Effect of Successful Parathyroidectomy on Hematopoietic Progenitor Cells and Parameters of Red Blood Cells in Patients with Primary Hyperparathyroidism

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Elevated levels of parathyroid hormone (PTH) in primary and secondary hyperparathyroidism inhibit hematopoiesis at the level of hematopoietic progenitor cells, mainly the burst forming units-erythroid (BFUe). Removal of parathyroid adenomas is associated with an increase in hematopoietic progenitor cells. In contrast, a certain amount of PTH and calcium is needed to correct anemia after bleeding demonstrating that PTH has also a stimulatory effect on the bone marrow.

We examined the effect of parathyroidectomy (PTX) in 10 patients with histologically proven primary hyperparathyroidism on hematopoietic progenitor cells and several parameters of red blood cells before and at 5, 30 and 90 days after PTX. After successful surgery serum levels of iPTH ($p < 0.01$) and calcium ($p < 0.001$) decreased significantly. Subsequently a steady increase in all hematopoietic progenitor cell classes was observed reaching significance for BFUe only ($p < 0.05$). Red blood cells and hemoglobin reached nearly pretreatment values within 90 days after PTX after they had decreased due to surgery associated blood loss. 8 patients undergoing hemithyroidectomy without PTX showed a similar decrease in red blood cells and hemoglobin followed by a rise after the operation. The changes of these parameters did not differ significantly from the patients with pHPT. In contrast to the patients with pHPT, no changes in hematopoietic progenitor cells during the 90 days were observed. The presented data provide further evidence that increased PTH concentrations might inhibit hematopoiesis in humans *in vivo*. The inhibition can be reversed following PTX by normalisation of PTH concentrations.

Key words: Primary Hyperparathyroidism – Parathyroidectomy – Hematopoietic Progenitor Cells

Introduction

Since anemia in primary hyperparathyroidism was first observed in the early 1930s, the relation between elevated PTH levels and anemia has been described in several studies (1,2). Patients with primary and secondary hyperparathyroidism associated with advanced bone disease and formation of fibrous tissue in the bone marrow exhibit normochromic, normocytic anemia, which is improved or reversed after removal of the abnormal glands (3,4).

These findings led to the suggestion of a relationship between serum PTH levels and anemia (5). Indeed, patients with chronic renal failure with bone marrow fibrosis, dependent on the severity of secondary hyperparathyroidism, need higher erythropoietin levels to maintain a normal hematocrit (6). Excessively high PTH levels in combination with other uremic toxins also induce anemia in uremic patients through other mechanisms: PTH can induce hemolysis (7), thereby promoting in part the shortened survival of erythrocytes.

In *in vitro* studies in human peripheral blood and mouse bone marrow, the inhibitory effect of PTH on erythropoiesis at the stage of BFU-e could be confirmed. Only intact PTH and the C-terminal fragments of PTH were active, whereas the N-terminal fragment was inactive. Increasing concentrations of erythropoietin could overcome this inhibitory action of PTH (8). Besides this inhibitory effect of elevated PTH concentrations on red blood cells and their hematopoietic progenitor cells in the bone marrow, some stimulatory effects of PTH in the regulation of erythropoiesis have also been described.

After acute blood loss, the presence of parathyroid glands with a physiological increase in serum PTH and calcium is essential for the normal restoration of red blood cells (9,10).

The aim of the present study was to determine the impact of elevated and normalized PTH and calcium concentrations before and after parathyroidectomy (PTX) on hematopoiectic progenitor cells and peripheral red blood cells in humans.

For this purpose 10 patients with primary hyperparathyroidism were investigated before, and at 5, 30 and 90 days after successful PTX and compared with 8 patients who had undergone a hemithyroidectomy to treat a thyroid adenoma.

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Patients and Methods

Patients: 10 patients (2 male, 8 female) with a mean age of 69.3 ± 9.8 (range 52 – 80) years and biochemically proven primary hyperparathyroidism (pPHT) were included in the study (Table **1**). Erythrocyte counts, hemoglobin, hematocrit, reticulocytes, erythropoietin levels, erythroid progenitor cells (BFUe, burst forming units-erythroid), myeloid progenitor cells (CFU-GM, colony-forming units granulocyte/macrophage) and pluripotent progenitor cells (CFU-Gemm, colony forming units-granulocyte/erythrocyte/macrophage/myelocyte) as well as serum calcium and intact PTH (Nichols Institute Diagnostics, San Juan Capistrano, California, USA, intra- and interassay coefficient of variation 7.5% and 6.8%, respectively) were measured before, and at 5, 30 and 90 days after parathyroidectomy. Blood loss associated with parathyroidectomy and thyroidectomy was 100 – 150 ml. None of the patients needed any red blood cell transfusions.

