treatment, we have no net increase in number of cells and, consequently, no increase in organ weights (Fig. 1). It should be noted that the lymph nodes showed the greatest ability to resist the lymphatic tissue effects of cortisol. This is due to the greater percentage of reticular cells present in this organ as compared to the thymus.

The increase in thymidine-2-^{14}C incorporation in the spleen, thymus, and lymph nodes in the saline treated animals (Fig. 2, 3, 4) cannot be fully explained at this time. However, it has been reported that a lymphocytosis in peripheral blood occurs following injection of saline owing to induced stress(16, 17). Injection of either saline or cortisol caused a small decrease in organ weights from the non-injected controls. This decrease can be attributed to the stress produced by the injection in non-adrenalectomized animals.

Summary. The data presented here show that immature cell populations produced by continued treatment with cortisol became resistant to its effects on nucleic acid synthesis after 3 days of treatment in thymus and lymph nodes. The spleen did not show this resistance.

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Extracellular β-Lysin and Muramidase in Body Fluids and Inflammatory Exudates.* (31785)

ROBERT S. JENSEN, JOHN G. TEW AND DAVID M. DONALDSON
Department of Bacteriology, Brigham Young University, Provo, Utah

β-lysin and muramidase (lysozyme) can be differentiated from each other even though they have similar bactericidal spectra, heat stabilities and adsorption properties(1). Muramidase is present in significant quantities in saliva, tears, plasma, polymorphonuclear cells, and macrophages(2,3). It is absent in aqueous humor, sweat, spinal fluid, and urine. β-lysin is found in both serum and platelets (4). In this study neutralizing anti-β-lysin serum was used in conjunction with conventional assay techniques to identify and quantitate free β-lysin and muramidase in various body fluids and inflammatory exudates of rabbits.

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TABLE I. Distribution of β-Lysin and Muramidase in Body Fluids of Rabbits.

Sample	β-lysin			Muramidase	
	No. of samples	Median, units/ml	Range, units/ml	Mean, μg/ml	Range, μg/ml
Serum	13	32	8–64	8.4	4.5– 9.5
Plasma	6	2	1– 4	8.6	4.6– 9.7
Saliva	6	2	1– 4	26.5	17 –38
Aqueous humor	6	2	1– 4	<1	<1
Vitreous humor	7	<1	<1	<1	<1
Spinal fluid	6	<1	<1	<1	<1
Urine	6	<1	<1	<1	<1
Peritoneal wash	6	<1	<1	<1	<1
Subcutaneous wash	6	<1	<1	<1	<1

Materials and methods. Blood was collected from the rabbits by cardiac puncture. Spinal fluid, urine, aqueous humor, and vitreous humor were collected asceptically from recently sacrificed animals. Peritoneal and subcutaneous washings were collected by injecting 10 to 20 ml of physiological saline (PSS), massaging and withdrawing a small volume of fluid using a hypodermic needle with multiple perforations near the bevel. Salivation was induced by a 10 ml intraperitoneal injection of 0.25% pilocarpine. The contaminated saliva collected from the mouth was sterilized by filtration through a 0.3 μ pore diameter Millipore filter. All samples were centrifuged immediately after collection, and the cellular and other particulate material were discarded.

Collection of inflammatory exudates. Peritoneal exudates were induced with 500 ml injection of 0.1% glycogen saline. At 6, 12, 18, and 24 hours, the exudates were collected, centrifuged, and the supernatant fluid tested for β-lysin, muramidase, and nitrogen content. An acute inflammation was induced by subcutaneously injecting 100 ml of either 0.1% glycogen saline or 0.1% aluminum silicate into the hind leg of a rabbit. A chronic inflammation of the subcutaneous tissue was induced by 6 daily injections of 50 ml of 0.1% aluminum silicate. Eighteen hours after the single subcutaneous injection or 48 hours after the last multiple injection, 10 ml of physiological saline was injected into the inflammatory site and an exudate wash of 3 to 5 ml was obtained.

Platelet extract. Thirty ml of blood was collected in 3 ml of 3.8% sodium citrate and centrifuged at 130 × *g* for 15 minutes. The platelet-rich plasma was separated and the platelets sedimented at 900 × *g* for 10 minutes. The platelets were washed twice in PSS. An aqueous extract was prepared by suspending the platelets in 10 ml of distilled water and freezing and thawing the suspension 5 times.

Leukocyte extract. Fourteen hours after an intraperitoneal injection of 500 ml of PSS the peritoneal fluid containing greater than 95% neutrophiles was withdrawn. The cells were sedimented at 700 × *g* and resuspended in 1/20 the initial volume of PSS. The cell suspension was frozen and thawed 5 times, centrifuged, and the sediment discarded.

Assay procedures. Techniques for the β-lysin assay and the neutralization of β-lysin by anti-β-lysin were described previously (4,5). Muramidase was assayed as outlined by Jollès(6) using three times crystallized egg white muramidase (lot L110B-078, Sigma Chemical Co.) as the enzyme standard. The micro-Kjeldahl method was used to determine the nitrogen concentration.

