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Over-additive Increase of Bacterial Mutations by Combined Action of Ultraviolet Light and Alkylation

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Over-additive increase of bacterial mutations by combined action of ultraviolet light and alkylation

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Abstract

Mutagenic agents added in combination may contribute to an overall biological effect in proportion to the effect they would have if given individually. The combined effect may, however, not just be additive but rather result in a response above or below expectation if the two mutagenic pathways interacted at some level. We report here on one such example. Ultraviolet light and subsequent treatment with the alkylating agent ethylmethanesulfonate (EMS) led to an over-additive increase of bacterial mutations. This is interesting with respect to unravelling the level mutagens interact. In addition such data may relate to the human situation which is only in the process of being assessed.

Überadditive Zunahme von Bakterienmutanten nach kombinierter Wirkung von ultraviolettem Licht und Alkylierung

Zusammenfassung

Mutagene Agenzien (Strahlung, Chemikalien) können bei kombinierter Einwirkung auf die biologische Zelle zu einem von der Additivität der Einzelwirkungen abweichenden biologischen Gesamteffekt führen. Dieser Effekt kann oberhalb (Synergismus) oder unterhalb (Antagonismus) der addierten Einzeleffekte liegen. In der vorliegenden Arbeit wird ein solches Beispiel an Colibakterien beschrieben. Ultraviolettes Licht und nachfolgende Behandlung mit alkylierender Äthylmethansulfonsäure (EMS) führt zu einem überadditiven Anstieg der Zahl von Mutanten. Diese Beobachtung verdient im Hinblick auf die Aufklärung der verschiedenen mutagenen Mechanismen der einzelnen Agenzien ein besonderes Interesse. Darüber hinaus können solche Kenntnisse zum besseren Verständnis vergleichbarer Situationen beim Menschen beitragen.

Results and Discussion

Survival and mutation rate of E.coli mutants after treatment with mutagens individually

According to current knowledge, the two mutagenic treatments selected (UV and alkylation) cause different primary lesions in the DNA (Setlow and Carrier 1964; Roberts 1978). The repair of these lesions seems to depend on different enzymes and the generation of mutations by these mutagens also differs (Kimball 1978; Roberts 1978). This is reflected in the survival curves and mutation rates shown in fig. 1 and 2.

While survival of bacteria after UV irradiation depended strongly on the presence of the rec-lex enzymes and uvrA, only the uvrA deficiency increased the mutation rate since rec + lex are required to induce mutations (Witkin 1969; Witkin and Wermundsen 1977, 1979). EMS treatment however led to more

mutants in uvrA and recA defective strains than in wildtype. The lexA mutant showed a slightly decreased rate compared to the wildtype. Survival and mutation curves suggest differences in the mutagenic pathways: While UV-induced mutagenesis depended on the presence and activity of recA, EMS did not require recA in order to induce mutations. UV is known to induce the expression of recA. EMS does not cause recA expression. On the other hand seem both mutagenic lesions, by either UV or EMS, to depend on uvrA and polA for repair. This may be a step of interaction relevant to combined mutagenic treatment.

Combined action of ultraviolet light and EMS caused above-additive number of mutations

In order to cover a wider range of dose-responses, we used two mutagenesis tests: the fluctuation test (Green and Muriel 1976) and a liquid incubation test (Mitchel 1978). From fig. 1 and 2, it is clear that only appropriate mutants could be used in our study. Thus rec-lex strains responded to combined treatment just as to EMS alone. This is expected since the UV induced mechanism of mutagenesis is defective. Wildtype E.coli showed a tendency to an overadditive mutation rate which was not significant with the number of mutants counted (not shown). The uvrA mutation, however, which causes increased sensitivity to both UV and EMS, enhanced strongly the synergistic effect of the combined treatment: fig. 3. For synergism was only achieved by irradiating first and treating with EMS subsequently, and not by treatment in the reverse order, simply the induction of recA could not cause the overadditive effect. This was confirmed by replacing UV by recA inducing doses of nalidixic acid (fig. 4).

