Virchows Arch. A Path. Anat. and Histol. 376, 145–157 (1977)

Virchows Archiv A

Fetal Development in Experimental Uremia *

Eberhard Ritz, Burkhard Krempien, Gabriele Klefisch, Theresia Ritter, and Eva Krause

Medizinische Univ.-Klinik (Direktor: Prof. Dr. G. Schettler) and Pathologisches Institut (Direktor: Prof. Dr. W. Doerr) der Universität Heidelberg

Summary. Uremic women on hemodialysis with metabolic bone disease (hyperparathyroidism, osteomalacia resulting from defective vitamin D metabolism) and anemia (erythropoietin deficiency) are known to give birth to infants without bone disease or anemia. Therefore, skeletal development (enchondral and desmal bone formation) and hepatic erythropoiesis were evaluated in fetuses of uremic rats. These fetuses failed to show defective mineralisation or evidence of bone disease. Bolus injection of high doses of exogenous PTH into the maternal or fetal organism did not affect fetal bone histology. In addition, no apparent defect of bone mineralisation or bone formation was found in fetuses of ricketic rats. Normal mineralisation in the offspring of uremic rats may be explained by fetal hyperphosphatemia and/or insensitivity of fetal (woven) bone mineralisation to vitamin D.

Absence of fetal anemia (normal hematocrits, normal density of hematopoietic cells in the liver) in the presence of maternal anemia is presumably due to the insensitivity of fetal erythropoiesis to erythropoietin.

Key words: Uremia – Uremic osteodystrophy – Parathyroid hormone – Vitamin D – Erythropoietin.

Zusammenfassung. Neugeborene urämischer hämodialysierter Mütter zeigen weder eine Knochenerkrankung noch eine Anämie, obwohl eine metabolische Osteopathie des mütterlichen Skelets (sekundärer Hyperparathyroidismus, Osteomalacie infolge gestörten Vitamin-D-Stoffwechsels) und eine Anämie (Ausfall der Erythropoetinsynthese) bestehen. An Feten urämischer Ratten wurden daher Knochenbildung und Erythropoese untersucht. Die Knochenentwicklung war in Skeletabschnitten mit enchondraler und desmaler Verknöcherung unauffällig. Die Mineralisation von Knorpel (präparatorische Verkalkungszone) und Osteoid erfolgte regelrecht. Nach einer Stoßinjektion

* With the support of "Deutsche Forschungsgemeinschaft"

For offprints contact: Prof. Dr. E. Ritz, Med. Univ.-Klinik (Ludolf-Krehl-Klinik), Bergheimer Straße 58, D-6900 Heidelberg, Federal Republic of Germany

hoher Dosen exogenen Parathormons in den mütterlichen oder fetalen Kreislauf wurde keine Abweichung des fetalen Knochenaufbaus beobachtet. In den Feten rachitischer Mutterratten konnte kein typischer rachitischer Mineralisationsdefekt nachgewiesen werden. Die unauffällige Mineralisation im Skelet von Feten urämischer Ratten – bei nachweisbaren Mineralisationsdefekten im Skelet ausgewachsener urämischer Ratten – wird auf die fetale Hyperphosphatemie und/oder die Fähigkeit des embryonalen Faserknochens, auch bei fehlender Vitamin-D-Einwirkung zu mineralisieren, zurückgeführt.

In recent years several pregnancies with successful outcome have been reported in dialysed uremic women (Herwig et al., 1965; Orme et al., 1968; Mitra et al., 1970; Confortini et al., 1971; Goldsmith et al., 1971; Parsons et al., 1975). The offspring did not show roentgenological signs of bone disease, although rapid development of the fetal skeleton occured in a biochemical environment that causes metabolic bone disease in the maternal skeleton, despite the comparatively slower turnover of the latter. In addition, anemia was not an outstanding feature in the newborn, although their mothers exhibited the characteristic anemia of endstage renal failure.

Uremia in the adult rat causes (1) parathyroid hyperplasia (as shown by Jastak et al., 1968, confirmed by ourselves), (2) a reduction in the rate of osteoid mineralisation (Krempien et al., 1972), (3) a depression of bone collagen synthesis (Krempien et al., 1972a) and (4) abnormal histology of growth apparatus and bone (Mehls et al., in press). Using this well established model of experimental uremia, we investigated whether impaired renal function in the mother animal affects osteogenesis and erythropoiesis in the fetus.

Table 1

	A[d] mosm/l	A (Na) mval/l	A (Urea) mg%	S[f] (Urea) mg%	ΔA–S (Urea) mg%	Length cm	Body weight (wet) g
Control[a]	370 ±8.12[e]	195 ±3.29	56.9 ±1.59	57.3 ±1.06	0.079 ±1.59[a] (a)	3.66 ±0.0233	3.58 ±0.041
Uremia[b]	417 ±9.81	193 ±4.40	113 ±3.48	106 ±3.17	7.53 ±2.01[b] (b)	3.51 ±0.0264	2.71 ±0.086
P[c]	0.001	N.S.	0.001	0.001	(a) N.S.[a] (b) 0.01[b]	0.001	0.001

[a] Fetuses of sham-op mother rats (7 litters; $n=49$)
[b] Fetuses of uremic mother rats (7 litters; $n=48$)
[c] Wilcoxon-test
[d] A = amniotic fluid
[e] Mean ± standard error ($\bar{x} \pm$ SEM)

