triculus was flaccid and contained excessive amount of fluid. The lining of the gizzard was loosely attached to the epithelial layer.

The pathology of laying hens fed histamine is similar to that of chicks fed histamine as described previously(2). The decrease in egg production might be related to the effect of histamine on the reproductive tract or on the endocrine system. These points require further studies.

The small number of hens used precludes definite conclusions from the results obtained from hens fed 0.1% histamine. It is possible that the decline in egg production was within the normal variation. However, the results obtained with the 0.25% histamine are unequivocal. The effect of histamine on feed consumption also seems to be unequivocal: there was no effect at 0.01% and 0.1% histamine and there was a very sharp drop in feed consumption with 0.25% histamine; a decrease which is beyond the normal variation in feed consumption. These results are in accord with previously published data on the effect of histamine on chicks(1).

Our results show that histamine can inhibit egg production and that under certain conditions the response of the hens to this drug is adaptive. In hens fed low concentration of histamine the effects of a larger dose were decreased or abolished. When these higher doses were given to unadapted birds there was complete inhibition of egg production. A diet containing 0.1% histamine was necessary to induce complete adaptation to 0.25%, since a diet containing 0.01% histamine did not induce this adaptation. Hens on 0.25% histamine followed with a normal diet for *30 days* were still refractive to a subsequent dose of 0.5% histamine in diet.

The effect of increased dosage of histamine on feed consumption differed from that on egg production: hens fed low histamine diets were not adapted to higher doses.

The 2 different responses to histamine might possibly be explained by the presence of 2 separate processes, one affecting egg production and one, feed consumption.

It would be of interest to determine if histamine, in nontoxic amounts, administered parenterally, would also have an effect on egg production. Further studies may provide information on factors for regulation of egg production in normal hens. The mechanism of adaptation to histamine is under study.

Summary. When laying hens were fed a diet containing 0.25% histamine, egg production stopped. When this diet was preceded by a diet containing 0.1% histamine, egg production was not affected. The adaptation of hens to histamine in their diets is discussed. The gross pathology of hens fed a diet containing 0.25% histamine is described.

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Increase in Lysozyme Activity in Kidneys of Irradiation Chimeras.* (28403)

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The wide distribution of lysozymes in plants and animals (tears, saliva, blood plasma, tissues, and secretions) and their lytic action against certain gram-positive and gram-negative bacteria(1) suggests a role for these enzymes in defense of plants and animals against bacterial invasion. The ob-

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servations(2-4) that lysozyme activity in serum and kidney of rabbits increases during sensitization to horse serum, after injection of heat-killed *Salmonella typhosa* or Newcastle-disease virus, during anaphylaxis and, in serum of children, after injection of adrenocorticotropin indicate that mobilization of lysozyme activity may be a rather general part of an animal's reaction to bacterial, immunological, and noxious insult. In this connection, the observations of Perri(5,6) on increased lysozyme activity in kidney of rats with certain tumors and those reported here showing increased lysozyme activity in kidney of homologous irradiation chimeras are of interest.

Materials and methods. Animals. One hundred male $(101/_{cum}xC_3H/_{Anf/cum})F_1$† mice, 12 to 20 weeks old, were used as bone marrow donors and 400 $(C_{57}L/_{cum}xA/He/_{cum})F_1$‡ male animals, also 12 to 20 weeks old, served as donors of isologous marrow or were irradiated and used as recipients. Animals in this series were killed 3 to 82 days after irradiation and treatment with bone-marrow cells. Four groups were investigated. Group I was normal mice not irradiated and not given bone marrow; Group II was mice given 950 r total-body irradiation but not bone marrow; Group III mice were similarly irradiated but were given 40×10^6 isologous bone marrow cells; and group IV mice were irradiated and given 40×10^6 homologous marrow cells.

For analysis of lysozyme activity in kidneys of older chimeras 110 LAF_1 females were used as donors for isologous marrow and irradiated recipients and 55 $1C3F_1$ females as donors for the homologous marrow. These were killed 244 to 322 days after irradiation. One group, killed on day 256, included 10 LAF_1 males as donors for isologous marrow and irradiated recipients and 5 $1C3F_1$ males as donors for the homologous marrow. In these experiments with older chimeras, recipient mice were divided into 5 groups: one group was irradiated without any bone-marrow treatment; 2 were irradiated and given 40×10^6 isologous bone-marrow cells, and 2 were irradiated and given 40×10^6 homologous bone-marrow cells.

