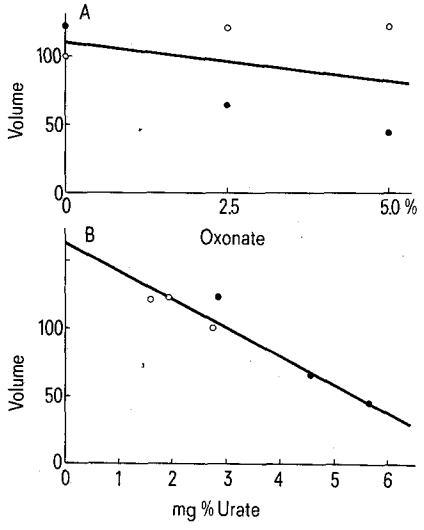
d'adjuvant de Freund (0,5 mg de *Mycobacterium butyricum* dans 0,1 ml d'huile minérale) dans le coussinet plantaire de la patte arrière gauche.

L'évolution de l'arthrite induite a été suivie pendant 50 jours par la mesure du volume des pattes arrière et d'un score arthritique[3]. L'œdème, ou augmentation du volume des pattes due uniquement à l'inflammation, est la différence de volume de pattes entre un groupe injecté et le groupe témoin correspondant non-injecté. Pour faciliter les comparaisons des résultats, nous avons déterminé les surfaces des différentes courbes entre le jour 9 (début de la réaction secondaire) et le jour 50 (fin de l'expérience) et calculé les surfaces relatives en prenant les valeurs du groupe injecté à alimentation normale (groupe 1) égales à 100%. L'acide urique sérique a été déterminé par une méthode colorimétrique au phosphotungstate[5], car la présence d'un inhibiteur de l'urate-oxydase dans de nombreux échantillons empêchait l'utilisation d'une méthode enzymatique.



Volume relatif de la patte arrière non-injectée en fonction du pourcentage d'oxonate dans le régime alimentaire (A) et en fonction de l'uricémie (B). Alimentations sans acide urique (groupes 1, 3, 5): ○, avec 1% d'acide urique (groupes 2, 4, 6): ●. Les droites de régression ont été déterminées par la méthode des moindres carrés.

Tableau 2. Influence de l'uricémie et de l'oxonate sur l'arthrite à adjuvant

Coefficients de corrélation* en fonction de	l'uricémie r	p	l'oxonate r	p
Score arthritique	−0,925	0,008	−0,558	0,25
Volume de la patte arrière injectée	−0,938	0,006	−0,335	0,52
Volume de la patte arrière non-injectée	−0,960	0,002	−0,366	0,48

* Les coefficients de corrélation r ont été calculés à partir des valeurs du tableau 1.

Résultats. Les rats injectés qui ont reçu une alimentation normale ou enrichie seulement en oxonate ou seulement en acide urique présentaient une inflammation intense des pattes arrière. Cette réaction inflammatoire n'était inhibée que chez les rats recevant une alimentation contenant à la fois de l'oxonate et de l'acide urique (groupes 4 et 6, tableau 1). L'alimentation enrichie seulement en oxonate a provoqué au bout de 2 mois une diminution de l'uricémie qui est associée à une légère aggravation de l'arthrite induite (groupes 3 et 5, tableau 1). Cette diminution de l'uricémie pourrait s'expliquer par une inhibition in vivo de la biosynthèse de novo des purines par l'oxonate[6].

Si on porte les valeurs relatives au volume de la patte arrière non-injectée en fonction de la concentration en oxonate dans l'alimentation (figure, A), on ne trouve pas de corrélation statistiquement significative: $r = -0{,}366$ et $p = 0{,}48$ (tableau 2). Par contre, si on porte ces mêmes valeurs en fonction de l'uricémie (figure, B), la corrélation est très significative avec $r = -0{,}960$ et $p = 0{,}002$ (tableau 2).

Les corrélations sont aussi très significatives ($p \leq 0{,}008$) quand on porte les valeurs relatives du volume de la patte arrière injectée ou du score arthritique en fonction de l'uricémie, alors que les corrélations ne sont jamais significatives ($p \geq 0{,}25$) quand on les calcule en fonction de la concentration en oxonate (tableau 2).

Ces résultats démontrent que l'inhibition de l'arthrite à adjuvant par un régime à l'oxonate est proportionnelle à l'uricémie et que l'oxonate n'a pas d'effet inhibiteur en tant que tel.

