Table I. Measured values of total extinction of standard C^{14}-Dansyl-chloride samples on the microautoradiogram

Amount of C^{14}-Dansyl chloride (M)	Total extinction
3×10^{-14}	2,300
6×10^{-14}	5,600
9×10^{-14}	7,400
15×10^{-14}	9,300
18×10^{-14}	17,000
21×10^{-14}	17,400
24×10^{-14}	22,600
27×10^{-14}	25,300
30×10^{-14}	24,000
40×10^{-14}	40,000

Tabelle II. Measured values and calculated amounts from the graph (Figure 2) of 7 amino acids in 60 nl of rat urine

Amino acid	Total extinction	Amount of amino acid (M)
1. Tryptophane	18,690	20×10^{-14}
2. Phenylalanine	10,668	$12{,}75\times10^{-14}$
3. Histidine	3,540	2.7×10^{-14} (5.4×10^{-14})
4. Leucine	7,350	9.3×10^{-14}
5. Valine	8,375	10.3×10^{-14}
6. Proline	14,433	14.5×10^{-14}
7. Hydroxyproline	27,900	30.4×10^{-14}

When there is a complete binding of amino acids with C^{14}-Dansyl chloride under these conditions, the molar ratio of amino acid to C^{14}-Dansyl chloride is one to one. (In the case of Histidine this ratio is 1:2. Histidine binds 2 Dansyl-molecules).

Figure 1 shows the measured intensities of one spot. The computer calculates the mean intensity of the total spot and the total area. The multiplication of total area by mean intensity gives the total intensity of the spot (cf. Table I). The background and extinction mainly depend on the sensitivity of the film, the time of exposure and the conditions of developing and fixation. The same background value is used for all spots on one autoradiogram. The diameter of the spot should not be more than 1 cm. Spots which are too close cannot be separated. When using higher amounts of radioactivity under these conditions, the calibration curve is no longer linear and reaches a maximum point of total intensity.

Results and discussion. Table I gives the measured data for total intensity of several C^{14}-Dansyl-chloride samples with a known concentration which have been transfered directly to the polyamide layer and subsequently autoradiographed. There is a linear correlation between total intensity and concentration ($r = 0.9842$, Figure 2). This good correlation is proof that the described technique can be applied to very small amounts of biological material. With higher radioactivities this linear relationship would not be found anymore and therefore a shorter exposure of the film would be necessary.

An autoradiogram of C^{14}-dansylated amino acids in 60 nl of rat urine was done. Single spots representing certain amino acids were measured with this new technique (Figures 3 and 4).

Table II gives the measured total intensity and the calculated concentration of 7 amino acids. It can be seen that these amino acids have a concentration in the range of 10^{-14} molar.

Zusammenfassung. Die beschriebene Methode stellt eine Kombination der Mikrochromatographie und der «Scanning-Mikroskop-Photometrie» dar und ermöglicht eine quantitative Bestimmung von C^{14}-markierten Dansyl-Aminosäuren in biologischem Material im Bereich von $10^{-14}M$.

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Direct Measurement of Interphase Shortening Produced by Kinetin plus Indol Acetic Acid in Meristematic Cells of *Allium cepa* L.

There are a number of papers dealing with different aspects of the effect of kinetin or kinetin plus indol acetic acid (IAA) on plant cells[1–4], but in the field of its action on the duration of the cell division cycle there is some confusion due to several contradictory reports.

The experiments here described intend to show, by using a synchronous binucleate cell population induced by caffeine in root meristems, whether these growth factors, at certain concentration, are able to shorten the duration of the cell division cycle.

The material used was the root meristem of *Allium cepa* L. The onion bulbs were grown in the dark at the constant temperature of $15° \pm 0.5$ °C in cylindrical receptacles of 70 cm^3 capacity, with tap water which was renewed every 24 h and aerated continuously by bubbling in 10–20 cm^3 of air/min. The bulbs were so placed that only their bases remained submerged in the water.

The roots were fixed in a 3:1 mixture of ethanol-acetic acid and the specimens were prepared by staining the roots with acetic orcein, according to the technique of Tjio and Levan[5].

Labelling with caffeine. The roots, attached to the bulbs, were submerged in 0.1% caffeine for 1 h. This drug inhibits cytokinesis in cells going through the telophase during this time and produces a binucleate population

[1] R. Guttman, J. biophys. biochem. Cytol. *3*, 129 (1957).
[2] W. A. Jensen, E. G. Pollock, P. Healley and M. Ashton, Expl. Cell Res. *33*, 523 (1964).
[3] L. Bergman, *The Effect of Kinetin on the Metabolism of Plant Tissue Culture* (Ed. P. R. White; McCutchan Publishing Corporation, Berkeley, California 1965), p. 171.
[4] R. D. MacLead, Chromosoma *24*, 177 (1968).
[5] J. H. Tjio and A. Levan, Anais. Estaç. exp. Aula Dei *2*, 21 (1951).

which initiates the interphase (Figure a) and goes through the whole cell cycle synchronously[6].

Treatments. The solutions for the various treatments were obtained with tap water, and the culture conditions already described were maintained throughout the period of treatment. The roots were submerged in the treatment solution in all cases without separating them from the bulbs at the actual moment when the caffeine labelling ended. Kinetin solutions at concentrations of 1, 0.5 and 0.1 ppm and kinetin at the same concentrations plus indole acetic acid at concentrations of 0.1, 0.01 and 0.001 ppm, were also tested.

