Brit. J. Pharmacol. (1949), **4**, 105.

ANTIMITOTIC ACTION OF MALEIMIDE AND RELATED SUBSTANCES

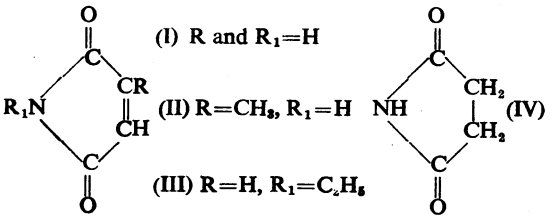
BY

E. FRIEDMANN, D. H. MARRIAN, AND (MRS.) I. SIMON-REUSS

From the Department of Radiotherapeutics, University of Cambridge

(Received October 2, 1948)

The results obtained by us (1948a) with maleic acid as an inhibitor of mitosis have been developed in an attempt to prepare other mitotic inhibitors related to maleic acid; in particular we have investigated the antimitotic activity of substances in which the maleic acid residue is part of an aliphatic ring. The imides of maleic acid (I) and of citraconic acid (II), N-ethylmaleimide (III), and in addition succinimide (IV) were chosen for this purpose.



The action of these substances on the growth of normal cells is reported in this paper.

METHODS

The experiments were carried out on tissue cultures of chick fibroblasts. The technique adopted by us has been described previously (1948a). The *hanging drop method* was used in all experiments except some with maleimide, in which the Carrel flask method was adopted in combination with the Kodak record film technique.

Carrel flask technique.—By this technique the tissues are grown in a solid coagulum of blood plasma at the bottom of a small flask. The embryo extract containing the compound is added as soon as the plasma has clotted.

Cine micrographical record.—A picture was taken every sixth minute on a Kodak recording film. The mitotic index was assessed by counting the mitoses occurring in each photograph and by making a total cell count at the end of every tenth hour. The percentage of hourly mitoses was calculated in terms of the number of cells present. Details of this technique have been described by Willmer and Jacoby (1936).

EXPERIMENTAL

Mitotic Disturbances

The values for mitotic inhibition and phase distribution obtained with compounds I, II, III, and IV are collected in the Table.

Maleimide (I; R and R_1 = H)

Maleimide has weak antimitotic activity. At $5 \times 10^{-6}M$ an inhibition of 21 per cent was observed. By raising the concentration to $9 \times 10^{-6}M$ no further increase of the inhibition has been observed.

At lower concentrations of maleimide 1×10^{-6} M (Table) and occasionally at $3 \times 10^{-6}M$, an increase in the mitotic count occurred after 1.5, 3, and 24 hours (Fig. 1). A number of experiments was performed to ascertain the nature of

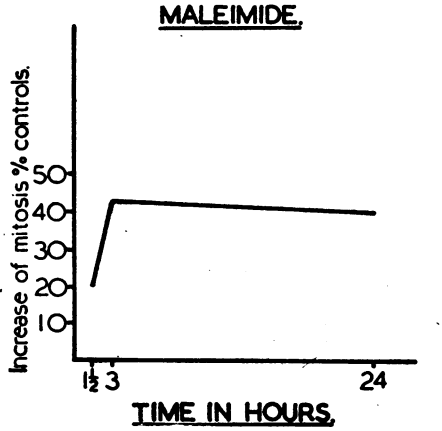


FIG. 1.—Increase in mitotic count with $3 \times 10^{-6}M$ maleimide at 1.5, 3, and 24 hours.

TABLE

TISSUE CULTURES: CHICKEN FIBROBLASTS, HANGING DROP METHOD, 4TH PASSAGE, 24-HOUR CULTURES, FIXED IN SUSA, STAINED IN HEIDENHAIN'S HAEMATOXYLIN

