A Method for Measuring Removal of Bacteria from the Blood by the Various Organs of the Intact Animal.*† (17333)

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(Introduced by E. A. Stead, Jr.)

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The ability of the body to remove foreign matter from the circulation has interested investigators since the nineteenth century. Wyssokowitch[1] in 1886 noted that the cells of the vascular endothelium rapidly cleared the blood of pathogenic and non-pathogenic organisms. Aschoff[2] considered these cells as a functioning unit which he called the reticuoendothelial system. By perfusing isolated organs Manwaring[3] found that the canine liver and spleen removed organisms much more rapidly than other organs. Studies on the rapidity of removal and the acceleration of removal by immunity were carried out by Bull[4] and Wright.[5] Cannon[6] and Sullivan[7] showed that after single intravenous injections, large numbers of organisms were found in the liver and spleen with few in the brain, lungs, muscle and thyroid. Beeson[8] in his study of subacute bacterial endocarditis noted the sparsity of organisms in hepatic venous blood. The object of the present report is to describe a method of quantitative estimation of the rate of removal of bacteria from the blood stream in the intact animal.

Methods. Healthy 7 to 23 kg dogs and 2.6 to 3.1 kg albino male rabbits were used. Light anesthesia was maintained throughout the experiment by the use of intravenous sodium pentobarbital.

The hemolytic *Micrococcus aureus* employed was of human origin, coagulase positive, and it fermented mannite. The encapsulated Klebsiella pneumoniae type B was obtained from Dr. R. J. Dubos. The inoculum used for infusion consisted of a suitably diluted saline suspension of bacteria washed from the surface of Bacto tryptose agar after 5 to 6 hours of incubation at 37°C.

A controlled bacteremia was maintained by a continuous infusion into the femoral vein, the inferior vena cava, or the right auricle of the dog of 1 to 5 x 10^5 hemolytic *Micrococcus aureus* organisms per minute by means of a number 6 or 8 ureteral catheter introduced into the veins. A similar infusion of approximately 1 x 10^4 Klebsiella pneumoniae type B organisms per minute was accomplished in the rabbit using the superior vena cava. A constant rate of inflow was maintained by the use of a standard intravenous drip set or by the use of a syringe activated by a motor-driven plunger with variable gears allowing control of the rate of movement of the plunger. An initial relatively large dose of organisms was given to each animal in order to attain rapidly the desired blood bacterial count which was maintained thereafter by the constant inflow of bacteria. After 5 to 30 minutes blood samples for culture were drawn from various sites as indicated in Tables I through III.

In the dog, femoral and jugular venous and femoral arterial blood samples were drawn at approximately 15 minute intervals through an arterial-type needle left *in situ*. Hepatic

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TABLE I.

Blood Levels of Bacteria and Percent Splanchnic Removal in Normal Dogs.

Duration i.v. admin. bact. (min.)	1				2				3				4			
	F.V.	F.A.	H.V.	%Eh	F.V.	F.A.	H.V.	%Eh	F.V.	F.A.	H.V.	%Eh	F.V.	F.A.	H.V.	%Eh
30	284	352	153	56.5	515	457	98	78.7	452	559	82	85.3	421	335	42	87.4
45	356	377	27	92.8	460	467	96	79.3		477	165	65.4	364	329	60	81.7
60	345	362	66	81.8	345	446	120	73.6		444	147	66.9	184	290	28	90.3
75		357	76	78.7	386	427	94	77.7		484	79	83.0	199	437	27	93.8
90	292	343	58	91.5	229	224	43	80.8		342	82	76.0	368	233		
105	309	352	75	78.7	402	372	77	79.4		404	25	93.8	249	468	17	96.4
120	238	247	60	75.7	278	389	104	73.3					405	480	28	94.2
135	214	251	73	70.9	243	282	72	74.5					353	446	20	95.5
150	244	292	61	79.1												
165	104	284	60	78.7												

