related to the virus-antibody complexes shown in these experiments(4).

The demonstration of infectious virus-antibody complexes in this common, but severe disease of mink suggests that similar mechanisms should be searched for in other diseases such as mouse mammary tumors(15) in which antibody to the causative agent has not been found by the usual tests.

Summary. Mink affected with Aleutian disease have viremia which persists until death. Removal of G immunoglobulin from infectious serum of affected mink markedly reduces the virus titer. The virus in the serum exists as a complex with immunoglobulin, in which *in vivo* infectivity is still present.

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Muramidase Activity in Leukemic Rats.*† (32540)

DAVID S. ROSENTHAL AND WILLIAM C. MOLONEY

Research Laboratory, Holy Ghost Hospital, Cambridge, Mass., and Tufts Hematology Laboratory, Boston City Hospital, Boston, Mass.

Muramidase, or lysozyme, discovered by Fleming in 1922 in human tears, was later found to be widely distributed in many tissues, including leukocytes(1). Recently Finch *et al* demonstrated markedly elevated muramidase levels in the serum of patients with monocytic and chronic granulocytic leukemia (2). Osserman confirmed these observations and found that large amounts of this enzyme were excreted in the urine of patients with monomyelocytic leukemia(3).

The presence of abnormally large amounts of muramidase in the serum and urine of patients with monocytic and granulocytic leukemia is of considerable interest; further investigations on the properties and functions of this enzyme may contribute important information on the characteristics and behavior of leukemic leukocytes.

In this laboratory studies have been carried out for the past 10 years on leukemia in the rat. Recent investigations have shown that high levels of muramidase activity occur in the serum, urine, ascitic fluid and myelocytic cells of rats with transplanted chloroleukemia.

Materials and methods. Muramidase activity was measured by a modification of the method of Smolelis and Hartsell(4). To 2 cc of lysozyme substrate (Difco) was added 0.2 cc of test serum, urine or homogenate and the optical density read over 90 seconds (ΔOD 540/90 sec). Units can be converted to micrograms of purified egg white lysozyme (Difco). Twenty-four hour urine samples were obtained from either individuals or pools of control and leukemic rats as well as various

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tissue homogenates. Protein electrophoresis was performed on cellulose acetate at pH 8.6.

Muramidase activity was determined on serum and urine of normal and leukemic Wistar-Furth (W/Fu) and Fischer rats. This leukemia is a unique mononuclear cell type which occurs spontaneously in these animals and which can be readily transplanted. Enzyme activity was also determined in the serum and urine of normal Sprague Dawley rats and animals of this breed bearing transplanted chloroleukemia. This chloroleukemia was originally obtained from Shay and since 1958 has been passaged 96 times in our laboratory(5,6). Passage has been carried out by injection of a 3 to 5 million chloroleukemic cell suspension subcutaneously to Sprague Dawley pups. During the past year local tumors (chloromas) appeared within 10 to 14 days and the majority of rats rapidly develop leukemia. The chloroma cells and those in the peripheral blood are myeloblasts and promyelocytes and show positive cytoplasmic esterase, myeloperoxidase and alkaline phosphatase activity on histochemical staining. At autopsy solid green chloromatous tumors are found at the site of injection or in the peritoneal cavity; spleen, liver, lymph nodes and bone marrow often show a greenish hue and all these tissues demonstrate a bright pink fluorescence when exposed to ultraviolet light.

Studies on serum muramidase activity were carried out following transplantation before and after local chloromas developed. In one animal muramidase activity was determined on ascitic fluid and in several rats this enzyme activity was investigated in extracts of homogenized chloroma cells. Similar studies were carried out on another chloroleukemia induced in Sprague Dawley rats by R. Jones with Sr^{90}(7). This chloroleukemia has undergone 14 passages in our laboratory and is similar in most respects to the Shay chloroleukemia.

Experimental results. As shown in Table I, serum and urine muramidase activity was similar in all 3 breeds of normal rats tested. W/Fu and Fischer rats with mononuclear cell leukemia showed no significant elevation of muramidase activity except in one animal. This rat had evidence of severe renal infection and extensive leukemic infiltration of the kidneys at autopsy. On the other hand, Sprague Dawley rats with either the Shay or Jones chloroleukemia uniformly showed markedly elevated muramidase levels in the serum, urine, and chloroma cell homogenates; and in the ascitic fluid in one animal. Following transplants of the chloroma, serum muramidase levels did not become elevated in the recipient until tumors were palpable.

Discussion. A number of investigators have studied serum muramidase levels in normal individuals and in patients with a variety of disorders and it seems well established that the highest elevations of serum muramidase activity are found in monocytic, monomyelocytic and chronic granulocytic leukemias (2,8). That muramidase is present in monocytes and granulocytes but absent from other cells of the blood forming organs has been

TABLE I. Muramidase Activity in Rat Serum and Urine.

Breed	Serum* No.	Serum* Avg	Serum* Med	Serum* Range	Urine† No.	Urine† Range
			Normal values			
Sprague-Dawley	57	17.4	17.0	10–22	6	0
Wistar-Furth	14	17.7	17.5	12–22	4	0
Fischer	9	17.2	15.0	12–25	6	<100
			Chloroleukemia			
Sprague-Dawley	27	280	290	70–410	9	2.5×10^5–8.0×10^5
			Mononuclear cell leukemia			
Wistar-Furth	16	18.9	16.0	10– 42	—	—
Fischer	8	41.1	28.5	13–110	—	—

* Units = ΔOD_{540}/90 sec/0.2 cc.

† Units = ΔOD_{540}/90 sec/24 hr.