The interaction of UV and EMS occurs during DNA repair or during establishment of mutations

We will consider briefly at what stage the interactions between the two mutagenic agents could occur:

i) Before reacting with DNA no interaction is possible since the UV lesions existed at the time of EMS treatment.

ii) EMS may, more effectively, react with guanine residues in the presence of rare pyrimidine dimers e.g. affinity to single strand regions around dimers or at arrested replication forks. This would be supported by the fact that alkylations seem to occur preferentially at the growing point (Cerdá-Olmedo et al. 1968) and that in single stranded nucleic acids the ratio of O-alkylation over N-alkylation is higher than in double stranded nucleic acids (Singer 1978). O-alkylation is considered to be the mutagenic lesion (Lawley and Martin 1975). We consider this the most likely possibility. Alkylation promotes or stabilizes changes of the DNA structure into the Z form (Santella et al. 1981) which may also occur with natural DNA and under physiologic conditions. Since in the order EMS - UV we could not observe the synergism, the assumed structural changes of DNA do not seem to alter the UV induced mutagenic pathway.

iii) Although the repair pathways seem rather different (Roberts 1978) an interaction during repair or establishment of mutation must be considered. This is supported by one of the pioneer investigations in the field of repair processes involved with combination of two physical agents: UV and x-rays (Bridges 1967). Since these agents depend partly on the same repair pathway a synergistic behaviour is not unexpected. In our case both

mutagens induce specific repair parthways: recA is induced by UV (Witkin 1975), while alkylating agents induces an enzyme preferentially removing O^6-alkylguanine (Samson and Cairns 1977; Jeggo et al. 1977; Sklar and Strauss 1980).

We can rule out that EMS led to an overinduction of recA. In the UV induction of λ-dependent galactokinase synthesis, the presence of EMS rather inhibited than increased the action of UV (fig. 5). This may be explained by an inhibition of translation exerted by EMS (Chen 1980) and would probably even add to the magnitude of the synergistic interaction in mutagenesis. By inhibiting protein synthesis, EMS may, however, hinder the induction of some other yet unknown UV induced protein which is involved in mutagenesis in the absence of uvrA. In view however of the higher susceptibility of single stranded regions to undergo alkylation (Singer 1978) one could also imagine that the mechanism of sister strand exchange during post-replication triggered by recA protein could be the source of further mutations. RecA protein initiates strand exchange from a nicked duplex, transferring the 3' OH terminus at the nick into the single stranded region of the gapped DNA leaving a new single stranded gap in the intact duplex (West et al. 1981).

The induction of a O^6-alkylguanine removing enzyme would diminish the rate of mutation by EMS. The relatively low dose of UV cannot have blocked the synthesis significantly. EMS is not an optimal inducer of the alkylguanine removing enzyme (Sklar and Strauss 1980). May be UV induces the enzyme. As a last possibility, EMS may interfer with dimer excision as does NAAF (Ahmed and Setlow 1979, 1980).

Summary

TRP^+ reversion and survival in E.coli WP2 influenced by UV-light, ethylmethanesulfonate (EMS) and by a combined action of both treatments were studied. Comparing repair-deficient mutant strains it was observed that UV and subsequent treatment with EMS led to an over-additive increase of mutations. The possible mechanism of this effect is discussed.

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Fig. 1a UV-inactivation of E.coli WP2 and its repair-deficient derivatives. All strains were trp^- (Bridges et al. 1967). Exponential phase bacteria grown in nutrient broth under aeration were diluted in saline buffer to 2-5x10^7 cells/ml and irradiated with a Hg-low pressure lamp (Quarzlampen GmbH, Hanau FRG) emitting light of 254 nm with a dose rate of 0.06 $Jm^{-2}s$ or 0.02 $Jm^{-2}s$, respectively. 0.1 ml samples were collected at various times, diluted in saline buffer and plated on nutrient agar. All procedures took place under subdued light. Surviving colonies were counted after an incubation period of 15 - 18 hrs at 37°C.

