Abstract
This study analyses the growth and the growth hormone secretion of children given various conditioning protocols before bone marrow transplantation (BMT). Twenty nine children (14 boys, 15 girls) given BMT were classified according to their conditioning protocol: total body irradiation (TBI) given as a single exposure of 10 Grays (Gy, group I, 11 cases), or 8 Gy (group II, four cases), 12 Gy given as six fractionated doses (group III, seven cases), or chemotherapy alone (group IV, seven cases). The arginine-insulin stimulated growth hormone peak, 2-7.5 years after BMT, was >10 μg/l in all patients except four from group I (6.9-8.9 μg/l). A second growth hormone secretion evaluation was performed in 10 group I patients because of persistent low growth velocity despite a normal growth hormone peak. There were no significant changes in the mean (SEM) stimulated growth hormone peak (18-4 (2-2) vs 20-1 (3-6) μg/l) at 3 (0-3) to 5-2 (0-6) years after BMT. The sleep growth hormone peaks and concentrations (n=6) were normal. The mean cumulative height changes (SD) during the three years after BMT were: -1.4 (0-2) in group I, -0.1 (0-4) in group II, -0.4 (0-2) in group III, and 1-5 (0-5) in group IV; this was significant in groups I and IV. The final heights of two monozygotic twins (BMT donor and recipient) had differed by 17.5 cm, despite them both having normal growth hormone peaks and puberty. Eight patients, treated for congenital immune deficiency syndrome, were growth retarded at the time of BMT. Of these, only those conditioned by chemotherapy alone had significantly catch up growth (2 (0-6) SD) while those conditioned by a single 8 Gy exposure did not (0 (0-4) SD).

It is concluded that the total radiation dose is critical for growth evolution, as is the fractionation schedule. For the TBI doses and the interval since BMT studied, there was no correlation between growth hormone peak and the height loss. The rapidity of decreased growth velocity after TBI and the comparison between the monozygotic twins suggest that radiation induced skeletal lesions are partly responsible for the decreased growth.

Patients and methods
PATIENTS
Twenty nine children (14 boys, 15 girls) given BMT were evaluated at the Paediatric Endocrinology Unit, Hôpital des Enfants Malades, Paris. The evaluations were performed after informed consent had been obtained from the children or from their parents. None of the patients had received any previous radiation. Patients were prepared for BMT by chemotherapy alone or by chemotherapy plus TBI. The patients were assigned to one of four groups, according to their conditioning protocol for BMT (Table I): 11 patients were given 10 Grays (Gy) TBI as a single four hour exposure (group I), four patients were given 8 Gy TBI as a single four hour exposure (group II), seven patients were given 12 Gy TBI as six fractions of 200 cGy over three consecutive days (group III). The remaining seven patients were prepared by chemotherapy alone (group IV). The mean (SEM) ages at BMT were 7-3 (1) years (group I), 3-1 (1-1) years (group II), 5-3 (3-1) years (group III), and 1-3 (0-4) years (group IV). The irradiation was delivered with a 5-5 or 18 MV linear accelerator. The dose rate was 4-2 cGy/min in group I and II and 50 cGy/min in group III. The conditioning chemotherapy varied depending on the initial disease. Twenty one patients were given cyclophosphamide (cases 1-9, 11-15, 23-29). Twelve patients were given etoposide (cases 4, 16-22, 26-29). Seven patients were given melphalan (cases 10, 16-22). Eight patients were given cyclosporin to prevent graft-versus-host disease (cases 1, 3-5, 9, 10, 26, and 28). Prednisone was terminated within six months after transplantation in all cases. Grade 1 acute grafted
versus-host disease occurred in two group I patients (cases 3 and 9) and in two group IV patients (cases 26 and 27). It had been terminated at time of the study and all patients with haemophaty were in remission. No patient had raised blood pressure or clinical evidence of chronic graft-versus-host disease. The serum creatinine concentrations were within normal limits.

**PROTOCOL**

During the study period, a total of 65 patients were evaluated in the Paediatric Endocrinology Unit after BMT. They included (1) all those conditioned by TBI and chemotherapy in the Paediatric Oncology Unit, Institut Curie and the Bone Marrow Transplantation Unit, Hopital St Louis during that period and (2) the first group of children conditioned by chemotherapy alone at the Immunology and Haematology Unit, Hopital des Enfants Malades. Twenty-nine of these patients were included in this study, and 36 were excluded because of factors other than conditioning for BMT interfering with growth or growth hormone secretion: previous cranial irradiation (n = 16), additional non-cranial irradiation (n = 4), disease relapse after a short time (n = 5), thalassaemia major (n = 1), interval since BMT less than two years (n = 6), BMT complications such as chronic graft-versus-host-disease (n = 2), renal insufficiency (n = 1), or scoliosis (n = 1). Patients conditioned for BMT by thoracoabdominal irradiation were not included.