Material and Methods

Experimental Uremia

200 g inbred Sprague.Dawley rats were given free access to food (Altromin 1324; 13,700 mg/kg Ca; 9350 mg/kg P; 1000 IU vitamin D3/kg) and distilled water throughout the experiment. 5 female rats and 2 male rats were housed in one cage. The onset of pregnancy was determined by monitoring for the presence of spermatocytes in vaginal smears (unstained; airdried; obtained daily at 8 h a.m.). Pregnant animals were subjected to 5/6 nephrectomy in the morning of the 13th day after conception, when skeletal mineralisation is known to begin. Since the aim of the investigation was to study possible abnormalities in skeletal mineralisation and development, this time was chosen for nephrectomy. Under ether anesthesia, the left kidney was decapsulated through a dorsal incision and removed after ligation of the renal artery. The right renal artery was clipped temporarily. The upper and lower poles of the right kidney were removed. Hemorrhage was prevented by topical application of Histoacryl® (Braun Co., Melsungen). Serum urea rose to 124 ± 17 mg%, when measured 7 days after the operation. Every other pregnant animal was sham-operated (decapsulation of both kidneys). On the morning of the 21st day (spontaneous delivery: afternoon of the 21st day) the fetuses were removed surgically under Nembutal® anaesthesia. Fetal blood was collected after incision of the neck. 0,2 ml amniotic fluid were removed with a suction pipette. Na, urea and osmolality were measured by micromethods. Hematocrit was determined by the use of a microcentrifuge. The fetuses were cleaned with a swab and weighed immediately on an electrical balance. Organs were weighed wet and after drying for 12 h under red light. Bones were fixed with 70% alcohol, parenchymatous organs with 5% formalin. The material was subsequently dehydrated, embedded in methylmetacrylate and cut using a Jung microtome K. The sections were stained after Masson-Goldner, hematoxilin-eosin, alcian blue – PAS and Krutsay (1963). Microradiographs were prepared from fetal tibiae using undecalcified ground sections (Kodak high resolution plates; 15 kV; 30 mA; fine focus Cr-anode; section thickness 100 μ). Sections of the liver (10 μ; embedded in methylmetacrylate; stained after Giemsa) were evaluated at × 1000 magnification using a measuring eye-piece with a counting grid (Wild u. Co., Heerbrugg). Nuclei of the hepatocytes and hematopoietic cells were counted in 100 fields (80×80 μ).

Hematocrit %	Kidneys mg dw	Kidneys mg/g[h]	Liver mg dw	Liver mg/g[h]	Adrenals mg dw	Adrenals mg/g[h]
37.4 ±0.072	4.11 ±0.072	1.15 ±0.014	71.5 ±1.37	18.3 ±0.354	0.654 ±0.204	0.189 ±0.0061
36.9 ±0.568	2.93 ±0.103	1.09 ±0.017	55.7 ±1.51	17.3 ±0.454	0.481 ±0.0231	0.181 ±0.0055
N.S.	0.001	0.01 (N.A.)[i]	0.001	N.S. (N.S.)[i]	0.001	0.01 $0.1 < P < 0.05$[i]

[f] S = fetal serum
[g] Wilcoxon test for paired differences (measurements in individual feti)
[h] Organ weight (dry weight) × 1000/body weight (wet weight)
[i] Difference between litter means