1 Cette recherche a été réalisée grâce à un octroi de la Société d'Arthrite du Canada.
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3 A. Lussier et R. de Médicis, J. Rheumat. *4*, 369 (1977).
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Effects of vinblastine on meristematic cells of *Allium cepa*, I[1]

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Summary. In meristematic cells of *Allium cepa*, vinblastine induces accumulation of prophase and metaphase stages, chromosome contraction and C-metaphases, appearance of bridges at anaphase, multipolar divisions at anaphase and telophase, micronucleus formation at telophase, and faint staining reaction and degeneration of nuclei or chromosomes at all stages.

Only one report on the effects of vinblastine in plants has been published, concerning *Hordeum sativum, Vicia faba* and *Nigella damascena*[2]. This report noted that C-mitosis and multipolar division were induced by vinblastine. The present study was carried out to give a more detailed account of the effects of vinblastine on plant cells.

Materials and methods. Root tips of *Allium cepa* were cut off 3 cm in length, and immersed in 20-µg/ml, 50-µg/ml and 100-µg/ml aqueous solution of vinblastine-sulphate (EXAL, Eli Lilly and Co, Indianapolis, USA; Sionogi and Co, Osaka, Japan) for 2, 4, 6, 12 and 18 h at room temperature. Control materials were treated in tap water. The