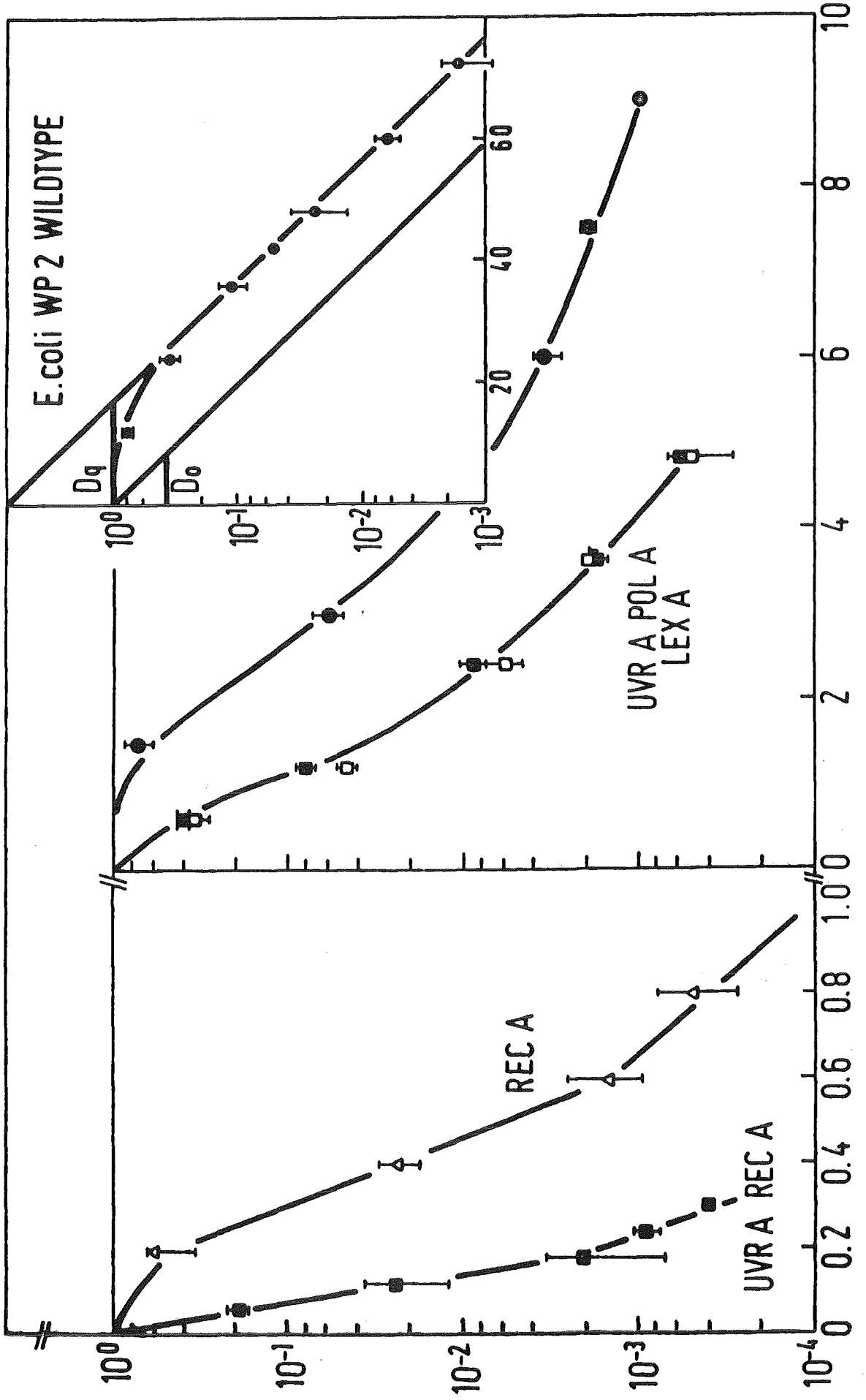
E.coli WP2 WILDTYPE
Dq
Do
UVR A POL A
LEX A
REC A
UVR A REC A
SURVIVING COLONY COUNT
UV-DOSE (J/m²)

Fig. 1a

Fig. 1b Induction of mutations in E.coli WP2 and its repair deficient derivatives after UV irradiation. Mutagenicity was tested in the low dose range (-a-) by a modified fluctuation test (Green and Muriel 1976) and, for higher doses (-b-) by a liquid incubation test (Mitchell 1978). Mutation rates were determined according to the calculation presented by Luria and Delbrück (1943). The data of the fluctuation test and the liquid incubation test were standardized. Results obtained with doses measured in both systems are in good agreement (■ E.coli WP2 uvr A, pol A; ● uvr A).

