Table 1. Sensitizer enhancement ratios (at a surviving fraction of 1%)

Concentration (mM)	Oridonin Aerobic	Hypoxic	Misonidazole Hypoxic
0.01	1.14	1.16	-
0.025	-	1.66	-
0.05	1.90	2.26	-
0.1	-	-	1.13
0.5	-	-	1.40
1.0	-	-	1.59
5.0	-	-	2.22

Table 2. Sensitizer enhancement ratios after the combined treatment (at a surviving fraction of 1%)

	Aerobic	Hypoxic
Oridonin (0.1 mM)	1.14	1.16
Misonidazole (1 mM)	1.0	1.59
Oridonin+misonidazole	1.14	1.92

oridonin, but when the concentration was increased to 0.05 mM, severe cytotoxicity appeared and the plating efficiency decreased to around 0.1. On the basis of these results 0.01 mM oridonin was used in the combination experiment.

Figure 3 shows the survival curves for combined drug-treated and untreated V79 cells irradiated in the absence or presence of oxygen. The combination of 0.01 mM oridonin and 1 mM misonidazole resulted in greater sensitization than with either drug alone under hypoxic conditions. On the other hand, under aerobic conditions no sensitization was obtained by the same combination of those drugs. Table 2 summarizes the ERs calculated from figure 3. An ER of 1.92 was obtained when 0.01 mM oridonin and 1 mM misonidazole were administered to hypoxic cells with radiation. Since the ERs of oridonin and misonidazole for hypoxic cells were 1.16 and 1.59 respectively, a supra-additive effect was obtained by combined treatment with these two drugs. Because there are no differences in the ER between the combination of oridonin with misonidazole and oridonin alone under aerobic conditions, it is clear that 0.01 mM oridonin had no effect on the radiosensitization of aerobic cells by misonidazole.

As mentioned above, it is important to develop a potentiator to enhance the effect of a hypoxic cell radiosensitizer. The present results suggest the possibility that oridonin can be used as a bifunctional agent which has an antitumor effect as a potentiator of misonidazole. Therefore, we propose the hypothesis that improvement in the radiosensitizing effectiveness of misonidazole would be caused by depletion of intracellular glutathione and nonprotein and protein thiol after the treatment with oridonin. This hypothesis can be supported by the facts that the center of physiological activity of oridonin is considered to be the α-methylene cyclopentanone function[9] and that the appearance of the physiological activity may be due to the deactivation of the SH enzymes (DNA and RNA polymerase) by oridonin[10]. More detailed investigations, e.g. measurement of nonprotein and protein thiols, the time schedule of administration of the combination and also in vivo experiments, are needed to clarify whether or not oridonin has a therapeutic potential.

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Collagen synthesis by human bone marrow fibroblasts[1]

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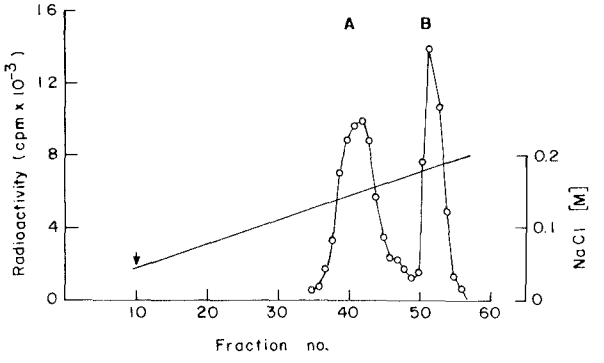
Summary. Collagen synthesis was measured in fibroblast cultures derived from normal and acute lymphoblastic leukemia (ALL) bone marrow. Collagen production was higher in normal than in ALL fibroblasts. These cells elaborate type I and type III procollagens in a ratio that depends on cell density and whether cells originate from normal or ALL bone marrow.
Key words. Collagen; bone marrow; acute lymphoblastic leukemia; fibroblasts.

Bone marrow stromal cells and their products (growth factors and extracellular matrix) form a hemopoietic microenvironment for stem cell proliferation and differentiation[2]. Among extracellular matrix (ECM) components, collagen, apart from its structural role, has numerous developmental and physiological functions. As judged by studies with hemopoietic long-term culture systems, collagen possibly affects growth factor production or has a direct influence on the hemopoietic stem cell[3].

Recent data have shown that bone marrow obtained from patients with acute lymphoblastic leukemia (ALL) either at diagnosis or during therapy, have a population of damaged stromal cells with impaired growth capacity[4]. Improvement after successful therapy suggests reversible damage[5]. Whether the damaged population of stromal cells in ALL also presents an impaired production of ECM components is not known. The current studies were undertaken to assess and compare the production and nature of collagen synthe-



DEAE-cellulose separation of labeled procollagens produced and secreted into the medium by normal fibroblasts. Radioactive proteins from the culture medium of a normal BMF culture were precipitated by ammonium sulfate and applied to a DEAE-cellulose chromatography column. Arrow indicates start of NaCl gradient. Peaks A and B correspond to procollagens type I and III, respectively.

sized by fibroblasts from normal and ALL bone marrow. In vitro these cells represent a major stromal phenotype[6].

