	Normal Balb/c		*nu/nu* Balb/c			'Hairless' mice	
	Controls	DMBA treated	Controls	DMBA treated	Thymus implant DMBA treated	Controls	DMBA treated
Initial No. of animals	6	12	10	16	6	6	10
No. of animals surviving 3 months	6	12	7	12	6	6	9
Animals with papillomas	none	10	none	none	none	none	none
Number of mast cells	160 (± 10)	420 (± 50)	610 (± 110)	557 (± 50)	470 (± 45)	380 (± 50)	355 (± 20)
Mast cell metachromasia	+++	+++	+++	+++	n.d.	+++	+++
Mast cell fluorescence	+++	+++	++	++	n.d.	+++	+++
Skin histamine	0.0139 (± 0.0035)	n.d.	0.0421 (± 0.0144)	n.d.	n.d.	0.0996 (± 0.0295)	n.d.
Passive cutaneous anaphylaxis	+		+			n.d.	

n.d. = not done.

Results. The first and striking observation on painting the 3 groups with DMBA was a complete lack of response of *nu/nu* and *hr/hr* skin to the surface application of the carcinogen. Normal Balb/c mice developed a local inflammatory skin reaction and epilation beginning at the end of the first week. Neither *nu/nu* nor *hr/hr* mice displayed these skin changes. By 3 months, 20 of 22 surviving Balb/c mice bore papillomatous skin tumors whereas exposure to DMBA was not followed by detectable changes in the skin of the other two groups (Table).

Histologically, the findings were equally unexpected for by far the largest numbers of mast cells in untreated skin were seen in athymic *nu/nu* mice (Table). Here, small elongated cells with characteristic granules lay parallel to the epidermis, and larger, more rounded cells were scattered throughout the deeper dermis. In the skin of the few *nu/nu* mice which had received a thymus implant, the number of mast cells and their variations in size were as in athymic *nu/nu* mice. The skin of normal Balb/c mice, rather poor in mast cells, reacted to DMBA by a marked increase in the number of mast cells under the now thickened epidermis, and their accumulation was most pronounced around the base of the skin tumors. Skin of *hr/hr* mice contained large numbers of well developed mast cells (Table).

Pharmacologically, the findings for histamine paralleled the mast cell content observed in the 3 groups of mice, taking into account the wide variations in size of the mast cells in *nu/nu* skin. Mast cells in all 3 groups displayed a golden-brown fluorescence in UVL, characteristic of 5-HT[5]. This was most obvious in mast cells clustered around the base of a papilloma[7].

Finally, the skin of *nu/nu* mice bound reaginic antibody to a comparable extent, as did normal Balb/c mice, thus indicating that the number of receptors for reaginic antibody is not diminished.

Discussion. Two points of interest emerge. The first concerns the striking lack of response to the surface application of DMBA in *nu/nu* and *hr/hr* mice. The carcinogen was effective, as shown by the development of skin tumors in the majority of Balb/c mice. Hair follicles are thought to provide the preferential portal of entry for topically applied hydrocarbons in mice[8]. The almost total lack of follicles in adult *hr/hr* mice, and their scarcity in *nu/nu* mice, together with a thickening of the interfollicular epidermis, may thus account for the early failure in both groups to show erythema and the subsequent development of papillomas.

The second point to be emphasized is the rich content of mast cells in the skin of congenitally athymic *nu/nu* mice. By generally accepted criteria, these are normal mast cells: they stain metachromatically, they contain histamine and 5-HT, and they participate normally in the PCA reaction. Whatever the origin and function of such cells may be, they can hardly be derived from the thymus. This fails to support the premise of BURNET[3,4] that the tissue mast cell is an end-stage of the T lymphocyte.

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Biological Activity of Vernoflexuoside on the Basis of *Allium* Test

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Summary. Biological activity of vernoflexuoside (Vf) a new sesquiterpene lactone glucoside, isolated from *Vernonia flexuosa* Sims was investigated by means of *Allium* test. Vf showed cytostatic activity and produced mitotic disturbances as well as symptoms of nuclear structure degeneration.

In the course of phytochemical investigation of *Vernonia flexuosa* Sims (*Compositae*), cultivated in the experimental plots of the Pharmacological Institute of Polish Academy of Sciences in Cracow, a guaianolide sesquiterpene lactone glucoside, so far unknown, was isolated by KISIEL[1] as the major constituent of the roots. This compound was named vernoflexuoside (Vf) by the author, who also established its structure (Figure 1).

The cytostatic activity of containing α-methylene-γ-lactone grouping was suggested, since the other representatives of this chemical group are known to be active[2]. Thus, an evaluation of the effect of this compound on the

The inhibitory effect of the water solutions of vernoflexuoside on the linear growth of the *Allium cepa* L. roots (%)

Concentration (*M*/l)	Time of treatment (h)		
	4	24	48
10^{-3}	100	100	100
10^{-4}	90	80	70
10^{-5}	10	10	8
10^{-6}–10^{-7}	0	0	0

C-concentrations of vernoflexuoside solutions.