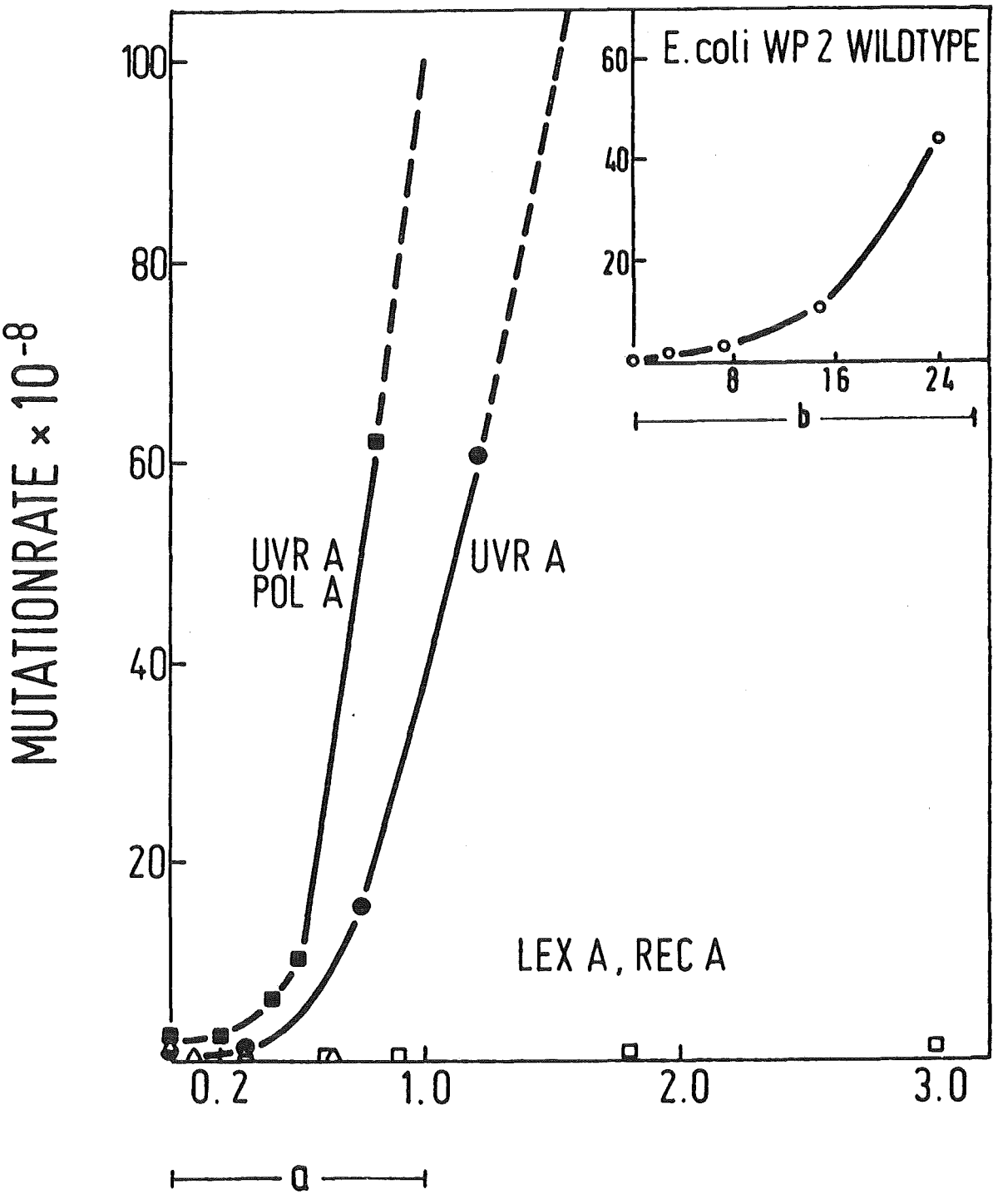
E.coli WP 2 WILDTYPE
MUTATIONRATE × 10⁻⁸
UVR A
POL A
UVR A
LEX A, REC A
UV-DOSE (J/m²)
a
b

Fig. 1b

Fig. 2a EMS-inactivation of E.coli WP2 and its repair-deficient derivatives. Samples of exponential phase bacteria were diluted to $1x10^7$ cells/ml in nutrient broth containing different concentrations of EMS and incubated at 37^oC in a rotory shaker bath. After 2 hours aliquotes were taken from every suspension and treated as described in Fig. 1a.

Fig. 2b Mutation induction of E.coli WP2 and its repair deficient derivatives after treatment with EMS. Mutagenicity was tested by the fluctuation test (10^{-5} - $2x10^{-3}$ M) and by the liquid incubation test (above 10^{-3} M) (see also Fig. 1b).

