which has been found typical for uncomplicated major surgery. The phosphorus diuresis is a consistent finding and is usually observed to occur in association with retention of calcium.

Small doses of calcium-45 (14 μc.) and phosphorus-32 (20 μc.) have been administered parenterally to patients 48 hr. before surgery and the specific activity of the urinary calcium and phosphorus plotted against time[2]. In three out of eight patients studied, marked deviations from the expected calcium specific activity curve have been observed in the 48 hr. following operation (Fig. 2*A*). Two out of two phosphorus specific activity curves have shown a similar effect of operation (Fig. 2*B*).

It is suggested that the above observations are consistent with a post-operative calcium mobilization which, in most cases, could be associated with an increase in parathyroid activity. It could follow that surgical trauma produces a condition where there is a local or systemic demand by the tissues for calcium. This possibility, and the further speculation that changes in the calcium concentration of tissues may favour the spread and establishment of metastases, are at present being investigated.

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Changes in the Sensitivity of Enzymes in the Dry State to Radiation

There is now considerable evidence that the sensitivity to radiation of enzymes in the dry state is influenced by the medium in which the enzymes are dried.

It was first noticed that different, commercially available invertase preparations had different radiation cross-sections in the dry state in a vacuum. This observation led us to prepare invertase solutions from commercial yeast using the purification methods described by Dieu[1], Fisher and Kohtes[2], and Sumner and O'Kane[3]. Although the enzyme solutions were prepared from the same yeast, the sensitivity of the enzyme showed variations even at different stages of purification. In Table 1 some of these results are given.

It can be seen that on dialysis of the Dieu extract the sensitivity of the enzyme increases considerably. If the dialysate from the dialysed extract of the Dieu preparation is added again to the residue, the cross-section returns to that of the crude extract. After free SH groups had been shown to be present in the yeast extract, cysteine and glutathione were tested for protective ability and were found to decrease the cross-section of several enzymes. Drying the enzyme from acetate buffer or from salt solutions in some cases led to greater sensitivity to radioactivity. In Table 2 the relative radiation sensitivities (for 4-MeV. deuterons) of four different enzymes dried down in solutions of various compounds are listed. In general, the effect of a compound on the radiosensitivity varied with its concentration. The results in Table 2 were obtained with a quantity of the added compound approximately one to ten times that of the enzyme used. Increasing the amount of compound added did not appear to change the values in Table 2 very much.

Table 1. Inactivation Cross-section ($A.^2$) of Dry Yeast Invertase. 40 MeV. α-particles. Cross-section in yeast cell, 2,000 $A.^2$

Purification method	Dieu	Sumner	Fisher
Crude extract	1,350 ± 50	1,400 ± 100	2,400 ± 100
Dialysed extract	2,500		2,300
Precipitation	3,200		1,900
Final product		2,550	3,800

Table 2. Relative Radiation Sensitivities of Enzymes in the Dry State

Additive	Invertase	Ribonuclease	Papain	Urease
Pure	1·0	1·0	1·0	1·0
Cysteine	0·5	—	—	0·5–0·7
Glutathione	0·5	0·52 ± 0·10	—	—
Yeast extract	0·45	0·52 ± 0·10	0·6–0·8	0·8
Ribonucleic acid	—	0·41 ± 0·07		
Acetate buffer	1·4–1·6	4·8 ± 1·4		
Sucrose		2·6 ± 0·5		
Sodium chloride	1·5–3·5	0·98 ± 0·21		

The conclusion to be drawn from these results is that the effect of ionizing radiation can either be increased or decreased. Reduction in radiation sensitivity of dry materials by added substances has recently been reported by Norman and Ginoza for catalase[4] and ribonucleic acid[5] and has been frequently observed in radiation studies on high polymers[6].

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Radioprotective Action of 5-Hydroxytryptamine

Tryptamine and its hydroxyl derivative are powerful radioprotective amines *in vivo*[1,2], but they also give rise to physiological changes. Bacq and Alexander[3] state that tryptamine is not a vasoconstrictor, but is nevertheless as good a radiation protector of mice as 5-hydroxytryptamine. However, tryptamine causes intense smooth muscle contraction, similar to its hydroxyl derivative[4,5]. While this pharmacological activity for tryptamine is much less, molecule for molecule[6], our studies indicate this also applies to its radioprotective properties in rats.

Both amines protect polymer irradiated in oxygenated solution *in vitro*[2], and Bacq and Alexander regard the protective action *in vivo* as one of competition for radicals (particularly $HO_2^\cdot$) from radiolysis of water. Others[4] have suggested that protection *in vivo* may be due to an 'oxygen effect'.

