Further Studies on Serum Protein Formation by Chimeras* (33990)
II. Induction of Chimerism with Sublethal X-Irradiation and Heterologous Antiorgan Serum

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(Introduced by C. A. Stetson)
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It has been shown that heterologous antiserum against lymphocytes (ALS) or thymocytes (ATS) is a potent immunosuppressive agent and significantly prolongs survival of allografts or xenografts of skin or other organs in animals so treated (1–4). However, its prolonged administration is accompanied by many untoward side effects many of which are incomptaible with host survival (5, 6). On the other hand, although allogeneic or xenogeneic bone marrow transfer protects lethally irradiated mice from radiation death, there is still a very high incidence of fatal secondary disease (7, 8). The present experiments were designed to induce xenogeneic chimerism using a combination of sublethal X-irradiation and the administration of small quantities of ALS or ATS in order to avoid the many systemic complications that may be encountered when either of these procedures is used alone.

The synthesis of donor type serum proteins by tissues *in vitro* from postirradiation rat-into-mouse chimeras has been demonstrated previously (9, 10) by the incorporation of ^{14}C-amino acids into serum proteins and subsequent autoradiography of immunoelectrophoretic patterns of concentrated culture fluids obtained from these tissues (11). The same method was applied in the present study to determine whether the combination of low dosage X-irradiation and ALS or ATS would be sufficient to permit the survival of the xenogeneic bone marrow cells in their hosts.

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Materials and Methods. Prepartaion of antimouse ALS and antimouse ATS. Lymph nodes from the axillary and cervical regions or thymus glands were obtained from $C_{57}B1/_6$ male mice (Millerton Labs., Millerton, N. Y.), weighing 25–30 g. The cell suspensions were prepared by gently pressing the tissue through a 60-mesh stainless steel wire screen into medium 199 (Microbiological Associates, Bethesda, Md.). These cells were washed twice and resuspended in 0.15 M saline containing 0.1% dextran (Pharmacia, Uppsala, Sweden) and 100 IU of heparin/10^9 cells. The cell suspensions were adjusted to the desired concentration.

Adult male New Zealand rabbits, weighing 2 kg were given 2 intravenous injections of 8–20 $\times$ 10^8 lymphocytes or thymocytes in a volume of 5 ml at weekly intervals. The rabbits were bled 1 week after the second injection. The antisera were inactivated at 56° for 30 min, absorbed twice with mouse red blood cells in a ratio 1:1.5 and sterilized through Millipore filters. The antisera were stored at —20° until used.

Production of chimerism. SJL/J male-mice (Jackson Memorial Laboratory, Bar Harbor, Me.) weighing 25–30 g were used as recipients of rat bone marrow cells obtained from Sprague-Dawley male rats, weighing 200–250 g (Blue Spruce Farms, Altamont, N. Y.). The bone marrow cells were collected in medium 199 from tibiae, femora, and humeri. The cells were washed once, filtered through layered gauze, and adjusted to the desired concentration. The 16 recipients (placed into 2 groups, ALS treated and ATS treated), were given intravenous injections of 9–10 $\times$ 10^7 bone marrow cells in a volume of 0.5 ml, within 4 hr of 450 R whole-body X-irradiation. The conditions of the X-irradiation have been described previously

TABLE I. Formation of Donor Type Serum Proteins by Tissues *in Vitro* at Various Intervals after ALS or ATS and Bone Marrow (BM) Transfer.

Tissues for culture	After BM-transfer (weeks)	Donor type IgG_2 synthesis			
		ALS group		ATS group	
		+	—	+	—
Spleen	2	2[a]	0	2	0
	4	2	0	2	0
	6	2	0		
	7			2	0
Mesenteric lymph nodes (pooled from 2 animals)	2	2	0	2	0
	4	2	0	2	0
	6	1	0		
	7			1	0

[a] Each value represents the number of cultures in which rat IgG_2 was found labeled.

(12). 3 days later, the mice received subcutaneous injections of 0.25 ml of ALS or ATS twice weekly for 7 weeks. The animals were given Terramycin in their drinking water and Aureomycin in their food (approx 6 mg/kg/day; Aurofac, General Biochemicals, Chagrin Falls, Ohio) starting 2 days before the X-irradiation for the duration of the experiments.

