The effects of some radioprotective agents upon mouse liver -SH and -SS- levels

THE PART played by tissue sulphydryl (-SH) groups in radiation response is still the subject of discussion. To help clarify this situation detailed measurements of total -SH, protein -SH and acid soluble -SH have been made after treatment with protective doses of known radioprotective agents. Additionally, disulphides (-SS-) have been measured because of the finding by Calcutt and Ting[1] that the more radioresponsive tissues have higher -SS- levels than the less radioresponsive ones. Because of their suggested role in radioprotection mixed disulphides were also measured. Liver has been used in the current experiments because of the requirement for adequate tissue samples for analytical purposes.

Balb/c mice, aged 12–14 weeks were used for all experiments. The choice of male or female animals for any experiment was based on the availability of animals. Radioprotectors used were: Cysteamine (M.E.A.) 150 mg/kg; Cysteine 1 mg/kg; 2 aminoethylisothiouronium bromide hydrobromide (A.E.T.) 300 mg/kg; serotonin (5HT) 95 mg/kg and sodium monofluoroacetate 4 mg/kg.

All experiments were started at 10.0 a.m., the chemicals being given by intraperitoneal injection.

Total -SH, protein bound -SH and acid soluble -SH were estimated by the methods described by Calcutt and Doxey,[2] and Calcutt, Doxey and Coates.[3] Disulphides were estimated as described by Calcutt and Ting.[1]
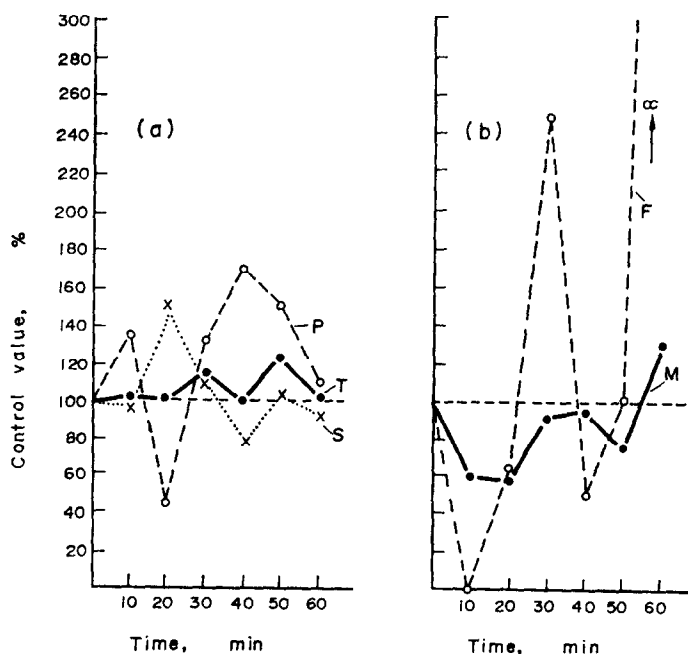