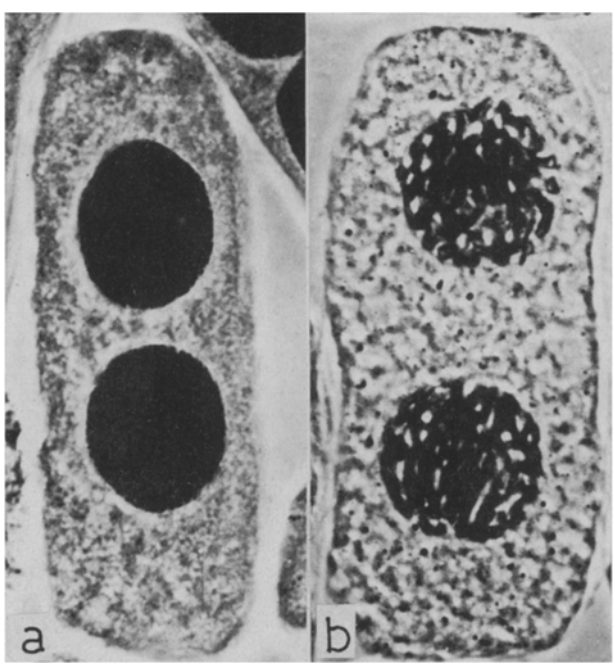
Of the 12 treatments tested (kinetin alone and kinetin plus IAA), only the 0.5 ppm concentration of kinetin plus 0.001 ppm of IAA had the effect of shortening the interphase, the first binucleate cells being observed to reach the prophase (Figure b) between the 18th and 19th h after the end of the caffeine treatment instead of the 22nd-23rd, as in the case of all control bulbs[7]. The fact that kinetin by itself has no demonstrable effect had already been observed by MILLER et al.[8].

Another experiment was carried out in which bulbs labelled with caffeine, as in the former case, were subjected to the shortener treatment with a solution of kinetin plus IAA from the 23rd h after the end of the caffeine treatment, at which hour the first biprophases were observed with a view to discovering the moment at which the first bitelophases appear. The results showed a shortening of mitosis, since in the control bulbs the bitelophase was detected at the 28th h while in the treated bulbs it made its appearance at the 25th.

In interpreting the results of his experiments with kinetin on the cell division cycle in *Allium cepa*, GUTTMAN[9] indirectly reached the conclusion that this drug shortened the interphase. On the other hand, VAN'T HOF[10] pointed out that kinetin plus IAA 'impaired' the entry of the cells upon the S period and their development in the course of this period, and that kinetin further increased the duration of the G_2 period in *Pisum* roots. Other investigators[11,12] have recorded a lengthening of the mitotic cycle in *Vicia faba* roots treated with IAA.

These contradictory results may be attributed to the different concentrations used of these growth factors, since it is well known that one and the same factor, at differnt concentrations, may produce opposite effects.

The use of a population labelled as binucleate has enabled us to demonstrate the shortening of the interphase induced by a solution of 0.5 ppm kinetin plus 0.001 ppm IAA and to measure it directly, and by the same procedure we have succeeded in detecting a shortening of mitosis itself.



Binucleate meristematic cells induced in a meristem of *Allium cepa* L. by treatment with caffeine at 0.1%. a) Binucleate cell in interphase; b) binucleate cell in prophase.

Resumen. El uso de una población binucleada sincrónia inducida por cafeína 0.1% en el meristemo de la raiz de *Allium cepa* L., nos ha permitido demostrar que una solución de kinetina 0.5 ppm mas ácido indol acético 0.001 ppm acorta tanto la interfase como la mitosis, y valorar este acortamiento por medida directa.

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[6] G. GIMENEZ-MARTIN, A. GONZALEZ-FERNANDEZ and J. F. LOPEZ-SAEZ, J. Cell Biol. *26*, 305 (1965).
[7] J. F. LOPEZ-SAEZ, G. GIMENEZ-MARTIN and A. GONZALEZ-FERNANDEZ, Z. Zellforsch. *75*, 591 (1966).
[8] C. O. MILLER, F. SKOOG, M. M. von SALTZA and F. M. STRONG, J. Am. chem. Soc. *77*, 1392 (1955).
[9] R. GUTTMAN, Chromosoma *8*, 341 (1956).
[10] J. VAN'T HOF, Expl. Cell Res. *51*, 167 (1968).
[11] R. D. MAC LEAD and D. DAVIDSON, New Phytologist *65*, 532 (1966).
[12] D. DAVIDSON and R. D. MAC LEAD, Chromosoma *18*, 421 (1966).

CONGRESSUS

USA

3rd Congress of the International Society on Thrombosis and Haemostasis, in conjunction with the Council on Thrombosis, American Heart Association

in Washington, D.C., 22–26 August 1972.

The Congress will be held at the Mayflower Hotel in Washington. The topics for the planary sessions include the following: Control mechanisms in hemostasis. Cell membranes: structure and function; platelets. Molecular biology and pathophysiology of fibrinogen. Vessel wall and thrombogenesis.

Further information by Dr. Harold R. Roberts, Chairman of the Organizing Committee, Box 630, Chapel Hill, N.C. 27514, USA.