is a constant feature of the disease. Furthermore, since porphyrinuria may indicate simply an hepatic insufficiency similar to that causing urobilinuria, the factor of liver dysfunction in pellagra warrants further study.

Summary. 1. Quantitative determinations of the excretion of porphyrin in a case of alcoholic pellagra are presented. 2. Both quantitatively and qualitatively abnormal excretion of porphyrin featured relapse and disappeared during the induced remission.

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Natural Meningococcal Agglutinins and Lysins in Human Serum.

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The study of the susceptibility-problem in cerebrospinal fever, a disease characterized by a highly selective incidence, has divided itself naturally into an analysis of the rôle played by the causative agent and the factors concerned with host-resistance. Investigations dealing with the organisms have centered chiefly around the carrier-condition as a source of infection and the significance of the serological types of meningococci; those dealing with the host have been mostly concerned with attempts to evaluate the humoral defences of the body against meningitic infection, it being unknown what causes the selective localization of the invading organisms in the meninges.

Together with phagocytosis, the bacteriolysin and agglutinin of human blood are regarded by some authors as the primary agents concerned with the removal of the meningococcus from the circulation following its initial invasion.[1, 2] It is generally accepted that not only the normal serum of man but also that of a number of animals (guinea pig, rabbit and monkey) are strongly meningococcidal *in vitro*.[3, 4] The normal bacteriolysin resembles other natural antibodies in that its titer is said to increase with age in man and animals, regardless of previously known exposure to the meningococcus; Silverthorne and Fraser,[5] however, stress the strong menin-

1 Murray, E. G. D., Medical Res. Council, spec. Rep't Series No. 124, 1929.

2 Heist, S., Solis-Cohen, S., Solis-Cohen, M., *J. Immunol.*, 1922, **7**, 1.

3 Flexner, S., *J. Exp. Med.*, 1907, **9**, 105.

4 Matsunami, T., and Kolmer, J., *J. Immunol.*, 1918, **3**, 177.

5 Silverthorne, N., and Fraser, D. T., *J. Immunol.*, 1935, **29**, 523.

gococcidal power of the blood of carriers as indicating its specific character. The same uncertainty prevails regarding the nature of the natural agglutinin which has variously been reported as being present or absent in the blood of normal persons, carriers, or convalescents.[6, 7, 8]

In view of the discrepancies cited above a study of normal human sera was undertaken in order to investigate not only the frequency of the occurrence of both lysin and agglutinin, but also to determine the relationship between the two antibodies and their specificity.

Methods. Three strains of meningococcus were used in this work: 2 of these represented stock strains of groups I-III and II-IV respectively, the third strain was freshly isolated from the nasopharynx of a healthy carrier and belonged to group II-IV. The human sera were used either unheated or heated to 60°C for ½ hour. The donors of these sera ranged in age from 14-72 years and gave no history of either a previous attack of meningitis or of unusual conditions of the upper respiratory tract. The agglutination-tests were set up with a variety of antigens, including suspensions of live cocci and of organisms killed by either formalin or phenol. The results were recorded after incubation at 37° for 2 hours and again after leaving the tubes in the icebox overnight. The lysin was determined by mixing 0.02 cc of undiluted serum plus 0.2 unit of guinea-pig alexin with 0.02 cc of dilutions of 1:1000, 1:10,000, and 1:100,000 of a freshly prepared heavy suspension of live cocci. After incubation for 24 hours, smears were made and examined microscopically for the presence of intact meningococci. This procedure gave results identical with the plating out of the serum-meningococcus mixtures and counting of developing colonies.

Results of the Agglutinative tests. 141 adult human sera and 26 sera from infants or children (ages 1 month to 9 years) were tested for agglutinins against the 3 strains of meningococcus representing the 2 broad serological groups. The results are given in Table I. It is apparent that about one-half of the adult sera agglutinated group I-III meningococcus whereas almost two-thirds of these sera contained agglutinins against group III-IV organisms. Further analysis of the individual reaction of each serum yielded the following data: 48 sera (34%) lacked agglutinins for both strains while 49 sera

[6] Stoevesandt, K., *Cent. f. Bakt. orig.*, 1908, **46**, 295.

[7] Gates, F. L., *J. Exp. Med.*, 1918, **28**, 449

[8] Worster-Drought, C., and Kennedy, A. C., *Cerebrospinal Fever*, London, A. and C. Black, Ltd., 1919.

TABLE I.
Incidence of Natural Meningococcal Agglutinins and Lysins in Normal Human Serum.

	Meningococcus I-III				Meningococcus II-IV					Meningococcus I-III				Meningococcus II-IV			
Agglutinin Titer*	Adult Sera		Infants' and Child. Sera		Adult Sera		Infants' and Child. Sera		Lysin Titer†	Adult Sera		Infants' and Child. Sera		Adult Sera		Infants' and Child. Sera	
1:160	32		0		36		0		Undiluted	0		0		0		0	
1:80	14		0		8		0		1:1,000	3	81(92%)	0	6(18%)	1	81(92%)	0	6(18%)
1:40	7	69(49%)	0	1(3.8%)	14	91(64.6%)	1	5(19.2%)	1:10,000	64		6		59		6	
1:20	5		0		10		1		1:100,000	14		0		21		0	
1:10	7		1		12		2		0	7	(8%)	28	(82%)	7	(8%)	28	(82%)
1:5	4		0		11		1			—		—		—		—	
0	72	(51%)	25	(96.2%)	50	(35.4%)	21	(80.8%)	Total	88		34		88		34	
Total	141		26		141		26										

*Indicates highest dilution of serum producing distinct clumping.
†Indicates lowest dilution of bacterial suspension lysed by serum.

