morula or in blastula stage. Differences between the percentage of ova with two to four blastomeres and the percentage of morulae and blastulas are not significant ($P < .25$), as in the first experiment.

The lower percentage of developing ova in the second experiment can be accounted for by the lower fertility of superovulated ova of the H strain. From the total of 76 ova from mice of the H strain isolated after a 36-hour cultivation, only 46 percent developed to two- to four-blastomere stage, and after an 84-hour cultivation only 49.2 percent developed to the morula and blastula stage from 67 isolated ova of the same animals.

Even though our experiment has not provided evidence for the dying of developing fertilized ova in the period before implantation, nor for their slowing in development, the relatively small number of experiments does not allow us to generalize these results. The disturbances in development of ova in the period before implantation may be manifested in some other inbred strains. Differences in the occurrence of the lethal gene t^{12} (*7*) may serve as example.

Part of the strain-H ova fertilized in vitro was left in organ culture for 144 hours. After 120 hours we observed in some blastocysts a herniation of their walls from one opening of the zona pellucida (Fig. 1B). Later this formation resembled a blastocyst (Fig. 1C). It was also possible to observe a migration of cells in the form of an unorganized cell mass, a part of which reminded one of the giant trophoblastic cells. An analogous development of blastocysts in vitro has been observed elsewhere (*8*). Dickson (*9*) observed the structural changes of blastocysts after their passage into the uterus, but he did not find similar forms.

The last test of the viability of oocytes fertilized in vitro and cultivated 72 to 84 hours was their transplantation to recipient animals. Eighty-three morulae and blastulas were transplanted to 19 female recipients that had been mated with fertile males. From nine pregnant females there were, however, only three with four developed transplanted embryos. Genetic control was assured by the use of pigmented recipients (C57/BL) and albinotic donors (H) (Fig. 2). Although only about 5 percent of transplanted morulae and blastulas implanted, the result may be regarded as being successful.

The relatively high fertility and normal development of the ova fertilized in vitro is a further advance on results of Brinster and Biggers (*1*) and made it possible to obtain, in some cases, normal embryos after transplantation.

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Serum Alpha Globulin Fraction: Survival-and-Recovery Effect in Irradiated Mice

Abstract. *An alpha macroglobulin fraction (19S) was isolated from the serum of rats and BC_3F_1 mice by zonal ultracentrifugation. Both the isologous and heterologous macroglobulin fractions increased survival among BC_3F^1 mice x-irradiated with 750 roentgens. The mouse macroglobulin fraction also enhanced radiation recovery of hematopoietic tissue as measured by colony-forming assay and iron-59 incorporation into erythropoietic cells. The overall difference in hematopoietic activity in the irradiated (400 roentgens) mice treated with the macroglobulin fraction, in comparison with this activity in the controls, was three- to fivefold in the bone marrow and nine- to tenfold in the spleen between days 4 and 7 after irradiation. This effect was not obtained with the isologous serum protein fraction containing proteins of smaller molecular weight.*

During the course of immunologic studies on mice injected with isologous and horse macroglobulin labeled with ^{125}I, radiation damage from the isotope and subsequent spleen recovery was observed. On day 7 after injection of isologous ^{125}I-macroglobulin with high specific activity, a two- to threefold increase in spleen weight was measured. Histologically, there was a marked hyperplasia of hematopoietic cells in the spleens of these mice. Normal animals injected either with horse macroglobulin labeled with ^{125}I of a comparable specific activity or with unlabeled isologous macroglobulin did not show these changes. Therefore, we investigated the effects of various macroglobulin preparations on irradiated mice, and found that isologous and rat macroglobulin isolated by zonal ultracentrifugation significantly enhanced radiation survival and accelerated the recovery rate of hematopoietic tissue.

Macroglobulins were prepared by centrifuging 25 to 30 ml of whole serum on a 1000-ml sucrose-density gradient in the B-IV zonal ultracentrifuge (*1*). The separation and characterization of the rat macroglobulin has been described (*2*). For separation of mouse and horse macroglobulins, the ammonium sulfate step cited in the procedure for rat macroglobulin was omitted. The gradient was monitored at 260 and 280 mμ as it passed through a 0.2-cm Oak Ridge flow cell; a Beckman B spectrophotometer, modified as described by Anderson (*3*) was used. The separation showed two peaks. The gradient was collected in 40-ml fractions. After four to six runs were made, the material in the second peak was pooled and concentrated. Analytical ultracentrifugation showed that it contained approximately 50 percent of 19*S* protein (about 750,000 molecular weight). An ultracentrifugally homogeneous 19*S* fraction was obtained by a second centrifugal separation in the B-IV zonal rotor. The 19*S* fractions were characterized as the alpha complex by acetate-strip electrophoresis in 0.05*M* barbital buffer at *p*H 8.8 (Fig. 1). Protein concentrations were estimated by the method of Lowry *et al.* (*4*).

