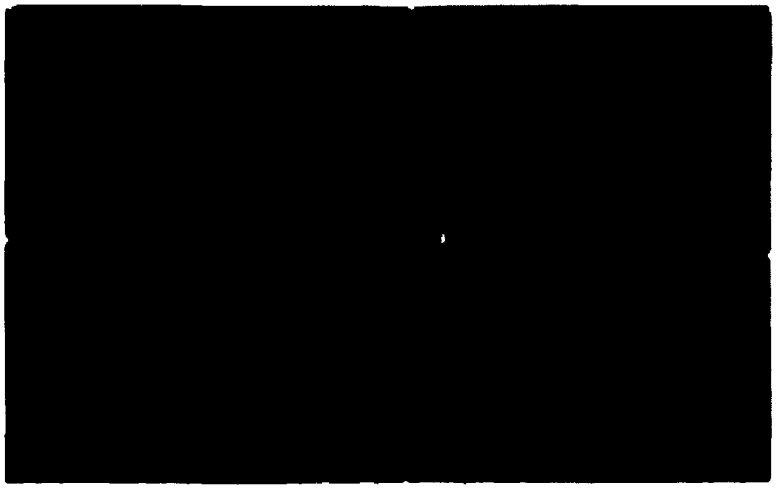
MBL 1976 - 11

# medisch biologisch laboratorium



TNO

MBL 1976 - 11

# IN VITRO EVALUATION OF SOME LATENT RADIOPROTECTIVE COMPOUNDS

O. Vos, G.A. Grant and L. Budke

Werkgebied 2 : Bescherming tegen (ioniserende) straling en chemische mutagentia

# IN VITRO EVALUATION OF SOME LATENT RADIOPROTECTIVE COMPOUNDS

O. Vos, G.A. Grant* and L. Budke

## Motivering en Toelichting

Bescherming tegen ioniserende straling is van belang i.v.m. het gebruik van kernwapens. Hoewel sinds vele jaren enige chemische stoffen bekend zijn, die in proefdieren goed beschermen tegen bestraling, is de practische toepassing van tegen bestraling beschermende stoffen niet goed mogelijk door de hoge toxiciteit van deze stoffen bij een effectieve dosering. Een ander bezwaar is de korte werkingsduur van de meeste stoffen. Om aan deze bezwaren tegemoet te komen heeft men een groot aantal derivaten van beschermende stoffen ontwikkeld. Bij experimenten in muizen blijken sommige van deze derivaten inderdaad minder toxisch te zijn bij een goede beschermende activiteit.

Omdat dierproeven wel een goed beeld geven van de totale beschermende werking maar geen informatie verschaffen hoe deze beschermende werking tot stand komt, zijn de experimenten in weefselkweek opgezet. Met behulp van deze *in vitro* proeven kan n.l. worden nagegaan of de bescherming tot stand komt door een directe werking op de cellen of dat er een algemene pharmacologische werking resulterende in een zuurstofgebrek aan ten grondslag ligt. Het is van belang dit te weten omdat men zo een beeld krijgt van de invloed van modificaties van de chemische formule op de activiteit van deze stof en zodoende beter kan bepalen of men met het introduceren van een bepaald type chemische modificaties op de goede weg is naar een effectiever stof.

Het gebruik van rattebloed werd geintroduceerd omdat gebleken is dat sommige stoffen op zich geen activiteit hebben, maar deze pas ontplooien nadat zij enzymatisch in de actieve stof zijn omgezet. De betrokken enzymen bevinden zich o.a. op de rode bloedcellen.

---

* Defence Chemical, Biological and Radiation Establishment, Ottawa 4, Ontario, Canada.

O. Vos, G.A. Grant* and L. Budke

Summary

Protection against X-irradiation by a number of cysteamine derivatives was studied in tissue culture and the results were compared with data obtained in mice. Compounds with a covered SH group like WR 638, cysteamine phosphate, WR 2721 and AE 48527, showed practically no protection when dissolved in tissue culture medium, but developed a protective activity when dissolved in rat blood. Thiol measurements demonstrated that in rat blood the compounds were partly hydrolysed to thiols. C511 was also hydrolysed in culture medium and was slightly less effective than cysteamine in culture medium. Cysteamine-phosphate was hydrolysed more easily than cysteamine sulphate and concordantly the protective activity in rat blood was better. WR 2721 was also partly hydrolysed in rat blood. The *in vitro* protection of this compound was disappointing when compared with results *in vivo*. Its SH form (WR 1065) also showed less protection than expected from *in vivo* experiments. Thus the little protection by WR 2721 *in vitro* in rat blood is not only due to its incomplete conversion into its thiol. Longer incubation times and the use of rat blood as a solvent brought the protective activity of WR 1065 up to almost the level of cysteamine. This may indicate that WR 1065 is poorly penetrating into the cells. WR 1065 was the only compound we studied which protective activity *in vitro* was improved appreciably by dissolving it in rat plasma.

* Defence Chemical, Biological and Radiation Establishment, Ottawa 4, Ontario, Canada.

# IN VITRO EVALUATION OF SOME LATENT RADIOPROTECTIVE COMPOUNDS

O. Vos, G.A. Grant* and L. Budke

## 1. Introduction

For many years, investigations to find a suitable chemical protector against ionizing radiation were centred on the synthesis of aminothiols. The biological screening and testing of the compounds was mainly performed using mice. When animal tests showed that the aminothiols were not well tolerated in large animals (Mundy, Heiffer and Mehlman, 1963; Rusanov, 1960) or man (Condit, Levy, Van Scott and Andrews, 1958), the emphasis in the synthesis programs was diverted to compounds in which the thiol group had a chemical group attached that could be removed by tissue enzymes. When a compound protects in vivo this action can be due to the chemical protector exerting its influence directly within cells, or to a systemic pharmacological effect resulting in anoxia. In vitro cellular studies are useful to differentiate between these modes of action. Furthermore, cellular studies are useful to relate protective activity to chemical structure because the concentration of the drug can be controlled in a cellular environment in vitro, whereas it cannot in in vivo studies. For these reasons, Vergroesen, Budke and Vos (1967) and Vos, Grant and Budke (1970a) investigated protection by a number of aminothiols under tissue culture conditions.

Previous experiments (Vergroesen et al., 1967) had shown that when the disulphide cystamine was added to cells in tissue culture no protection against ionizing radiation was observed, contrary to the earlier results obtained in animals (Bacq, Herve, Lecomte, Fischer, Blavier, Deschamps, Lebihan and Rayet, 1951). Further experiments in which whole blood was added to kidney cells in tissue culture (Vos, Grant and Budke, 1970b; Grant, Vos and Budke, 1975) showed that the cystamine was reduced to a thiol by glutathione reductase of the red cells and protection against x-ray radiation was

* Defence Chemical, Biological and Radiation Establishment, Ottawa 4, Ontario, Canada.

afforded to the kidney cells. These results supported the proposal that the thiol group was essential to provide a compound with good radio-protective properties. It was apparent that if the new generation of candidate protective agents were to be tested in the cell culture system, modifications to the biological environment would be required to supply tissue enzyme systems capable of releasing aminothiols from the potential protective compounds. This paper describes the results of adding whole blood to the cell medium in which a variety of phosphorothioates, thiosulfates and other compounds were evaluated for protective activity. Also, dose reduction factors were determined using mice.

## 2. Materials and Methods

### 2.1. Chemicals

All the compounds had a purity of at least 98 per cent as determined by elementary chemical analyses. The structure of the compounds (Table 1) was determined from infra-red spectra and from physical constants.

### 2.2. Analytical method

Aminothiol liberated in the culture medium was determined by a method described by Grassietti and Murray (1967). The thiol concentrations present in the control culture fluid and plasma were used as the blanks. The thiol concentration was determined 10 min after the compound was dissolved; when blood was used the erythrocytes were removed from the cell medium by centrifuging and the thiol concentration was determined in the plasma.

## 2.3. Tissue culture

A heteroploid human kidney cell-line (T-cells) with a cell-cycle of about 24 hours was used. The cells were maintained in glass bottles. Survival after irradiation and/or protection was determined by seeding the cells in Petri dishes, which had a Melinex bottom (Barendsen and Beusker, 1960). Cells forming a colony of at least 50 cells within 13 days after seeding were considered as survivors. All Petri dishes were supplied with a "feeder layer" of lethally-irradiated cells. The growth medium consisted of Dulbecco's phosphate buffered salt solution to which 0.5 per cent lactalbumin hydrolysate and 6 per cent new-born calf serum were added. Furthermore, the medium contained 100 IU penicillin and 0.1 mg streptomycin per ml. If necessary the pH of the medium was adjusted to 7.3 by the addition of sodium hydroxide.