After X-irradiation and marrow treatment, when this was given, animals were kept 10 in a cage and allowed free access to food and water. In all experiments the sex of donor and recipient mice was the same.

Irradiation. A constant potential X-ray machine was used. Radiation factors were 250 kilovolts, 15 ma; inherent filtration, 1 mm Al; half-value layer, 0.5 mm Cu; target-object distance, 60 cm; dose rate in air approximately 157 r/min.

Mice were placed in a circular, perforated lucite container attached to a revolving turntable and given a single total-body exposure of 950 r or 900 r.

Bone-marrow treatment. Marrow was obtained from the femurs of donor mice and suspended in Tyrode's solution by flushing through a 22- and 27-gauge needle. The nucleated cells were counted in a hemocytometer and the volume was adjusted with Tyrode's solution to give 40×10^6 cells/ml of suspension. Recipient mice were given 1 ml intravenously within 5 hours after irradiation.

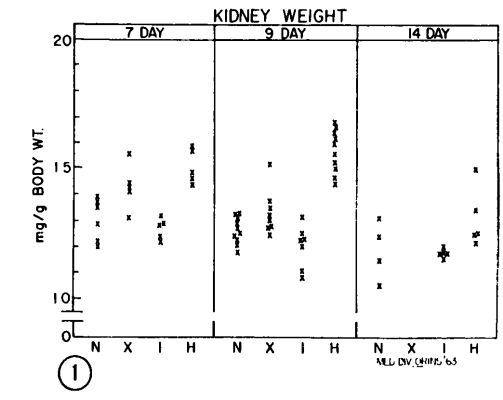
Sacrifice. One to 3 animals from each group were weighed then killed by decapitation 3, 7, 10, 12, 15, 18, 20, 28, 36, 60, 68, 82, 244, 256, 294, 302, 312, 314, and 322 days after irradiation and injection of bone marrow. Animals from the control groups were killed along with the irradiated and bone-marrow-treated mice. Spleen and kidneys were removed, freed of adjacent connective tissues and fat, and then weighed.

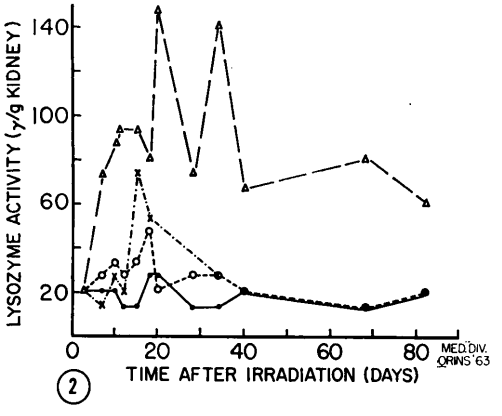
Enzyme activity measurement. Both kidneys of mice from each group were homogenized 5 minutes with 1/15 M pH 7.3 phosphate buffer(5). The mixture was then centrifuged for 50 minutes at 2400 rpm in a refrigerated centrifuge. The supernatant was collected and its lysozyme activity measured; the sediment was discarded.

The procedure of Shugar(7) standardized against Worthington lysozyme, batch No. 616, was used in the lysozyme assay. Worthington lyophilized *Micrococcus lysodeikticus* cells were used as substrate. A Zeiss PMQ II spectrophotometer was used for absorbance measurements (wave length was 450 mμ

† Hereafter referred to as $1C3F_1$.

‡ Hereafter referred to as LAF_1.





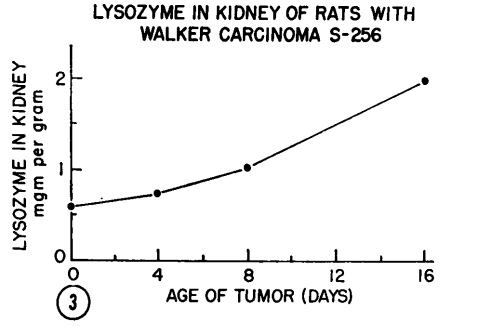


FIG. 1. Weight of kidneys at 7, 9, and 14 days after 950 r total-body irradiation (X), irradiation and 40 × 10^6 isologous (I) or homologous bone-marrow cells (H) given intravenously after irradiation compared to normal unirradiated controls (N).

