Crossreactivity of antisera (calculated on a weight basis as equivalence at 50% displacement of ^{3}H-melatonin)

Antigen	Melatonin-M-BSA
Melatonin	100.0
N-Acetylserotonin	1.3
6-Hydroxymelatonin	< 0.1
5-Methoxytryptamine	< 0.1
N-Acetyl-1-tryptophan	< 0.1
Bufotenine monooxalate hydrate	< 0.1
N-Acetyl-1-tryptophanamide	< 0.1
Serotonin Creatinine sulphate	< 0.1
5-Methoxyindoleacetic acid	< 0.1
5-Hydroxy-N-methyltryptamine oxalate	< 0.1
N-Methyltryptamine	< 0.1
5-Methoxytryptophol	< 0.1
Tryptamine hydrochloride	< 0.1
5-Methyltryptamine hydrochloride	< 0.1
N-Methylserotonin hydrogen oxalate	< 0.1

antibody technique, the tissues were prepared according to the method described in a previous paper[11]. The first antibody, a highly specific anti-melatonin antibody (table), has been prepared according to Grota and Brown[14]; the second antibody, a fluorescein labelled antibody, was used as described by Coons et al.[15]. Comparable serial sections were stained by cresyl violet for identification of cellular details. The specificity of the staining was determined in 3 separate tests: a) the antimelatonin serum was saturated with melatonin; b) the different specific antiserum (antitestosterone) were used for comparison; and c) the reaction was performed with second antibody, without using the specific antimelatonin serum[11].

No specific fluorescence was found in the liver, spleen or pancreas. However, fluorescence indicating melatonin was observed throughout the whole digestive system. In the esophagus, melatonin is mostly present in the basal epithelium but some also is found in the circular muscles. In the stomach, the fluorescence was registered in the glandular portion of the wall. One of the highest amounts of melatonin was found in the duodenum, mostly in the Lieberkühn's crypts and the Brunner's glands but a considerable amount was also localized in the villi. On the other hand, the jejunum was found to be almost melatonin free, with only a few fluorescent particles scattered in the glandular portion. More melatonin than in the jejunum, but far less than in the duodenum, was registered in the ileum. Fluorescence was distributed mostly in the Lieberkühn's crypts and villi. In the caecum, colon and the rectum, the distribution of fluorescence was almost identical but there was a rising quantity of melatonin toward rectum, where it reached the highest concentration. Most fluorescence was observed in the higher and apical portions of the Lieberkühn's crypts (figure, A, B). The exact localization of melatonin in respect to the type of cell containing the N-acetylated indolealkylamines will require a further study.

The distribution of melatonin (higher in the stomach and duodenum, low in the jejunum and ileum and rising again toward rectum) corresponds to the distribution of serotonin-producing argentaffin cells[16,17]. Moreover, the localization of fluorescence in the Lieberkühn's crypts (higher and apical portion) corresponds closely to the localization of argentaffin cells[16]. Serotonin is known to facilitate the peristalsis[16]. It is therefore possible that melatonin, a derivative of serotonin, also participates in some aspect of intestinal physiology. Physiological studies investigating the role of melatonin in the digestive processes are in progress.

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Cytological effects of some medicinal plants used in the control of fertility[1]

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Summary. The effects induced upon the cell cycle of Allium cepa meristems by 2 medicinal plants used in the control of fertility were studied. Infusions of Aristolochia triangularis induces typical c-mitotic figures. On the other hand, Stevia rebaudiana have no specific toxicological effects upon the cell cycle.

Rural and indigenous peoples of Paraguay employ several medicinal plants in the control of fertility[4]. The effectiveness of these treatments has not been confirmed yet, and many international institutions are highly interested not only in the effects of these plants upon fertility, but in research concerning their general biological effects[5]. Chaudhury[6] found that several plants used by primitive peoples of India in order to prevent pregnancy significantly decrease the fertility of adult female albino rats. Many drugs with specific toxicological effects upon mitosis (i.e. colchicine, vinblastine and podophyllotoxine) are isolated from plants which have been employed by primitive people for generations[7]. Finally, Wiesner and Yudkin[8] and Robson[7] have proposed that the so-called

