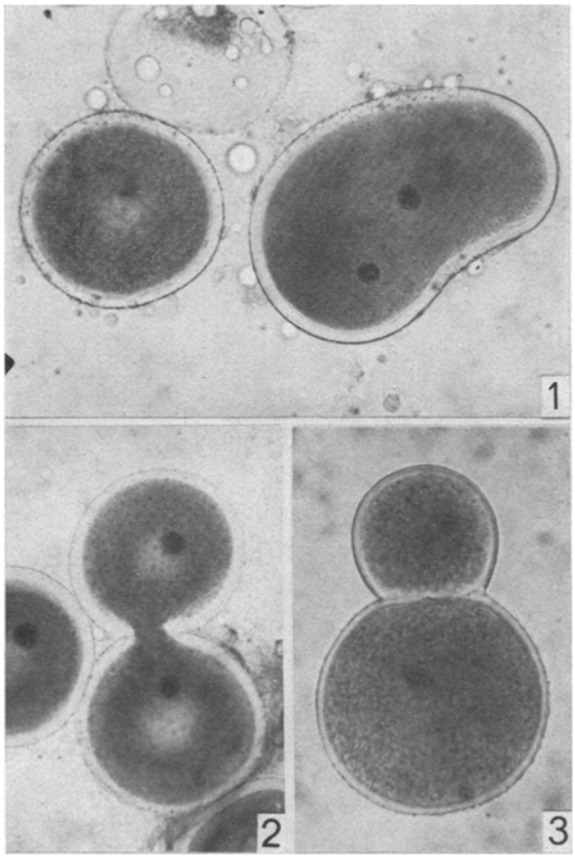

Figs. 1–3. Developing microspores of *Heliconia bihai* L. × 350. 1. The small round microspore is the one developed from a normal tetrad, with its nucleus remaining undivided. The larger elongated microspore is the one developed from a diad with its nucleus divided into two. 2. One of the large elongated binucleate microspores undergoing symmetrical furrowing of its wall. 3. Another large microspore undergoing asymmetrical furrowing of the wall producing a small bud.

nucleate microspores elongate, and a large proportion of them start furrowing of their wall across the middle region, which grows progressively deeper thereby separating the 2 nuclei to the 2 halves of the constricted microspore (Figure 2). The furrowing becomes complete in about 48 h after its initiation, finally dividing the developing microspore into 2 more or less equally sized 'daughter pollen'. In a small proportion of such microspores, furrowing of the wall is found to be asymmetrical resulting in the formation of variously sized buds (Figure 3). Frequency of the diads as well as incidence of the abnormal phenomenon showed considerable increase (25 to 30%) in materials subjected to cold treatment.

The abnormality observed in the present species is clearly a departure from the normal course of development of the microspore. A phenomenon comparable to this is known in *Petunia*[5], in which asymmetrical furrowing of the wall is reported to occur in a small proportion of the normal microspores, while in the present species the furrowing is predominantly symmetrical resulting in a 'binary fission' of the developing microspores that have developed from the diads, a phenomenon not so far known in angiosperms. The abnormality described here occurs spontaneously in plants growing in normal field conditions, and it is consistently noticed in materials collected from different localities in this region, and hence this may be under genetic control. However, it is interesting that the frequency of its incidence increases considerably in cold-treated materials which indicates that the phenomon is greatly influenzed by the effect of environment.

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Kinetics of Lymphocyte Division in Blood Cultures Studied with the BrdU-Giemsa Technique[1]

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Summary. Normal human lymphocytes were cultured for 72 h with different doses of BrdU. The analysis of metaphases processed with the BrdU-Giemsa method shows that in leukocyte cultures 3 different lymphocyte populations coexist which are able to perform 1, 2 or 3 rounds of replication in vitro. Moreover, it was concluded that 5 μg/ml is the minimal dose of BrdU inducing good differentiation in the areas of sister chromatid exchanges.

It is well known that lymphocytes cultured for 72 h in the presence of PHG may perform one or more mitotic divisions[2,3]. However, the percentage of cells in the 1st, 2nd or 3rd division at the moment of harvesting the culture has not been determined yet with accuracy.

It has been demonstrated that chromosomes which have incorporated 5-bromo-2 deoxyuridine (BrdU) into its DNA have decreased Giemsa stainability or quenched fluorescence with Hoechst 33258 which are directly proportional to the amount of BrdU in the DNA molecule[4–7].