Duration i.v. admin. bact. (min.)	5				6			7			8				18		
	F.V.	F.A.	H.V.	%Eh	F.A.	H.V.	%Eh	F.A.	H.V.	%Eh	F.V.	F.A.	H.V.	%Eh	F.A. ×10³	H.V. ×10³	%Eh
30					68	62	8.8	152	76	50.0	145	129	24	81.4	110	<1	>90.9
45					153	23	85.0	171	79	53.8	143	132	24	81.8			
60	785	861	331	62.0	79	20	74.7	132	38	71.2					81	17	79.0
75	826	903	426	53.0	113	45	60.2	129	56	56.6		80	18	44.4	105	27	74.3
90	1065	946	473	50.0							92	139	9	93.5	101	24	76.2
105															97	28	71.1
120	841	973	372	62.0											101	19	81.2
135	905	858	342	60.0							92	92	33	64.1			
150	729	788	278	65.0											104	21	79.8

F.V.—Femoral venous.
F.A.—Femoral arterial.
H.V.—Hepatic venous.
%Eh—% splanchnic removal $\frac{\text{(F.A.-H.V.} \times 100)}{\text{F.A.}}$.

TABLE II. Blood Levels of Bacteria and Percent Removal by Various Organs in Normal Dogs.

Duration i.v. admin. bact. (min.)	Splenic vein						Pulmonary artery				Jugular vein				
	9			10			11		12		13				
	F.A.	S.V.	%Es	F.A.	S.V.	%Es	F.A.	P.A.	F.A.	P.A.	J.V.	F.A.	H.V.	%Eh	
15											139	123	14	89.0	
30							40	62			249	246	46	81.3	
45	73			208			46	41	103	129	400	356	85	76.1	
60	63			152	26	82.9	41	32	123	111	673	342	190	44.1	
														6′ 30″ after	
75							28	28			166,000	56,000	1500	97.4	240 × 10⁵
90	32			115	22	80.0	25	27	90	103				18′ after	
105	70	14	80.0	126	6	95.4	52	45	322	327	27,000	25,000	2600	89.6	240 × 10⁵
120	79	40	49.4				46	38	426	411					
135							41	44	364	411					
150							28	16							

S.V.—Splenic venous.
P.A.—Pulmonary arterial.
J.V.—Jugular venous.

$$\%Es = \%\text{ splenic removal } \left(\frac{F.A. - S.V.}{F.A.} \times 100\right).$$

Other abbreviations as in preceding table.

venous, renal venous and pulmonary arterial blood samples were obtained through indwelling number 6 or 8 ureteral catheters introduced into the vessel via a jugular vein under fluoroscopic visualization. The technic of venous catheterization used in these experiments has been described by Cournand and Ranges.[9] In each instance the right lobe of the liver was catheterized. Splenic venous blood samples were obtained by repeated venipunctures or from an arterial-type needle left *in situ* under direct visualization after opening the abdomen.

In the rabbit mixed blood samples were obtained at approximately 7 minute intervals in initial preparations by repeated cardiac punctures and in subsequent preparations through an indwelling number 6 ureteral catheter introduced via a jugular vein into the inferior vena cava to the lower lumbar level. In each instance the left lobe of the liver was catheterized.

In numerous experiments the animals were sacrificed at the close of the observation period, and the position of the catheters was checked then by direct visualization.

Experiments revealed that the preliminary withdrawal of 4 ml of blood sufficed to clear the catheter of its contents so that the subsequent sample represented the blood present in the lumen of the catheterized vessel. Such clearance was done in every instance prior to the withdrawal of a blood sample for study.

Duplicate pour plates were made with blood samples of 0.5 to 1.0 ml volume in Bacto tryptose agar, serial saline dilutions of the blood being used when necessary to obtain countable plates.

Results. In Fig. 1 a representative experiment on a dog is charted. The bacterial counts in the femoral venous and femoral arterial blood varied somewhat during the course of each experiment. This is attributed to a slight variation in the rate of inflow of organisms and to the fact that the number of viable bacteria in the saline suspension which was administered decreased during the course of each experiment, as shown by a comparison of the

[9] Cournand, A., and Ranges, H. A., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 462.