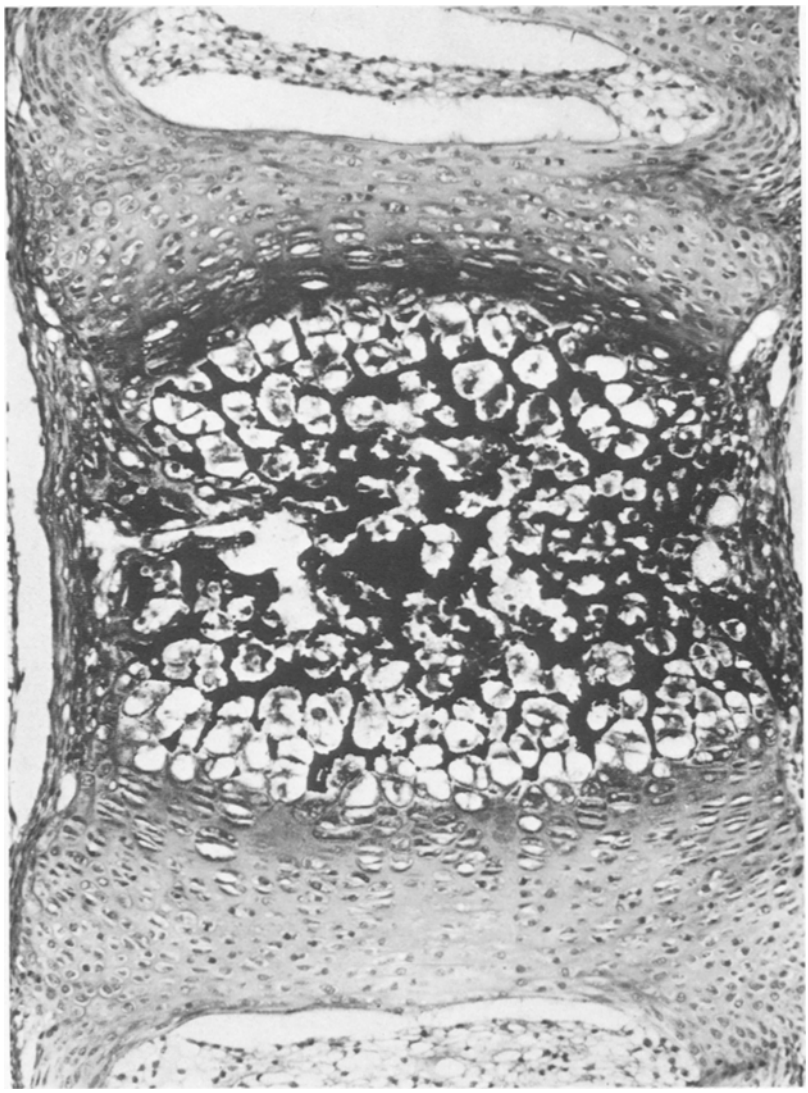

Fig. 1. Vertebral body of a fetus of a uremic rat (undecalcified section, Krutsay-stain, microphotograph ×110). Prospective intervertebral discs on top and bottom; growth cartilage underneath intervertebral discs; ossification center in the middle of the vertebral body. Note the orderly mineralisation of cartilage between hypertrophic cartilage cells and of the primary spongiosa trabeculae

Tetracycline Labelling. In additional experiments, pregnant rats were given 1.0 mg/100 g tetracycline (Reverin®) intraperitoneally on the 20th and 21st day of their pregnancies. The 2nd injection was administered 3 h before the fetuses were removed surgically.

Injection of PTH to Fetuses. On the 19th day of pregnancy, fetuses of pregnant SD rats (320 g) were given 0.3 ml, 0.6 ml or 0.9 ml PTH Lilly (1 ml = 100 IU USP) intrahepatically. The abdomen of the mother animal was opened surgically; the fetuses could be identified through the myometrium. Those in the right horn of the uterus were given saline and those in the left horn were given

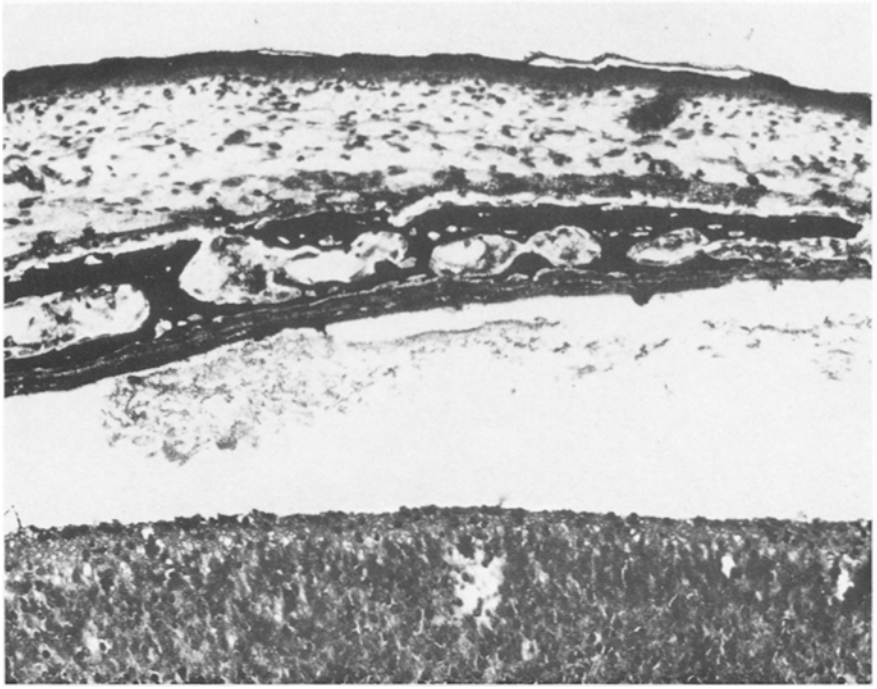

Fig. 2. Calvaria of a fetus of a uremic rat (undecalcified section, Krutsay-stain, microphotograph $\times 120$). Formation of the calvaria directly from fibrous tissue (in membrane) without intervention of a cartilaginous mould (desmal bone formation). Note the well mineralized bone trabeculae in the anlage of the calvaria (skin on top anlage of the calvaria in the middle, brain on bottom)

