onset of rigor but decreased by 50% at 24 hours post-mortem. However, under conditions of low pH with temperatures above 35°C at 2 hours post-mortem (Group A) the myofibrillar protein solubility decreased by 56% at onset of rigor and by 74% at the 24-hour period. These data show that the solubility of both sarcoplasmic and myofibrillar proteins is markedly altered by post-mortem conditions of pH and temperature in the muscle. To obtain muscle protein extractability consistent with *in vivo* composition, it is essential to freeze the muscle samples immediately after death in a cryogenic liquid. Loss of protein solubility is closely associated with rate of glycolysis and fluid retaining properties of the post-rigor muscle.

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Lysozyme Activity in Radiation Chimeras. (29049)

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It was shown(1) that a marked increase occurred in lysozyme activity in the kidneys of irradiated mice treated with homologous bone marrow. This investigation was repeated, and lysozyme activity in different organs was determined.

Our interest in lysozyme stems from the problem of secondary disease or graft-against-host reaction in homologous bone marrow chimeras. In this immunologic disorder there appears to be a "metabolic starvation" that follows the immune reaction(2-4). The metabolic problem in secondary disease may be the important feature in causing death after treatment with forgein homologous bone marrow.

Even though the biological significance of lysozyme is unknown, it seems to be increased in mammals during immunological and other defense reactions.

A study of the striking changes in lysozyme in homologous bone marrow chimeras might contribute either to an understanding of the biochemical role of lysozyme in tissues or to the mechanism of metabolic alterations during the foreign bone marrow reaction.

Materials and methods. Animals. Approximately 1000 male mice, 12 to 20 weeks old, were used in these experiments. Hybrid animals (101/Cum ♀ × C3H/Anf Cum ♂)F_1 were used as recipients or donors of isologous bone marrow, and (C57BL/6 Cum ♀ × DBA/2 Cum♂)F_1 as donors of homologous bone marrow. They are referred to hereafter as $IC3F_1$ and $B6D2F_1$. These animals were divided into 4 groups: Group I were normal control mice, Group II were mice given 950 r total-body irradiation, Group III were mice irradiated (same dose) but given 40×10^6 isologous bone marrow cells (IBM), and Group IV were mice irradiated (950 r) and given 40×10^6 homologous bone marrow cells (HBM). The cells were given intravenously by tail vein within 5 hours after irradiation. They were kept 10 per cage and allowed free access to food and water.

The conditions of irradiation and technique for preparing bone marrow suspensions were the same as described previously(1).

Procurement of tissues. The animals were

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weighed and decapitated. The organs were then removed, freed of connective tissue, and weighed. Both kidneys and lungs of each group were used for the enzyme assay. Skeletal muscle was excised from the thigh and leg. Lymph nodes from the axillary, inguinal, thoracic, and abdominal regions were pooled for assay. Small intestine, from the pylorus to the caecum, was used after removing the contents of the lumen. The intestine was not washed, since Litwack(5) showed that 5% of the enzyme activity was lost in washing. In the lysozyme assay of plasma, mice were anesthetized intraperitoneally with Nembutal, and blood was aspirated into heparinized syringes by cardiac puncture.

Enzyme activity measurement. The tissues were homogenized 5 minutes with 1/15 M phosphate buffer at pH 7.3 with a glass-Teflon homogenizer(6) except for muscle, where a glass homogenizer (Ten Broeck type) was used. The mixture was then centrifuged 30 minutes in the cold at 14,000 rpm. The sediment was discarded and the supernatant used for the lysozyme assay. In part of this study, in an attempt to obtain more complete disruption of the tissue cells (to release all of the enzyme activity), the suspensions were frozen in dry ice plus acetone and thawed at 37°C four consecutive times. The cells were rehomogenized 2 minutes, then centrifuged as above.