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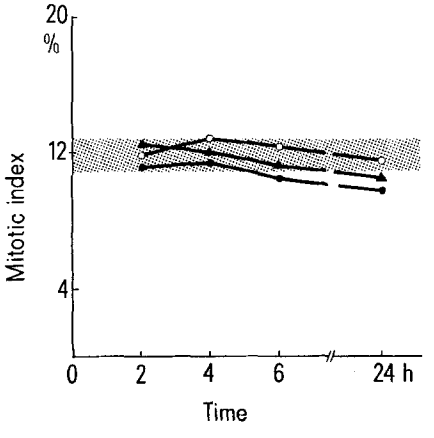
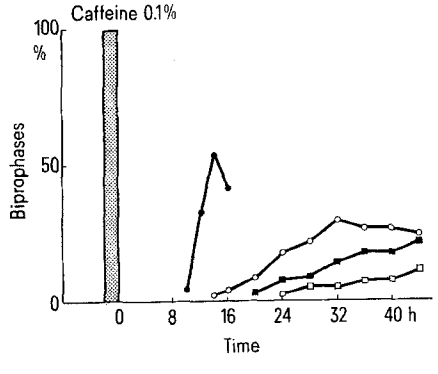
Evolution of phase indices, expressed as a frequency of mitotic cells observed in each phase, after 2, 4, 6 and 24 h treatment with different infusions of A. triangularis

Treatments		2 h	4 h	6 h	24 h
1% of the original infusion	Prophase	0.50	0.52	0.46	0.42
	Metaphase	0.13	0.12	0.17	0.17
	Anaphase	0.08	0.08	0.08	0.13
	Telophase	0.29	0.28	0.29	0.28
10% of the original infusion	Prophase	0.45	0.44	0.23	0.19
	Metaphase	0.13	0.20	0.61	0.77
	Anaphase	0.09	0.06	0.04	0.02
	Telophase	0.33	0.30	0.12	0.02
Original infusion	Prophase	0.46	0.47	0.12	0.11
	Metaphase	0.14	0.21	0.70	0.85
	Anaphase	0.09	0.08	0.04	0.00
	Telophase	0.31	0.24	0.14	0.04

Each percentage count is based on 500 mitotic cells scored per root.



A Evolution of mitotic indices after 2, 4, 6 and 24 h treatment with different infusions of S. rebaudiana: Original infusion (▲); 10% of the first (●); 1% of the first (○). The stippled area indicates the control range. Note that treatments do not significantly modify the mitotic indices.



B Cell cycle development of a synchronous subpopulation in control conditions (●) and in the presence of different infusions of S. rebaudiana: Original infusion (□); 10% of the first (■) and 1% of the first (○). The synchronous subpopulation was induced by means of a short pulse with 0.1% caffeine which blocks cytokinesis labelling as binucleate, all cells traversing cytokinesis during the pulse.

'mitotic poisons' could act against fertility by inhibiting the development of the fertilized egg.

In the present study, analyses of the effects produced upon the cell cycle of Allium cepa L. meristems by infusions of 2 medicinal plants (Aristolochia triangularis Cham. and Stevia rebaudiana Hemsl.) used by rural and indigenous populations of Paraguay in the control of fertility[4], were carried out.

Material and methods. Infusions of both plants were prepared according to instructions provided by rural people[9]. Original infusions were made at a concentration of 0.4 g/ml using dry branches of A. triangularis (Aristolochiaceae) and dry leaves in the case of S. rebaudiana (Compositae). Lower concentrations at 10% and 1% of the original ones, were also tested.

Bulbs of A. cepa were grown in tap water, in the dark at a temperature of 25 °C ± 0.5 °C, and continually aerated. Roots were submerged in the infusions without separating them from the bulbs, and in the same conditions previously described for the time specified in each case. In the first set of experiments, fixations were made after 2, 4, 6 and 24 h treatment, then bulbs were returned to tap water in order to observe their recovery. In the second set of experiments, roots previously treated with 0.1% caffeine for 1 h, in order to label as binucleate cells a natural synchronous subpopulation[10], were submerged in the different infusions and fixations were made at successive hours later. Finally, in the third set of experiments, roots which had been submerged in the infusions for 12 h, were exposed to ^{3}H-thymidine (10 μCi/ml) for 15 min and immediately fixed.

