Albumin/globulin ratio was nearly the same in all the cases. Beta-globulin fraction is the highest in all toads in both the seasons. This is similar to the findings reported earlier[5,6] for other animals. Gamma-globulin was lowest in all the cases.

The levels of alpha-globulin in male and female toads were significantly different ($P > 0.01$) in both seasons. Changes in globulin fractions were seen in female toads: beta- and gamma- globulin fractions increased significantly ($P > 0.05$ and $P > 0.01$ respectively) in summer. This is similar to the results of Ashton[7] for sheep. The alpha-globulin fraction was significantly higher during winter in female toads ($P > 0.001$). Perk and Loble[8] had found a direct genetic control on plasma protein fractions.

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RADIOMODIFYING EFFECT OF CHAMPHOR AS REVEALED BY SISTER CHROMATID EXCHANGE

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SISTER chromatid exchange (SCE) represents reciprocal exchanges between replicated sister chromatids at homologous points and its analysis is a sensitive means of monitoring genotoxicity[1-3]. Camphor has been reported to enhance radiation-induced damage in bacterial cells under hypoxic conditions[4], in mouse transplantable mammary adenocarcinoma[5], and in spermatogonia of adult mice[6]. In view of the potential utility of camphor in cancer therapy, studies were undertaken to evaluate the radiomodifying effect of camphor on normal tissues by SCE analysis. In this paper we report the radioprotective effect of camphor in mouse bone marrow.

Three-to-four-month-old inbred Swiss albino mice were randomly assorted into 15 groups (table 1), each with four animals. Each animal, except controls, received camphor (0.5 μmol/g body wt) dissolved in olive oil intraperitoneally. Whole-body gamma irradiation was given 30 or 45 min after administration of camphor. One day before sacrificing the animals, 9 injections of BrdU (10^{-2} M), spaced hourly, were administered intraperitoneally. Each injection contained deoxycytidine (5×10^{-3} M) to reduce the toxicity to BrdU. Seventeen hours after the last BrdU injection, colchicine (0.4 mg/ml) in distilled water was injected intraperitoneally (4 mg/100 g body wt). Two hours later, animals were sacrificed by cervical dislocation. Chromosome preparations were made and stained with Hoechst 33258 and Giemsa[7]. Animals were kept at 25±2°C and food and water were given *ad libitum*.

Table 1 presents the frequency of SCE in bone marrow cells after various doses of gamma irradiation in the presence or absence of camphor. In the different control groups (first three rows in table 1) the frequency of SCE was within normal limits. Animals given camphor and no radiation showed enhancement in SCE frequency but that was not statistically significant. Animals exposed to increasing doses of whole body gamma radiation showed significantly higher incidence of SCE.

Animals administered camphor prior to irradiation showed a marked reduction in SCE compared to the correspondingly irradiated group that did not receive camphor (table 1 and figure 1). Compared to 30 min interval between camphor treatment and irradiation, 45 min interval resulted in a slight decrease in number of SCE. The difference between the 30 min and 45 min groups is significant only at 2.0 Gy radiation dose. Further, the reduction in SCE frequency due to camphor became more pronounced with increasing levels of radiation exposure. Maximum reduction in frequency of SCE by camphor was at 2.0 Gy. The radioprotective potential of camphor is thus evident from this study.

The bone marrow cells are well oxygenated and oxygen is an effective radiation sensitizer. The present findings agree with previous reports[1]. Goel *et al.*[6] studied radiosensitizing effects of camphor in the testicular system, which is often used as a hypoxic model for *in vivo* studies. They observed

Table 1 *Radiomodifying effect of camphor as assessed by effect on frequency of sister chromatid exchanges in bone marrow cells of Swiss albino mice*

Treatment	No. of metaphases scored	Total no. of SCEs	SCEs per metaphase Mean ± SE
Control (no treatment)	180	497	2.76 ± 0 08
Olive oil only	189	535	2.89 ± 0 07
Camphor + olive oil (0 5 μmol g body wt)	250	866	3 40 ± 0 04
0.25 Gy only	300	1490	4 73 ± 0.06
0 25 Gy + camphor (30 min)	150	597	3.98 ± 0 06
0.25 Gy + camphor (45 min)	155	589	3 83 ± 0 06
0.50 Gy only	199	1118	5 61 ± 0.06
0.50 Gy + camphor (30 min)	222	926	4 23 ± 0.05
0 50 Gy + camphor (45 min)	190	792	4.20 ± 0 05
1.00 Gy only	165	960	5.85 ± 0.06
1 00 Gy + camphor (30 min)	170	767	4.65 ± 0.06
1.00 Gy + camphor (45 min)	145	618	4.19 ± 0 07
2.00 Gy only	5*	36	7.20 ± 0.65
2.00 Gy + camphor (30 min)	50	247	4 94 ± 0 12
2 00 Gy + camphor (45 min)	58	249	4.29 ± 0.10

*The low number is due to nonavailability of sufficient second division metaphases.