Results. Distribution of β-lysin and muramidase in normal body fluids. Serum, plasma, and saliva contained measurable levels of both muramidase and β-lysin (Table I). Serum had relatively more β-lysin than saliva, and saliva had relatively more muramidase than serum. In contrast to the high serum and low plasma concentrations of β-lysin, the muramidase levels in serum and plasma were not significantly different from each other. Aqueous humor contained detectable levels of β-lysin but no muramidase. Vitreous humor, spinal fluid, urine, peritoneal washings, and subcutaneous washings lacked

TABLE II. Neutralization of Serum, Aqueous Humor, and Saliva by Anti-β-Lysin.

Sample	β-lysin, units/ml	Muramidase, μg/ml
Rabbit serum	32	8.4
Serum + anti-β-lysin	<2*	9.6
Serum + guinea pig serum	32	8.0
Aqueous humor	4	<1
Aqueous humor + anti-β-lysin	<2*	—
Aqueous humor + guinea pig serum	8	—
Saliva	4	27
Saliva + anti-β-lysin	<2	27
Saliva + guinea pig serum	4	28

* The lowest number of bactericidal units that could be measured was 2 units/ml since equal volumes of sample and anti-β-lysin were used in the neutralization test.

both β-lysin and muramidase. The assumption that the active agent against *Bacillus subtilis* was β-lysin in serum, aqueous humor, and saliva was confirmed by the observation that anti-β-lysin of guinea pig origin neutralized the bactericidal activity of these body fluids against *Bacillus subtilis* (Table II). In contrast, the muramidase activity of serum and saliva was not significantly altered by anti-β-lysin.

Distribution of β-lysin and muramidase in peritoneal and subcutaneous exudates. In view of the importance of leukocytes and platelets in the inflammatory process and the observation that both subcutaneous and peritoneal washings of normal tissues were lacking in both β-lysin and muramidase, it was considered warranted to determine if extracellular β-lysin and muramidase are found in inflammatory exudates. During the first 24-hour period after an intraperitoneal injection of glycogen, the non-cellular nitrogen, β-lysin and muramidase concentrations all increased in the peritoneal exudates (Table III). The activities per unit nitrogen also increased with time indicating that both β-lysin and muramidase are selectively concentrated during inflammation.

Some idea of how selectively these bactericidal agents are accumulated in the peritoneal cavity can be gained by comparing the specific activity of exudates with that of serum (Table III). During the glycogen-induced inflammation the muramidase in the peritoneal cavity increased at a faster rate and to relatively higher levels than the β-lysin. At 24 hours the specific activity of the peritoneal exudates was 8.4 times that of serum for β-lysin and 22 times that of serum for muramidase.

The bactericidal activity of the 6-, 12-, and 18-hour peritoneal exudates against *Bacillus subtilis* was neutralized by anti-β-lysin. The bactericidal activity of the 24-hour exudate was decreased from 32 to 4 units per ml by anti-β-lysin. The high muramidase levels of the 24-hour exudates could account for the lack of complete neutralization by anti-β-lysin, since *B. subtilis* is susceptible to these concentrations of muramidase.

Both glycogen and aluminum silicate solutions were injected to induce inflammatory reactions at subcutaneous sites. The subcutaneous exudates induced with a single injection of either glycogen or aluminum silicate did not cause the marked increase in β-lysin (Table IV) seen in the peritoneal exudates.

TABLE III. Extracellular β-Lysin and Muramidase Levels in the Glycogen-Induced Peritoneal Exudates.

Sample	Nitrogen content, mg N/ml	β-lysin				Muramidase			
		Median, units/ml	Spec. act.,* avg	Rel. act.†	P value‡	Mean, μg/ml	Spec. act.,* avg	Rel. act.†	P value‡
Serum	9.44	16	2.5	—	—	8.4	.91	—	—
0-time wash	—	<1	—	—	—	<1	—	—	—
6-hr exudate	.55	2	2.9	1.2	.5	3.1	7.1	7.8	*<.01*
12-hr "	.91	8	9.9	4.0	*<.001*	9.1	12.8	14	*<.001*
18-hr "	1.54	16	8.4	3.4	*<.01*	18.6	14.7	16	*<.001*
24-hr "	2.31	32	21	8.4	*<.01*	40.0	20.1	22	*<.001*

* Specific activity = units β-lysin or μg muramidase/mg N from 6 or more rabbits.
† Relative activity = specific activity sample/specific activity serum.
‡ P value calculated with student's t test. The specific activities of exudates were compared to those of sera.

TABLE IV. Extracellular β-Lysin and Muramidase in Subcutaneous Exudates.

