pentobarbital sodium anesthesia. Care was taken to avoid damage to the sublingual gland and surrounding lymphoid tissues. Sham-operated mice of matched age were used as controls. Each mouse was injected i.v. with 0.1 ml of a 20% suspension of SRBC (4×10^8 cells) on day 0. Some of the SMG-ectomized male mice were injected i.p. with the extracts described below at a dose of 50 µg protein/g b.wt daily 4 times from day 0 to day 3. Mice were killed by cervical dislocation on day 4 and the spleen was removed. It was minced in a petri dish containing 2.5 ml of ice-cold Eagle's medium with heparin. The material in the dish was transferred to a tube, allowed to settle for 10 min, and then decanted into another tube. The cell suspension was diluted to contain 10^6–10^7 cells/ml. Plaque-techniques were carried out by the method of Jerne and Nordin[4].

The extracts were prepared as follows; the SMG from 12-week-old male, female and castrated male mice (castration was performed at the age of 8 weeks) were homogenized with saline and the supernatants obtained by centrifugation at $105{,}000\times g$ for 1 h were designated as saline extracts. Protein content in the extracts was measured by the method of Lowry et al.[5].

Results. The removal of SMG caused suppression of the PFC response to SRBC in male but not female mice at 8 weeks after the operation. The number of PFC in the SMG-ectomized males was about $^1/_3$ of the value in the control males (figure 1). The administration of the saline extract from male mice to the SMG-ectomized males brought the suppressed response back to the levels seen in sham-operated controls. The extracts from females and castrated males did not affect the suppressed response (figure 2). It seems unlikely that the suppression of PFC response observed in the SMG-ectomized males is due to the nonspecific effect caused by SMG-ectomy because the administration of the SMG-extract from normal males restored the suppressed response to the levels seen in sham-operated controls. Thus the results presented above suggest that the SMG of male mice contains a possible endocrine factor or factors which influences immune response to SRBC and that the factor is androgen-dependent.

Discussion. The SMG of mice is an interesting organ, rich in biologically active proteins such as nerve growth factor (NGF) and epidermal growth factor (EGF). Concerning its effects on immunological cells, Kongshavn and Bliss[6] have demonstrated that extracts from male mouse SMG prolong the survival of H-2 incompatible skin allografts in mice. Koch and Rowe[7] have reported on the effect of SMG-extract on antibody formation in response to SRBC. There is no report, however, examining the possibility that the removal of the SMG in mice influences the immune response. Our previous[3] and present results suggest that the SMG of male mice contains a possible endocrine factor or factors capable of influencing cells involved in immune responses and that the factor(s) is androgen-dependent. But the divergence of findings between delayed type hypersensitivity and the SRBC response is still inexplicable.

A marked sexual dimorphism of the mouse SMG is well known[8,9]; NGF, EGF and proteolytic enzyme levels are much higher in males than in females and these proteins are induced by androgens[10–12]. Therefore the possibility that one or several of these proteins may be involved in the regulation of the immune response is worth considering. Further investigations are needed to clarify whether 1 or different factors regulate cell-mediated and humoral immune responses and to study the target cells of the factor(s).

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Adenosine deaminase activity in peripheral blood cells from SJL/J mice[1]

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Summary. Adenosine deaminase (ADA) activities were measured in peripheral blood cells of SJL/J mice, and compared with those of C57BL/6J animals. No association was observed between the levels of lymphocyte or erythrocyte ADA and immunologic abnormalities in SJL/J mice nor was evidence obtained to suggest a relationship between ADA activity and tumorigenesis in this strain.

In 1972, it was reported that the blood cells of 2 patients with severe combined immunodeficiency disease were deficient in adenosine deaminase (ADA), an enzyme of the purine salvage pathway[2]. This discovery was soon confirmed and then extended by other investigators to include findings relevant to the role of genetics and to the mechanism of the defect[3].

Recently, several investigations have failed to demonstrate an association between ADA deficiency and immunodeficiencies in animals such as the marmoset, mouse and horse[4,5]. This paper describes a related study with SJL/J mice, an inbred strain characterized by immune disorders and by spontaneous development of reticulum cell neoplasms[6].

Materials and methods. Animals. Female SJL/J mice were supplied from our breeding colony in the Animal Services Center, University of Alabama in Birmingham. Strain C57BL/6J females, obtained commercially (The Jackson Laboratory, Bar Harbor, Maine), were used as controls.