FIG. 1. Cysteamine on livers of female mice. (a) Effects on thiols. (b) Effects on disulphides. In this and successive diagrams curves are designated as: T, total -SH. S, acid soluble -SH. P, protein bound -SH. F, free disulphides. M, mixed disulphides.
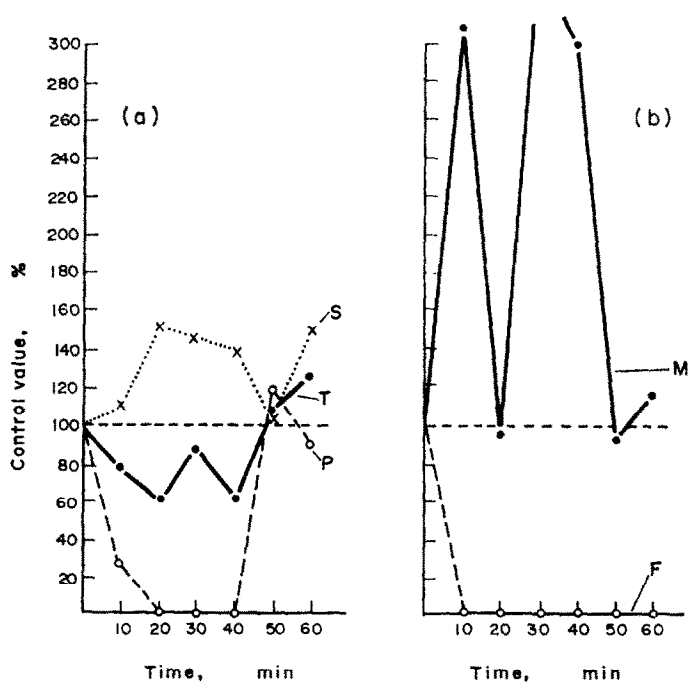
FIG. 2. Cysteine on livers of male mice. (a) Effects on thiols. (b) Effects on disulphides.
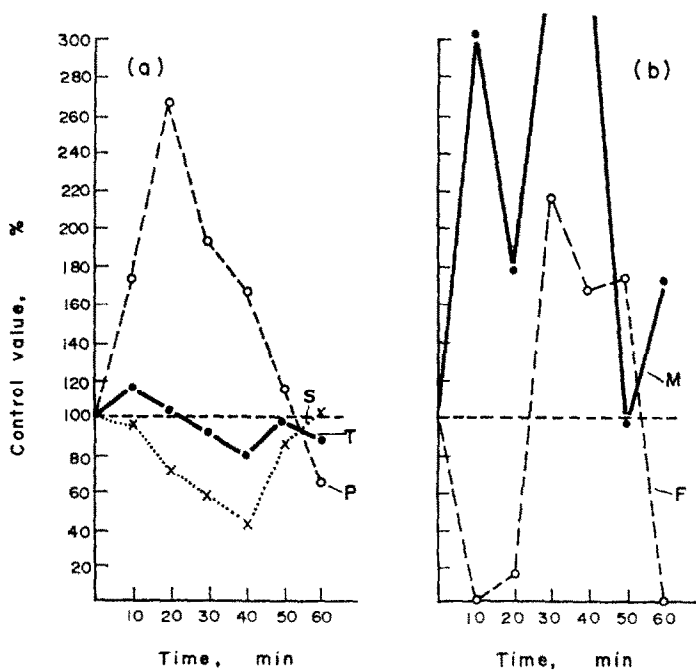

FIG. 3. A.E.T. on livers of male mice. (a) Effects on thiols. (b) Effects on disulphides.
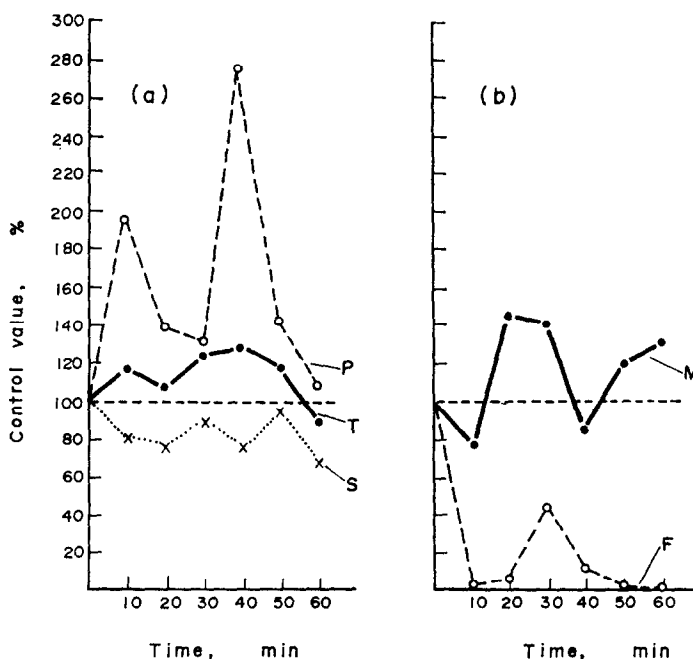
FIG. 4. Serotonin on livers of female mice. (a) Effects on thiols. (b) Effects on disulphides.

