Foetal Development in Experimental Uraemia

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Successfully terminated pregnancies in dialysed uraemic women have been reported in recent years (Confortini et al, 1971). The newborns did not show roentgenological signs of bone disease, although rapid development of the foetal skeleton occurred in a biochemical environment that causes metabolic bone disease in the maternal skeleton despite the comparatively slower turnover of the latter.

It was therefore the purpose of the present study to investigate whether impaired renal function in the mother animal affects osteogenesis and erythropoiesis in the foetus.

MATERIAL AND METHODS

Inbred Sprague-Dawley rats (200 g) were given free access to food (Altromin 1324; 13,700 mg Ca; 9,350 mg P; 1000 IU vit D3/kg) and water throughout the experiment. Five female rats and 2 male rats were housed in one cage. The onset of pregnancy was determined from the presence of spermatocytes in vaginal smears obtained daily at 8 am. The pregnant animals were marked and subjected to 5/6 nephrectomy in the morning of the 13th day after conception, when skeletal mineralisation in foeti is known to begin. Under ether anaesthesia the left kidney was decapsulated and removed through a dorsal incision after ligation of the renal artery. The right renal artery was clipped temporarily. Then the upper and lower poles were removed and haemorrhage prevented by topical application of HistacrylR. Serum urea rose to 112 ± 18.4 mg/100ml (at term). Every other pregnant animal was sham operated (decapsulation of both kidneys). On the morning of the 21st day (spontaneous delivery expected afternoon of the 21st day) the foeti were removed surgically. Foetal blood was collected after incision of the neck. Material for histology was fixed with 5% buffered formalin, dehydrated, embedded in paraffin or methylmetacrylate, cut and stained with Masson-Goldner, H. E., v. Kossa and alcian blue-PAS.
RESULTS

The maximal length of the extended foeti (hind foot-snout) was only slightly, albeit significantly, diminished in foeti of uraemic rats, pointing to an almost unimpaired longitudinal growth; in contrast, their body weight was considerably reduced (possibly an indication of placental malfunction). While the absolute weights of the kidneys, livers and adrenals were considerably diminished, the reductions were roughly proportional to the reduction of body weight, the ratio of organ weight/body weight being almost normal. Specifically there was no compensatory hypertrophy of the kidneys.

Skeletal Development

The degree of uraemia encountered in the uraemic mother animal causes changes of intermediary metabolism (Krempien & Ritz, 1971) and a marked disturbance of mineralisation rats, (Krempien et al, 1972) in bones of adult rats as described elsewhere.

In foeti of uraemic rats cartilage growth in the epiphyseal plate was unimpaired as shown by the almost normal foetal length. Histological analysis of enchondral (vertebrae tibia metaphysis) and desmal (calvaria) bone formation centres, as well as studies of dental development revealed essentially no difference between foeti of uraemic and sham-operated rats (Figures 1-4). Bone mass seemed to be somewhat reduced and bone maturation to be less advanced in foeti of uraemic rats, presumably a consequence of slower foetal development (also indicated by lower birth weights). Studies of the ossification centres with v. Kossa stain gave no evidence of defective mineralisation of the provisional zone of calcification in epiphyseal cartilage. Similarly, there was no excess of osteoid in metaphyseal spongiosa. It must be kept in mind, however, that v. Kossa stain (strictly a phosphorous stain) gives only information about the onset and not about the final intensity of mineralisation. The transformation of epiphyseal growth cartilage into primary spongiosa was orderly, unlike that seen in post-natal rickets or in growing uraemic children (Ritz et al, 1973).

A suggestive increase of focal cellular activity at the endosteal surface was seen in the bones of foeti after intrahepatic injection of 3 IU PTH (Lilly Co) on the 18th day, but not in the foeti of rats after maternal intraperitoneal injection of 100 IU/day from the 18th-21st day, pointing to the relative impermeability of the placenta to exogenous PTH. Parathyroid glands are already functioning in the foetus (Norris, 1946) and undoubtedly react to hyper- or hypocalcaemia (Bodansky & Duff, 1941; Garel et al, 1971). No such effect was seen in foeti of uraemic rats.

It has been stated that in human beings defective mineralisation is seen in the newborns of vitamin D deficient mothers (Maxwell & Turnbull, 1932); yet we failed to see abnormalities of mineralisation with the v. Kossa stain
in the foeti of rachitic mother rats raised on a McCollum diet. This finding might indicate that woven foetal bone mineralises in the absence of vitamin as does woven bone in adults (Ball & Garner, 1966).