Protective compounds were dissolved in growth medium a few minutes before exposure of the cells to the solution. Cells attached to the bottom of the dishes were incubated in medium containing the protective agent 10 min before irradiation by replacing normal growth medium by medium containing the agent. Cells were washed three times with normal growth medium within a few minutes after irradiation to avoid toxicity of the protective drugs. A time schedule of the various steps in the protection procedure has been reported previously (Vergroesen et al., 1967).

## 2.4. Irradiation

Tissue cultures were irradiated with a Philips 250 KV X-ray machine. The radiation constants were 250 KV; 15 mA; h.v.l. 1.9 mm Cu; filtration 1.5 mm Cu (corrected); dose rate 200 rad/min; maximal backscatter was employed by placing the dish on a layer of hardboard of 13 cm thickness.

A Baldwin Ionex MK3 ionization gauge was used for monitoring the dose during each experiment.

The mice were irradiated in a special cesium$^{134}$ irradiator (Cunningham, Bruce and Webb, 1965) at a dose rate of 95 rad/min. The mice were maintained, 5 or 10 per cage, in wire-bottomed cages in an air-conditioned room. The drinking water was chlorinated. The compounds were dissolved in pH 7.2 phosphate buffer and 0.1 ml was injected intraperitoneally into mice. The compounds were administered 15 min or 30 min prior to irradiation. The mice were from Bio-Breeding Laboratories, Ottawa and were SPF COBS white females, weighing 25 to 28 g.

The $LD_{50/30}$ and $LD_{50/7}$ values were obtained by irradiating 5 or 6 groups each containing 20 mice with graded doses of radiation and counting the number of dead mice during a 30-day holding period.

The $LD_{50}$ values and other statistical parameters were calculated with a computer program for probit analysis. The dose reduction factor $DRF = \left( \frac{LD_{50} \text{ treated}}{LD_{50} \text{ untreated}} \right)$ was calculated if the slopes of the response curves for the untreated and treated groups were approximately parallel.

## 3. Results

Dose-survival curves obtained in tissue culture medium with cysteamine-phosphate (WR 638), cysteamine-sulphate, cystamine and cysteamine, in concentrations of 4 and 16 mM - except for cystamine, which was used at 2 and 8 mM concentration - are presented in figures 1 and 2. The results with cysteamine were published earlier (Grant, Vos and Budke, 1975), but are presented here also to enable direct comparison. Cystamine is used in half the concentration of the other compounds because after conversion it gives double the concentration of cysteamine. It is shown that cystamine,

cysteamine-phosphate and cysteamine-sulphate, when dissolved in tissue culture medium, provide practically no protection, whereas cysteamine elicits a good activity. Figures 3 and 4 demonstrate that rat blood activates the protective ability of the compounds, but none of the compounds attained the protective level of cysteamine. The best protection found with cysteamine, is followed by cystamine, cysteamine-phosphate and cysteamine-sulphate, in that order. The unexpected enhancing effect of rat blood on protection by cysteamine has been published in an earlier paper (Grant et al., 1975).

The dose-reduction factors calculated from the data at the 1 per cent survival level are presented in table 2. The thiol concentrations found in the various solutions are presented in table 3. From these data it is evident than when cysteamine-phosphate, cysteamine-sulphate or cystamine were dissolved in tissue culture medium or rat plasma, no increase in thiol concentration occurred in the solutions. However, when the same compounds were dissolved in rat blood the thiol concentration was increased. The data show also that the conversion of the substances into cysteamine was incomplete. Conversion into a thiol was most effective for cystamine followed by cysteamine-phosphate and cysteamine-sulphate, in that order.

In figure 5, the DRF's obtained with the addition of various concentrations of cysteamine to tissue culture medium and to rat blood are presented. Also presented in this figure are DRF's at two concentrations of cystamine, cysteamine-phosphate and cysteamine-sulphate in rat blood. The DRF's obtained are plotted against the thiol concentrations determined (Table 3) when rat blood was used as incubation medium to convert the compounds to cysteamine (thiol). Since the data points lie below the expected protection for cysteamine incubated in rat blood, it is possible that rat blood did not completely convert the compounds to cysteamine, but that the thiol concentration measured was partly due to other thiols with a smaller protective capacity.

Dose-survival curves obtained with WR 2721, WR 1065, AE 48527 and C 511 are depicted in figures 6 - 13. The dose-reduction factors calculated from these data are presented in table 2 and 5. It is evident from figures 6 and 7 that in comparison to cysteamine, WR 2721 afforded very poor protection to kidney cells in vitro. When dissolved in tissue culture medium practically no protection is obtained, while even in the presence of rat blood, only a small DRF of 1.3 is observed at both concentrations of 4 or 16 mM. The results of the thiol determination (Table 3) demonstrate that even in the presence of rat blood, only a very small fraction of WR 2721 was hydrolyzed to the thiol product and this provides an explanation for its lack of protective activity in vitro.

The investigation was extended to WR 1065, the thiol hydrolysis product of WR 2721, in order to examine whether this compound was able to protect, in which case the protective activity of WR 2721 in mice did not need to be explained by a pharmacological mode of action. The toxicity data presented in table 4 suggests a minor toxicity in the 1 - 4 mM range similar to the other thiols (Vos et al., 1970a). The dose survival curves obtained with WR 1065 at concentrations of 2.6 and 10.4 mM dissolved in growth medium, rat blood or plasma are shown in figures 8 and 9 respectively. It is evident that surprisingly little protection is afforded at a concentration of 2.6 mM when WR 1065 is dissolved in growth medium, while at 10.4 mM protection is apparent. However, when dissolved in rat plasma or rat blood, the WR 1065 demonstrated appreciable protective activity. The dose-reduction factors obtained with the compound when dissolved in the various media are given in table 5. A DRF of 3.7 is observed with 10.4 mM of WR 1065 dissolved in rat blood. Although these levels of DRF can be considered to demonstrate good protective activity, cysteamine under the same conditions provides a DRF of approximately 4.8.

Since it was evident the rat blood did not efficiently convert the WR 2721 to the thiol analogue WR 1065 during the incubation time employed, the conversion was studied employing longer incubation times. With 1.3 mM of WR 2721 dissolved in rat blood incubation medium for 40 min, a DRF of 2.7 was obtained. This level of protection is comparable to that obtained with cysteamine.

The results obtained with AE 48517 are not differing importantly from those obtained with WR 2721. The alteration of the place of the NH group in the molecule did not improve the protective activity.

A series of thiazolidine carboxylate and glycolate esters of 2-amino-ethanethiol and 2-cysteine were synthesized by Fourneau, Efimovsky, Gaignault, Jacquier and Le Redant (1971). One of the compounds, methyl(2-aminoethylthio) glycolate hydrochloride (C 511) was reported by Jezequel, Frossard, Perles and Poutrain (1971) to have radioprotective properties equal to cysteamine and to be much less toxic. These animal studies were confirmed in our strain of mice. Administration of 450 mg/kg of the compound 15 min prior to radiation produced an $LD_{50/30}$ of 1138 (1109 - 1167) rads (95 per cent Fudicial Limits) compared to a control value of 790 (740 - 881) rads. The data of these experiments are presented in table 6 together with data obtained with some other compounds. A dose-reduction factor of 1.44 was found which is approximately the DRF (1.5) obtained with cysteamine when 200 mg/kg is administered i.p. 15 min prior to irradiation. Since the compound represents a new class of radioprotective agents because of the new covering function on the sulphur atom, it was considered worthwhile to investigate it under in vitro conditions.

In vitro 4 mM C 511 afforded protection almost equal to that of cysteamine (figure 12). At 16 mM protection afforded by C 511 was slightly less than that of cysteamine (figure 13). Determinations of the thiol concentration in solutions of C 511 have shown that in tissue culture fluid

the compound is hydrolyzed to the thiol form (cysteamine) almost immediately after it is dissolved (table 3). Therefore, the protection afforded by C 511, both in vivo and in vitro, is probably due to conversion into cysteamine. No enzymes seem to be required for this hydrolysis. The relatively small protection at the 16 mM concentration may be due to an incomplete conversion into cysteamine at higher concentrations due to the short incubation time.

