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Concentration and Purification of Antityphoid Horse Serum.

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Extremely severe hemorrhagic necrosis appears in rabbits at the skin sites prepared by an injection of a bacterial filtrate when the injection is followed 24 hours later by an intravenous injection (*i. e.*, reacting factors) of a culture filtrate of the same or another bacterium.[1, 2, 3, 4] Among the various phases of this phenomenon of local skin reactivity, the specific neutralization of the reacting factors by homologous immune sera was observed. A method has been described for quantitative measurement of the neutralizing antibodies of immune antimeningococcus and antityphoid horse sera. Inasmuch as the titer of these antibodies did not run parallel to the agglutination titer of the same sera, advantage was taken of this method[2, 3] in order to develop highly potent neutralizing horse sera for ultimate therapeutic application. The work reported below was undertaken in order to concentrate and purify the antityphoid neutralizing antibodies.

Concentrated and purified antibody solutions should contain a maximum amount of antibodies associated with a minimum of total solids, especially of protein, as indicated by the nitrogen content. Gibson and Banzhaf[5, 6] were able to obtain three- to fourfold concentration of diphtheria and of tetanus antitoxins from antisera. Preparations of pneumococcus antibody solutions by methods of Banzhaf,[7] and Banzhaf and Sobotka,[8] and by other methods, yield concentrations up to 7-10 times the amount of protective units found in the original serum. It should be noted that only solutions containing the identical amount of total solids or nitrogen may be directly com-

[1] Shwartzman, G., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 560; xxvi, 131, 207; *J. Exp. Med.*, 1928, xlviii, 247; 1929, xlix, 593; 1930, li, 571; *J. Inf. Dis.*, 1929, xlv, 232; *Klin. Wsch.*, 1930, *Ein neues immunologisches Phaenomen*, in press.

[2] Shwartzman, G., *J. Exp. Med.*, 1929, i, 513; *J. Am. Med. Assn.*, 1929, xciii, 1965.

[3] Shwartzman, G., "The effect of bacterial variations upon the factors necessary for the phenomenon of local skin reactivity" (in preparation).

[4] Shwartzman, G., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 843.

[5] Gibson, R. B., *J. Biol. Chem.*, 1906, i, 161.

[6] Gibson, R. B., and Banzhaf, E. J., *J. Exp. Med.*, 1910, xii, 411.

[7] Banzhaf, E. J., *Weekly Bull. N. Y. Dept. of Health*, 1927, xvi, 17.

[8] Personal communication.

pared. By the following method we are able to reach a much greater concentration as demonstrated by the phenomenon of local skin reactivity to *B. typhosus.*

The plasma or serum was diluted to one and a half times its original volume with distilled water. Instead of a saturated ammonium sulfate solution, a 47.5% sodium-magnesium sulfate solution was used as the protein precipitant to facilitate the nitrogen analysis. Twenty-eight parts of this solution were added to 72 parts of the diluted serum. The precipitate which contains fibrinogen and part of the euglobulin was discarded. Then a sufficient amount of the salt solution was added to the filtrate to bring the sulfate concentration up to 19%. The precipitate obtained was allowed to become almost dry on the filter paper and was then transferred to a dialyzing bag in running water. After about 6 hours the white pasty mass became a clear brown solution which gradually grew turbid and later showed a white precipitate. The dialysis was continued for 72 to 96 hours until all the sulfate ion had disappeared and no more precipitate seemed to form. The globulin solution was centrifuged and the precipitate was made up with physiological salt solution to a convenient volume, *e. g.,* one-tenth of the original serum. Extraneous matter was removed by centrifuging, and the supernatant fluid filtered through a Berkefeld "V" candle.

The neutralization experiments were performed as follows: (a) Titration of *B. typhosus* reacting factors. *B. typhosus* culture filtrates employed in this work were prepared according to the method previously described.[4] The rabbits used for titrations were each injected intradermally with 0.25 cc. of the undiluted filtrate* and divided into groups of 4. Twenty-four hours later a single intravenous injection of the filtrate diluted in 0.85% NaCl solution (one cc. per kilo of body weight) was given to each rabbit. Each group received intravenously a different dilution of the filtrate. The local reactions were read 4 to 5 hours after the intravenous injection. The titrations were continued until the lowest dilution was found which gave no reactions in the 4 rabbits tested. The highest dilution which gave reactions in one or more rabbits of the group was also ascertained. The minimal dose of reacting factors was then considered to be between these 2 figures.

