haemoglobin was absent. Both fractions proved to be homogeneous in disc electrophoresis at pH 4.3. For all fractions the following lethal activities were determined by s.c. injection into mice:

	Acute toxicity approximate LD_{50} mg/kg	Minimum dose for myoglobinuria mg/kg
I	-	-
II	4.5	0.5
III	0.8	-
IV	4.3	2.9
V	1.2	-

Besides acute toxicity characterized by respiratory paralysis within 2–3 h, fractions II and IV (to a lesser extent fraction III, probably due to contamination with fraction II) produce myoglobinuria at a lower dose level. In the latter case the mice die in an emaciated state after 3–4 days which might be due to muscle degradation and renal failure as a result of massive myoglobin excretion. Whereas both fractions exhibited nearly the same LD_{50}-value in acute toxicity, fraction II, which was less basic, was 6 times more active in causing myoglobinuria, indicating that there is no strict relationship between basicity of the protein and muscular damaging effect. Furthermore, quite different pathogenic mechanisms, paralysis and myoglobinuria, seem to be associated with the same molecule.

The amino acid composition of fractions II and IV shows close similarities to that reported for *Enhydrina schistosa* myotoxin[4] considering the number of Asx, Ala, Gly, Lys and the 12 (II) and 14 (IV) half-cystine residues.

Australian snake venoms show a great variety of phospholipases A[7] which may have high neuromuscular blocking activity like taipoxin[8] (from *Oxyuranus scutellatus* venom) and notexin[9] (from *Notechis scutatus* venom), or being less toxic (in terms of LD_{50}) may directly affect muscle tissues, resulting in a massive release of myoglobin. Other toxic phospholipases A of minor specificity in action (sometimes of postsynaptic blocking activity) are also present. Phospholipase A is, perhaps, the most versatile enzyme, having a primarily digestive function and evolving to toxins with high affinity for various membrane structures, i.e. those of nerve and muscle.

Amino acid composition of fractions II and IV from *Pseudechis colletti* and of the myotoxin from the sea snake *Enhydrina schistosa*[4]

	Fractions II	Fractions IV	Myotoxin
Asx	22	17	21
Thr	8	8	4
Ser	5	7	5
Glx	10	6	6
Pro	1	7	3
Gly	12	12	9
Ala	11	11	10
1/2 Cys	12	14	14
Val	4	4	6
Met	2	3	2
Ile	4	4	3
Leu	5	5	4
Tyr	8	7	12
Phe	4	4	2
His	2	2	2
Lys	10	14	10
Trp	2	2	0
Arg	5	3	7
Total	127	130	120
Formula weight	14,170	14,200	13,500

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The induction of chromosome aberrations in human lymphocytes by negative π-mesons under conditions of anoxia and oxygenation

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Summary. After a dose of 3.0 Gy in the peak position of the pion depth-dose curve, the ratios between observed chromosome aberration yields under conditions of oxygenation and of anoxia were obtained for lymphocytes exposed at 3 depths in a plastic phantom. These ratios were 3.7, 1.9 and 1.3 in the plateau, peak and post-peak positions, respectively, suggesting a corresponding decrease in the oxygen enhancement ratio.

Calculations by Fowler and Perkins[2] showed that negative π-mesons (pions) possess several advantages from the point of view of radiotherapy. Their ideal depth-dose profile and advantageous LET distribution combine to give a very useful distribution of biological damage within the treatment volume. Low LET radiations do have the advantage of allowing normal tissue to recover during the interval between treatment fractions but with the disadvantage that the oxygen enhancement ratio (OER) for this type of radiation is of the order of 3. Thus anoxic tumor tissue may remain resistant to the levels of radiation dose achievable without causing intolerable damage to adjacent healthy tissues.

Pion induction of chromosome abnormalities was first examined by Richman et al.[3] working with *Vicia faba* root meristem cells. Gnanapurani et al.[4] analyzed chromatid aberration in the same material and estimated the OER of peak radiation at 1.8. Further OER estimations range from 1.35 for *Vicia faba* root growth[5] to 2.2 for V79 Chinese hamster cell survival[6].

The characteristics of the pion beam available from the NIMROD accelerator have been examined using a variety of biological systems[7]. An OER of 1.8 was obtained for *Vicia faba* root growth compared with a value of 3 with cobalt-60 γ-rays[8]. OERs of the order of 1 were obtained for mouse testis weight loss and spermatogonial cell survival in

Table 1. Induction of chromosome aberrations by negative π-mesons under different conditions of oxygenation at 3 positions in a plastic phantom