## 4. Discussion

### 4.1. General

When rat blood is used in the incubation medium the protective ability of disulphides, thiophosphates and thiosulphates can be demonstrated. The effect of rat blood will depend on whether it contains enzymes which can hydrolyze efficiently the compounds to release a thiol compound. However, in addition the rat blood has also an effect on the protective activity of the released thiol as demonstrated by previous studies with cysteamine (Grant, Vos and Budke, 1975). Unpublished data showed that the effect was primarily due to erythrocytes as washed blood taken from a rat a few days after lethal irradiation, which contained only a few white cells, exerted the same effect as whole blood from an unirradiated rat. Unpublished data also showed that homogenates of liver and kidney tissues improved protection by cysteamine. However, large amounts of homogenate tissue caused the medium to become anoxic and thus the interpretation of the results became more complicated. Since normal rat blood was a convenient medium to use the experiments described in the present paper were done with blood. Primary experiments done with rat plasma showed that it did not influence the protective activity of cysteamine, cystamine and cysteamine-phosphate. However, a slight improvement of protective activity of WR 1065 was noted.

## 4.2. Cysteamine-sulphate

Cysteamine-sulphate was first shown to afford protection against X-ray radiation in animals by Sorbo (1958) and Holmberg and Sorbo (1959). Sorbo (1962) also suggested that cysteamine-sulphate could be converted to cysteamine *in vivo* by reaction with glutathione followed by reduction of glutathione-cysteamine disulphide by glutathione reductase.

The present studies show that the conversion of cysteamine-sulphate to cysteamine by use of rat whole blood *in vitro* is very poor. This is in agreement with the poor protection against X-ray radiation afforded by the compound to the kidney cells. Cysteamine-sulphate can be classed as a radioprotective agent as it has been shown to provide protection to whole animals. With our strain of mice administration i.p. of 450 mg/kg of the compounds 30 min prior to radiation afforded protection at the level of a DRF of 1.4 which is comparable to the level of protection found for cysteamine (DRF 1.5) after administration of 200 mg/kg.

Since it is known that thiosulphate esters react with thiols to form sulphides and disulphides (Walley, 1959; Swan, 1957) and neither of these chemical functions can directly provide protection, it is unlikely that a thiosulphate derivative will produce efficient radioprotective agents. The protective properties found are most likely due to the reduction of the mixed disulphide to cysteamine, and this is not an efficient process in most tissues. In the duodenum Kelly, Hamilton and Friedman (1967) found most of the sulphydryl was bound as disulphide to the protein and that glutathione reductase in blood was not very efficient in reducing the mixed disulphide. This is in agreement with results in our own laboratory. Thus, it is unlikely that an efficient radioprotective agent with a high DRF can be produced by the addition of the sulphate group to a thiol, which is in agreement with the large number of such compounds in the thiosulphuric acid series that have been synthesized, tested and proven to be able of little or no protection

(Westland, Holmes, Monk, Marsh, Cooley and Dice, 1968; Klayman and Shine, 1968 and unpublished results).

## 4.3. Cysteamine-phosphate (WR 638)

Previous studies have shown that cysteamine-phosphate is a potent radioprotective agent in mice (Hansen and Sorbo, 1961) and that it releases substantial concentrations of cysteamine into a number of tissues in the rat (Kelly, Herrington, Ward, Meister and Friedman, 1967). It has been suggested that the protective effect of cysteamine-phosphate is due at least in part to its conversion to cysteamine in vivo (Akerfeldt, 1963). However, no direct experimental evidence is available to support the Akerfeldt proposal. The in vitro studies demonstrate that when the cysteamine-phosphate is added alone to a kidney cell culture, no release of cysteamine is evident and the compound does not afford protection to the T-cells against X-ray radiation. However, when whole blood or red cells are added to the tissue culture medium with WR 638, cysteamine is released and the T-cells are protected against the lethal effects of the ionizing radiation. These findings provide direct experimental evidence that the protective effect of cysteamine-phosphate is due to its enzymatic hydrolysis to cysteamine and orthophosphate.

## 4.4. WR 2721 and WR 1065

One of the most potent radioprotective drugs in animals, WR 2721, uncovered in recent years was first reported by Piper, Stringfellow, Elliot and Johnston (1969). Dose reduction factors over 2.0 were reported by Yuhas and Storer (1969) and Yuhas (1970). With our strain of mice a DRF of 2.14 is obtained by administrering 350 mg/kg i.p. 15 min prior to irradiation.

Studies by Harris and Phillips (1971) indicate that the protection afforded to animals by WR 2721 may be due to the thiophosphate entering the cell by passive diffusion and being dephosphorylated to a free thiol. However, they failed to find an increase in the thiol level in the spleen and liver after administration of the drug. This is unusual as these tissues usually show an increase in thiol after administration of WR 638, a compound with a similar chemical structure containing the phosphate group as a covering function (Sorbo, 1958; Herrington et al., 1967). These findings indicate that the differences in chemical structure cause an appreciable difference in hydrolysis rates by the alkaline phosphatases. Harris and Phillips (1971) demonstrated that bone marrow cells of WR 2721 treated mice contained levels of acid-soluble thiol but examination of bone marrow cells treated in vivo with WR 2721 failed to reveal the release of thiol. Also, measurement of intracellular WR 2721 confirmed the absence of the intracellular protective agent. These findings are in agreement with our observation that incubation of T-cells with WR 2721 alone failed to release a free thiol and hence the T-cells were not protected.

Harris and Phillips (1971) found that WR 638 was dephosphorylated faster than WR 2721. In the present cellular system when whole rat blood was used as incubation medium, WR 638 was a superior protector to WR 2721, which is the converse of what is to be expected from observations in mice.

An alternative explanation for the lack of agreement between the in vivo and in vitro protective studies is that a pharmacological mode of action plays a role in protection observed with WR 2721 or its hydrolysis products. In this regard it has been demonstrated that WR 2721 and analogs of it induce vasodilation in mice, especially in the spleen. Removal of the spleen from mice reduces both toxicity and protectiveness of WR 2721. The protective effect of hypoxia and WR 2721 are more nearly additive in splenectomized mice than in normal animals (Yuhas, Proctor and Smith, 1973).

These observations have been interpreted by Yuhas et al. (1973) as indicating that the pronounced vasodilation alters the blood supply in such a way that tissue oxygen tensions are reduced and radiation resistance is thereby elevated. However, the fact that with WR 2721 optimum protection is observed within 15 or 30 min, while spleen weight increases very little in 15 min does not support the proposal of Yuhas et al. (1973). If anoxia occurs in radiosensitive tissue by a blood volume change in the spleen, this may account for the length of time that protection is observed with WR 2721. Thus initial protection over a short time interval is due to chemical and biochemical repair processes, while protection observed from 45 min to 2 hours after administration of WR 2721 may be due in part to hypoxia in tissue as well.

## REFERENCES

AKERFELDT, S., 1963, Acta Radiol., 1, 465.

BACQ, Z.M., HERVE, A., LECOMTE, J., FISCHER, P., BLAVIER, J., DECHAMPS, G., LEBIHAN, H. and RAYET, P., 1951, Archs. Int. Physiol., 59, 442.

BARENDSEN, G.W. and BEUSKER, T.J.L., 1960, Radiat. Res., 13, 832.

CONDIT, P.T., LEVY, H., VAN SCOTT, E.J. and ANDREWS, J., 1958, J. Pharmacol. Exptl. Therap., 122, 13A.

CUNNINGHAM, J.R., BRUCE, W.R. and WEBB, H.P., 1965, Phys. Med. Biol., 10, 381.

FOURNEAU, J.P., EFIMOVSKY, O., GAIGNAULT, J.C., JACQUIER, R. and LE REDANT, C., 1971, Comptes. Rendus, 272, Series C 1515.

GRANT, G.A., VOS, O. and BUDKE, L., 1975, Int. J. Radiat. Biol., 28, 45.

GRASSIETTI, D.R. and MURRAY, J.F., 1967, Anal. Biochem., 21, 427.

HANSEN, B. and SORBO, B., 1961, Acta Radiol., 56, 141.

HARRIS, J.W. and PHILLIPS, T.L., 1971, Radiat. Res., 46, 362.

HERRINGTON, K.A., SMALL, C.J., MEISTER, A. and FRIEDMAN, O.M., 1967, Cancer Research, 27, 148.

HOLMBERG, B. and SORBO, B., 1959, Nature, 183, 832.

JEZEQUEL, E.J., FROSSARD, H., PERLES, R. and POUTRAIN, P., 1971, Comptes. Rendus, 272, Series D, 2826.

KELLY, J.J., HAMILTON, N.F. and FRIEDMAN, O.R., 1967, Cancer Research, 27, 143.

KELLY, J.J., HERRINGTON, K.A., WARD, S.P., MEISTER, A. and FRIEDMAN, O.M., 1967, Cancer Research, 27, 137.