Lysozyme was assayed according to Shugar (7) using *Micrococcus lysodeikticus* cells (Worthington batch No. 606-4) as substrate and following the change in absorption at 450 mμ at 25°C with a Zeiss PMQ II spectrophotometer. Worthington lysozyme (twice crystallized), batch No. 616 was used in the construction of a standard curve for comparison with activity of tissue lysozyme. One-tenth ml of the supernatant was added to 2.9 ml of the substrate for each assay. Differences in buffer volume per gram of tissue were taken into account in the final calculations for enzyme activity. Absorbance was read at 30-second intervals, and the course of the reaction was recorded for 2½ minutes.

Results. Fig. 1 is a plot of lysozyme activity (referred to a standard enzyme preparation) in kidney homogenates of the 4 groups of mice. In the homologous chimeras, during the first 6 days after irradiation and treatment with bone marrow, the lysozyme was almost at the normal value, but at day 7 there was a marked increase which reached a peak at 9 days and then rapidly decreased and was only slightly above the normal value during the 3rd week. Data from kidney homogenates of homologous chimeras at 100, 150, and 200 days after irradiation and bone marrow treatment showed a normal value of lysozyme activity as had been presumed in our previous work(1). There was a slight increase in lysozyme activity in the irradiated mice and in mice irradiated and treated with isologous bone marrow during the 2nd week. As the dose of irradiation was lethal (950 r total-body irradiation), a large number of mice was needed to get survivors for the X-ray control groups, and only a few were available for the later time intervals of this experiment. Occasional mice survived 950 r, indicating that this exposure was not supralethal.

The kidney homogenates referred to in Fig. 1 were not frozen and thawed.

TABLE I. Effect of Freezing and Thawing of Homogenate on Lysozyme Activity of Different Organs of $1C3F_1$ Normal Male Mice.

Organs taken from 3-7 mice were pooled to obtain each value.

Pooled tissue homogenate	Lysozyme activity/(μg/g tissue)	
	Not frozen	Frozen
Kidney	20	40
Spleen	14	28
Bone marrow	108	135.5
Lung	25	34
Liver	8	12
Lymph node	12.8	12.8
Thymus	7.6	7.6

Table I shows the effect of freezing and thawing the homogenates on the lysozyme activity of different organs of normal mice. The cells that were frozen and thawed after homogenization were resuspended. Kidney, spleen, bone marrow, lung, and liver, by this method gave a lysozyme activity of 1½-2 times higher than with the method used previously. The freezing-thawing technique has also been used by several authors(8-11).

Table II shows lysozyme activity in dif-

ferent organs of mice killed at 9 days after irradiation and treatment with bone marrow. Normal and X-irradiated mice (950 r) were killed as control groups along with the irradiated bone marrow-treated animals. The values were obtained from a frozen-thawed pooled homogenate.

Lysozyme assay of the different organs of normal mice indicated highest values in bone marrow, small intestine, lung, kidney, spleen, and colon. In contrast, no activity was found in brain and skeletal muscle. In X-irradiated mice not given bone marrow a decrease in activity was found in 7 of the 11 organs ex-

TABLE II. Lysozyme Activity in Different Organs of Normal Mice, and of Mice 9 Days After Irradiation (950 r), Irradiation and Injection of 40 × 10^6 Isologous Bone Marrow Cells or 40 × 10^6 Homologous Cells.*

Tissue	Lysozyme activity (μg/g tissue)			
	Normal control	Irradiated control	IBM	HBM
Bone marrow	180 (11)	29 (10)	350 (13)	459 (13)
Small intestine	51 (3)	81 (3)	57 (3)	53 (3)
Lung	48 (4)	36 (3)	56 (4)	64 (3)
Kidney	28 (3)	21 (3)	42 (3)	196 (3)
Spleen	17.3 (5)	3.8 (13)	39 (4)	56 (3)
Colon	17 (3)	4 (6)	6 (3)	4 (4)
Lymph node	8 (5)	0 (11)†	22 (8)	25 (3)
Submaxillary gland	4.6 (5)	5.2 (5)	2.6 (5)	2.4 (5)
Thymus	3.4 (11)	0 (34)†	3.8 (22)	6.6 (23)
Liver	2.2 (3)	4.4 (3)	4.0 (3)	6.6 (3)
Plasma	1 (3)	1 (7)	0 (4)†	1 (3)

* No. of mice is given in parentheses.