FIG. 2. Lysozyme activity in kidneys of normal control mice (●——●) and of animals given 950 r total-body irradiation (×-·-×), irradiation and 40 × 10^6 isologous bone-marrow cells (○- - -○), or 40 × 10^6 homologous cells (△— —△). Each point is avg of data from 1-3 mice.

FIG. 3. Increase in lysozyme activity in kidneys of rats inoculated at 150 to 200 g body wt with Walker 256 carcinoma. Enzyme assay was done as indicated in text. Activity is plotted in terms of equivalent weight of standard enzyme per g of fresh kidney against time after inoculation of tumor.

and temperature was controlled at 25°C). The reagents were kept 30 minutes in a constant temperature bath at 25°C before measurement of enzyme activity. One-tenth milliliter of the supernatant was used for each lysozyme assay. Absorbance was read at 30-second intervals and the course of the reaction was recorded for 2½ minutes.

Results. Fig. 1 shows kidney weight, calculated as milligrams per gram body weight, increased by 10 to 28% in X-irradiated mice at 7 and 9 days unless they were given isologous bone-marrow cells after irradiation. This increase is greatest at day 9 in mice given homologous marrow. Incomplete time-course data suggest that kidney weight is normal after day 40.

Fig. 2 is a plot of lysozyme activity (referred to a standard enzyme preparation) in kidney homogenates of irradiated mice treated with bone-marrow cells. There is great increase after treatment with homologous cells and in an occasional animal, surviving 950 r without treatment with bone marrow, there was also a large increase. The increase in mice given isologous cells is slight. It should be noted that these data refer to *activity* only since the enzyme was not isolated from the homogenate and its amount, in terms of protein molecules, was not determined.

Data in Table I indicate that lysozyme activity of kidney homogenates from late irradiation chimeras is normal unless major pathologic changes were present of a type associated with immunologic stimulation.

Discussion. The increase in lysozyme activity in kidney of the occasional mouse that survives 950 r without bone marrow treatment is due probably to bacterial infection and associated response in its recovering immunological system. This view is supported by the observation of elevated lysozyme in sera of patients with infectious renal disease but not in those with renal pathology of noninfectious nature(8) and in serum of rabbits infected with group A Streptococcus (9). In irradiated mice given isologous marrow, recovery is sufficiently rapid that post-

TABLE I. Lysozyme Activity in Kidneys of Mice 244 to 322 Days After Irradiation and Treatment with 40 × 10^6 Isologous Bone Marrow Cells (IBM) or 40 × 10^6 Homologous Cells (HBM). All animals were given 900 r total-body X-irradiation.

Time after irradiation (days)	Activity of lysozyme (γ/g kidney)					
	X-ray control		IBM		HBM	
244			47*	(3)†	20	(3)†
256			20	(2)	20	(2)
			340‡	(1)	67‡	(1)
294			13	(3)	13	(3)
			13	(3)	13	(3)
302			20	(3)	20	(3)
			13	(3)	13	(3)
312			20	(3)	27	(2)
					33‡	(1)
			20	(3)	13	(3)
314			13	(3)	27	(3)
			20	(3)	20	(3)
322	20	(1)†	27§	(3)	40§	(3)
			13	(3)	20	(3)

* Spleen weight in these animals was elevated.
† No. of mice in parentheses.
‡ Abscess and increased spleen weight.
§ One of these animals had increased spleen weight.

irradiation infection is minimal and lysozyme activity is only slightly increased.