KLAYMAN, D.L. and SHINE, R.J., 1968, Quarterly Reports on Sulfur Chemistry, 3, 228.

MUNDY, R.L., HEIFFER, M.H. and MEHLMAN, B., 1963, Amer. J. Physiol., 204, 997.

PIPER, J.R., STRINGFELLOW, C.R., ELLIOT, R.D. and JOHNSTON, T.P., 1969, J. Med. Chem., 12, 236.

RUSANOV, A.M., 1960, Diagnosis and Treatment of Acute Radiation Injury. IAEA and WHO Scientific meeting, Geneva, pp. 347-359.

SORBO, B., 1958, Acta Chem. Scand., 12, 1990.

SORBO, B., 1962, Arch. Biochim. Biophys., 98, 342.

SWAN, J., 1957, Nature, 180, 643.

VERGROESEN, A.J., BUDKE, L. and VOS, O., 1967, Int. J. Radiat. Biol., 13, 77.

VOS, O., GRANT, G.A. and BUDKE, L., 1970a, Int. J. Radiat. Biol., 18, 111.

VOS, O., GRANT, G.A. and BUDKE, L., 1970b, Radiation Protection and Sensitization, Ed. H.L. Moroson and M. Quintiliani, London (Taylor and Francis Ltd) p. 211.

WALLEY, S.G., 1959, Biochem. J., 71, 132.

WESTLAND, R.D., HOLMES, J.T., MONK, M.L., MARSH, D.D., COOLEY, R.A., Jr. and DICE, J.R., 1968, Med. Chem., 11, 1190.

YUHAS, J.M. and STORER, J.B., 1969, J. Natl. Cancer Inst., 42, 331.

YUHAS, J.M., PROCTOR, J.O. and SMITH, L.H., 1973, Radiat. Research. 54, 222.

YUHAS, J.M., 1970, Radiat. Research, 44, 621.

| Designation | Chemical name | Structure |
|---|---|---|
| Cysteamine (MEA) | S-2-aminoethylmercaptan | $NH_2CH_2CH_2SH$ |
| Cystamine | 2,2"-dithiobis(ethylamine) dihydrochloride | $[NH_2CH_2CH_2S]_2 \cdot 2HCl$ |
| Cysteamine-phosphate (WR638) | Sodium hydrogen S-2-amino-ethyl phosphorothioate | $NH_2CH_2CH_2SPO_3HNa$ |
| Cysteamine-sulphate | Sodium S-2-aminoethanethio-sulphate | $NH_2CH_2CH_2SSO_3Na_2$ |
| C 511 | Methyl(2-aminoethylthio) glycolate hydrochloride | $HCl \cdot NH_2CH_2CH_2SCH(OH)COOCH_3$ |
| WR 1065 | 2-(3-aminopropylamino) ethyl mercaptan | $NH_2(CH_2)_3NHCH_2CH_2SH$ |
| WR 2721 | S-2-(3-aminopropylamino) ethyl phosphorothioic acid | $NH_2(CH_2)_3NH(CH_2)_2S-PO_3H_2 \cdot H_2O$ |
| AE 48527 | S-3-(2-aminoethylamino) propyl phosphorothioic acid | $NH_2(CH_2)_2NH(CH_2)_3SPO_3H_2$ |

Table 1. Chemical structure of compounds. Some WR compounds were also supplied by Colonel W. Rothe, Division of Medical Chemistry, Walter Reed Army Institute of Research; C 511 was also supplied by Dr. H. Frossard, Centre de Recherches du Service de Santé des Armées, Clamart, France.

| Compound | 4 mM concentration | | 16 mM concentration | |
|---|---|---|---|---|
| | Tissue culture medium | Rat blood | Tissue culture medium | Rat blood |
| Cysteamine | 1.9 | 3.6 | 3.4 | 5.0 |
| Cystamine (2 and 8 mM) | 1.1 | 2.6 | 1.1 | 2.9 |
| Cysteamine-phosphate | 1.1 | 1.8 | 1.0 | 2.3 |
| Cysteamine-sulphate | 1.0 | 1.2 | 1.0 | 1.3 |
| WR 2721 | 1.1 | 1.3 | 1.1 | 1.3 |
| AE 48527 | 1.1 | 1.3 | 1.3 | 1.5 |
| C 511 | 1.7 | 3.1 | 2.2 | 3.8 |

Table 2. Dose-reduction factors calculated from data at the 1 % survival level in tissue culture.

| Compound added | Determination of thiol concentration | | |
|---|---|---|---|
| | Tissue culture medium | Rat plasma | Rat blood |
| Cysteamine 4 mM | 4.1 | 3.9 | 4.4 |
| Cysteamine 16 mM | 15.5 | 13.5 | 16.6 |
| Cystamine 2 mM | < 0.1 | < 0.1 | 2.6 |
| Cystamine 8 mM | 0.2 | < 0.1 | 3.4 |
| Cysteamine-phosphate 4 mM | < 0.1 | < 0.1 | 0.6 |
| Cysteamine-phosphate 16 mM | < 0.1 | < 0.1 | 1.0 |
| Cysteamine-sulphate 4 mM | < 0.1 | < 0.1 | 0.2 |
| Cysteamine-sulphate 16 mM | < 0.1 | < 0.1 | 0.4 |
| WR 2721 4 mM | < 0.1 | 0.3 | 0.3 |
| WR 2721 16 mM | < 0.1 | 0.4 | 0.3 |
| AE 48527 4 mM | 0.2 | 0.8 | 1.2 |
| AE 48527 16 mM | 0.2 | 1.0 | 1.3 |
| C 511 4 mM | 3.5 | 2.0 | 3.3 |
| C 511 16 mM | 13.6 | 9.0 | 12.8 |

Table 3. SH-concentrations (mM)* in solutions of cysteamine, cystamine, cysteamine-phosphate and cysteamine-sulphate in tissue culture medium and rat blood.

| No. experiment | Concentration (in mM) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0.16 | 0.33 | 0.65 | 1.3 | 2.6 | 5.2 | 10.4 |
| 1 | | | | | 43 | | 106 |
| 2 | 96 | 93 | 88 | 62 | 46 | 79 | 85 |
| 3 | 112 | 120 | 103 | 88 | 74 | 97 | 106 |

Table 4. Toxicity of S-3(2-aminopropylamino)ethyl mercaptan (WR 1065) on cells in culture. Figures represent surviving cells expressed as percentage of non-treated controls. Exposure was at room temperature and lasted 20 minutes.

| Concentration (mM) | Growth medium | Solution in rat plasma | Rat blood |
|---|---|---|---|
| 2.6 | 1.2 | 1.7 | 1.9 |
| 10.4 | 2.0 | | 3.7 |

Table 5. Dose reduction factors obtained with WR 1065, when dissolved in various media. DRF values are calculated from data presented in fig. 8 and 9 (Calculations at 1 % survival level).

| Compound | Dose mg/kg* | $LD_{50/30}$ rads | Dose reduction factor |
|---|---|---|---|
| Cysteamine | 200 | 810 ( 776- 842) | 1.53** |
| | 150 | 1102 (1080-1124) | 1.4 |
| Cysteamine-sulphate | 450 | 770 ( 734- 810) | 1.4 ** |
| C 511 | 450 | 1138 (1109-1167) | 1.44 |
| WR 2721 | 350 | 1594 (1557-1628) | 2.14 |
| | 200 | 1595 (1571-1617) | 2.10 |

Table 6. Dose-reduction factor provided by some protective agents in mice.

* The compounds were injected 15 min before the beginning of irradiation, except cysteamine sulphate which was injected 30 min before irradiation.

