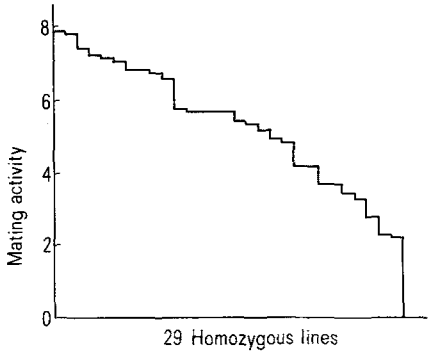

Fig. 2 Male mating activity of 29 homozygous lines.

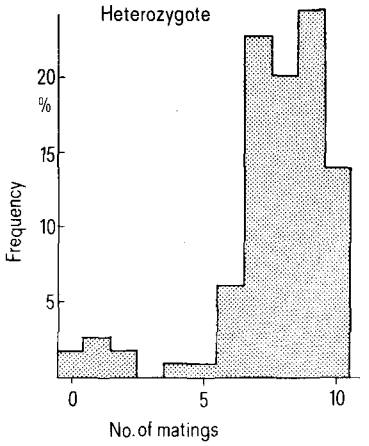

Fig. 3 Frequency distribution of mating activity in heterozygous males.

within 24 h. The mean mating activity expressed as the number of matings on an individual basis was calculated to be 5.348 ± 0.142. Figure 2 represents the male mating activity of 29 homozygous lines. The male mating activity over the array of 29 lines was 5.261 ± 0.311. The number of copulations in the highest line (7.83 ± 0.37) is more than 3 times as large as that in the lowest one (2.17 ± 0.73). Analysis of variance disclosed that the genetic difference among 29 lines is significant at the 1% level (table). If we include the sterile lines, then genetic variability becomes even more distinctive. Male mating activity in heterozygous individuals, that is, progenies from the natural population is given in figure 3. They are assumed to be random heterozygotes for the 2nd chromosome and to have the same genetic background as homozygotes. The distribution of mating activity in heterozygotes differs greatly from that in homozygotes (fig. 1), essentially in that mating activity is enhanced. The majority of heterozygote males mated at least 6 times within 24 h. The frequency of 'low' individuals who mated less than 6 times is very low in comparison with homozygous males. 16 out of 109 males (14.7%) mated at least 10 times. Mean mating frequency in heterozygous males was 7.670 ± 0.207, and this is significantly higher than that in homozygous ones. The coefficient of variation in homozygous males is much smaller, indicating perhaps a homeostatic nature of this behavioural character. The observed variation in mating activity suggests that mate selection may be unexpectedly intense in natural populations. It may also imply that fertility including mate selection, in a broad sense, is a more important fitness component than are fitness variants in preadult stages[5].

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Submandibular gland-conditioned medium enhancement of bone marrow colony-forming cells in tumor-bearing murine recipients[1,2]

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Summary. Soft-agar clonogenic assay techniques were used to examine 2 sources of colony-stimulating activity (CSA), submandibular gland-conditioned medium (SMG-CM) and pregnant mouse uterine extract (PMUE), for potentiation of granulocyte-macrophage (GM-CFUc) or monocyte-macrophage (M-CFUc) progenitor cell populations. The femoral populations being examined were aspirated from normal mice and from those bearing 1 of 2 types of tumor: Lewis lung carcinoma (3LL) or thymic lymphoma ascites tumor (EL-4). When PMUE was used as a CSA source, normal animals showed a greater clonogenic response per population than either of the tumor-bearing groups. When SMG-CM was used as a CSA source, the pattern of GM-CFUc response was much different: GM-CFUc magnitudes increased by fourfold to sixfold over normal levels in tumor-bearing animals. M-CFUc response patterns were also significant, being similar in response but smaller.