Other factors known to influence erythropoiesis were excluded by normal plasma concentrations of haptoglogin, iron, transferrin, ferritin, vitamin B12 and folic acid. No patient showed signs of renal dysfunction and none had any signs of subperiostal bone resorption or bone cysts. In all patients a parathyroid adenoma was removed.

A group of 8 patients with a mean age of 69. ± 9.7 (range 52 – 80) years, which was not significantly different from the age of the hyperparathyroid patients, had undergone a hemithyroidectomy to treat a thyroid adenoma and served as controls.

Progenitor cell assay: Peripheral mononuclear cells (PMNC) were harvested after a Ficoll-Hypaque gradient centrifugation (400 g, 40 min, 1.077 g/ml). Pluripotent (CFU-Gemm) as well as committed progenitor cells (BFU-e, CFU-GM) were assayed using a modification of the clonal assay described by *Fauser* and *Messner* (11). Each plate contained 0.9% methylcellulose, 30% fetal calf serum, 10% bovine serum albumin (Behring, Marburg, FRG), 1 U/ml erythropoietin (Toyobo, Osaka, Japan) alpha-thioglycerol (10^4 mol/l), 10 U/ml IL-3 and 100 U/ml GM-CSF and Iscove's modified Dulbecco's medium (IMDM, Gibco, Paisly, Scotland). PMNC were plated in duplicate at $1.0 \cdot 10^5$/ml. After a culture period of 14 days (37 °C, 5% CO_2, full humidity), cultures were examined under an inverted microscope. Aggregates with more than 50 translucent, dispersed cells were counted as CFU-GM. Bursts containing more than 100 red colored cells were scored as BFU-e. CFU-MIX were identified by their heterogenous composition of translucent and hemoglobinized cells. Individual colonies suspected to be CFU-MIX were picked, transferred to glass slides and stained with May-Grünwald-Giemsa for cytological examination under a light microscope. Our results were expressed as colonies per $1.0 \cdot 10^5$ PBNC plated.

Erythropoietin assay: Erythropoietin levels were measured by a commercially available ELISA (monoclonal enzyme-immunoassay, standardized against the 2nd international preparation of erythropoietin for bioassays, MEDAC, Hamburg, FRG). The serum levels for a healthy population ranged from 5 to 25 mU/ml.

Furthermore, results were correlated with a modified specific radio-immunoassay. The exact procedure has already been published (12).

Statistical methods: Variables of interest were described by median, upper and lower quartiles, range, coefficient of variation and correlation coefficient (Kendall Tau).

To assess the relationship of the variables with time (baseline, day 30 and 90) Kendall's Tau was calculated per patient. Then these values were tested with Wilcoxon's signed rank test. All p-values are results of two-sided tests. The statistical software package SAS (SAS Institute Inc., Cary, NC, USA, 1990) was used for calculations.

Results

The characteristics of the 10 patients with primary hyperparathyroidism and the 8 patients with thyroid adenoma are shown in Table **1**. Serum levels of intact PTH and serum calcium levels were above the normal range in 7 and in the upper normal range in 3 patients (PTH: median 157, interquartile range 94 – 177 pg/ml; Ca^{++}: median 2.9, interquartile range 2.6 – 2.95 mmol/1; Table **2**). After parathyroidectomy, a significant decrease was observed in serum levels of intact PTH and calcium over 90 days (median PTH: 35.5, interquartile range 24 – 56 pg/ml, median Ca^{++}: 2.27 interquartile range 2.2 – 2.4 mmol/l, $p < 0.01$, Table **2**) with a significant correlation between both parameters (R: 0.75, $p < 0.001$).

5 days after parathyroidectomy (PTX), the absolute number of red blood cells, hemoglobin and hematocrit decreased in a similar way, but increased again 30 days after PTX and reached nearly pretreatment values after 90 days (Fig. **1**).