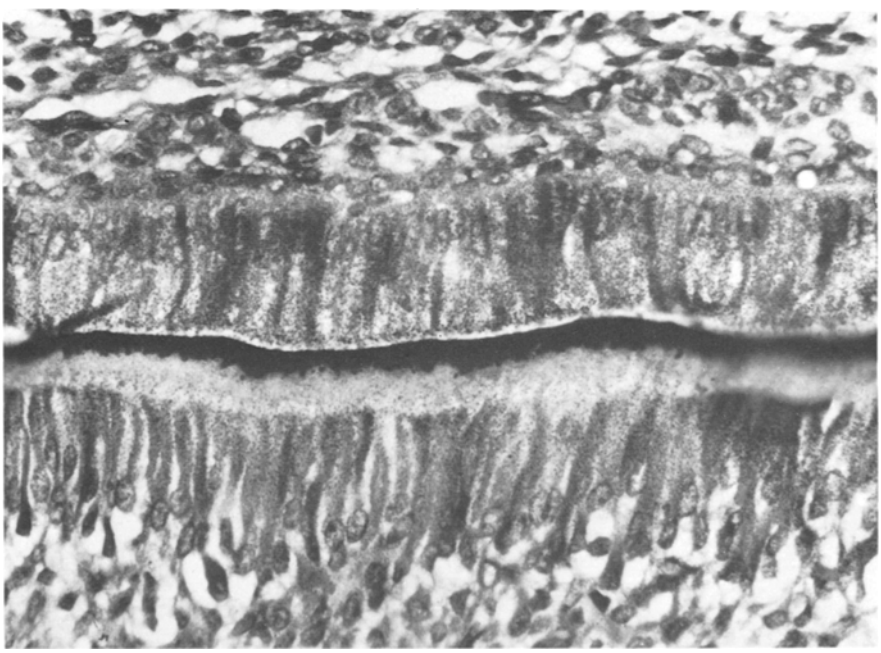

Fig. 3. Dental development in a fetus of a uremic rat (undecalcified section, Krutsay-stain, microphotograph $\times 300$). Ameloblasts (forming enamel) on top, odontoblasts (forming dentin) on bottom. Note the presence of mineral to the right of the ameloblasts

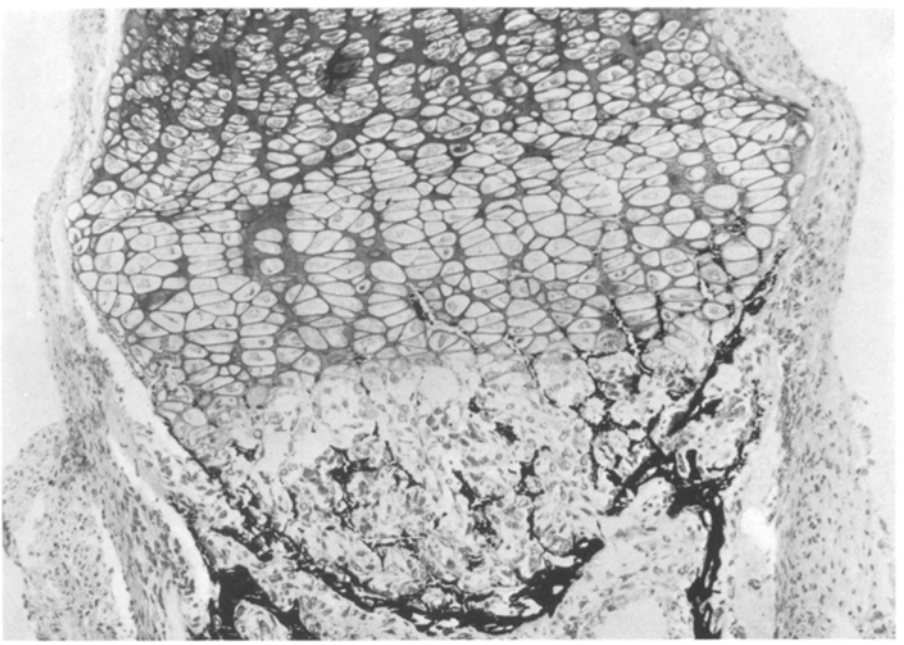

Fig. 4. Tibia of fetus of uremic rat (undecalcified section, Krutsay stain, microphotograph ×78)

rising doses of PTH. The puncture site was closed with Histoacryl® to prevent leakage of the amniotic fluid. In the morning of the 21st day of pregnancy, the fetuses were removed and worked up as described above.

Administration of PTH to Pregnant Rats. Pregnant rats were given 5 ml (=500 IU USP) PTH Lilly intraperitoneally. Fetal and maternal material was removed as described above in the morning of the 21st day of pregnancy.

Fetuses of Ricketic Rats. The animals studied were the f 3 generation of rats who had been put on vitamin D3 deficient diet (Altromin C 1017; Ca/P-ratio 1.1). The respective mother/animals (f 2 generation) were weaned 20 days after birth; they were kept on vitamin D3 free diet and housed in closed boxes without access to UV-light. The animals were handled by one person wearing gloves throughout the experiment. These vitamin D deficient animals were mated with male rats kept under identical conditions. Fetuses (f 3 generation) of the ricketic mother rats (f 2 generation) were removed on the 21st day of pregnancy as described above.

Results

Body and Organ Size (Table 1)

The maximal length of the extended fetuses (hind foot-snout) was only slightly, although significantly, diminished in those from uremic rats, pointing to almost unimpaired longitudinal growth. In contrast, body weight was considerably reduced, possibly an indication of placental malfunction. Placental wet weight was 371 ± 51.4 mg in control and 314 ± 44.3 mg in uremic rats ($P < 0.01$), whereas placental dry weight was 62.4 ± 9.33 mg in control and 52.9 ± 6.71 in uremic