Materials and methods. Fibroblasts were obtained from the bone marrow of 6 patients (4–12 years of age) undergoing orthopedic surgical procedures and from 6 children with acute lymphoblastic leukemia, undergoing marrow aspiration at the time of diagnosis (morphologic and immunophenotype classification). After informed consent, samples were collected and processed as described[4]. Briefly, bone marrow cells were suspended in alpha MEM (minimum essential medium, Gibco) containing 100 U/ml penicillin, 100 µg/ml of streptomycin and 10% fetal calf serum (FCS), seeded and incubated at 37 °C in a 5% CO_2 atmosphere.

After two passages[7], cultures of bone marrow fibroblasts (BMF) were grown to confluency and then labeled for 24 h with 10 µCi/ml of ^{3}H-proline (20 Ci/mmol, Amersham) in a medium consisting of α MEM containing 1% FCS, 50 µg/ml each of ascorbic acid and β-amino propionitrile. Cultures were incubated at 37 °C in a 5% CO_2 atmosphere. The medium was harvested and processed for determination of total labeled protein and for collagen synthesis by analysis of radioactive collagenase-sensitive material[8]. The cell layer was extracted (0.25% trypsin, 5 min at 37 °C) and used for cell count. Since cultures employed for these experiments were growth-arrested by confluency[7], the cell count on each plate before and after the 24-h labeling period was constant (approximately 120,000 cells/cm^2).

The type and ratio of procollagens synthesized by low and high density cultures of BMF were estimated by DEAE-cellulose chromatography[9]. For these studies, cells (10,000–40,000 cells/cm^2) were seeded and cultured for different periods of time (2–7 days) so that after the 24-h labeling period with ^{3}H-proline prior to harvesting, the low and high density cultures contained 40,000 and 120,000 cells/cm^2, respectively. Proteins from the culture medium were precipited by ammonium sulfate (300 mg/ml), dissolved and dialyzed overnight in 25 mM Tris-HCl buffer, pH 7.5, containing 2 M urea and 1 mM EDTA. The dialyzed material was applied to a DEAE-cellulose column (2.2 × 16 cm) equilibrated with the buffer used for dialysis. The columns were eluted with the dialysis buffer followed by a linear NaCl concentration gradient (up to 0.22 M) in the same buffer. Fractions (8 ml) were collected and radioactivity measured. Radioactive peak A (procollagen type I) and peak B (procollagen type III)[9] were dialyzed, lyophilized and used to measure collagen content by determination of collagenase-sensitive material.

Results. Collagen synthesis by bone marrow fibroblasts derived from hematologically normal and ALL patients was measured after a labeling period with tritiated proline. In both types of cells, the amount of collagen synthesized and found in the culture medium increases with the cell count until confluency is reached. At confluency, collagen in the medium represents approximately 60% of total collagen synthesized by bone marrow fibroblasts (data not shown). Collagen synthesis, expressed as percent of labeled collagen with respect to the total labeled proteins in the medium, differs depending on the origin of the cells. Collagen synthesis by normal fibroblasts is 33%, whereas in ALL fibroblasts it represents 23% (table, A).

The nature of the collagen synthesized by normal and ALL fibroblasts was estimated by separation of type I and type III procollagens by DEAE-cellulose column chromatography. Results in the figure show a typical elution profile of labeled proteins produced and secreted into the medium by normal fibroblasts. Various radioactive elution peaks (not shown) were resolved by the salt gradient before reaching 0.12 M NaCl. At higher salt concentrations, two main peaks (A and B) were obtained which correspond to type I and type III procollagens, respectively. Labeled proteins from the culture medium of ALL fibroblasts showed similar elution profiles. The ratio of procollagen type I/procollagen type III is shown in the table, B. This ratio in fibroblasts from normal bone marrow was 1, irrespective of whether the labeled-collagenous material proceeded from low or high density cultures. In fibroblasts from ALL marrow, however, the ratio of procollagens depends on the cell density. At low cell density a ratio of 3 was obtained, which decreases (1.3–2.0) at high cell density.

Discussion. Both normal and ALL bone marrow fibroblasts produce collagen and secrete it into the cell culture medium. We observed that the amount of labeled collagen with respect to total labeled material in the medium was higher in normal than in ALL fibroblasts. This result agrees with a lower net synthesis of collagen on a per cell basis observed in ALL fibroblasts as compared to normal cells[10].