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Percentage of cells in 1st, 2nd or 3rd mitosis in blood cultures

Donor	No. of mitosis	BrdU (10 μg/ml)	BrdU (5 μg/ml)		BrdU (3 μg/ml)
		Percent of cells	Percent of cells 1st series	2nd series	Percent of cells
A	1st mitosis	46.5	45	42	63
	2nd mitosis	46.5	47.5	50	35
	3rd mitosis	7	7.5	8	2
B	1st mitosis	63	50	67	75
	2nd mitosis	30	46	33	23
	3rd mitosis	7	4	0	2
C	1st mitosis	68	46	78	73
	2nd mitosis	30	47.5	21	25
	3rd mitosis	2	6.5	1	2
D	1st mitosis	27	39	–	50
	2nd mitosis	58	60	–	47
	3rd mitosis	23	11	–	3
E	1st mitosis	40	47	–	64
	2nd mitosis	55	47.5	–	34
	3rd mitosis	5	5.5	–	2

Thus, by analyzing the staining properties of metaphase chromosomes, it is possible to determine how many cycles in the presence of the base analogue the cell has had before entering mitosis. In this report we shall analyse 3-day human blood cultures labeled with BrdU in order to determine the number of cell cycles undergone by mitotic cells.

Material and methods. Blood samples from 5 normal donors were employed to set up a total of 15 lymphocyte cultures. A group of 5 cultures (1 culture from each donor) was treated with 3 μg/ml of BrdU. Another group of 5 cultures received 5 μg/ml of BrdU. The remaining 5 cultures received 10 μg/ml of the base analogue. All cultures were kept in complete darkness. After 72 h of incubation at 37 °C, the cultures were harvested and the chromosome spreads were treated with the BrdU-Giemsa technique of KOREMBERG and FREEDLANDER[5]. 1 week later, a second series of blood cultures were set up, treated with 5 μg/ml of BrdU and processed as described before.

Results and discussion. A total of 200 mitosis randomly selected were analyzed in each culture. The number of DNA synthesis periods performed by the cell during the 72 h of culture was determined for each metaphase and recorded (Figures 1 and 2).

The Table shows for each concentration of BrdU the percentage of cells which had gone through 1, 2 or 3 rounds of replication in the presence of the base analogue. The analysis of this Table shows that the percentage of cells in the 2nd and 3rd mitosis is lower with 3 μg/ml than with 5 or 10 μg/ml of BrdU. On the other hand, the figures corresponding to 1st mitosis show a reverse situation. The most likely explanation for this phenomenon is the difficulty in identifying the differences of stainability between chromatids when the dose of 3 μg/ml of BrdU is employed. With this concentration, the amount of BrdU incorporated into the DNA is probably not enough to produce a quenching in the intensity of the Giemsa stain equivalent to those induced by 5 and 10 μg/ml.

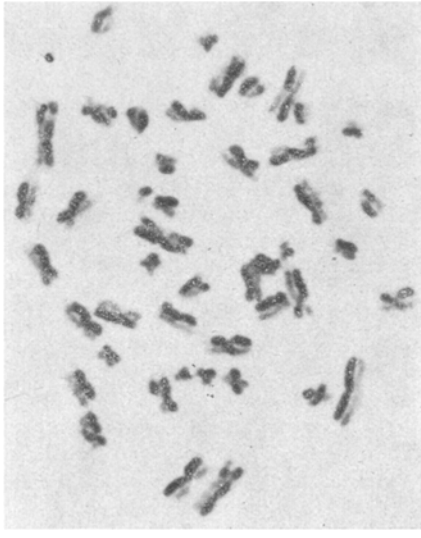

Fig. 1. Second division. BrdU-Giemsa method. ×1100.

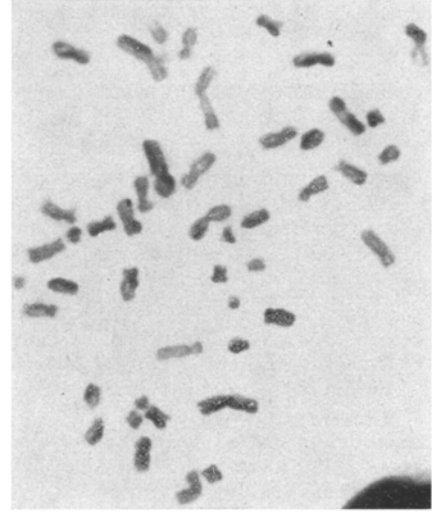

Fig. 2. Third division. BrdU-Giemsa method. ×1100.

