twice at 100 °C for 30 min. The latter mode of sterilization was also applied to the media where sucrose was replaced by sugar mixtures.

The placentae were cultivated at 25 °C in the dark for 12 weeks after pollination. The experiments were performed in 2 sets, each variant involving 20 ovaries. The results were evaluated statistically using the analysis of variance and Duncan's test.

Results and discussion. Germinating seeds in situ appeared as early as 6 weeks after pollination, but only in those media, which contained a mixture of glucose and fructose without sucrose (Figure 1). During further 2 weeks of cultivation, the number of germinated seeds considerably increased (up to 32% in the case of medium with invert sugar) and some germinating seeds also occured in the media with sucrose. The highest germination during the whole period from the 6th to the 12th week after pollination was registered on the medium containing a mixture of equal parts of glucose and fructose (medium 4). Shifting this proportion either in favour of glucose (medium 5) or fructose (medium 6) caused a decreased in the rate of germination. Statistical analysis of the percentage of germinated seeds 12 weeks after pollination revealed significant differences between the 3 media with sucrose and media 4, 5 and 6 and showed the highest germination on medium 4 (Figure 2B). There is no significant difference between media 5 and 6 and between the autoclaved and non-autoclaved sucrose media. It is of interest that invert sugar did not affect the germination when applied in a mixture with 2 parts of sucrose. On the other hand, glucose or fructose when applied alone, had a detrimental effect on the formation of seeds: in most ovaries no seeds developed, quite exceptionally a few seeds were set (unpublished data).

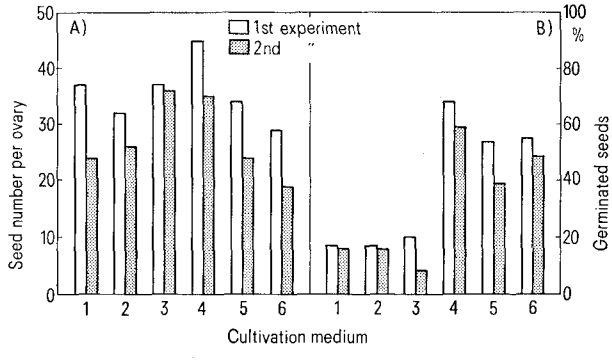

Fig. 2. Mean number of seeds per ovary (A) and percentage of in situ germinated seeds (B) 12 weeks after pollination of excised placentae in vitro. For media description see Figure 1.

Regarding the mean number of seeds per ovary, a similar situation was found as in the case of seed germination in situ (Figure 2A). However, the statistical treatment of the data showed that only medium 4 containing invert sugar significantly stimulated seed set with respect to the control media containing only sucrose.

Although the 2 experiments were performed within 1 week, the number of seeds per ovary, as well as seed germination was higher in the first experiment than in the second one (Figure 2). We assume that this may be related to some differences in the physiological state of the ovaries caused by a variation of environmental conditions such as luminosity and temperature.

It is generally accepted that sucrose is the best sugar component for media used in plant cell, tissue and organ culture. This is based on many experiments made with various plant parts and species in which the growth effect of different sugars was compared. The excized embryos of most plants[9] and young seedlings of tobacco[10] were found to grow in vitro better in sucrose-containing media than in the presence of other sugars, including glucose and fructose. Our results coincide with these observations in that sucrose cannot be replaced either by glucose or by fructose. They show, however, that each of these monosaccharides has a specific importance for the nutrition of placentae and for the development of ovules into mature seeds, and when supplied in a mixture as invert sugar, they bring about even better seed formation than the medium with sucrose. On the other hand, in pollen tube culture[11] and in barley embryos[12], sucrose could not be fully replaced by the mixture of glucose and fructose.

Regarding the stimulatory effect of invert sugar on seed germination on placentae, no similar observations have to our knowledge been made so far, and for its understanding further investigations are needed.

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'Binary Fission' and Budding in Microspores of *Heliconia bihai* L.

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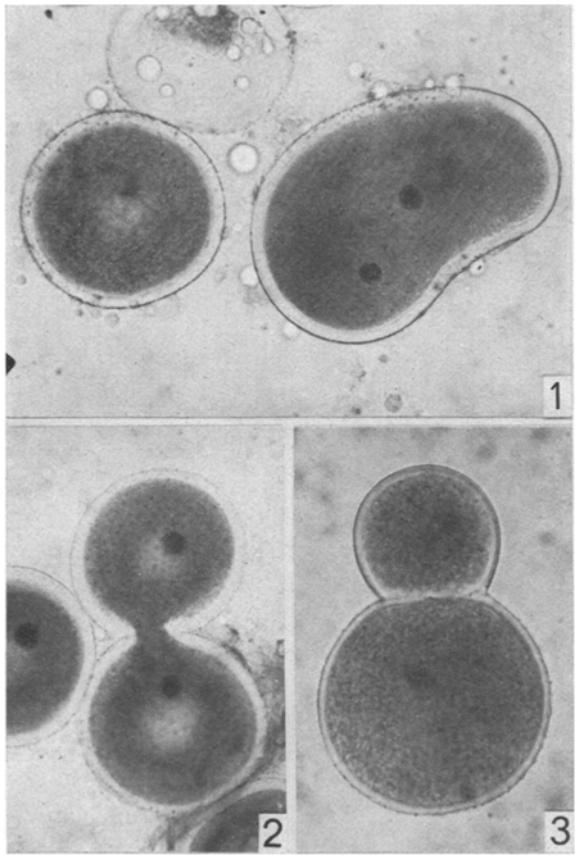
Summary. In *Heliconia bihai* L. 'binary fission' and budding occur in vivo in microspores which arise from diads, by furrowing of their wall. Frequency of this abnormal phenomenon considerably increased in materials subjected to cold treatment.

The course of development of the male gametophyte is remarkably uniform in angiosperms[2], barring a few unusual situations as in *Hyacinthus*[3], *Ornithogalum*[4], *Petunia*[5] and *Ophiorrhiza*[6]. This note deals with the occurrence in vivo of certain developmental abnormalities in microspores of *Heliconia bihai* L. (Musaceae).

Meiosis and stages of development of microspores were studied from flower buds collected from plants growing in field conditions as well as from inflorescences exposed to cold treatment (10–15 °C) for 24–36 h. Meiosis in the species is found to be normal with the formation of 12 bivalents at M I, except for failure of second division in 10–15% of the PMC's, and as a result, both tetrads and diads are formed at the end of meiosis. The microspores that have developed from the diads are larger in size (45 µm in diameter) than those developed from the normal tetrads (32 µm). About 30 h after meiosis, the nucleus of the larger microspores divides to form 2 equally sized daughter nuclei, while that of the normal microspores remain undivided (Figure 1). Following this, the larger bi-



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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