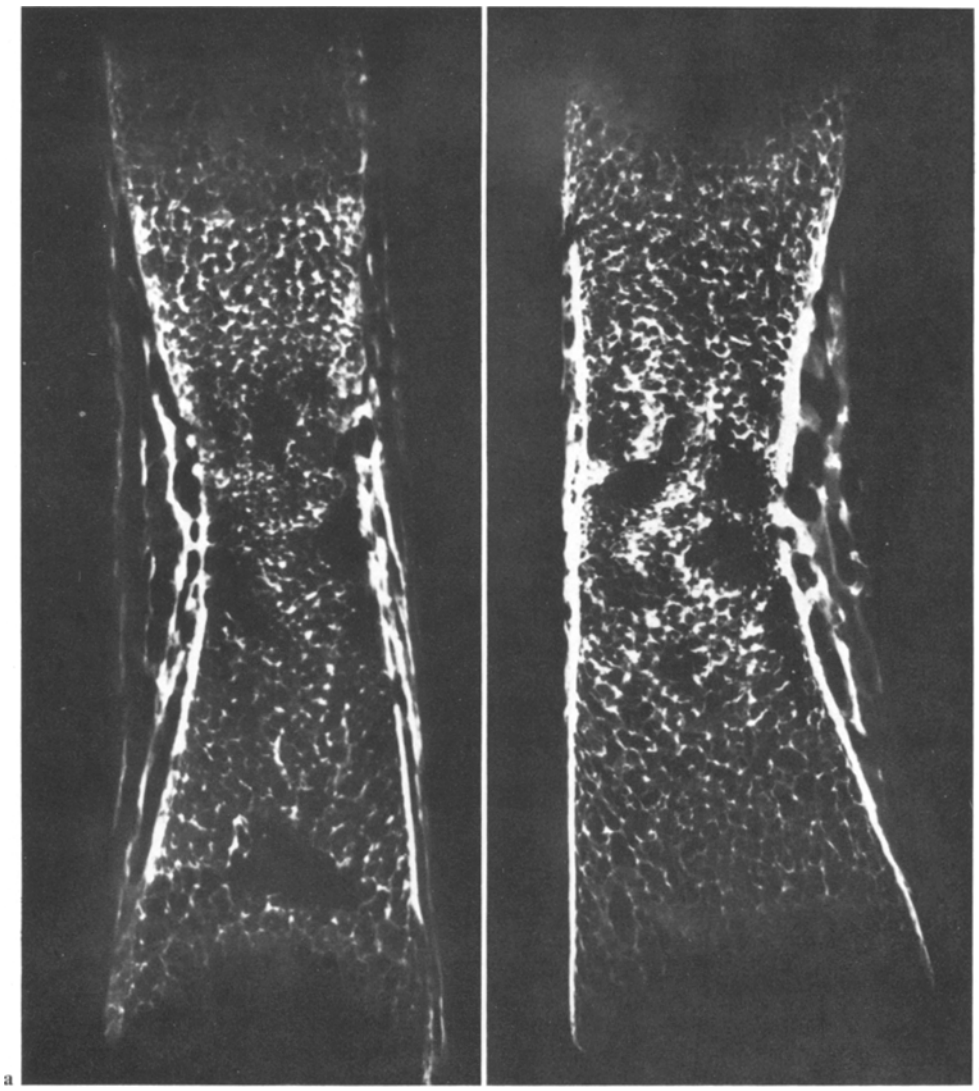

Fig. 5a and b. Fetus of control rat (**a**) and fetus of uremic rat (**b**). Microradiograph of tibia. (Embedding in methylmetacrylate, undecalcified section (100 μ), microradiograph ×40). Enchondral ossification with formation of metaphyseal spongiosa (on top and bottom); diaphyseal collar (cortical bone) in the middle. No difference of cortical width or cortical mineralisation and no difference of enchondral ossification

rats ($P<0.01$). In normal animals, the relation between organ weight and body weight was linear (r [kidneys]$=0.807$). While the absolute weight of the kidneys, livers and adrenals was considerably diminished in uremic animals, the reduction was 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.

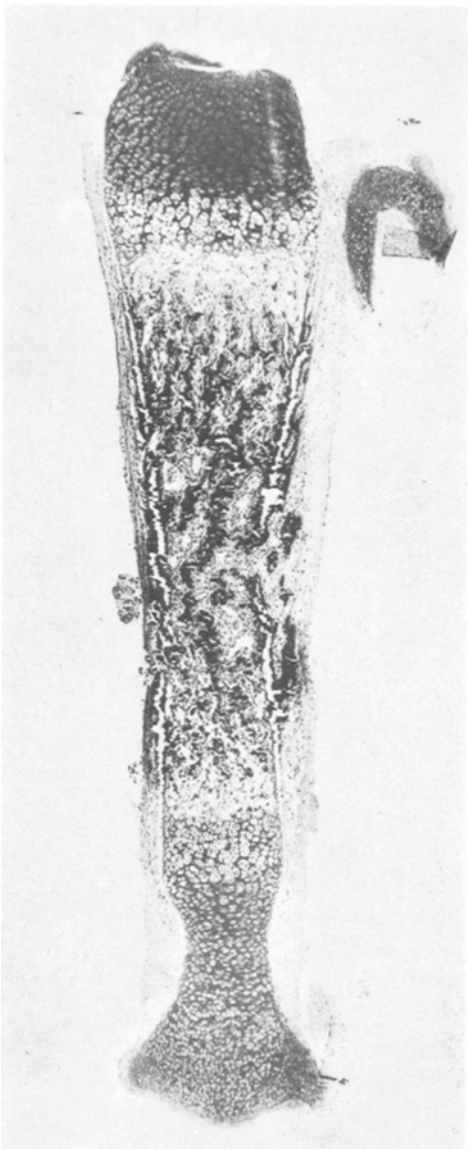

Fig. 6. Fetus of ricketic rat. Proximal tibia. (undecalcified section (5 μ), Masson-Goldner-stain, microphotograph × 60). The zone of proliferating and hypertrophic growth cartilage is not enlarged; no accumulation of chondroosteoid

