

**Material and methods.** Donor  $^3\text{H}$ -labelled DNA was isolated from L-cells grown in the medium containing  $^3\text{H}$ -thymidine ( $3\ \mu\text{Ci/ml}$ , 20 h) using the modified method of Marmur, employed already in earlier studies<sup>6</sup> (specific activity of isolated  $^3\text{H}$ -DNA  $5.64\ \mu\text{Ci/mg}$ , m.w.  $3.15 \times 10^7$  daltons). The  $^3\text{H}$ -DNA was preincubated for 20 h at  $37^\circ\text{C}$  with GPAG prepared from calf or sheep sera<sup>7</sup>; subsequently the complex of  $^3\text{H}$ -DNA + GPAG was added to Eagle's MEM in a concentration of  $25\ \mu\text{g } ^3\text{H}$ -DNA/ml of medium and 0.8 mg GPAG/ml of medium. As the host cells, L-strain mouse fibroblasts grown in a monolayer in Eagle's MEM supplemented with 10% calf serum were used. For experiments, cells were plated on cover-slips placed in Petri dishes; in the logarithmic phase of the growth, cover-slips containing cell layer were washed with Hanks' solution and cells grown for additional 30 or 60 min in Eagle's MEM without serum, containing  $^3\text{H}$ -DNA + GPAG or  $^3\text{H}$ -DNA preincubated for 20 h at  $37^\circ\text{C}$ . As a control, non-preincubated  $^3\text{H}$ -DNA was used. After this period, cultures were rinsed and transferred for 30 min into DNase solution ( $37^\circ\text{C}$ ,  $20\ \mu\text{g/ml}$  DNase N.B.C. 2x cryst. in Hanks' solution, pH 7.0). One set of cultures from each experimental series was fixed im-

mediately (ethanol and concentrated acetic acid 3:1); the second set was cultivated for an additional 6, 24 or 48 h in Eagle's MEM supplemented with 10% calf serum. After this period, cells were fixed, the acid-soluble cell material removed by 1% perchloric acid, and the preparations further processed using autoradiographic technique (Stripping film KODAK AR 10, exposure of 2 to 3 weeks).

**Results and discussion.** The results of our experiments showed that the uptake of isologous exogenous  $^3\text{H}$ -DNA into L-cells is markedly stimulated by GPAG. Stimulatory effect of GPAG prepared from sheep serum was higher than that of calf serum GPAG (figure). Preincubation of  $^3\text{H}$ -DNA at  $37^\circ\text{C}$  did not affect the labelling of cells, which proved that no significant  $^3\text{H}$ -DNA degradation occurred. That the  $^3\text{H}$ -DNA retains its macromolecular structure in the host cell is also supported by the pattern of labelling, and especially by the identical ratio of grain distribution between the cytoplasm and the nucleus of cells grown in the presence of control and preincubated  $^3\text{H}$ -DNA.

In accordance with results described previously<sup>5</sup>, the uptake of exogenous  $^3\text{H}$ -DNA by host cells is increased in the presence of GPAG; at the same time, intracellular transport of  $^3\text{H}$ -DNA into the nucleus is accelerated. Under the conditions given, radioactivity in the cell nucleus is retained simultaneously in a quantity three times higher than for control cultures even after 48 h of additional incubation in the medium without  $^3\text{H}$ -DNA (table). Increased quantities of incorporated DNA in nuclei, which are caused by GPAG, thus creates better conditions for DNA integration with recipient cell genome and may be properly utilized in model experiments on exogenous DNA incorporation into mammalian cells under physiological circumstances.