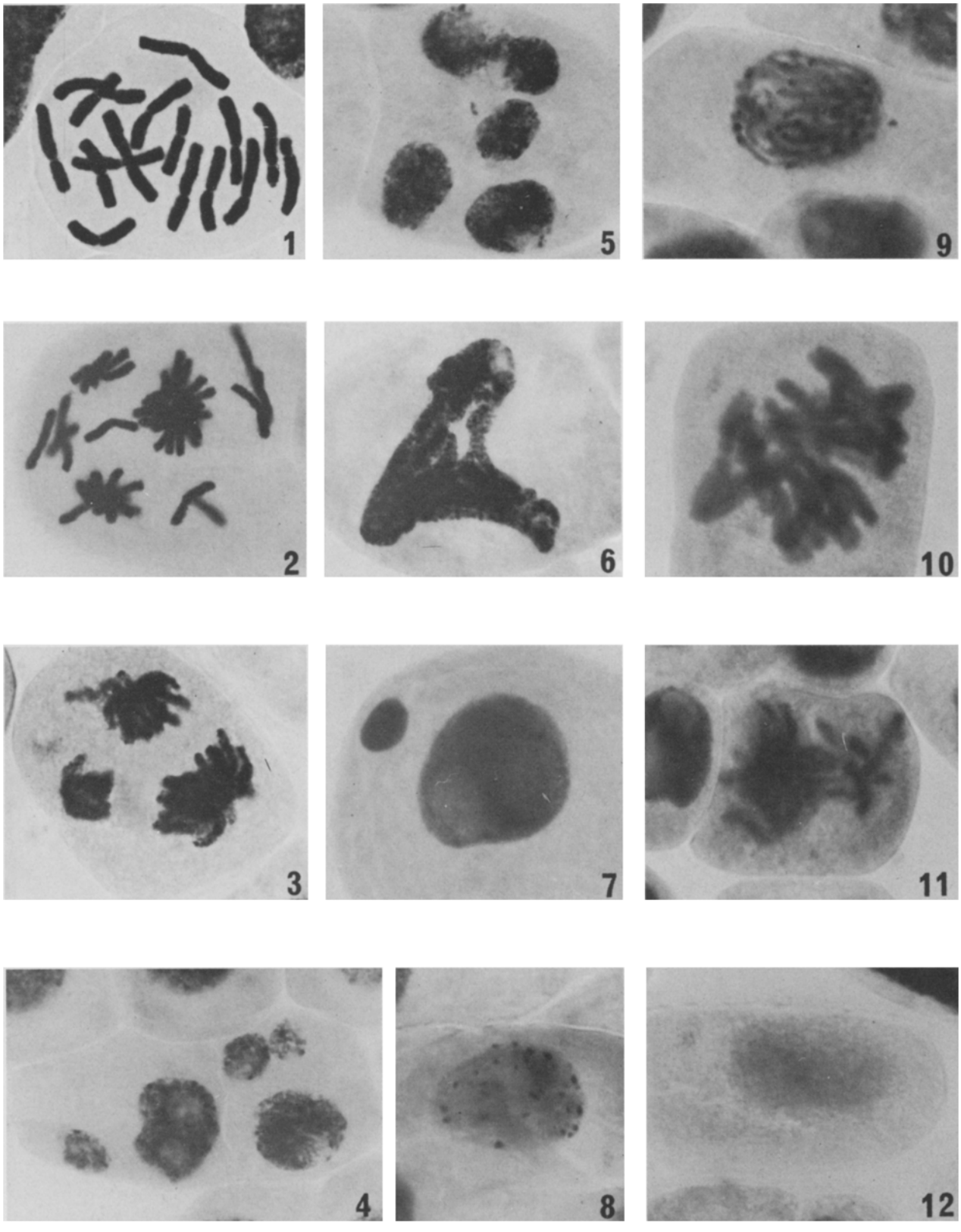

Figure 1–12. Various aberrations in meristematic cells of *Allium cepa* treated with vinblastine. *1* Chromosomal contraction (20 µg/ml, 2 h). *2* Multipolar division (20 µg/ml, 4 h). *3* Tripolar division (20 µg/ml, 4 h). *4* Daughter cells with micronuclei (20 µg/ml, 4 h). *5* Cell with 5 nuclei (20 µg/ml, 6 h). *6* Sticky bridge (50 µg/ml, 4 h). *7* Faintly staining nucleus and micronucleus (50 µg/ml, 12 h). *8* Faintly staining nucleus at interphase (100 µg/ml, 6 h). *9* Faintly staining nucleus at prophase (100 µg/ml, 6 h). *10* Faintly staining and swollen chromosomes at metaphase (100 µg/ml, 6 h). *11* Faintly staining chromosomes at anaphase (100 µg/ml, 6 h). *12* Cell without nucleus (100 µg/ml, 6 h).

Mitotic frequencies and aberrations of root-tip cells treated with aqueous solution of vinblastine. The numbers show the percentages of cells to all cells given in same treatment

Time (h)	Stage	Effect	Control	Treatment (µg/ml) 20	50	100	Time (h)	Stage	Effect	Control	Treatment (µg/ml) 20	50	100
2	P	NOR	1.10	1.25	2.24	1.32		T	NOR	2.30	1.93	1.38	
		ABN		0.08	0.06	0.14			ABN		0.49	0.35	0.52
	M	NOR	0.89	1.17	1.73	1.54			MIN		1.99	0.03	
		ABN		0.14	0.35	0.52		I	NOR	95.00	92.08	93.81	66.51
	A	NOR	3.02	0.03	0.06	0.11			ABN				30.75
		ABN				0.08			CWN				0.57
		MLP			0.03	0.08	12	P	NOR	0.93			
	T	NOR	5.59	0.92	1.86	1.49			ABN		0.04	0.65	0.76
		ABN				0.11		M	NOR	1.39			
	I	NOR	89.41	96.41	93.67	94.60			ABN		0.21	1.89	0.12
								A	NOR	0.67	0.04		
4	P	NOR	0.36	0.66	0.78	0.10			ABN		0.04	0.08	0.02
		ABN		0.80	0.78	0.93			MLP		0.14	0.38	
	M	NOR	1.64	0.77	1.30	0.05		T	NOR	1.96	1.21	0.22	
		ABN		0.30	0.52	1.31			ABN		0.04	0.62	0.93
	A	NOR	1.78	0.05	0.03				MIN		1.04	0.03	
		ABN		0.42	0.16	0.10		I	NOR	95.05	97.24	96.08	53.47
		MLP		0.57	0.75				ABN			0.05	34.70
	T	NOR	2.18	1.22	0.49	0.23			CWN				10.00
		ABN		1.25	0.10	0.26	18	P	NOR	0.81			
		MIN		0.63	0.03				ABN		0.23		
	I	NOR	94.05	93.33	94.98	96.56		M	NOR	0.91			
		ABN			0.09				ABN		0.96		
		CWN				0.46		A	NOR	0.88			
6	P	NOR	0.77	0.31	0.64				ABM		0.23		
		ABN		0.43	0.41	0.57		T	NOR	2.50	0.75		
	M	NOR	1.41	0.15	0.47				ABN		0.51		
		ABN		0.37	0.20	0.99			MIN			0.26	
	A	NOR	0.51	0.04	0.09			I	NOR	94.90	57.87	53.85	25.02
		ABN		0.06	0.06	0.09			ABN		36.56	25.39	57.48
		MLP		2.15	2.57				CWN		2.89	20.50	17.50