**Erythropoiesis**

Both haematocrit of foetal blood and qualitative histological evaluation of

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**Figure 1.** Vertebral body of a foetus of a uraemic rat (undecalcified section, v. Kossa stain, microphotograph x 110). Prospective intervertebral discs on top and on bottom; growth cartilage underneath intervertebral discs; ossification centre in the middle of the vertebral body. Note the orderly mineralisation of chondroid between hypertrophic cartilage cells and of the primary spongiosa trabeculae (mineral stained black with v. Kossa). (Enchondral bone formation)
extramedullary erythropoiesis in liver sinusoids did not give any evidence of grossly impaired erythropoiesis in the foeti of uraemic rats.

Renal Function
The reaction of the foetal kidneys to the uraemic environment was reflected both by the composition of the amniotic fluid relative to foetal serum (excretion

Figure 2. Calvaria of a foetus of a uraemic rat (undecalcified section, v Kossa, microphotograph x 150). Formation of the calvaria directly from fibrous tissue (in membrane) without intervention of a cartilaginous mould (desmal bone formation). Note the well mineralised bone trabeculae in the anlage of the calvaria. (Skin to the right, anlage of calvaria in the middle, brain to the left)
of urea, Table I) and by the histological finding of an increased number of patent proximal tubuli with well differentiated epithelium, presumably as a consequence of increased diuresis.

In conclusion, evidence of hyperparathyroidism or mineralisation defects was absent in the skeletons of foeti of uraemic rats. This could indicate either that the foetal kidney takes over the metabolic role of the maternal organ in
Figure 4. Metaphyseal spongiosa in a foetus of a uraemic rat (undecalcified section, v. Kossa stain, microphotograph x 375). Note the good mineralisation of the trabeculae and the absence of broad osteoid seams.

the synthesis of 1,25 dihydroxycholecalciferol (transplacental transfer of vitamin D is known to occur, Hassad et al, 1971), or that mineralisation of primitive foetal woven bone may occur even in the absence of vitamin D metabolites.

The lack of foetal anaemia or the lack of a gross disturbance of extramedullary foetal erythropoiesis may similarly be explained either by erythro-
Table I

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<th>A (urea)</th>
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(1) Foeti of sham-op mother rats (7 litters; n=49)
(2) Foeti of uraemic mother rats (7 litters; n=48)
(3) Wilcoxon-test
(4) A = amniotic fluid
(5) mean + standard error (x + SEM)
(6) S = foetal serum
(7) Wilcoxon test for paired differences (measurements in individual foeti)
(8) Organ weight (dry weight) x 1000/body weight (wet weight)
(9) difference between litter means in brackets
poietin formation on the foetal kidney or by the ability of foetal erythroblasts to proliferate in the absence of erythropoietin.

ACKNOWLEDGMENT

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OPEN DISCUSSION

G SCHUTTERLE (Giessen): You do not make any remarks about the duration of the uraemia in the maternal rats, and none about their anaemia, urea and creatinine.

RITZ: As a matter of fact I mentioned that the mother animal was nephrectomised on the 13th day of the 21 days pregnancy. The serum urea levels at term in the mothers was 112mg/100ml, so they are quite uraemic. Uraemia started at the moment when foetal mineralisation is known to begin.

M KAYE (Montreal): Do you hypothesize that the foetus either synthesises vitamin D or does not need it? What evidence do you have that non-pregnant uraemic rats in your model are unable to synthesise the active form of vitamin D?

RITZ: This is a very tricky question. There is no direct evidence, of course, but there is indirect evidence if you accept the data of the decrease of the rate of mineralisation that we presented in the tibial diaphysis. One could
even hypothesize that if there is impairment of mineralisation in the mother, one would expect it to occur in the foetus because by comparison its rate of turnover is so incredibly high. Thus you should be able to pick up even minimal impairment histologically. I did not present additional data from other reports we have done, but we have also studied the foeti of rachitic animals raised on a McCollum diet. To our great surprise and in contrast to reports in the literature, we did not find any osteoid in these foeti either. As things are at the moment, I am more in favour of the second hypothesis that vitamin D is not required to mineralise foetal bone. This is currently under study.

SCHUTTERLE: How does the weight loss of the maternal uraemic rats compare with normal or sham operated rats?

RITZ: I am afraid that we did not measure this. The experiment was not done with pair feeding, since the male rats were terribly non-enterprising and did not impregnate our female rats at predicted intervals, so that we could never have two corresponding pregnant animals on which to do pair feeding studies.