Initially, we studied survival of mice after whole-body irradiation. Random-

ized groups of 12- to 13-week-old male BC_3F_1, [(C_{57}Bl ♀) × (C_3H/AN ♂)F_1 Cum], mice were irradiated at approximately 145 roentgens per minute for a cumulative exposure of 750 r. The cumulative 30-day mortality figures from seven separate experiments (10 to 35 mice per group) are shown in Fig. 2. Group 1 consisted of 205 mice receiving 750 r and no treatment after irradiation; the cumulative mortality was 83.4 percent. Group 2 consisted of 180 mice given 750 r and, within 2 hours after irradiation, intravenously injected with 3 or 6 mg of proteins from the first peak. The cumulative mortality for this group was 75.3 percent. Group 3 was composed of mice given 750 r plus 3 or 6 mg of isologous macroglobulin (second peak). A total of 191 mice was used in this group. In the first two experiments these animals received 3 mg of the purified macroglobulin (homogeneous 19*S*). The groups in the other experiments received 6 mg of the crude macroglobulin preparation (approximately 50 percent was 19*S*). There was no significant difference in survival between these two groups. The cumulative mortality for the seven different experiments was 31.9 percent. Group 4 consisted of 100 mice given 750 r and injected intraperitoneally with 25 μg of *Salmonella typhimurium* endotoxin (Difco) within 2 hours after irradiation. The cumulative mortality for these mice was 21.0 percent. Thus, isologous macroglobulin and endotoxin both caused a significant and comparable increase in survival after 750 r ofx-irradiation.

Histologic studies were made of spleens from unirradiated mice injected with 6 mg of the fraction containing isologous macroglobulin or with 25 μg of *S. typhimurium* endotoxin. There was no morphological effect in either spleen white or red pulp during the first 10 days in mice treated with macroglobulin. However, within the first 24 hours after the mice were given the endotoxin injection, there was a marked atrophy of the red pulp in the spleens and hyperplasia of the white pulp. Thus, whereas endotoxin appears to be very cytotoxic in unirradiated mice, isologous macroglobulin does not produce any detectable histological effect.

Changes in spleen weight were measured in animals irradiated with 750 r, followed either by no injection, intravenous injection of 6 mg of isologous first peak material, 6 mg of second peak material (isologous macroglobulin), or intraperitoneal injection of 25 μg *S. typhimurium* endotoxin. Five mice from each group were killed at various times during the 30-day interval after irradiation. At 15 days there was a twofold increase in the ratio of spleen weight to body weight (means) in the mice treated with macroglobulin and endotoxin, as compared with mice injected with the fraction from the first peak. At 20 days after irradiation, the ratio of spleen weight to body weight of the macroglobulin-treated animals was approximately 50 percent above that of the uninjected and the first-peak–injected controls. The ratio of spleen weight to body weight of the endotoxin-treated mice was no different from that of the controls during this interval. Also at this time, there was an approximate twofold increase in peripheral blood white cell count in the macroglobulin group, as compared with the two control groups. Increase in spleen weight in the macroglobulin-treated mice was a result of hematopoietic recovery.

In order to determine the magnitude of the enhanced recovery after macroglobulin treatment, we used the colony-forming assay (*5*) and measured incorporation of ^{59}Fe (*6*). Mice were irradiated with 400 r, and within 2 hours they were injected intravenously with either 6 mg of second peak (isologous macroglobulin) or 6 mg of isologous protein from the first peak, or they were not injected. The mice were killed on day 4 or day 7 after irradiation. Spleen weights and the number of nucleated cells per spleen and femur were determined. The bone marrow cells obtained from femurs of five donors were pooled. Cells obtained from the spleens of the same five donors were also pooled. Suspensions of bone marrow and spleen cell were then adjusted to the desired cell concentrations, and portions were transferred to lethally irradiated recipients. On day 9 after transfer ^{59}Fe (0.1 μc) was intraperitoneally injected into the recipients,