Erythropoietin, the main regulator of erythropoiesis, showed a steady increase shortly after PTX and rose marginally during the whole period from 10 U/L (interquartile range 5 – 25 U/L) to 19.5 (U/L (interquartile range 6 – 34 U/L, n.s.). A similar change was observed for reticulocytes (data not shown).

On day 30 and 90 after PTX all hematopoietic progenitor cells showed a tendency to rise as compared to pretreatment values, but only the BUFe (median pretreatment value $313 \cdot 10^5$, interquartile range $177 – 519 \cdot 10^5$ PMNC) reached significance after 90 days (median posttreatment value $562 \cdot 10^5$, interquartile range $439 – 920 \cdot 10^5$ PMNC, $p < 0.03$, Fig. **2**).

Moreover, a strong negative correlation was seen only between PTH, but not serum calcium concentrations and BFUe levels ($r = -0.5$, $p < 0.03$). In contrast, no correlation could be detected between PTH and the other progenitor precursors cells.

In the control group of patients with hemithyroidectomy, PTH and serum calcium remained in the normal range at all time points before and after operation, displaying a slight decrease of PTH after surgery (Table **2**). In parallel, the absolute number of erythrocytes, hemoglobin and hematocrit dropped in a similar way as in the patients with pHPT, already reaching preoperative values after 30 days. The changes of these parameters did not differ significantly from the patients with pHPT (Fig. **1**). In contrast to the patients with pHPT, their hematopoietic progenitor cells stayed in the upper range of normal

Table 1 Patients' characteristics

	Patient	Age	Sex	PTH (pg/ml)	Ca^{++} (mmol/l)	Protein (g/l)	HB (g/dl)	HK (%)
pHPT	1	67	F	152	2.6	74.1	12.8	38.6
	2	75	M	162	3.05	65.0	13.6	41.9
	3	69	F	78	2.43	64.0	14.8	44.4
	4	80	F	143	2.9	77.9	13.5	41.8
	5	70	F	226	2.9	72.8	14.8	44.5
	6	53	F	177	2.9	73.3	12.7	37.6
	7	75	F	179	2.95	66.7	11.7	34.7
	8	75	M	175	2.97	71.2	14.5	43.3
	9	52	F	95	2.5	62.5	14.0	42.3
	10	80	F	94	2.93	64.0	14.0	43.0
TA	1	74	M	25.3	2.53	69.4	14.3	40.8
	2	75	M	39.2	2.41	71.5	15.0	43.2
	3	68	F	26.3	2.41	73.2	12.1	35.6
	4	69	F	35.3	2.25	69.5	14.9	43.3
	5	79	F	24.3	2.34	68.5	13.4	38.4
	6	80	F	12.6	2.34	68.1	14.7	43.2
	7	55	F	28.3	2.33	68.7	14.0	41.3
	8	52	F	14.7	2.25	71.8	12.0	35.8
	normal range			(10–65)	(2.1–2.6)	(65–85)	(12–17)	(40–53)

pHOT: Patients with primary hyperparathyroidism
TA: Patients with thyroid adenoma

Table 2 Time course of iPTH and serum calcium

		0 days	5 days	30 days	90 days
PTX	PTH	157 (94–117)*	14 (8–23)	57 (27–69)	35.5 (24–56)
	Calcium	2.9 (2.6–2.95)*	2.14 (1.95–2.3)	2.35 (2.2–2.4)	2.27 (2.2–2.4)
TX	PTH	27 (24–35)	17.5 (12–34)	11.1 (8–34)	13.5 (10–25)
	Calcium	2.34 (2.33–2.4)	2.23 (2.21–2.24)	2.31 (2.3–2.35)	2.41 (2.34–2.43)

* $p < 0.05$; median ± interquartile range
PTX: Patients before and after parathyroidectomy
TX: Patients before and after hemithyroidectomy

and showed no change during the observation period of 90 days (Fig. **2**).

Erythropoietin levels rose slightly from 9 U/L (interquartile range 5–23 U/L) to 16 U/L (interquartile range 7–29 U/L, n.s.) in the control group and the values were not significantly different from the values of the patients with pHPT (data not shown).

