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The Stathmokinetic Agents

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The word *stathmokinesis* has two roots of Greek derivation: σταθμός (*stathmos*) which means arrest and κίνησις (*kinesis*) which means movement. The general meaning of the term stathmokinesis is "arrest of movement". In cytology this term has been used to indicate the arrest of mitosis and, in particular, the metaphasic arrest of mitosis, which can be induced by various means. All the agents which possess the ability of arresting cell division at the metaphase stage are called *stathmokinetic agents*. Fig. 1 shows a growing leukemic cell population, which was exposed for a period of 12 hours, to the action of a stathmokinetic agent (*vincristine*): a great number of arrested metaphases, have progressively accumulated because of the mitotic block caused by the stathmokinetic agent. Post-metaphase figures are absent because no mitosis was able to progress further than the metaphase stage.

The metaphasic arrest of cell division is, generally, the result of inactivation or destruction of the mitotic spindle.

Every mitotic cycle builds up anew the spindle fibers. The formation of the spindle, in a generalized animal cell, can be schematized as follows: the centrioles separate and migrate to the opposite poles of the prophasic nucleus; between the centrioles then appears an array of oriented fibres which represent the spindle. Cytologists distinguish two sets of fibres in the spindle (Fig. 2): 1) The *continuous fibres* (also called exterior or centrosomic spindle) which appear to run from pole to pole, and 2) the *chromosomal fibres* (also indicated as interior or centromeric spindle) which are attached to the chromosomes and run from the chromosome centromere to each pole. Radiating out from each centriole into the cytoplasm are more fibres forming the aster and known as astral rays.

Our knowledge of the mechanism of action of the spindle fibres is rather incomplete. It is generally accepted, however, that the chromosomes change their position as the result of a co-ordinated activity of the spindle fibres and the centromeres (Bayer, 1961).

The movement factors which produce the separation of daughter chromosomes during anaphase and telophase can be schematized according to Östergren *et al.* (1960) as follows:

1. A movement of the centromeres on the spindle toward the poles (pulling force? pumping activity?).

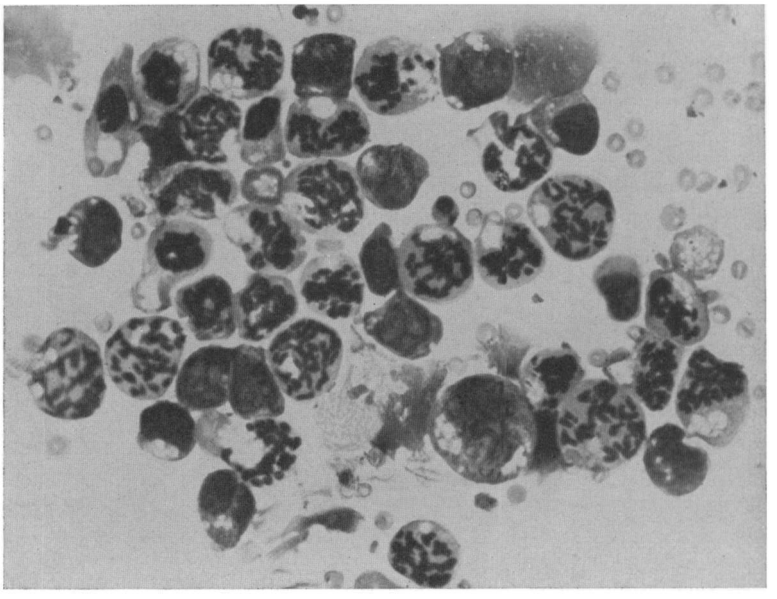

Fig. 1. Dividing leukemic cells (L1210 leukemia) arrested at the metaphase stage by the effect of vincristine

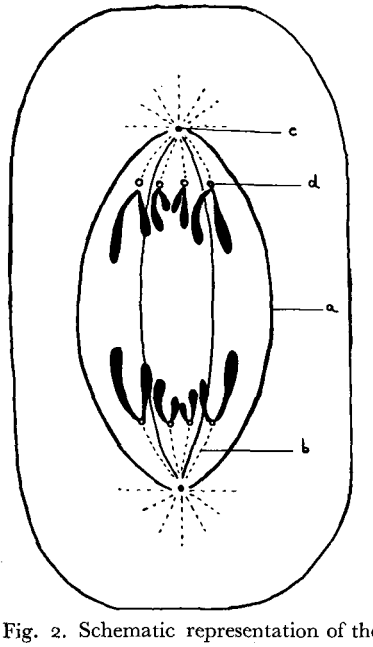

Fig. 2. Schematic representation of the mitotic spindle
a) Continuons fibre; *b*) chromosome fibre; *c*) centriole; *d*) centromere

2. A stretching *of the spindle* that increases the distance between the two poles, contributing to the separation of the daughter chromosomes (pushing body action?).