† "0" refers to no decrease in optical density after 2½ min. incubation of extract with substrate.

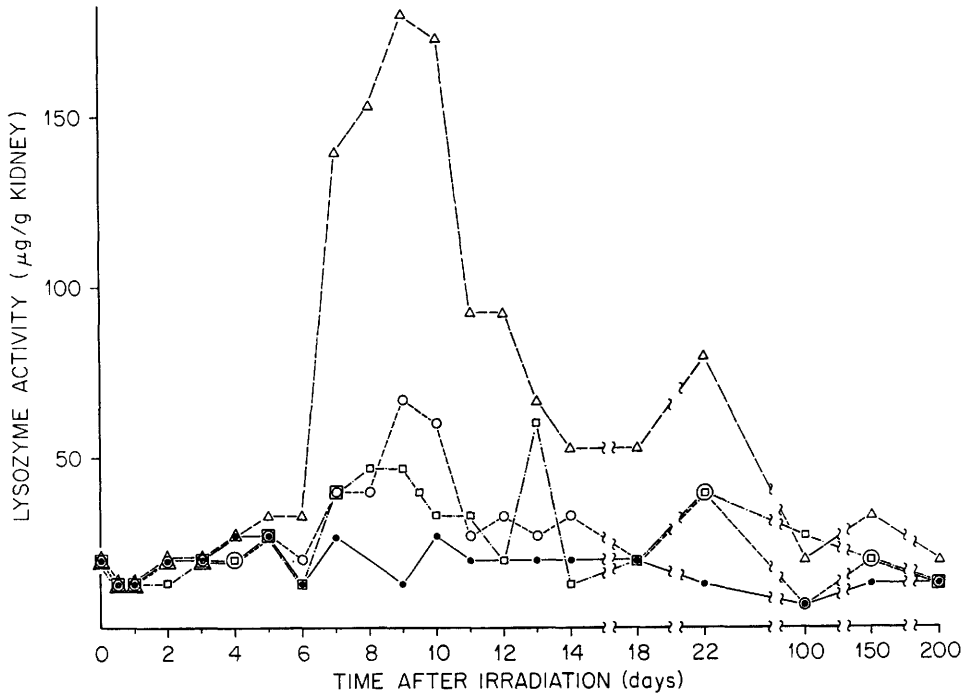

FIG. 1. Lysozyme activity in kidneys of normal control mice (●); of animals given 950 r total-body irradiation (□); irradiation and 40 × 10^6 isologous bone marrow cells (○); or 40 × 10^6 homologous cells (△). $1C3F_1$ males, 12-20 wk of age. Each point is the value obtained from the pooled homogenate of 3 mice.

amined. Four organs showed no change or an increase.

In the chimeras, greatest lysozyme activity was present in the bone marrow, the HBM group being higher than the IBM. The next highest activity was in kidneys of the HBM group.

Discussion. The present work confirms our previous finding that kidney lysozyme activity is markedly elevated in HBM mice compared to IBM and irradiated control animals. Greatest activity was seen during the 2nd week after HBM injection, whereas in the earlier report(1) the increase in kidney lysozyme was spread out over a greater period. The reason for these differences is not known, however, because different strains of mice were used in the two investigations.

The increase in kidney lysozyme activity is found during the time when greatest cellular proliferation is observed in the lymphatic tissues(4). These cellular changes are thought to be the primary morphological expression of the graft-against-host reaction, and they precede the clinical appearance of secondary disease.

Our data showed that bone marrow in normal mice has the greatest lysozyme activity. This fits well with the observation frequently reported that granulocytes contain lysozyme activity(8-10,12,13).

Much greater than normal enzyme activity in the bone marrow of IBM and HBM mice 9 days after transplantation of marrow would be expected if the regenerating marrow was predominantly granulopoietic. In HBM-treated mice, hyperplasia of granulopoiesis has been reported(14).