(b) Measurement of neutralizing antibodies. The technique employed for the neutralization experiments was similar to one described before.[2, 3] One area of the skin of the abdominal wall of

* The standardization of this procedure will be discussed in detail in a subsequent publication.

the rabbit was injected with 0.25 cc. of the undiluted filtrate. Twenty-two to 24 hours later a single intravenous injection was made of a mixture of the same filtrate (diluted previously in 0.85% NaCl solution to the desired degree) with a given undiluted serum in the proportion 4:1. The mixtures prepared on the morning of the experiment were incubated in a water bath at 37.5°C. for one hour. The precipitate in the mixtures was broken up by shaking immediately before the injection. The intravenous dose was 1.25 cc. per kilo of body weight. Each mixture was tested in 4 rabbits. The

TABLE I.
Concentration and Purification of Anti-typhoid Horse Sera.

Sample	Vol. in cc.	N mg. per cc.	Agglutinins	Precipitins	Neutral units per cc.	Neutral units per mg. nitrogen	Total No. neutralizing units	Yield of neutralizing antibodies
Original horse serum No. 110A	200	11.3	10240	32	580	50	116000	52%
Antibody solution recovered	75	0.3	6400	16	800	2650	60000	
Concentration per mg. N			23X	19X		53X		
Original horse serum No. 110A	480	11.4	10240	32	580	50	278400	40%
Antibody solution recovered	96	0.3	20480	64	1200	4000	115200	
Concentration per mg. N			76X	76X		80X		
Original horse serum No. 133	450	11.5	10240	32	580	50	261000	45%
Antibody solution recovered	45	1.1	102400	128	2600	2350	117000	
Concentration per mg. N			105X	42X		47X		
Original horse serum No. 133	350	11.5	12800	16	360	30	126000	44%
Antibody solution recovered	35	1.3	25600	64	1600	1200	56000	
Concentration per mg. N			18X	36X		40X		
Original horse serum No. 133	350	11.5	12800	16	360	30	126000	44%
Antibody solution recovered	35	1.9	25600	64	1600	850	56000	
Concentration per mg. N			12X	24X		28X		

amount of serum (0.25 cc. per kilo of body weight) was kept constant. The titrations were continued until the lowest dilution of the filtrate was found which was still consistently neutralized (*i. e.*, in all rabbits tested) by this amount of serum. The number of reacting units present in this lowest dilution of the filtrate was taken to indicate the same number of neutralizing units in 0.25 cc. of the serum.

The volume, nitrogen content, number of neutralizing units, agglutinins and precipitins of 2 batches of antityphoid horse sera and of 5 representative concentrated preparations are compared. The total yield in neutralizing antibodies was from 40 to 50% of that in the original serum. The concentration as indicated by the quotient Neutralizing Units/mgm. N varied from 28 to 80 times. Thus a great part of the neutralizing antibodies was recovered in a very small globulin fraction. The range of magnitude of this concentration exceeds by far any concentration attained by previous authors by chemical separation of antibodies of various antibody and antitoxin solutions. There is apparently no relation between the concentration of the agglutinins and the neutralizing antibodies. Further observations will decide whether a parallelism exists between neutralizing antibodies and precipitins.

Further work on antityphoid as well as on antimeningococcus and anticoli horse sera is under progress.

Preliminary chemical analysis of this highly specific serum fraction seems to indicate the preponderance of proteins in its constitution. Dr. Harry Sobotka and one of the authors will report this phase of the work in another communication.

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Chorio-allantoic Grafting Followed by Direct Transplantation in the Chick.*

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The present note calls attention to the possibilities of a new procedure in tissue grafting which promises to prove useful in investigation of problems in development and which should be made avail-

* From the Department of Anatomy, Harvard Medical School; to which the author is indebted for the material and facilities.