Role of the Kidney in Accumulation of Egg White Muramidase in Experimental Animals.* (28866)

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Muramidase† activity is highest in the kidney and the lung of normal animals(1,2); however, lung muramidase activity is not significantly changed under experimental conditions which evoke marked increases of kidney muramidase(3,4,5). The reason for this increase in muramidase activity is not known. *De novo* biosynthesis by the kidney, or accumulation of the enzyme from the circulatory system could be possible explanations.

Evidence is presented here to demonstrate that the kidney is capable of accumulating from the blood stream large amounts of either endogenously produced or exogenously administered muramidase.

Experimental. Animals used in these experiments, Sprague-Dawley rats (Charles River) and guinea pigs (Rockland Farms) were maintained on standard laboratory diet (Purina Laboratory Chow) with free access to water. Animals were sacrificed under nembutal narcosis, and organs were rapidly excised, blotted, weighed and homogenized as previously described(3,6) to prepare extracts for muramidase determinations. Blood plasma or urine was tested after dilution with the proper buffer. The enzymatic assay for muramidase activity, a modification of Litwack's method(7) has been previously described(8). Enzyme activity was calculated from a standard curve obtained with egg white muramidase‡ (EWM) and results expressed as mg equivalents of EWM per gram of wet tissue.

The purity of the injected muramidase was checked by chromatography on 1RC50 resin at pH 6.90 with 0.2 M phosphate buffer. The UV absorption of the effluent was directly recorded at 280 μm using continuous flow cells§ of 0.3 ml volume and 1 cm light path in a Beckman DB spectrophotometer against buffer in the reference cell.

Exp. 1. Fifty mg of EWM dissolved in 0.2 M phosphate buffer pH 7.2 were injected daily i.p. in a group of 10 rats (average body weight 135 g). In a second group the dosage was doubled. Littermate controls for each group(10) received daily i.p. injections of buffer. Muramidase content of kidney, spleen and lung was determined, after 13 days of treatment.

Exp. 2. Enhanced production of endogenous muramidase was induced by implanting Jensen-Sarcoma. The animals were kept in metabolic cages, and the urine level of muramidase activity was determined. At the end of experiment, blood and organs were analyzed for muramidase.

Exp. 3. The disappearance rate of EWM from blood was studied in 4 rats (body weight 400-600 g) under nembutal, whose jugular vein and carotid artery were cannulated with Polyethylene tubing (PE 60). Blood samples were taken prior to i.v. injection of 100 mg of EWM dissolved in rat plasma, and at intervals during the next 60 minutes. In a second group of 4 animals both kidneys were excluded from the circulation by ligation of the vascular peduncle before EWM administration. At the end of experiment kidney, spleen and lung muramidase activity was determined.

Exp. 4. A similar experiment (as Exp. 3) was performed on guinea pigs (700-800 g).

The individual kidney extracts of the rats from Exp. 1 were pooled and muramidase precipitated with 6 volumes of ethanol(6), collected by centrifugation, dissolved in H_2O

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† New name recommended by Commission on Enzymes of International Union of Biochemistry, Muramidase intead of Lysozyme (E.C.3.2.1.17).

‡ Worthington Lot 597.

§ Furnished through the courtesy of Pyrocell Co.

TABLE I. Distribution of Muramidase Activity After Administration of EWM in Normal Rats. mg of EWM equivalents/g of wet tissue.

	Controls	Dose, 50 mg/day	Controls	Dose, 100 mg/day
Kidney	.384 ±.072*(9)	1.439 ±.314 (10)	.404 ±.097 (10)	3.804 ±1.910 (10)
Spleen	.103 (pooled)	.180 (pooled)	.041 ±.016 (6)	.408 ± .020 (4)
Lung	—	—	.334 ±.064 (10)	.357 ± .075 (10)

* Mean ± S.D.

and absorbed on a 1RC50 column buffered at pH 6.2. Muramidase was then eluted by pH gradient (pH 6.2-7.2) with 0.2 M phosphate buffer. The fractions showing activity were pooled, freeze-dried and desalted on a Sephadex G25 column with pyridine acetic acid buffer at pH 5.5(9). The enzyme fraction was lyophilized and used for direct chromatography on 1RC50 at pH 6.90 using 0.2 M phosphate buffer(10).