	Position	Dose (Gy)	Number of cells	Total unstable aberrations	Aberrations per cell	Peak:plateau ratio ± SE
Anoxic	Plateau	0.65	500	18	0.036	
	Peak	1.0	447	53	0.119	3.3 ± 0.9
	Post peak	0.63	442	24	0.054	
Venous	Plateau	0.65	200	20	0.100	
	Peak	1.0	141	51	0.362	3.6 ± 1.0
	Post peak	0.63	200	20	0.100	
Oxygenated	Plateau	0.65	200	29	0.145	
	Peak	1.0	500	141	0.282	1.9 ± 0.4
	Post peak	0.63	200	17	0.085	
Anoxic	Plateau	1.94	700	63	0.090	
	Peak	3.0	514	285	0.554	6.2 ± 0.8
	Post peak	1.89	200	80	0.400	
Venous	Plateau	1.94	200	76	0.380	
	Peak	3.0	438	564	1.288	3.4 ± 0.4
	Post peak	1.89	200	93	0.465	
Oxygenated	Plateau	1.94	500	167	0.334	
	Peak	3.0	500	537	1.074	3.2 ± 0.3
	Post peak	1.89	200	105	0.525	

vivo compared with a value of 1.6 with cobalt-60 γ-rays[8]. In this note the effect of alterations in the oxygen tension of human blood on the yield of pion-induced chromosome aberrations in lymphocytes is described.

Materials and methods. 1. Physical: Pions with a momentum of 160 MeV/c and a 15% spread were selected from particles produced by the interaction of the 7 GeV proton beam from the NIMROD proton synchrotron and a 10 cm tungsten target[10]. These were focused along a beam line of approximately 6 m leading into the shielded experimental room. This beam gave an absorbed dose of between 0.80 and 1.50 Gy/h over a useful volume of $2.5 \times 2.5 \times 3$ cm at the 80% isodose contour. In the peak position, doses given were either 1.0 or 3.0 Gy corresponding to concurrent doses of 0.65 or 1.94 Gy in the plateau and 0.63 or 1.89 Gy in the post peak position. Ionization measurements were made with a 0.2 ml Baldwin-Farmer chamber, calibrated with cobalt-60 γ-rays.

2. Biological: Blood from a healthy male donor was maintained at 37 °C and irradiated in 0.8 ml aliquots placed in 2 ml sterile polypropylene ampoules (Sterilin) set in a plastic phantom. The phantom composition was $C_{43}O_8N_2H_{67}$ with a density of 1.15 g/cm^3. Pairs of ampoules were placed at distances of 5.2, 13.5 and 15.0 cm within the phantom corresponding to the plateau, peak and post-peak regions of the Bragg ionization curve.

3 conditions of oxygen tension were used: equilibration with 100% oxygen; 'venous' oxygen tension (40 mm Hg measured on an IL 213 blood-gas analyzer); and equilibration with oxygen-free nitrogen with less than 2 ppm oxygen contamination. The techniques and apparatus for alteration of the oxygen tension have been described elsewhere[11]. Because the process of equilibrium takes about 45 min and of necessity was carried out within the pion irradiation facility, control samples were removed from the apparatus prior to irradiation and analyzed for any damage possibly caused by the high background radiation level in the working area around the beam line. Blood samples were transferred to ampoules within the phantom without contact with the atmosphere and the equilibrating gas was passed through the apparatus during the exposure.

After irradiation, whole blood microcultures were set up according to standard procedures[12]. Chromosome preparations were made after 48 h and scored for unstable aberrations (dicentrics, rings, fragments and minutes).

Results and discussion. In control samples removed before irradiation with pions, the observed number of aberrations was not above background. Table 1 shows the numbers of aberrations observed in blood irradiated under different experimental conditions together with the numerical ratio between the number of aberrations per cell observed in the peak sample and the number of aberrations per cell observed in the sample exposed concurrently in the plateau. This latter ratio does not differ significantly between pairs of samples except in the case of anoxic blood receiving a dose of 3.0 Gy in the peak position. A large number of cells were scored from the corresponding plateau to improve the statistical errors and the value of 6.2 ± 0.8 is significantly different from the ratio of peak:plateau aberrations observed with oxygenated or venous blood.

Corresponding values of peak:plateau ratios calculated from yields after a peak dose of 1.0 Gy are based on low yields of aberrations particularly in the plateau. Significant data are confined therefore to those obtained as a result of doses of 3.0 Gy in the peak. Using these 3.0 Gy data, the decrease in the peak:plateau ratio from 6.2 after irradiation under anoxia to 3.2 under oxygenated conditions can be seen to result from a differential increase in aberration yield at the 2 positions. As shown in table 1, aberration yield in the plateau increases more than that in the peak. Such differences in the effect of oxygen at different depths in the phantom are expected if, as LET differences indicate[13–15], the OER is greater in the plateau than in the peak. In table 2 damage ratios for human chromosomes under

Table 2. The ratio between numbers of aberrations observed in samples irradiated in the same position in the phantom under different conditions of oxygenation