3. A pushing effect exerted (during telophase) by the phragmoplast on equatorially directed chromosome arms, moving them in the two opposite poleward directions.

4. A strong contraction of the chromosomes, at the end of metaphase, which contributes in pulling equatorially directed arms closer to the polar groups.

The 1st listed movement was considered to be a consequence of a pulling force exerted by the chromosomal fibres of the spindle. Östergren, however, has produced evidence against the idea of a simple elastic pull. According to this Author the movement of the centromeres on the spindle is due to *a special transporting mechanism working on the spindle substance.* There is a kind of shifting or sliding force which moves the chromosomal spindle fibres relative to the background material of the spindle. The mechanism causing this effect is called by Östergren the *pumping activity* of the spindle.

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The second movement factor listed is a *stretching of the spindle* during metaphase. This increase in lenght of the whole spindle is usually decribed as the *pushing body action* or stemmkörperwirkung (Bêlâr, 1929).

It seems, however, that there is no pushing action in the true meaning of the word (Östergren *et al.*, 1960). The spindle stretching does not act by pushing the chromosomes from behind, but it acts by increasing the distance between the two poles, as emphasized by Ris (1949). The last two movement factors, by moving in the two opposite poleward directions the equatorially directed chromosome arms, reduce the risk that they may form connections between the two daughter nuclei.

The spindle mechanism is so delicately coordinate that it can be easily disturbed by many physical and chemical agents. Colchicine is certainly the best known stathmokinetic agent, but spindle poisons were known long before colchicine. Many physical agents (heat, cold, ultraviolet light, hydrostatic pressure, etc.) can cause metaphase arrest of dividing cells.

As far as chemical agents are concerned, there is a large number of compounds which are known to possess the ability of blocking mitosis at the metaphase stage. No evident relationship has been shown to exist between chemical constitution and stathmokinetic activity.

In spite of the extensive studies conducted on various classes of spindle poisons, we do not know how exactly they react with the spindle. The work conducted with sulphydryl reactants seems to suggest that, at least some compounds, may interact with the SH groups of which the spindle seems to be rich.

Among the first stathmokinetic agents known there is sodium cacodilate. In 1947 Dustin showed that the metaphasic arrest caused by *sodium arsenite* could be reversed by *dimercaptopropanol* or *British-Anti-Lewisite* (BAL). Other sulphydryl reactants such as monoiodiacetate (Gompel, 1952), chloroacetophenone, organic mercury compounds (Sentein, 1957), cadmium chloride (Dustin, 1943) and other heavy metal compounds have been shown to be effective in blocking cell division at the metaphase stage. The observation that dimercaptopropanol itself (Dustin, 1947) and sodium diethyldithiocarbamate (Dustin, 1949) are spindle poisons is, according to Dustin, an other fact suggesting that the thiol groups play an important role in the spindle mechanism (Dustin, 1963).

Experiments conducted with colchicine and its derivatives have given information on the process of spindle inactivation caused by stathmokinetic agents.

Sauaia and Mazia (1961) have shown that *colcemide* attacks first the asters and then progressively disorganizes the spindle. Isolation procedures of the mitotic apparatus from the sea urchin eggs, which were exposed for thirty minutes to the action of colcemide at the concentration of 0.001%, yielded instead of a recognizable spindle, large spherical bodies which possibly corresponded to the hyaline globules reported by Gaulden and Carlson (1951) in grasshopper neuroblasts blocked by colchicine. However, no direct action of colcemide or colchicine on the isolated mitotic apparatus could be observed at any concentration. This finding would suggest that the action of colchicine is indirect, mediated by processes taking place in the intact cell.

