Serum Alkaline Phosphatase in Dogs with Experimental Splenic and Renal Infarcts and with Endocarditis. (23137)

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In studies of experimental endocarditis in dogs(1), infarcts of kidney and spleen were encountered frequently. Since these organs contain abundant alkaline phosphatase, and since the enzyme was depleted in the infarcted areas, studies were made on the serum alkaline phosphatase values. To aid in interpretation of these data, studies of alkaline phosphatase were made also on dogs with bland infarcts produced by vascular ligation. As will be shown, the development of infarcts of kidney and spleen, whether produced by vascular ligation or by emboli in the course of bacterial endocarditis, may be indicated by a sharp rise in serum alkaline phosphatase.

Materials and methods. Eighteen mongrel dogs of both sexes weighing 10 to 15 kg were selected, after quarantine at our animal hospital. Infarcts of the spleen were produced in 6 dogs by tying off multiple branches of the splenic artery at the hilus. Infarcts of the kidney were produced in 7 dogs by tying off several branches of the left renal artery at the hilus (dog 13, Table I), the left renal artery (dogs 1, 4, and 16), or the left renal artery and vein (dogs 5, 15, and 18). The renal vein was ligated to determine if this procedure would alter the elevation of serum alkaline phosphatase, perhaps by slowing absorption of the enzyme from the infarct. In 5 dogs used as controls, an exploratory laparotomy was performed, but vessels to the spleen and kidney were not ligated. Blood for determination of serum alkaline phosphatase values was obtained before (usually within an hour) and at intervals after sur-

TABLE I. Serum Alkaline Phosphatase in Bodansky Units in Operated Control Dogs and in Dogs with Infarcts following Ligation of Splenic and Renal Vessels.

Dog	Preop.*	Immed. postop.†	Days after operation 1‡	2	3	4	5	6	7	9	10	11
Operated control dogs												
6	2.0	.4	2.7	2.4	2.9	2.9		2.9				
8	1.0	.7	.3	1.4	1.4	1.4	1.1		.7			
9	.5	1.2	1.0	1.3	1.2	1.2	.9					
12	1.8		.3	.1	.8	.8	.7			.3		
14	1.3	1.3	2.7	1.5	2.2	2.2	2.0		1.3			
Dogs with splenic infarcts												
2	.7		5.4	6.0	4.7	3.5	2.8			1.3		.6
3	1.1	1.3	2.1	2.2	1.5				.0			
7	3.4	3.6	3.5	3.7	3.9	4.6			3.5			
10	.4	1.2	1.1	1.1	1.1	1.3			1.2			
11	1.8		3.4	3.5	2.2	2.9	2.0			2.3	1.7	
17	.9		3.4	3.1	2.2			1.7				
Dogs with renal infarcts												
1	.2		6.8	6.2	2.7	3.3	1.7			1.2		1.8
4	.5	.5	6.9	4.9	3.2				2.0	1.5		
5	1.5	0	4.6	5.0	3.9			1.6				
13	.6		4.0	4.0	3.8	3.2	2.4			.7	1.4	
15	.2	.4	1.2	.9	.4	.4			.0			
16	3.0	2.1	5.6	4.8	2.6			2.6	2.4			
18	3.0	.8	5.7	13.5	12.1				10.1	6.4		

* Blood usually taken 1 hr before surgery. Earlier additional samples, taken in some dogs, showed similar values.

† Blood taken 3 to 6 hr after surgery except 1 hr for dog 16.

‡ " " 18 to 24 " " " .

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TABLE II. Serum Alkaline Phosphatase in Bodansky Units in Dogs with Aortic Insufficiency before and after Inducing Endocarditis by Intravenous Bacterial Injections.

Dog	Before* inj. bact. alk. phos.	After inj. bacteria: Days†	After inj. bacteria: Alk. phos.	Postmortem findings: Days†	Postmortem findings: Infarcts‡
Dogs given *Streptococcus mitis*					
63	1.1	5	3.7	7	R
		7	4.4		
66	1.7	7	2.2	11	R
67	1.3	6	4.2	11	R
		11	3.5		
108	.8	6	3.1	37	R, O
		14	1.6		
		20	1.1		
		28	1.5		
		35	7.6		
Dogs given *Staphylococcus aureus*					
71	3.2	4	6.4	6	R
		6	5.9		
114	.5	2	5.7	4	R
Dogs given *Staphylococcus aureus* and antibiotic therapy					
74	1.0	21	.8	37	—
70	3.0	7	8.0	17	O
78	2.4	4	2.3	28	O
		11	1.3		
		17	1.3		
75	1.0	11	4.5	28	O
		13	5.3		
		17	2.9		
		27	1.5		

* Blood for alkaline phosphatase was usually taken one to several days before inj. bacteria; earlier additional samples, taken in some dogs, showed similar values.