Peak dose	Total aberration yield			
	1.0 Gy		3.0 Gy	
Position	Venous anoxic	Oxygenated anoxic	Venous anoxic	Oxygenated anoxic
Plateau	2.8	4.0	4.2	3.7
SE	0.87	1.17	0.71	0.54
Peak	3.0	2.4	2.3	1.9
SE	0.59	0.38	0.17	0.14
Post peak	1.7	1.5	1.2	1.3
SE	0.53	0.47	0.18	0.19

conditions of oxygenation and of anoxia are given for the different sample positions and pion doses. This ratio between the damage observed after the same dose under 2 conditions is not a value of OER but in the absence of complete dose-response curves provides a realistic approximation[16]. In the 1.0 Gy peak and plateau positions results are inconclusive because of the limited number of cells which can be analyzed. However for the 3.0 Gy samples the plateau value of 3.7 for the oxygenated:anoxic ratio is significantly greater than the peak ratio of 1.9. This peak ratio of damage agrees very well with a value of 1.7–1.9 obtained for the ratio of chromatid aberrations in *Vicia faba* root meristem cells irradiated with pions under similar conditions[3].

Positions in the phantom were chosen such that plateau and post-peak doses were approximately equal. The average peak:plateau ionization ratio was 1.55 and the average peak:post peak ionization ratio was 1.59. At the dose levels achieved in the plateau (1.94 Gy and 0.65 Gy) OER values for 250 KVp X-rays of 3.2 and 4.1 would be expected[11]. Comparison of these expected values with the observed damage ratios in table 2 (3.7 and 4.0) leads to the conclusion that the plateau radiation is of comparable OER to 250 KVp X-rays implying a similar LET to X-rays. The ratios given in table 2 show that damage in the post-peak however is significantly less affected by the presence of oxygen; suggesting that the radiation in this part of the beam is of high LET as observed with *Vicia faba* at CERN[13] and, on the pion beam from NIMROD, with HeLa cells[14,15].

1 Acknowledgments. We wish to thank Dr D.H. Reading, Mr M.A. Hynes and Mr W. Spinks for their assistance at the Rutherford Laboratory's π-II beam line. This work was partly supported by Euratom Contract 171-76-1 BIO-UK.

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Normal osteoclast number and function in rat pups lacking parathyroid hormone[1]

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Summary. In parathyroidectomized suckling rats, bone modeling and the number and activity of cells in the osteoclast population are normal. These findings are at variance with observations in older animals and suggest that factors other than parathyroid hormone influence osteoclast formation and function in the neonate.

Levels of circulating parathyroid hormone are low before birth[2–5] yet fetal bone contains substantial numbers of osteoclasts which appear active in the modeling process in vivo, and are responsive to parathyroid hormone in vitro[6]. By contrast, hypoparathyroidism in young and adult animals results in a pronounced decrease in osteoclast number and function[7–10]. Precisely when during development the osteoclast population becomes dependent upon parathyroid hormone has not been established. The present study was undertaken to determine whether the transition to hormone dependency occurs during the suckling period.

Materials and methods. Parathyroid glands were removed by dissection from 1-day-old rat pups under cold anesthesia. Sham-operated littermates served as controls. Following surgery, the pups were returned to their mothers and nursed until sacrificed at 14 or 19 days of age. At sacrifice, blood was collected for analysis of calcium[11] and phosphorus[12], and femurs and trachea-thyroid complexes were removed for histological evaluation. Tissues were fixed in 10% neutral formalin, and stored in 5% neutral formalin until embedded. For electron microscopy, bones were demineralized in 7.5% EDTA in 0.1 M phosphate buffer overnight, placed in 3% gluteraldehyde in phosphate buffer for 1 h, and postfixed in OsO_4 in 0.1 M cacodylate buffer for an additional h. They were then washed in buffer, dehydrated in alcohols, and embedded in Araldite 502. Thin sections were cut on an LKB ultramicrotome and stained with uranyl acetate and lead citrate. For light microscopy, femurs were demineralized in 7.5% EDTA in 5% formalin, and embedded in paraffin. Trachea-thyroid complexes were similarly embedded and serially sectioned to ascertain completeness of parathyroidectomy. The 5 µm sections were stained with hematoxylin and eosin. Osteoclast quantitation was done using a Merz-Schenk grid at a magnification $\times 450$. The metaphyseal region of each bone was sequentially scanned and the number of osteoclasts and the amount of bone per field was determined. The results were expressed as osteoclasts/mm^2 bone. The number of nuclei/osteoclast was also established.

Results. Successfully parathyroidectomized (Ptx) animals showed no evidence of residual parathyroid tissue in trachea-thyroid sections, and were characteristically hypocalcemic and hyperphosphatemic. Mean blood calcium for Ptx pups was 7.1 mg/dl (range 6.4–8.4) as compared to 10.9 mg/dl (range 9.8–12.6) for sham-operated littermates; mean Ptx blood phosphorus was 17.9 mg/dl (range 15.6–21.0) vs 10.4 mg/dl (range 8.8–11.6) for controls. Despite the absence of parathyroid hormone, histomorphometric analysis of bone sections revealed that osteoclast number and size (i.e., No. nuclei/cell) were comparable in normal