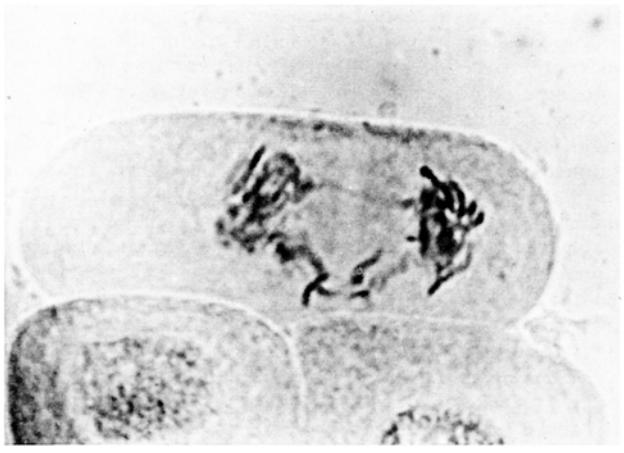

Fig. 1. Chromosomenbrücken quer durch eine sich bildende Zellwand nach Kultur in 100 µg/ml Aflatoxin B_1 (46,5 h). ×1100.

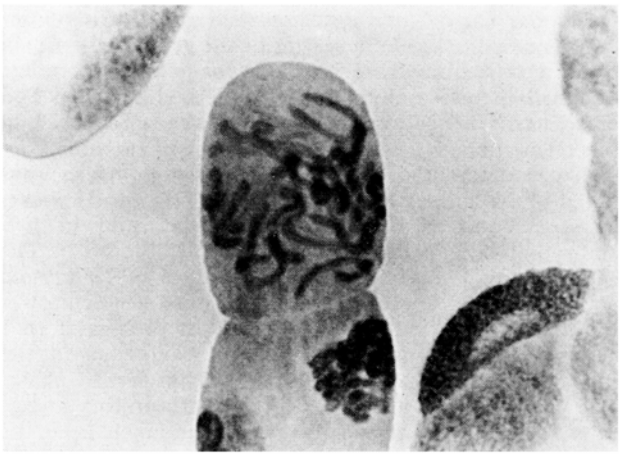

Fig. 2. C-mitotische Chromosomen nach Kultur in 200 µg/ml Aflatoxin B_1 (47 h). ×1100.

formamid (DMF) (Serva) eingetaucht und nach 4,24 und 48 h entnommen. Zur Kontrolle wurden die Wurzeln mit 0,3% DMF ohne Zusatz behandelt. Die Chromosomen in den meristematischen Zellen der Wurzelspitzen wurden mit Karminessigsäure (Chroma, Stuttgart) durch Erwärmen und Quetschen[13] dargestellt.

Ergebnisse. Verklebungen einzelner Chromosomen während der Anaphase, die sogar quer durch neu gebildete Zellwände verfolgt werden konnten (Figur 1), waren die auffälligsten Schädigungen. Daneben zeigten einzelne Zellen auch C-mitotische Chromosomen während der Metaphase (Figur 2). Alle Aberrationsformen traten gehäuft auf, wenn die Zwiebelwurzeln 48 h lang mit 200 µg/ml Aflatoxin B_1 behandelt worden waren (etwa 50% aller Meta- bzw. Anaphasen geschädigt) und nahmen in dem Maße ab, wie Toxinkonzentration und Einwirkungszeit herabgesetzt wurden. Zusätzlich wurde bei *A. cepa* die allgemeine Mitoserate herabgesetzt: Mitoseindex (Anzahl der Mitosen in Prozent der Gesamtzellzahl): DMF-Kontrollen: 12%, 200 µg/ml Aflatoxin B_1: 3%. Ähnliche Verminderungen der Mitosehäufigkeit unter dem Einfluss von Aflatoxin B_1 wurden auch bei menschlichen Lungenzellen[10], menschlichen Leukozyten[9] sowie bei Wurzelzellen von *Vicia faba*[8] festgestellt. In der DMF-Kontrolle entstanden bei *A. cepa* keine Chromosomenschädigungen.