Exp.	Molar conc.	Mitoses as % of mitoses of controls	Per cent inhibition	Phase distribution in % of mitoses							
				Prophase		Metaphase		Anaphase		Telophase	
MALEIMIDE (9,619 mitotic cells investigated)											
1	Controls	—	—	15.4		47.2		6.2		31.2	
2	1×10^{-6}	128.4 ± 7.9%	0		14.0		34.0		6.8		45.2
3	3×10^{-6}	99.0 ± 5.4%	0		19.0		25.0		3.0		53.6
4	Controls	—	—	12.7		39.1		5.7		42.5	
5	5×10^{-6}	79.0 ± 6.1%	21.0		22.2		29.3		2.1		46.4
6	7×10^{-6}	76.7 ± 8.2%	23.3		16.8		30.3		4.9		48.0
7	9×10^{-6}	79.0 ± 4.8%	21.0		28.3		28.9		4.9		37.9
8	Controls	—	—	16.8		30.9		3.6		48.7	
9	3×10^{-6}	139.0 ± 1.3%	0		17.2		22.0		3.4		57.4
CITRACONIMIDE (4,634 mitotic cells investigated)											
1	Controls	—	—	23.4		20.8		4.3		51.5	
2	3×10^{-6}	38.2 ± 2.8%	61.8		19.5		31.6		4.5		44.4
3	Contro s	—	—	10.0		45.6		6.5		37.9	
4	1×10^{-6}	66.7 ± 2.1%	33.3		6.3		34.9		13.6		45.2
5	6×10^{-7}	80.6 ± 3.2%	19.4		14.1		43.1		6.8		36.0
6	4×10^{-7}	89.8 ± 4.9%	10.2		7.5		42.8		9.4		40.3
N-ETHYLMALEIMIDE (5,070 mitotic cells investigated)											
1	Controls	—	—	22.1		31.8		1.6		44.5	
2	4×10^{-7}	82.0 ± 2.9%	18.0		16.3		36.1		5.1		42.5
3	6×10^{-7}	72.7 ± 3.7%	27.3		9.1		39.5		11.3		40.1
4	1×10^{-6}	67.9 ± 3.0%	32.1		19.5		34.9		5.9		39.7
5	Controls	—	—	12.2		40.8		8.6		38.4	
6	2×10^{-6}	49.2 ± 2.4%	50.8		10.7		45.4		1.6		42.3
7	4×10^{-6}	52.9 ± 1.9%	47.1		10.5		43.2		5.2		41.1
8	6×10^{-6}	47.9 ± 2.3%	52.1		16.1		41.1		3.7		39.1
SUCCINIMIDE (5,373 mitotic cells investigated)											
1	Controls	—	—	18.8		27.0		6.2		48.0	
2	3×10^{-6}	97.6 ± 3.5%	2.4		19.1		30.2		6.1		44.6
3	Controls	—	—	15.6		27.1		3.4		53.9	
4	5×10^{-6}	84.3 ± 3.4%	15.7		17.8		41.2		2.6		38.4
5	7×10^{-6}	96.5 ± 3.0%	3.5		15.0		41.0		2.1		41.9
6	9×10^{-6}	93.6 ± 2.1%	6.4		17.3		40.0		1.8		40.9

this increase. The Carrel flask method in combination with the Kodak record film technique made it possible to count the mitoses hourly in unstained preparations, whereas in the hanging drop method stained cells are counted only after 24 hours. The film showed that some cells remained in metaphase for 8–12 hours and that other cells never completed cell division. In the 24-hour period used in the hanging drop method, therefore, not only the cells which are going into division are counted but in addition those which persist in metaphase. Thus the Kodak record film technique gave clear evidence that the increase of the mitotic count after 24 hours consisted in an accumulation of metaphases.

Abnormal cells were found at all concentrations; they were confined mostly to clumped metaphases. Enlarged and vacuolated cells were

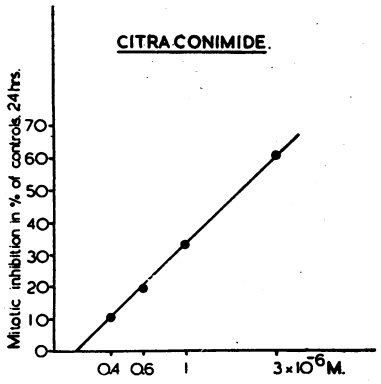


FIG. 2.—Mitotic inhibition plotted as percentage of controls against the logarithm of the concentration of citraconimide.

present. At $1 \times 10^{-6}M$, the lowest concentration investigated, a few tripolar cells (3 in 470 metaphases), fragmented chromosomes, and chromosome bridges (10 in 142 anaphases) were observed.

Citraconimide (II ; R = CH_3, R_1 = H)

Citraconimide had the strongest antimitotic properties of the substances of this group. The mitotic inhibition increased with rising concentration. At $3 \times 10^{-6}M$ the inhibition was 61.8 per cent. The increase followed closely a logarithmic graph when plotted against the $\log_{10}$ of the concentrations (Fig. 2). Microscopically no abnormalities were found in significant quantities.

N-ethylmaleimide (III ; R = H, $R_1 = C_2H_5$)

N-ethylmaleimide showed increasing antimitotic activity from $0.4 \times 10^{-6}M$ to $2 \times 10^{-6}M$; at the latter concentration 50.8 per cent inhibition was observed. A further rise in concentration did not lead to greater inhibition. Microscopically, no abnormalities in significant numbers are present.

Succinimide (IV)

Succinimide showed no mitotic inhibition and no abnormal cells were observed.

Sulphydryl Uptake

In a previous paper (1948b) we drew attention to a parallelism between mitotic inhibition and –SH uptake, which was clearly evident in the maleic acid series. In order to ascertain whether a similar parallelism could be established in the maleimide series, we investigated the –SH uptake of maleimide, citraconimide, and of N-ethylmaleimide, using thiolacetic acid and glutathione as –SH donors and following the directions given by Morgan and Friedmann (1938) for maleic acid and these two –SH compounds.