Chloroma cell homogenate—102 units/mg tissue.
Chloroleukemia ascites—480 units/0.2 cc.

demonstrated by various techniques(9,10). Osserman and Lawlor have reported on serum and urinary muramidase in cases of monomyeloid leukemias(3). They present evidence that the muramidase found in these leukemic patients was normal enzyme produced in excessive amounts. These authors have shown that muramidase is a basic cationic protein of small molecular weight, which can be demonstrated by electrophoresis in urine but not in serum. Osserman has recently reported on the successful crystallization of human muramidase and has noted that muramidase is located in the lysosomes of leukemic monocytes(11,12).

Many of the properties and characteristics of muramidase have been established, but the question of whether both monocytes and granulocytes elaborate muramidase is, according to Osserman, not clearly resolved(3). However, it seems quite evident that in the rat chloroleukemia, where there is no question about the myelogenous nature of the leukemic cells, high levels of muramidase activity were noted in serum, urine and homogenates of chloroleukemic cells. The presence of large amounts of this enzyme in the urine and in homogenates of chloroma cells make these animals excellent sources of muramidase for further studies. It will be of great interest also to investigate the lysosomal localization of muramidase in chloroleukemic cells.

In our experience with 144 primary leukemias encountered in 1299 control, irradiated and methylcholanthrene treated Wistar, W/Fu and Fischer rats, all but 15 were of the acute mononuclear (AMN) cell type. The leukemic cells are difficult to classify. In the majority of cases the cell is large with an indented, sometimes "folded" nucleus; the chromatin, however, is of lymphoid type except in younger cells where it has more of a reticulum cell appearance. The cytoplasm is clear blue but usually contains coarse to medium sized red granules. This leukemia does not have the distribution histologically of either lymphatic or granulocytic leukemia. As shown in Table I, only 2 rats with AMN leukemia showed increased serum muramidase levels and only one was in the range of chloroleukemic animals. In this rat with a serum level of 110 units, there was evidence at post mortem of extensive renal infections as well as infiltration of the kidneys by the leukemic process. Osserman and Lawlor(3) have confirmed the occurrence of moderately elevated muramidase levels in chronic pyelonephritis noted previously by Prockop and Davidson(13). Of significance was the finding of normal serum enzyme levels in 14 W/Fu rats made leukemic by injection of spleen homogenates from rats with acute mononuclear cell leukemia. The lack of significant elevation of serum and urine muramidase levels in rats with acute mononuclear cell leukemia is very suggestive evidence against a monocytic derivation of this form of leukemia.

Summary. 1. Greatly elevated serum and urine muramidase levels were observed in rats with transplanted chloroleukemia. 2. In one chloroleukemic rat with ascites, the fluid had a very high muramidase content. 3. Homogenates of chloroma cells consisting of myeloblasts and promyelocytes had very high muramidase activity. 4. In rats with acute mononuclear cell leukemia, no significant elevation of serum or urinary muramidase activity was noted, suggesting rat mononuclear leukemia is not myelogenous or monocytic in origin.

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The Reproductive Performance of the Laboratory Mouse: Maternal Age, Litter Size and Sex Ratios.* (32541)

ROBERTS RUGH AND MARLIS WOHLFROMM
Radiological Research Laboratory, Dept. of Radiology, Columbia University, New York City

The description of the normal reproductive activity of mice cannot be generalized because it varies from strain to strain(1,2). Age and previous breeding experience are also contributing variables. Nevertheless, there is merit in presenting statistical data for a particular strain of mouse used frequently in research laboratories. The strain here described is known as the CF1 from Carworth Farms, but the data would probably be quite similar for other strains.

Experimental procedure. Virgin females of 3 months of age were earmarked for individual identification and 350 were selected for continuous matings (except for 3-5 days after delivery) with adult males of the same strain. The offspring were removed at birth, analyzed and discarded so that the female could soon become pregnant again. Data were collected through the first 4 pregnancies and since no nursing was allowed, the time-lapse for the 4 pregnancies was reduced.

At birth the members of each litter were counted, including those born dead, those that had persistent amnions, or showed anomalies of the central nervous system, or were eaten by the mother. Subtracting these delivered but abnormal mice from the total, the litter size of "normal" mice was established for each mouse and for each of the 4 pregnancies. In addition, the sex of each mouse was determined at birth so that the sex ratio of successive litters could be evaluated. Thus, the data herein presented come from 4 successive pregnancies of 350 selected female CF1 mice.

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In addition 557 litters from 4 different groups of females were analyzed. The females were young and mature virgins, multipara females and ex-breeders of 10-12 months of age. Since these pregnant females were dissected at 18 days the data include resorptions, anomalies, and information on total implantations.

Experimental data. Aside from abnormal or destroyed newborn mice, some 13,508 "normal" newborn mice were available for this study. In other studies it has been established that the average implantation number for this strain of mouse is close to 11. When the average litter size is determined for all of the 350 mice it is shown that the first litter had an average of 8.40 and the 4th litter an average of 10.28, with increments for the 2nd and 3rd litter over the 1st. The 5th litter of 128 of these mice averaged 10.35 offspring. Subsequent litters tend to decrease in number.

If one lists those pregnancies which result in litters of different sizes, it becomes apparent that among the primipara mice the largest group have litters numbering 9, while in the 2nd, 3rd, and 4th litters, the greatest number of mice produced 11 offspring. Thus, not only is the average litter size increased with reproductive activity (or successive litters), but all litters after the 1st have their greatest size frequency at 11 offspring. Individual records vary greatly, however, with some primipara mice having a low litter size of 1 and others with a high of 14, while mice having their 4th litters varied from a low of 1 to a high of 18. The data indicate that the 1st litter of these mice is, on the average, the smallest.

The total percentage of anomalous mice at birth (dead, with amnion, with gross anomalies, or eaten by the mother for whatever