morphic forms and particularly the bacillary forms were least stable, while the Gram positive diplococcus and the large opaque colony form was most stable.

In regard to the fermentation of sugars, it was noted that the typical Gram negative diplococcus fermented dextrose; the extreme "R" or tiny colony form failed to ferment sugars, but as dissociation progressed, first dextrose alone, and then maltose, sucrose, levulose and lactose were utilized. The Gram negative micrococcus failed to ferment carbohydrates, but when the typical form was attained fermented dextrose. Therefore, reversion was accompanied by return to fermentation type.

The agglutination for the homologous and typical cyclostage attained a titer of 1:5120, while the titer for the opaque form (polar-body and Gram positive diplococcus) was 1:160 to 1:320. It was difficult to obtain a homogeneous suspension for the "R" form or the tiny Gram negative micrococcus, so that no determinations were made for these stages.

7367 C

Accumulation of Bacteriophage in Spleen and Liver Following Its Intravenous Inoculation.

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The rôle of the reticulo-endothelial system in removing microorganisms from the blood stream has been generally recognized in recent years. The earlier work of Bull[1] in this field has been supported and extended by others, particularly Cannon[2, 3] and his coworkers. The question arose as to whether or not a readily filterable biological principle such as bacteriophage, which is probably particulate and of the general order of magnitude of the filterable viruses, would be removed from the blood stream by organs of the reticulo-endothelial system to a greater degree than by organs with few fixed phagocytic cells.

[1] Bull, Carroll G., *J. Exp. Med.*, 1915, **22**, 475, 484.

[2] Cannon, Paul R., Sullivan, F. L., and Neckermann, E. F., *J. Exp. Med.*, 1932, **55**, 121.

[3] Sullivan, F. L., Neckermann, E. F., and Cannon, Paul R., *J. Immunol.*, 1934, **26**, 49.

Y. Shitate[4] reported on the fate of bacteriophage in the body. Since he made no attempt to obtain quantitative results, his findings do not aid in answering the above question. Smirnow and Goldin[5] made a similar investigation on the fate of phage in the guinea pig's body after inoculation into the blood stream. Unfortunately they selected a 24-hour period for their first examination of the organs. Phage was found in low concentrations in blood, liver, spleen and kidneys. Sullivan, Neckermann and Cannon[3] have recently pointed out that although bacteria are present in much greater numbers in the spleen than in the blood 1 hour after an intravenous inoculation, that organ may be sterile in 24 hours. This may explain why Smirnow and Goldin failed to find any significant difference in bacteriophage titer between the blood and the reticulo-endothelial organs after so long a period as 24 hours.

Method. 15 albino rats (150-200 gm.) were etherized and injected in the exposed femoral vein with 2 cc. of staphylococcus phage. (1 cc. was injected in rats 11, 12 and 13.) In the case of rats 12-15 0.5 cc. of blood was collected from the clipped tail within 5 minutes. Two hours later the animals were again anesthetized and 0.5 to 1.0 gm. of various tissues were removed, ground with sand and 5 cc. of saline in a test tube. This material was diluted in steps of 1-10 with plain nutrient broth. A fresh sterile pipette was used for each dilution. One drop of a broth culture of the susceptible organism was seeded into each tube. Inhibition of growth was noted after 48 hours' incubation. Occasional doubt as to inhibition due to resistant secondary growth was removed by subculturing from the doubtful tube to an agar slant and noting the presence or absence of lytic areas. An additional procedure, the plaque enumeration test, was used in the case of rats 11-15. 0.1 cc. of material from the undiluted ground-up material or an appropriate dilution, as experience suggested, was placed on the surface of an agar plate with 0.1 cc. of a heavy suspension of staphylococcus. (5.0 cc. saline to an agar slant.) This was carefully spread with a bent glass rod. The number of plaques per plate was estimated after 24 hours' incubation.

Ten rats were injected in a similar manner with a saline suspension of staphylococcus. After 2 hours the animals were killed and the number of viable bacteria in the various tissues was estimated by using the serial dilution method described above, noting the highest dilution showing growth after incubation. A plate count was made on material from rats 6-10.

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[4] Shitate, Y., *Orient. J. Dis. Infants,* 1931, **10**, 1.

[5] Smirnow, P., and Goldin, M., *Cent. f. Bak.,* Orig. I, 1931, **122**, 512.

TABLE I.

Titration of Phage in Inoculum and Tissues.

Rat No.	Final Tube to Show Evidence of Phage in Decimal Dilution Series. 5 min. Inoculum	5 min. Blood	2 hr. Blood	2 hr. Spleen	2 hr. Liver	2 hr. Brain	2 hr. Kidney	2 hr. Muscle
1	8		4	5	6			0
2	9		3	6	5			0
3	9		4	4	5			2
4			4	6	6			0
5	9		5	6	5			3
6	9		4	6	5			3
7	8		4	6	6			2
8	9		3	3	6			0
9	9		4	5	5			0
10	8		4	5	5			0
11	10		4	5	5	1	2	
12	10	6	2	5	4	2	3	
13	10	6	1	5	5	0	3	
14	10	7	2	6	4	2	4	
15	9	7	2	6	5	2	3	
Mean	9.0	6.5	3.3	5.3	4.9	1.4	3.0	1.0
Phage units per gm. as determined by plaque numeration on 0.01 gm. or less of sample.								
11	100 mil.		200	20,000	2,500	0	300	
12	1000 mil.	135,000	0	54,000	1,000	0	20	
13	100 mil.	40,000	0	3,600	30,000	0	20	
14	100 mil.	2,400	0	60,000	300	0	50	
15	1000 mil.	600,000	0	2,400	240	1	20	
Mean	460 mil.	194,350	40	28,000	6,800	0	82	