** $LD_{50/30}$ of control mice in these experiments was also low (530 rads).

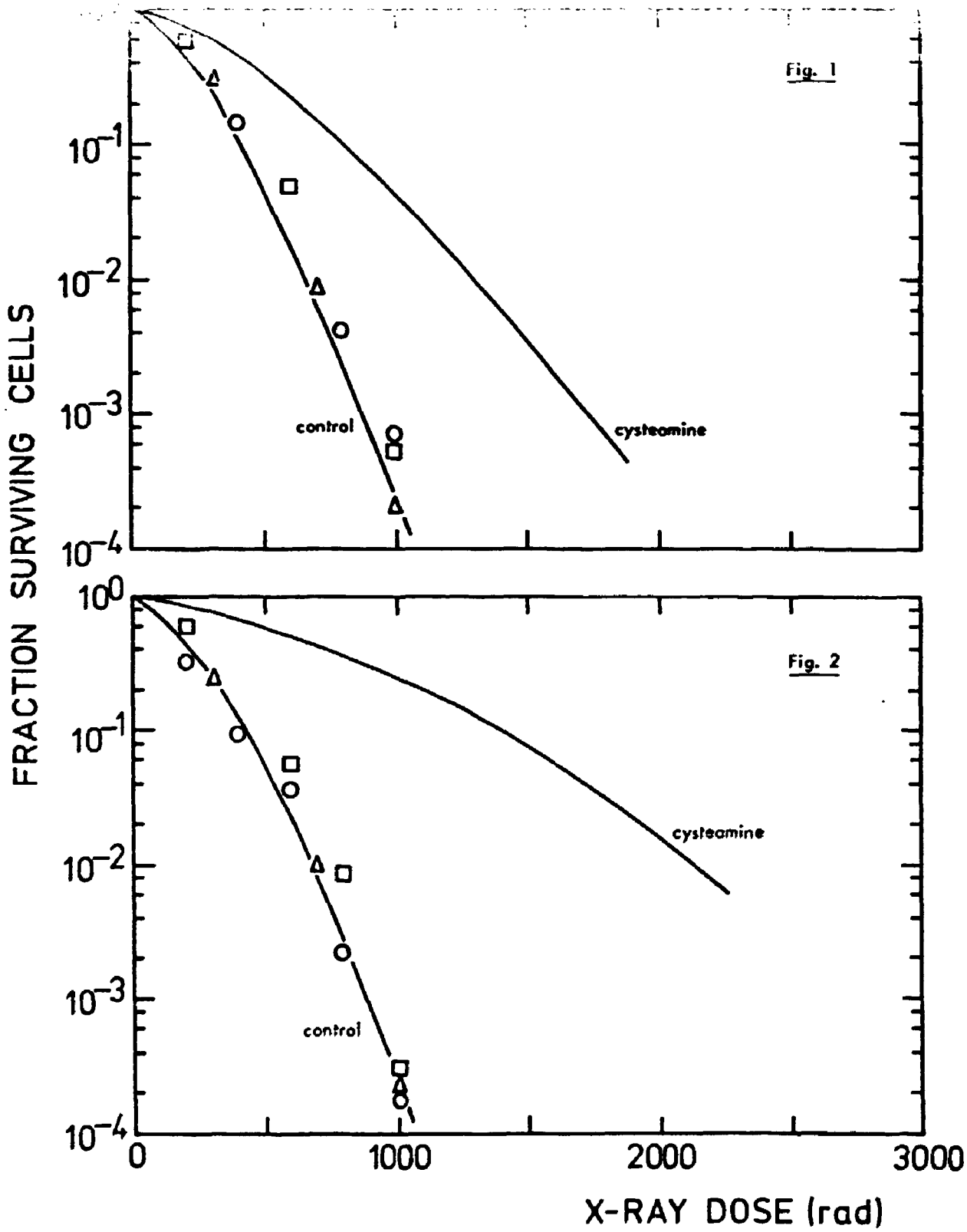



Figure 1. Protection of T-cells by 4 mM cysteamine, cysteamine-phosphate (O) and cysteamine-sulphate (Δ) and 2 mM cystamine (□) in tissue culture medium. The experimental points for the survival curves in culture medium (control) and in cysteamine dissolved in culture medium are shown in figure 12.

Figure 2. Protection of T-cells by 16 mM cysteamine, cysteamine-phosphate (O) and cysteamine sulphate (Δ) and 2 mM cystamine (□). The experimental points for the survival curves in culture medium (control) and in cysteamine dissolved in culture medium are shown in figure 12.

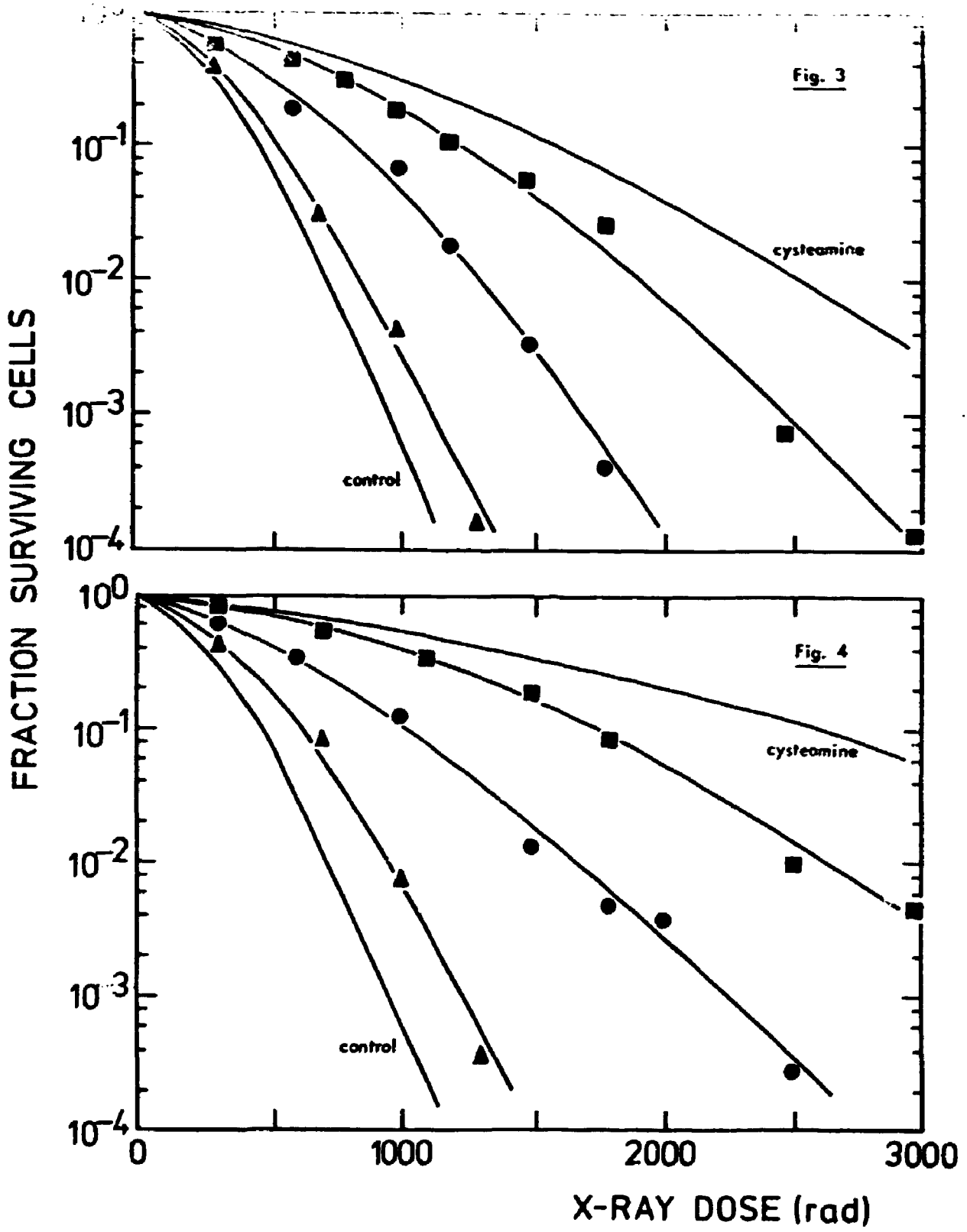



Figure 3. Protection of T-cells by 4 mM cysteamine, cysteamine-phosphate (●), cysteamine-sulphate (▲) and 2 mM cystamine (■) in rat blood. The experimental points for the survival curves in rat blood (control) and for 4 mM cysteamine in rat blood are shown in figure 12.

Figure 4. Protection of T-cells by 16 mM cysteamine, cysteamine-phosphate (●), cysteamine sulphate (▲) and 8 mM cystamine (■) in rat blood. The experimental points for the survival curves in rat blood (control) are shown in figure 12 and for 16 mM cysteamine in rat blood in figure 13.