Accordingly, some of the metaphases in the 2nd or 3rd division are not recognized as such, and they are consequently recorded as cells in the 1st division.

A second series of cultures from 3 of the donors previously employed were set up and treated with 5 μg/ml of the base analogue (Table). The comparison of the results from the two series of cultures shows that the percentage of cells in 1st, 2nd or 3rd division may vary from culture to culture independently of the fact that cultures may stem from the same donor. This has to be taken into account to explain the variability of results obtained in simultaneous cultures of the same donor treated with 5 and 10 μg/ml of BrdU (only the donor A shows equivalent results in both sets of leukocyte cultures).

It has been known since long that PHG-stimulated human blood cultures comprise mixed lymphocyte subpopulations which start DNA synthesis at different moments or which have different lengths of their cell cycles[3, 8–11]. The experiments from BENDER and BREWEN[2] suggest that human blood cultures have 2 lymphocyte subpopulations with different radiosensitivities and with different rates of progression through the process of DNA synthesis to cell division; the fastest and the slowest cells to reach mitosis would exhibit high and low radiosensitivity respectively.

Our results show that the BrdU-Giemsa technique is a useful method to identify with accuracy the percentage of lymphocytes which have gone through 1, 2 or 3 divisions in 72 h blood cultures. It is tempting to speculate that these 3 different rates of division correspond to 3 different lymphocyte subpopulations. However, before accepting this assumption, it will be necessary to correlate the sensitivity to clastogenic agents exhibited by lymphocytes which have entered mitosis one, two or three times during the culture period.

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The Seasonality of Mid-Day 'Zero' and 'Minus' low Tides on the Tropical Shores of Hawaii and their Effects on Intertidal Seaweed Populations[1]

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Summary. This study has revealed that mid-day 'zero' and 'minus' low tides in Hawaii show a definite seasonality and that their occurrence causes considerable bleaching and killing of intertidal *Hypnea* populations.

Tides are the major factors limiting the upper and lower limits of intertidal seaweeds[3, 4]. In the tropics the time of the occurrence of the low tides is especially important.

In Ghana, LAWSON'S[5] studies revealed that the seasonal variation in the abundance and vertical zonation of intertidal seaweeds, such as *Hypnea musciformis* (Wulfen) Lamouroux, was significantly correlated with the seasonal changes in the time of the occurrence of lowest low tides. In the season of their day-time occurrence, the intertidal populations declined. When occurring at night, the seaweed populations increased and also extended higher up the shore.

Table I. Number of days per month in the Hawaiian Islands with predicted mid-day 0.0 cm and 'minus' low tides[a]

Year	Months											
	J	F	M	A	M	J	J	A	S	O	N	D
1972	8	13	14	8	7	3	1	0	0	0	0	3
1973	9	13	11	5	6	3	2	0	0	0	0	0
1974	6	12	10	7	7	3	0	0	0	0	0	3
$\bar{x}$	7.7	12.7	11.7	6.7	6.7	3.0	1.0	0.0	0.0	0.0	0.0	0.0
SD.	1.5	0.6	2.1	1.5	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.7

[a] Source: Tide calendars for the Hawaiian Islands published by the Dillingham Corporation, Honolulu.

Since different shores do not necessarily have identical tidal rhythms[6] it is erroneous to use LAWSON'S results in predicting the effects of the tides on intertidal seaweeds in other tropical regions of similar latitude. The local tidal characteristics of many regions must be studied before broad generalizations can be made. However, extremely scanty literature has been written on this subject. In Hawaii there seems to be no published account showing whether or not there is any seasonality in tidal behaviour. The present study was therefore focused on this aspect.

In this investigation, attempts have also been made to demonstrate actual physical damage and death of intertidal seaweeds during mid-day tide-induced emersion.

Material and methods. The number of days per month with predicted mid-day 'zero' (0.0 cm) and minus (e.g., −0.8 cm) low tides in the Hawaiian Islands for 1972, 1973 and 1974 (Table I) were counted. These were taken as tides occurring between 11.00 and 14.00 h.

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