(35%) agglutinated both strains; 11 sera (7.8%) agglutinated I-III strains only and 33 sera (23%) agglutinated II-IV strains only. It is also clear that organisms belonging to group II-IV were agglutinated, almost uniformly, to higher titers. In contrast to these results, only 3.8 and 19.2% of the sera from infants and children showed agglutinins of a low titer against groups I-III and II-IV meningococci, respectively. The agglutinin is evidently heat-stabile since heating for ½ hour at 60°C did not appreciably change the titer of the serum.

Results of the Bacteriolytic Tests. 88 normal adult sera and 34 sera from infants and children (ages ranging from 12 days to 4 years) were tested for lytic activity against the 2 serological groups of meningococci. As may be seen from Table I, fully 92% of the adult sera showed some bacteriolytic effect against either group of meningococci, no sera being lytic for one strain and not for the other. Moreover, the end-titers were approximately the same for both groups of organisms. On the other hand, 82% of the infants' and children's sera were devoid of meningococcidal power under the conditions of our test. The bacteriolytic complex requires "complementation" as shown by the absence of lysis when heated sera were brought together with cocci without the addition of alexin.

Relationship Between Agglutinin and Lysin. As regards the coexistence of the natural agglutinin and lysin in the same serum, our data indicate that 19 of 95 lytic sera—20% of all sera examined for the simultaneous presence of the 2 antibodies—failed to agglutinate either serological group of cocci. However, in no case was the reverse encountered, *i. e.*, absence of lysin in an agglutinating serum. Again, if one compares the agglutinative titer with the lytic strength against any one group of meningococci, no quantitative relationship becomes evident as indicated by the fact that a given serum may agglutinate only feebly but be markedly lytic, and *vice versa.*

Specificity of the Natural Agglutinin and Lysin. Experiments to determine the degree of immunological specificity of the 2 antibodies were carried out by absorbing pooled normal adult serum with homologous and heterologous organisms and testing the sera after absorption for agglutination and lysis of the various members of the genus *Neisseria.* The results indicated that the natural meningococcal agglutinin possesses well marked type- and species-specificity, only absorption with the homologous type meningococci causing complete removal of the respective agglutinins. The same experiment, however, demonstrated a complete lack of immunological specificity for the natural meningococcal lysin, as evidenced by

the fact that this substance may be removed not only by absorption with the heterologous type meningococci but by contact with *B. coli* as well. In a similar absorptive test in which specific rabbit-anti-meningococcal serum was substituted for normal human serum, both the immune agglutinin and lysin exhibited a high degree of immunological specificity, contact with *B. coli* causing no change in the agglutinative titer and only a minor diminution of the lytic activity.

Animal Sera. A small number of normal sera from various animals, including the monkey, rabbit, guinea pig and rat were examined for their content of natural meningococcus agglutinins and lysins. Suffice it to state that all sera tested showed marked meningococcidal power with no or negligible agglutinin titers.

Conclusions. Our data permit certain deductions regarding the nature and origin of the natural meningococcal agglutinin and lysin. It is clear that both antibodies increase in intensity and extensity with age and since conditions are generally favorable for the successive absorption from the nasopharynx of antigenic material belonging to the genus *Neisseria* one might be led to believe that both are due to "occult" immunization. However, as will appear below, it is more than likely that we are dealing with two separate entities.

The natural meningococcal agglutinin, according to our data, possesses a well marked immunological specificity. This is suggested not only by the occurrence of type-specific agglutinins in a certain percentage of normal human sera but more particularly by the fact that such agglutinins can be exhausted only by absorption with the specific antigens. In this respect the natural agglutinin resembles the immune agglutinin. Our observation that natural agglutinins occurred more often against group II-IV than against group I-III strains of meningococci is further suggestive evidence in favor of assuming their specific origin inasmuch as Branham[9] had found a preponderance of II-IV strains among cultures obtained from carriers whereas a majority of cultures isolated from cases of meningitis belonged to group I-III. In contrast to what has been said about the natural agglutinin, our data show that the power of normal human serum to lyse the meningococcus is invariably directed against both serological groups, and absorption-experiments revealed a complete lack of immunological specificity. The natural meningococcal lysin, therefore, appears to be quite different from the marked increase in meningococcidal power which is obtained following immunization with the specific organisms. This is also

[9] Branham, S., *J. Am. Med. Assn.*, 1937, **108**, 692.

borne out by the fact that the lytic property in normal human serum may exist entirely apart from its agglutinating capacity. The conclusion, therefore, seems justified that the normal meningococcidal power of human and animal blood is wholly unrelated to any previous immunizing experience with the specific antigen. While the possibility cannot be excluded that in those instances in which natural agglutinin and lysin occur together both antibodies reflect the result of common antigenic stimulation, it is more than likely that this relationship is merely fortuitous and that their coexistence should be interpreted as a chance occurrence of 2 different substances, one a specific antibody and the other a nonspecific factor of the blood.

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Citrate Solutions for Preservation of Fluid Blood.

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Solutions of sodium citrate have been used to prevent coagulation of blood for many years and these solutions have become increasingly important in preserving the fluid blood for transfer from one human being to another. The citrate solutions are also employed in various laboratory procedures, particularly in the preservation of leukocytes for study of phagocytosis. The present study was undertaken because of the suspicion that some untoward reactions observed following citrate transfusions might be related to the citrate.

We obtained hermetically sealed ampules of the citrate solutions of 4 different manufacturers. All of these were perfectly clear. A test of the hydrogen-ion concentration of these 4 specimens revealed the following results:

Specimen	pH
A	8.5
B	8.0
C	8.1
D	8.2

We also prepared some solutions in our own laboratory and found that one solution of sodium citrate, 2.5% in 0.6% sodium chloride, had a pH of 8.7, higher than that observed in any of the 4 commercial samples.