Generally speaking and regardless of etiology, cancer is a known disruptor of normal hemopoiesis even though the blood-forming tissues (as we know them) are not directly involved. It is generally accepted that lymphopoiesis is suppressed[3-5] and myelopoietic homeostasis is disturbed[6]. The degree and direction of disruption may depend on the time and type of tumor being investigated, since both myeloid increases[7,8] and decreases[6,9] have been reported. Divergences in the literature may be due to a number of factors, among which may be: a) regulatory factors are being produced by the tumors mass(es)[8,10] or b) an altered bone marrow response pattern could be due to specific cellular metabolic prerequisites[11] or a change in makeup of the femoral matrix pattern, i.e., microenvironment.

Materials and methods. Recipient mice were (C57BL/6×CBA)F1 Cum Br hybrid (B6CBF1) male mice (Cumberland View Farms, Clinton, TN), 10–12 weeks old. 3LL is a transplantable metastatic tumor that is initated by s.c. implantation of 0.2 ml tumor brei. Tumor brei was obtained by excising tumors 12–13 days after implantation and passing them through a tissue press. The resultant separated cell suspension was layered on a Ficoll-Hypaque (Pharmacia) density gradient spun at 2200×g for 20 min. The cell layer was aspirated off, washed in RPMI-1640, resuspended, counted, and examined for viability via trypan blue exclusion (90%–99%). 2×10^5 3LL cells resulted in 100% tumor incidence. EL-4 is an ascites tumor passaged by i.p. injection of 2×10^4 cells and harvested at 4 days. EL-4 cells underwent handling procedures similar to 3LL cells.

Femoral target cell populations were obtained from mice 12–14 weeks old. Mice with palpable tumors 18 days postinjection were used as marrow donors. Our rationale for use of the 18-day donor model stemmed from the determination (table 1) that despite early quantitative reductions of 10–15%, marrow cellularity returned to essentially normal level by day(s) 18/19. Femurs were aseptically removed from mice killed by cervical dislocation. They were cleaned of excess tissue, and the marrow plugs were aspirated out. The bone marrow/plug suspension was then pushed through 6 layers of nylon mesh to disperse cells into a single cell suspension.

Colony determations were conducted in 35×10 mm sterile culture dishes. The bottom layer consisted of 0.5% nutrient-supplemented agar and 10% (v/v) CSA source (PMUE, SMG-CM). The overlayer was 0.33% nutrient-supplemented agar and 2.5×10^4 bone marrow cells. Plates were kept in a 37 °C humidified incubator and gassed with 5% CO - 95% air. Plates were scored for GM-CFUc on day 10, and M-CFUc arising later were scored on day 22[12].

Results. GM-CFUc on day 10 of culture are shown in table 2. In the absence of a source of CSA, no background clonal growth was detected in either the normal or tumor-bearing marrow populations. In the presence of PMUE, 14 ± 0.4 colonies were seen in normal marrow cell cultures, and fewer arose from tumor-bearing animals: 11 ± 0.7 from 3LL and 9 ± 0.3 from EL-4 ($p<0.01$). When SMG-CM was used as a source of CSA, a much different clonogenic picture was seen. Normal marrow cell cultures demonstrated 9 ± 0.4 colonies, and cultures from marrow of 3LL-bearing mice gave 57 ± 1.7 colonies ($p<0.001$). EL-4-bearing mice similarly demonstrated an enhanced response pattern, with 45 ± 1.2 ($p<0.001$) colonies. Colonies were aspirated and stained with Giemsa. Morphological appearance was that of granulocytes.

M-CFUc colonies on day 22 of culture are shown in table 3. Nonstimulated normal bone marrow cells showed no colony growth, and marrow from tumor-bearing animals showed infrequent colony growth. In the presence of PMUE, normal marrow demonstrated 16 ± 1.0 colonies compared to 10.2–10.8 colonies ($p<0.005$) from marrow of tumor-bearing animals. When SMG-CM was used as a source, no M-CFUc growth was observed in cultures of normal marrow cells. Applied to marrow derived from tumor-bearing animals, SMG-CM resulted in the formation of 4–6 colonies ($p<0.001$). Aspirated colonies morphologically resembled macrophages and actively phagocytized *E. coli.*

Discussion. Spontaneous or transplanted tumors[13,14] are assumed to perturb hemopoietic homeostasis by affecting the distribution and dynamics of progenitor cells. This situation arises even in instances where hematopoietic tissue is not directly involved.

