Meiner Assistentin Frl. L. RIESTERER danke ich für ihre wertvolle Mitarbeit.

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Summary

Based on former experiments it is shown that the venous pulse on the isolated vein of the membrane of *Chiroptera* (preparation of the venous sack) is caused by a humoral factor, which is isolated in the serum dialysate of bats and other mammals as L-*arginine*. This amino-acid is responsible for the increasing of amplitude, tonus, and frequency.

Antagonism between Tropolone and Colchicine in Rat Fibroblast Cultures

In extension of previous work on the mechanism of the metaphase arrest by colchicine and on agents antagonizing this effect in cultures of rat fibroblasts[1], we should like to present here a report on the influence of the simplest structural analogue of colchicine, namely tropolone (2-hydroxy-2,4,6-cyclo-heptatrien-1-one)[2], on this system.

In an earlier study it was established that three cycloheptene derivatives, tropolone, its methylether and 4,5-tetramethylene tropolone, did not appear to act as spindle poisons and, in contrast to colchicine, failed to produce any significant metaphase arrest[3]. Further analysis of these observations suggested that tropolone influenced the mitotic phase percentage in a direction opposite to the colchicine effect, and tests were run to determine the action of these two compounds, when applied simultaneously.

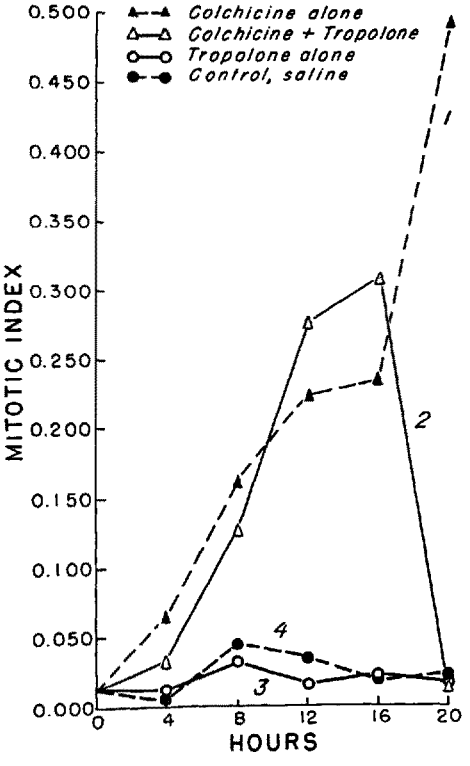
The results indicate that tropolone is a very potent antagonist of colchicine: it favors the formation of the spindle and reverses the metaphase arrest produced by colchicine. This action of tropolone takes effect quite suddenly in dividing rat fibroblasts after 16 h of exposure to the two agents. At this point in the cycle, tropolone, if applied alone, reduces the percentage of metaphase figures and raises the percentage of both pre- and post-metaphase figures, as compared with the untreated cultures. These findings are of special interest because of the structural relationship between colchicine and tropolone.

Fibroblasts, obtained from the subcutaneous areolar connective tissue of 3 to 4 months old male rats, were carried as stock cultures in roller tubes for 10 days and then transferred to MAXIMOW slides, where they were allowed to grow for 5 days before the experiments were begun. The slides for the various experimental groups were carefully matched to insure uniformity of tissue and equal distribution of explants from the different tubes. The experimental series consisted of 4 groups containing:

(1) 2×10^{-6} M colchicine;
(2) 2×10^{-6} M colchicine + 10^{-4} M tropolone;
(3) 10^{-4} M tropolone;
(4) saline (control).

The feeding mixture used in the control experiments (group No. 4) consisted of one volume of human placental serum, one volume of beef serum ultrafiltrate, one volume of chick embryonic extract, and two volumes of SIMMS' balanced saline solution. The latter served, in the experimental groups 1–3, as the solvent of colchicine, tropolone or both. These agents were introduced in quantities sufficient to give the effective final concentrations listed in the Table.

In our previous work on the reversal of the colchicine effect[1] it was noted that the metaphase arrest reached its peak, in terms of the mitotic index, and leveled off, at 20 h after application of the agent. When *meso*-inositol was included at the same time with colchicine, the rising curve broke sharply at 12 h, and by 20 h had descended to a level close to that of the controls. A similar arrangement was followed in the present study involving colchicine and tropolone, whose results are represented graphically in the Figure; one-fifth of the cultures in each group was, at 4 h intervals up to 20 h, fixed in HELLY's fluid. These were later stained with HARRIS' haematoxylin, and cell-counts were made according to the method previously described[1].



Mitotic indices, determined at 4-hour intervals, of rat fibroblast cultures exposed to colchicine and tropolone. (Compare with the Table).

As shown in the Figure, the mitotic index of the group receiving colchicine plus tropolone (No. 2) rises above that of the group receiving colchicine alone (No. 1) between 8 and 16 h after exposure, but descends rapidly thereafter to a point below that of the controls (No. 4), at 20 h. An analysis of the phase percentages of the 4 groups at 20 h (Table) shows that the presence of tropolone has reduced the percentage of metaphases to 72, a figure half way between 99% for the colchicine group,

[1] M. R. MURRAY, H. H. DE LAM (BENITEZ), and E. CHARGAFF, Exper. Cell. Res. *2*, 165 (1951).

[2] We are indebted to Prof. W. v. E. DOERING, Yale University, for this substance.

[3] H. BENITEZ, M. R. MURRAY, and E. CHARGAFF, Anat. Rec. *112*, 309 (1952).

[1] M. R. MURRAY, H. H. DE LAM (BENITEZ), and E. CHARGAFF, Exper. Cell. Res. *2*, 165 (1951).

