Alterations of Alkaline Phosphatase in Mouse Tissues after Experimental Infection. (31859)

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The intracellular function of alkaline phosphomonoesterase (AP; EC 3.1.3.1) is presently not well defined. The enzyme has a relatively wide substrate specificity and is found in high concentrations in the small intestines, bone, and kidney. Other tissues have appreciably less, with brain and muscle having very low activity. Changes in serum activity of AP have had extensive diagnostic application in medicine(1,2). Serum activity is elevated in diseases affecting the skeletal system, and the hepatobiliary system as, for example, in viral hepatitis or obstructive jaundice.

The level of AP in leukocytes is known to be increased after infection(3-7). In patients with leukemia leukocytic AP was variable, being elevated in some cases(4) and markedly depressed in others(1). Generally, there was no correlation between the serum and the leukocytes AP. However, in a report on malignant lymphoma an increased granulocytic AP did correlate with an increase in the serum enzyme(8).

In a recent study of virus-infected mice no significant change of plasma AP was noted by Mahy *et al*(9). A reduction was noted by Garg and Sharma(10) in the liver of virus-infected rabbits. In unpublished studies in our laboratory, liver AP of chickens was elevated, but serum and intestinal activity were depressed during Newcastle disease virus infection. Serum AP was markedly elevated by culture filtrates of *Bacillus anthracis*(11); a rapid increase in serum AP has been reported to accompany experimental staphylococcal sepsis in mice(12), but otherwise, serum or tissue AP levels during acute bacterial infections have received relatively little attention. This report presents data on sequential alterations of AP in several mouse tissues and serum during acute pneumococcal infection.

Materials and methods. Male white mice (CD-1 strain) weighing 25-35 g were obtained from the Charles River Mouse Farms, Inc., North Wilmington, Mass. Adrenalectomized mice of the same weight and strain, maintained with normal saline as drinking water, were studied 1 week postoperatively. Sodium *beta*-glycerophosphate substrate was purchased from the Hartman-Leddon Co., Philadelphia, Pa.; amino acids from Nutritional Biochemicals Corp., Cleveland, Ohio; sterile hydrocortisol sodium succinate from Upjohn Co., Kalamazoo, Mich., and inorganic reagents from Fisher Co., Silver Spring, Md.

Stock cultures of *Diplococcus pneumoniae* type I (strain A_5) were kept at —70°C in brain-heart-infusion broth, pH 7.4, containing defibrinated sheep red blood cells and rabbit serum. The culture was mouse-passed at monthly intervals and remained fully encapsulated and mouse virulent. A transfer culture was grown in the same medium for 18 hours. Subsequently, a 4-hour culture was prepared which was diluted serially in tryptose-phosphate broth, pH 7.8. A 10^{-8} dilution normally contained between 10-25 organisms per ml. Blood-agar plates were used to determine the count.

Mice were injected subcutaneously in the back with 0.5 ml of the 10^{-8} bacterial dilution. This dose caused death in 48-72 hours. Two groups of 10 mice each—uninfected controls and infected—were sacrificed and the tissues prepared as follows. Animals were bled from the right brachial artery under ether anesthesia. The blood was allowed to clot, centrifuged, and the serum removed. Femurs and sternum were scraped clean; the kidneys were freed of all surrounding fat. Lungs, spleens, and livers were rinsed in 0.9% saline to remove adherent blood. The upper small intestine (about 15 cm in length) was freed of its mesentery and rinsed thoroughly in 0.9% saline. It was not necessary to remove the extraneous fluid of the lumen, since this did not appear to influence the enzyme activity. Pooled bile was studied. The tissues and fluids were stored at —20°C

until the time of enzyme assay. The entire study was repeated on 4 separate occasions in intact mice and twice in adrenalectomized mice.

When adrenalectomized mice were used, groups of 5 animals were included at each point. Three sets of animals were used: controls were given 0.1 ml normal saline per mouse intraperitoneally; the second set, 0.1 mg cortisol per mouse; and the third, 3.0 mg cortisol per mouse. Cortisol was injected at 12-hour intervals beginning 3 hours before injection of pneumococci.