All fixations were made in ethanol-acetic acid (3:1). For cytological analysis, preparations were made from the first and second sets of experiments, according to Tjio and Levan's staining technique[11]. Roots from the third set of experiments were stained by the Feulgen method on gelatined slides and processed for autoradiography by Mak's technique[12]. Finally, cytophotometric studies were performed under a Vickers M85 microdensitometer.

Results and discussion. Onion roots grown under fixed environmental conditions are in steady-state kinetics, a situation characterized by a constant growth rate and a reliable mean duration of the cell cycle[13]. Under these conditions, the meristematic population is found to be uniformly distributed throughout the cell cycle, and the percentage of cells found at any given stage of the cycle remains constant over a long period.

Mitotic phase indices obtained after 2, 4, 6 and 24 h of treatment with infusions of A. triangularis show a typical c-mitotic action. Recoveries were quite normal in all cases. The table shows how prophases, anaphases and telophases decrease with time while c-metaphases increase in relative terms. This effect appears to be common to more than one species of this genus, since Barnard[14] demonstrated that extracts of A. elegans also have a c-mitotic action. It is probable that an active principle common to

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all Aristolochiaceae could be responsible for this effect, and Schvartzman et al.[15] reported that aristolochic acid I, which is found in almost all the species of this genus[16], has c-mitotic effects.

No mitotic alterations were observed in roots treated with infusions of S. rebaudiana, as can be deduced from the figure A. The evolution of mitotic indices after 24 h treatment is quite normal in all cases. Nevertheless, cell cycle duration is prolonged by treatments with these infusions, as can be seen in the figure B, which shows the entrance in mitosis of a synchronous binucleate subpopulation in control and treated roots. Finally, cytophotometrical studies showed that the distribution of cells in G_1, S and G_2, amounting to 26%, 45% and 16% respectively in the controls, was not significantly modified by these treatments. Extracts of S. rebaudiana were found to have contraceptive properties in rats[17]. Our experiments demonstrate that infusions of this plant have no specific toxicological effects upon the cell cycle of A. cepa meristems, suggesting that the contraceptive properties may not be connected with the chromosome cycle[15].

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Ventral motor neuron alterations in rat spinal cord after chronic exercise[1,2]

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Summary. The observed differences in the soma and nuclear diameters reflect chronic changes specific to each exercise regimen used.

A dearth of information exists concerning the effects of physical activity on the size of the cell body and the nucleus of the ventral motor neuron. Acute bouts of swimming or running have been reported to increase[4,5], decrease[6–8], or not change[9] the volume of the soma. Similar conflicting results have been found for changes in nuclear sizes[4,5,9]. An inspection of the exercise protocols used indicates that the inconsistency in these results may be attributed to the use of acute exercise programs of different types, intensities, and durations. Conversely, chronic programs of physical activity have consistently resulted in no changes in the volumes of the soma and nucleus[9,10,11]. These findings have been interpreted as reflecting an adaptation of the motor neuron to the new level of neuromuscular activity[12]. However, to date, all longitudinal studies have used an endurance swimming program of light to moderate intensity as the mode of exercise. It seemed logical to determine if these structural adaptations would be consistent using different intensities of training. Therefore, this investigation was designed to study the chronic effects of 2 well-defined interval running programs of different intensities on the soma and nuclear diameters of the working motor neurons.

Materials and methods. Normal, 72-day-old, male albino rats (Sprague-Dawley strain) were brought into the laboratory and assigned randomly to one of the following

Table 1. Summary of χ^2-values for soma and nucleus distribution differences between groups after 12 weeks of training

Comparison		df	12 weeks χ^2
CON* vs SPT*	Soma	30	73.44**
	Nucleus	14	77.87**
CON vs END*	Soma	30	53.39**
	Nucleus	14	42.83**
SPT vs END	Soma	30	44.94**
	Nucleus	14	39.40**

*n, 4 animals. ** Significant distribution differences at the 0.05 level.

Table 2. Summary of χ^2-values for soma and nucleus distribution difference between pooled-zero** and 12-week animals for each treatment group

Comparison	CON		Sprint		Endurance	
	df	χ^2	df	χ^2	df	χ^2
Soma	30	166.08*	30	29.04	30	106.79*
Nucleus	14	133.02*	14	12.97	14	34.49*

* Significant distribution differences at the 0.05 level. ** All zero-week animals were pooled into one distribution since there were no significant differences between groups at the start of the study (n = 12).

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