Previous CFU-s and CFU-C progenitor cell assays have shown that neoplastic growth(s) result in altered bone growth kinetics[6,9]. Presence of Ehrlich ascites tumor in mice has been noted to result in a decrease of both CFU-s[9] and CFU-C[6]. The colony-forming cells were found to be at low levels on days 3–5 postinjection, followed by gradual recovery to normal levels by day 10. However, other investigators, using other tumor lines, reported that tumor presence resulted in an augmentation of colony formation. Baum and Fisher[7] demonstrated an increase in the numbers of macrophage colonies in animals bearing spontaneous mammary tumors. Milas and Basic[8] showed an increase in myeloid colonies in the CFU-s pool found in mice bearing a transplantable fibrosarcoma. We previously reported that SMG-CM contains colony-stimulating activity[15]. We report here that the femoral marrow of animals bearing either 3LL or EL-4 possesses a significantly greater percentage of progenitor types responsive to SMG-CM than cells responsive to PMUE, a known positive source of colony-stimulating activity.

A possible explanation of the differences seen in colony numbers may be the presence of a specific type of colony-stimulating activity within the SMG-CM. Ledney et al.[11] have proposed that subpopulations of CFC may exist in 3LL-bearing animals, which require specific growth media-

Table 1. Cellularity per femur in 3LL hosts

Groups	Cellularity $\times 10^7$	SEM	Percent of control value
Control	2.2	±0.09	
Days post tumor			
7	1.9	±0.17	86
9	1.9	±0.02	86
12/13	2.0	±0.06	91
18/19	2.2	±0.30	100
27	1.5	±0.01	68

Table 2. GM-CFUc responses of tumor-bearing mice to SMG-CM and PMUE

Treatment	Cell type	Colonies per plate (2.5×10^4 cells)						Mean	SEM	p
Control	Norm BM	0	0	0	0	0	0	0	0	
	3LL BM	0	0	0	0	0	0	0	0	
	EL-4 BM	0	0	0	0	0	0	0	0	
PMUE	Norm BM	16	14	13	13	14	15	14.17	0.4	
	3LL BM	10	8	13	13	12	11	11.17	0.7	<0.008
	EL-4 BM	8	8	10	9	9	9	8.83	0.3	<0.000
SMG-CM	Norm BM	11	8	9	9	10	9	9.33	0.4	
	3LL BM	64	57	56	56	54	57	57.40	1.7	<0.000
	EL-4 BM	43	47	42	50	43	46	45.17	1.2	<0.000

Table 3. M-CFUc responses of tumor-bearing mice to SMG-CM and PMUE

Treatment	Cell type	Colonies per plate (2.5×10^4 cells)						Mean	SEM	p
Control	Norm BM	0	0	0	0	0	0	0	0	
	3LL BM	1	0	0	0	1	0	*	0	
	EL-4 BM	0	0	0	0	0	0	0	0	
PMUE	Norm BM	21	15	14	16	14	16	16.00	1.0	
	3LL BM	9	10	11	12	11	12	10.83	0.4	<0.005
	EL-4 BM	11	9	10	8	11	12	10.17	0.6	<0.000
SMG-CM	Norm BM	0	0	0	0	0	0	**	0	
	3LL BM	5	6	7	6	7	7	6.20	0.3	<0.000
	EL-4 BM	4	3	4	4	6	5	4.33	0.4	<0.000

* Occasional colonies were noted in a small fraction of plates (1 colony each in 2 of 11 plates); ** colony numbers on day 22 decreased from day-10 counts.