P, prophase; M, metaphase; A, anaphase; T, telophase; I, interphase; NOR, normality; ABN, aberration; MLP, multipole; MIN, micronucleus; CWN, cell without nucleus.

tissues were fixed in acetic alcohol and the nuclei stained according to the aceto-orcein squash method. The data of the mitotic frequencies and aberrations were based on examination of the first 3000 cells in each control and experimental batch. Aberration means all stages of degenerative breakdown either via pycnosis and liquefaction or by swelling and gradual dissolution.

Results. The results are shwon in the table and in figures 1–12. No significant damage in the control materials was observed in each test. 2-h treatment: The first effect noticed, compared with the control, was the accumulation of prophasic and metaphasic cells. In all treatments of vinblastine, contraction of chromosomes was observed (figure 1). The percentage of anaphasic and telophasic stages in treated materials is lower than that of the control. In the group treated with vinblastine, degenerative breakdown of nuclei and chromosomes at prophase and metaphase was observed. In the 50-µg/ml and 100-µg/ml treatments, multipolar division was also observed.

4-h treatment: The frequency of multipolar divisions at anaphase was 0.57% in 20-µg/ml and 0.75% in 50-µg/ml treatment (figures 2 and 3); the frequency of micronuclei at telophase was 0.63% in 20 µg/ml and 0.03% in 50 µg/ml. At 50-µg/ml treatment, tripolar condition and sticky bridges were often observed at telophase. By treatment in 100 µg/ml some degenerating nuclei and cells without nuclei were observed (0.46%).

6-h treatment: The number of mitotic cells, compared to the control, was markedly reduced in this treatment. In the treatment with 20 µg/ml and 50 µg/ml, we could see high frequencies (2.15 and 2.57%) of multipolar division at anaphase. At telophase in the 20-µg/ml treatment, 2% of the cells contained micronuclei (figures 4 and 5). In the treatment with 100 µg/ml, the nuclei at interphase and prophase and chromosomes at metaphase and anaphase were often faintly staining (figures 8–11). Also, swollen chromosomes at metaphase and cells without nuclei at interphase were observed (figures 10 and 12).

12-h treatment: In the cells treated with vinblastine, no metaphase was observed. Faintly staining nuclei, and micronuclei were often recognized in the cells treated with 50 µg/ml (figure 7). In the materials treated in 100 µg/ml normal mitotic features were not observed. The frequency of cells with abnormal and faintly staining nuclei was 34.7%, and that of cells without nuclei was 10% at interphase.

18-h treatment: In the treatment with 20 µg/ml vinblastine normal anaphasic features were observed, although the proportion of interphase cells without nuclei was increased. In the cells treated with 50 µg/ml and 10 µg/ml, mitotic

divisions were not observed except for telophase figures in the 50-µg/ml treatment. Among the treated cells, many had faintly staining nuclei or were lacking a nucleus altogether.

Discussion. The effects of vinblastine on meristematic cells vary with the concentration of the drug. With treatments of 20–100 µg/ml over 2–18 h, frequent abnormalities of increasing severity were observed. Among these, we note especially: a) accumulation of mitotic cells at prophase and metaphase; b) chromosome contraction and C-metaphases; c) appearance of sticky bridges at anaphase; d) multipolar divisions at anaphase and telophase; e) micronucleus formation at telophase; f) faint staining reaction of nuclei or chromosomes at all stages; g) surviving cytoplasts without a nucleus; and h) degenerative breakdown of nuclei and chromosomes at all stages. Among these aberrations, C-metaphases and multipolar divisions have already been reported[2]. If it is admitted that vinblastine chiefly inhibits the function of the spindle poles, we nevertheless believe that we have shown that other lethal effects, not directly connected with the spindle poles, are mediated by this compound.