At room temperature and at a final concentration of $M/50$ for each component, maleimide took up 100 per cent of thiolacetic acid and 100 per cent of glutathione within 2 min. Citraconimide took up 70 per cent of thiolacetic acid and 75 per cent of glutathione within 3 min. After this quick uptake the reaction slowed down and the curves representing it become asymptotic. N-ethylmaleimide behaved like maleimide and showed 100 per cent –SH uptake from thiolacetic acid and glutathione within 1 min.

Fig. 3 demonstrates the –SH uptake from glutathione as –SH donor with maleimide and citraconimide as –SH acceptors at room temperature.

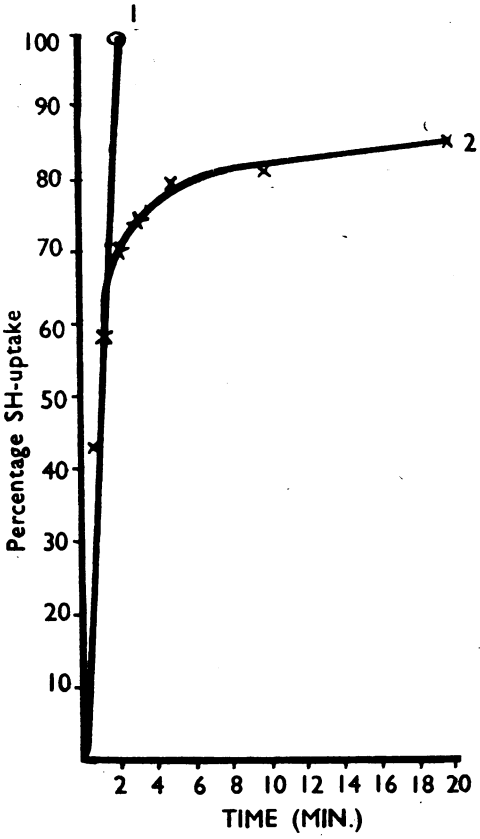


FIG. 3.—SH uptake of maleimide and citraconimide. Curve 1: maleimide and glutathione, final concentration $M/50$. Curve 2: citraconimide and glutathione, final concentration $M/50$.

Discussion

Of the four substances investigated, three are unsaturated and only one, succinimide, is saturated. The three unsaturated substances, maleimide, citraconimide, and N-ethylmaleimide, have antimitotic properties, whereas the saturated compound, succinimide, is devoid of antimitotic activity. These mitotic inhibitors share with the other mitotic inhibitors, encountered previously—i.e., maleic acid and the 1 : 4-naphthoquinones —the property of forming –SH adducts.

The –SH uptake of the maleimides is extremely rapid compared with the –SH addition to maleic acid: $M/25$ maleic acid needs 6 hours for a 50 per cent –SH uptake from $M/50$ thiolacetic acid and $M/50$ glutathione, whereas maleimide and N-ethylmaleimide accomplish a 100 per cent –SH uptake from the same substances in 1–2 min. and citraconimide a corresponding uptake of 70 per cent in 3 min. It will be seen that the introduction of a methyl group in maleimide decreases the –SH uptake as shown by citraconimide.

The mitotic inhibition of the imides exhibits a picture which is very different from the mitotic inhibition observed in the maleic acid group. Maleimide is less active than maleic acid, whereas citraconimide is quite active, although the free acid was inactive. The readiness of the maleimides to add sulphydryl compounds and the presence of glutathione in the medium used for the tissue cultures (Berger and Peters, 1933) may show the way for a reasonable explanation of these results. Experiments in this direction are in progress.

Summary

1. The antimitotic activity of maleimide (I), citraconimide (II), N-ethylmaleimide (III), and of succinimide (IV) has been tested in tissue cultures of chick fibroblast. The unsaturated imides (I, II, III) were active in the concentration range of $10^{-6}M$. The highest activity was shown by III, the methyl derivative of maleimide. The saturated imide (IV) was inactive.

2. The –SH uptake of the unsaturated imides has been determined. The introduction of a methyl group in maleimide decreases its reactivity towards –SH compounds.

One of us (E. F.) is indebted to Messrs. May and Baker, Ltd., Dagenham, Essex, for financial support.

References

Berger, G., and Peters, T. (1933). *Hoppe-Seyl. Z.*, **214**, 91.
Friedmann, E., Marrian, D. H., and Simon-Reuss, I. (1948a). *Brit. J. Pharmacol.*, **3**, 263.
Friedmann, E., Marrian, D. H., and Simon-Reuss, I. (1948b). *Brit. J. Pharmacol.*, **3**, 335.
Morgan, E. J., and Friedmann, E. (1938). *Biochem. J.*, **32**, 733.
Willmer, E. N., and Jacoby, F. (1936). *J. exp. Biol.*, **13**, 237.