The enzyme preparation was made in the following manner: Temperature was maintained between 0-5°C. Thawed tissues were finely minced with scissors and 10% (W/V) suspension of each was prepared with 0.25 M sucrose. These were homogenized for 1 minute in a Potter-Elvehjem apparatus with a teflon pestle, bones in a Waring blendor. Each tissue homogenate was centrifuged at 12,000 $\times$ *g* for 15 minutes in a Spinco Model L centrifuge. The supernatant solution was removed by pipetting, filtered through glass wool to remove fat, and subsequently used as the enzyme solution without further treatment. These preparations were not contaminated by the infecting bacteria. Thawed serum was used directly.

The enzyme assay was patterned after the method of Shinowara, Jones, and Reinhart (13). The substrate solution contained 0.02 M sodium *beta*-glycerophosphate, 0.02 M Veronal buffer, pH 9.5, and 0.005 M $MgCl_2$. Specimens of intestine, kidney, sternum, and femur were incubated in a final volume of 3.1 ml at 37°C for 15 minutes, liver, lung and spleen for 30 minutes and serum, 60 minutes. After incubation the enzymatic reaction was stopped by addition of 2 ml of trichloroacetic acid. A reagent and tissue blank was included in each experiment. The inorganic phosphate (Pi) liberated was determined by the method of Fiske and Subbarow(14). Protein was determined by the Biuret method(15) using fraction V bovine serum albumin as a standard. Inhibitors were added at a concentration resulting in maximal inhibition.

Results. The infected animals became noticeably ill after 12 hours and all were dead by 72 hours. As infection progressed, an increased quantity of bile was secreted, as evidenced by the presence of a brilliant dark-yellow color of the intestinal content independent of food intake. The bile at no time contained more than minimal AP activity.

Results were expressed in international enzyme units (IU) μmoles Pi liberated per minute, per mg protein(16). Under the experimental conditions used, no significant change from control AP activity was found in the femur (7.30×10^{-2} IU), kidney (3.50×10^{-2} IU), lung (3.00×10^{-3} IU), spleen (3.00×10^{-3} IU), or sternum (6.50×10^{-2} IU). However, in the liver, small intestine and serum, alterations were found as the infection proceeded. These 3 tissues were subsequently investigated more intensively.

The control value obtained from the uninfected animals during each experiment was arbitrarily assigned a value of 100%; changes were expressed as per cent of control (Fig. 1). Thus, a 38% decrease, 7.52×10^{-3} to 4.72×10^{-3} IU, occurred in the liver in 64 hours. The enzyme level in serum increased 40%, from 1.10×10^{-3} to 1.55×10^{-3} IU. The greatest change was found in the small intestine, where a 65% increase in activity was observed, 5.22×10^{-1} to 8.90×10^{-1} IU.

A constant feature of the AP activity was the marked activation seen in the presence of Mg^{++}. Therefore, 0.005 M $MgCl_2$ was added to all assay vessels. The effects of other ionic compounds and certain amino acids are shown in Table I. Fishman *et al*(17) showed in rats that Zn^{++} inhibited liver, but not intestinal, AP activity; this was confirmed. $AlCl_3$ inhibited liver, serum and intestinal enzyme almost completely, while NaF had relatively little effect. NaCN inhibited intestinal and serum activity strongly; the liver enzyme was partially resistant. Histidine, cysteine, leucine, and methionine strongly inhibited the intestinal and serum enzyme while sparing the liver enzyme. Phenylalanine and p-fluorophenylalanine inhibited the intestinal but not the liver enzyme. Serum enzyme behaved in a similar way to the intestinal en-

TABLE I. Effect of Various Inhibitors on Alkaline Phosphatase Activities of Two Mouse Tissues and Serum.*

Inhibitor	Concentration, M	Liver	Serum	Intestine
		Source of enzyme		
		% Inhibition†		
$AlCl_3$	.01	96	94	96
NaF	.01	6	7	3
NaCN	.005	37	98	98
$ZnCl_2$	.01	88	6	9
L-cysteine	.005	43	88	93
L-histidine	.01	53	89	90
L-leucine	.01	49	86	89
L-methionine	.01	55	90	90
DL-phenylalanine	.01	8	58	64
DL-fluorophenyl-alanine	.01	10	62	65

* Tissues were obtained from mice infected for 60 hr.

† Percent inhibition is expressed as:

$$100 - \left[\left(\frac{\text{I.U.} + \text{inhibitor}}{\text{I.U. normal}}\right) 100\right].$$

zyme. The data of Table I were obtained using mice infected 60 hours; however, the tissues from uninfected control mice showed the same behavior.