Results. Rats injected with either 50 or 100 mg of EWM (Exp. 1) showed increased muramidase activity mainly in the kidney (Table I) and levels of kidney muramidase were proportional to amount of EWM administered. No change was found in spleen or lung in the one group of animals tested. The increased kidney muramidase activity observed could represent either accumulation of injected EWM or increased production of endogenous muramidase.

Rat kidney muramidase (RKM) was eluted from an IRC-50 column ahead of the EWM (Fig. 1). Extracts of the rat kidneys from Exp. 1 when chromatographed showed 2 distinct peaks of enzyme activity corresponding to the positions expected for RKM and EWM respectively (Fig. 2).

In animals bearing Jensen-Sarcoma, muramidase activity was not demonstrable in the urine until about the 10th day after transplantation. Increasing amounts of enzyme are thereafter excreted as the tumor grows and the kidney muramidase level increases (Fig. 3).

When EWM was injected into the guinea pig blood stream (Exp. 4) its level fell rapidly (Fig. 4), and within approximately 20 minutes 50% of the administered dose had disappeared. However, when kidney function was eliminated by ligation of the renal vascular peduncle the blood level of the injected EWM remained unchanged for more than 40 minutes and then fell. This latter fall could be associated with the appearance of edema in the animals. Similar results were obtained in normal and kidney ligated rats where the enzyme activity disappeared from the blood at a rapid rate and the ½ time disappearance values were of the order of 10 minutes (Fig. 5). In normal animals the enzyme activity appeared rapidly in the urine, but the amount in urine varied for each animal.

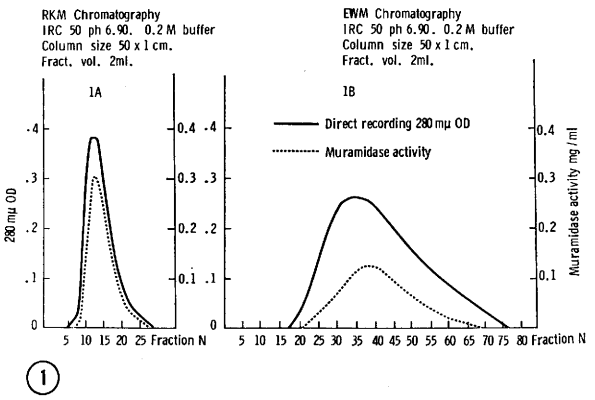
From Fig. 4 it is possible to calculate the blood volume of the animal by extrapolation to time zero. The values obtained are in good agreement with those calculated by considering the blood volume equal to 7% of the body weight(11). In this experiment the figures were respectively 41.5 ml (from body weight) and 42.0 ml from muramidase extrapolation values. Sixty-eight per cent of the injected EWM was recovered in the urine 60 minutes after injection. Total EWM recovery was 78% (blood, urine and tested organs). The 22% unaccounted for, could represent EWM trapped in the rest of the carcass, or degraded by the tissues.

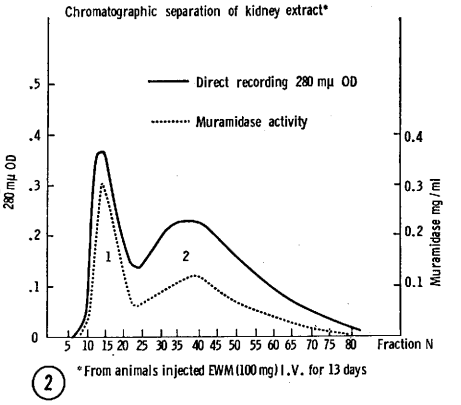
Discussion. The results demonstrate that injected EWM rapidly disappears from the blood stream, accumulates in the kidneys and is eliminated in urine. This rapid fall does not occur when the kidneys are excluded from the circulation.