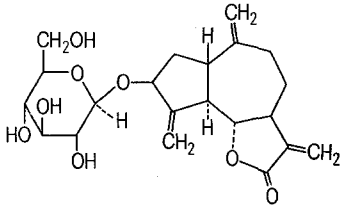

Fig. 1. Vernoflexuoside.

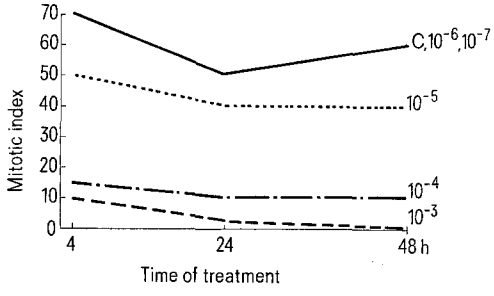

Fig. 2. The relationship between the mitotic index and the time of treatment of meristematic cells of *Allium cepa* L. roots with vernoflexuoside solutions (10^{-3}–10^{-7} *M*/l). C, control; h, time of treatment.

meristematic cells was tested by means of *Allium* test according to LEVAN[3].

Material and methods. Allium cepa L. bulbs were kept for 3–4 days in boiled tap water at 18 °C (day light), until the length of roots reached 2–3 cm. Then the onion bulbs were halved. One of them was placed in the Vf-solution to be tested, the other one in tap water, as control. 10^{-3} to 10^{-7} *M*/l concentrations of Vf were used. To avoid the influence on mitoses of secretions of saprophytic bacteria living on the bulb roots[4]; every 12 h the water was changed.

The length of roots (treated and controlled) was measured before experiment and after 4, 24 and 48 h, respectively. At the same time intervals, the growing points of roots were taken out and placed in the MURIN's fixative[5]. The squashes were prepared according to MURIN[5] after 2 weeks of maceration. The growing points were cut into short lengths on the microscope slides smeared with Haupt's adhesive. Subsequently the material was covered with cellophane stripes as well as filter paper and a pressure was applied. The squashes were placed for 45 min into Coplin jar saturated with formalin vapours. Afterwards they were washed in running tap water for 12 h in order to remove the formalin as well as the cellophane stripes and then stained with aqueous 1% gentiana violet, dehydrated by means of alcohol and mounted in a few drops of Canada balsam dissolved in xylene. For the material investigated, the mitotic index values; a number of dividing cells per 1,000 meristematic cells (Figure 2) were calculated and mitotic disturbances or phenomena of nucleus structure degeneration (Figures 3–6) were established.

Results and discussion. Macroscopical examinations of the onion roots treated with Vf solutions revealed only differences in length in comparison with the control values. Percentage inhibition of linear growth of onion roots at 10^{-3} and 10^{-4} *M*/l concentrations was well marked and related values attained 70 to 100% (Table). The lower concentrations (10^{-5}–10^{-7} *M*/l) of Vf produced no significant changes of the root's length.

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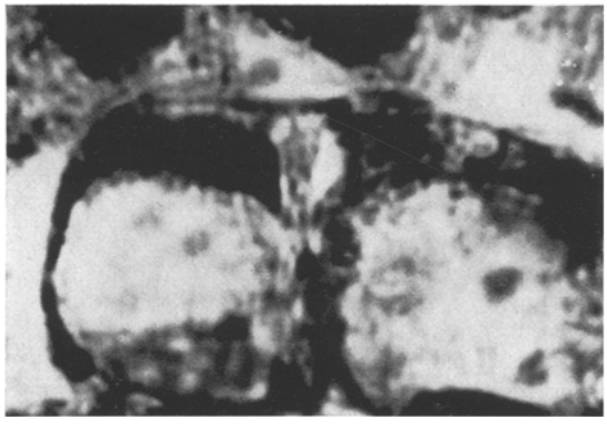

Fig. 3. Margination of chromatin induced by 24 h treatment with vernoflexuoside solution (10^{-3} *M*/l).

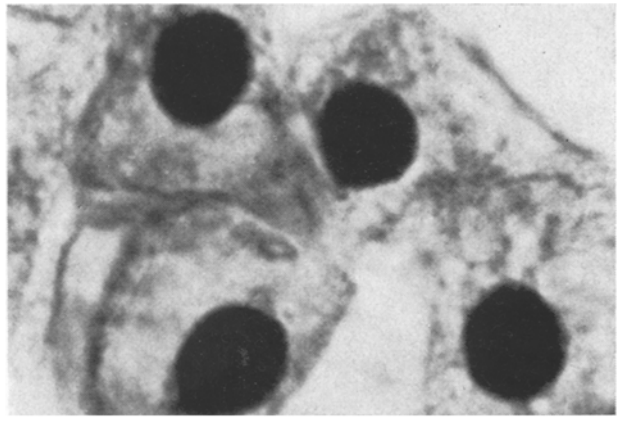

Fig. 4. Pycnosis induced by 24 h treatment with vernoflexuoside solution (10^{-4} *M*/l).