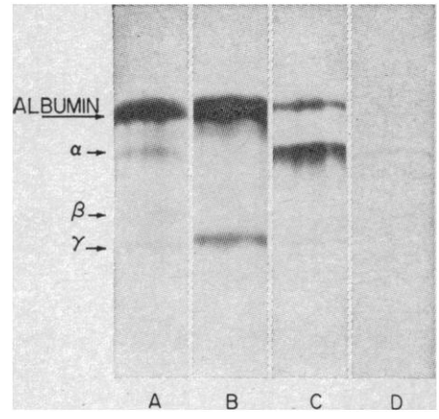

Fig. 1. Electrophoretic patterns of: (A) Normal BC_3F_1 male mouse serum; (B) ultracentrifuge fraction of mouse serum first peak (83.6 mg protein per milliliter); (C) ultracentrifuge fraction of mouse serum second peak (macroglobulin 48.4 mg protein per milliliter); and (D) reconcentrated second peak (macroglobulin 6.9 mg protein per milliliter).

Table 1. Effect of serum protein fractions on recovery of CFU and ^{59}Fe incorporation after 400-r x-irradiation. The transplanted cells were obtained from pooled spleen or femurs of five donor animals. Each CFU point represents the mean of 10 to 12 mice.

Treatment groups	Time after x-irradiation (days)	Recovered donor cells × 10^6 (No.)	Cells transferred (No.)	Colonies* per recipient spleen (Av. No.)	CFU/10^6 donor cells	CFU/femur or spleen
			Cells from femur			
None	4	3.2	1.5×10^5	3.2 ± 0.4	21.30	68.2
First peak	4	3.1	1.5×10^5	3.7 ± 0.5	25.00	77.5
Macroglobulin	4	2.9	1.5×10^5	9.3 ± 0.8	62.50	181.3
None	7	10.3	5.0×10^4	1.3 ± 0.4	26.60	274.0
First peak	7	18.6	5.0×10^4	1.1 ± 0.4	22.00	409.2
Macroglobulin	7	20.4	5.0×10^4	3.4 ± 0.3	68.40	1395.4
			Cells from spleen			
None	4	22.9	1.0×10^7	0.70 ± 0.3	0.07	1.6
First peak	4	25.2	1.0×10^7	2.50 ± 0.7	0.25	6.3
Macroglobulin	4	33.8	1.0×10^7	3.20 ± 0.5	0.32	10.8
None	7	32.4	1.0×10^6	0.89 ± 0.2	0.89	28.8
First peak	7	37.7	1.0×10^6	1.88 ± 0.6	1.88	70.8
Macroglobulin	7	51.0	1.0×10^6	5.75 ± 0.6	5.75	293.2

* One standard error of the mean.

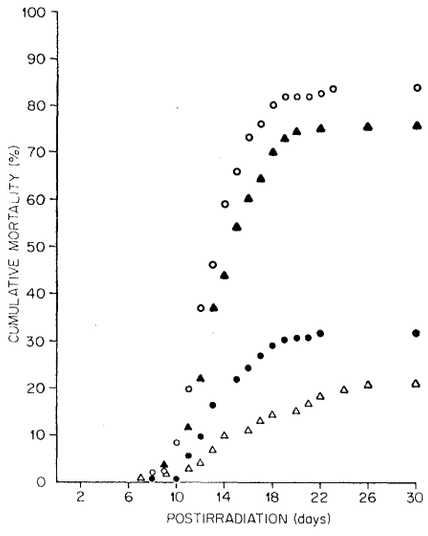

Fig. 2. Cumulative mortality (percent) of x-irradiated (750 r) BC_3F_1 male mice after: group 1 (○), no treatment; group 2 (▲), 3- or 6-mg first-peak fraction; group 3 (●), 3- or 6-mg second-peak (macroglobulin) fraction; group 4 (▲), 25 μg of *S. typhimurium* endotoxin.

which were killed 6 hours later. Spleens were fixed in Bouin's fluid, and radioactivity was measured in a Packard automatic gamma-counter; the number of colonies per spleen was then determined. A preliminary experiment with bone marrow and spleen cells from unirradiated mice and mice irradiated with 400 r was carried out to establish the relation between the number of cells injected and the number of colonies or percentage of ^{59}Fe incorporation for the various time points after irradiation. To test the activity of serum fractions, the cell dose transferred at various times after irradiation was determined from the above study. The results of studies of hematopoietic recovery after injection of serum protein fractions are given in Table 1 and Fig. 3. In Fig. 3, the hematopoietic activity in the femur and spleen from irradiated mice with the different treatments is expressed as percentage of hematopoietic activity of unirradiated controls. On day 4 after irradiation, there was no significant difference obtained in the mean number of nucleated cells recovered per femur in the three groups of donor mice (Table 1). However, at this time there was about a threefold difference in the number of colony-forming units (CFU) per 10^6 donor bone marrow cells and the number of CFU per femur of the irradiated mice injected with macroglobulin, as compared with groups receiving other treatments. The same relative difference is detected when the CFU per femur of the macroglobulin-treated mice are expressed as percentage hematopoietic activity of femurs of nonirradiated mice (Fig. 3).

