transfilter cultures the nitrogenium fixed by the bacteria migrates in the form of NH_4^+ to the plant cells. This assumption, of course, needs further confirmation.

Portulaca cells associated with rhizobia showed an increased accumulation of glutamic acid compared with control callus (figure 1). The stronger ninhydrin coloration of glutamate was even visible to the naked eye. None of the other free amino acids, including glutamine, showed a comparable increase. The glutamine synthetase - glutamine oxo-acid aminotransferase pathway represents the predominant route for ammonia assimilation in higher plants[9], glutamate being both the acceptor of ammonia and the product of its assimilation[10]. Apparently the ammonia transferred into the *Portulaca* cells follows this pathway, causing an increase in glutamate. That no comparable increase in glutamine could be detected, might be due to a low stationary concentration.

As for the further fate of the transferred N, it seemed sufficient to check just 2 classes of substances: the betacyanins, representing N-containing secondary plant products, and the proteins, representing N-containing substances of primary importance.

Rhizobia are unable to synthesize betacyanins. Therefore it was not absolutely necessary to separate plant cells and bacteria. In any case, *Portulaca* cells associated with rhizobia showed an increased betacyanin accumulation, whether we used the transfilter technique or not (figure 2).

Total protein from callus associated with rhizobia was somewhat, but statistically insignificantly higher than from control callus. That no marked difference could be detected, could perhaps be due to the comparatively rough colorimetric procedure. ^{15}N analysis, however, revealed differences between experiment and control. *Portulaca* callus associated with bacteria and control callus not associated with rhizobia were kept in an atmosphere containing $^{15}N_2$. The ^{15}N content of proteins extracted from control callus was within the limits of the method. Proteins from callus associated with rhizobia, however, showed a more than 10-fold ^{15}N-enrichment (figure 3). About 10% of the ^{15}N incorporated into the callus material were found within the proteins.

This ^{15}N analysis confirmed the interpretations of the results based on accumulation studies; in associations of rhizobia with cells of the nonlegume *Portulaca*, nitrogenium fixed by the bacteria was transferred to the plant cells and channelled into the normal pathway of ammonia utilization. Cells of nonleguminous plants are able to induce nitrogenase activity in rhizobia, and vice versa are able to profit from the nitrogenium fixed by the bacteria.

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Nonrandom association of acrocentric chromosomes in human epithelial cells[1]

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Summary. In diploid, aneuploid and polyploid cells of a human epithelial finite cell line, a statistically significant higher frequency in the involvement of D's than G's in acrocentric chromosome associations was found.

Studies on acrocentric chromosome associations have been very contradictory, revealing either randomness[2-4] or specific variations in different individuals[5-9], regarding the frequency of participation of different acrocentric chromosomes as well as the percentage of metaphases containing associations. Moreover, the almost exclusive utilization of PHA-stimulated lymphocytes rather limits the significance of chromosome associations, so that there is no information on a possible relationship with a) gene functional activation or suppression in other types of normal or neoplastic cells, and b) the obvious rearrangements of the genetic material in the chromosomes of neoplastic cells. In this work, we examined the frequency of associations and the involvement of D's and G's in acrocentric chromosome associations in diploid, aneuploid and polyploid cells of a human epithelial finite cell line.

Methods. The finite cell line KOS-ROV was derived by trypsin dissociation from a sterile tissue specimen removed from the right ovary of a 43-year-old female on October 27, 1978. Both ovaries of the patient were removed prophylactically, when the patient exhibited extensive osteolysis and pains in the pelvis; 18 months before this operation, the patient had been subjected to a breast tumor removal and radiotherapy. McCoy's 5a medium supplemented with 15% fetal bovine serum, penicillin 100 IO/ml, streptomycin 100 µg/ml and kanamycin 100 µg/ml and buffered with Hank's salts at pH 7.4 was used. Cells were proved to be free of mycoplasma and other contaminations, as shown by ^3H-thymidine labelling and autoradiography[10].