† Days after first bacterial inj.

‡ Infarcts are designated R if recent and O if they appeared to be older than 1 wk. Dog 70 had no infarcts and 78 had only a renal infarct; all others had renal and splenic infarcts.

gery (Table I). Values were determined with the aid of a Leitz photrometer using the methods of Fiske and Subbarow and of Bodansky(2). We found serum alkaline phosphatase in normal dogs to be under 2 Bodansky units, in most instances, and rarely above 3.5 units. Preliminary studies showed that, while serum alkaline phosphatase values may vary considerably in 2 different dogs, daily fluctuation in values in the same healthy dog is not great (see values after 2 days in controls, Table I). Dogs were sacrificed 7 to 12 days after surgery. Blocks of tissue from the infarcted organs were fixed in 65% ethanol and modification of the Gomori method(3) was used to demonstrate alkaline phosphatase. Other blocks were fixed in 10% buffered (pH 7.0) aqueous formalin and stained routinely with hematoxylin and eosin.

Dogs with aortic insufficiency have proved highly susceptible to endocarditis(1). Bacterial endocarditis was produced consistently in more than 80 such dogs by single or multiple intravenous injections of cultures of *Staphylococcus aureus* or *Streptococcus mitis*. The insufficiency was induced, usually about 2 weeks before injecting bacteria, by perforating an aortic leaflet with a punch(5). The endocarditis was more fulminating in dogs given staphylococci, but was prevented, arrested, or modified in some by early or delayed treatment with antibiotics (Table II). Determinations of the alkaline phosphatase level in the blood of 44 of these dogs were made at various intervals, particularly when certain clinical features such as increasing malaise and fever suggested possible infarction. The findings in 10 of these dogs, selected as a representative sample, are recorded in Table II.

Results. Postmortem studies revealed infarcts of the spleen (Fig. 1) or kidney (Fig. 2) in all the operated dogs except the controls. Since the dog kidney receives some blood from the ureteral artery, capsular vessels, and, in the female, from the ovarian artery(4), renal infarction was incomplete even after ligation of the renal artery and vein.

As shown in Table I, there was a significant elevation in serum alkaline phosphatase in 3 of the 6 dogs with splenic infarcts (dogs 2, 11, and 17), in 6 of the 7 with renal infarcts, but in none of the operated controls. The elevation developed within 24 hours and gradually dropped to normal in about a week. Some dogs showed a drop in values immediately after surgery. After ligation of both renal artery and vein, the enzyme level did not rise significantly in dog 15 (male), but was elevated in dogs 5 (female) and 18 (male).

Histologic studies revealed absence of alkaline phosphatase in the infarcted areas, except

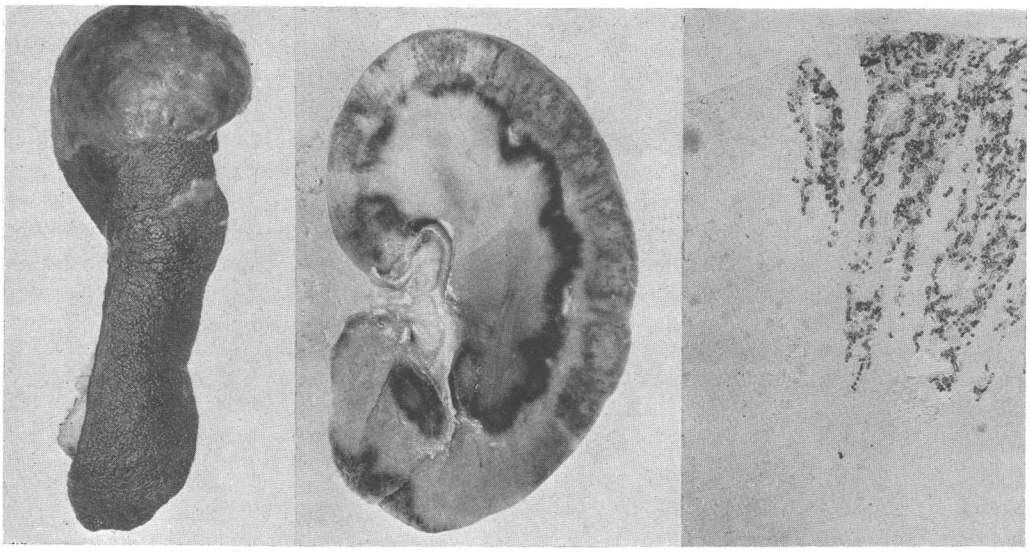

Fig. 1 Fig. 2 Fig. 3

FIG. 1. Infarcts in broad portion of spleen of dog 11 produced by ligating branches of the left splenic artery at the hilus.

FIG. 2. Infarcts of left kidney of dog 5 following ligation of left renal artery and vein.