On day 7 (Table 1) there was approximately a twofold difference in mean number of nucleated cells per femur between the first peak and macroglobulin-injected groups, as compared with the mice that received radiation only (400 r). The colony counts were approximately threefold higher in the macroglobulin-treated mice. Thus, a four- to fivefold difference is obtained in CFU per femur.

In the hematopoietic recovery of the spleen at 4 and 7 days after irradiation there was a 50 to 60 percent increase in mean number of recovered donor spleen cells in the macroglobulin group. This corresponded to a 50 to 60 percent increase in mean spleen weight. In terms of CFU per 10^6 spleen cells and CFU per spleen, there was a significant increase when the group receiving the first peak fraction was compared with the controls that were irradiated only. However, this increase was not nearly as great as that obtained when the macroglobulin group was compared with these controls. A five- to sixfold difference was measured in CFU per 10^6 cells, and a nine- to tenfold difference was measured in CFU per spleen at the 4- and 7-day intervals.

Our studies show that isologous macroglobulin significantly enhances the rate of hematopoietic recovery in bone marrow and spleen of BC_3F_1 male mice irradiated with 400 r. In the bone marrow of the control groups between days 4 and 7, there was a four- to fivefold increase in CFU. The doubling time (T_2) of the CFU was approximately 36 hours. In contrast, there was an eightfold increase (T_2 ~24 hours) in macroglobulin-treated animals. The spleen of the control groups increased about 15-fold in CFU (T_2 ~18 hours), and the macroglobulin-treated mice had a 27-fold increase ($T_2 < 18$ hours) between 4 and 7 days.

We then tried to determine whether the 19*S* fraction from serums of different species had the same recovery-enhancing properties in irradiated mice. The 19*S* macroglobulins were isolated from serums of horse and specific pathogen-free Sprague-Dawley rats. The cumulative survival of animals in two separate 30-day studies was recorded for groups of 25 mice given 750 r and then injected with 6 mg of rat macroglobulin, 6 mg of horse macroglobulin, and 6 mg of horse first-peak serum protein fraction, respectively. In comparison with the 86.0 percent cumulative mortality obtained in the controls that received 750 r only, 32.0 percent mortality was obtained with rat macroglobulin. Injection of the horse macroglobulin after irradiation resulted in 64.0 percent mortality, whereas 6 mg of first-peak fraction from horse serum was ineffective in providing enhanced survi-

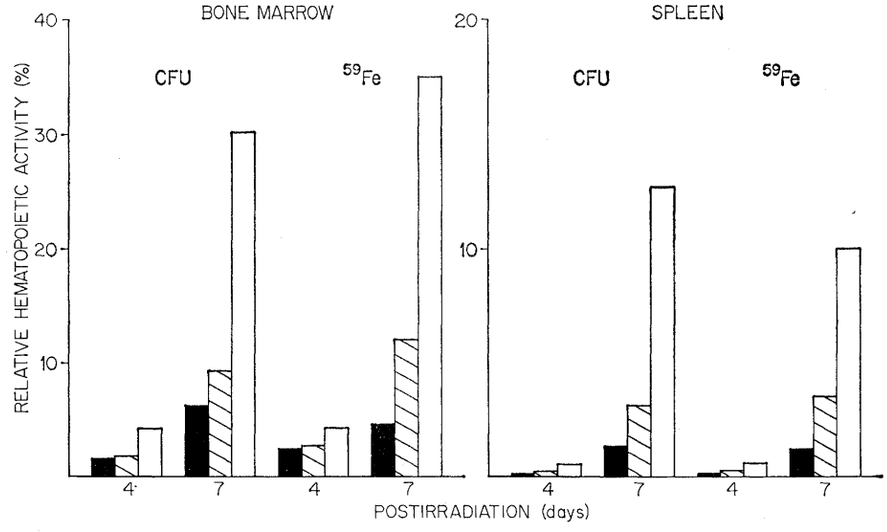

Fig. 3. Relative hematopoietic activity in bone marrow and spleen of BC_3F_1, mice 4 and 7 days after 400-r x-irradiation. Treatment after irradiation: ■, none; ▨, 6-mg first-peak fraction; □, 6-mg second-peak (macroglobulin) fraction.

val (80.0 percent cumulative mortality). Thus, rat macroglobulin is equally as effective as mouse macroglobulin in producing enhanced survival in irradiated BC_3F_1 mice. Horse macroglobulin was only marginally effective.