The findings are shown graphically in Figs. 1–5 inclusive. For the aminothiols and serotonin they cover a period of 1 hr after injection of the radioprotector. With sodium monofluoroacetate the period covered has been extended to 5 hr since protection has been found by Bacq, Liebecq-Hutter and Liebecq[4] to occur between 2 and 5 hr after injection.

All results are shown as a percentage of the corresponding figure for untreated controls taken at the same time. This has been done to overcome the difficulties arising from the rapid and extensive diurnal fluctuations known to occur in -SH levels[5,6] and in -SS-levels.[1] Each point is based on mean values of three to five independent measurements, this applying to both control and experimental series.

In the case of sodium monofluoroacetate the values for free disulphides have been omitted since so many zero values were encountered in both treated and control series that no reasonable assessment of the data was possible.

Although liver is generally a non radioresponsive tissue and can thus only be taken as a model the main interest in these results is in relation to mechanisms of radioprotection. So the general findings in respect of all components measured at times when radioprotection could be expected to be effective are summarized in Table 1. The additional time of 1 hr in the case of sodium monofluoroacetate has been included because of Novak's[8] finding of whole body protection at this time after injection of the protector.

The present results are in keeping with the finding of Calcutt, Connors, Elson and Ross[9] that cysteine raised the acid soluble -SH levels of rat liver, spleen and thymus shortly after injection. Bacq[10] has quoted Zins et al. to the effect that A.E.T. causes a loss of tissue glutathione and this is confirmed. The finding by Sorbo[11] that serotonin caused an increase in liver -SH levels and a fall in free disulphide levels is also confirmed.

There is no consistency of behaviour among the -SH levels after treatment with the aminothiols, let alone serotonin or sodium monofluoroacetate. Thus no support is found for the view of Revesz[12] that protectors give rise to an increased intracellular thiol level and thereby mediate protection. The data, also, do not support the role of radical scavenging by way of increased acid soluble or protein bound -SH.