Blood samples for immunoelectrophoretic analysis of sera and alkaline phosphatase reaction of peripheral leukocytes for identification of rat cells were obtained from the retroorbital plexus of individual mice at weekly or biweekly intervals. The sera were stored at —20° until analyzed by microimmunoelectrophoresis according to the method of Scheidegger (13). Blood smears were stained according to the method of Kaplow (14) for alkaline phosphatase activity.

Cultures and analysis of culture fluids. The mice were sacrificed biweekly and their spleens and mesenteric lymph nodes were removed aseptically. Approximately 80–100 mg of spleen tissue from individual animals and 50–70 mg of mesenteric lymph nodes pooled from 2 animals were minced and cultured for 24–48 hr at 37° in roller tubes with 1–2 ml of medium as previously described (10). ^{14}C labeled lysine and isoleucine (100–200 μCi/mmole; Schwarz Bioresearch, Orangeburg, N. Y.) were added to a concentration of 1 μCi/ml each. The analysis of concentrated culture fluids and the identification of mouse or rat serum proteins, including transferrin (Tr), by means of autoradiography of immunoelectrophoretic patterns were performed as described previously (10, 11).

Results. The mean survival time is difficult to evaluate because of the small numbers of animals in each group which were sacrificed periodically for organ cultures. Three mice from the 2 groups died during the first 2 weeks after the experimental procedure. The spleens of these animals ranged in size from normal to enlarged and all 3 animals exhibited a marked anemia. The others survived well but showed some evidences of secondary disease beginning 2 weeks after bone marrow injection. Most of their spleens and mesenteric lymph nodes were found to be enlarged when sacrificed.

The majority of animals in both groups were chimeras as demonstrated by the synthesis of rat IgG_2 by their lymphoid tissues *in vitro*. Radioimmunoelectrophoresis of culture fluids from spleens and mesenteric lymph nodes of mice treated with sublethal X-irradiation and with either ALS or ATS showed definite rat IgG_2 synthesis at all intervals studied 2–7 weeks after bone marrow injection (Table I, Fig. 1). The intensity of staining and frequency of appearance of precipitation lines with spleen and mesenteric lymph node cultures from the ALS group were similar; whereas in the ATS group, the