Sample	Nitrogen content, mg N/ml	β-lysin				Muramidase			
		Median, units/ml	Spec. act., avg	Rel. act.	P value	Mean, μg/ml	Spec. act., avg	Rel. act.	P value
Serum	9.44	16	2.5	—	—	8.4	.9	—	—
Zero-time wash	—	<1	—	—	—	1	—	—	—
Glycogen exudate*	.18	0	—	—	—	18.1	102	112	*<.001*
Aluminum silicate exudate*	.57	2	3.5	1.4	.2	25	44	49	*<.001*
Chronic aluminum silicate exudate†	.63	8	14	5.7	*<.01*	52	83	91	*<.001*

* Exudates collected from 6 or more rabbits 18 hr after a single subcutaneous injection of either glycogen saline or aluminum silicate.

† Exudates collected from 6 rabbits 48 hr after the sixth daily injection of aluminum silicate.

However, the specific β-lysin activity of the exudate of a chronic inflammatory reaction induced by daily injections of aluminum silicate was approximately 6 times higher than that of serum.

Muramidase was present in high concentrations in all subcutaneous exudates regardless of the type of stimulus used to induce the inflammatory response. The specific muramidase activities of these different subcutaneous exudates were from 49 to 112 times more active than those of sera.

In an effort to determine possible sources of the free muramidase and β-lysin observed in the inflammatory exudates, a study was carried out on the comparative concentrations of these two bactericidal compounds in leukocytes and platelets. The results of this investigation are shown on Table V. The aqueous extract of blood platelets had no muramidase but was bactericidal for *Bacillus subtilis*. This bactericidal activity was neutralized by anti-β-lysin. The aqueous extract of leukocytes containing 2,640 μg muramidase per ml killed *B. subtilis*. This lethal activity against *Bacillus subtilis* was attributed to the high concentrations of muramidase in the leukocyte extracts since the bactericidal activity was not altered by anti-β-lysin. These results indicate that the β-lysin rich leukocytes do not serve as a muramidase source and that β-lysin rich platelets do not contribute muramidase to these exudates.

Discussion. The differences between the β-lysin concentrations of serum and plasma can be accounted for by the fact that β-lysin is released during coagulation of blood(7). The primary source of the serum β-lysin is the platelet(4,8,9) which ruptures during the coagulation process. In all probability the bactericidal substance, plakin, which has been studied extensively by Amano *et al*(10,11) is the same compound as β-lysin. The origin of β-lysin in saliva and aqueous humor is unknown. Since the concentrations of β-lysin in both saliva and aqueous humor were comparable to that of plasma, the possibility exists that β-lysin in the saliva and aqueous humor could reach the reported levels due to simple diffusion from the plasma. However, diffusion alone can not account for all of the muramidase in saliva since saliva contained

TABLE V. β-Lysin and Muramidase in Aqueous Extracts of Blood Platelets and Peritoneal Leukocytes.

Sample	Nitrogen content, mg N/ml	Bactericidal assay*		Muramidase assay	
		Median, units/ml	Specific activity	Mean, μg/ml	Specific activity
Platelet extract	.21	32	152	0	—
Idem + anti-β-lysin	—	<2	—	—	—
Leukocyte extract	3.46	32	9.2	2,640	760
Idem + anti-β-lysin	—	32	—	2,640	—

* Bactericidal test ran against *Bacillus subtilis*.

3 to 4 times as much muramidase as plasma and serum. The observation that β-lysin is found in an active form in plasma, saliva, and aqueous humor indicates that this compound may function as a host defense mechanism in the absence of blood coagulation.

The high levels of free β-lysin and muramidase found in the inflammatory exudates emphasized the potential significance of these non-specific bactericidal substances in the control of infection at inflammatory sites. Since neither muramidase nor β-lysin was detected in the subcutaneous tissue or the peritoneal cavity of non-stimulated animals, it is evident that the inflammatory process is of importance in the release of these agents. The high specific muramidase activity (as high as 112 times that of serum) could be due to its release as a result of leukocyte autolysis at the inflammatory site or degranulation which takes place during phagocytosis(12). Although the β-lysin differences in the inflammatory exudate did not appear to be as great as the muramidase differences, it should be emphasized that the comparisons were always made with serum. The muramidase level of plasma is similar to that of serum whereas the β-lysin level of plasma is only approximately 1/16 that of serum. Consequently, the increases in β-lysin and muramidase in inflammatory exudates are comparable in respect to the plasma levels. It is speculated that the high levels of β-lysin observed at the sites of inflammation are due to the disruption of platelets during the coagulation that takes place at these sites.

Summary. β-lysin was found in an active form in plasma, saliva, and aqueous humor at approximately 1/16 the serum concentration. Muramidase was present in serum, plasma, and saliva but was not found in aqueous humor. Neither of these bactericidal agents were detectable in vitreous humor, spinal fluid, urine or in normal tissue washings. Inflammation resulted in the accumulation of high concentrations of extracellular β-lysin and muramidase at tissue sites where these compounds are not normally found. Evidence was presented implicating leukocytes as the source of free muramidase and platelets as the source of extracellular β-lysin at inflammatory sites.

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