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Use of Sodium Azide to Control Bacterial Destruction of Rh Agglutinating Antibodies.

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(Introduced by Maurice Landy.)

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Numerous authors have stated that errors in blood grouping, particularly false positive reactions, may result from the use of contaminated blood grouping serum.[1,2,3,4] With the increasing use of anti-Rh serums in routine diagnostic tests as well as in numerous phases of investigative work it becomes increasingly important to have a satisfactory preservative for such serums. Waller[5] reported that merthiolate inhibited anti-Rh agglutinins, but was without effect on the other hemagglutinins tested. Diamond and Abelson,[6] however, noted that exposure to merthiolate "while inactivating anti-Rh agglutinins, appears not to affect" Rh "blocking" antibodies. Cameron and Diamond[7] found that anti-Rh serum containing 0.1% carboxylmethoxylamine hemi-hydrochloride ("compound I") showed some loss of both titer and avidity after storage at room temperature, but it is not clear whether the slight loss of potency was due to the storage temperature or to the presence of "compound I". No additional reports on the use of preservatives specifically in anti-Rh serums have appeared, although Favour[8] suggests that "compound I" can be used "in the preservation of blood-typing serum, . . . and other preparations with a high protein content."

The successful use of sodium azide (NaN_3) in preserving liquid complement,[9] and its broad spectrum of bacteriostatic and fungistatic activity[10,11,12] suggested the possibility of its use in the preservation of anti-Rh serums, and is the subject of this investigation.

Materials and Methods. For the experiments reported here a high-titered human serum was selected containing anti-Rh_0 (anti-D) saline agglutinins. These antibodies are known to be more labile than are the "blocking" (univalent) type of Rh antibodies and thus should provide a more sensitive measure of any deleterious action of a preservative. The serum was diluted to a titer† of 128, using as the diluent pooled normal serum containing neither Rh nor other irregular isohemagglutinins. The serum was sterilized by filtration and divided into three portions. To two of these NaN_3 was added to a concentration of 0.1% and 0.05% respectively. The third portion was retained as the untreated control. Each of these three portions was then further subdivided; half was kept sterile and the other half was seeded with a mixed suspension of bacteria composed of five strains originally isolated as common

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2 Davidsohn, I., and Toharsky, B., *J. Immunol.*, 1942, **43**, 213.

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4 McGraw, J. J., Vaubel, E. K., and Elliott, J., *Surg. Clin. N. America*, 1945, **25**, 1042.

5 Waller, R. K., *Am. J. Clin. Path.*, 1944, **14**, 116.

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8 Favour, C. B., *J. Bact.*, 1948, **55**, 1.

9 Richardson, G. M., *Lancet*, 1941, **241**, 696.

10 Lichstein, H. C., *J. Bact.*, 1941, **42**, 293.

11 Lichstein, H. C., and Soule, M. H., *J. Bact.*, 1944, **47**, 221.

12 Herrick, J. A., and Kempf, J. E., *ibid.*, 1944, **48**, 331.

† Reciprocal of greatest dilution giving macroscopic agglutination of test cells.

TABLE I.

Agglutinin Titers of Sterile Anti-Rh_0 Serum (Duplicate Samples) Preserved with Graded Quantities of NaN_3 and Stored at 37°C. Test Cell Suspensions and Serum Dilutions Made in 0.85% NaCl.

CONCENTRATION OF NaN_3	WEEKS OF STORAGE	SERUM DILUTION							
		2	4	8	16	32	64	128	256
0.1%	0	++++	++++	++++	++++	++++	+++	+	—
	8	++++	++++	++++	+++	++	—		
		++++	++++	++++	+++	++	—		
	10	++++	++++	+++	+++	+	—		
		++++	++++	+++	++	—			
	14	++++	+++	++	—				
		+++	+++	++	—				
0.05%	0	++++	++++	++++	++++	++++	+++	+	—
	8	++++	++++	+++	++	+	—	—	
		++++	++++	++++	+++	++	—	—	
	10	+++	+++	+++	+++	++	—	—	
		++++	+++	+++	+++	++	—	—	
	14	+++	+++	—					
		++	++	—					
NONE (CONTROL)	0	++++	++++	++++	++++	++++	+++	++	—
	8	++++	++++	+++	++	+	—		
		++++	++++	+++	++	+	—		
	10	+++	+++	+++	++	—			
		++++	+++	+++	++	+	—		
	14	+++	++	—					
		+++	+++	—					