Response of the mixed disulphide levels to the different protectors has been variable. This would cast doubt on the general validity of any theory of the formation of mixed disulphides as an immediate protective mechanism. The fact that there is a decline in level in some cases suggests that even if binding
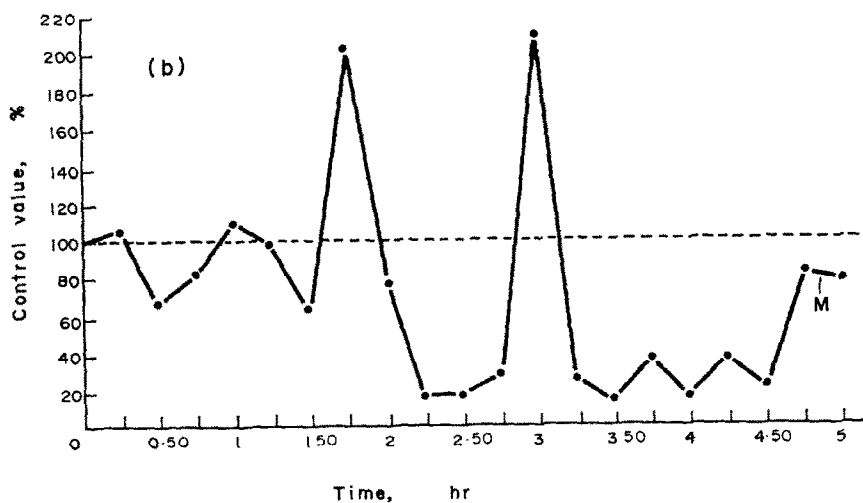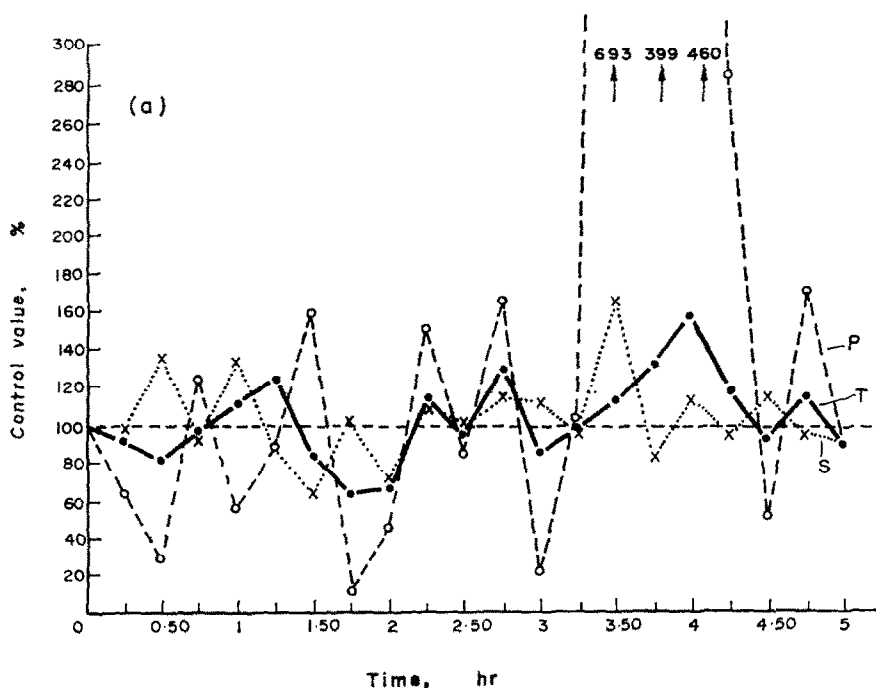
FIG. 5. Sodium monofluoracetate on livers of male mice. (a) Effects on thiols. (b) Effects on disulphides.

TABLE 1. STATUS OF LIVER COMPONENTS AT TIME OF PROTECTION

Agent	Time after injection	Total -SH	Acid soluble -SH	Protein bound -SH	Free -SS-	Mixed -SS-
Cysteamine	10 min	No change	Down	Up	Down	Down
Cysteine	10 min	Down	Up	Down	Down	Up
A.E.T.	10 min	Up	Down	Up	Down	Up
Serotonin	10 min	Up	Down	Up	Down	Down
Na. monofluoracetate	1 hr	Down	Up	Down		Down
Na. monofluoracetate	2–5 hr	Varies	Varies	Varies		Varies

of the protector to cellular protein occurs it is not via -SH groups or that an immediate compensatory, or over compensatory, loss of preexisting mixed disulphides occurs.

The four agents for which data were obtained all caused a fall in free -SS- levels at a time when protection would be expected. This is consistent with the previous finding[1] of higher -SS- levels in radioresponsive than in radioresistant tissues. Whilst under present circumstances it is not possible to envisage any critical role for a molecule such as -GSSG- (oxidized glutathione) in irradiation response it is evident that the problem of tissue disulphides demands further attention.

Radiotherapy Research Unit,   G. CALCUTT
Barossa Place,   S. M. TING
Bristol 1

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Inhibition of oligomycin sensitive and insensitive fish adenosine triphosphatase activity by chlorinated hydrocarbon insecticides*†

IN PREVIOUS reports[1-3] we have shown that chlorinated hydrocarbon pesticides cause inhibition of both Na^+-K^+ ATPase and Mg^{2+} ATPase activities. The inhibition occurred in a wide variety of