The 29 patients were followed up from the time of BMT. Height, weight, and genital maturation, basal plasma thyroid stimulating hormone and free thyroxine were recorded each year. The duration of this clinical follow up varied from three to 10 years. All patients but two (cases 7 and 11) were prepubertal throughout this follow up period. The first growth hormone evaluations, using arginine-insulin stimulation and plasma insulin-like growth factor-I (IGF-I) evaluation, were performed at least two years after transplantation (range 2-6-6 years, mean 3.1 (0-2) years). This interval was not significantly different from one group to another. A second growth hormone evaluation using arginine-insulin stimulation was performed in 10 patients. Five of these patients were also evaluated for their growth hormone secretion during sleep the night before the stimulation test. This second growth hormone evaluation was performed in all patients who continued to show a decreased growth velocity, in spite of a normal growth hormone peak response to the first stimulation and normal basal plasma thyroid stimulating hormone and thyroxine. In this
METHODS
Height was measured using a Harpenden stadiometer. The changes were expressed as the SD score for height based on the chronological age, and compared with the normal values for the French population. Growth hormone secretion was evaluated by arginine-insulin stimulation.7 Sleeping growth hormone concentration was measured on blood samples taken every 20 minutes, from 2200 to 0400 hours. Plasma cortisol was measured at 0800 hours and after insulin induced hypoglycaemia. Blood specimens were drawn and kept frozen at −20°C until assayed for plasma IGF-I, free thyroxine, thyroid stimulating hormone, cortisol, and (in pubertal age boys) testosterone. Plasma growth hormone peak values over 10 µg/l were considered as normal as it is the limit set by the French Pituitary Agency below which human growth hormone treatment is given. Growth hormone concentrations during sleep of over 3 µg/l were considered normal. The normal limits for plasma free thyroxine were 12-28 pmol/l and those for plasma thyroid stimulating hormone were 0-6-5 mU/l. Plasma IGF-I was determined in unextracted plasma collected over EDTA by the non-equilibrium technique of Furlanetto.8 The endocrine parameters were measured by radioimmunoassay.

The height changes within each group were analysed using the Wilcoxon matched paired test. All other comparisons were performed by the unpaired Wilcoxon two sample test. All results are expressed as mean (SEM).

RESULTS
GROWTH HORMONE SECRETION AND PLASMA IGF-I
At the first evaluation (table 1), the mean growth hormone peak values after arginine-insulin stimulation (µg/l) were 16-1 (2-5) in group I, 20-2 (4-2) in group II, 20-8 (3-6) in group III, and 27-4 (3-6) in group IV. Only the group I patients (TBI, 10 Gy×2) had a significantly lower growth hormone peak value than that of group IV patients (chemotherapy alone, p<0-02). Three patients from group I had growth hormone peak values (µg/l) below 10-75 (case 1), 6-9 (case 10), and 8-9 (case 11). The concomitant growth hormone peak and concentration during sleep of case 1 were normal (11-6 and 3-6 µg/l respectively). The plasma IGF-I values were interpreted according to the sex and age of patients.4 Eleven of the 29 patients had plasma IGF-I values below the lower limits of normal. These low values of plasma IGF-I were found in the two patients who were low growth hormone responders (cases 1 and 10) and in nine normal growth hormone responders (cases 3, 5, 12, 16, 18, 22, 24, 27, and 28). All had normal plasma thyroxine values. Plasma IGF-I values were not correlated with the body mass index (height/weight).4 The mean values of this parameter in the whole population and in group I were normal (46 (5) centile).

At the second growth hormone evaluation (table 2), performed 2-2 years after the first evaluation, the mean growth hormone peak after arginine-insulin stimulation had not changed significantly (18-4 (2-2) v 20-1 (3-6) µg/l, n=10). This evaluation confirmed the low growth hormone peak in case 11 and showed a lower growth hormone peak in case 3. Five patients with normal stimulated growth hormone peaks had normal growth hormone peaks and concentrations during sleep. The mean plasma IGF-I value did not change significantly (0-7 (0-1) v 0-9 (0-1) U/ml). Three of the four patients having low IGF-I values at the first evaluation had normal value at the second.