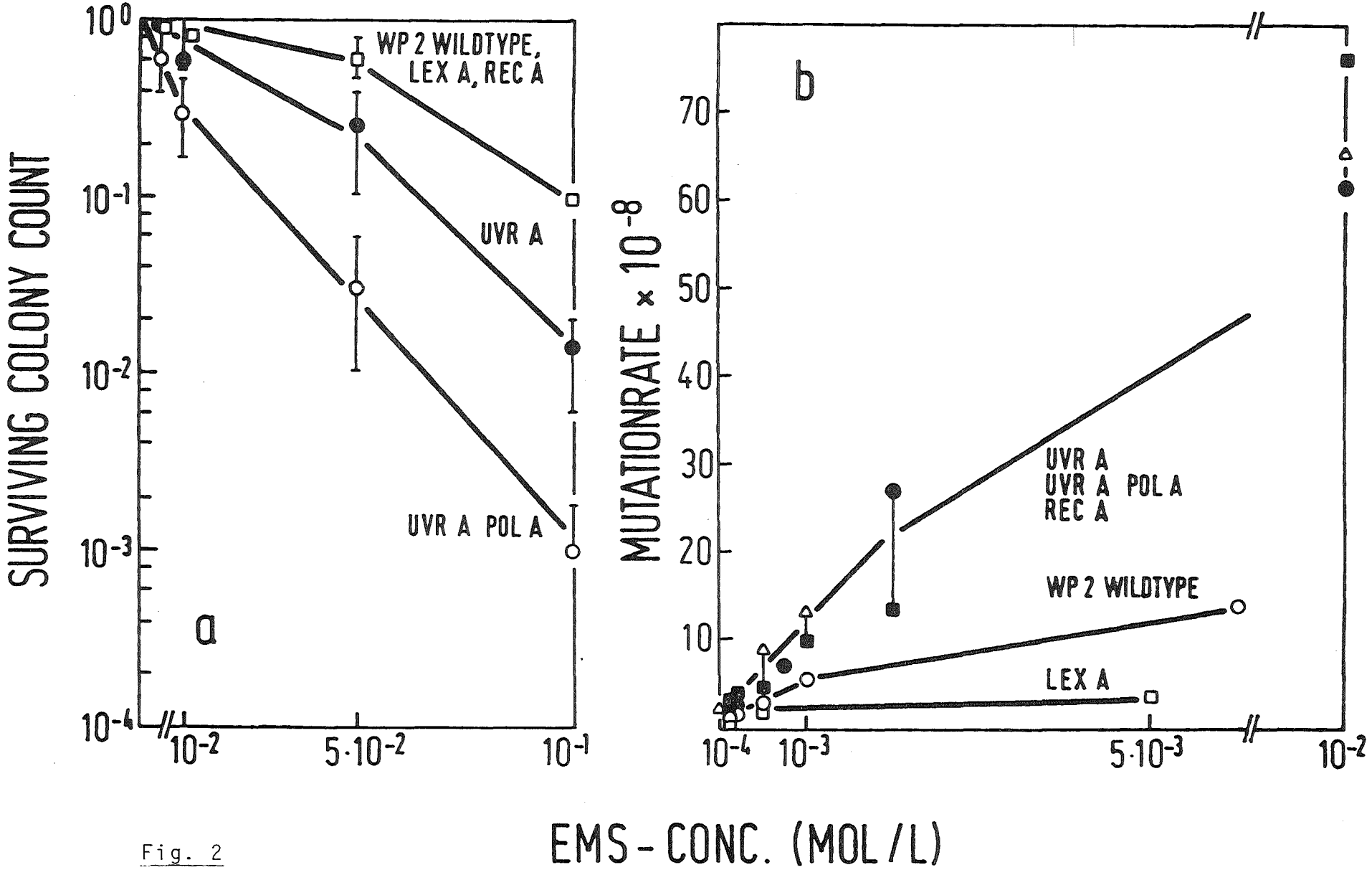
SURVIVING COLONY COUNT
10^0
10^-1
10^-2
10^-3
10^-4
WP 2 WILDTYPE, LEX A, REC A
UVR A
UVR A POL A
a
10^-2
5·10^-2
10^-1
MUTATIONRATE × 10^-8
70
60
50
40
30
20
10
b
UVR A
UVR A POL A
REC A
WP 2 WILDTYPE
LEX A
10^-4
10^-3
5·10^-3
10^-2
EMS - CONC. (MOL/L)

Fig. 2

Fig. 3 Overadditive effect on mutation induction by combined treatment with UV and EMS in E.coli WP2 uvr A.

a) Mutation induction was determined by the liquid incubation test (Mitchell 1978) modified to permit investigation of combined effects. Exponential phase bacteria were contrifuged (3000 g) adjusted to $1x10^8$ cells/ml in saline buffer and irradiated as described in fig. 1a. Samples were diluted 1:10 in incubation medium (Mitchell 1978) containing EMS and kept for 2 hrs in a rotory shaker bath. Suspensions were washed by centrifugation, the pelltes incubated in nutrient broth for another 2 hrs followed by washing and resuspension in saline buffer (10 x conc.) 0.1 ml samples were plated undiluted on selection agar and diluted on nutrient agar. Surviving colonies were counted after 18 hrs, mutant colonies after 40 hrs. Treatment of E.coli WP2 uvrA with UV 1.2 J/m^2 or $5x10^{-2}$ EMS alone resulted in surviving colony counts of 75 % and 40 %, respectively. Combination of treatments reduced surviving fraction to 22 %.

b) The order of treatments was reversed. After 2 hours in EMS, the cells were harvested and irradiated, then plated as above. Significance of the combined effects was calculated by the t-test.