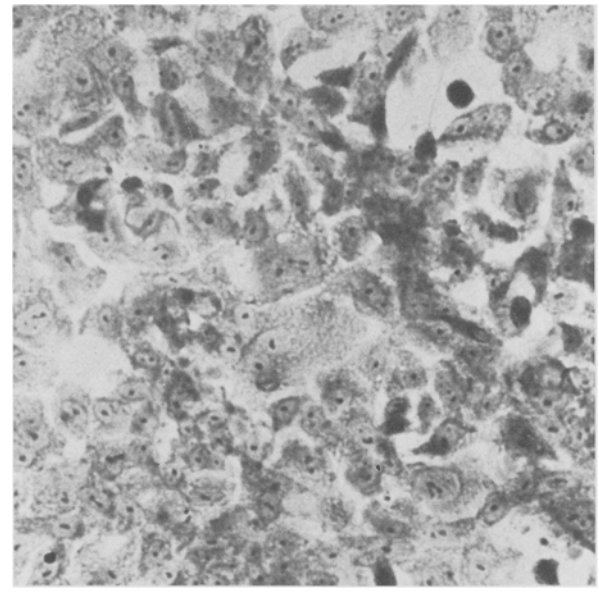
Cells in the logarithmic phase of growth were treated with colcemid 0.1 µg/ml for 4 h, harvested by trypsinization, subjected to hypotonic treatment with 0.95% sodium citrate pH 7.5 for 30 min and fixed in methanol-glacial acetic acid (3:1). Metaphase preparations were stained with Giemsa.

Results. A KOS-ROV monolayer showed a pavement-like arrangement with anaplastic and pleomorphic features, and cell overlapping during confluency (figure). Each cell was polygonal epithelial-like with abundant, well expanded cytoplasm. The ultrastructure of KOS-ROV cells revealed considerable similarities with benign Brenner tumour cells, regarding the plasma membrane foldings, the morphology of nuclei, mitochondria and secretory granules and the

presence of lysosomal dense bodies containing amorphous materials[11,12]. The distribution of chromosome number is given in the table. Aneuploid cells exhibited a tendency towards loss or excess of chromosomes of the E or F groups, very limited inconsistent translocations, unidentified small acrocentrics and chromosomal fragments like those characterized as 'double minutes'[13]. 7 cells (6.48%) with 'double minutes' were found among 108 metaphases examined. Most metaphases exhibited intensely stained satellites on most of the acrocentric chromosomes. Of the metaphases examined, 73.14% contained formations of 2, 3 or 4 associated chromosomes of the D and/or G group (table). Associations of 2 (83.6%), 3 (13.1%) and 4 (3.3%) chromosomes were scored. The associations of 2 chromosomes, showed a nonrandom distribution in the involvement of D and G chromosomes. Supposing that participation of D and G group chromosomes in associations occurs randomly, the combinational analysis in order to determine the expected values of (D–D), (D–G) and (G–G) combinations gives a ratio of 34:54:14 for the total of 102 associations of 2 chromosomes observed. The corresponding observed ratio is 46:53:3. Application of the χ^2 test indicated that the increase of (D–D) associations is significant ($p < 0.05$) and the decrease of (G–G) associations is highly significant ($p < 0.01$), while (D–G) associations are within the expected values. Additionally, the increase in the participation of D group chromosomes in associations of 3 or 4 chromosomes is also highly significant. The ratio D:G of all D's and G's involved in associations observed is 194:74, and supposing a random involvement, the ratio should be 161:107; the difference is highly significant ($p < 0.01$), and, therefore, it supports the contention that D chromosomes are more actively involved in associations than G chromosomes. No significant difference was found between observed and expected frequencies of associations between diploid, aneuploid and polyploid cells.

Discussion. The present results show that D group chromosomes participate more actively in chromosome associations than G group chromosomes. This observation, in connection with previous reports which show a nonrandom chromosome participation in associations in cells with D or G chromosome abnormalities[7,9,14], point to the necessity of data from other types of cells with or without obvious structural chromosomal abnormalities. As acrocentric chromosome association in metaphase has been correlated with NOR activity and nucleolus organization[15,16], nonrandomness of the phenomenon may indicate a genetic heterogeneity among different satellites.

The percentage of PHA-stimulated lymphocytes containing acrocentric chromosome associations (satellite associations) in c-metaphase varied, as previously reported[17], from 2.8 up to 46.3%; using the same strict criteria of Arditto et al.[17], the



KOS-ROV cells on coverslip culture after the 10th subculture. Giemsa staining. × 200.