Blood cell preparations. Blood samples were collected by tail bleeding into heparinized tubes from groups of 12–16 mice at 12, 22, 32, 42, and 52 weeks of age. The lympho-

Adenosine deaminase activity in blood cells of mice*

Age (weeks)	Adenosine deaminase (units/mg protein)** Lymphocytes		Erythrocytes	
	SJL/J	C57BL/6J	SJL/J	C57BL/6J
12	0.43±0.074	0.20±0.027	0.093±0.009	0.089±0.012
22	0.59±0.062	0.23±0.035	0.091±0.008	0.080±0.004
32	0.52±0.077	0.24±0.047	0.096±0.008	0.093±0.005
42	0.48±0.038	0.17±0.022	0.081±0.009	0.078±0.005
52	0.52±0.054	0.16±0.013	0.074±0.010	0.083±0.008

* Values determined from samples of 12–16 mice and expressed as means ± SE. ** 1 unit of activity is defined as the amount of enzyme which deaminates 1.0 µmole of adenosine per min at 25 °C.

cytes were separated by a method which was developed to allow for their isolation from small volumes of peripheral blood[7]. Then, they and the resultant erythrocyte fractions were washed in cold Hanks' balanced salt solution, resuspended in phosphate-buffered saline (pH 7.4), and lysed by freezing and thawing at −60 °C.

Enzyme assay. Lymphocyte and erythrocyte ADA activities were determined spectrophotometrically in a final volume of 1.0 ml by a method described previously[8]. The reaction mixture contained 0.2 mM adenosine in 0.05 M phosphate buffer (pH 7.4), 0.1 unit of nucleoside phosphorylase (or present in erythrocyte lysates), and 0.2 units of xanthine oxidase (Sigma Chemical Corporation. St. Louis, Missouri). After addition of the sample and incubation at 25 °C for 6 min, the absorbance of the solution was recorded at 293 nm for 4 min. ADA activity is expressed as µmoles of adenosine deaminated per min per mg of protein at 25 °C. Protein was determined by the Bio-Rad Assay (Bio-Rad Laboratories, Richmond, California).

Results and discussion. The results, which are summarized in the table, indicate that lymphocyte ADA activities were higher in SJL/J mice than C57BL/6J animals. Further, both strains exhibited little variation in enzyme activity throughout the test period. These findings suggest that a deficiency of lymphocyte ADA is not associated with immune disorders of SJL/J mice or with the development of reticulum cell neoplasms in this strain.

As also shown in the table, ADA activities in erythrocyte lysates were similar in strains SJL/J and C57BL/6J at all ages tested. Thus, it would appear that neither modifications in the immune status of SJL/J mice nor spontaneous tumor development are reflected by alterations in the levels of erythrocyte ADA.

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Local leukocyte mobilization in irradiated or cyclophosphamide-treated rats[1]

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Summary. Sprague-Dawley rats made neutropenic by ^{60}Co irradiation or cyclophosphamide treatment retained a limited capacity for mounting a local leukocyte mobilization (LLM) response. Rats irradiated with 700 rad ^{60}Co lacked an LLM. Rats treated with 100 mg/kg cyclophosphamide showed no LLM following an initial low response when assayed originally.

The plastic skin chamber technique has been developed to study local leukocyte mobilization (LLM) to a skin abrasion site[2,3]. A flexible plastic cup is glued over a skin abrasion and filled with serum or other osmotically buffered fluid. The cup contents are removed at selected time intervals, and the cells are counted or microscopically assayed. Since neutrophils constitute over 95% of the cells found in chambers during the first 24 h, the cells counted are a measure of neutrophil migration to the skin abraison[4]. Also, assay of skin chamber fluid at 24 h provides a superior comparative assessment of an animal's LLM capacity due to kinetic variations[5]. The plastic skin chamber technique has been used to study in vivo LLM as a function of fluid contents in the chamber or various disease conditions in the animal[3–8].

This report is an examination of LLM in female Sprague-Dawley rats, weighing 250–325 g, following exposure to ^{60}Co radiation or cyclophosphamide (Sigma). Rats were irradiated with ^{60}Co at 40 rad/min or administered cyclophosphamide by tail vein injection prior to installing skin chambers. When circulating neutrophil counts in blood drawn from rat tail veins dropped to a nadir 3 or 4 days after treatment, skin chambers were installed on the animals. The chambers were glued over skin abrasions on the right or left lateral thoracic surfaces of the rats. Blood neutrophils were determined by counting white blood cells on a Coulter Counter Model F (Coulter Electronics, Inc.) and making differential counts of blood smears. The plastic skin chambers were filled with sterile physiological saline having 250 µg/ml *Salmonella typhosa* lipopolysaccharide W (Difco). Approximately 24 h later, fluid was removed from the skin chambers, and cells in the fluid were counted and sized with the aid of a Coulter Counter equipped with a Coulter Channelyzer Model H4. Skin chamber fluids were also examined microscopically. Following removal of a chamber from a rat, another plastic skin chamber was installed over a skin abrasion on the opposite thoracic surface of the animal and assayed as described above approximately 24 h later. Control and experimental animals were assayed for the LLM response concurrently, and no attempt was made to determine the LLM of treated rats before irradiation or cyclophosphamide injection.

The number of neutrophils migrating to plastic skin chambers on control rats was reproducible when the fluid used to