Skeletal Development

In fetuses from uremic rats the rate of cartilage growth in the epiphyseal plate was unimpaired, as shown by almost normal fetal length. Histological analysis of enchondral (vertebrae, tibiae, metaphyses) and desmal (calvaria) bone formation centers, as well as studies of dental development revealed essentially no difference between fetuses of uremic and sham-operated rats (Figs. 1–3). Bone

mass seemed to be somewhat reduced in fetuses of uremic rats, presumably a consequence of slower fetal development (also indicated by lower birth weights).

Studies of ossification centers with Krutsay stain gave no evidence of defective mineralisation of the provisional zone of calcification in epiphyseal cartilage (Fig. 4). Similarly, there was no excess of osteoid in metaphyseal spongiosa. The presence of normal amounts of mineral in the provisional zone of calcification and in the primary spongiosa could also be demonstrated directly by microradiography (Fig. 5a–b).

It was impossible to judge mineralisation with the tetracycline labelling technique, since tetracycline was diffusely taken up throughout the entire skeleton ("diffuse staining") presumably because mineral content and diffusion impedance of the skeleton were both low.

Histological examination of the proximal tibia of mothers with uremia did not show evidence of uremic bone disease, i.e. fibroosteoclasia or disturbed mineralisation.

In contrast to preliminary impressions in non-quantitative studies (Ritz et al., 1973) semiquantitative evaluation did not show any abnormality of skeletal histology in either the fetuses of rats who had received PTH i.p. or in those who had received PTH intrahepatically.

In fetuses of ricketic mother rats, although bones were rarefied, provisional calcification and mineralisation of primary spongiosa proceeded in an orderly fashion without demonstrable accumulation of chondro-osteoid which is typically seen in postnatal rickets of the rat (Park, 1954). Impaired mineralisation was neither found with the Krutsay stain nor with microradiography (Fig. 6).

Hepatic Hematopoiesis (Table 2)

Uremic mother animals were definitely anaemic (U: $28 \pm 2.6\%$ Hkt; Co: $35 \pm 1.5\%$ Hkt). However, hematocrit values did not differ significantly between the fetuses of uremic and of normal mother animals. In accordance with this hematopoietic cells in the livers of uremic fetuses when expressed both as number of nuclei per unit area (cell density) and as number of nuclei per 100 hepatocytes (cell fraction), did not differ significantly between fetuses of uremic rats and normal rats.

Table 2. Hepatic erythropoiesis in fetuses of uremic rats

	Hematopoietic cells (cells/6400 μ^2)	Hepatocytes (cells/6400 μ^2)	Ratio hematopoietic cells/hepatocytes
Uremic ($n=12$)	1858 ± 193	918 ± 72	2.03 ± 0.23
Control ($n=17$)	1652 ± 196 N.S.	908 ± 88 N.S.	1.83 ± 0.23 N.S.

Discussion

This study demonstrates that fetal osteogenesis is little affected by uremia of the mother animal with its attendant hyperparathyroidism, lowering of ionised plasma Ca levels and defective vitamin D metabolism (Massry, 1972). This result explains the failure of newborns of uremic hemodialysed mothers to develop metabolic bone disease.

The absence of fibroosteoclastic or osteomalacic changes in the maternal skeleton is hardly surprising, since the growth and turnover rates of the maternal skeleton are far below those of the fetal skeleton. It appears that the duration of uremia was too short to permit the appearance of bony changes. However, in short term uremia, although histological abnormalities were not demonstrable by light microscopy, histodynamic studies (Krempien et al., 1972) revealed a decrease in mineralisation rate. Definite bone disease was shown in long term uremia (Mehls et al., 1977).

In uremic rats, parathyroid glands are hyperplastic (Raisz, 1969) and circulating plasma parathyroid hormone levels must be elevated (Mehls, 1976). However, the placenta seems to be relatively impermeable to maternal PTH as shown by Hoskins and Snyder (1933), Krukowski and Lehr (1963) and Garel et al. (1971). An elevation of maternal PTH levels can therefore not be transmitted to the fetus.

In addition, the depression of ionised serum Ca levels in the maternal circulation is unlikely to stimulate fetal PTH secretion. In the rat, fetal parathyroid glands have been shown to be definitively functional at term (Pic et al., 1965; Garel et al., 1971). Yet it is questionable whether maternal hypocalcemia of moderate intensity is transmitted into amniotic fluid. Active transport of Ca has been demonstrated in the placenta (Alexander et al., 1973) so that fetal plasma Ca levels are maintained in the presence of large fluctuations of maternal plasma Ca levels (Bawden et al., 1965; Bawden and Wolkoff, 1967; Greeson et al., 1968). This is in agreement with the finding that transplacental isotopic exchange of Ca is slow (Plumlee et al., 1952; Wassermann et al., 1957). A homeostatic mechanism within the placenta was suggested by the finding of Alexander et al. (1973) that plasma Ca returned to the fetal level within short time when the fetus was replaced with a pump and a reservoir of maternal blood. These compensatory mechanisms may fail in the presence of prolonged severe maternal hypocalcemia. Sinclair (1941) found stimulation of fetal parathyroid glands when parathyroidectomised pregnant rats were given low Ca diets. Intrauterine hyperparathyroidism has also been observed in the newborns of patients with untreated hypoparathyroidism (Aceto et al., 1966). Hypocalcemia of such intensity does not occur, however, in experimental uremia (Mehls et al., 1976) and in dialysed uremic women (Confortini, 1971).