OTHER ENDOCRINE DATA
Plasma free thyroxine was low in three patients (cases 2, 6, and 19). The basal plasma thyroid stimulating hormone values were raised (>5 mU/l) in seven patients (cases 1, 2, 4-6, 10, 17). Six of them belonged to group I and one to group III. These patients were therefore given l-thyroxine replacement treatment (50–100 µg/m²/day) from that time. This treatment did not improve their growth rate. Basal and insulin stimulated plasma cortisol concentrations were normal in all cases (data not shown). Only two boys were pubertal (cases 7 and 11) with plasma testosterone values of 2-9 and 9-7 nmol/l respectively.

GROWTH
The growth evolution of each group was different. All the patients were followed up for at least three years after BMT. The mean cumulative height changes (SD) during this period were: −1-4 (0-2) (p<0.001) in group I (compared with height at BMT), −0-1 (0-4) (not significant) in group II, −0-4 (0-2) (not significant) in group III, and 1-5 (0-5) (p<0.05) in group IV (table 1). As shown in fig 1, the mean SD score for height decreased until the fourth year after irradiation in group I. In contrast, there were no changes in

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<th>Table 2</th>
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Mean (SE) 3 (0-3) 18-4 (2-2) 0-7 (0-1) 5-2 (0-6) 20-1 (3-6) 1-0 (0-1)

*After arginine-insulin stimulation.
†Peak (concentration).
groups II and III, and catch up growth was observed until the third year in group IV. The evolution of the individual data is shown in fig 2. The growth of the patients with growth hormone peak <10 μg/l (cases 1, 3, 10, and 11) was similar to that of the other patients in group I having normal growth hormone secretion. Three of them were treated with human growth hormone.

The growth velocity was normalised in one and increased for age in a second (fig 2). The extent of growth retardation after a single 10 Gy exposure despite normal growth hormone secretion was documented by comparing the growth evolution of monozygotic twins: the case 7 boy had a final height 17.5 cm below that of his brother who donated the bone marrow for transplantation. This occurred in spite of case 7 having a normal growth hormone peak, plasma IGF-I concentration, and a similar age at pubertal development (fig 3). Growth was further analysed in the eight patients treated for congenital immune deficiency syndrome who were growth retarded at the time of BMT. The height changes after three years of those given TBI (8 Gy single exposure, cases 12–15) were compared with those of the patients given chemotherapy alone (cases 23–26) (fig 2). The TBI group had no change in mean height (0 (0·4) SD) but the chemotherapy group had a mean height gain of 2 (0·6) SD indicating strong catch up growth. The difference between these two groups was significant (p<0·01). Data of upper segment length, measured as the sitting height, were collected at the first evaluation (3·8 (0·7) years after BMT) in 14 patients. When expressed as the SD score, it was found to be a mean (SD) −1·7 (0·4) while the concomitant height was −1·7 (0·4).

**Discussion**

Short stature could be a severe side effect in children treated by BMT. We therefore asked two questions. First, what is the risk of growth hormone deficiency due to irradiation of the hypothalamus and pituitary as part of TBI? Patients who had previously been
given cranial irradiation were not included in this study. Second, is the growth related to the conditioning protocol? Only the group of children who had been exposed to a single exposure of 10 Gy failed to grow, while patients given single dose of 8 Gy or a fractionated dose of 12 Gy grew normally and those conditioned by chemotherapy had catch up growth.

Growth hormone deficiency induced by TBI is one of the potential causes of growth retardation. Its frequency is difficult to evaluate as the published studies were performed after various protocols of TBI, times after TBI, and methods of growth hormone stimulation. The growth hormone peak (µg/l) after one stimulation in patients without previous cranial irradiation was found to be 7–10 µg/l in 6/18 patients evaluated by Sanders et al.,8 below 8 µg/l in 10/18 patients evaluated after 10 Gy by Borgström et al.,14 below 10 µg/l in 1/5 patients evaluated after 9–10 Gy by Papadimitriou et al.,15 and also below 10 µg/l in 12/25 patients evaluated after 11–15-2 Gy by Ogilvy-Stuart et al.12 The spontaneous growth hormone secretion was found to have a below normal number of peaks and amplitude in 7/9 patients evaluated by Borgström et al.8 and only below normal amplitude in 9/10 evaluated after 10 or 12 Gy by Hovi et al.16 This last study included three patients who had previously received cranial irradiation. It showed that the mean nocturnal growth hormone concentrations decreased with the length of the follow up period. In these studies, the TBI was delivered as a single dose or in six fractions and the duration of follow up extended from one to 14 years. In the present study, the growth hormone secretion was evaluated after a post-transplant interval longer than two years. Among the 22 patients conditioned by TBI, only four had growth hormone peak below 10 µg/l. All belonged to group I (10 Gy in a single exposure), which included 11 patients. The patients who had a second growth hormone evaluation performed 2–2 years after the first evaluation because of a persistent decrease in their growth velocity, showed no change in this parameter.