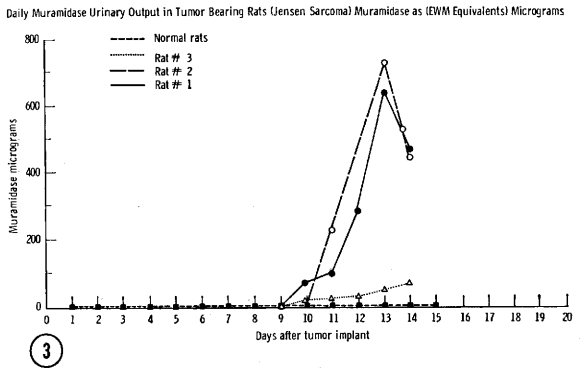
EWM for rats and the guinea pig is a foreign protein and therefore, may be handled by the kidney in a manner different from endogenously produced muramidase. However, other evidence supports the concept that both exogenous and endogenous muramidase are handled by the kidney in the same fashion. For example, animals whose endogenous muramidase production has been enhanced by growth of transplantable tumors

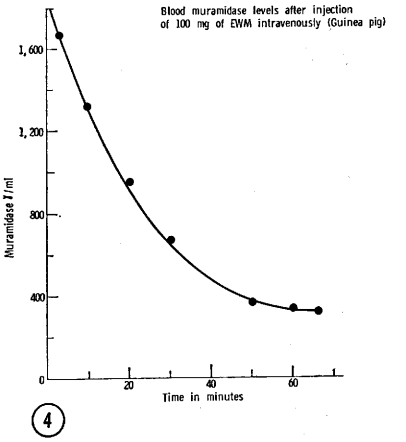
show a maximum increase of kidney muramidase activity and the appearance of muramidase in urine. Muramidase activity is not found in the urine of normal animals but appears in urine under conditions of enhanced production. This is in accord with the suggestion of a kidney threshold for muramidase in man(12) and rabbits(13).

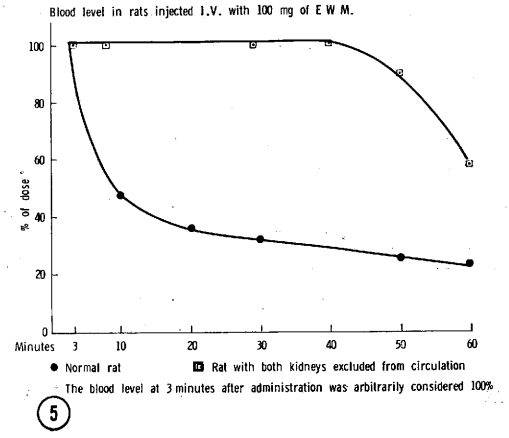
Muramidase activity has been shown to increase in conditions accompanied by RES stimulation; chronic infections(14) implants of transplantable tumors(3,4) BCG infection(15) or zymosan injection(16). Furthermore, macrophages and polymorphonuclear leucocytes have been shown(17,18) to be rich in muramidase. On this evidence one











could postulate that muramidase is produced by phagocytic cells as a response to stimuli of the host defense mechanism and when released from such cells would be available for accumulation by the kidney and partial elimination into the urine.

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A Virus Inhibitor in Pharyngeal Washings from Patients with Influenza.* (28867)

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Several investigators have recently suggested that interferon(1) plays a role in recovery from viral infection(2,3,4,5,6,7). Isaacs and Hitchcock demonstrated interferon in the lungs of mice experimentally infected with influenza virus(2) and correlated its presence with virus content and eventual recovery. Baron and Isaacs failed to find interferon in human lung tissue obtained from fatal cases of viral influenza(8) and speculated that its absence may have been "related to the fatal outcome." To the best of our knowledge, the presence of interferon or similar virus inhibitors has not been demonstrated in *patients* with viral infection.

Widespread A_2 influenza occurring in the winter of 1962-3 afforded the opportunity to look again for a virus inhibitor in human disease. The nasopharynx was chosen as a source of such an inhibitor for two reasons: 1) influenza virus multiplies in the human upper respiratory tract(9), and 2) a virus inhibitor had been previously demonstrated in pharyngeal washings obtained from a young boy with upper respiratory illness due to parainfluenza II virus infection(10).

This communication reports the presence of an inhibitor of Sindbis virus, exhibiting certain of the properties of an interferon, in pharyngeal washings from patients with A_2 influenza infection.

Methods and materials. Epidemic. Localized outbreaks of febrile respiratory illness occurred in New England early in 1963. Influenza A_2 virus was isolated from hospitalized university students in early February, and similar strains were recovered repeatedly during the next 4-6 weeks.

Thirteen patients with influenza were selected for inclusion in the present study on the basis of comparability of clinical specimens and severity of illness. This group was composed of 12 males and 1 female ranging from 17-34 years of age. For comparison, 5 patients with rubella (female, 17-23 years), 2 patients with infectious mononucleosis (female, 19 and 21 years), 5 patients with leu-

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