Post-incubation (h)	No. of grains/cell		No. of grains/nucleus	
	$^3\text{H}$ -DNA	$^3\text{H}$ -DNA + GPAG	$^3\text{H}$ -DNA	$^3\text{H}$ -DNA + GPAG
0	15.32	21.45	10.27	16.52
24	11.23	14.33	5.32	11.16
48	6.86	9.51	2.05	7.30

0:60 min incubation of cells with exogenous  $^3\text{H}$ -DNA or  $^3\text{H}$ -DNA + GPAG. 24 (48): 60 min incorporation of cells with exogenous  $^3\text{H}$ -DNA or  $^3\text{H}$ -DNA + GPAG and subsequent 24 (48) h postincubation without exogenous  $^3\text{H}$ -DNA. (GPAG was prepared from calf serum.)

6 J. Kepřtová, *Neoplasma* 20, 671 (1973).

7 J. Michl, *Exp. Cell Res.* 23, 324 (1961).

## A plant species of suspected accumulator behaviour

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**Summary.** 17 plant species growing near Bombay, India, were analysed for 26 trace elements by neutron activation analysis. Out of these only *Alternanthera sessilis* was found to be an accumulator plant for Al, V, Ti and Sc.

Accumulator organisms are characterized by their capacity to absorb and store large amounts of specific elements which are not taken up by the population at large growing in the same environment. They are also called indicator or collector species. Several examples of such species have been given by BOWEN<sup>1</sup> and UNDERWOOD<sup>2</sup>. These plants also serve as useful geochemical indicators for identification of the underground mineral deposits.

We recently undertook a study to determine the elemental concentrations of a number of plant species and to compare the effects of air pollution on trace element content of the plants. During the course of this study, we came across some plant species which looked promising as accumulator species.

**Materials and methods.** The plant samples were collected from the Institute of Science Botanical Gardens,

Bombay. The leaves, stems and where possible roots, inflorescence and fruits were collected. The material was washed, dried at  $85^\circ\text{C}$  and powdered. The analysis for trace elements was performed by the technique of neutron activation analysis, the details of which are given elsewhere<sup>3</sup>.

**Results and discussion.** 17 plant species were analyzed for 26 trace elements. Most of the species were within a close range for a given element. Only *Alternanthera ses-*

<sup>1</sup> H. J. M. BOWEN, in *Trace Elements in Biochemistry* (Academic Press, London 1966).

<sup>2</sup> E. J. UNDERWOOD, in *Trace Elements in Human and Animal Nutrition* (Academic Press, New York 1971).

<sup>3</sup> R. A. NADKARNI and G. H. MORRISON, *Anal. Chem.* 45, 1957 (1973).

*sis* was found to accumulate 4 elements – Al, V, Ti, and Sc. The data is given in the table. The range of values found for 16 other plant species growing in that area for these particular elements is also included.

Aluminium is accumulated by *Alternanthera sessilis* up to about 10 times more than other plants in the same area. Roots accumulate more Al than shoot portions. Normal Al concentration in the land plants is given by BOWEN<sup>1</sup> as 500 ppm. CHENERY<sup>4</sup> has documented certain

Trace Elements in *Alternanthera sessilis*

Element, ppm	Roots		Shoots	
	<i>A. sessilis</i>	Others	<i>A. sessilis</i>	Others
Aluminium	7100	212–730	2910	200 – 750
Vanadium	31	–	11	0.3 – 4.5
Titanium	550	20–51	93	27 – 43
Scandium	2.1	–	2.5	0.06– 1

Al accumulators which contain up to 15%  $\text{Al}_2\text{O}_3$  in leaves, though more common Al accumulators have about 0.2%. HESS<sup>5</sup> found 5000 ppm Al in the leaves of the mangrove *Rhizophora harrisonii*.

*Alternanthera sessilis* also seems to accumulate V and Ti, again the roots storing more than the leaves. Degree of enrichment in the leaves here is not as great as in the case of Al. Other plant species have been reported to contain less than 1 ppm V and 1–2 ppm Ti by BOWEN<sup>1</sup> and UNDERWOOD<sup>2</sup>.

Scandium is accumulated by *Alternanthera sessilis* to a lesser degree compared to other plants in that area. Roots and shoots seem to store the same amount of Sc. BOWEN<sup>1</sup> gives the average Sc concentration in the land plants as only about 0.008 ppm, which makes *Alternanthera sessilis* accumulating property about 2000 times more than average. To the best of our knowledge, there are not Ti and Sc accumulator plants reported so far.

