

in answering this question. Numerous photographs of streptococci growing in plain broth showed the normal appearance of the organism by this method to be quite constant (Plate 1). The examination of cells grown in broth containing 10 mg % of either sulfanilamide or its isomers and washed only once or not at all showed characteristic crystals surrounding the organisms (Plate 2). However, when the organisms were washed 2 or 3 times, the appearance of the streptococci was similar to normal organisms, the crystals having been washed away (Plate 3). Referring to the first experiments, this evidence insofar as it may apply suggests that the drugs diffuse into the organism rather than that they are adsorbed onto the surface of the bacterial cell.

Figure 1 shows the blood concentration time curves of sulfanilamide and its ortho and meta isomers as obtained in mice following oral administration of 10 mg of the respective compounds. All of these substances are well absorbed from the gastrointestinal tract and are therefore available for action *in vivo*.

In vivo and *in vitro* studies on the ortho and meta isomers prove them to be completely inert as chemotherapeutic agents in streptococcal and pneumococcal infections whereas the activity of the para isomer, sulfanilamide, is well known. It must be concluded, therefore, that availability to the host by absorption and distribution, and to the bacteria by strong adsorption or diffusion through the cell, is not a criterion of chemotherapeutic activity of any given compound. Inactive substances may manifest the properties of availability to the host and diffusion into or adsorption onto the bacterial cell but do not inhibit reproduction or otherwise destroy the organism by participating in the metabolism, in the manner that has been suggested for active substances such as sulfanilamide.

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Preservation of *Bartonella muris* in the Frozen State.

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Bartonella muris has been cultivated only with difficulty and but few workers have reported success.^{1, 2, 3} All used Noguchi's lepto-

spira medium or some minor modification of it. Recently two new methods for the cultivation of the human bartonella, *Bartonella bacilliformis*, have been described. One⁴ involves the use of the chorioallantoic membrane of the fertile hen's egg; the other does not require living tissue. Geiman⁵ reports good growth of *Bartonella bacilliformis* on a serum-tryptone medium to which small amounts of ascorbic acid and glutathione are added. Whether these media are suitable for the growth of *Bartonella muris* of the rat has not yet been demonstrated. Preliminary attempts by the author to cultivate *Bartonella muris* by inoculating infected blood directly into the yolk sac of the fertile hen's egg, have as yet yielded negative results.

In view of the difficulty involved and the meager success obtained in cultivating *Bartonella muris*, a simple method of preserving this infective agent over long periods is desirable. Alsted,⁶ when testing the effect of storing *Bartonella muris* at various temperatures, noted no change in infectivity after the preservation of infective blood at 0°C for 24 hours. Weiman⁷ reported success in maintaining the infectivity of a related species, *Eperythrozoon coccoides*, for 5 days when infective blood was stored at 5°C. However, when tested after storage for 10 days the material was no longer infective. It occurred to the author that if the organisms were stored at extremely low temperature they might survive storage for prolonged periods. This method of storage is not new, having been used successfully by Turner⁸ for the preservation of the spirochetes, *Treponema pallidum* and *Treponema pertenuis*, as well as by Coggeshall⁹ for the malarial organisms, *Plasmodium knowlesi* and *Plasmodium inui*.

Method. By means of cardiac puncture, blood was drawn from a series of splenectomized rats at the peak of their infection with *Bartonella muris*, when a large percentage of their erythrocytes showed the presence of the parasite. The blood was defibrinated and pooled. After a portion of the blood was set aside to serve as a control upon the infectivity of the fresh blood, 0.5 cc samples were

¹ Noguchi, H., *J. Exp. Med.*, 1928, **47**, 235.

² Marmorston-Gottesman, J., and Perla, D., *J. Exp. Med.*, 1932, **56**, 763.

³ Lawkowitz, W., *Trans. Roy. Soc. Trop. Med. and Hyg.*, 1939, **32**, 601.

⁴ Jiminez, J. F., and Buddingh, G. J., *PROC. SOC. EXP. BIOL. AND MED.*, 1940, **45**, 546.