It is possible that colchicine-like drugs may interact with enzymatic systems active in the formation and preservation of the integrity of the spindle. Attention has been drawn to a specific ATPase, which seems to be associated with the fibrous components of the mitotic apparatus. However, Chaffee and Mazia (1961) have found that spindles, isolated from dividing cells after a short treatment with colcemide, retain their ATPase activity. Furthermore, colchicine, at concentration of 5×10^{-4}, which is sufficient to arrest mitosis and disrupt the spindle in *vivo*, has no effect at all on the ATPase of the isolated mitotic apparatus in *vitro*. These findings are against the hypotesis that the antimitotic effect of colchicine depends on an inhibition of ATPase.

The discovery of new stathmokinetic agents which possess also an antitumor or antibiotic activity has increased in the last few years the interest in this group of antimitotics. The most important of these new agents are:

1. Some alkaloids extracted from a pantropical plant: *Catharanthus roseus*. The best known of these alkaloids are *vinblastine* and *vincristine*.
2. Some new derivatives of *podophyllotoxin*.
3. *Griseofulvin*.

Both vinblastine and vincristine are effective antitumor agents. Vinblastine is a drug active against the Hodgkin disease. Vincristine is a very useful agent in the treatment of acute leukemia especially in children. Details on the experimental and clinical research on these two compounds will be presented by the other speakers.

As regards podophyllin derivatives, their stathmokinetic activity was well known. Podophyllin is a mixture of various components, of which the most important is podophyllotoxin. Podophyllin, however, is insoluble in water. In the last few years some glucosidique derivatives have been isolated from the rhizome of podophyllum which are hydrosoluble. The most important of such derivatives are the *podophyllic acid ethyl-hydrazide* (SPI-77) and the compound SPG 827 (Fig. 3), which is the benzylidene derivative of the podophyllotoxin glycoside complex isolated from the roots of podophyllum emodi; the principal constituent (ca 90%) is the *Benzylidene-β-D-glucoside of podophyllotoxin* (Stahelin and Cerletti, 1964).

We have studied the effect of SPI-77 on the mitotic activity of normal bone marrow and leukemic cells.

At the dose level of 200 to 300 mg/kg the compound caused a complete metaphase arrest of all dividing cells in the bone marrow of C57BL/6 mice. The number of arrested metaphases increased progressively from ½ hour to 4 hours after the treatment (Tab. 1).

Fig. 4 shows arrested metaphases in a leukemic cell population 16 hours after treatment with the SPI-77. It is of interest that the two compounds SPI-77 and SPG 827 possess some antitumor activity but have little toxic effect on the bone marrow.

Among the antibiotics which have a stathmokinetic activity we just mention the griseofulvin, an antifungal antibiotic isolated from Penicillium griseofulvin (Paget and Walpole, 1958) and the Patulin, isolated from Penicillium patulum (Astaldi, 1965).