The pattern of altered AP activity was essentially similar in infected adrenalectomized mice. As shown in Table II, during infection in adrenalectomized control mice given only saline, the enzyme activity still increased in the small intestine, though somewhat less than in normals (Fig. 1). In adrenalectomized animals receiving cortisol in physiologic replacement doses or in pharmacologic amounts increased intestinal AP activity also occurred during the course of infection. Increasing the concentration of cortisol administered appeared to overcome the decrease in hepatic AP activity seen in normal mice; at the higher dose there was actually a stimulation of the activity.

Discussion. Since enzyme variations in tissues during infectious diseases have not been investigated extensively, our observation that intestinal AP activity increases during infection was a new finding. Alterations in liver and intestine were reproducible, progressive with time, and appeared to be specific with respect to each tissue.

The major change was an increase in activity in intestinal tissue unrelated to excretion of AP in bile, for despite the apparent increase in bile production no increase in its AP activity could be detected. Furthermore, the enzymatic activity decreased in the liver as the infection progressed. On the basis of inhibition studies it appeared that the slight increase in serum AP represented enzyme arising in the gut mucosa. Increased bile may have solubilized the intestinal enzyme in greater quantity. In support of this concept, it had previously been demonstrated by Fishman *et al*(18) that serum AP of intestinal origin decreased markedly following bile duct ligation in the rat.

Since intestinal mucosal cells have a turnover time second only to the leukocytes, it may be that the observed increase in intestinal AP is related in some manner to a more rapid cell turnover during infection(19). This may be similar to the elevated leukocyte AP associated with an increased white blood cell production during infection. The rate of intestinal mucosal cell turnover was not measured directly, but by another approach it was shown that the rate of C^{14}-leucine incorporation into proteins by intestinal mucosal cells was greater during pneumococcal infection (20).

Adrenal glucocorticoid hormones are known to increase protein and RNA synthesis in liver and intestines of mice and to increase AP activity in several tissues. Although graded doses of cortisol could induce corresponding increases of hepatic protein syn-

TABLE II. Changes in Alkaline Phosphatase Activities of Tissues of Infected Adrenalectomized Mice.

Tissues	Uninfected control,* %	% Change by hours after infection: 18	42
Saline control			
Small intestine	100	112	127
Liver	100	80	61
.1 mg cortisol			
Small intestine	100	119	135
Liver	100	91	86
3.0 mg cortisol			
Small intestine	100	124	144
Liver	100	108	114

* Control values obtained from uninfected animals were assigned a rating of 100%.