In recent years, the serum alpha globulins fractionated by various procedures have been investigated. Mowbry and Hargrave (*7*) described an immunosuppressive effect of heterologous alpha globulins in mice injected with rat erythrocytes (RRBC). Repeating the experiments of Mowbry and Hargrave with our macroglobulin fractions (mouse, rat, and horse), we found no effect on the serum hemagglutinin response to RRBC as compared with controls that were not treated with macroglobulin.

The increased concentration of a serum alpha-2 protein, often designated "acute phase macroglobulin," has been described in man, monkey, dog, rabbit, rat, and chicken under various physiological and pathological conditions (*8*). Several hypotheses have been advanced to explain the appearance of this macroglobulin. One hypothesis is that the etiological agent stimulates a synthesis of the protein present ordinarily in trace amounts (*9*). A survey of conditions in which the protein was detected reveals the common factor of tissue injury or cell death, or both. It is interesting to note that 24 hours after *Salmonella* sp. endotoxin injection into rats (*8*) and mice (*10*), an increase in this protein has been detected. Thus, endotoxin is one of the many agents that can stimulate the increased concentration of this alpha-2 macroglobulin. It has also been well-established that endotoxin injected 24 hours prior to irradiation promotes optimum changes of survival (*11*).

Since the active material which we have isolated is associated with the alpha macroglobulin fraction, further investigations are warranted to determine if indeed we are concentrating the trace amounts of "alpha-2 (acute phase) macroglobulin" considered normally present in rat and mouse. Also, this may prove to be a means of determining whether the radiation-survival activity of the endotoxin is mediated through this macroglobulin.

The recovery-enhancing effect of serum macroglobulin in mice shown in these experiments has proved to be highly reproducible. The seven survival studies with isologous macroglobulin were carried out over a period of 1 year from four individual macroglobulin preparations obtained from separate batches of fresh mouse serum. We are confident that the active material is an intrinsic serum constituent associated with the 19*S* protein of mice and rats, and functions across species barriers.

Bioassay for erythropoietin, in polycythemic C_3H mice (*12*), showed that no detectable erythropoietin (> 0.05 units) was present in 3 mg of either the first or second peak of BC_3F_1 mouse-serum protein preparation.

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Serotonin Release from Brain Slices by Electrical Stimulation: Regional Differences and Effect of LSD

Abstract. *Slices of rat brain which had accumulated tritiated serotonin either in vivo or in vitro were superfused and electrically stimulated. There occurred a marked release of the exogenous amine and, to a lesser extent, its deaminated metabolites, which varied with the region of brain tested and was inhibited by lysergic acid diethylamide.*

The status of serotonin as a transmitter substance within the mammalian central nervous system remains uncertain despite its occurrence within the synaptic vesicles of certain nerve terminals (*1*) and the effects of its microelectrophoretic application upon the activity of some neurons (*2*). Although biochemical and histochemical evidence of depletion of serotonin after electrical stimulation of specific neural pathways has been reported (*3*), there has been no direct demonstration of release of serotonin in response to nerve cell stimulation in the brain of the living animal. The difficulties associated with the study of serotonin release in vivo have prompted the development of various techniques in vitro. Anden and co-workers (*4*) reported the enhanced efflux of endogenous serotonin from isolated spinal cord by prolonged electrical excitation. The electrical stimulation of brain slices has been shown to liberate exogenous catecholamines (*5*). We now report on the release of tritiated serotonin under similar conditions.

Brain slices, when incubated in a suitable medium, maintain their bioelectrical and biochemical activity for several hours (*6*). Electrical stimulation of such tissues has been shown to displace resting membrane potentials (*7*). Labeled serotonin is accumulated by brain slices (*8*) and, when administered in low concentrations, appears to be largely concentrated within nerve terminals which normally contain serotonin (*9*). There is also electron-microscopic-autoradiographic and fluorescent histochemical evidence suggesting the uptake of serotonin within the synaptic vesicles of presumably serotonergic nerves when the amine is introduced into the cerebrospinal fluid of the living animal (*10*). Accordingly, we attempted to release exogenous serotonin from slices derived either from animals which had received an intracisternal injection of labeled serotonin or from animals that were incubated with the amine in vitro. Similar results were obtained in both cases.

Tritiated serotonin (20 μc, 2 c/