●—● 2 hrs treatment with EMS only

o—o combined treatment with UV (1.2 J/m^2) and EMS 2 hrs

dotted aerea: no significant deviation from additivity of the single effects ($p = 0.05$).

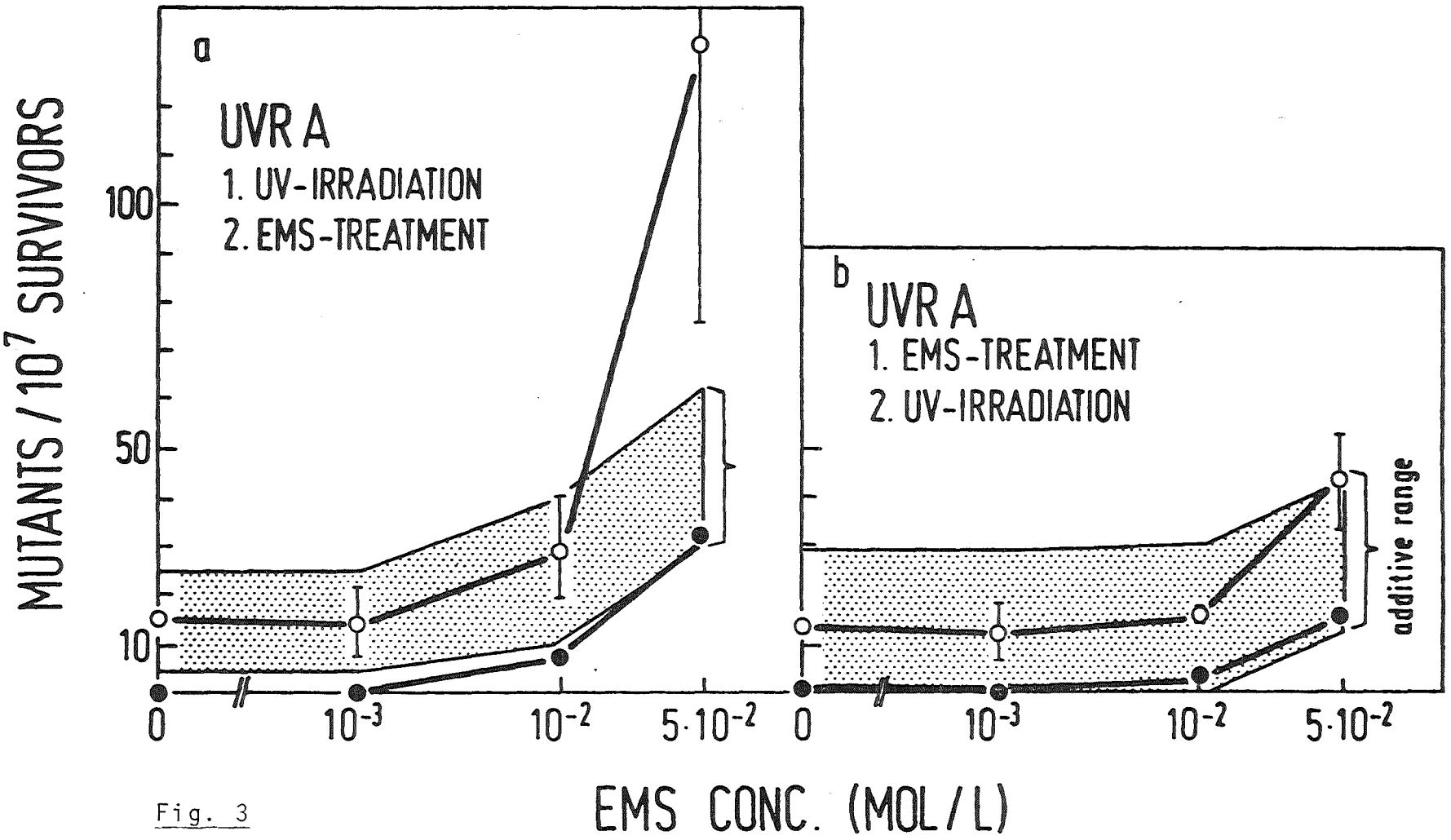
a
UVR A
1. UV-IRRADIATION
2. EMS-TREATMENT
b
UVR A
1. EMS-TREATMENT
2. UV-IRRADIATION
additive range
MUTANTS / 10^7 SURVIVORS
100
50
10
0
10^{-3}
10^{-2}
$5 \cdot 10^{-2}$
EMS CONC. (MOL/L)

Fig. 3

Fig. 4 Mutation induction after combined treatment with EMS and Nalidixic acid.