Distribution of combinations of acrocentric chromosome associations observed among 108 KOS-ROV metaphases

Combinations observed	Chromosome number										
	45	46	47	48	92	93	94	95	98	137	
(DD)		10	1			1					
(DG)	1	17	1	1	1						
(DDD)		2									
(DDG)	1	1			1						
(DGG)		2	1								
(DDDD)		1									
(DDDG)		1						1			
(DD) (DD)		3	1		2						
(DD) (DG)		7			1	2					
(DG) (DG)	1	2			1						
(DD) (GG)		2									
(DD) (DDD)		2	1								
(DG) (DDD)		2									
(DD) (DGG)		1									
(DG) (DDDG)		1									
(DD) (DG) (DG)		1	1								
(DG) (DG) (DG)		1									
(DD) (DG) (DDG)					1						
(DD) (DD) (DG) (GG)					1						
(DD) (DG) (DG) (DDD)									1		
											Total
Cells with associations	3	56	6	1	8	3	0	1	1	0	79
Cells without associations	3	16	2	0	4	1	2	0	0	1	29
Number of metaphases	6	72	8	1	12	4	2	1	1	1	108

percentage of KOS-ROV cells with associations was significantly higher (73.14%). These variations may indicate that chromosome associations involve a very delicate structural process, influenced by several factors, such as sample manipulations in different laboratories[16,18-20], subjective judgement of the observer[3], age and sex of the subject[20]. These parameters, however, would seem to be unlikely to affect the nonrandom participation of specific chromosomes in the association figures. The kind of tissue used may be a factor influencing both frequency and nonrandomness in chromosome associations.

1 Acknowledgments. This work is supported by grant 0044-331 from the National Research Foundation of Greece. We thank Miss M. Margaronis for technical assistance.

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Kaurene biosynthesis in intact *Phaseolus coccineus* suspensors

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Summary. Intact suspensors from *Phaseolus coccineus* seeds incorporate 2-(^{14}C)-MVA into a number of radioactive compounds, among which kaurene was identified by GC-MS. This result confirms that the kaurene biosynthetic pathway previously shown for cell-free extracts is active in intact tissues as well.

Studies of GA biosynthesis in higher plants have been mainly performed in cell-free systems. The pathway from mevalonic acid (MVA) to GA_{12}-aldehyde was firstly demonstrated by Graebe[1] in *Cucurbita maxima* and has been ascertained also in *Marah macrocarpus*[2] and *Pisum sativum*[3]. Although this pathway has been repeatedly suggested to be active also in intact tissues, very little information is available as to the direct biosynthesis of gibberellins in intact higher plants. One of the few reports available deals with the incorporation of 2-(^{14}C)-MVA into kaurene by leaves of *Stevia rebaudiana*[4].

We have previously described the pathway from MVA to kaurene[5] and from kaurene to ent-7α-hydroxy-kauren-19-oic acid in a cell-free system of *Phaseolus coccineus* (Lamarck) suspensors[6].

The biosynthesis of gibberellins by the same extracts fed with ent-7α-hydroxy-(^{14}C)-kauren-19-oic acid, previously biosynthesized by cell-free extract of *Phaseolus coccineus* suspensors, was demonstrated recently[7].

To test whether the pathway shown by cell-free extracts is actually active also in intact tissues, we incubated 2-(^{14}C)-MVA with suspensors freshly taken from *Phaseolus coccineus* seeds of 5–8 mm length. 1000 suspensors were incubated with phosphate buffer (0.05 M, pH 7.5), $MgCl_2$ (2.5 µM), ATP (5 mM) and 2-(^{14}C)-MVA (22.500.000 dpm at a sp. act. of 22 µCi/µmole) in a final volume of 450 µl, and shaken for 2 h at 28 °C. The reaction was terminated by adding 1 ml acetone, and after boiling for 2 min the tissue was homogenized, adjusted to pH 3 and extracted 3 times with EtOAc. The extract was then reduced to a small volume and chromatographed on silica TLC plates using hexane for development.

Radioactivity scanning revealed the separation of 2 peaks removed from the origin. The 2 fractions were then scraped, eluted and investigated by analytical and preparative GLC. The less polar fraction showed only 1 radioactive peak which co-chromatographed with an authentic kaurene standard. The identity of this compound as (^{14}C)-kaurene was definitively assessed by GC-MS and its specific activity, calculated on analytical GLC base, was 2.9 µCi/µmole. The MS-spectrum of the biosynthesized sample corresponds well with the standard kaurene MS spectrum obtained under the same conditions. We were unable to determine the identity of the other compound, separated by hexane, possibly due to the low level of the pool present originally in the tissue, as well as the nature of polar compounds that did not move from the origin.

In conclusion the incorporation of 2-(^{14}C)-MVA into kaurene shows that at least the early pathway of gibberellin biosynthesis is active in the intact suspensor, at the same developmental stage previously used to demonstrate the synthesis of GAs in a cell-free system.

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