Chromosomenbrücken wurden auch nach Einwirkung von Aflatoxin auf *Vicia faba*-Wurzeln[8] sowie nach Behandlung von Zwiebelwurzeln mit dem chemisch verwandten Cumarin[11,12] beobachtet. Zumindest für *A. cepa* kann angenommen werden, dass die beobachteten Aberrationen nicht für Aflatoxin B_1, sondern vielmehr für eine Gruppe chemisch verwandter Substanzen spezifisch ist.

Summary. After treatment of roots of *Allium cepa* with aflatoxin B_1 in 0.3% dimethylformamide, chromosome bridges, C-mitose chromosomes and a reduction of the mitotic index were observed. The aberrations occurred especially frequently when the roots had grown in 200 µg/ml toxin for 48 h. In its cytotoxic effect on *Allium cepa* root tips, aflatoxin B_1 acts similarly to the chemically related coumarin.

J. REISS

Mikrobiologisches Laboratorium,
Grahamhaus Studt K.G.,
D-655 Bad Kreuznach (Deutschland), 1. April 1971.

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The Phase Numbers and Their Variation under Different Treatments

In order to study the dynamics of a meristematic population, indices have been introduced which relate the number of cells in mitosis to the number going through the interphase[1,2]. Theoretically, the partial mitotic indices, apart from the phase indices, do not always express the kinetics of a cell population going through the division cycle, and for this reason we need a figure which will relate one type of index to the other, giving a resultant which expresses variation or normality in the proportional relationship between the cells at different stages in the division cycle.

The idea of the phase number is that it should represent the percentage of cells in each of the phases of the division cycle within the meristematic population, and it must necessarily reflect any modification that can be detected in the dynamics of that population. It may be determined by a direct count, to find out the percentage of cells in each one of the actual phases within the

population, or by relating the product of the mitotic index and each of the phase indices as a percentage according to the formula

$$\text{Ph N} = \frac{\text{MI} \times \text{Ph I}}{100}; \quad \text{Ph N} = \text{phase number};$$

MI = mitotic index; Ph I = phase index, so that the phase number would reflect the development of a cell population within the division cycle more satisfactorily. The material used was the meristem of *Allium cepa*. The bulbs were grown in the dark at a constant temperature of 15 ± 0.5 °C in cylindrical receptacles of 70 cm³ capacity, with tap-water which was renewed every 24 h and with constant bubbling, at the rate of 10 to 20 cm³ of air/min. Squash preparations of root tips were made after being stained with acetic orcein[3].

Phase numbers in untreated root meristems. The phase numbers studied hourly in *Allium* root meristems remain roughly constant throughout the experiment. The variation from bulb to bulb is not larger than within the bulb.

Phase numbers in the course of an interphase blockage. By using the drug 5-aminouracyl (5-AU) at 0.5 m*M* concentration, at which it has a blocking effect on S period[4] and studying the phase numbers obtained from 2 h before we began the treatment up to 6 h after, we have been able to draw up a table of phase number values which is shown in Figure 1. From this we observe a drop in the phase numbers from the 2nd h after the beginning of the treatment. Now, since the phase number can be kept constant only if the same number of cells enter the phase as leave it, this drop in the number of prophases from the 2nd h onwards can only be interpreted as a decrease in the number of cells entering upon mitosis, and as the duration of the cycle at the temperature tested is 30 h[5] and the duration of G_2 is about 4 h (unpublished data), the phase number curve also indicates a depressive effect on G_2.

The heavy drop in the number of prophases between the 4th and 6th h would appear to point to a strongly inhibitive effect of the drug in the interphase, which would be in agreement with the finding of a period particularly sensitive to 5-AU[4].

