

So-called hybrid vigor, or heterosis, usually finds its expression in physical strength, in fertility, or in longevity, in which characters the hybrids surpass both parent strains. In the case of the mice described above no difference in size has been noticed and about the duration of life nothing is known, but there is no doubt that a cross between the 2 strains gives more viable young than either one of the pure strains. The birthrate in this case has not been studied, because, especially in the case of E L T M where the mothers destroy so many young, it would require the closest observation to state accurately how many young are born. On the other hand the observation as to how many survive with the mother one month after birth is easily made. Hybrid vigor certainly does not occur in all crosses between mice and the term is used for a variety of phenomena. Most often the explanation is lacking but sometimes hybrid vigor is explained by the fact that genetical factors which promote each other are combined in the hybrid. A more complete analysis will be necessary to understand the case reported here.

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A Simplified Technique for the Study of Pneumococcus Growth Inhibition.

CHAO-JEN WU AND RICHARD H. P. SIA.

From the Department of Medicine, Peiping Union Medical College.

Various techniques have been employed to determine the pneumococidal power of serum, plasma or whole blood of naturally or artificially immune animals. Robertson and Sia¹ used serum-leucocyte-cocci mixture placed in small paraffin corked tubes and agitated during incubation in a manner simulating the circulating blood. The procedure is complex and time-consuming. Bull and Tao² claimed that whole blood prevented from coagulation by sodium citrate served just as well as the serum-leucocyte mixture in demonstrating the pneumococidal property of the sera of chicken and immune rabbits and that agitation was not necessary as shown to be important by the former workers. The present report deals with the results obtained while looking for a simplified technique of the original method.

¹ Robertson, O. H., and Sia, R. H. P., *J. Exp. Med.*, 1924, **40**, 467.

² Bull, C. G., and Tao, S. M., *Am. J. Hyg.*, 1927, **7**, 648.

Since the citrated whole blood is so easy to obtain and has been claimed to give satisfactory results we repeated the experiments according to the details set forth by Bull and Tao. 1% by volume of saturated sodium citrate (Merck) solution (determined to be nearly 100%) was used. A virulent Type I pneumococcus was grown in broth for 16 hours. At the same time the original method of Robertson and Sia was carried out as control. Many experiments were done at different times and the results are summarized in Table I. It will be noticed from columns A and C that while the

TABLE I.
Comparison of pneumococcus growth inhibition tests by the method of Bull and Tao with that of Robertson and Sia.

Dilution of Pneumococci	Citrated Whole Blood						Serum-leucocyte Mixture	
	A			B			C	
	Rabbit	Cat	Dog	Chicken	Dog	Chicken	Cat	Dog
Undiluted			+	+				+
1:10			+	+		+		+
1:100		+	+	+	+	0	+	+
1:1,000		+	+	+	+	0	+	+
1:10,000		+	+	+	+	0	0	0
1:100,000		+	+	+	0	0	0	0
1:1,000,000	+	+	+	+	0	0	0	0
1:10,000,000	+	+	+	+	0	0	0	0
			Plasma	Control			Serum	Control
1:1,000,000		+	0	+	0	0	+	+
1:10,000,000		+	+	+	0	0	+	+

+ = Growth in 48 hours by smears. 0 = No growth.

original method clearly demonstrates the pneumococcal property of the blood of the resistant animals, this property failed to appear in the citrated blood of the same animals. On the other hand, as shown in column B, it was found in a few instances that there was no growth in the control tubes with the citrated plasma of the naturally resistant animals as well as the citrated whole blood series in the tubes seeded with the higher dilutions of the culture. This result might be due to pneumococcal power of the blood, but the absence of growth in the plasma led us to suspect the inhibitory effect of sodium citrate upon the growth of pneumococci. Experiments were then done to determine this effect of the anti-coagulant, and the results are shown in Table II. Similar experiments with blood and plasma of cat and chicken gave identical results with that of the dog. Three facts present themselves. First, sodium citrate in the strengths tested had a definite detrimental effect upon the organisms. The margin of safety is so very small that a difference of even 0.1% brought out a marked change in the reaction of dog's blood. Second, with equal strength of sodium citrate, pneumococcus

TABLE II.
Effect of varying amounts of sodium citrate upon the growth of pneumococci in whole blood and plasma of rabbit and dog.

Sodium Citrate %		1.0	1.0	1.1	1.2	1.2	1.3	1.4	1.4	1.5
Dilution of pneumococci		Blood	Plasma	Blood	Blood	Plasma	Blood	Blood	Plasma	Plasma
Rabbit	1:100				+	+		+	+	0
	1:1,000				+	+		+	+	0
	1:10,000				+	+		+	+	0
	1:100,000	+	+	+	+	+		+	0	0
	1:1,000,000	+	+	+	+	+		+	0	0
	1:10,000,000	+	0	+	+	0		0	0	0
Dog	1:10	+	+	+	+		+	Few		+
	1:100	+	+	+	0		0	0		0
	1:1,000	+	+	0	0		0	0		0
	1:10,000	+	0	0	0		0	0		0
	1:100,000	+	0	0	0		0	0		0
	1:1,000,000	+	0	0	0		0	0		0
1:10,000,000	+	0	0	0		0	0		0	