Symbols: ++++ Single large clump.
+++ Breaks into several large clumps.
++ Breaks into numerous small clumps.
+ Many fine macroscopic clumps.
— Negative.

laboratory contaminants.‡ The suspensions were standardized photometrically to contain approximately 100 million organisms per cc and a sufficient quantity was added so that the serums contained approximately one million organisms per cc. Each of the six samples was tested for sterility by inoculation of tubes of veal infusion broth, titrated for serological activity, and dispensed aseptically into 5 cc vaccine bottles fitted with rubber diaphragm sleeve stoppers. Duplicate samples of each series of treated and untreated (NaN_3), sterile and contaminated serums were then stored at 37°C. At periodic intervals all samples were titrated against a 2% saline suspension of pooled Group O, Rh_0 positive (D positive) cells, and tested for sterility. Results of these serological titrations are presented in Tables I and II.

Results. It was found that NaN_3 in the concentrations tested was bacteriostatic, though not bactericidal for the test organisms. Viable organisms were found at all test periods. The only strain which was recovered consistently from the NaN_3 treated samples was the streptococcus. Only Gram negative bacilli were recovered from the untreated contaminated control specimens although microscopic examination of direct smears revealed the presence of Gram negative bacilli, Gram positive bacilli and streptococci.

Periodic titrations revealed a gradual decrease in potency (loss of titer) in both the NaN_3 treated and untreated sterile samples. This decrease in potency in the two series was approximately parallel, indicating that NaN_3 had no observable influence on the thermal stability of the antibody being studied.

In the contaminated series, however, a marked difference in potency was noted after 3 weeks storage: the samples containing NaN_3 showed a slight loss of titer, comparable

‡ The cultures used were *Bacillus cereus, Aerobacter cloacae, Streptococcus liquefaciens, Pseudomonas ovalis,* and *Staphylococcus epidermidis.* These were obtained from the National Institute of Health through the courtesy of Dr. Margaret Pittman.

TABLE II.

Agglutinin Titers of Contaminated Anti-Rh_o Serum (Duplicate Samples) Preserved with Graded Quantities of NaN_3 and Stored at 37°C. Test Cell Suspensions and Serum Dilutions Made in 0.85% NaCl.

CONCENTRATION OF NaN_3	WEEKS OF STORAGE	SERUM DILUTION							
		2	4	8	16	32	64	128	256
0.1%	0	++++	++++	++++	++++	++++	+++	++	–
	3	++++	++++	+++	++	++	+	–	
		++++	++++	+++	++	+	+	–	
	8	++++	++++	+++	+++	++	–		
		++++	++++	+++	++	+	–		
	10	++++	+++	+++	++	+	–		
		++++	++++	+++	++	+	–		
	14	++++	+++	++	–				
		++++	++	+	–				
0.05%	0	++++	++++	++++	++++	++++	+++	+	–
	3	++++	++++	+++	++	++	+	–	
		++++	++++	+++	++	++	+	–	
	8	++++	++++	+++	++	–			
		++++	+++	+++	+	+	–		
	10	++++	++++	+++	++	+			
		++++	++++	+++	++	+			
	14	++++	+++	+	–				
		++++	+++	+	–				
NONE (CONTROL)	0	++++	++++	++++	++++	++++	+++	++	–
	3	++++	+++	++	+	–			
		++++	+++	++	+	–			
	8	+++	+	–					
		++	–	–					
	10	–	–						
		–	–						
	14	–	–						
		–	–						

SYMBOLS USED HAVE THE SAME MEANING AS GIVEN IN THE LEGEND TO TABLE 1.

to that found in the sterile samples, whereas the contaminated samples stored without NaN_3 showed a more striking and rapid loss of titer.

Complete inactivation of the Rh antibody in the untreated contaminated samples was observed after 10 weeks storage whereas the sterile samples and contaminated samples containing NaN_3 were still very active, although showing some loss of titer due to the storage temperature.