●—● 2 hrs treatment with EMS only (see Fig. 3a)

o—o combined treatment with EMS and Nalidixic acid: addition of 50 µg/ml Nalidixic acid 30 min before the end of the 2 hours - EMS-treatment (procedures as in fig. 3a).

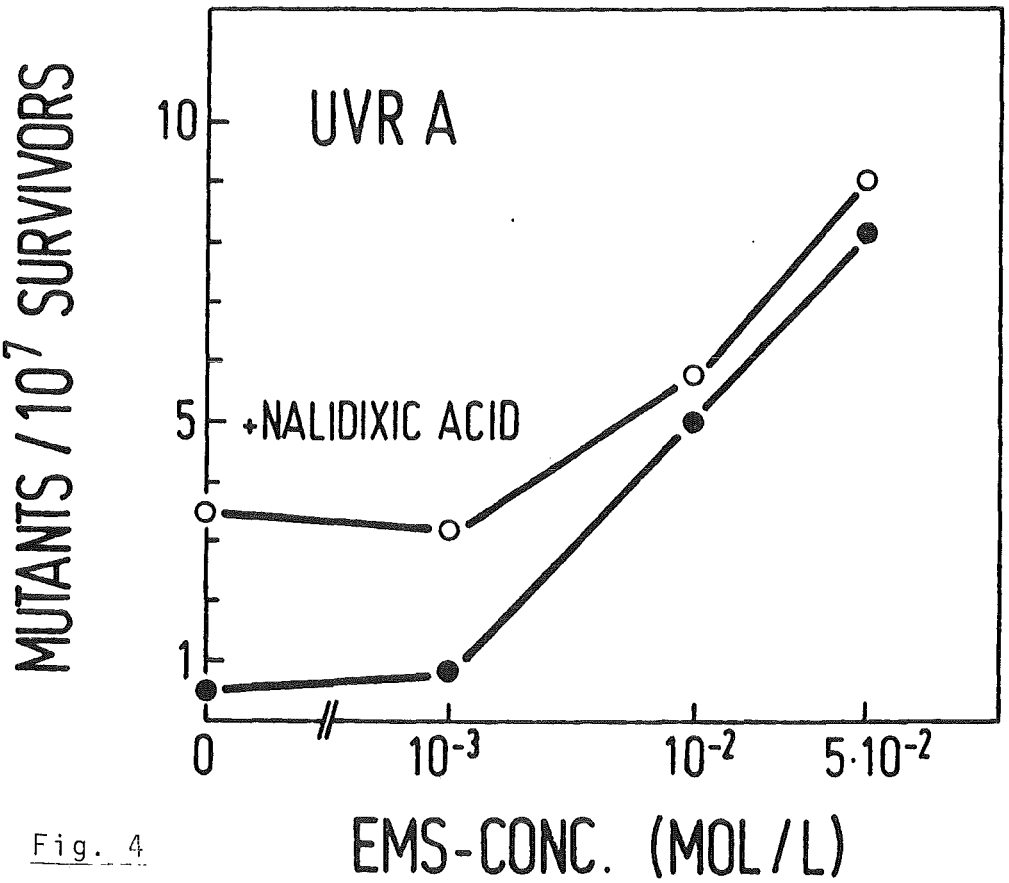
MUTANTS / 10^7 SURVIVORS
10
5
1
UVR A
+NALIDIXIC ACID
0
10^{-3}
10^{-2}
$5 \cdot 10^{-2}$
EMS-CONC. (MOL/L)

Fig. 4

Fig. 5a Induction of λ dgal prophage escape synthesis after combined treatment with UV and EMS. Prophage was induced by UV according to Levine et al. (1978). Late exponential phase bacteria E.coli K12-SA 1512 (λCI) (Devoret, Gif sur Yvette) (7 x 10^8 cells/ml) grown in Luria broth were centrifuged (3000 g) and resuspended in Saline buffer. 1 ml samples were exposed to UV followed by a 2 min high speed centrifugation. Pelletes were resuspended in Luria broth containing EMS and incubated at 37°C for 2.5 hrs. At the indicated times 0.1 ml aliquots were tested for galactokinase activity according to the procedure of Wetekam et al. (1971).

●—● Control

■—■ UV-irradiation only (6 J/m^2); combined with EMS 10^{-4} M (□); 10^{-3} M (o); 10^{-2} M (▲) and 5 x 10^{-2} M (△)

insert: EMS induction of λ-prophage

●—● Control; EMS 10^{-3} M (o); EMS 10^{-2} M (□); EMS 5 x 10^{-2} M (△)

Fig. 5b Induction of the gal-operon in E.coli B_{s-1} with fucose in absence or presence of EMS, measured as galactokinase activity after different incubation times. Exponential phase bacteria (1 x 10^8 cells/ml) were diluted 1:10 into glycerol M9-medium. Cells were induced with 5 mM fucose in absence or presence of 10^{-2} M EMS in a rotory shaker bath at 37°C after reaching a titer of 7 x 10^8 cells/ml 0.1 ml aliquots were tested for galactokinase activity after the indicated times (see also fig. 5a).

