

ture heated for one-half hour at 80°C. there appeared symptoms of upper respiratory irritation which lasted for less than 24 hours. We realize the difficulty of a final judgment concerning the successful cultivation of an invisible agent and simply present the facts as we have observed them.

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Relationship of Sera and Spinal Fluids to Agglutination and Flocculation by Dyes.

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It has been known for a long time¹ that human red blood cells and bacteria could be agglutinated by solution of basic dyes. In addition M. Gutman² showed that whole serum could be flocculated by the same dyes. In our experiments we found that these phenomena were much more complex.

The human red blood cells used in the experiments were washed 3 times in normal saline (0.85%) in order to free them from all traces of serum. A 2% suspension of red blood cells was used. Whenever human serum was used in conjunction with the red blood cells the same types were employed in order to avoid agglutination of the cells by the serum. The standard dye used was a gentian violet solution 1:2500 in normal saline. The ingredients were mixed in equal amounts in narrow glass tubes by thorough shaking. Agglutination and flocculation could easily be observed macroscopically. For microscopic examination drops of the same ingredients were placed on glass slides and mixed with glass rods.

Results. 1. *Flocculation of human sera:* The flocculation of whole human serum by basic dyes noted by Gutman takes place only if relatively strong solutions are added. (*e. g.*, ½-1% aqueous solutions of safranin or gentian violet). But by using weaker solutions different results were obtained. Whole human serum, or serum diluted with normal saline (0.85%) up to 1:6 is not flocculated by our standard solution of gentian violet. Flocculation here first occurs with a serum diluted 1:8 and increases in strength with higher dilutions of sera. In dilutions of serum of 1:64 floccula-

¹ Brossa, G. A., *Z. f. Immunitätsforsch.*, 1923, **37**, 221.

² Gutman, M., *Centralbl. f. Bakt.*, I Abt., Orig., 1928, **56**, 68.

tion decreases, and it does not occur when the serum is diluted beyond 1:128.

2. *Agglutination of human red blood cells and bacteria.* In accordance with the work of Gutman and others we found that solutions of basic dyes agglutinate red blood cells and bacteria.

3. *Protective power of human serum.* Whole human serum added to a 2% suspension of human red blood cells or bacteria and thoroughly mixed with them acted as a protective and prevented their agglutination upon the subsequent addition of the gentian violet solution. The serum exerted this protective power in dilutions up to 1:64. In higher dilutions the serum no longer protected and agglutination occurred as if no serum were added.

4. *Desagglutination by serum.* A 2% suspension of human red blood cells was agglutinated by our solution of gentian violet. Equal amounts of whole human serum, or serum diluted up to 1:8 added to the cells agglutinated by the dye caused prompt desagglutination.

5. *Flocculation of spinal fluids.* Our solution of gentian violet added to undiluted spinal fluid or its dilutions up to 1:5 caused their flocculation. In dilutions higher than 1:5 flocculation decreases and does not occur when the spinal fluid is diluted beyond 1:10.

6. *Protective power of spinal fluid.* Undiluted spinal fluid added to a 2% suspension of human red blood cells or to bacteria protects them against agglutination upon subsequent addition of the gentian violet solution.

There are individual variations in the reactivity of human red blood cells, sera and spinal fluids towards dyes. We are now carrying on further experiments to determine whether there are any differences produced by pathological conditions in the human body.

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Demonstration of a Tumor-Inhibiting Substance in Filtrate of Rous Chicken Sarcoma and in Normal Chicken Sera.

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In a previous communication¹ we reported that the active agent

¹ Sittenfield, M. J., and Johnson, B. A., *PROC. SOC. EXP. BIOL. AND MED.*, 1930, **28**, 206.