The sustained and very great increase in lysozyme activity in kidneys of irradiated mice given homologous marrow (Fig. 2) and the progressive increase in rats with Walker Carcinoma S-256 (Fig. 3)§ may be more complex. First, lysozyme activity in tumor-bearing rats is correlated with tumor size (or time after inoculation as in Fig. 3) and decreases as the tumor regresses(5); it seems unlikely that infection in the host would be so closely correlated with tumor growth. Second, if the increased lysozyme in the irradiation chimera is due to response to infection, it must be related to reactivity of donor cells, since the increase is already pronounced at 7 days after irradiation when X-ray controls show normal lysozyme activity. The sustained nature of the increase in the irradiation chimeras could be taken as evidence of the presence of chronic infection in these animals and chronic infection has been offered as a causal explanation for homologous disease(10). The randomness of elevation of lysozyme activity in older irradiation chimeras and enzyme increase in occasional mice given isologous marrow could be taken as further evidence for this view. Although infection no doubt plays a role in these experiments, it seems unlikely that it is the only, or even the chief, cause of elevation of lysozyme activity.

Several observations point to other factors. First, the animals given homologous marrow cells appear to recover from irradiation as well as the controls given isologous marrow until the onset of secondary weight loss. Yet, as Fig. 2 shows, lysozyme activity in homologous chimeras is rapidly increasing during the 3- to 14-day interval and during this time controls given isologous cells show no significant increase. Second, other biochemical observations from this laboratory have indicated certain metabolic similarities between mice with homologous disease and rats with Walker S-256 tumors. As shown in Fig. 3, in agreement with the observations of Perri and Anigstein(5), enzyme activity increases progressively with tumor size. It seems more likely that this result is to be interpreted in immunological rather than bacteriological terms. The increase in activity of lysozyme in kidneys of homologous chimeras, beginning after the 3rd day, rising rapidly during the 1st and 2nd week to a peak in the 20- to 40-day interval, and returning to normal sometime between 100 and 200 days, would also fit with present views regarding the immunological basis for secondary disease. Third, in other work to be reported it was found that fecal nitrogen excretion and gross weight of feces was not different in mice given homologous marrow from animals given isologous cells. Thus, infectious or irradiation diarrhea is not a factor to be considered in our experiments. Fourth, data on adrenal weight suggest that for the first 20 to 30 days at least, the pituitary-adrenal axis is not more active in the homologous chimeras than in the controls given isologous marrow. Finally, the observations of Kerby(11), showing that lysozyme leaks from cells exposed *in vitro* to endotoxin, suggest the possibility that cytotoxic activity in irradiation chimeras and animals with inoculated tumors, as well as necrosis within the malignant mass in the latter animals, may be releasing lysozyme

§ The tumor-bearing rats were generously supplied by Dr. F. Comas.

from injured or dying cells. However, in work to be published, the lysozyme activity was not elevated at 12 hours after irradiation so that release from dying cells is probably not an important factor.

The precise extent to which these various factors, individually or in combination, contribute to the observation of increased lysozyme activity in irradiation chimeras reported here must await further work for definition.

Increased weight of kidneys and liver has been noted by Frei *et al.*, in leukemia(12). In our experiments, the animals given homologous marrow had definitely increased kidney weight that became maximal at 9 days but was nearly normal by 14 days after treatment. This weight increase was not, therefore, correlated with the increase in lysozyme activity, the peak of which came later. The relationship in time after treatment, between the increase in liver weight that we have previously found(13) in mice given homologous cells and the increase in kidney weight reported here is of interest, although the mechanism involved is unknown.

Summary. Assay of lysozyme activity in homogenates of kidney of irradiation chimeras has shown that a marked increase in activity is associated with the presence of grafted homologous bone marrow. This increase began about 3 days after injection of the marrow cells, reached its peak 20-40 days and returned to normal 100-200 days after treatment. A significant increase in weight of kidney, maximal at 9 days after treatment, was observed in the animals given homologous bone-marrow cells.

Dr. D. G. Doherty and Amleto Castellani helped in setting up the assay procedure.

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Prolactin Activity in Blood During the Normal Human Menstrual Cycle.* (28404)

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Because of various difficulties in bioassay techniques there have been scanty previous data on prolactin activity in the blood or urine of normal men and women. In a previous paper mostly concerned with presenting proofs of the validity of our bioassay procedure, we(1) found no prolactin activity in the blood of children, normal young men and normal young women in the first half of the menstrual cycle. However, prolactin activity was present in the blood of normal women during the second-half of the cycle. These assays were performed on single specimens of blood obtained from different individuals

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