TABLE III.
Blood Levels of Bacteria and Percent Splanchnic Removal in Normal Rabbits.

Duration i.v. admin. bact. (min.)	7			8			10			12		
	C	H.V.	%Eh	C	H.V.	%Eh	C	H.V.	%Eh	C	H.V.	%Eh
8	141	89	36.9							261	216	17.2
15	140	120	14.3							214	151	29.4
20	160	117	26.9							400	185	53.8
29	172	153	11.0	386	214	44.6	480	393	18.2	283	214	24.1
36	190	156	17.9				490	417	14.9	257	212	17.5
43				701	496	29.2	647	505	21.9	348	242	30.5
50				699	642	8.2	695	533	23.3			
57				889	789	11.2	867	640	26.2			
64							904	803	11.2			

Duration i.v. admin. bact. (min.)	15			17			28		
	C	H.V.	%Eh	C	H.V.	%Eh	V.C.	H.V.	%Eh
8	651	520	20.1	360	338	6.1			
15	549	445	18.9	352	268	23.9	475	389	18.1
20	628	483	23.1				564	494	12.4
29	615	486	21.0	384	283	28.0	733	545	25.6
36	545	481	11.7	365	287	21.4	726	632	12.9
43	606	549	9.4	386	367	4.9	787	665	15.5
50	758	613	19.1						
57	993	828	16.6						

C—Intracardiac.
H.V.—Hepatic venous.
V.C.—Vena caval.
%Eh—% splanchnic removal $\left(\frac{\text{C or V.C.—H.V.}}{\text{C or V.C.}} \times 100\right)$

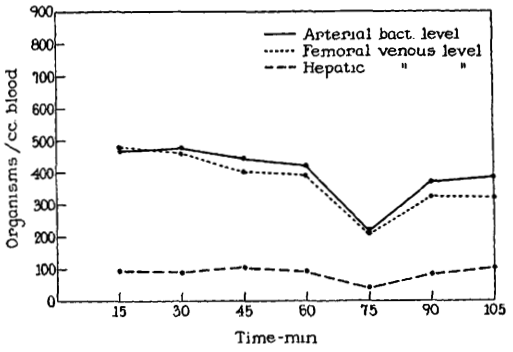

FIG. 1.

Chart showing bacteremia maintained over 105 minutes with arterial levels plotted against femoral and hepatic venous levels.

inoculum titres at the beginning and at the end of the observation period. The data permit comparison of counts on samples drawn simultaneously from different sites, however.

Table I records the counts of hemolytic *Micrococcus aureus* per ml of blood in a group of nine dogs, including one animal (No. 18) in which an arterial blood level of 1 x 10^5 bacteria per ml was maintained throughout the experiment. The rate of removal of organism by the splanchnic circulation is recorded in percent calculated from the simultaneous hepatic venous and the femoral arterial counts. A high percentage of organisms was taken out by the splanchnic circulation, the average removal in 57 observations on nine dogs being 74 ± 16% (S.D.). An analysis of femoral venous and femoral arterial bacterial counts based on 35 observations in 6 dogs showed that the removal of organisms in the peripheral circulation of the hind extremity was variable within wide limits, averaging 12 ± 14% (S.D.).

Table II presents observations in a group of 5 dogs in which various other organs were studied for their ability to remove hemolytic *Micrococcus aureus* from the blood stream. In two dogs (Nos. 9 and 10) the bacterial counts per ml of blood from the femoral artery and the splenic vein were used to calculate the percent removal in the splenic circulation. The average removal by the spleen was 78 ± 17% (S.D.) a rate which approximates closely that of the splanchnic circulation as a whole. In 2 animals (Nos. 11 and 12) no significant difference was found on comparison of the pulmonary arterial and the femoral arterial bacterial counts. In 6 observations on a single animal (No. 13), the jugular venous counts were 24 ± 27% (S.D.) higher than the simultaneously determined femoral arterial counts. Preliminary studies in several dogs not included in the present series have indicated no consistent trend in the relationship of renal venous and superior mesenteric venous counts to femoral arterial counts.