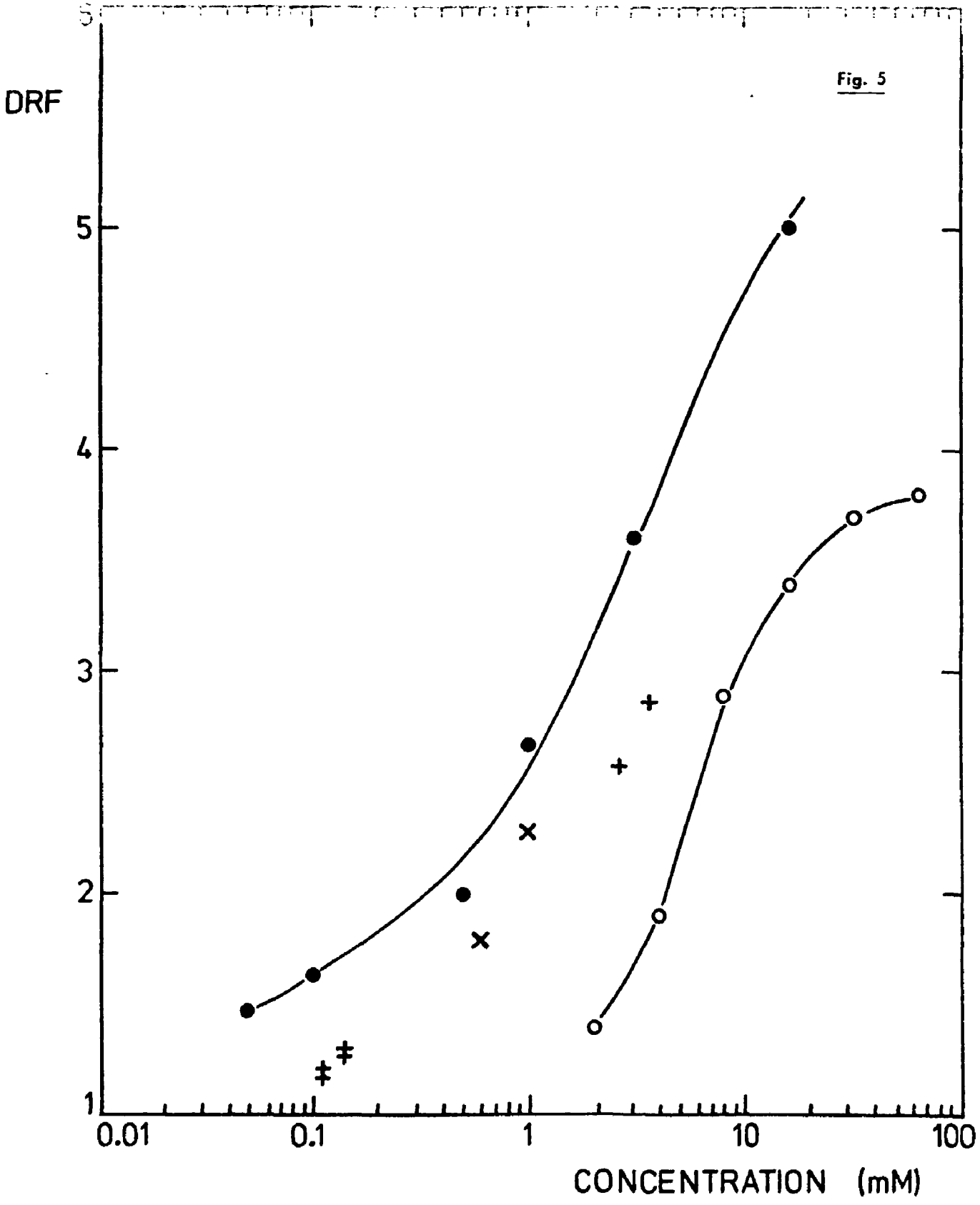



Figure 5. Dose reduction factors obtained with cysteamine in culture medium (O) and in rat blood (●). Also plotted are DRF's of 2 and 8 mM cystamine (+), 4 and 16 mM cysteamine-phosphate (×) and 4 and 16 mM cysteamine-sulphate (ǂ) in rat blood. The latter DRF's (see table 2) are plotted against the thiol concentration determined under these conditions (see table 3).

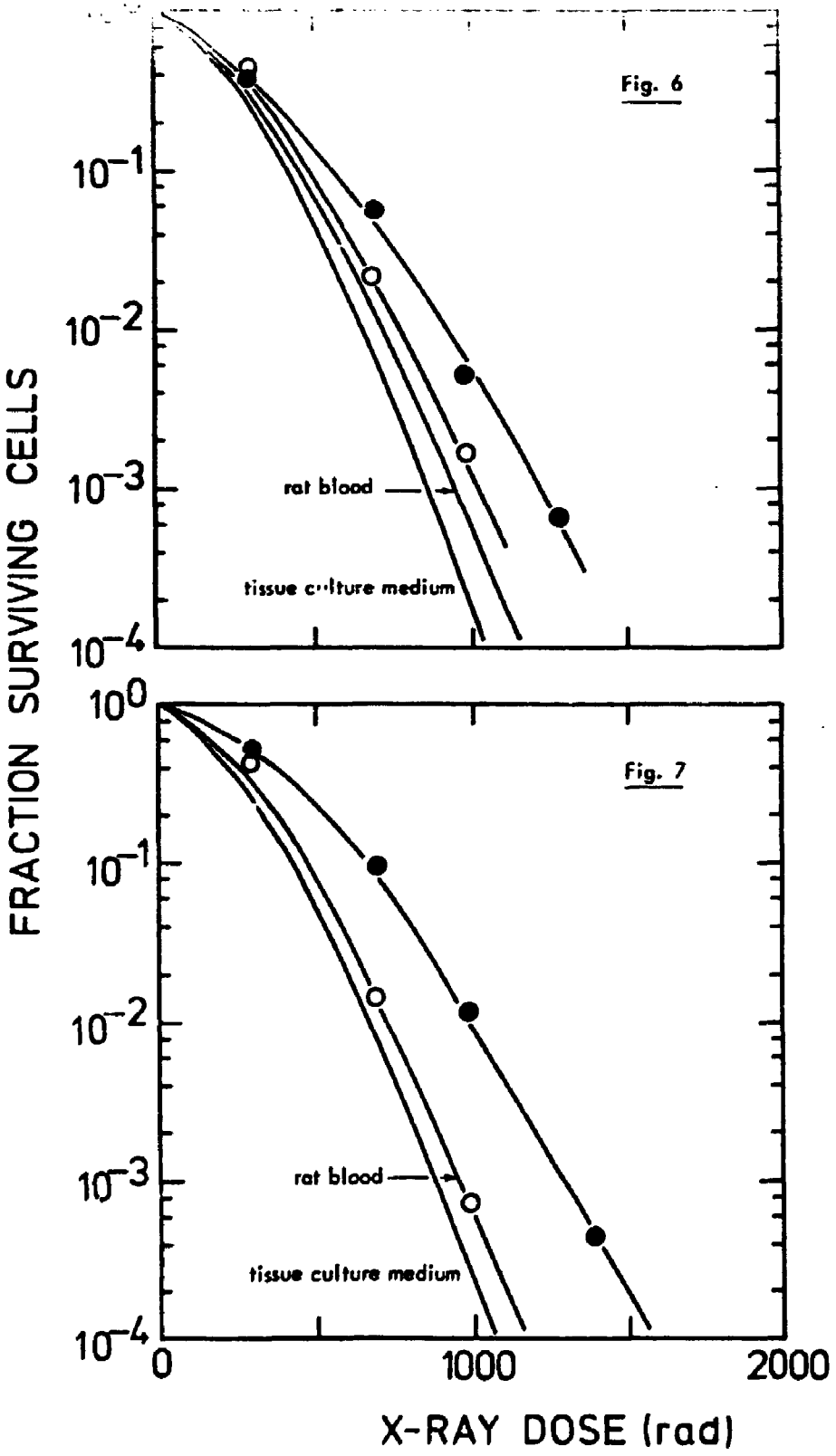



Figure 6. Protection of T-cells by 4 mM WR 2721; ●——● in rat blood, O——O in culture medium. The experimental data points belonging to the survival curves of the cells irradiated in culture medium and rat blood without addition of protective compounds are presented in figure 12.

Figure 7. Protection of T-cells by 16 mM WR 2721. Symbols and control curves as presented in figure 6.