FIG. 3. Kidney of dog 16 showing loss of alkaline phosphatase (black) in infarct on left. Alkaline phosphatase stained by modified Gomori method. × 10.

in occasional renal tubules (Fig. 3). Occasional tubules contained casts and debris reacting like alkaline phosphatase. The left kidney of dog 15, which did not develop an elevated serum alkaline phosphatase, nevertheless showed a massive infarct which was depleted of alkaline phosphatase.

In the endocarditis studies, nearly all the untreated dogs developed an elevation in serum alkaline phosphatase and died with endocarditis and multiple septic infarcts of the kidney and spleen (Fig. 4). The age of the infarcts was estimated by their gross and microscopic appearance(4) and corresponded closely with the estimated age based on a beginning rise in serum alkaline phosphatase. There were only occasional dogs with infarcts, illustrated by dog 66, Table II, in which no significant elevation in serum alkaline phosphatase was detected. The infection was prevented in some dogs by early antibiotic treatment. These dogs, illustrated by dog 74, did not develop a rise in serum alkaline phosphatase and did not show infarcts at autopsy. Therapy modified the course of endocarditis in others such as dogs 70 and 78 (Table II). Dog 78, which developed no apparent elevation in serum alkaline phosphatase, had only one small depressed yellow infarct in the right kidney. Generally, however, the development of infarcts in dogs receiving treat-

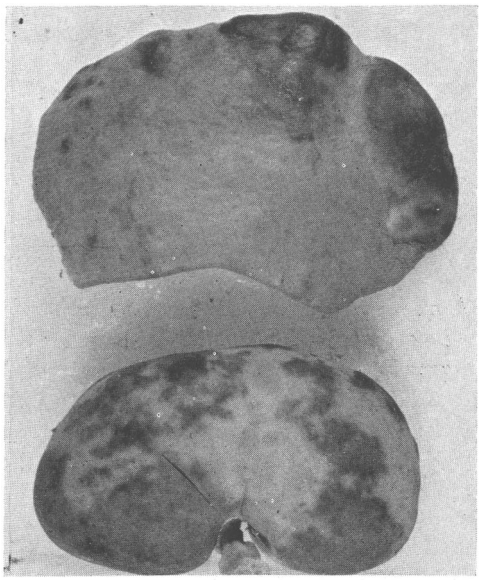

FIG. 4. Old pale yellow and recent dark red infarcts of spleen and kidney of dog 108 (Table II) which died 37 days after the first of 4 inj. of *Streptococcus mitis*.

ment was indicated by a rise in alkaline phosphatase and confirmed at autopsy (dogs 75 and 78, Table II).

Discussion. Our findings in operated dogs show that infarcts in spleen and kidney may cause a significant elevation in serum alkaline phosphatase level. The elevation is considered to be due to absorption of the enzyme from the infarcted areas. This elevation may aid in diagnosis of such infarcts and in following their course. The absence of such an increase, however, does not completely rule out the presence of a renal or splenic infarct. In our studies, some animals with splenic and occasionally with renal infarcts, such as dog 15 in Table I and 66 in Table II, failed to develop an elevation in serum alkaline phosphatase. To determine the causes of these failures requires further investigation.

We have found serum alkaline phosphatase values most useful in experimental endocarditis, in determining during life the occurrence of infarcts and their course. The value of serum alkaline phosphatase studies in man, in endocarditis and in the differential diagnosis of infarcts of spleen or kidney has not been determined.

Summary and conclusions. Infarcts of spleen and kidney were produced by vascular ligation in 13 dogs. The level of serum alkaline phosphatase increased significantly in 3 of 6 dogs with splenic infarcts and in 6 of 7 with renal infarcts. The level reached a maximum in about 24 hours and gradually returned to normal in about a week. An increase in serum alkaline phosphatase level was noted also in dogs with experimental endocarditis. This increase correlated well with the occurrence of infarcts in spleen and kidneys and proved to be of great diagnostic and prognostic value.

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Effect of Beta-Propiolactone on Complement-Fixing Antigens of St. Louis Encephalitis Virus.* (23138)

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A number of laboratory-acquired infections have occurred among individuals processing antigens for use in diagnosis of virus diseases (1,2). The only reported laboratory infection with St. Louis Encephalitis virus(3) occurred in an individual who was preparing complement-fixation antigen by lyophilization and extraction with benzene using the procedure described by Espana and Hammon(4, 5). More recently, a fatal infection with the virus of Russian Spring-Summer Encephalitis occurred as a result of an accident while processing antigens with the same procedure (6). An inapparent infection occurred in a laboratory worker who was working at the same laboratory bench. The present study is concerned with efforts to limit the hazards involved in preparing viral antigens. A recent study by LoGrippo and Hartman(7) has shown that Beta-propiolactone (BPL) promptly inactivates certain viruses without seriously reducing their ability to elicit neu-

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