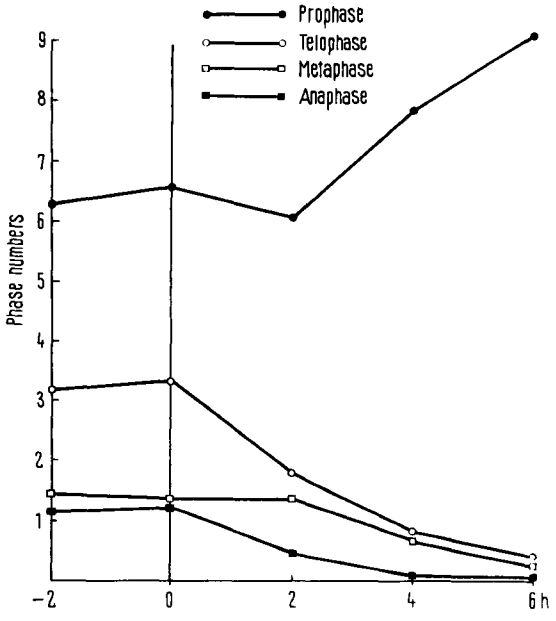

Fig. 2. Phase number from 2 h before and during the first 6 h of treatment with a drug blocking the transition from prophase to metaphase (ethidium bromide 100 µg/ml).

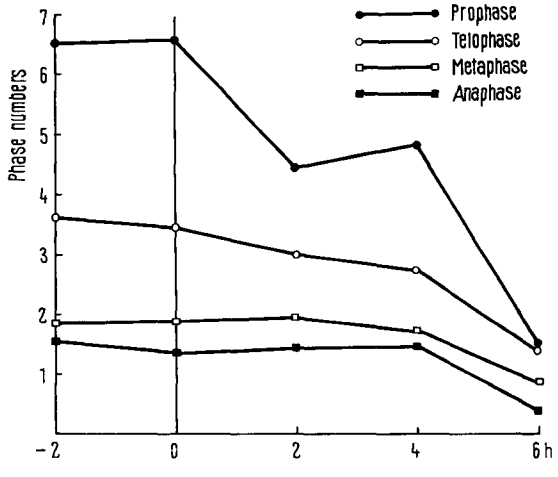

Fig. 1. Phase number from 2 h before and during the first 6 h of an interphase blockage (5-aminouracilo, 0.5 m*M*).

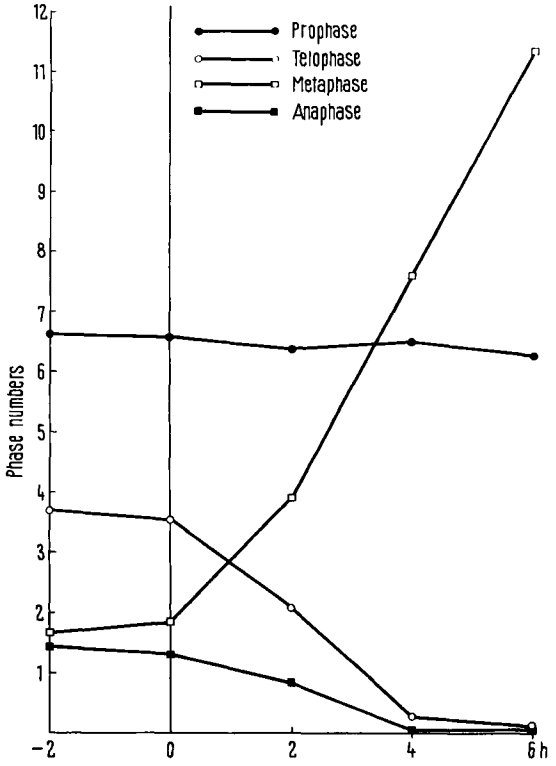

Fig. 3. Phase numbers from 2 h before and in the course of a metaphase blockage (0.05% colchicine).

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Phase numbers in the course of a blockage in the transition from prophase to metaphase. Using various inhibitors of nucleic acid synthesis in meristems, it was found that ethidium bromide at 10 µg/ml[6] increased the prophase index during the treatment, while the mitotic indices remained notably constant.

