ters. In our experience, the reliability and reproducibility of virus penetration studies are considerably enhanced if the following experimental conditions can be satisfied: low multiplicity infection, ability to make separate measurements of attachment from penetration, efficient methods for inactivating virus on the cell surface, and at least 100-fold increments in formation of infective centers at optimal conditions.

Summary. The kinetics of infective center formation was studied as a parameter of herpes simplex virus penetration into suspended HEp-2 cells. Similar rates of penetration were obtained when antibody or acid was used to inactivate virus attached to the cell surface, although the acid method was far more efficient and reliable. The data indicate that virus penetration is strictly temperature dependent and is virtually complete by 7 minutes at 37°, but the reaction does not conform to simple first order kinetics.

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Muramidase Activity of Kidney and Spleen in Swiss Mice Challenged with B.C.G., Zymosan and Bacterial Endotoxins.* (29393)

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Increased muramidase activity in the kidneys and spleens of tumor-bearing mice, rats and hamsters has been demonstrated in our laboratory(1,2,3). Elevated enzyme levels in tissues other than kidney have been reported for non-neoplastic pathological conditions(4, 5,6). Granulomatous tissue of the lungs of rabbits infected with *Bacillus Calmette-Guerin* (BCG)(7), plasma of animals infected with Newcastle's disease virus(8), and blood of animals treated with bacterial endotoxins (9), also show increased muramidase activity. It is our purpose to demonstrate that after specific challenges to the host defense mechanism, by BCG, zymosan and bacterial endotoxins, muramidase activity in the kidney and spleen is elevated.

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Materials and methods. Swiss, female, ICR mice (18-22 g) were maintained on Purina laboratory chow and water *ad lib.* Zymosan† (lot #7B-171), the bacterial lipopolysaccharides of *Escherichia coli,*‡ of *Serratia marcescens,*‡ of *Salmonella enteritidis,*‡ of *Staphylococcus aureus,*‡ and of *Pseudomonas,*‡ were obtained from commercial sources. BCG was prepared from the Phipps strain of tuberculobacillus *Calmette-Guerin* using pooled 14 day old cultures.

The mice given BCG received a single intravenous injection (i.v.) of 0.2 mg wet weight of cells suspended in 0.2 ml of sterile 0.85% saline. Mice treated with zymosan were given a single i.v. injection of 1.0 mg and those given *E. coli* endotoxin received a single i.v. injection of 0.2 mg. All intravenous injections were given into the lateral tail vein. The remaining bacterial endotoxins were administered in single intraperitoneal doses of 0.45 mg.

At the end of each experiment the mice were killed by cervical fracture. The kidneys and spleens were rapidly excised, weighed and the muramidase extracted. The method for extraction which has been described(1), briefly, consisted of homogenization of mouse tissues in 0.15 M citrate-phosphate buffer pH 4.0. After standing overnight at 4°C the homogenates were centrifuged at 2000 rpm for 30 minutes; supernatants were collected and retained for assay. The assay for muramidase was carried out by a modification of Litwack's method(10) described previously (1,2).

Results. The muramidase activity in the kidneys and spleens of mice treated with BCG, zymosan and *E. coli* endotoxin is shown in Table I. Infection with BCG produced a marked elevation of renal muramidase activity. Elevation of splenic activity was less pronounced. The enzymatic activity in the kidneys increased slowly, reaching a peak at 21 days after infection at which time the treated mice showed a 20-fold increase in activity compared with the controls. The activity then began to decrease gradually, but a 5-fold increase was still detectable on day 42. Splenic activity, on the other hand, reached a peak on day 28 when a 14-fold increase in muramidase activity occurred.

† Standard Brands, Inc.

‡ Difco Laboratories, Inc.

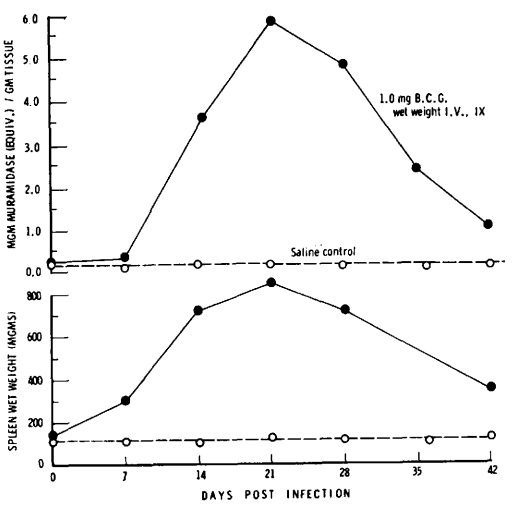

FIG. 1. Kidney muramidase activity and splenic hyperplasia in B.C.G. infected mice.

The relationship of splenic hyperplasia and muramidase activity in the kidneys of BCG infected mice is shown in Fig. 1. As splenic weights increased with time, a concomitant increase of muramidase activity was noticed. Both parameters reached a peak 21 days post infection, then decreased slowly.

An elevation of muramidase activity also occurred in zymosan-treated mice (Table I). On day 14 renal muramidase activity reached a peak indicating a 15-fold increase over the controls. Simultaneously, splenic muramidase activity showed only a 2-fold increase. At the end of the test period (21 days), a 6-fold increase in renal muramidase was measurable, while the enzyme activity in the spleen had returned to normal.

Unlike BCG and zymosan, the endotoxin of *E. coli* evoked a rapid but less marked rise in muramidase activity in both the spleen and kidney (Table I). There was a 3-fold increase in renal muramidase and a 2-fold elevation in the spleen on day 2. The level of muramidase in the kidney remained twice that of the controls 24 days after treatment, whereas muramidase activity in the spleen returned to normal by day 21.