Table III records the bacterial counts per ml of blood in a group of 7 rabbits receiving an encapsulated Klebsiella pneumonia Type B. The percent removal by the splanchnic circulation was calculated from the hepatic venous and the intracardiac or the inferior vena caval bacterial counts. Average splanchnic removal in 39 observations on 7 rabbits was 20 ± 10% (S.D.), a degree of removal which is highly significant ($p = < 0.01$).

Discussion. A constant intravenous bacterial infusion combined with the technic of venous catheterization provides a convenient method of determining the rate of removal of microorganisms by the organs draining directly into the caval system. The spleen must be studied by some method allowing puncture of the splenic vein. The method used here may be useful in studying the rate of removal of antigens and other particulate matter.

Cannon[6] studied the removal of the micrococcus from the blood stream by culturing weighed amounts of various organs. He showed the remarkable capacity of the animal to concentrate the injected organisms in the liver and spleen as well as the ability of these organs to destroy the micrococcus. The lungs did not concentrate the organisms after intravenous injection. Manwaring[3] found the extraction in the liver and spleen to be 80 and 60%, respectively. Beeson[8] found an average of 85% extraction in hepatic venous blood in human subacute bacterial endocarditis.

The figure for the total splanchnic removal of the hemolytic *Micrococcus aureus* in our dogs was 74 ± 16% (S.D.) and the splenic removal 78 ± 17% (S.D.). The hepatic venous and the splenic venous blood were

cleared to the same degree. The absolute efficiency of these organs cannot be determined, as the blood flow per unit of tissue is not known in either instance. The percent removal of the bacteria remained high even when the arterial levels were maintained at 50,000 and 100,000 per ml. When one considers that the splanchnic circulation receives one-fifth of the cardiac output and that approximately 75% of the organisms are removed in a single circulation, it becomes clear why such a large number of bacteria is required to maintain a blood level of 100 organisms per ml.

The tissues of the hind limb showed no consistent ability to remove organisms from the circulating blood. The percent removed in a single circulation through the hind extremity was 12 ± 14% (S.D.). No organisms were lost in circulation through the lungs of the dog.

In the present series of rabbits the total splanchnic removal of Klebsiella pneumoniae type B averaged 20 ± 10% (S.D.). The effect of variation in the parasite on the rate of removal by the host will be discussed in a later communication.

Other studies[4,5] have shown that in the course of some experimental bacterial infections, the organisms are cleared partially or completely from the blood stream after a variable period of 5 to 48 hours, the variation depending apparently on the virulence of the infecting organism and the susceptibility of the host. After a period of several hours, the blood culture may become positive again and may be associated with a progressively unfavorable clinical course leading to the death of the animal. The reason for the ineffective clearing of the blood stream in the later stage of the infection has not been determined. It may be that the removal mechanism is blocked by accumulation of organisms in the cells of the reticuloendothelial system, but this seems unlikely because the mechanism still functions efficiently at a level of 100,000 organisms per ml. The organisms may proliferate in the cells and so alter their physiologic functions. Observations on the sites from which the bacteria return to the blood stream and the state of the reticuloendothelial system at that time are needed.

Summary. 1. A method of constant intravenous infusion of bacteria combined with the determination of bacterial counts in the circulation at various sites by venous catheterization is described, which provides a means of determining the site and of quantitating the rate of removal of bacteria from the blood stream of the intact animal.

2. The total canine splanchnic removal of hemolytic *Micrococcus aureus* averaged 74 ± 16% (S.D.); splenic removal, 78 ± 17% (S.D.). No organisms were lost in circulation through the lungs.

3. The total rabbit splanchnic removal of encapsulated *Klebsiella pneumoniae* type B averaged 20 ± 10% (S.D.).

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The Reversible Inactivation of Calcification *in vitro*.* (17334)

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Some aspects of normal calcification can be explained by physico-chemical concepts. For example, new calcification depends on the concentration of calcium and phosphate ions in excess of a critical product.[1-10] Furth-

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