Cell counts, mitotic indices and phase percentages

Group No.	Agent	Duration of exposure	Total cells	Total mitoses	Mitotic index	Phase percentage			
						Prophase	Metaphase	Anaphase	Telophase
		hours				%	%	%	%
1	Colchicine	16	12,432	2,982	0·240	3·2	95·2	0·6	0·9
		20	13,304	6,606	0·496	0·6	98·9	0·1	0·3
2	Colchicine + tropolone . .	16	15,822	4,922	0·311	3·4	95·6	0·7	0·3
		20	13,384	166	0·012	0·6	72·3	6·0	21·1
3	Tropolone	16	8,416	172	0·020	10·5	31·4	12·8	45·3
		20	12,853	310	0·024	4·5	44·5	13·2	37·7
4	Control	16	10,342	230	0·022	2·6	48·2	16·5	32·6
		20	7,248	144	0·020	2·1	50·0	9·0	38·9

and 50% for the controls. A striking shift is also registered in anaphase and telophase figures. In fixed preparations of cultures exposed to tropolone alone, the spindle is more frequently and more clearly visible than it is in the controls. In the colchicine preparations it is rarely if ever discernible. These and related observations, which will be presented elsewhere in more extended form, suggest that tropolone is able to shorten the duration of the metaphase, perhaps by exerting an influence on factors that govern the formation of the spindle.

The only agent discovered heretofore capable of blocking the action of colchicine has been *meso*-inositol[1]. The experiments presented here show tropolone to be even more effective. Though it is unknown whether seven-membered ring compounds occur in animal cells and whether the similarity in effect of tropolone and inositol is more than accidental, it is not unattractive to ascribe the action of colchicine in producing metaphase arrest to its functioning as an antimetabolite in a chain of reactions required for nuclear division.

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Résumé

Il a été démontré ici que la tropolone, analogue simple de la colchicine, est capable d'agir comme antagoniste puissant de celle-ci. Dans une culture de fibroblastes du rat soumise à l'action d'un mélange de ces deux agents, cette action – consistant dans la disparition soudaine de l'arrêt métaphasique produit par la colchicine – commence à se manifester après 16 h.

[1] M. R. MURRAY, H. H. DE LAM (BENITEZ), and E. CHARGAFF, Exper. Cell. Res. *2*, 165 (1951).

Antagonistes de l'iodure de décaméthonium à la jonction neuro-musculaire

La Prostigmine accentue l'inhibition de la transmission neuro-musculaire due à l'iodure de décaméthonium et l'ésérine est inefficace, contrairement à ce qu'on observe pour le tubocurare. Les principaux antagonistes connus du composé dont la propriété est de dépolariser la membrane musculaire sont l'iodure de pentaméthonium[1] et le tubocurare. Dans ce dernier cas, l'antagonisme est réciproque[2].

Nous avons constaté que toute une série de substances appartenant à des séries chimiques diverses étaient capables, dans une mesure plus ou moins marquée, de réduire le bloc par dépolarisation provoqué par l'iodure de décaméthonium. C'est le cas pour la Paludrine, le chlorhydrate de bis-(2 heptyl)amine (1637 L), le chlorhydrate d'une butylamine substituée (1745 L), la Stilbamidine, la Pentamidine, le 48–80 et le Tween 20.

Parmi ces composés, la Paludrine est elle-même un inhibiteur faiblement actif de la transmission neuro-musculaire[3]; son influence qui accuse un caractère cumulatif très marqué, est fortement accentuée tant par l'intervention préalable du tubocurare que par celle de l'iodure de décaméthonium. Elle ajoute ses effets à ceux du premier et se montre antagoniste du deuxième quand elle est administrée (8 mg de base/kg) pendant que la transmission est entravée par le dépolarisant. Son action inhibitrice propre n'est pas modifiée par l'ésérine.

La bis-(2 heptyl)amine (1637 Labaz) qui a fait l'objet d'une étude pharmacologique de PHILIPPOT[4] est antagoniste de l'iodure de décaméthonium et elle réduit nettement la sensibilité du chat à ce composé. Elle additionne ses effets à ceux du tubocurare; quand l'animal a reçu une injection de ce dernier, elle se montre à son tour curarimimétique. Après action de la bis-(2 heptyl)-amine, celle du décaméthonium chez le chat est levée par l'ésérine et par l'adrénaline. Ce composé paraît donc intervenir au niveau des plaques motrices du chat pour empêcher le développement de la dépolarisation: chez le chat soumis à son influence, l'iodure de décaméthonium devient compétitif comme le tubocurare.

Le 1745 Labaz, dont les propriétés hypotensives ont fait l'objet d'une étude préliminaire effectuée par CHARLIER[5] possède une action tout à fait semblable (1 mg/kg); cependant, il exerce plus difficilement une influence inhibitrice par lui-même. Il n'empêche toutefois pas l'amyl-triméthylammonium, autre composé

[1] W. D. M. PATON et E. J. ZAIMIS, Brit. J. Pharmacol. *4*, 381 (1949).

[2] M. J. DALLEMAGNE et E. PHILIPPOT, Brit. J. Pharmacol. *7*, 601 (1952). – O. F. HUTTER et J. E. PASCOE, Brit. J. Pharmacol. *6*, 691 (1951).

[3] J. R. VANE, Brit. J. Pharmacol. *4*, 14 (1949).

[4] E. PHILIPPOT, J. Pharm. Belge *7*, 202 (1952).

[5] R. CHARLIER, Arch. Int. Pharmacodyn. (sous presse).