It is apparent from these experiments that bacterial contamination of anti-Rh serum can, under some circumstances, result in complete inactivation of Rh antibodies. NaN_3 in concentrations of 0.05% and 0.10% inhibited this inactivation. It is doubtful that the inactivation of hemagglutinins by bacteria observed here is specific for Rh antibodies,§ rather it is probably due to gradual destruction of the immune globulins of the serum, possibly through proteolysis.

Despite the toxicity‖ which may limit its

§ In preliminary experiments the same effect of bacterial contamination has been demonstrated on "blocking" Rh antibodies. Similar studies are being carried out with the alpha and beta isoagglutinins. The identity of the strain or strains of organisms which caused deterioration of the antibodies has not been established.

‖ Although NaN_3 is highly toxic,[13,14,15] the small amount present (1 to 5 mg) in the volume of anti-Rh serum usually dispensed would scarcely represent any danger to the technician. Cf. Graham *et al.*[16] who recommends further investigation of this chemical as a therapeutic agent.

[13] Kempf, J. E., and Nungester, W. J., *Science*, 1944, **100**, 411.

[14] Fairhall, L. T., *et al.*, *Pub. Health Reports*, 1943, **58**, 607.

[15] Smith, L., and Wolf, C. G. D., *J. Med. Res.*, 1904, **7**, 451, cited by Richardson.[9]

[16] Graham, J. D. P., Rogan, J. M., and Robertson, D. G., *J. Indust. Hyg. & Toxicology*, 1948, **30**, 98.

usefulness, NaN_3 could be of value in the preservation of anti-Rh serums. It possesses certain advantages over "compound I" in that it is active against fungi, and is effective in heavily contaminated materials. Like "compound I" the effectiveness of NaN_3 is not impaired by high concentrations of protein. In experiments now in progress it is being found that the bacteriostatic effectiveness of NaN_3 is not impaired in anti-Rh serums fortified with 20% bovine albumin. Through their action as specific metabolic inhibitors, compounds such as NaN_3 and "compound I" may find wider use in the preservation of diagnostic biologicals.

Summary. The effectiveness of NaN_3 in concentrations of 0.1% and 0.05% as a preservative for anti-Rh agglutinating serums has been investigated. It was found that in these concentrations the agent was markedly bacteriostatic, but had no apparent effect on the titer or avidity of the antiserum. Anti-Rh serum deliberately contaminated with common bacteria but not preserved with NaN_3 rapidly lost potency when stored at 37°C.

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Effects of Ovarian Hormones on Certain Cytoplasmic Reactions in the Vaginal Epithelium of the Mouse.*†

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Since the classical work of Allen and Doisy[1] many investigators have studied the effects of the various gonadal hormones on the growth and differentiation of the vaginal epithelium. With the exception of the numerous studies on glycogen in the monkey and human and mucin in the rodent, little work has been directed toward the alterations in intracellular constituents. Recently Jeener[2] stated in a brief report that estrogen evoked a marked increase in both alkaline phosphatase and cytoplasmic ribonucleic acid in the vaginal epithelium of the mouse, but few cytological details were given.

In view of this paucity of information, the present study was designed to examine the effects of estrogen and progesterone on the amount and distribution of cytoplasmic ribonucleic acid, alkaline phosphatase and mucin in the vaginal epithelium of the mouse.

Methods. Twelve young adult mice of the Swiss albino strain were bilaterally ovariectomized. A control group of 3 animals received no further treatment and was sacrificed 3 to 4 weeks after operation. At this time the remaining mice were divided into 3 equal groups. One group received 1.0 μg of estradiol banzoate; the second, 1.0 mg of progesterone, and the third received simultaneously 1.0 μg of estradiol benzoate and 1.0 mg of progesterone daily for 3 to 5 days. Crystalline hormones were used. The daily dose was dissolved in 0.05 to 0.1 cc of sesame oil and was injected subcutaneously. The animals were sacrificed on the day following the last injection and were autopsied immediately. The vaginas from both the control and experimental groups were fixed in chilled 80% ethyl alcohol for at least 24

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† The estradiol benzoate used in this work was supplied through the courtesy of Schering Corporation, the progesterone by the Warren-Teed Products Company.

1 Allen, E., and Doisy, E. A., *J.A.M.A.*, 1923, **81**, 819.

2 Jeener, R., *Nature*, 1947, **159**, 578.