

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<sup>3, 8-11</sup>. The experiments from BENDER and BREWEN<sup>2</sup> 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 radio-sensitivity 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.

<sup>8</sup> N. O. BIANCHI and M. S. BIANCHI, *Chromosoma* 17, 273 (1965).  
<sup>9</sup> A. MICHALOWSKI, *Expl. Cell Res.* 32, 609 (1963).  
<sup>10</sup> R. SANTOSMELLO, D. KWAN and A. NORMAN, *Radiat. Res.* 60, 482 (1974).  
<sup>11</sup> J. A. STEFFEN and W. M. STULZMANN, *Expl Cell Res.* 59, 453 (1969).

**The Seasonality of Mid-Day 'Zero' and 'Minus' low Tides on the Tropical Shores of Hawaii and their Effects on Intertidal Seaweed Populations<sup>1</sup>**

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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<sup>3, 4</sup>. In the tropics the time of the occurrence of the low tides is especially important.

In Ghana, LAWSON's<sup>5</sup> 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.

Since different shores do not necessarily have identical tidal rhythms<sup>6</sup> 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.

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

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

<sup>a</sup>Source: Tide calendars for the Hawaiian Islands published by the Dillingham Corporation, Honolulu.

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<sup>3</sup> M. S. Doty, *Ecology* 27, 315 (1946).  
<sup>4</sup> M. S. Doty, in *Treatise on Marine Ecology and Paleoecology* (Ed. J. W. HEDGPETH, Ecol., Geol. Soc. Am., Washington 1957), vol. 1, p. 535.  
<sup>5</sup> G. W. LAWSON, *J. Ecol.* 45, 831 (1957).  
<sup>6</sup> J. R. LEWIS, *The Ecology of Rocky Shores* (English Universities Press, London 1964), p. 323.

In assessing the effects of the tides on intertidal seaweed populations, cloudless days which occurred towards the end of a series of such tides were selected. Diamond Head reef on Oahu Island (Hawaii) was selected as the study area and 3 species of *Hypnea* were used as research material (Table II).

Table II. Percentages of *Hypnea* thalli with killed and bleached apices during mid-day 0.0 cm and 'minus' low tides

Species	Date	Distance seaward from shore (m)			
		0	5	10	15
<i>H. cervicornis</i>	10. 3. 1973	100±0	67±6	53±4	0±0
	6. 5. 1973	100±0	69±8	48±3	0±0
<i>H. chordacea</i>	10. 3. 1973	—	100±0	56±4	7±2
	6. 5. 1973	—	83±5	60±6	0±0
<i>H. nidifica</i>	10. 3. 1973	—	25±3	8±1	0±0
	6. 5. 1973	—	33±4	19±2	0±0

—, No thalli grew in the area.

Table III. Percentages of *Hypnea* thalli with killed and bleached apices during early morning 0.0 cm and 'minus' low tides

Species	Date	Distance seaward from shore (m)			
		0	5	10	15
<i>H. cervicornis</i>	28. 4. 1973	4±0.5	0±0	0±0	0±0
	29. 7. 1973	0±0	3±0.5	0±0	0±0
<i>H. chordacea</i>	28. 6. 1973	—	18±2	0±0	0±0
	29. 7. 1973	—	8±1	5±1	0±0
<i>H. nidifica</i>	28. 6. 1973	—	7±2	0±0	0±0
	29. 7. 1973	—	0±0	0±0	0±0

—, No thalli grew in the area.

Sampling was done along a transect across the intertidal belt. A 45 cm diameter brass sampling ring was randomly tossed 6 times at each of 0, 5, 10 and 15 m seaward from a gently sloping shore. Where the ring fell, thalli of the selected species showing signs of killing through tide-induced emersion were counted. The affected plants had bleached, killed and disintegrated tips. The percentages of affected *Hypnea* plants were subsequently calculated and the data tabulated (Table II).

The procedure was repeated on selected dates (Table III) when the intertidal seaweeds were emersed during early morning — (06.00 to 09.00 h) 'zero' and 'minus' low tides, i. e., when the light intensity, temperature and desiccation would be expected to be less destructive.

**Results and discussion.** The data obtained (Table I) show that, in the Hawaiian Islands, there is definite seasonality in tidal behaviour. *Hypnea* thalli showed considerable damage (Table II) during the days with mid-day 'zero' and minus low tides. More bleaching and killing of the thalli was observed for *H. cervicornis* and *H. chordacea* which grow higher up intertidally than for *H. nidifica* which is more subtidal. On the days with early morning low tides (Table III), algal killing by tide-induced emersion was negligible.

From the literature<sup>5</sup> and from these results, one can predict that the season when intertidal seaweed populations in Hawaii would be minimal is towards the end of the seasons (June and July) with the highest frequency of mid-day 'zero' and 'minus' low tides. During August to November, when such tides are non-existent, intertidal seaweed populations would be expected to increase steadily to a maximum. Indeed the seasonal standing crop variations of the above species of *Hypnea* show this predicted pattern<sup>7</sup>. DEWREDE's<sup>8</sup> findings on the seasonal variations of *Sargassum* populations in Hawaii (showing maxima during November and December and minima in May to July) also seem to fit this predicted pattern. These results suggest that in our attempts to determine the causal factors for seasonal changes in seaweed populations in the tropics, tidal behaviour is one of the most important factors to be examined.

<sup>7</sup> K. E. MSHIGENI, Ph. D. dissertation, Univ. Hawaii (1974).

<sup>8</sup> R. E. DEWREDE, Ph. D. dissertation, Univ. Hawaii (1973).

## Nuclear Fusion and Irregular Cytokinesis in Binucleate and Tetraploid Cells of *Vicia faba* after Caffeine Treatment

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**Summary.** By means of 3 h treatment with 0.2% caffeine solution, binucleate and tetraploid cells were obtained in the lateral root meristem of *Vicia faba*. During recovery changing rates of fused interphases were noticed. Cell walls were formed in the equatorial plane of the preceeding division of binucleate and tetraploid cells at interphase and in the course of bimitosis or 4n-mitosis at prophase or metaphase; during bitelophase a constriction of the fused nuclei could be seen. The conclusion is that the basic requirements of cytokinesis are not affected by caffeine.

The effect of caffeine on cytokinesis<sup>1</sup> leads to binucleate cells that divide synchronously<sup>2</sup>, or to tetraploid cells<sup>3</sup>. During division of binucleate cells, there may be formed tetraploid nuclei by fusions<sup>3,4</sup>. In the present paper, the further development of these cells is described.

**Materials and methods.** *Vicia faba* seedlings were grown in moist sand and, after cutting off the main root up to 3

cm, they were transferred into aerated (3 ml/sec) Hoagland's general solution No. 3 in permanent light at 25 ± 1°C. Lateral roots were treated 3 h with 0.2% caffeine solution (DAB 7, Merck, Darmstadt) in distilled water and fixed at different times during recovery<sup>3</sup>. Bimitoses and 4 n-mitoses were studied at Feulgen stained squash preparations.