Table 1. EFFECTS OF 5-HYDROXYTRYPTAMINE AND 5-HYDROXYTRYPTAMINE ANTAGONISTS ON LETHALITY, LOSS OF WEIGHT AND DIARRHŒA IN IRRADIATED RATS

Group* (Treatment)	No. of animals: mean weight (gm.)	No. of survivors (30 days)	Mean survival (days)	Weight loss, initial 10 days	Incidence of diarrhœa at fourth day (per cent)
Experiment 1†					
Controls (saline)	15 (181)	0	8·07 (± 4·69)	35	100
5HT ($0·5 \times 10^{-5}$ mole)	15 (180)	9	21·40 (± 9·93)	11	13
LSD ($0·6 \times 10^{-7}$ mole)	15 (189)	0	9·93 (± 3·83)	48	64
LSD ($0·6 \times 10^{-7}$ mole) + 5HT ($0·5 \times 10^{-5}$ mole)	15 (178)	1	11·33 (± 6·68)	14	50
Experiment 2					
Controls (saline)	10 (145)	0	7·10 (± 2·03)	—	100
5HT ($0·5 \times 10^{-5}$ mole)	10 (145)	9	22·90 (± 6·17)	17	0
BAS-phenol ($1·7 \times 10^{-5}$ mole)	10 (148)	0	5·90 (± 2·65)	- -	100
BAS-phenol ($1·7 \times 10^{-5}$ mole) + 5HT ($0·5 \times 10^{-5}$ mole)	10 (151)	0	7·10 (± 3·48)	- -	100
BAS-phenol ($0·3 \times 10^{-5}$ mole) + 5HT ($0·5 \times 10^{-5}$ mole)	10 (146)	0	12·30 (± 1·97)	17	0
Experiment 3					
Controls (saline)	10 (149)	0	5·10 (± 2·58)	—	100
5HT ($0·5 \times 10^{-5}$ mole)	10 (149)	4	20·70 (± 9·12)	30	10
BOL 148 (3×10^{-7} mole)	10 (150)	0	6·10 (± 2·38)	—	30
BOL 148 (3×10^{-7} mole) + 5HT ($0·5 \times 10^{-5}$ mole)	10 (153)	0	8·80 (± 3·40)	—	71

* All injections given intraperitoneally; 5-hydroxytryptamine 5 min. before irradiation. For *d*-lysergic acid diethylamide, 1-benzyl-2-methyl-3-(2 aminoethyl) 5 methoxy indole hydrochloride (BAS-phenol) and BOL 148, single injections given 5 min. before irradiation, or 10 min. before irradiation were followed by 5-hydroxytryptamine.

† In experiments 1 and 2, Canberra Black stock rats were used in proportions of 2 males to 1 female for each group. In experiment 3 Wistar hooded female rats were used. All animals received 1,000 r. whole-body irradiation.

We have investigated the effect of pretreating rats with antagonists and specific anti-metabolites, preceding administration of 5-hydroxytryptamine and tryptamine, and its effect on acute lethality from total-body irradiation (1,000 r. X-rays). Results of some of these experiments are set out in Table 1, for the 5-hydroxytryptamine anti-metabolites, *d*-lysergic acid diethylamide (LSD), its brominated-derivative (BOL 148), and the anti-serotonin, 1-benzyl-2,5-dimethyl serotonin (BAS phenol)[6]. Acute lethality was assessed for a 30-day period following irradiation, as in previous experiments[7]. The loss of weight and incidence of diarrhœa in survivors was also charted.

Antagonists and anti-metabolites of 5-hydroxytryptamine alone had no significant protective action, but inhibited that of 5-hydroxytryptamine and tryptamine. Anti-histamines failed to influence the protective action of 5-hydroxytryptamine but the radioprotective action of histamine[8] was inhibited.

Atropine and dibenzyline, which respectively block two types of tryptamine receptors[9], in doses of 0·25 mgm. failed to influence the protective action of 5-hydroxytryptamine.

Tranquillization of rats with reserpine (20 mgm. per kgm.), 18 hr. before irradiation, did not influence lethality significantly. Given 5–10 min. before irradiation, a slight protection resulted which could be increased by pretreatment with iproniazid (100 mgm./kgm.) given 12 hr. before reserpine. However, administration of 5-hydroxytryptamine 5 min. before irradiation to reserpinized rats resulted in a radioprotective effect, comparable to 5-hydroxytryptamine alone in similar doses. Reserpine exerts a non-specific antagonism to 5-hydroxytryptamine and other amines in smooth-muscle preparations[10], and besides releasing and depleting these amines by a change from bound to free form, apparently reduces their capacity to react with cell receptors. An anti-metabolite (for example, 1-benzyl-2,5-dimethyl serotonin) is pictured as acting by excluding a structurally related metabolite (5-hydroxytryptamine) from combination with its normal specific reactant (5-hydroxytryptamine receptor)[11].

The results obtained point to a close correlation between the radioprotective action of certain amines *in vivo*, and their respective pharmacological actions. However, the narrow range of tissue oxygen tension, which governs the oxygen effect in radioprotection *in vivo*[12], seems an inadequate explanation for some of the results obtained, and in particular, the protection which results from the endogenous release of 5-hydroxytryptamine by combining reserpine with an amine oxidase inhibitor. The conception of radioprotection *in vivo* as largely one of competition for free radicals, also fails to afford a convincing explanation for the specificity of antimetabolites, in modifying the protective action of certain aromatic amines.

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