the Sprague-Dawley and Osborne-Mendel strains are quite vulnerable to the induction of mammary cancer by intragastric instillation of 7,12-dimethylbenz(*a*)anthracene and rats of Fischer, Marshall, and Long-Evans strains are relatively resistant, the fatty acid composition of mammary glands from rats of these five strains were comparable. A sex difference was noted in three strains with males having a somewhat greater proportion of 18:2 (linoleic acid) and a lower proportion 16:1 (palmitoleic acid) than females. The phospholipid levels of the breasts tissue of both male and females in all five strains were quite low relative to the total lipid.

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Effect of Mithramycin on Kidney Transamidinase of C3H Mice*† (32633)

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Mithramycin is an antibotic derived from a culture of *Streptomyces tanashiensis* (1) In the treatment of neoplastic diseases, clinical improvement has been observed in embryonal cell carcinoma of the testis, glioblastoma multiforme, and hypernephroma (2,3). These antitumor effects were associated with severe clinical toxicity including hepatocellular and renal disturbances. Hepatic injury was characterized by a marked increase in serum enzymes. Renal injury was evidenced by proteinuria, blood cells in the urine, and uremia. Acute tubular cell damage in patients dying of drug toxicity and atrophy of tubules in long surviving patients have been observed. Initial treatment studies were associated with a high mortality (2).

In the investigation of the mechanism of action of mithramycin, it was found to inhibit the synthesis of RNA in a mouse ascites tumor with little or no immediate effect on that of DNA (4). A similar inhibitory phenomenon occurred in tissue cultures of HeLa cells treated with mithramycin.[1] In a study of the differential effect of this agent on various organs of the mouse, there was a profound inhibition of the capacity for RNA synthesis in the organs studied (5). Measurement of the distribution of tritium-labeled mithramy-

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cin revealed concentrations in the liver and kidneys, correlating closely with the observed clinical toxic effects (6). Pathological studies revealed central lobular necrosis in the liver and renal tubular damage terminating in atrophy of the tubules.

Among the biochemical signs of hepatic toxicity were the increase of serum lactic dehydrogenase, serum glutamic oxalacetic transaminase and glutamic pyruvic transaminase, and serum ornithine carbamyl transferase. In view of those striking enzymes changes, a study of a renal enzyme, transamidinase, was undertaken in mice.

Transamidinase catalyzes the transfer of the amidine group of arginine to the amino group of glycine to form guanidinoacetic acid, a precursor of creatine. The transamidinase activities of kidney and pancreas are much greater than the activities found in other tissues of the rat (7). Rat kidney transamidinase activities have been investigated under a great variety of experimental conditions. Kidneys from the following experimental animals have very low transamidinase activities *in vitro*: rats fed a protein-free diet, or a complete diet supplemented with creatine (7); hypophysectomized rats (8); rats given doses of a mercurial diuretic (9); and tumor bearing mice or rats (10).

In this study, transamidinase activities were determined in kidneys from mice that had been given injections of mithramycin.

Methods and Materials. C3H mice, age 12 weeks and weighing approximately 25 gm, were used. Tissue transamidinase activity was determined by a simple assay method (11). The enzyme activity was expressed as mg of guanidinoacetic acid formed per hour per gm of kidney. RNA synthesis was measured by the incorporation of phosphate-^{32}P expressed as the relative specific activity (12). Mithramycin was administered ip in a dosage of 1500 μg/kg. The dietary intake of the treated mice was measured daily. Pair-fed mice received the amount of food consumed the day before by the mithramycin treated animals.

Results. Twenty mice were given injections of 1500 μg/kg of mithramycin and 20 mice were given injections of physiological saline. One half of each group of mice were sacrificed 12 hours after the injections and the other half were sacrificed 24 hours after the injections. There was no significant alteration in the kidney transamidinase activity in the mithramycin injected mice (Table I, Series I).

A group of 20 mice were next injected with 1500 μg/kg of mithramycin i.p. daily for 3 days, and in groups of 5, the injected mice were sacrificed every 24 hours over a 3 day period. The transamidinase activities remained constant until the 4th day after the 1st injection. Four days after the injection, 4 out of the group of 5 mice had died. At that time, the activity of the 1 mouse was 50% of normal.

Next, 20 mice were given 1500 μg/kg of mithramycin i.p. daily for 3 days. The daily food consumption of these mice was recorded and a group of 10 pair-fed control mice were given as much food per day per mouse as was consumed by the mithramycin injected mice the preceding day. The pair-fed group excluded any differences in transamidinase activity due to reduced dietary intake during mithramycin therapy. Of the 20 mithramycin treated mice, 7 died during the experiment. On the morning of the 4th day after the 1st injections of mithramycin, 10 control mice and 13 mithramycin injected mice were sacrificed. The transamidinase activities of the pair-fed control mice and of the mithramycin injected mice were 1.64 and 0.72 mg guanidinoacetic acid formed per gm of kidney per hour, respectively. The probability that this difference was due to chance was less than 0.001 (Table I, Series II).

In the final study, the RNA synthesis in the kidney was measured on the 4th day after the 1st injection of mithramycin following the same procedure as in the previous study. Morphological studies of the kidneys at this time interval were studied. Three groups of animals were studied: (a) a control group that was fed *ad libitum* and received no mithramycin, (b) a mithramycin treated group, and (c) a pair-fed control group. On the morning of the 4th day, phosphate-^{32}P was injected ip in the treated group and the control group that was not pair-fed. The pair-fed group was managed in the same manner on the 5th day. Two hours later, the animals

TABLE I. Transamidinase Activities *in Vitro* of Kidneys from Mice Treated with Mithramycin.