tors. SMG-CM may supply the growth requirements necessary for cellular proliferation of progenitor cell subpopulations in the tumor-bearing animal. Another alternative may be that the tumors may act directly or indirectly to reduce total marrow cellularity. Clerici et al.[16] found that administration of 8.5×10^5 tumor cells reduces total marrow cellularity by 60–80%. Although our decreases were of considerably less magnitude and despite the fact that normal quantitative levels were reached by day(s) 18/19, it remains possible that a tumor-induced marrow depletion selective for cells that are unreactive to CSA may explain the inordinate increase noted in the responsive cellular elements reported here. Lastly, it cannot be ruled out that the tumorigenic states may in themselves mediate a leukemoid type augmentation, such as that seen by Burlington et al.[10] in the case of a spontaneous mammary adenocarcinoma.

This report demonstrates that a change does take place in the CSA response pattern of the femoral marrow cells taken from a tumor-bearing animal. There is a profound increase in the proportion of progenitor cells that are responsive to colony-forming factors found in SMG-CM and that are evident in the marrow populations of tumor-bearing animals. Further work is planned to ascertain whether the effects reported here are attributable to the SMG elaboration of 'unique' growth-regulating substance(s) or whether the effects reflect tumor-induced changes in the murine medullary progenitor cell compartment.

1 Research was conducted according to the principles enunciated in the 'Guide for the Care and Use of Laboratory Animals', prepared by the Institute of Laboratory Animal Resources, National Research Council.
2 Supported by Armed Forces Radiobiology Research Institute, Defense Nuclear Agency, under Research Work Unit MJ 00041. Views presented in this paper are those of the authors. No endorsement by the Defense Nuclear Agency has been given or should be inferred.
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Glutathione peroxidase, superoxide dismutase and catalase in the red blood cells of GSH-normal and GSH-deficient sheep[1]

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Summary. Levels of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase were measured in the red blood cells of glutathione(GSH)-normal and GSH-deficient sheep. There were no significant differences in any of the 3 enzyme activities measured in the 2 groups of sheep. Also, there was no relationship between GSH level and the enzyme activity. These results suggest that inspite of large differences in GSH levels, the red blood cells from GSH-normal and GSH-deficient Merino sheep appear to have similar response to oxidative stress against which GSH is credited to play a major role.

GSH is a widely occurring peptide that is found in relatively large amounts in the liver, kidney, red cells and the lens[3]. The contributions of GSH to the metabolic economy of the red blood cells have been extensively studied and it has been suggested that GSH protects intracellular components from oxidative attacks and thus plays a significant part in maintaining the viability of the red blood cells[4]. Considerable variation exist in red cell GSH concentrations both within and between domestic mammalian species[5]. Because these variations are not always associated with haemolytic disease, the comparative biochemical analysis of GSH metabolism in different species has provided an insight into the relative significance of the different aspects of red cell GSH metabolism[5].

GSH metabolism in sheep red cells has been well studied[5]. Sheep exhibit 2 biochemically distinct forms of inherited GSH deficiency known as the Merino type and the Finn type. The biochemical lesion responsible for the Merino type GSH deficiency is a diminished activity of γ-glutamyl cysteine synthetase, the first enzyme of GSH biosynthesis. The Finn type GSH deficiency is due to defective amino acid transport across the red cell membrane; the enzymatic machinery required for GSH synthesis in these sheep cells appears to be normal. Based on haematological investigations, it appears that the red blood cells with the Merino type GSH deficiency are not detrimentally affected under normal conditions. However, studies on Finn type GSH deficiency suggests that these red cells may be more susceptible to oxidative stress than normal red cells[5].

We have studied several physiological and biochemical parameters of GSH-deficient and GSH-normal Merino sheep[5]. In continuation of these studies, we now report our results on the levels of 3 enzymes (GSH-Px, SOD and catalase) that are associated with GSH metabolism and the