Patients undergoing parathyroidectomy had significantly higher levels of serum calcium ($p < 0.01$) and iPTH ($p < 0.005$) (Table **2**) and significantly lower levels of CFU-GM ($p < 0.003$) and BFUe ($p < 0.05$) than the patients with hemithyroidectomy at the basal state (Fig. **2**).

Discussion

Anemia has been reported in patients with primary hyperparathyroidism and advanced bone disease, where parathyroidectomy led to an increase in red blood cell counts (2). *In vitro* studies supported the hypothesis that PTH inhibits erythropoiesis at the stage of progenitor cells in both human and murine BFUe, but not CFUe (8), but adequate amounts of EPO could overcome this inhibitory action of PTH.

In accordance with these previous data, this study demonstrates a rise in the number of hematopoietic progenitor cells after successful parathyroidectomy with a significant negative correlation of BFUe with iPTH, but not with serum calcium. After PTX red blood cells, hemoglobin and hematocrit dropped due to blood loss during surgery and due to rehydration by fluid replacement during and after operation, but was restored almost entirely after 90 days. It is noteworthy that none of our patients initially had marked anemia, and thus special preoperative precautions such as preparation of additional red blood cell packs did not appear to be necessary. However, hematopoietic progenitor cell numbers prior to PTX were significantly lower when compared to normal controls. One of the reasons for the lack of substantial suppression of erythropoiesis in our patients might be the short duration of PTH excess or mild disease without marked bone alterations in the x-rays