Only one patient with a normal growth hormone peak at the first growth hormone evaluation became growth hormone deficient at the second evaluation. The plasma IGF-1 concentration was low in several patients at the first evaluation without any relationship with the body mass index. Some of the low values became normal at the second evaluation. This normalisation suggests that the low initial values may have been due to factors other than decreased growth hormone secretion, such as initial chemotherapy or disease. There was not a good agreement between the growth hormone peak and plasma IGF-1 values. These data, taken together, suggest that TBI at a single dose of 10 Gy or more may partially reduce growth hormone secretion. The growth hormone secretion of patients given 8 Gy was normal in the present study. Longer follow up of more homogeneous cohorts are required to evaluate the frequency and the delay of growth hormone deficiency after various doses of TBI.

What is the relationship between growth hormone secretion and growth after TBI? Factors other than growth hormone deficiency, such as chronic graft-versus-host disease, corticosteroid treatment, or renal insufficiency may be responsible for the growth retardation after BMT. Any patient who presented with these complications was excluded from this study. The mean body mass index was normal and thyroid insufficiency was compensated. A rapid decrease in growth velocity occurred only in the patients given 10 Gy TBI in a single exposure. Conversely, the patients given 12 Gy in six fractions had normal growth velocity. Among the patients given irradiation, only those conditioned by chemotherapy had catch up growth. Those conditioned by 8 Gy single exposure did not, in spite of a normal growth hormone peak after stimulation. This lack of catch up growth suggests that skeletal lesions may have occurred in irradiated patients. This finding must be confirmed on a larger group, however, as the difference may be due in part to the patients conditioned by chemotherapy alone being younger. There was no correlation between the growth hormone peak and the height loss in the 10 Gy TBI group; this was also found by Hovi et al.16 Borgström et al. found a decreased growth rate in patients given a single 10 Gy dose.17 The slowdown began during the first year after transplant and they suffered a total height loss of about 2 SD after 5 years.

Bushouse et al. found a mean height loss of 0-67 SD during the two years after a single 7.5 Gy TBI dose.18 Sanders et al. found that the type of TBI (10 Gy × 1 or 2 or 2.25 Gy × 6 or 7) did not appear to affect height until three years or more after transplant,4 at which time those who had received fractionated TBI grew significantly taller than those who had received a single exposure. These data and the comparison between the two twins suggest that the decrease in growth velocity secondary to TBI may be partly due to cartilage and bone lesions. This is comparable with the data observed in children who received spinal irradiation for medulloblastoma. A significant failure to grow occurred with the mean growth hormone secretion before the decrease in growth hormone secretion.19 Neuhauser et al. described roentgenogram vertebral body changes after spinal irradiation.17 They showed the importance of the total radiation dose, duration of treatment, and age at irradiation. Doses of 10 Gy delivered in one week produced changes in children younger than 2 years.17 Tefft showed that these changes occurred after lower doses when delivered to children less than 1 year old.19 Willi et al. found a persistent decreased growth velocity in patients who had transplants for neuroblastoma.10 The role of chemotherapy, especially melphalan, was discussed. However, their patients also received local radiotherapy, which may decrease growth velocity. Papadimitriou et al. have found that sitting height SD scores had been affected more than subischial leg length in 13 children evaluated after a mean interval time of 3-2 years.10 In the present study, sitting height and total height, evaluated 3-8 years after BMT, were similar.

In conclusion, the decrease in growth velocity occurred only in patients conditioned for transplantation by 10 Gy delivered in a single
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exposure. The mechanism of this decrease is unknown. Four patients had growth hormone peaks in the lower range. The normalisation of their growth velocity on standard doses of human growth hormone treatment favours the role of this growth hormone decrease in the decreased growth. However, the absence of agreement between growth hormone peak, plasma IGF-I concentrations, and growth in this group indicates that the contribution of growth hormone deficiency to the decreased growth is not clear. Other mechanisms for growth retardation were ruled out. As other studies showed that single dose induced complications more frequently than fractionated doses, our data are a further argument favouring conditioning by fractionated schedule or chemotherapy.

Growth and growth hormone secretion after bone marrow transplantation.

R Brauner, M Fontoura, J M Zucker, A Devergie, J C Souberbielle, C Prevot-Saucet, J Michon, E Gluckman, C Griscelli and A Fischer

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