Although pharmacological doses of exogenous PTH raise plasma Ca levels in the fetus (Hoskins and Snyder, 1933; Garel et al., 1971), a high intrahepatic dose of exogenous PTH failed to affect the development of fetal bone tissue. However, this negative finding may merely result from the short half life of PTH when it is given as a bolus injection. There is suggestive evidence that the fetal skeleton is responsive to prolonged PTH excess; for instance prolonged

stimulation of fetal parathyroid glands has been shown to result in osteitis fibrosa of the skeleton (Aceto et al., 1966).

It is remarkable that in this study no mineralisation defects or defective transformation of cartilage into bone were found. This finding is the more remarkable since clearcut ricketic change are found in 200 g Sprague-Dawley rats with uremia resulting from 5/6 nephrectomy (Mehls, 1976). In human beings defective mineralisation (rickets) is observed in newborn infants of mothers with vitamin D deficiency osteomalacia (Maxwell and Turnbull, 1932; Ford et al., 1973; Moncrieff, 1974; Russel, 1974). In this study, fetal rat bone mineralised even when the mother animal was severely vitamin D deficient; in addition, epiphyseal cartilage of the fetus was transformed into primary spongiosa in an orderly fashion.

Several hypotheses may be advanced to explain the paradoxical absence of rickets in the fetuses of both vitamin D deficient and uremic mother animals.

When ricketic rats are fasted, hyperphosphatemia ensues and rickets heal as evidenced by the reappearance of mineral deposits in the provisional zone of calcification (Park, 1954). Fetal Ca and phosphorus levels are kept above maternal levels, presumably by placental transport processes (Alexander, 1973), and this may contribute to the preservation of mineralisation.

Lamellar bone with an ordered suprafibrillar collagen structure requires vitamin D for mineralisation. In contrast, woven bone with an irregular suprafibrillar collagen structure has been shown to mineralise even in the absence of vitamin D (Ball and Garner, 1966). Since the fetal skeleton consists of very immature woven bone, it is likely that the fetal skeleton depends less on vitamin D for mineralisation than does the adult skeleton.

Finally, we cannot exclude the possibility that in fetuses of uremic rats the kidney is able to synthesize 1.25-dihydroxycholecalciferol. The precursor, 25-hydroxycholecalciferol, is present in normal concentrations in uremic rats (Mehls et al., 1976) and is available to the fetus by transplacental transfer (Haddad et al., 1971).

The absence of anemia in fetuses of uremic rats is in striking contrast to its presence in the uremic mother animals. Fetal erythropoiesis has been shown to be relatively independent of stimulation by erythropoietin (Zanjani, 1973). Fetal erythropoietin production can be stimulated by experimentally induced haemolytic anemia when the erythropoietin produced appears to be of extrarenal origin, since its production is not affected by nephrectomy. It is less likely, therefore, that the absence of fetal anemia is due to erythropoietin synthesis in the kidneys of the feti of uremic mother rats.

In addition to the fall of erythropoietin levels, retention of toxic substances has been implicated in the pathogenesis of the anemia of renal insufficiency. This study does not document toxic inhibition of erythropoiesis.

Acknowledgements. This paper contains results of a doctoral thesis of Z. Strobel and H. Zimmermann.

The help of Professor Kriz (Heidelberg) in preparing and interpreting sections of fetal kidneys is gratefully acknowledged.

References

Aceto, T., Batt, R.E., Bruck, E., Schultz, R.B., Perez, Y.R.: Intrauterine hyperparathyroidism: a complication of untreated maternal hypoparathyroidism. J. Clin. Endocr. **26**, 487–492 (1966)

Alexander, P.D., Britton, H.G., Nixon, D.A., Cameron, E., Foster, C.L., Buckle, R.M., Smith, F.G.: Calcium, Parathyroid hormone and Calci in the foetus. In: Foetal and neonatal physiology, p. 421–429. Proc. of Sir Josef Barcroft Centenary Symposium. Cambridge Univ. Press, 1973

Ball, J., Garner, A.: Mineralization of woven bone in osteomalacia. J. Path. Bact. **91**, 562–567 (1966)

Bawden, J.W., Wolkoff, A.S., Flowers, C.E.: Maternal-fetal blood calcium relationships in the sheep. Obst. Gynaec. **25**, 548–552 (1965)

Bawden, J.W., Wolkoff, A.S.: Foetal blood responses to maternal calcium infusion in the sheep. Am. J. Obst. Gynaecol. **99**, 55–60 (1967)

Confortini, P., Galanti, G., Ancona, G., Giongo, A., Bruschi, E., Lorenzini, E.: Full term pregnancy and successful delivery in a patient on chronic hemodialysis. Proc. Europ. Dial. Transpl. Ass. **8**, 74–78 (1971)