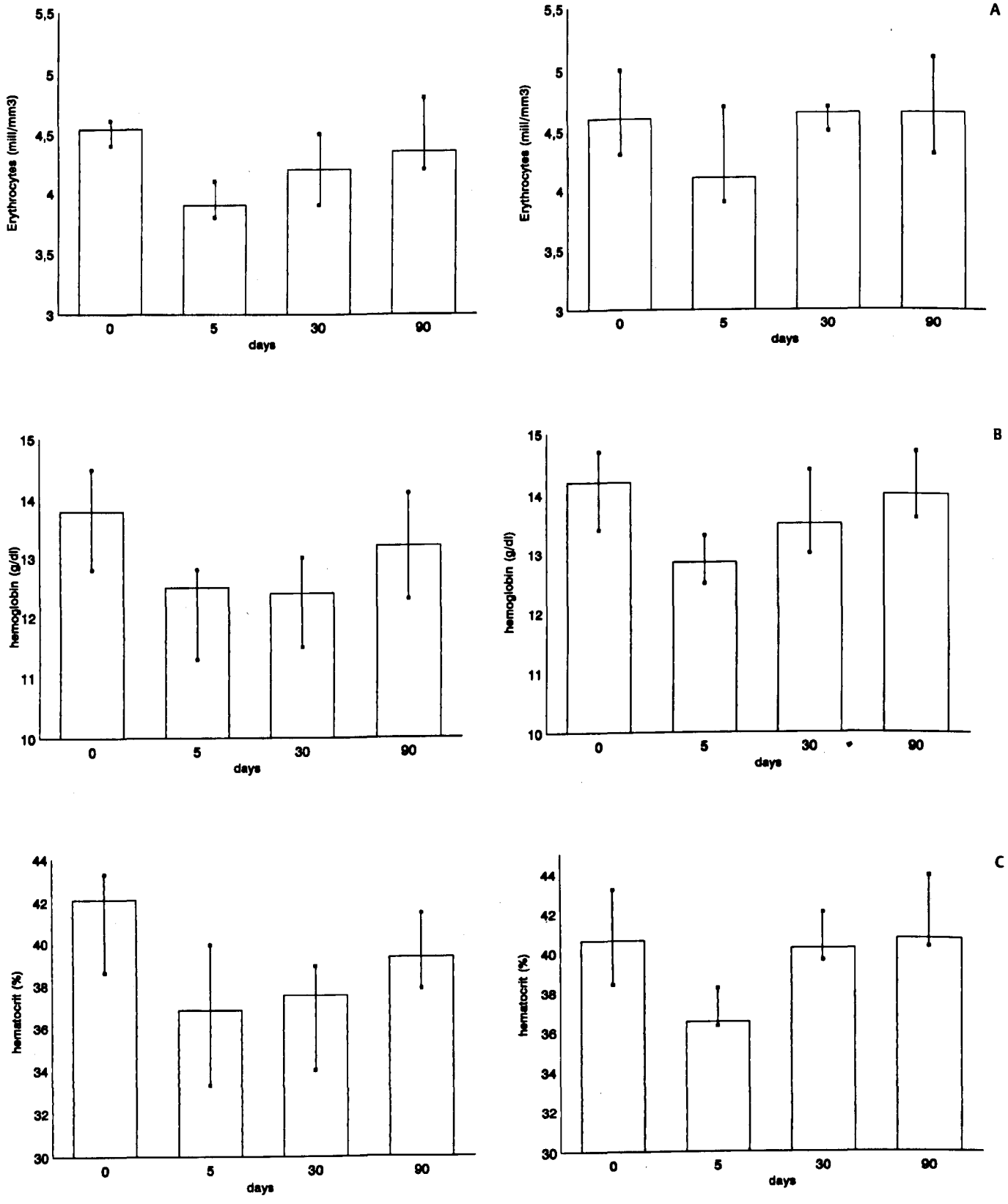

Fig. 1 **A** Erythrocyte counts, **B** hemoglobin and **C** hematocrit values before and 5, 30, and 90 days after parathyroidectomy (left panel) and before and 5, 30 and 90 days after hemithyroidectomy (right panel) (data are expressed as median with interquartile range)

