erythropoiesis, an effect which was lacking in the mice treated with the 11α,9α-EPA substituted at C-9. In fact it was very difficult to evaluate the 11α,9α-EPA due to its very potent smooth muscle constrictor activity causing respiratory problems in the mice. The 9α,11α-EPA was also found to be more potent than naturally occuring PGE_2. The 9α,11α-EPA has been shown in other biological systems to primarily mimic the effects of PG-endoperoxides, whereas the 11α,9α-EPA has been found to mimic the effects of thromboxane A_2. Therefore, the erythropoietic effects of the EPA appear to be related to the endoperoxide-like activity rather than the constrictor activity associated with thromboxanes which might produce renal ischemia and enhance Ep production from a hypoxic kidney.

It is of interest that all the PG we evaluated in vivo for its ability to stimulate erythropoiesis possessed oxygen at the C-9 position. It therefore appears that a structure-activity-relationship probably exists, with regard to the ability of PG to interact with a potential PG-receptor which modulates Ep production and hence erythropoiesis. This appears to be confirmed by the observation that PG having some other group at position C-9, e.g., hydroxyl ($PGF_{2\alpha}$) or methyl (11α,9α-EPA) apparently lack the ability to stimulate in vivo erythropoiesis.

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Haemopoietic stem cell concentration and CFUs in DNA synthesis in bone marrow from different bone regions

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Summary. The concentration of colony-forming cells (CFUs) is about 40% less in sternal marrow than in the marrow of lumbar vertebrae and femora. Marrow of trabecular bones in lumbar vertebrae contains fewer mitotically active CFUs than marrow of trabecular bones in the femoral distal epiphysis and metaphysis, or the peripheral marrow near the cortical bone in the femoral diaphysis. Only a minor part of the variability of the results in the CFU-assay is due to differences in CFU-concentrations between individual donor mice; pooling of the cell suspensions does not substantially decrease variability. Specific pathogen-free mice yield the same results as BALB/c mice from conventional breeding.

Colony-forming stem cells (CFUs) are primitive haemopoietic cells with a multiple differentiation capacity[2] and capable of extensive self-replication[3]. The macrophage - granulocyte committed stem-cells (CFUc) have a restricted differentiation capacity, a high mitotic activity[4] and little or no self-replicative ability[5]. Both populations show a high radiosensitivity[2,6]. It has been suggested that colony-forming cells may be accumulated rather near the endosteal surfaces[7-9], although not all experiments confirm this[10,11]. Localization of CFUs in bone marrow is important, e.g. when histogenesis of haemopoietic cells or radiotoxic effects from short-range bone-seekers on the stem cells are studied. The concentrations of CFUs in marrow regions of selected cortical and trabecular bones as well as in a marrow cell population not close to the bone surface, are described. The proportion of CFUs in DNA synthesis is also measured; and an analysis of different variance components is used to distinguish between the individual variability of host mice and other experimental errors.

Materials and methods. Experiments were performed with BALB/c mice from conventional breeding and with specific pathogen free (SPF) mice, both from S.C.K.-C.E.N. breeding. 5 male 3-month-old mice were used per observation. They were killed by cervical transection; the right femur, 4 lumbar vertebrae and the sternum were removed and the outsides thoroughly cleaned. Trabecular bone from epiphysis and metaphysis at the distal and proximal end of the femur were separated from the cortical bone of the diaphysis, with a miniature saw-file. The axial marrow of each diaphysis was flushed out twice with 1 ml of α-MEM (Flow Laboratories, UK) under a constant flushing pressure of 250 g and collected in a plastic tube, yielding a cell population not close to the endosteum. Microscope sections of the flushed bones showed that peripheral marrow remained and that central marrow was absent. Nucleated cells remaining in the peripheral shafts, as well as those of the lumbar vertebrae, of the sterna and of the distal femoral ends, were obtained by grinding the bones separately in a mortar and then washing them in a fixed volume of α-MEM. To estimate individual variability, the marrow suspensions from each mouse were kept separately. In other experiments the cells of 5 donor mice were pooled for each bone marrow region. After homogenization of the cell suspensions and haemolysis of red blood cells, the cell concentration was measured with an electronic counter (Coulter counter ZF). The suspensions were preserved in plastic tubes under aseptic conditions on melting ice and each was assayed for CFUs and CFUc concentrations. Each experiment included, as a control, the femoral marrow from 3 3-month-old male BALB/c mice, prepared by grinding the whole, cleaned right femora. In each experiment all the data were normalized to that of the femoral control cell suspension.