Ford, J.A., Davison, D.C., McIntosh, W.B., Fyfe, W.M., Dunnigan, M.G.: Neonatal rickets in Asian immigrant population. Br. Med. J. **1973/3** (5873): 211–212. Summary in: Calcified Tissue Abstracts – April 1974 6: 93

Garel, I.M., Pic, P., Jost, A.: Action de la parathormone chez le foetus de rat. Ann. Endocr. (Paris) **32**, 253–262 (1971)

Goldsmith, H.J., de Boer, C.H., Meuzier, D.N., Caplan, W., Mc Candless, A.: Delivery of healthy infant after five weeks' dialysis treatment for fulminating toxaemia of pregnancy. Lancet **1971 II**, 738–741

Greeson, C.D., Crawford, E.G., Chandler, D.C., Bawden, J.V.: Foetal blood Ca response to maternal hypercalcemia in guinea pigs. J. Dent. Res. **47**, 447–449 (1968)

Haddad, J.G., Boirseau, V., Avioli, L.V.: Placental transfer of vitamin D3 and 25-hydroxycholecalciferol in the rat. J. Lab. Clin. Med. **77**, 908–915 (1971)

Herwig, K.R., Merrill, J.P., Jackson, R.L., Oken, D.E.: Chronic renal disease and pregnancy. Am. J. Obstet. Gynaec. **92**, 1117–1121 (1965)

Hoskins, F.M., Snyder, F.F.: The placental transmission of parathyroid extract. Am. J. Physiol. **104**, 530 (1933)

Jastak, J.T., Morrison, A.B., Raisz, L.G.: Effects of renal insufficiency on the parathyroid gland and calcium homeostasis. Am. J. Physiol. **215**, 84–89 (1968)

Krempien, B., Ritz, E., Ditzen, K., Hudelmaier, G.: Über den Einfluß der Niereninsuffizienz auf Knochenbildung und Knochenresorption. Virch. Arch. Abt. A. **355**, 354–366 (1972)

Krempien, B., Ritz, E., Schmidt, G.: Experimentelle autoradiographische Untersuchung zum Kollagenstoffwechsel des Knochens bei Uraemie. Z. Orthop. **110**, 25–34 (1972)

Krukowski, M., Lehr, D.: Parathyroid hormone and the placental barrier. Arch. Int. Pharmacodyn. **146**, 245 (1963)

Krutsay, M.: Methode zur Darstellung einzelner Kalziumverbindungen in histologischen Schnitten. Acta Histochemica **15**, 189–191 (1963)

Massry, S.G.: Divalent ions in renal failure. Kidney International **4**, 71–173 (1972)

Maxwell, J.P., Hu, C.H., Turnbull, H.M.: Foetal rickets. J. Path. Bact. **35**, 419–439 (1932)

Mehls, O., Ritz, E., Gilli, G., Krempien, B., Kourist, B., Schmidt-Gayk, H., Wesch, H., Prager, P.: Skeletal changes and growth in experimental uremia nephron (submitted for publication) (1976)

Mitra, S., Vertes, F., Roza, O., Berman, L.B.: Periodic hemodialysis in pregnancy. Am. J. Med. Sci. **259**, 333–339 (1970)

Moncrieff, M., Fadahunsi, F.O.: Congenital rickets due to maternal vitamin D deficiency. Arch. Dis. Childhood **49**, 810–811 (Oct. 1974)

Orme, B.M., Veland, K., Simpson, D.P., Scribner, B.H.: The effect of hemodialysis on fetal survival and renal function in pregnancy. Trans. Am. Soc. artif. Intern. Organs **14**, 402–404 (1968)

Park, E.A.: The influence of severe illness on rickets. Arch. Dis. Childhood **29**, 369–380 (1954)

Parsons, F.M., Brunner, F.P., Burck, H.C., Gräser, W., Gurland, H.J., Härlen, H., Schärer, K., Spiess, G.W.: Statistical Report. Proc. Europ. Dial. Transpl. Ass. **11**, 3–68 (1975)

Pic, P., Maniey, J., Jost, A.: Facteurs endocriniens réglant la calcémie foetale. Indications sur le rôle des parathyroides. C.R. Soc. Biol. (Paris) **159**, 1274 (1965)

Plumlee, M.P., Hansard, S.L., Comar, C.L., Beeson, W.M.: Placental transfer and deposition of labeled calcium in the developing bovine foetus. Amer. J. Physiol. **171**, 678–686 (1952)

Raisz, L.G.: Parathyroid gland metabolism. Arch. Intern. Medicine (Chicago) **124**, 389–396 (1969)

Ritz, E., Krempien, B., Strobel, Z., Zimmermann, H., Mehls, O.: Foetal development in experimental uremia. Proc. Europ. Dial. Transpl. Ass. **10**, 127–135 (1973)

Russell, J.G., Hill, L.F.: True foetal rickets. Br. J. Radiol. **47** (562), 732–734 (Oct. 1974)

Sinclair, I.G.: Size of the parathyroid glands of albino rats as effected by pregnancy and controlled diet. Anat. Rec. **80**, 479–496 (1941)

Wasserman, R.H., Comar, C.L., Nold, M.M., Lengemann, F.W.: Placental transfer of calcium and strontium in the rat and rabbit. Am. J. Physiol. **189**, 91–97 (1957)

Received April 28, 1977