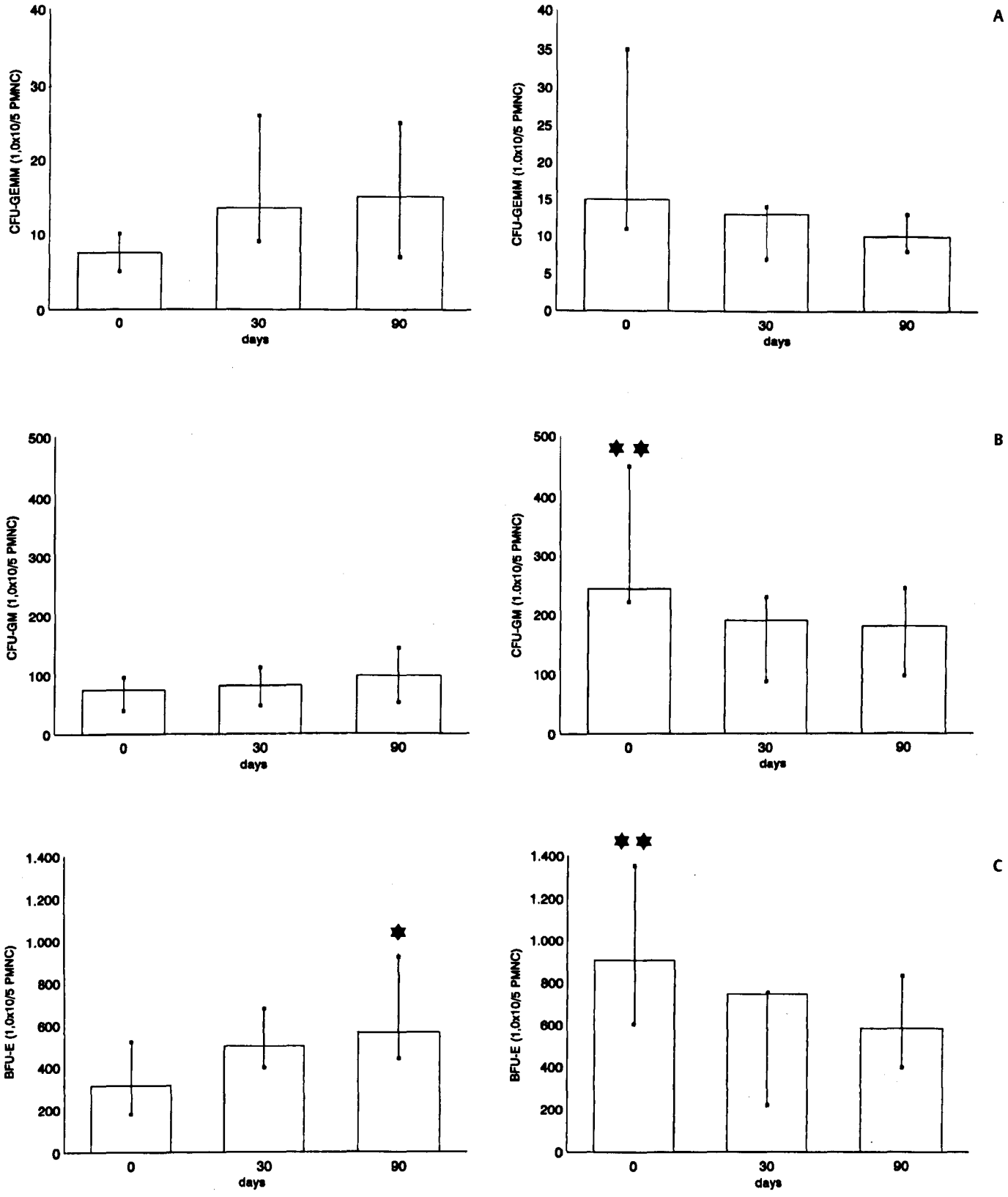

Fig. 2 Hematopoietic progenitor cells, **A** CFU-GEMM (colony forming units – granulocyte / erythrocyte / macrophage / myelocyte, normal range 4–77), **B** CFU-GM (colony-forming units granulocyte/macrophage, normal range 50–936), **C** BFUe (burst forming units-erythroid, normal range 120–1862) before, 30 and 90 days after parathyroidectomy (left panel) and before, 30 and 90 days after hemithyroidectomy (right panel) (data are expressed as median with interquartile range)

* $p < 0.05$ = significance within one group in comparison to basal state

** $p < 0.05$ = significance between the two groups in the basal state

of the bone and normal kidney function and thus normal EPO production.

However, the rise in BFUe numbers after correction of PTH overproduction and the significant negative correlation between BFUe and iPTH, but not serum calcium, indicates a causal role of PTH in the observed changes in erythropoiesis, which remains subclinical unless considerable bone involvement or marrow fibrosis occurs.

In contrast to the patients after PTX, the control group displayed normal serum concentrations of PTH and calcium, showed hematopoietic progenitor cells in the upper normal range without any changes after red blood cells, and hemoglobin were normalized after operation.

The importance of intact parathyroid glands producing adequate levels of PTH and calcium in correcting anemia after bleeding has been demonstrated (9,10). The factor that induces the rise of iPTH and serum calcium after acute blood loss is not hypovolemia, but the loss of blood cells which stimulates the mitotic activity in bone marrow. The cellular mechanism through which PTH affects erythropoiesis might be the stimulation of calcium entry into erythroid cells.This regulation is abolished in thymoparathyroidectomized rats (9,10).

Studies in serum free cultures of fetal mouse liver cells showed that PTH stimulated erythropoiesis at low concentrations in a dose dependent way only in the absolute absence of exogenous erythropoietin (13,14). The effect of PTH and erythropoietin is calcium dependent, and calcium is known to be required for both extracellular and intracellular action of EPO at its target cells (15). In higher concentrations (250 times of normal) PTH suppresses erythropoiesis with a significant impairment of the EPO response (5,6), yet it was not possible to demonstrate a significant relationship between PTH and anemia or inhibition of erythropoiesis in patients with uremia.

These experiments indicate a biphasic action of PTH with both stimulatory and inhibitory effects on the EPO responsive cells as a function of its concentrations.

After PTX, there was an expansion of hematopoietic progenitor cells (mainly the BFUe) by approximately 200%. This could be due to the abolishment of direct inhibition of PTH on the progenitor compartment, as we know from *in vitro* studies that PTH inhibits erythropoiesis at the stage of BFUe (8). Alternatively, rising erythropoietin levels could be responsible, since we have shown previously that administration of recombinant EPO to patients with endstage renal failure led to a signifcant increase in the number of BFUe in these patients (16). If the blood loss with the decrease of hemoglobin concentrations had been the main cause for the changes in progenitor cells, one would have expected the erythropoietin concentrations to react in a different pattern with a strong increase immediately after blood loss followed by a decrease.

In summary, the inhibitory effect of PTH on hematopoietic progenitor cells might be abolished after PTX in humans. Thus, normalisation of PTH and calcium serum concentrations after successful PTX may induce an increase in the erythroid progenitor cells.

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