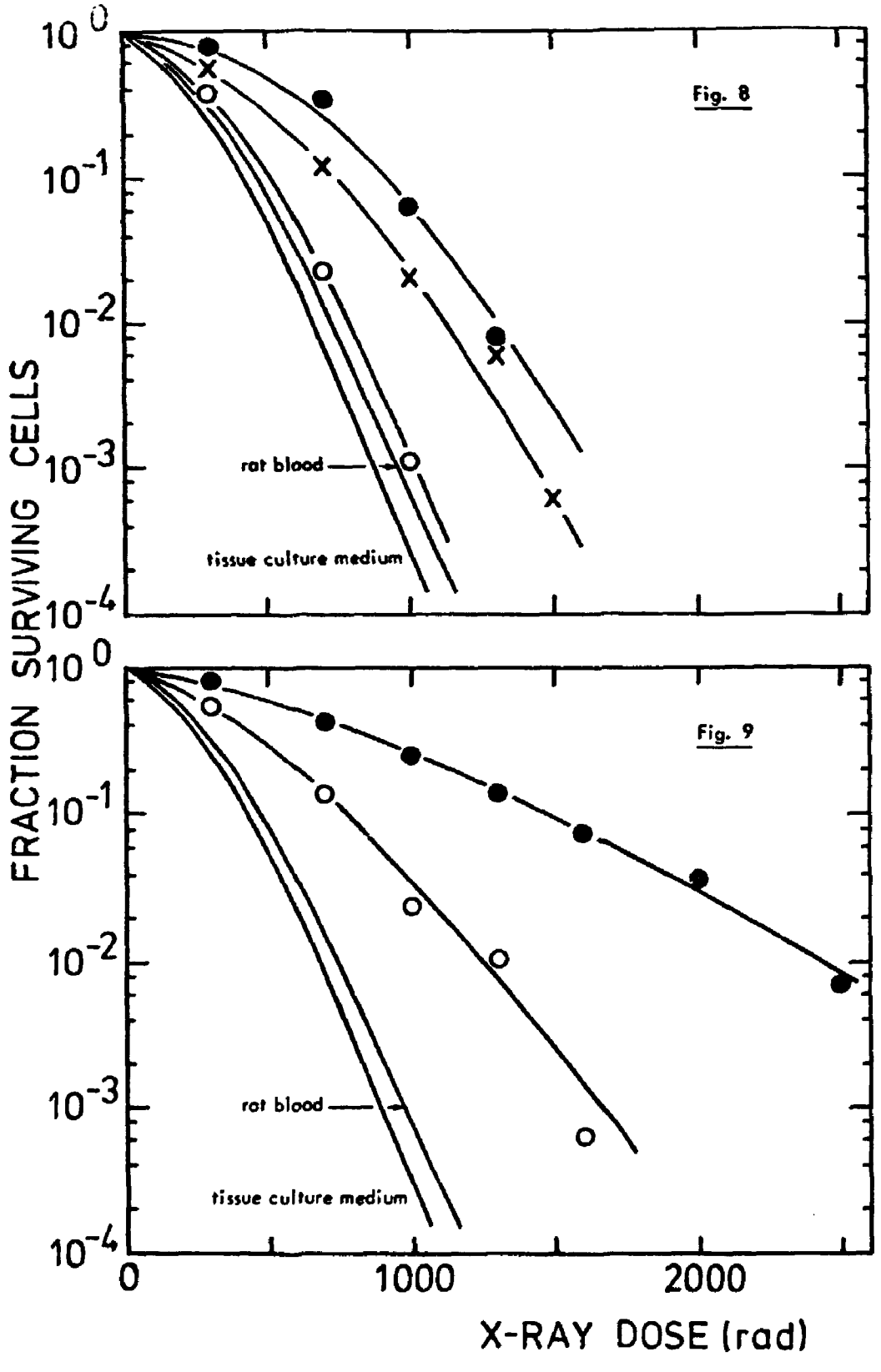



Figure 8. Protection of T-cells by 2.6 mM WR 1065; X———X in rat plasma, other symbols and control curves as presented in figure 6.

Figure 9. Protection of T-cells by 10.4 mM WR 1065; symbols and control curves as presented in figure 6.

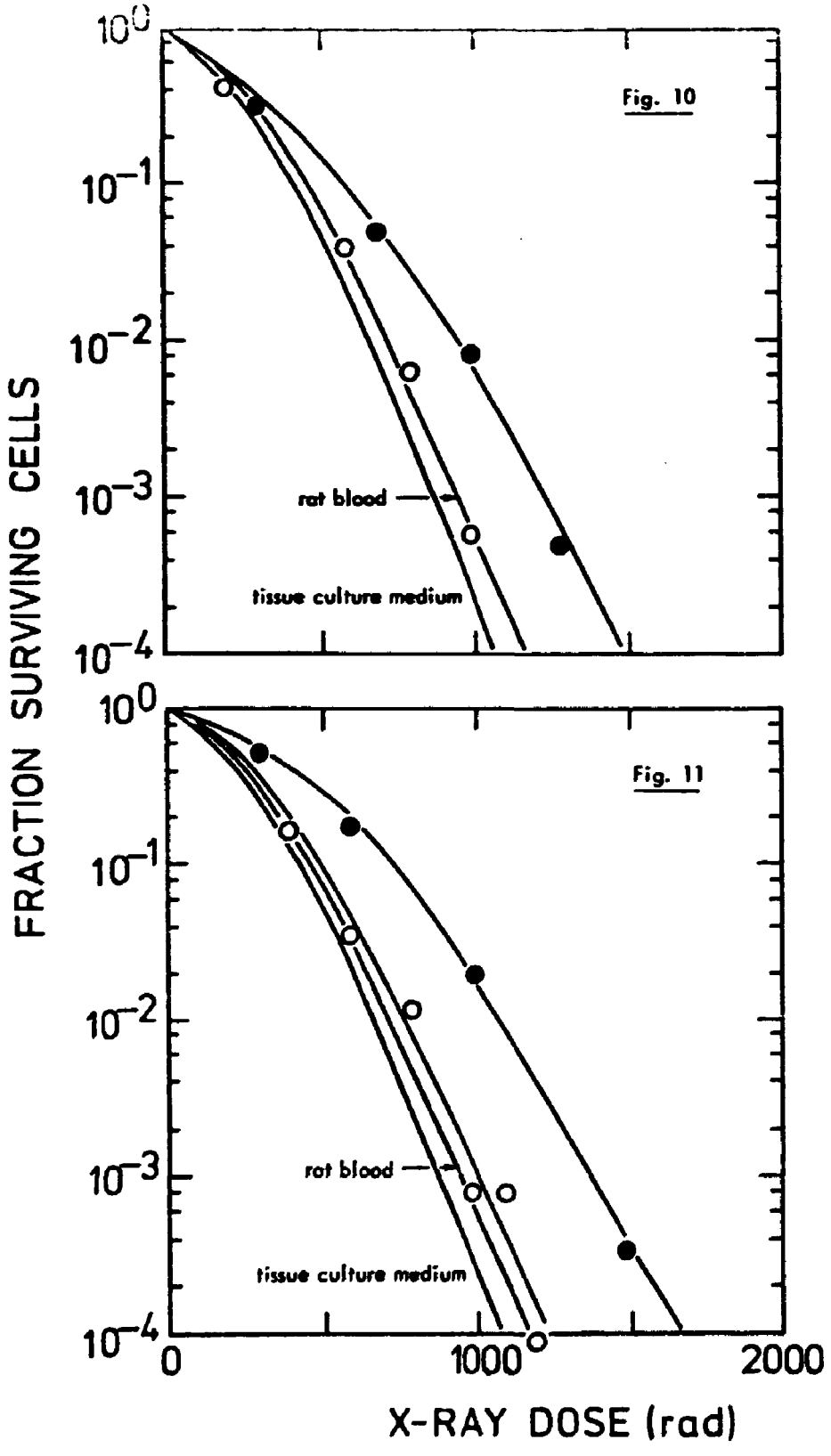



Figure 10. Protection of T-cells by 4 mM AE 48527; symbols and control curves as presented in figure 6.

Figure 11. Protection of T-cells by 16 mM AE 48527; symbols and control curves as presented in figure 6.