⁵ Geiman, Q. M., *PROC. SOC. EXP. BIOL. AND MED.*, 1941, **47**, 329.

⁶ Alsted, G., *Acta. Path. et Microbiol. Scandinav.*, 1938, **37**, 37.

⁷ Weiman, D., *Les Parasites Érythocytaires Révélés par la Splénectomie: Bartonella et Eperythrozoon*, 1935, Paris, Amédée Legrand.

⁸ Turner, T. B., *J. Exp. Med.*, 1938, **67**, 61.

⁹ Coggeshall, L. T., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **42**, 499.

placed in Wassermann tubes and sealed with rubber stoppers. The tubes were immersed in a freezing mixture of dry ice and alcohol and rotated rapidly to insure uniform and rapid freezing of the infected blood. They were then packed in dry ice and stored in a thermos jug of one gallon capacity, the dry ice being replenished as required. The frozen blood was tested for infectivity at various intervals thereafter. Immediately before use the blood was liquefied by immersing the tubes in warm water (40-45°C). Melting occurred rapidly. Shortly thereafter, 0.1 cc samples were inoculated intraperitoneally into splenectomized bartonella-free rats. The test animals were all from 45 to 50 days of age and had been splenectomized immediately before infection.

Results. Six rats were injected with the control, fresh blood (Series I). Two days later Bartonella-infected red cells were present in Giemsa stained films of the tail blood of all the animals. Five of the rats succumbed on the fourth day and one on the sixth day.

The frozen blood was tested after storage for 3, 5, 9, and 11 weeks. After storage for 3 weeks (Series II) there was no signifi-

TABLE I.
Infectivity of *Bartonella muris* Preserved in the Frozen State.

Series No.	No. wks blood stored	Rat No.	Days after infection						
			1	2	3	4	5	6	
I	0	1	0	+	++	D			
	0	2	0	+	++	D			
	0	3	0	+	++	D			
	0	4	0	+	++	D			
	0	5	0	+	++	D			
	0	6	0	+	+	++	++	++	D
II	3	7	0	0	+	D			
	3	8	0	0	+	D			
	3	9	0	+	++	D			
III	5	10	0	0	+	D			
	5	11	0	0	++	D			
	5	12	0	0	+	++	D		
	5	13	0	0	+	++	++	++	D
IV	9	14	0	0	0	+	D		
	9	15	0	0	0	+	D		
	9	16	0	0	0	+	D		
V	11	17	0	0	0	+	++	D	
	11	18	0	0	0	+	++	D	
	11	19	0	0	0	+	++	D	

All rats infected with 0.1 cc of blood.

0 = no cells infected.

+ = less than 50% of red corpuscles infected.

++ = more " 50% " " " " "

D = dead.

cant change in the infectivity of the frozen material. Two of the 3 rats injected with this material showed a prepatent period of 3 days. Yet, all the animals died on the fourth day. Two of the 4 animals injected with blood stored for 5 weeks (Series III), died on the fourth day after infection; the others succumbed on the fifth and sixth days respectively. Animals infected with blood stored for nine weeks (Series IV), showed a further increase in the prepatent period to four days. However, there was little or no loss in the virulence of the parasite, for all the animals succumbed on the fifth day. Rats injected with blood stored for 11 weeks (Series V) showed the same prepatent period as those given blood stored 9 weeks, but death occurred on the sixth day.

At the time the last series was tested the virulence of the parasites in the frozen blood had not changed, using the death of the infected animals as the criterion. The gradual increase in the length of the prepatent period of the infected animals, which resulted from longer storage, was probably due to the destruction of some of the *Bartonella* with preservation. The infective dose thus representing fewer infective units than the original stock, although these were of undiminished virulence. Doubtless the 11-week period during which the blood was stored by no means indicated the maximum length of time during which *Bartonella muris* may be preserved in the frozen state.

Summary. The infectivity of *Bartonella muris* can be preserved for at least 11 weeks when infected defibrinated rat blood is rapidly frozen in a mixture of dry ice and alcohol and then preserved in dry ice.

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