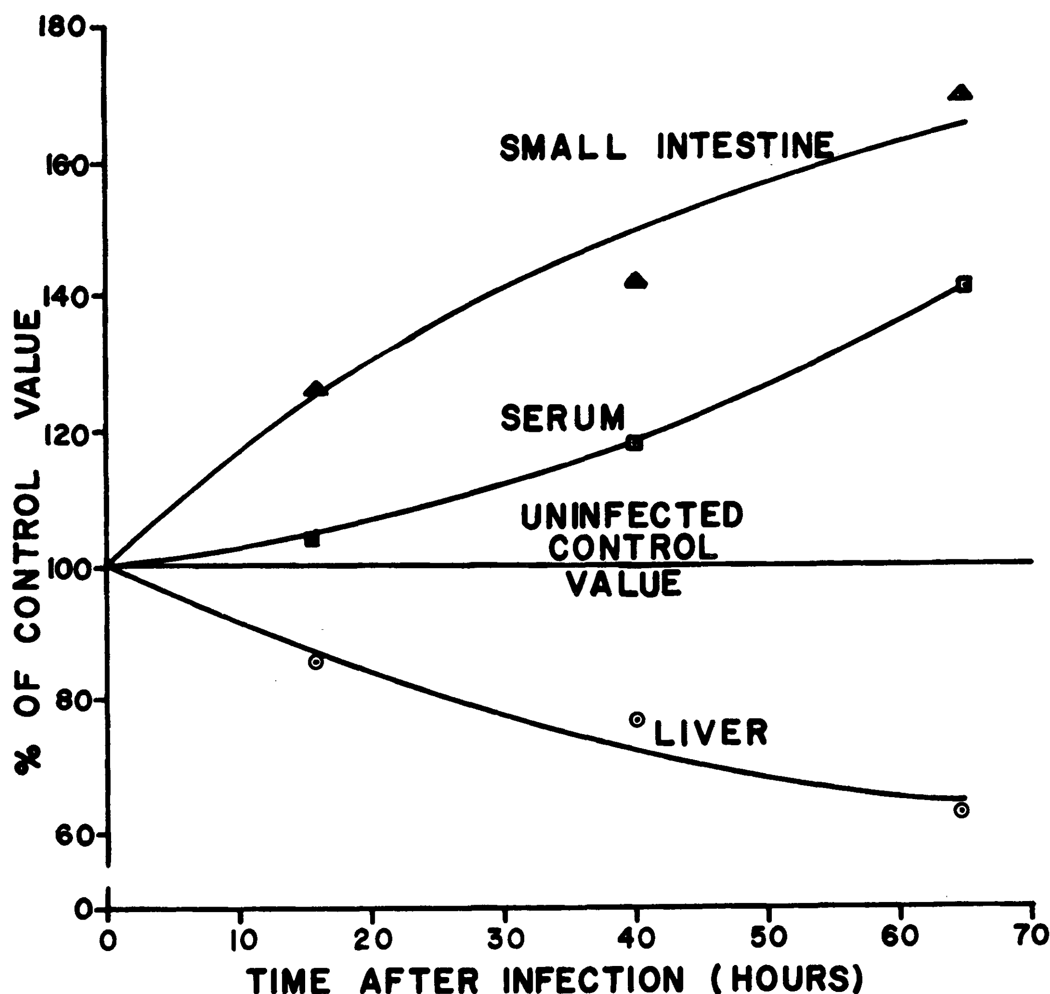

Fig. 1. Alteration of alkaline phosphomonoesterase activity in 2 mouse tissues and serum as a function of time after infection with *D. pneumoniae*.

thesis in adrenalectomized mice, no additional increment of synthesis accompanied pneumococcal infection(20). This implied that the increase of hepatic protein synthesis seen during infection in intact mice required the presence of functioning adrenal tissue and was not merely dependent upon a permissive effect contributed by circulating glucocorticoid hormones. In contrast, the infection-related changes in tissue AP activity occurred in the absence of the adrenals and included an apparently typical rise of intestinal and a fall in hepatic activity. The infection-related depression of hepatic AP was reversed by a pharmacologic dose of cortisol.

From evidence now available, it would appear that patterns of altered tissue enzyme activity during various infectious illnesses may be dependent on both the host species as well as the nature of the invading microorganism.

While these studies still leave the specific intracellular function of AP unanswered, they nevertheless provide additional information that AP alterations do occur in mouse tissues during an acute bacterial infection. The reason for the increase in AP activity in the small intestine, as well as in the leukocyte, during infection is being sought in additional investigations.

Summary. The alkaline phosphomonoesterase activity of several mouse tissues and

serum has been investigated in uninfected controls and animals infected with *Diplococcus pneumoniae.* No significant change of enzymatic activity was found in kidney, lung, sternum, femur, and spleen. As the infection progressed a decrease in activity was observed in the liver enzyme, while a marked increase occurred in small intestine. The enzymatic activity of the serum also increased during infection, but less strikingly. In organ-specific inhibition experiments it was shown that serum and intestinal enzyme behaved identically. The data support the concept that a major portion of serum AP may originate in the small intestine. Neither the infection-related increase in intestinal AP nor the fall in hepatic AP was dependent upon an intact adrenal gland in the presence of circulating glucocorticoid hormones.

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The Effect of Hypovolemia on Drinking in Rats With Lateral Hypothalamic Damage.* (31860)

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Bilateral lesions of the lateral hypothalamic area of rats result in adipsia and aphagia. However, with appropriate post-operative care a partial recovery from these deficits may occur so that a rat with these lesions eventually begins to ingest sufficient water and food to maintain life(1,2). Such rats have been termed "recovered laterals." The physiological stimuli which induce drinking in recovered laterals are not known. Intracellular dehydration, the best known stimulus for normal thirst, is ineffective, as are simple water deprivation and hyperthermia(3). It appears that recovered laterals may drink water only while they eat dry food(3).

Rats normally show a close relationship between food and water intakes. Gregersen (4) has emphasized the pronounced reduction in blood volume (hypovolemia) during feeding which is caused by the rapid secretion of digestive juices. Since a decreased plasma volume can be an important stimulus

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