CFUs concentrations were determined using the spleen colony assay technique[2] and CFUc were assayed by culturing in a single-layer soft agar system. Complete medium consisted of α-MEM, 0.22% $NaHCO_3$, gentamicin (100 µg/ml α-MEM, Shering Corp., USA), 10% fetal calf serum and 10% horse serum (Flow Laboratories, UK). Colony formation was stimulated by conditioned serum[12] derived from

mice injected with endotoxin (lipopolysaccharide B from *Salmonella abortus* Equi, Difco Lab. USA), and by β-mercaptoethanol. Quadruplicate cultures were made of each cell suspension and the colonies were counted after 7 days. In order to study the proportion of CFUs in S-phase in each cell suspension, 1 ml paired aliquots, each of 10^6 cells, were incubated at 37 °C in a mixture of 5% CO_2 and air. After 10 min 200 µCi methyl-^{3}H-thymidine (3H-TdR) (IRE, Belgium) of 15 Ci/mmole in 0.2 ml isotonic medium was added to one tube of each pair to kill mitotically active cells by the incorporation of the radioactive precursor in their DNA. The other tube, serving as control, received an equal amount of cold thymidine. After 30 min of further incubation the suspensions were diluted in ice-old α-MEM and 7.5×10^4 cells in 0.25 ml were injected into irradiated recipient mice for CFUs assays[13]. The percentage loss in spleen colonies between the controls and the ^{3}H-TdR treated suspensions was taken as a measure of the proportion of CFUs in DNA synthesis.

Results and discussion. Obviously the CFUs- and CFUc-concentrations did not show large fluctuations between the different bone marrow sites (tables 1 and 2). Only the sternal marrow contained significantly less CFUs and CFUc per 10^5 nucleated marrow cells compared with other sites. For the CFUs- and CFUc-concentrations in the axial marrow of the femur, a correction factor of 0.8 was introduced, to make these data comparable to those of other compartments in which about 20% cell killing occured due to bone crushing[14]. If differences in CFUs- and CFUc-concentrations per 10^5 nucleated cells were compared, one must take into account that the nucleated cells, according to the bone region, may contain more differentiated blood-forming cells or more cells committed to other cell lines, e.g. stromal cells or bone cells. Also different marrow sites may contain different amounts of red blood cells. The density of CFUs and CFUc per unit volume of marrow in marrow cavities of different bones is given in table 1. The total amount of colony-forming stem cells was divided by the marrow volume of that particular bone fragment. Bone volume was measured by determining the volume of water replaced by immersion of the bone (6 repetitions per bone fragment); the proportion of marrow and spinal cord was measured with an automatic image analyser, the Quantimet 720 (unpublished results, the mea-

Table 1. Comparison of different bone marrow sites

	Axial diaphysis	Peripheral diaphysis	Distal epiphysis	Lumbar vertebra	Sternum
Number of nucleated cells $\times 10^7$	1.3±0.2	0.3±0.1	0.9±0.1	1.2±0.1	1.6±0.2
Marrow volume (mm^3)	6.1		6.7	7.9	21
Mean CFUs per 10^5 cells for each experiment	16.4–19.2–14.3–19.6	22.0–21.5–13.3–20.0	20.3–17.0–16.3–17.0	14.4–23.1–14.4–16.7	8.5–11.6–12.2–13.9
Mean number of recipient mice	8	10	9	10	9
Group mean ± pooled SEM	17.4±1.5	19.2±1.2	17.6±1.3	17.2±1.4	11.6±1.0
Variance due to differences among pools	5	16	2	13	5
Variance due to other experimental errors	10	21	15	13	14
CFUs per mm^3 marrow	465		236	261	88
% CFUs in S-phase ± 95 % value					
a) in conventional mice	10±20	40±12	44±13	13±25	11±25
b) in SPF mice	22±17	40±27	40±23	0±38	10±25
Mean CFUc per 10^5 cells for each experiment	166–194–137–212	82–185–115–188	149–80–112–160	149–191–114–170	54–54–80–136
Group mean ± pooled SEM	177±26	142±22	125±10	156±27	81±11
CFUc per mm^3 marrow	4470		1679	2370	617

Pooled samples of 4 replicated experiments of CFUs and CFUc concentrations in marrow of conventional BALB/c mice. 95% values were calculated using the Student t-distribution[15]. % CFUs in S-phase ±95% value were derived from Finney[16]. The variance components were derived by an analysis of variance for unequal sample sizes[17].

Table 2. Comparison of conventional and SPF mice

	Axial diaphysis	Peripheral diaphysis	Distal epiphysis	Lumbar vertebra	Sternum
Conventional mice					
Mean CFUs/10^5 cells in each individual	17.2–16.3–19.9–22.4–20.0	16.3–18.9–16.2–21.9–22.7	18.6–19.4–17.6–21.1–20.5	13.8–20.3–17.3–21.2–18.2	11.2–12.7 12.9–15.4–11.8
Group mean ± pooled SEM	19.2±1.6	19.2±2.1	19.4±1.8	18.2±1.3	13.8±1.3
Variance due to differences among individuals	3.7	4.9	0.7	6.7	0.9
Variance due to other experimental errors	13.5	25.4	19.1	10.5	9.7
SPF mice					
Mean CFUs/10^5 cells in each individual	16.8–16.7–13.7–20.0–19.3	19.1–14.4–12.2–12.3–14.4	20.5–21.7–21.9–23.1–19.3	24.1–21.6–24.7–17.6–20.5	13.4–13.3–15.9–16.4–13.4
Group mean ± pooled SEM	17.3±1.2	14.6±2.4	21.3±2.4	21.7±1.3	14.6±1.4
Variance due to differences among individuals	4.7	2.5	3.5	6.6	0.4
Variance due to other experimental errors	9.1	31.7	33.7	9.8	11.1