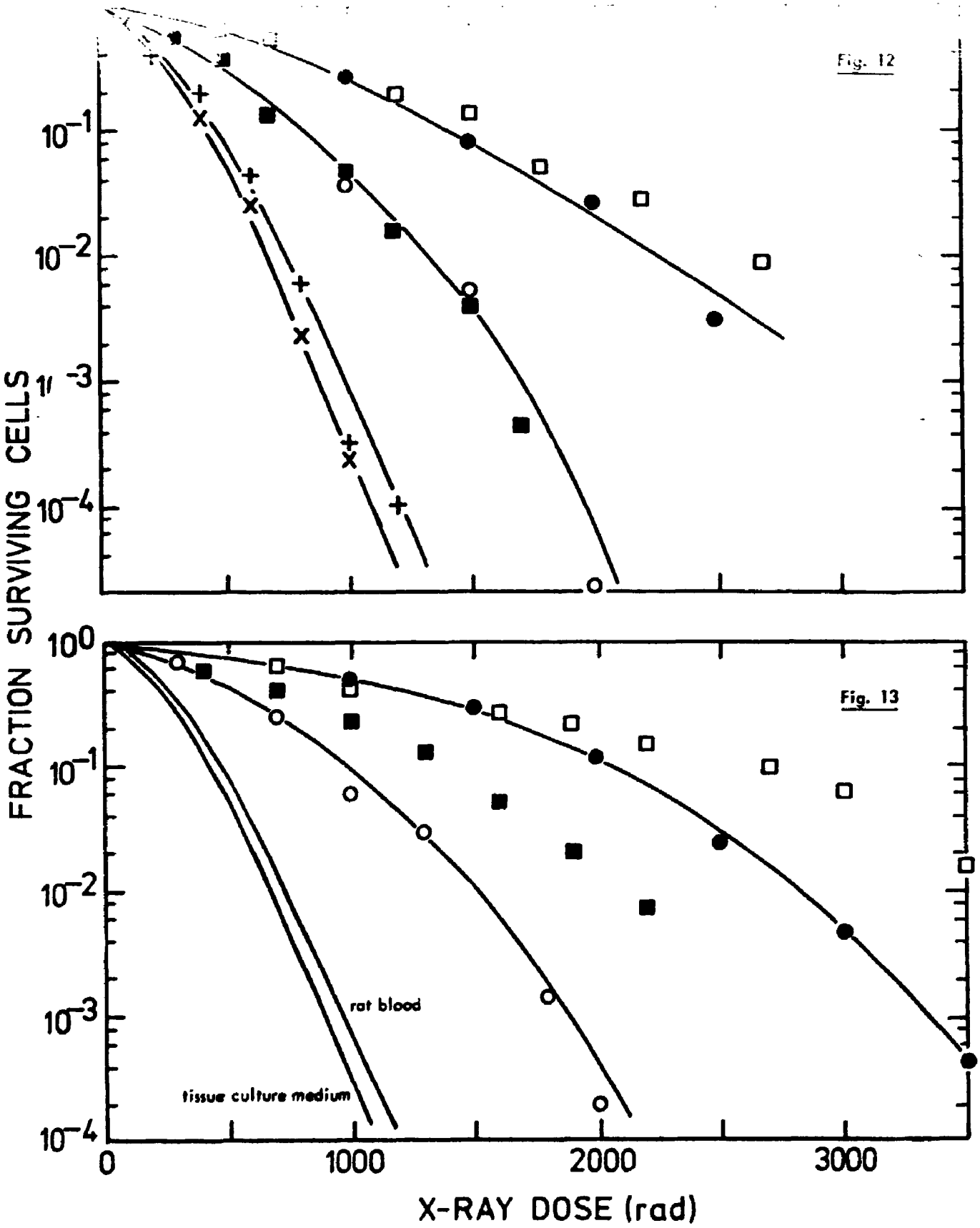



Figure 12. Protection of T-cells by 4 mM C 511; O——O in culture medium and ●——● in rat blood. Experimental data points obtained with 4 mM cysteamine (■ in culture medium and □ in rat blood) are added for comparison. Experimental data points for the control survival curves in tissue culture medium (x) and rat blood (+) without the addition of a protective compound are presented in this figure.

Figure 13. Protection of T-cells by 16 mM C 511; symbols and control curves as presented in figure 12.

DISTRIBUTIELIJST

DE AFDRUKKEN ZIJN ALS VOLGT GENUMMERD EN BESTEMD VOOR:

1. Voorzitter RVO-TNO
2. Gedelegeerde van de Minister van Defensie bij de RVO-TNO, Brig.gen. Ir. H. Doup
3. Plv. Gedelegeerde van de Minister van Defensie bij de RVO-TNO, Brig.gen. Ir. L. Barneveld Binkhuysen
   Lid (KL) van het bestuur van de RVO-TNO, Lt.Kol.Ir. A.K. v.d. Stal
   Plv. lid (KL) van het bestuur van de RVO-TNO, Lt.Kol. Ir. A. Kamphuis
4. Lid (KLu) van het bestuur van de RVO-TNO, Kol. P.J. Wamsteker
5. Plv. lid (KLu) van het bestuur van de RVO-TNO, Lt.Kol. Ir. H. Kreuning
6. Lid (KM) van het bestuur van de RVO-TNO, Ir. E. Atsma
   Plv. lid (KM) van het bestuur van de RVO-TNO, KTZ Ir. N. de Groote
7. Tijdelijk lid van het bestuur van de RVO-TNO, Brig.gen. b.d. Ir. C.J. van Tatenhove
8. Lid van het bestuur van de RVO-TNO, alsmede Voorzitter van de Militair Geneeskundige Raad, Schout bij Nacht-arts R. Kooper
9. Inspecteur Geneeskundige Dienst (KL), Brig.-Generaal arts J.W. Hartman
10. Inspecteur Geneeskundige Dienst (KLu), Cdre vliegerarts T.B. Kölling
11. Inspecteur Geneeskundige Dienst Zeemacht

Leden van de Contact Commissie NBC-bescherming RVO-TNO:

12. KLTZT C.C. Brandon Bravo
13. LTZT1 P.F.J. Brunsmann
14. Kol. Ir. R.J. van Erven
15. Lt.Kol. H.L.J. van der Grinten
16. Maj. J. Huivenaar
17. KLTZ Ir. C.J.M. de Keijser
18. Dr. J. Koster
19. KTZT F.J. Mittertreiner
20. Kol.-arts R.G. Nypels
21. KLTZAR J.W. Pinke
22. Lt.Kol. Ir. C.J.B.H. van der Pols
23. Kol.-vliegerarts H.J. Wiggers
24. Secretariaat RVO-TNO (oningebonden exemplaar)

25. Hoofd van de Afdeling Medisch Wetenschappelijk Beleid van de Inspectie Geneeskundige Dienst (KL)

26. Chef van de Pharmaceutische Dienst van de Inspectie Geneeskundige Dienst (KL)

27. Commandant NBC-school (KL), Breda

28. Commandant NBC-school (KLu), Rijen

29. Commandant NBCD-school, Den Helder

30. Adviseur voor Interne Zaken van het MBL-TNO, Prof. Dr. Joh. Blok

31. Adviseur voor Interne Zaken van het MBL-TNO, Prof. Dr. C. van der Meer

32. Adviseur van het MBL-TNO, Prof. Dr. D. Bootsma

33. Adviseur van het MBL-TNO, Prof. Dr. O. Vos

34. Adviseur van het bestuur van de RVO-TNO, t.b.v. het MBL-TNO, Prof. Dr. K.C. Winkler

35. De Hoogleraar in de Moleculaire Genetica, Rijksuniversiteit te Leiden

36. De Directeur van het Laboratorium voor Stralengenetica, Rijksuniversiteit te Leiden

37. De Directeur van het J.A. Cohen Interuniversitair Instituut voor Radiopathologie en Stralenbescherming, Leiden

38. De Directeur van de Stafafdeling Epidemiologie en Informatica, Ministerie van Volksgezondheid en Milieuhygiëne, Leidschendam

39. De Directeur-Generaal van het Rijksinstituut voor de Volksgezondheid, Bilthoven

40. Bibliotheek van de Gezondheidsraad, Rijswijk

41. Instituut voor de Toepassing van Atoomenergie in de Landbouw, t.a.v. de Heer H.J. Sonies, Wageningen

42. Bibliotheek van de Centrale Organisatie TNO

43. Voorzitter van de Gezondheidsorganisatie TNO

44. Directeur van het Radiobiologisch Instituut GO-TNO, Rijswijk

45. Prof. Dr. D. de Wied, Rudolf Magnus Instituut voor Farmacologie, Utrecht

46. Directeur van het Chemisch Laboratorium TNO, Rijswijk

47. Directeur van het Technologisch Laboratorium TNO, Rijswijk

48. Adj. Directeur Centrale Diensten der Prins Mauritslaboratoria TNO, Rijswijk

49. Bibliotheek van de Prins Mauritslaboratoria TNO, Rijswijk

| | |
|---|---|
| 50 t/m 52. | Hoofd van het Wetenschappelijk en Technisch Documentatie- en Informatiecentrum voor de Krijgsmacht |
| 53. | Plv. Directeur van het Medisch Biologisch Laboratorium TNO |
| 54. | Conservator van het Medisch Biologisch Laboratorium TNO |
| 55. | Mej. L. Budke, Medisch Biologisch Laboratorium TNO |
| 56. | Dr. J.J. van Hemmen, Medisch Biologisch Laboratorium TNO |
| 57. | G.A. Grant, Defence Chemical, Biological and Radiation Establishment, Ottawa 4, Ontario, Canada |
| 58. | Archief Medisch Biologisch Laboratorium TNO |
| 59. | Archief Medisch Biologisch Laboratorium TNO |
| 50 t/m 65. | Reserve exemplaren |