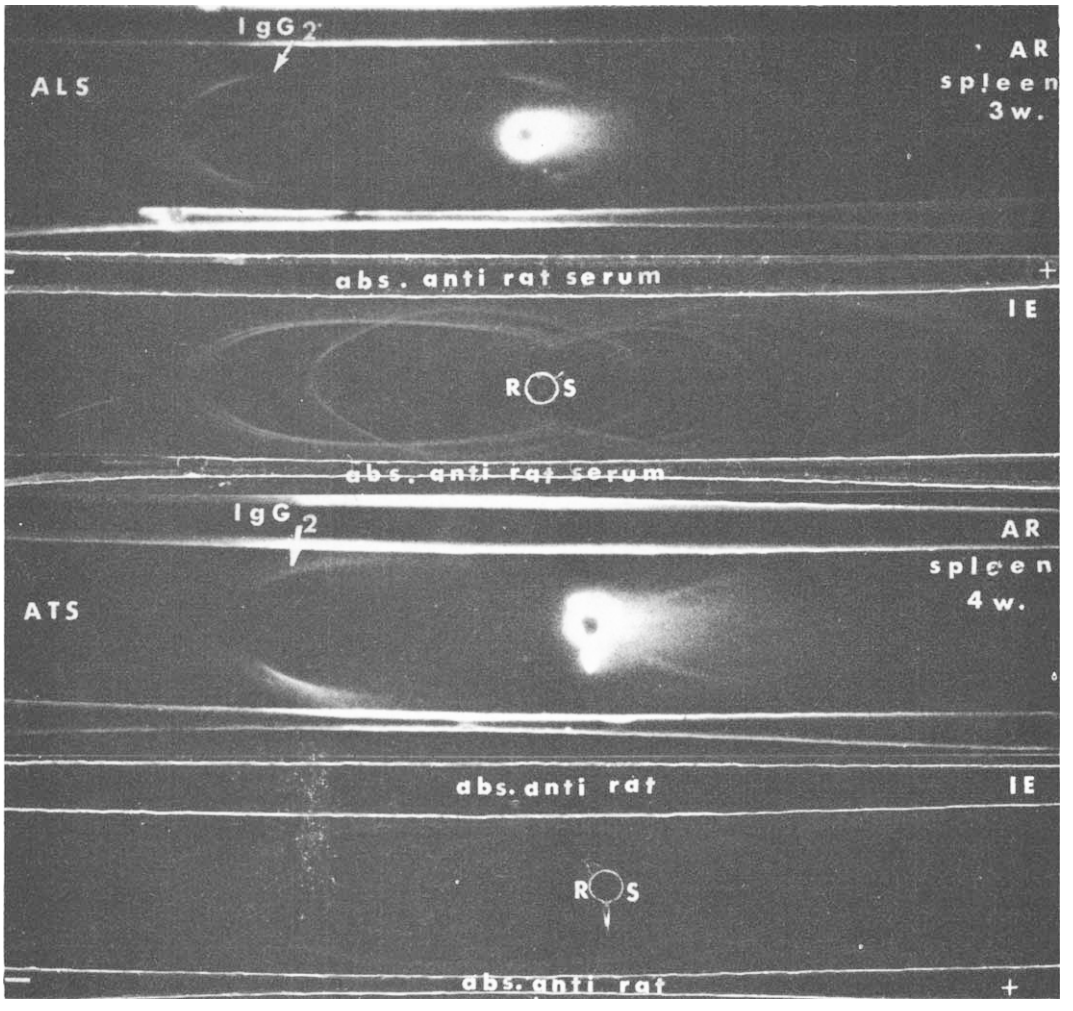

FIG. 1. Autoradiograph (AR) of immunoelectrophoretic (IE) patterns developed with rat serum (RS) as a carrier and culture fluids obtained from spleens removed 3 weeks (ALS group) and 4 weeks (ATS group) after bone marrow transfer. The intensity of labeling of IgG_2 with culture fluids obtained from the ATS treated animals is stronger than that from the ALS-treated animals.

labeling obtained with spleen culture fluids was more pronounced than with the mesenteric lymph nodes from the same animals and the spleen cultures from the ALS group of animals. No other rat serum proteins were synthesized by the host lymphoid tissue *in vitro* other than rat Tr which was clearly labeled in the 2 spleen cultures and 2 mesenteric lymph node cultures specifically examined for it.

Synthesis of mouse serum proteins *in vitro* was clearly demonstrated at all intervals studied. There was definite labeling of mouse immunoglobulins at 2, 4, 6, or 7 weeks (Fig. 2). Culture fluids were not specifically examined for mouse Tr. Alkaline phosphatase positive cells were not found in the smears of their peripheral blood and donor type serum proteins could not be identified in their sera.

Discussion. It was shown that radiation death of lethally irradiated mice can be prevented by the protection of these animals with isogeneic (IBM), allogeneic (ABM) or xenogeneic (XBM) bone marrow grafts