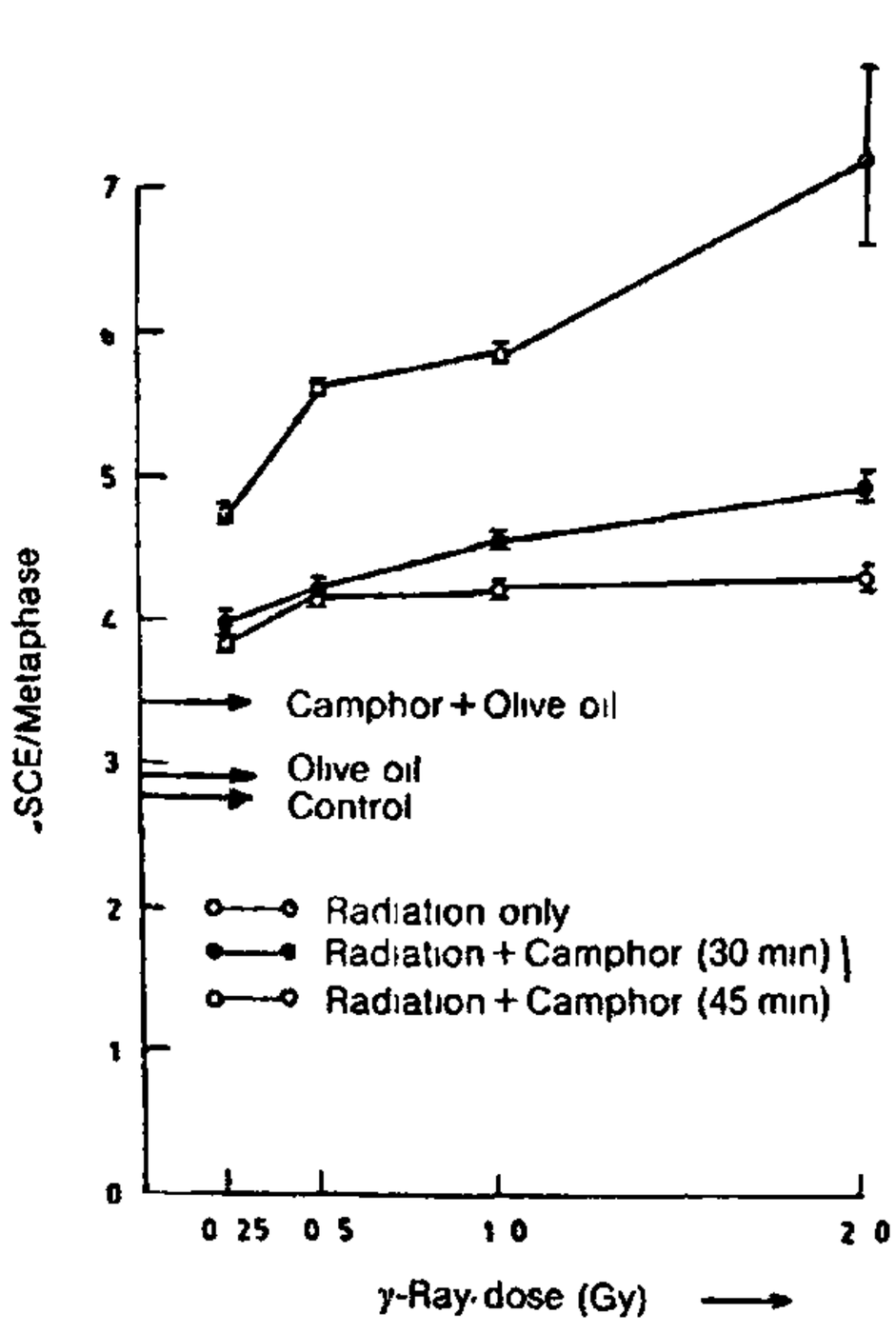

Figure 1. Effect of camphor on gamma irradiation-induced SCE in bone marrow cells of Swiss albino mice.

accelerated damage to resting primary spermatocytes in mouse testis due to irradiation in presence of camphor. Camphor has been shown to act as hypoxic sensitizer in bacterial cells[4] and also in mammary adenocarcinoma in C_3H Jax mice[5].