Analysis of the phase numbers in the course of continuous treatment with this drug shows an increase in the prophase number compared with other phase numbers, which all tend to be reduced to 0 (Figure 2). The fact that the mitotic index remains normal during the first 6 h of the treatment seems to indicate that all the cells are blocked at the transition from prophase to metaphase, i.e. that they accumulate in the prophase. This seems to be proved clearly by the observed kinetics of the prophase number.

Phase numbers in the course of a metaphase blockage. It has been known since 1934[7] that colchicine acts upon dividing cells by accumulating them in the metaphase, and the increase in the number of cells in metaphase per unit of time has been used by several authors as a way of measuring the speed of the cell division cycle. Analysis of the mitotic indices under 0.05% colchicine treatment[8] shows them to be normal during the first 4 h of treatment (Figure 3), while they increase subsequently through the accumulation of dividing cells. If we analyse the phase numbers under this treatment, we find that the prophase number remains constant, and that the increased mitotic index is due to the larger number of metaphases. The number of cells initiating mitosis is practically constant throughout the treatment, but 4 h after it has begun there are practically none issuing from it. The dynamics of the metaphase number clearly show the accumulating effect of colchicine from the first hour of treatment.

In short, the 3 effects studied – blockage in the interphase, in the prophase and in the metaphase – are easily detectable by the use of phase numbers and their analysis in sequence. The information thus obtained may be of use when a new drug is to be tested[9,10].

Resumen. Se determinan los números de fase en poblaciones meristemáticas en equilibrio dinámico, donde permanecen constantes a 1 o largo del tiempo. Se estudia su evolución en presencia de tres tratamientos que producen bloqueo en tiempos diferentes del ciclo de división celular – interfase, profase y metafase.

G. GIMÉNEZ-MARTÍN, A. GONZÁLEZ-FERNÁNDEZ, C. DE LA TORRE and M. E. FERNÁNDEZ-GÓMEZ

Departamento de Citología, Instituto de Biología Celular, C.S.I.C., Velázquez 144, Madrid 6 (Spain), 27 January 1971.

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In vitro Culture of *Toxoplasma* Infected Cat Intestine

Recent investigations by HUTCHISON et al.[1], FRENKEL et al.[2] and SHEFFIELD and MELTON[3] have shown that *Toxoplasma gondii* undergo schizogony and gametogony in the cat ileum giving rise to oocysts in the faeces of the infected animal. The oocysts are highly resistant to chemical treatment and can, therefore, be obtained in bacteriologically sterile condition by washing the faeces with various chemicals. The other intestinal forms of the parasite are, however, not resistant to chemicals and as such cannot be obtained in sterile condition from the lumen of the bowel like the oocysts. To overcome this problem we have used this method of organ culture involving the *Toxoplasma* infected cat ileum and find that the method is particularly suitable for obtaining bacteria-free merozoites in large numbers.

The strain of *Toxoplasma* used in this work was first isolated from a Malayan tree shrew (ZAMAN and GOH[4]). The cats used varied in age from 5–8 weeks and were reared in the laboratory, under pathogen free conditions. The infective material consisted of pooled mice brain containing a large number of cysts. The brain tissue was first chopped into small bits with scissors and fed to each cat by intragastric inoculation using a polythene tube. The faeces from each cat were collected daily and examined microscopically after zinc sulphate flotation. 6 cats passing a heavy concentration of oocysts, 7 days after the date of infection, were used for the study.

Laporotomy was performed on these animals and 4–5 inches of lower ileum was removed under sterile condi-

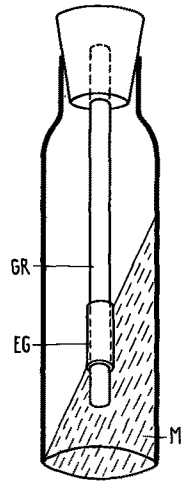

Fig. 1. GR, glass rod; EG, everted gut; M, medium. The container is kept at an angle in the roller drum to allow the everted gut to come in contact alternately with the medium and air in the tube.

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