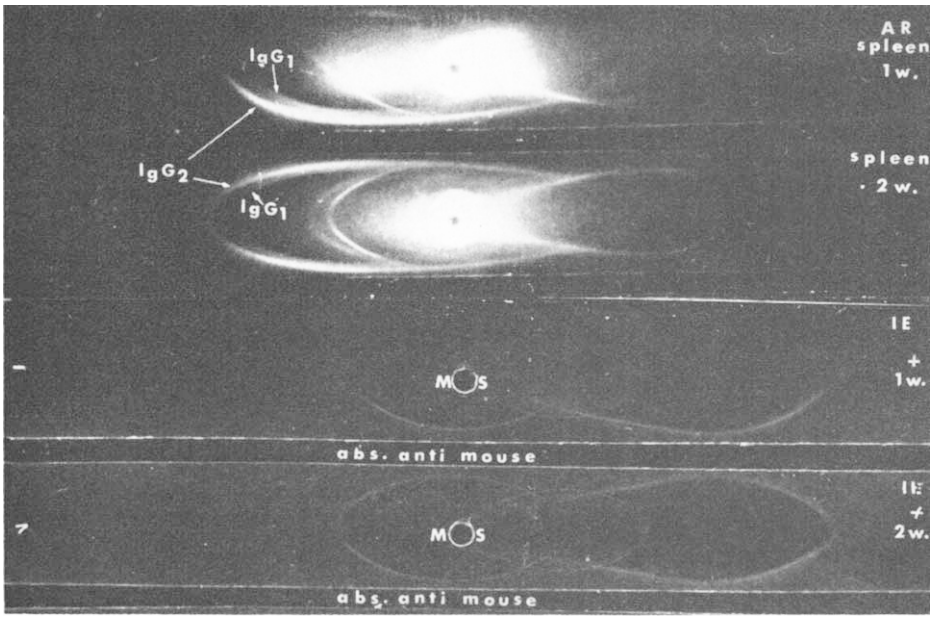

FIG. 2. Autoradiograph (AR) of immunoelectrophoretic (IE) patterns developed with mouse serum (MS) as a carrier and culture fluids obtained from spleens of chimeric animals 1 and 2 weeks after bone marrow injection. There is strong labeling of mouse immunoglobulin at both intervals.

(15). In contrast to the results obtained with lethal X-irradiation, X-ray dosages in the range of 500 and 600 r and subsequent protection with rat bone marrow grafts, increased the 30-day mortality above that of the control groups receiving no treatment of IBM (14). No effect was apparent at 400 r. This midlethal dose effect was attributed to an acute rejection of the transplanted marrow cells by the host (7, 8). The incidence of acute rejection of the graft is dependent on the radiation dose and can only occur if recovery of the host's immunological defense system is sufficiently rapid.

Xenogeneic chimeras, although protected from radiation death, show an incomplete recovery with an increased incidence of mortality 2–4 weeks after treatment as a consequence of the graft-versus-host reaction. Our preliminary experiments (16) to produce xenogeneic chimerism with ALS or ATS alone or combined with sublethal X-irradiation failed because of excessive doses of ALS or ATS given too frequently resulting in severe anemia, leukopenia, generalized wasting and early death of the animals.

In the present experiments, successful induction of chimerism and increased survival were obtained by treatment with and less frequent administration of small doses of ALS or ATS, previously absorbed with mouse red blood cells, combined with sublethal X-irradiation. These findings are in accord with those of Van Bekkum *et al.* (17) who demonstrated a suppression of both acute and delayed secondary disease in allogeneic mouse and monkey chimeras following treatment of the recipients with ALS. The results obtained in the present study can be attributed to the marked reduction in the side effects on the host (from lethal X-irradiation or large doses of ALS) that were incompatible with survival and also to the establishment of adequate central and peripheral immune suppression afforded by a fortuitous combination of these 2 treatments, permitting xenogeneic graft survival.

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The Effect of Changes in Arterial pCO_2 on Isogravimetric Capillary Pressure and Vascular Resistances* (33991)

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Total and prolonged arrest of the circulation can result in an increased vascular permeability (1–3). Among the factors that might change capillary permeability under the condition of absent or reduced blood flow, the first to be considered are those associated with continued metabolism of tissues such as reduced oxygen tension, local pH changes due to accumulation of metabolites such as lactic acid, and increased pCO_2.

The primary object of the present investigation was to determine if changes in carbon dioxide tension causes an alteration in capillary permeability in the canine forelimb. In studies on single mesenteric capillaries of frogs, Landis (4) found that changes in pCO_2 within the physiological range do not affect capillary permeability. Since no studies have been carried out in a mammalian species, it was considered to be of value to devise experiments using a method which permitted measurement of changes not only in a single capillary but in a complete organ and to also determine the effects of changes in pCO_2 on pre- and postcapillary vascular resistances.

Methods. Experiments were performed on mongrel dogs of either sex anesthetized with sodium pentobarbital, 30 mg/kg of body weight. Sixteen experiments were carried out in which one forelimb of a small dog (5–7 kg of body wt) was surgically removed above the elbow. Leg tissue was severed between double ligatures. Cutaneous bleeding was controlled by cautery and that from marrow cavity stopped with bone wax. All veins were dissected free for several centimeters above the joint. All animals were given heparin (5 mg/kg of body wt) at completion of surgery.

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