and remodelling of bone whatever the cause (Gillman, 1958; Lalich, Grouper, and Jolin, 1965; Severson, 1969), and it is possible that the presence of excess mast cells in azotaemia is a secondary manifestation

of bone resorption and formation. The precise relationship between mast cells and bone resorption and formation does not appear to have been resolved. Local effects of heparin may be responsible since it is known that the administration of heparin and similar but synthetic polysaccharides to experimental animals is associated with bone resorption and impaired ossification (Gillman and Naidoo, 1957; Ellis, 1965). Osteoporosis has also been reported following the long-term administration of heparin to patients (Griffith *et al*, 1965; Jaffe and Willis, 1965; Schuster *et al*, 1969). The mechanisms involved are unknown although it has been shown that heparin stimulates collagenase activity (Asher and Nichols, 1965; Sakamoto *et al*, 1973) and that heparin is capable of releasing lysozymal enzymes which might be involved in bone resorption (Sledge and Blackburn, 1968). It has been shown (Ellis and Peart, 1970) that heparin increases the resorption of diaphyseal bone in cultured mouse limb bones, suggesting that there is a direct effect of heparin on bone. This effect occurred, however, only with higher concentrations of heparin than are reached in the blood and tissues of intact animals even after prolonged administration. For the *in vivo* changes to be the result of a direct toxic effect on bone there would have to be a selective accumulation of heparin in bone tissues. The excess marrow mast cells in the present cases are, of course, already near to the bone, and there might be localized zones of high heparin concentration. Others have suggested that the bone resorptive effect of heparin is an indirect one and mediated through hyperparathyroidism resulting from the hypocalcaemia following the binding of serum calcium ions by heparin (Hahnemann, 1965). This hypothesis is not borne out by the observations that calcium heparin is equally effective as sodium heparin in inducing osteoporosis (Ellis, 1965) and that parathyroidectomy does not prevent development of heparinoid osteopathy (Ellis and Peart, 1971b). There is also evidence from tissue culture experiments to suggest that heparin may enhance bone resorption by acting as a parathormone co-factor (Goldhaber, 1965).

In discussing the possible local effects on bone of mast cells, one has to consider the relatively rare condition of systemic mastocytosis where there is infiltration of the skin and other tissue by mast cells (Sagher and Even-Paz, 1967). Bone sections have been reported as showing resorption of trabeculae related to collections of mast cells, deposition of fibrillary lamellar bone, and trabecular thickening, marrow fibrosis, and osteosclerosis (Stark *et al*, 1956; Jensen and Lasser, 1958; Havard and Scott, 1959; Lerner and Lerner, 1960). In azotaemic renal osteodystrophy osteosclerosis may occur when the

amounts of osteoid and mineralized woven bone formed exceed the loss of mineralized lamellar and woven bone due to excess osteoclasts (Ellis and Peart, 1973). Woven bone is particularly rich in sulphated polysaccharides but we could not find any definite relationship between the numbers of marrow mast cells and the total amount of bone or the amount of woven bone in the present cases.

In conclusion, our results indicate that there is an increase in the number of mast cells in the marrow of patients with chronic renal failure. Although there are commonly numerous mast cells within the fibrous parts of the marrow forming part of the osteitis fibrosa reaction, we have been unable to explain the increased mast cells in renal failure solely on the basis of hyperparathyroidism. In addition, there does not appear to be any clear relationship in the present cases between the number of marrow mast cells and the presence or severity of a bone mineralization defect.

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