We cannot close this paper without mentioning the importance of stathmokinetic

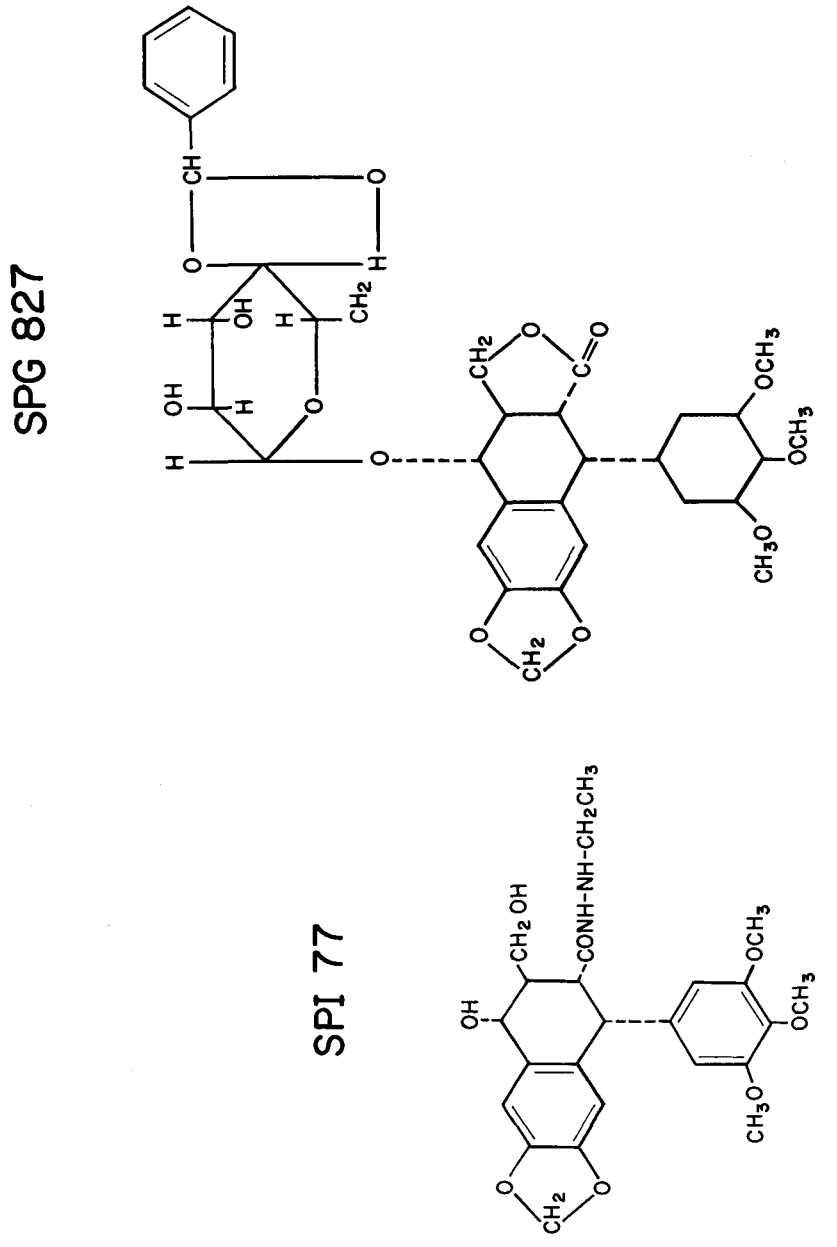

Fig. 3

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Tab. 1. Accumulation of arrested metaphases in C57BL/6 mice 4 hours after I.P. injection of SPI-77

Dosage (mg/kg)	Bone marrow mitotic index (per thousand)
100	20.7
200	117.7
300	122.1
Controls (treated with the vehicle)	12.6
Controls (untreated)	12.0

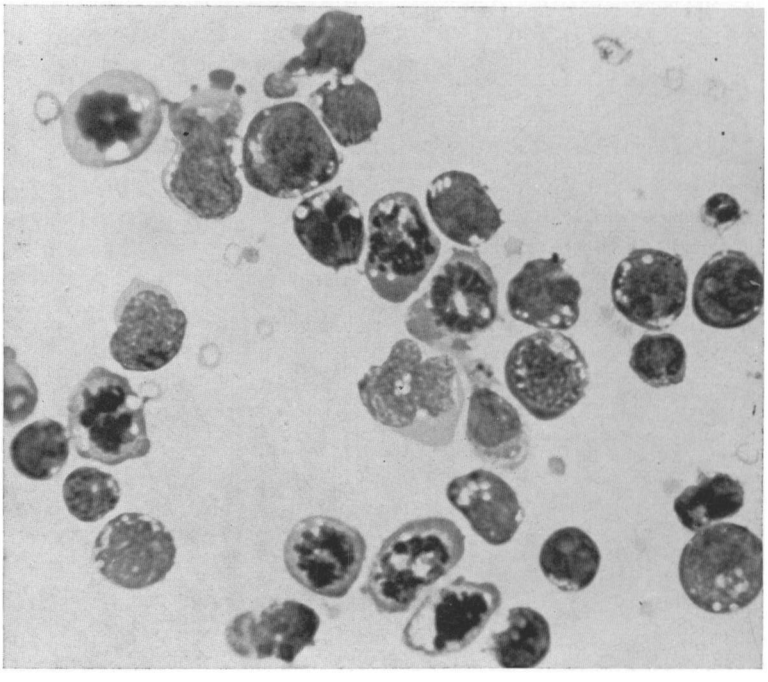

Fig. 4. Dividing leukemic cells (L1210 leukemia) arrested at the metaphase stage by the effect of SPI-77

agents as a tool for investigating some biological problems. One of the most important applications is that of colchicine and vinblastine for the study of cell proliferation. In the last few years colchicine and its derivatives have been also used as tools for chromosomal studies.