Individual CFUs concentrations in marrow of 3-month old BALB/c mice. 5 or 6 recipient mice were used for each marrow suspension.

surements were performed in the laboratory of Dr E. Polig; Kernforschungszentrum, Karlsruhe). 2 sections of 2 animals were analysed per bone fragment. The density of colony-forming stem cells was highest in the femoral diaphysis, 50% less in the lumbar vertebrae and the distal femoral end, and more than 5 times less in the sternum (table 1). With the ^{3}H-Tdr suicide technique the mitotic activity of the CFUs in several bone marrow sites may be compared. Corroborating earlier findings[8], about 40% of CFUs were in S-phase in the peripheral part of the femoral shaft, whereas in the axial femoral marrow only a few of them were mitotically active. In the distal end of the femur, about 40% of the CFUs were in S-phase. It is an open question why the proportion of mitotically active cells is important in the peripheral diaphysis and the distal epiphysis of the femur and why it is not in the lumbar vertebrae, the sternum and the axial femoral marrow. No relationship with bone structure exists since both the distal epiphysis and the lumbar vertebrae are characterized by a large ratio of endosteal surfaces per unit volume of marrow.

Variation in CFUs concentrations between individual mice (table 2) was of the same magnitude for the different marrow sites, and about the same in SPF mice (between 0.4 and 6.6) and in conventional mice (between 0.7 and 6.7). Individual variability was small ($p < 0.05$) compared with other experimental errors (between 9.1 and 33.7 for SPF mice and between 9.7 and 25.4 for conventional mice). In the pooled experiments more recipient animals were used (10 instead of 6) but the variance was not influenced. Pooling cell suspensions instead of working with marrow suspensions of individual mice did not influence the total variance (table 2).

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Tissue distribution of juvenile hormone hydrolytic activity in *Galleria mellonella* larvae

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Summary. Juvenile hormone (JH) hydrolytic activity was determined in different tissues of day-4 last instar larva of *Galleria mellonella*. Midgut, gonad, imaginal wing discs and fat body contain higher JH hydrolytic activity than hemolymph, while silk gland and body wall have lower activity. JH esterase activity in imaginal wing discs exhibits a pattern of age-related changes different from that of the hemolymph.

Juvenile hormone (JH) inhibits metamorphosis of juvenile insects and differentiation of adult characteristics[3]. In several lepidopteran, coleopteran and orthopteran insects endogenous JH is inactivated by hemolymph JH esterase (JHE) just before initiation of a metamorphic molt[4-8]. Although it was shown that in *Manduca*[9] and *Galleria*[10] fat body is the source of hemolymph JH esterase, distribution of JH hydrolytic activity in other tissues of insect larvae has not been studied extensively. Furthermore, inactivation of JH prior to metamorphosis is critical for normal post-embryonic development of insects[3,11], and it has been shown that hemolymph JHE may play an important role in regulation of differentiation of tissues in larval insects.[6]. Hence it is important to know the tissue distribution of this enzyme activity in last instar larvae. In the present report data on JHE activity in extracts of fat body, gonad, midgut, body wall (containing cuticle, some muscle tissue, and the chitogenous epithelium) and imaginal wing discs from last instar larva of *Galleria* and age-related changes in JHE activity in the wing discs are presented.

Wax moths, *Galleria mellonella*, were reared in the laboratory according to procedures described earlier[12]. Last instar larvae were collected within 8 h after ecdysis into the instar and were aged for use in these studies. Larvae less than 8 h after ecdysis into the last stadium are designated as day-0 larvae. Larvae aged for 24 h after collection are designated as day-1 larvae and so on. When raised under optimum conditions of density the rate of growth of different larvae is nearly uniform, all pupating in 8 days $\pm$ 12 h.

Specific tissues were dissected from larvae and cleaned of attached tissues. They were rinsed first in Ephrussi-Beadle Ringers and then in 0.05 M phosphate buffer pH 7. The tissue was homogenized in the buffer and centrifuged at $10{,}000 \times g$ for 10 min, and the supernatant was used for all assays. JHE activity in the supernatant was determined by procedures described in the literature[4,6]. In brief, this procedure consists of incubations of an aliquot of the supernatant (50 - 500 µg protein) with labelled JH for 10 to 30 min at 30 °C. The time was varied depending on the total amount of JH hydrolyzed. All incubations were controlled such that only 20 to 30% of JH in the incubation mixture was hydrolyzed by the end of the incubation period. At the end of the incubation the unhydrolyzed JH, JH acid, JH diol and JH acid-diol were separated by TLC and were quantitated by scintillation counting. At least 1 sample of each series was measured in the presence of DFP which inactivated general carboxylesterases. In the studies using gonads, penultimate larval midgut and fat body, JHE