The radioprotective and radiosensitizing behaviour of camphor for euoxic and hypoxic cells respectively during irradiation show its potential for meeting the most ideal requirement of a chemical radiomodifier for improving clinical radiotherapy. However, the differential behaviour of camphor for oxic and hypoxic tissues is yet not well understood and further investigations in this direction are in progress.

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A NOVEL METHOD FOR CLONING CHROMOSOMAL DNA IN *HAEMOPHILUS INFLUENZAE* Rd

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PLASMID pRSFO885, genetically marked with an ampicillin-resistance marker[1], transforms *Haemophilus influenzae* very poorly[2]. However, if sequences homologous to chromosomal DNA are spliced to its only *Pvu*II site, transformation frequency increases a thousand-fold for the *amp*^{r} marker[3]. The enzyme *Pvu*II produces blunt ends in the DNA. Consequently, ligation to DNA segments is not as efficient as with a sticky-end-cut DNA vector and insert. A vector, pJ1–8, has been derived from pD7 (consisting of a chromosomal DNA segment spliced to pRSFO885) which has only one *Eco*R1 site (and no *Pvu*II site)[4]. Like pRSFO885, pJ1–8 gives little or no transformation for the *amp*^{r} marker, but when *Eco*R1-generated segments of chromosomal DNA are spliced to it, the frequency of Amp^R transformants increases by 3–4 orders of magnitude. Using this plasmid, several DNA clones carrying alleles of *nov*, *str*, *nal* and *uvrI* genes have been cloned[5–7]. However, most of the clones (except pKuvr1) are lost in the absence of selection pressure. A similar observation has been made[8] with DNA fragments cloned in pRSFO885. A new type of cloning system was serendipitously discovered. Plasmid pRSFO885 has two *Bam*HI sites, generating fragments designated here as A and B. The *amp*^{r} marker is on fragment A. In an attempt to clone DNA in one of the two sites by partial digestion, a few stable clones were obtained, although at a very low frequency. Analysis of some of these revealed that all such clones should carry a duplication of the 2.45 kb *Bam*HI fragment of pRSFO885. This report describes the construction and isolation of such clones.

A wild-type strain of *H. influenzae* Rd was used. Plasmids used were pJ1-8 (ref. 4), pRSFO885 (ref. 1), pJ1-8Str^{R}38, pJ1-8Str^{R}14 and pJ1-8Nal^{R}33 (ref. 6).

H. influenzae cells were grown at 37°C with aeration in BHI (brain heart infusion, Difco) broth containing 2 μg/ml NAD and 10 μg/ml haemin. Cells were grown to exponential phase, before freezing at −73°C in the presence of 15–20% glycerol.

Cells were made competent by the aerobic–anaerobic method[9]. A transformation mixture normally consisted of 0.1 ml competent cells + 0.1 ml DNA + 0.8 ml BHI broth. DNA uptake was allowed for 10–15 min before plating. Plates were challenged with ampicillin (5 μg/ml) after 1.5–2 h of incubation by overlaying with 10 ml BHI agar containing ampicillin.

Chromosomal DNA was extracted by the method of Marmur[10]. Plasmid DNA was extracted by the method of Hirt[11], with minor modifications. Plasmid DNA was purified by cesium chloride–ethidium bromide equilibrium density gradient centrifugation[12].

For electrophoresis of DNA, 0.7–1.4% agarose gels were used in Tris–acetate buffer (pH 8). Electrophoresis was carried out at 4 V/cm for 4–5 h[13].

Restriction enzymes and T4 DNA ligase were purchased from Bethesda Research Laboratories and used according to their instructions.

Plasmid loss was measured[8] by growing the cells overnight without ampicillin, and then plating on plates without ampicillin. Colonies were replica-plated on plates with or without ampicillin. Colonies that grew on plates without ampicillin but failed to grow on plates with ampicillin were considered to be those of cured cells.

pRSFO885 DNA has two *Bam*HI sites and it was considered possible that either of the two sites may be used for the cloning of DNA. Accordingly, *Bam*HI-digested chromosomal DNA was spliced with a partial digest of pRSFO885. Upon exposing a competent wild-type cell culture to the ligated DNA, a large number of Amp^R transformants were obtained (approximately 2×10^4 per ml). Five hundred Amp^R colonies were picked and analysed. Transformation using clear lysates obtained from these clones revealed that most of them give little or no transformation for the *amp*^{r} marker (less than 1×10^3 per ml) when reused for transformation. Only four clones were exceptions in that they gave high transformation frequency (table 1). These clones were designated pS43, pS121, pS322 and pS436. Agarose gel electrophoresis of the clear lysates showed that all the plasmids that fail to give *amp*^{r} transformation fall into any of the 3–4 groups of particular sizes (data not shown). Apparently,