Properdin System and Nonspecific Inhibitor of Hyaluronidase.* (23167)

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There are a number of striking similarities between the nonspecific inhibitor of hyaluronidase in normal serum (NSI)(1) and some components of the properdin system(2). NSI is heat labile, protease sensitive and can be precipitated quantitatively from a solution of low ionic strength at pH 5.2 to 5.5(3). Its action on hyaluronidase requires the presence of magnesium or cobalt ion(4) and is inhibited in solutions of high ionic strength(1). The biological significance of NSI is unknown. It is ineffective against many bacterial enzymes(5) and against those that it inhibits is not as effective *in vivo* as the specific inhibitors in serum(1). On the basis of its known properties NSI could possibly be identified with properdin or C'1. The work to be described was undertaken to test these possibilities.

Materials and methods. The methods for determination of hyaluronidase and NSI activity and the units (U) for their measurement have been described(1). Complement activity was measured by adding 0.5 ml of 1.25% sensitized sheep cell suspension to 0.5 ml saline containing the varying amounts of serum to be tested, and determining the extent of hemolysis at the end of 30 min. Zymosan (Nutritional Biochemicals) was pretreated as described(6). Human, guinea pig or rabbit serum was added to an equal volume of zymosan suspension containing .05M sodium chloride and .002M magnesium chloride and incubated for 90 min. at 37°C. The NSI and complement activity of this serum was compared with the activity of serum incubated under identical conditions without zymosan. Blood samples were taken from rabbits before and after intravenous injection of testicular hyaluronidase(7) and tested for NSI and complement activity. Blood samples obtained from guinea pigs before and 72 hr after the injection of bacterial lipopolysaccharides were tested for NSI activity. The lipopolysaccharides were from the following sources: *Salmonella typhosa, Serratia marcescens* and *Escherichia coli.*†

Results. Zymosan experiments. When serum is incubated with zymosan under proper conditions, properdin, C'3 and possible other components of complement are removed(2). In 12 experiments at pH ranging from 6.9 to 7.8 and zymosan concentrations varying from 2 to 8 mg per ml of serum there was no measurable loss in the NSI activity due to zymosan. In every instance there was a loss of complement activity. Four of these experiments are presented in Table I.

Hyaluronidase injection. Under proper conditions NSI can be removed from rabbit serum *in vivo* by intravenous injection of testicular hyaluronidase(7). Sera of such animals taken 30 min after the injection show no loss of complement activity as compared with samples taken before the injection; the NSI activity has usually disappeared completely (Table II). Furthermore, serum used in hemolysis of sensitized sheep cells does not lose any of its NSI activity.

Lipopolysaccharide injection. Eighteen guinea pigs were injected with bacterial lipopolysaccharides. Each of the 13 animals which survived showed a significant increase in the NSI titre of its serum 72 hr after injection. The increases ranged from 12% to 100% over the control values, with an average increase of about 55% (Table III). Ten days after the injection the titres were still elevated. These increases are highly significant, since in laboratory animals we have found no significant change in the serum titre of NSI from day to day. The animals that died were injected with lipopolysaccharides of

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TABLE I. Effect of Incubation with Zymosan on NSI and Complement Activity of Serum. Incubation was for 90 min. at 37°C.

Source of serum	pH	Zymosan, mg/ml serum	NSI activity, units/ml serum: Without zymosan	NSI activity, units/ml serum: With zymosan	Complement activity: ml serum producing 100% hemolysis: Without zymosan	Complement activity: ml serum producing 100% hemolysis: With zymosan	Complement activity: ml serum producing no hemolysis: Without zymosan	Complement activity: ml serum producing no hemolysis: With zymosan
Rabbit	7	2	120*	120*	.05		<.025	.05
Human	7	2	27	29	.05		"	.1
"	6.9	8	27	29	.05		"	.1
Guinea pig	7.2	2	106	106	.025	.05		.025

* This activity was measured against the hyaluronidase of *Streptococcus mitis* #19; all others against testicular enzyme.

S. typhosa and *S. marcescens*.

Discussion. The properties of NSI enumerated in the introduction led us to consider the possibility that it may be identified with properdin or C'1. The experiments with zymosan show that it cannot be identified with properdin and the experiments with hyaluronidase-injected rabbits make it seem highly improbable that it can be identified with C'1. On the other hand the increase in the titre of NSI following lipopolysaccharide injection provides an additional point of similarity between NSI and properdin. All the components of the properdin system are not known, and it is probable that NSI may yet prove to be a component of this system.

Summary. The non-specific hyaluronidase inhibitor of serum (NSI) is not inactivated when serum is incubated with zymosan for 90 min at 37°C. Serum depleted of NSI does not lose complement activity. Thus it is unlikely that NSI is one of the known components of the properdin system. However, injection of lipopolysaccharides which increase the serum titre of properdin increases the titre of NSI.

TABLE II. Effect of Depletion of NSI on Complement Activity of Serum. Rabbits were given 500 to 1000 U/kg testicular hyaluronidase intrav.; serum samples were taken before and 30 min. after injection.

NSI activity, units/ml serum: Before inj.	NSI activity, units/ml serum: After inj.	Complement activity: ml serum producing: 100% hemolysis: Before inj.	Complement activity: ml serum producing: 100% hemolysis: After inj.	Complement activity: ml serum producing: No hemolysis: Before inj.	Complement activity: ml serum producing: No hemolysis: After inj.
42	0	.2	>.2	<.05	.05
40	0	.2	.2	.05	.05
42	0	.1	.1	.03	.03
37	0	.1	.1	.04	.04
100	8	.4	.5	<.02	<.04
44	0*	.1	.1	.04	.04

* This serum contained 2.5 U/ml hyaluronidase.

TABLE III. Effect of Lipopolysaccharide Injection on NSI Activity of Guinea Pig Serum. Injections were given intraper. to animals weighing about 300 g. Serum samples were taken before and 72 hr after injection.

Lipopolysaccharide Source	Lipopolysaccharide Amt, mg	NSI activity, units/ml Before inj.	NSI activity, units/ml After inj.
E. coli	1.0	111	125
	1.0	90	160
	.8	78	120
	.8	80	122
	.8	33	66
	.8	50	91
	.8	74	102
S. typhosa	1.0	46	92
	.4	74	120
S. marcescens	1.0	100	150
	.4	42	54
	.4	51	69
	.4	50	76

1. Hadidian, Z., Mahler, I. R., and Murphy, M. M., *J. Bact.*, 1957, v73, 484.

2. Pillemer, L., *Ann. N. Y. Acad. Sci.*, 1956, v66, 233.

3. Hadidian, Z., *ibid.*, 1950, v52, 1105.

4. Mathews, M. B., and Dorfman, A., *Physiol. Rev.*, 1955, v35, 381.

5. Hadidian, Z., Murphy, M. M., and Mahler, I. R., *J. Bact.*, 1957, v73, 491.

6. Pillemer, L., Blum, L., Pensky, J., and Lepow, I. H., *J. Immunol.*, 1953, v71, 331.

7. Hadidian, Z., and Murphy, M. M., *J. Gen. Physiol.*, 1955, v39, 185.

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