has a better chance of growth in the whole blood than in the plasma of the same animal. Third, sodium citrate in the blood or plasma of resistant animals exerts a greater injurious effect upon pneumococci than in blood or plasma of a susceptible animal. Cheer³ demonstrated that sodium citrate in strengths of 0.8 and 1.0% exerted a growth inhibitory action upon pneumococci. Therefore, it seemed to us that the pneumococidal power of the citrated blood as reported by Bull and Tao could be explained by the inhibitory effect of sodium citrate upon the growth of organisms themselves rather than by the interaction of serum and leucocytes. And similarly, it would also explain the fact that agitation during incubation was claimed to be not necessary. If they had controlled their experiments with citrated plasma as we did in ours, they might have arrived at a similar interpretation of their results. We also believe that sodium citrate in a strength too weak to affect the organisms would injure the leucocytes when they come into contact with each other for 24-48 hours as this test requires. This may explain our results shown in column A of Table I where there was growth in every tube of the citrated blood of resistant animals as well as that of susceptible animal.

The modification of the original method consists of either of the following:

A. "Reconstituted blood": A desired amount of blood is sterily withdrawn and divided into 2 portions. One portion is allowed to

³ Cheer, S. N., *J. Imm.*, 1930, **18**, 187.

coagulate to give serum. The other portion is well mixed with 1-2 parts of 1% sodium citrate in normal saline, centrifuged and the supernatant fluid discarded. The cells are then washed once in gelatin salt solution and the washings discarded. Enough homologous serum is finally added to make up to the original volume of the blood. This non-coagulable reconstituted blood is deprived of its fibrin and spared of its normal active leucocytes since they are in contact with citrate for only a very short time. B. "Defibrinated blood": This is accomplished by shaking a sample of blood thoroughly in a flask with glass beads. The results with the defibrinated blood have been very satisfactory and in a way confirm the contention of Todd⁴ and Ward⁵ that complete defibrination does not

TABLE III.
Growth inhibition test by "Reconstituted" and Defibrinated Blood.

	Dilution of pneumococci	Reconstituted blood	Serum control	Defibrinated blood	Plasma control
Rabbit	1:100,000	+		+	
	1:1,000,000	+		+	+
	1:10,000,000	+		+	+
Dog	1:10	+		+	
	1:100	+		+	
	1:1,000	+		+	
	1:10,000	0		0	
	1:100,000	0		0	+
	1:1,000,000	0	+	0	
	1:10,000,000	0	+	0	
Cat	1:10	+		+	
	1:100	+		+	
	1:1,000	+		+	
	1:10,000	0		0	
	1:100,000	0		0	+
	1:1,000,000	0	+	0	
	1:10,000,000	0	+	0	
Chicken	1:10	+		+	
	1:100	+		+	
	1:1,000	0		+	
	1:10,000	0		0	
	1:100,000	0		0	+
	1:1,000,000	0	+	0	
	1:10,000,000	0	+	0	
Human*	Undiluted	+		+	
	1:10	+		+	
	1:100	+		0	
	1:1,000	0		0	
	1:10,000	0		0	
	1:100,000	0		0	
	1:1,000,000	0	+	0	
	1:10,000,000	0	+	0	

*Type I pneumonia convalescent. Blood taken 9th day after crisis.

⁴ Todd, E. W., *Brit. J. Exp. Path.*, 1926, **7**, 368; 1927, **8**, 1.

⁵ Ward, H. K., *J. Exp. Med.*, 1930, **51**, 675.

alter the phagocytic activity of the leucocytes. The number of leucocytes present in the defibrinated blood is apparently not markedly reduced. In one or two instances actual counts on the different lots of the blood were made. In that of dogs the count varied from 11,800 to 13,600 per c.mm.

In actual tests, 0.5 cc. of blood (0.4 or 0.3 cc. have been found to be quite satisfactory) prepared according to either A or B is placed in each of a series of small tubes, and 0.1 cc. of the various dilutions (in broth) of pneumococcus is added. The tubes are corked and agitated according to the method of Robertson and Sia. Rotation was found essential. The results obtained by these 2 modifications (Table III) were so consistently good and they compared so favorably with those of the original method that we consider it worthwhile to propose their use as a simplified technique. The method has been used to measure the potency of an immune serum and to detect the pneumococidal power of human convalescent blood. It was found applicable. While the "reconstituted blood" offers one the means of studying serum and leucocytes from different individuals the defibrinated blood has the distinct advantage of being the simpler. However, in view of some inhibitory effect of dog plasma upon the growth of pneumococci* the latter modification is to be preferred only in selected instances.

Summary. Citrated whole blood has been shown to be inadequate for the study of pneumococcus growth inhibition on account of the detrimental effect of sodium citrate upon the organisms on the one hand, and upon the leucocytes on the other. A modification of the original method of Robertson and Sia is proposed. Because of the simplicity and the satisfactory results it gives, this technique may find a wider application for similar studies with other organisms.

* The inhibitory effect of dog plasma upon the growth of pneumococci will be taken up in detail in a further communication.