	No. mice	Injection	Sacrifice (hours after infection)	Transamidinase[a] Mean ± SE
Series I	10	Saline	12	2.08 ± 0.063
	10	Mithramycin 1500 μg/kg	12	2.08 ± 0.047
	10	Saline	24	1.98 ± 0.063
	10	Mithramycin 1500 μg/kg	24	2.21 ± 0.057
Series II	10	Pair fed controls	24	1.64 ± 0.017
	13	Mithramycin 1500 μg/kg daily for 3 days	24	0.72 ± 0.053

[a] Mg guanidinoacetic acid formed per gm kidney (wet weight) per 2 hours.

were sacrificed. The kidneys of the mithramycin treated animals were large and pale, and the livers appeared normal. The right kidney was removed for pathological study, and the left kidney was removed for RNA isolation. There was no difference in the rate of RNA synthesis in the 3 groups. There was no evidence of RNA synthesis inhibition 24 hours after the last dose of mithramycin.

Morphological examinations revealed extensive damage of the tubular cells with normal appearing glomeruli.

Discussion. Mithramycin is an effective antitumor agent that produces severe clinical and biochemical toxicity. It has been demonstrated to be an inhibitor of RNA synthesis (4). Because of the striking enzyme changes associated with hepatic injury during mithramycin therapy, it seemed possible that mithramycin might alter transamidinase activities.

A significant lowering of kidney transamidinase activities did occur in mithramycin treated mice compared to pair-fed control animals, but only after 3 days of injections. This decrease of transamidinase activity could have been due to inhibition of enzyme synthesis or to leakage of the enzyme from the damaged tubular cells. It is apparent that in measuring the enzyme, the cumulative toxic effect of mithramycin is measured. There was no evidence of inhibition of RNA synthesis after 24 hours, since the inhibition of RNA synthesis is of only a short duration (5).

In view of the apparent lack of glomerular damage, and the intensive tubular damage, the localization of transamidinase activity may be in the tubular cells with lesser amounts in the glomerulus, if any. Also, the data are interpreted to indicate that mithramycin had no effect on the rate of synthesis of the enzyme transamidinase per se, since under the previously studied experimental conditions that produce low kidney transamidinase (i. e., protein-depletion or creatine supplementation), the results are apparent 24 hours later. In these experiments with mithramycin, the low kidney enzyme values did not appear until 4 days later, at which time, extensive kidney tubular damage had occurred. It is suggested that the loss of enzyme activity with mithramycin is the result of the loss of the integrity of the kidney tubule cell and the physical loss of the enzyme from the kidney and/or loss of the cells capability to synthesize this enzyme.

Summary. Mithramycin is an antitumor agent known to inhibit the synthesis of RNA and to produce severe renal toxicity. After 3 days of administration of mithramycin to normal mice, there was a significant decrease in kidney transamidinase activities compared to pair-fed control animals. It appears that transamidinase activity is localized in the tubular cells and the loss of enzyme activity is the result of the loss of the integrity of the kidney tubule cells.

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Inhibition of Antigen–Antibody Reactions *in Vitro* by Nonsteroidal Anti-Inflammatory Agents (32634)

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Although the etiology of rheumatoid arthritis and other connective tissue diseases remains unknown, an increasing body of evidence suggests that these may be autoimmune diseases (1). Even though no specific antigen capable of initiating rheumatoid arthritis has been isolated, the possibility exists that the disease could result from an interaction of an altered gamma globulin with rheumatoid factor on synovial and connective tissue cells. Furthermore, Rawson and Torralba demonstrated that injections of complexes of heat-aggregated gamma globulin and rheumatoid factors into normal rabbit joints induce lesions similar to those observed in rheumatoid arthritis in humans (2). Thus, agents useful in treating rheumatoid arthritis might exert their therapeutic effects, at least in part, by preventing the interaction of these immunologic species.

Investigations with salicylates indicate that these compounds affect immunologic phenomena (3). Friend (4), using *in vitro* anti-egg albumin and antipolysaccharide systems with appropriate antigens, observed that sodium salicylate and its analogs inhibit antigen–antibody precipitation reactions. In this study we compare other nonsteroidal anti-inflammatory drugs in their ability to inhibit similar antigen–antibody reactions.

Materials and Methods. Four albino rabbits (2–4 kg) were injected twice weekly for 3 weeks with 1.0% egg albumin (EA) in isotonic saline. Initially each rabbit received 2.0 ml of EA solution intravenously followed by 1.0 ml of a 1:1 suspension of EA solution and corn oil subcutaneously in the foot pads. Subsequent intravenous doses were 1.0 ml of EA solution so that each animal received a total of 75 mg protein. Seven days after the last injection the animals were anesthetized (25 mg/kg pentobarbital Na, iv) and bled by catheterization of the carotid arteries. Blood was pooled and allowed to clot overnight in the refrigerator before centrifuging at 5°C for 15 min at 1470 *g* in a RC-2 Sorvall centrifuge. A gamma globulin fraction was prepared from the pooled sera as outlined below.

After precipitation at 33.3% saturation with ammonium sulfate, pH 7.0, the gamma globulin (Anti-EA) fraction was dissolved in 0.005 *M* sodium phosphate buffer, pH 7.0, and dialyzed in the same buffer. The dialyzate (about 60 ml) was divided into 5-ml aliquots and frozen until used. Each milliliter contained 19.1 mg protein. Maximum precipitation was obtained after incubation at 37°C for 1 hour using 0.5 ml Anti-EA mixed with 0.5 ml of a 1:100 dilution of 1.0% EA solution.

In inhibition studies, 0.5 ml of a 1:100 dilution of 1.0% EA was added to 0.5 ml of Anti-EA followed immediately by 2.0 ml