1 The 2nd article of this series will be published in Mem. Fac. Integrated Arts and Sci., Hiroshima Univ., Ser. IV, vol. 3.
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Remarks on the karyotype of the Polypteriformes. The chromosomes of *Polypterus delhezi, P. endlicheri congicus* and *P. palmas*

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Summary. The chromosomes of 3 species of bikirs (*Polypterus delhezi, P. endlicheri congicus* and *P. palmas*) were studied in somatic metaphases. The diploid number was found to be 2 n = 36 in all the species and a basic morphological identity of the karyotype emerges from karyogram comparison not only in the 3 species described herein, but also in the other Polypteriformes already studied.

Two papers in Experientia, by Denton and Howell[1] and from our group[2], presented the karyotype of 2 Polypteriformes (*Polypterus palmas* and *Calamoichthys calabaricus*), a group of bony fishes for which no karyological information had previously been available. Both papers stressed the fact that as regards chromosome number, shape and size, the Polypterine karyotype was closer to that of the Dipnoans and Amphibians than to that of the primitive Actinopterygians (Chondrostei) with which the Polypteriformes are normally associated[3,4]. The karyological evidence thus supports the view of those workers[5–8] who have given the Polypteriformes a quite peculiar place in the phylogenesis of the bony fishes, occasionally assigning them to a separate subclass (Brachiopterygia), acknowledging that they are closer to the radiation of the Sarcopterygia (Coelacanthidae)[9]. Schaeffer's position is very interesting in this regard[10]; he hypothesizes 'a close relationship between the Polypterids and the generalized Devonian Paleonisciforms. Such a hypothesis implies a separate lineage extending back into the Paleozoic, for the Polypterids'. All these considerations have led to a more thorough analysis being made of Polypterid karyology with a view to giving, through the study of the karyotype of other species, a general value to evidence based on 2 species alone.

During an expedition on the Zaire river, the opportunity presented itself of examining 10 bikirs belonging to 3 different species, i.e. *Polypterus palmas* Ayres (4 immatures and 1 female from Mbandaka), *Polypterus endlicheri congicus* Boulanger (1 immature, 1 male and 1 female from Mbandaka) and *Polypterus delhezi* Boulanger (1 immature and 1 female from Kinkole, Kinshasa). The material utilized for this paper has been deposited with in the National Museum La Specola in Florence (Italy). Normal air-drying technique[11] was used for the somatic metaphases, although owing to the difficulties involved in on-the-spot preparation, the quality of the slides is not the best. Nevertheless the interest presented by the species examined warrants a brief description of them. In any case, it has been possible to examine at least 20 good quality metaphases for each specimen.

In all species examined (figure 1) the diploid number was found to be 2 n = 36. No morphological differences were observed in *P. endlicheri* in comparisons made between karyotypes of individuals of both sexes. For the purpose of karyotype characterization, the chromosomes were divided into 3 size groups, as was done in our previous paper[2], i.e. a) large chromosomes with an average length of > 4 µm; b) medium size chromosomes with an average length between 3.5 and 2.5 µm; c) small chromosomes with a length of < 2.5 µm.

In the case of the large and medium size chromosomes, in which centromeric index evaluation did not involve serious

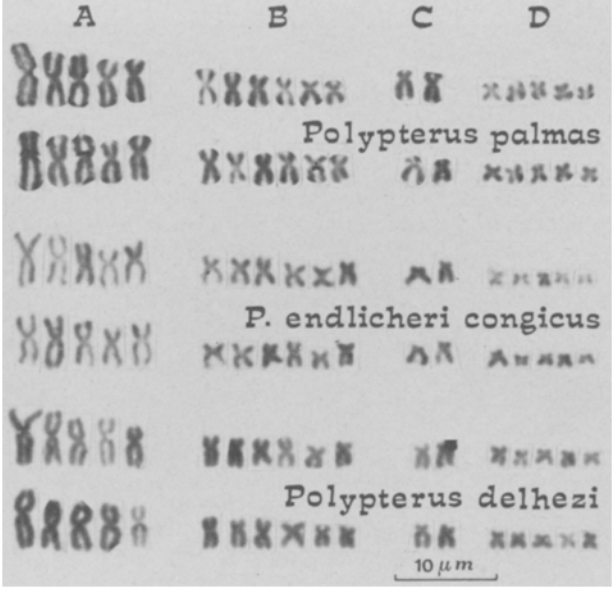

Fig. 1. Karyotypes of the 3 species of bikirs analyzed in the present paper. *A* Large metacentric chromosomes, *B* medium size metacentrics, *C* medium size submetacentrics, and *D* small chromosomes.