In conclusion the stathmokinetic agents are a group of antimitotic agents which present many aspects of interest both to the biologist and the clinician.

Summary

Stathmokinetic agents are a class of antimitotic agents which possess the ability of arresting cell division at the metaphase stage.

They are also called *spindle poisons* because they act by inactivating or distructing the mitotic spindle. Some new discovered stathmokinetic compounds, like vinblastine and vincristine, are effective anti-tumour agents. The mechanism of action of spindle poisons is briefly discussed.

References

ASTALDI G. (1955). Azione antimitotica di alcuni antibiotici. *Atti I Simposio sugli Antimitotici*, 271-287.

BAYER A. (1961). Note on the behaviour of spindle fibres at mitosis. *Chromosoma*, 12: 64-71.

BÊLÂR K. (1929). Beiträge zue Kausalanalyse der Mitose. II Untersuchungen an den Spermatocyten von Chrthippus (Stenobothrus) lineatus Panz. *Arch. Entwicklungsmech.*, 118: 359.

CHAFFEE and MAZIA, *cit.* by Sauaia H. and Mazia D.

DUSTIN P. jr. (1947). Some new aspects of mitotic poisoning. *Nature*, 159: 794-97.

— (1949). Mitotic Poisoning at Metaphase and -SH proteins. *Exp. Cell Res. Suppl.*, **1**: 153-155.

— (1963). New aspects of the pharmacology of antimitotic agents. *Pharm.*, 15: 449-480.

GAULDEN M. E., CARLSON J. G. (1951). Cytological effects of colchicine on the grasshopper neuroblast *in vitro* with special reference to the origin of the spindle. *Exp. Cell Res.*, 2: 416-433.

GOMPEL C. (1952). Sur l'inactivation du fuseau chez la souris par les substances thioloprives. *Rev. Belge Path.*, 22: 85-92.

ÖSTERGREN G. *et al.* (1960). An interpretation of transport phenomena at mitosis. *Ann. N. Y. Acad. Sci.*, 90: 381-408.

PAGET G. E., WALPOLE A. L. (1958). Some cytological effects of griseofulvin. *Nature*, 182: 1320-1321.

RIS H. (1949). The anaphase movement of chromosomes in the spermatocytes of Grasshopper. *Biol. Bull.*, 96: 90.

SAUAIA H., MAZIA D. (1961). Action of colchicine on the mitotic apparatus. *Path. Biol.*, 9: 473-476.

SENTEIN P. (1957). Action antimototique de l'Ethylmercurithiosalicylate de sodium. *Acta Anat.*, 30: 787-801.

STAHELIN H., CERLETTI A. (1964). Experimentelle Ergebnisse mit den Podophyllum-Cytostatica SP-I and SP-G. *Schweiz. Med. Wschr.*, 94: 1490-1502.

RIASSUNTO

Gli *agenti statmocinetici* costituiscono una classe di antimitotici che hanno in comune la proprietà di arrestare la divisione cellulare in metafase. Essi sono anche chiamati veleni del fuso perché agiscono inibendo la formazione del fuso mitotico. Alcuni nuovi agenti statmocinetici, quali ad esempio la vinblastina e la vincristina, possiedono una marcata attività antitumorale. Il meccanismo d'azione degli agenti statmocinetici viene discusso dagli autori.

RÉSUMÉ

Les agents stathmokinétiques constituent une classe d'antimitotiques arrêtant la division cellulaire en métaphase. Ils sont aussi appelés « poisons du fuseau », à cause de leur action inhibitrice de la formation du fuseau mitotique. Quelques nouveaux agents stathmokinétiques, tels que la vinblastine et la vincristine, par example, possèdent une remarquable activité antitumorale. Le mécanisme d'action des agents stathmokinétiques est discuté.

ZUSAMMENFASSUNG

Die statmokinetischen Wirkstoffe stellen eine Art der antimitotischen Präparate dar, welche die Eigenschaft gemeinsam haben, die Zellteilung in der Metaphase anzuhalten. Man nennt sie auch « Spindelgifte », denn ihre Aktion besteht in der Inhibition der Mitosespindel. Einige neue statmokinetische Wirkstoffe, wie z. B. Vinblastina und Vincristina, besitzen eine ausgesprochene antitumorale Wirkung. Die Verf. besprechen den Wirkungsmechanismus der statmokinetischen Wirkstoffe.

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