+ — + Control; Fucose induction (●); Fucose induction in presence of EMS 10^{-2} M (o).

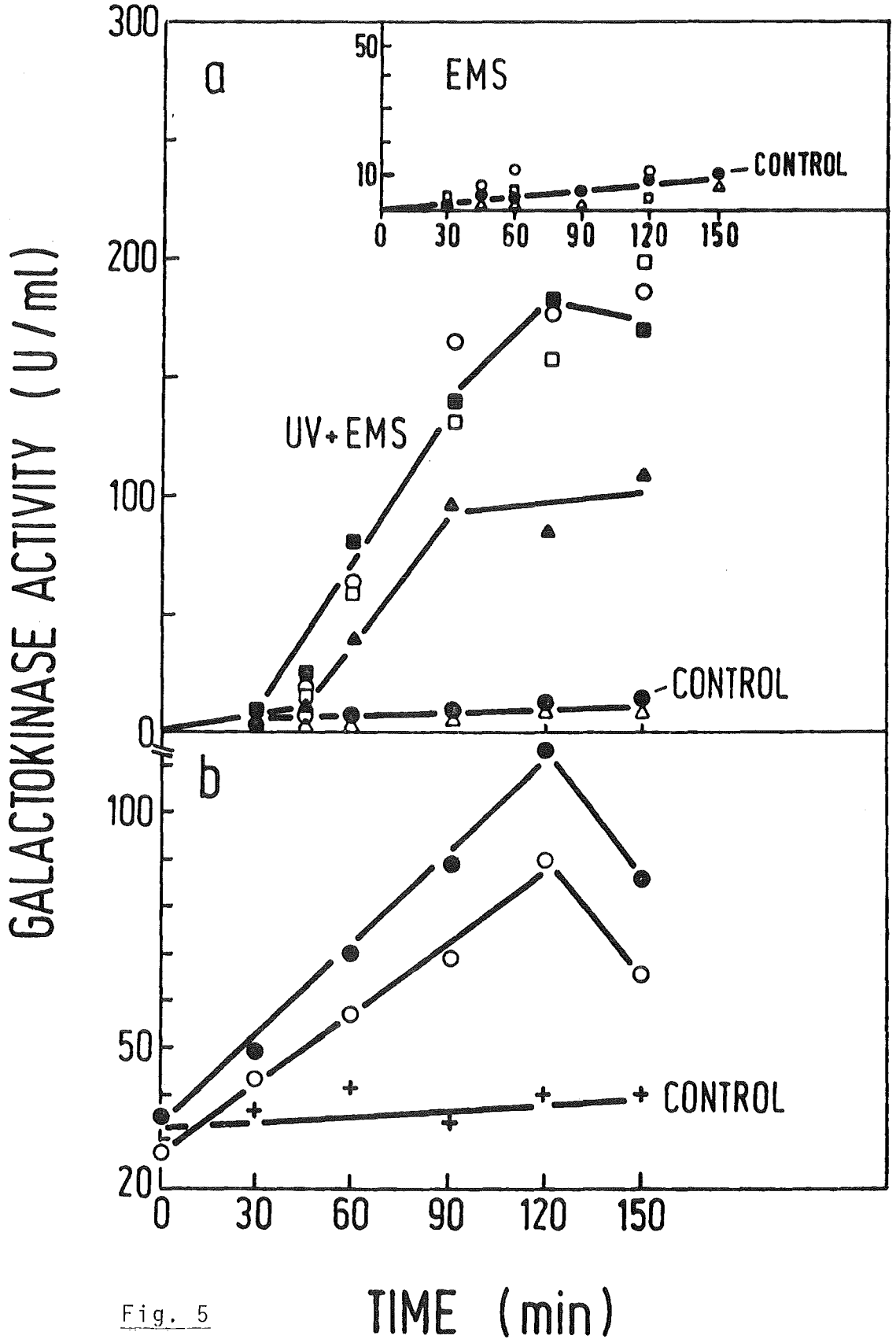
GALACTOKINASE ACTIVITY (U/ml)
300
200
100
0
a
50
10
EMS
CONTROL
0 30 60 90 120 150
UV+EMS
CONTROL
b
100
50
20
+ CONTROL
0 30 60 90 120 150
TIME (min)

Fig. 5