These data suggest that in acute lymphoblastic leukemia, less collagen is available for deposit in the bone marrow stromal compartment, and this may result in the development of a defective extracellular matrix. The latter is consistent with the observation that ALL fibroblasts synthesize and release less fibronectin than normal cells[10]. The diminished synthesis of collagen by ALL fibroblasts might represent another intrinsic property of this damaged stromal phenotype[4,5] or a sort of down-regulation of collagen synthesis to match a diminished rate of deposition of ECM molecules. Decreased synthesis of collagen and other extracellular matrix proteins have also been reported to occur after viral transformation[11]. Normal and ALL bone marrow fibroblasts elaborate type I and III procollagens. The expression of these two types of collagens depends on the origin of the fibroblasts and on the cell density of the cultures. The constant ratio of procollagens found in normal bone marrow fibroblasts indicates that

Collagen synthesis and ratio of procollagens produced by bone marrow fibroblasts

Fibroblasts	A Collagen* (%)	B Procollagen ratio** Cell density	
		Low	High
Normal	33.2 ± 4.0 (13)	1.0; 1.0○	1.0●
ALL	22.6 ± 4.2 (11)	3.0●	1.3; 2.0○

* %Collagen: (labeled collagenase-sensitive material in the medium/total labeled material in the medium) × 100. Values are expressed as mean ± SD. Numbers in brackets indicate number of determinations performed in BMF cultures from 6 normal subjects and 6 ALL patients, respectively. ** Ratio of procollagen type-I/procollagen type-III after DEAE-cellulose chromatography separation. Ratio was determined in a single strain each of a normal and an ALL BMF culture. ○, Individual values for a duplicate culture dish. ●, Values for a single culture dish.

these cells are probably less affected by crowding and do not reduce the synthesis of type I collagen at confluency[12] as may occur in ALL fibroblasts. Density-dependent changes in the types of collagens synthesized by fibroblasts from different origins have been reported[12,13].

The ratio of collagen types here reported for normal human bone marrow stromal cells differs to that previously informed for murine stromal cells[14]. The discrepancy could be attributable to the different origin of the stromal cells or to the fact that in our studies cells were labeled for collagen synthesis at a low serum concentration[15].

These studies give additional support to previous observations on the existence of a population of damaged stromal cells in the bone marrow of ALL patients at diagnosis or during the early stages of therapy[5,16]. One may speculate that defects in the establishment of the extracellular matrix, such as those described in the murine bone marrow system[3], may contribute to an altered hemopoiesis.

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Puff activity after heat shock in two species of the *Drosophila obscura* group

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Summary. When individuals of *Drosophila guanche* are submitted to heat shock, five new puffs are induced. These puffs usually do not appear during normal development. Comparing these results with those obtained in *Drosophila subobscura*, also belonging to the *obscura* group, differences between the induced puffing pattern of both species have been found.
Key words. Drosophila guanche; Drosophila subobscura; puffs; heat shock.

It is well established that living organisms respond to heat shock by important changes in their gene expression[1-3]. Drastic alterations in the puffing pattern level after heat shock treatment have been observed in several *Drosophila* species: *D. busckii*[4], *D. melanogaster*[5,6], *D. hydei*[7]. These reports show that two effects clearly characterize the heat shock response at the puffing level: first, a new set of specific puffs is induced and second, the majority of puffs active during normal development (developmental puffs) decrease their activity. However, this model appears to be not so simple. *D. subobscura* displays some variability in the puffing response depending on the heat shock conditions[8]. Different responses were found depending on treatment temperature and an important number of developmental puffs do not decrease in their activity. Some of them maintain and some others increase their expression. This behavior contrasts with the remarkable conservation of the response described in a very wide variety of organisms, from *E. coli* to man[9].

In this work we compare data about the heat shock puffing response in *D. guanche* with those obtained by Pascual and de Frutos[8] in *D. subobscura*.

D. guanche, like *D. subobscura*, is considered to be a member of the *obscura* group of *Drosophila*[10-13]. Because of their cytogenetic characteristics both species are closely related and belong to the same cluster. A high degree of homology between the banding patterns of polytene chromosomes in the two species is evident, except for the sexual chromosome[14]. While *D. subobscura* exhibits a rich inversion polymorphism, *D. guanche* seems to be a monomorphic species. We used the *D. guanche* TF2 strain, descending from individuals captured in the Canary Islands. The TF2 strain differs from *D. subobscura* in at least six inversions[15].

Individuals synchronized at the beginning of prepupa formation (O-h prepupa), and incubated at 19 ± 1 °C, were exposed at 31 °C or 37 °C, during a period of 30 min. After temperature treatment all prepupae were dissected in Drosophila Ringer solution. For experimental treatments the method described by Pascual and de Frutos[8] and for cytological procedures the method described by de Frutos and Latorre[16] were used. Controls were carried out with synchronized prepupae which remained at 19 ± 1 °C, during the incubation period.

Five nuclei were sampled from each of the 60 individuals analyzed (20 individuals for each temperature, 31 °C or 37 °C, and control). Only two levels of activity were taken into account for each locus: (+) puff of maximum or medium size and (O) small size or no puff. For each experimental treatment, the number of type (+) observations related to the total observations, gives the frequency of appearance of each puff. Consequently, a high frequency of puffing would indicate a high gene activity.

For the location of the puffs, the chromosome map of