With the other bacterial endotoxins results were similar to those obtained with *E. coli*

(Table II). Peak activity occurred 2 to 3 days after initiation of treatment. Both endotoxins of *S. aureus,* and *S. enteritidis* produced a 5-fold increase in renal muramidase while *Pseudomonas* and *S. marcescens* endotoxins caused a 2- and 3-fold increase respectively.

Discussion. When the reticuloendothelial system (RES) of non-tumor bearing mice was stimulated by BCG, zymosan and bacterial endotoxins, elevation of muramidase activity occurred in the spleens and marked elevation in the kidneys (Tables I, II). The level of splenic muramidase, although elevated, was less marked than the enzymatic activity associated with the kidney. Renal muramidase activity following BCG infection or zymosan treatment reached a peak on days 21 and 14 respectively, whereas bacterial endotoxins produced a moderate but rapid

TABLE I. Effect of *Bacillus Calmette-Guerin* (BCG) Infection on Muramidase Activity of Kidney and Spleen Tissue in Swiss Mice.*

BCG, .2 mg/mouse I.V.	mg muramidase (equivalent)/g tissue			
	Kidney		Spleen	
Days post infection	Treated	Control	Treated	Control
0	.27 ± .04	.26 ± .93	.14 ± .01	.15 ± .03
7	.38 ± .07	.17 ± .05	.20 ± .02	.17 ± .02
14	3.64 ± .6	.13 ± .05	.22 ± .07	.15 ± .03
21	5.74 ±1.5	.29 ± .04	.25 ± .04	.14 ± .01
28	5.04 ± .25	.27 ± .01	.21 ± .02	.16 ± .03
35	2.50 ± .96	.21 ± .01	.20 ± .04	.17 ± .02
42	1.28 ± .19	.27 ± .04	.16 ± .06	.13 ± .05
Zymosan, 1.0 mg/mouse I.V.				
Days post treatment	Treated	Control	Treated	Control
0	.59 ± .02	.44 ± .03	.17 ± .02	.15 ± .05
7	1.03 ± .08	.32 ± .02	.32 ± .01	.16 ± .04
14	7.06 ± .2	.46 ± .04	.31 ± .02	.18 ± .03
21	3.28 ± .1	.56 ± .09	.20 ± .04	.17 ± .01
E. coli, .2 mg/mouse I.V.				
Days post treatment	Treated	Control	Treated	Control
0	.34 ± .07	.28 ± .01	.17 ± .02	.16 ± .01
1	.35 ± .04	.38 ± .03	.18 ± .02	.15 ± .01
2	1.10 ± .1	.37 ± .03	.36 ± .01	.15 ± .09
3	.79 ± .1	.28 ± .02	.29 ± .02	.18 ± .09
7	.75 ± .08	.36 ± .05	.24 ± .04	.16 ± .01
14	.65 ± .08	.30 ± .02	.23 ± .03	.17 ± .02
21	.64 ± .03	.36 ± .02	.20 ± .04	.19 ± .02

* Banks, male 18-22 g, 10 mice/group.

TABLE II. Effect of Various Bacterial Endotoxins on Kidney Muramidase Activity in Swiss Mice.*

Endotoxin	Route	mg/mouse	Peak activity, days post-treatment	mg muramidase (equivalent)/g tissue	
				Treated	Control
Staphylococcus aureus	I.P.	.45	2	.85 ± .09	.17 ± .02
Pseudomonas	"	.45	2	.76 ± .07	.33 ± .01
Serratia marcescens	"	.45	3	.80 ± .05	.29 ± .02
Salmonella enteritidis	"	.45	3	1.27 ± .14	.29 ± .02

* Banks, male, 18-22, 10 mice per group.

rise in renal and splenic muramidase activity.

Enhanced muramidase activity has been demonstrated in many pathological conditions(6) and in different types of tissues (7,14). A survey of several rodent tumor systems made in our laboratories has shown that muramidase in the kidney increases with the progression of tumor growth, and decreases with drug therapy, surgical intervention, or spontaneous tumor regression(1,2,3). Our present data, however, suggest that augmentation of muramidase activity may be the result of RES stimulation of the host.

Stimulation of the RES during the growth of various transplantable tumors has been amply demonstrated(12,13). One index of RES stimulation is splenic hyperplasia(11, 12).

We found in mice infected with BCG that spleen weight and muramidase activity increased concomitantly, reached a peak at 21 days and then decreased (Fig. 1). This sequence and degree of enzymatic activity corresponds exactly with the time-course, sequence and degree of RES stimulation previously demonstrated for BCG-infected mice (11).

Although the mechanism responsible for enhanced renal muramidase activity is obscure, stimulation of the RES may be an explanation for the phenomenon. Old *et al* (12) have reported the RES stimulation in mice is accompanied by increased production of macrophage with enhanced phagocytic activity. It has also been shown that macrophages(14) and polymorphonuclear leucocytes(15) possess muramidase and are known to function as part of the reticuloendothelial system. Hence it becomes plausible that muramidase activity is associated with cells capable of phagocytic activity and that the enzyme level increases when the hosts defense mechanism is challenged by specific or nonspecific stimuli. A possible explanation for elevated muramidase activity in the kidney has been proposed by Perri *et al*(16), who suggest that phagocytic cells release muramidase into the circulatory system, the enzyme accumulates in the kidney, and it is then partially eliminated in the urine.

Summary. Elevation of muramidase activity occurs in the spleens and kidneys of normal mice treated with RES stimulators such as BCG infection, zymosan and bacterial endotoxins. This confirms that the increase in muramidase level in kidneys and spleens of tumor-bearing animals is not directly associated with tumor growth but is the result of stimulation of the RES.

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