Results. Table I shows the results obtained in the phage experiment involving 15 rats. The mean of the values obtained by the inhibition test indicates that phage was present in the spleen 2 hours after injection in approximately* 100 times the concentration found in the blood. The liver contained about 40 times as much as the blood, while the kidney titer was about the same as that of the blood. The brain and skeletal muscle yielded only about 1/100 as much phage as the blood. Blood collected immediately after injection contained over 10 times as much phage as did the spleen at 2 hours. The results obtained by the plaque method indicated that the spleen contained approximately 700 times and the liver 170 times as much phage as the blood at the period examined. Yet immediately after injection the blood contained around 7 times as much phage as did the spleen at 2 hours.

* Inasmuch as a difference of 1 tube in the decimal dilution series employed represents a tenfold difference in phage content, the antilogarith (base 10) of the number of the final tube showing phage gives the numerical representation of titer.

The fate of microscopic particles including bacteria injected intravenously has been well established by other workers. Nevertheless the results of a set of such experiments on the fate of intravenously injected staphylococci are recorded in Table II. These data, ob-

TABLE II.
Titration of Staphylococcus in Inoculum and Tissues.

Rat No.	Final Tube to Show Growth in Decimal Dilution Series.		2 hr.		
	Inoculum	Blood	Spleen	Liver	Brain
1	8	3	4	5	3
2	7	4	6	6	4
3	6	4	5	4	4
4	5	4	5	5	4
5	5	3	5	4	3
6	9	4	6	6	4
7	8	4	5	6	3
8	8	4	7	8	4
9	7	3	6	5	3
10	7	3	7	6	3
Mean	7.9	3.6	6.1	5.5	3.5
	Organism per gram as determined by plating 0.01 gm. or less of sample.				
6	36 mil.	0	80,000	60,000	0
7	100 mil.	50	22,000	16,400	0
8	100 mil.	50	60,000	14,000	50
9	30 mil.	0	1,080	1,320	0
10	30 mil.	0	720	1,200	0
Mean	59 mil.	20	32,760	18,584	10

tained under conditions similar to those existing in the phage experiments, are therefore better adapted for comparison with the latter results. By the dilution method the spleen contained 300 times as many bacteria as did the blood 2 hours after injection. The liver contained about 100 times as many, while the brain contained about the same number as the blood. Plating methods indicated that the spleen contained approximately 1600 times and the liver 1000 times as many bacteria as were found in the blood. The brain and blood had about the same number by this method. It is to be noted that there is a general similarity between these results and those obtained with bacteriophage.

The plaque method for phage and the plating method for bacteria gave more striking results than the serial decimal dilution methods. In the phage experiments plaques were seen on but one plate streaked with blood drawn at 2 hours. On the other hand it was often necessary to use dilutions of the splenic substance to avoid complete lysis on the plate.

Significance. Aside from any direct bearing on the fate of bac-

teriophage in the body these results raise several questions of somewhat wider interest. Does the mechanism of removal of phage units from the blood stream by the spleen and liver or by the circulating phagocytes as reported by Eliava[6] involve the mechanism of phagocytosis as has been observed for microscopic particles? A direct answer to this question is desirable but how to obtain such evidence does not appear obvious. The second question is concerned with the possible existence of an analogy between the fate of phage in the blood stream and that of filterable viruses which may get into the blood of the host. An answer to this query would be of value in a better understanding of those diseases caused by the filterable viruses.

Summary. It has been demonstrated that bacteriophage injected intravenously into rats accumulates in the spleen and liver. These organs accumulate phage to the same general degree as they do bacteria.

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Effect of Acidosis upon Production of Agglutinins and on Blood Proteins in Rabbits.

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A few reports in the literature indicate that acidosis hinders the production of agglutinins. Various substances have been used to produce an acidosis. Ammonium chloride produces a prolonged acidosis in man.[1, 2] Davesne and Haber[3] thought they obtained a decrease in agglutinin production while administering hydrochloric acid. Moen and Reimann[4] obtained a poor response or no agglutinin production to typhoid vaccine in uncontrolled diabetic patients with acidosis.

In the experiments reported here, rabbits were given three 0.5 cc. intravenous injections of suspensions of killed typhoid bacilli on alternate days. In 12 normal rabbits the agglutinin titer averaged

6 Eliava, G., *Comp. rend. Soc. de Biol.*, 1930, **105**, 829.

1 Burdon, J. B., and Haldane, S. H., *J. Physiol.*, 1921, **55**, 265.

2 Lui, S. H., and Hastings, A. B., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 781.

3 Davesne, J., et Haber, P., *Annales de l'Institute Pasteur*, 1932, **49**, 220.

4 Moen, J. K., and Reimann, H. A., *Arch. Int. Med.*, 1933, **51**, 789.