<sup>4</sup> E. M. CHENERY, J. Soil Sci. 2, 97 (1950).

<sup>5</sup> P. R. HESSE, Pl. Soil 19, 205 (1963).

## Lengthening of lobster muscle fibres by two age-dependent mechanisms<sup>1</sup>

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**Summary.** Fibres of the lobster accessory flexor muscle elongate by 2 mechanisms: an increase in sarcomere length, which is restricted to their early development and by the addition of serial sarcomeres of a relatively constant size, which prevails throughout the life of the animal.

Vertebrate muscle fibres, after their early developmental stages, grow in length by adding sarcomeres of a constant size to the ends of the muscle fibres<sup>2–5</sup>. In fact adult muscle fibres can change in length not only by adding sarcomeres but also by removing them from the ends of muscle fibres<sup>6,7</sup>.

On the other hand crustacean muscle fibres grow longer by the continuous lengthening of individual sarcomeres during early developmental stages<sup>8,9</sup> and throughout adult life<sup>10</sup>. On this basis, crustaceans such

as lobsters which continue to grow in mass might therefore be expected to have unusually long sarcomeres to account for the increase in fibre length. But if the sarcomeres maintain a fairly constant length throughout adult life then the lengthening of fibres must be attributed to the addition of sarcomeres. We find the latter to be the case for fibres of the limb accessory flexor muscle in lobsters.

**Material and methods.** Lobsters (*Homarus americanus*) were held in running sea water tanks at ambient temperature (ca. 23°C) at Woods Hole. The accessory flexor muscle in the first walking leg was exposed and fixed at rest length in aqueous Bouins solution in which it was also stored. All measurements were made with an ocular

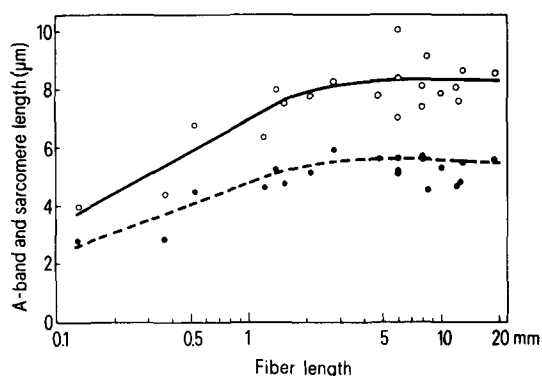


Fig. 1. Relationship between the mean A-band (closed circles) and sarcomere (open circles) lengths of a proximal fibre from the distal head of the accessory flexor muscle and the log of its fibre length. The curve fitting the points for the A-band (dashed line) and the sarcomere (continuous line) lengths is drawn by eye.

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<sup>2</sup> D. A. FISHMAN, J. Cell Biol. 32, 557 (1967).

<sup>3</sup> G. GOLDSPIK, J. Cell Sci. 3, 539 (1968).

<sup>4</sup> G. E. GRIFFEN, P. E. WILLIAMS and G. GOLDSPIK, Nature 232, 28 (1971).

<sup>5</sup> P. E. WILLIAMS and G. GOLDSPIK, J. Cell Sci. 9, 751 (1971).

<sup>6</sup> G. GOLDSPIK, C. TABARY, J. C. TABARY, C. TARDIEU and G. TARDIEU, J. Physiol., Lond. 236, 733 (1974).

<sup>7</sup> Z. F. MUHL and A. F. GRIMM, Experientia 31, 1053 (1975).

<sup>8</sup> G. D. BITTNER, J. exp. Zool. 167, 439 (1968).

<sup>9</sup> C. K. GOVIND, H. L. ATWOOD and F. LANG, J. exp. Zool. 189, 395 (1974).

<sup>10</sup> G. D. BITTNER and D. L. TRAUT, Personal communication (1976).