The increased kidney lysozome in the radiation chimera seems to parallel the finding of increased activity in the kidney of certain kinds of animals with transplantable tumors (1,15,16). There is no explanation for the increase in activity, although it has been suggested that the kidney filters the lysozyme from the blood(16) without excreting it into the urine(12). Speece(17) found activity only in the kidney cortex, and concentrated mainly in the proximal convoluted tubules.

Increased lysozyme in spleen and lymph nodes of the day 9 chimeras might be associated with the active proliferation of lymphatic tissue elements(3); however, granulopoiesis can also be present at this time in these tissues.

Some increase was found in the lung and possibly also in the thymus and liver of the chimeras. It is not known if these changes are significant.

Reduced lysozyme activity in the colon of irradiated control animals and the two irradiated bone marrow-treated groups was observed. One would expect a decreased activity in the marrow, spleen, lymph nodes, and thymus at day 9 in the irradiated control group since the parenchyma of these organs is destroyed. The increase or decrease in other organs in the irradiated control group is of unknown or uncertain significance.

In general, these findings probably support the idea that lysozyme is involved in the biochemistry of the immune response. The lysozyme changes might be a part of the host's response to the proliferation of lymphatic tissues; it could also be an integral aspect of the cells taking part in the proliferative process.

In addition, Litwack(18) found that kidney but not spleen lysozyme activity could be changed by altered hormonal states, particularly those related to hypophysis and thyroid.

We have no evidence for a primary or secondary hormonal imbalance in homologous bone marrow transplantation, but further study based on Litwack's observations is needed.

Summary. Lysozyme activity in the kidneys of lethally irradiated mice treated with homologous bone marrow (HBM) was markedly elevated during the 2nd week after irradiation and transplantation compared to the normal, isologous bone marrow (IBM)-treated or controls which were irradiated only. Measurement of lysozyme activity on day 9 in the normal, IBM, HBM, and irradiated control groups showed levels of 180, 350, 459, and 29 μg/g of bone marrow, respectively. In the same groups on day 9 the kidney levels were 28, 42, 196, and 21 μg/g of tissue, respectively. Measurements were also made on small intestine, lung, spleen, colon, lymph

node, submaxillary gland, thymus, liver, and plasma. The changes in lysozyme activity in some of the organs are probably associated with transplantation of granulopoietic elements. Biochemical significance of lysozyme is not known, but it may reflect immunological activity. In some of our lymphatic tissue homogenates examined microscopically not all cells were disrupted, so that the experimental procedure of freezing and thawing the homogenate, then rehomogenizing, was carried out. This increased the enzyme activity yield in nearly every organ studied.

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Production of 7S and 19S Antibodies to the Somatic Antigens of *Salmonella typhosa* in Rabbits.* (29050)

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Numerous reports indicate that antibodies to most antigens appear in both the 19S and 7S globulins. These two molecular species of antibodies have been demonstrated in rabbits immunized with erythrocytes(1,2), bovine γ-globulin(3), human serum albumin(4), hemocyanin(4), diphtheria toxoid(4) and bacteriophage(5). Chickens produced both kinds of antibody to bovine serum albumin(6) and to bacteriophage(7). In frogs and goldfish both rapidly and slowly sedimenting antibodies were found(7). In human serum, antibodies to diphtheria toxoid(8) and *Brucella suis*(8) and certain antibodies to erythrocytes(9,10) have been found in both 19S and 7S globulins. Although both 7S and 19S antibodies to *Salmonella* flagella antigens were demonstrated in human serum(11) and in rabbit serum(4), antibodies to the somatic antigens of *S. typhosa* appeared only in the 19S fraction of human serum after immunization with TAB vaccine(11) and only in the 19S fraction of rabbit serum after foot pad inoculation(4). Since the implication that antibodies to lipopolysaccharide antigens may be entirely macroglobulins is a matter of considerable significance, we have studied further by cellulose chromatography, mercaptoethanol sensitivity and ultracentrifugation the O agglutinins in the serum of rabbits hyperimmunized with *S. typhosa*. Evidence that 7S as well

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