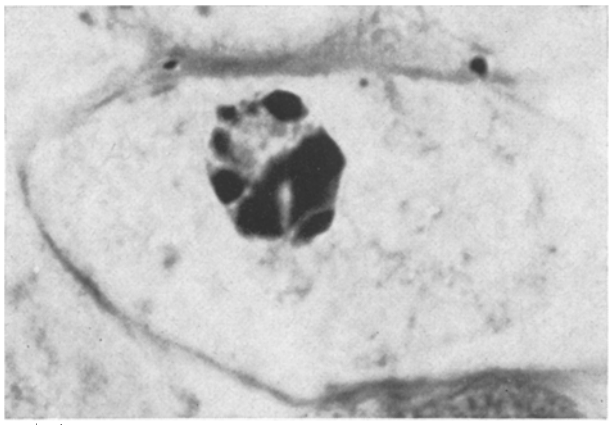

Fig. 5. Karyorhexis induced by 48 h treatment with vernoflexuoside solution (10^{-3} M/l).

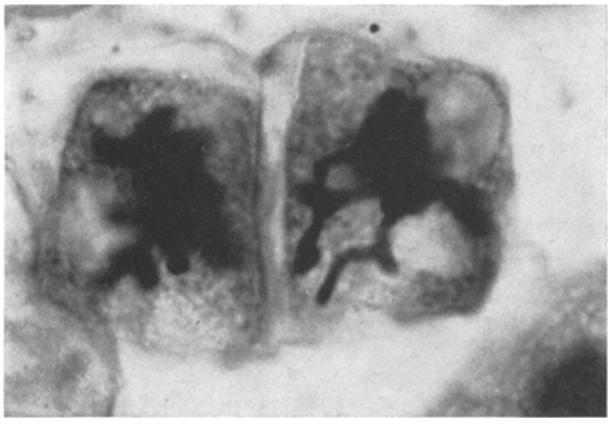

Fig. 6. Irregular congregation and partial agglutination of chromosomes in metaphase induced by 24 h treatment with vernoflexuoside solution (10^{-5} M/l).

The onion roots treated for 48 h with Vf solutions retained the ability to continue their growth and normal development when they were washed and transferred into tap water again. This may be proof that Vf does not show cytotoxic effect in the concentrations investigated.

On the basis of microscopical analysis of the squashes, the mitotic index values for each concentration of Vf solutions were calculated (Figure 2).

The following concentrations of Vf aqueous solutions produced mitotic disturbances or nucleus structure degeneration symptoms: 1. chromatine margination (Figure 3), at 10^{-3}–10^{-4} M/l concentration of Vf after 24 and 48 h; 2. pycnosis (Figure 4) at 10^{-4}–10^{-5} M/l concentration after 4, 24 and 48 h; 3. karyorhexis (Figure 5) at 10^{-3}–10^{-4} M/l concentration after 48 h; 4. irregular chromosomes congregation and partial agglutination in metaphase (Figure 6) at 10^{-4}–10^{-6} M/l concentration after 24 and 48 h.

Among these mitotic disturbances and symptoms of nucleus structure degeneration, pycnosis was the most frequent phenomenon. According to GULJAJEV[6], pycnosis occurs after chromatin margination and its final step is karyorhexis. These three phenomena were very distinct in the material investigated when treated with Vf in 10^{-3}–10^{-5} M/l concentrations. These observations lead to the conclusion that Vf shows some of the chromatoclassical properties[7].

The calculated mitotic indexes confirm the very significant cytostatic activity of Vf in concentrations of 10^{-3} and 10^{-4} M/l (Figure 2). This activity may be attributed to the conjugated α-methylene-γ-lactone grouping, which occurs in Vf structure. KUPCHAN[8] underlined the fact that these groupings may inhibit phosphofructokinase in cells by binding the active SH-group of the enzymes. The interaction of sesquiterpene α-methylene-γ-lactones with thiol groups is well established and it may explain biological activity of these compounds.

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Bakterien im Ovar aquariengehälterter Meeresfische

Bacteria in the Ovary of Marine Fishes Held in Aquaria

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Summary. In the ovary of the ovoviviparous teleost *Zoarces viviparus* L. kept in aquaria, gram-negative bacteria are found. These penetrate the tissue up to the basement membrane which separates the follicle epithelium from the theca.

In Aquarien gehälterte Meeresfische sind auch dann zahlreichen negativen physischen und physiologischen Einflüssen ausgesetzt, wenn der derzeit übliche technische Standardaufwand getrieben wird (Kühlung, Belüftung, Abschäumung usw.). Es ist seit langem bekannt, dass die ♀♀ vieler mariner Fische in Gefangenschaft Schwierigkeiten haben, ihre Eier abzusetzen und dann an Legenot zugrundegehen können. Dieses Problem wird sich auch bei künftigem «fish farming» in grösserem Ausmass stellen.

Bei den ♀♀ lebendgebärender (ovoviviparer) Fische wie der Aalmutter (*Zoarces viviparus* L.) verzögern sich im Aquarium die Oogenese und die Embryonalentwicklung. Die meisten Embryonen sterben vor der Geburt. Früh absterbende Embryonen können offensichtlich – wie degenerierende Oocyten – resorbiert werden, während in