factor is not present (in adequate amounts) in rat, mouse, and chicken plasma(10). Our experience, cited above, shows that FPF precipitates rat and mouse plasma. Chicken plasma reacts with neither coagulase nor FPF; however, addition of normal rabbit serum renders chicken plasma clottable with coagulase, but does not impart FPF reactivity.

The significance of the fibrinogen precipitating factor in streptococcal infections and the non-suppurative sequalae of such infections is the subject of current investigations.

Summary. 1) An active factor was found in streptococci which precipitates various mammalian plasmas, and human and bovine fibrinogens. Fibrinogen precipitating factor (FPF) was found in crude acid extracts of several strains of group A, and one strain of group G streptococci; extracts of staphylococci, pneumococci, and viridans streptococci have proven non-reactive. 2) Streptococcal M protein and FPF appear inseparable, although presence of hyaluronic acid in a suspension of streptococcal cells prior to acid extraction may inhibit the fibrinogen precipitating reactivity of the extract, without significantly affecting the typing reaction. FPF and staphylocoagulase share certain properties, but they are not analogous.

1. Swift, H. F., Wilson, A. T., Lancefield, R. C., *J. Exp. Med.,* 1943, v78, 127.

2. Lancefield, R. C., *ibid.,* 1928, v47, 91.

3. ———, *ibid.,* 1943, v78, 465.

4. Lancefield, R. C., Perlmann, G., *ibid.,* 1952, v96, 71.

5. Pakula, R., *J. Gen. Microbiol.,* 1951, v5, 640.

6. Laki, K., *Blood Clotting and Allied Problems,* Josiah Macy Jr. Fn., N. Y., 1951, 223.

7. Hale, J. H., Smith, W., *Brit. J. Exp. Path.,* 1945, v26, 209.

8. Wiley, G. G., Wilson, A. T., *J. Exp. Med.,* 1956, v103, 15.

9. Duthie, E. S., Lorenz, L. L., *J. Gen. Microbiol.,* 1952, v6, 95.

10. Rammelkamp, C. H., Jr., Lebovitz, J. L., *Ann. N. Y. Acad. Sci.,* v65, 144.

Received July 9, 1959. P.S.E.B.M., 1959, v102.

Properdin Levels in Splenectomized Persons. (25173)

HAROLD N. CARLISLE AND SAMUEL SASLAW

Dept. of Medicine, College of Medicine, Ohio State University, Columbus

Our previous studies(1) showed that antibody response to tularemia vaccine in 92 of 105 splenectomized persons was similar to that observed in 47 normal controls. Failure of 13 persons to exhibit antibodies was attributed to the underlying disease for which splenectomy was performed rather than to absence of the spleen. This present study is concerned with determination of properdin levels in the same 105 splenectomized subjects.

Materials and methods. The 105 splenectomized persons included 35 males and 70 females, 2 to 77 years old and 2 months to 18 years post-splenectomy. Blood samples for properdin determinations were obtained prior to tularemia vaccination(1). Healthy non-splenectomized controls consisted of 100 males and 13 females, 18 to 40 years of age. All blood samples were centrifuged 5-7 minutes at 2000-2200 rpm in an International, Size 2 centrifuge and the serum frozen and stored at −22°C until tested. Properdin levels were determined by the Pillemer zymosan assay technic(2). Serum reagents RP and R3 were prepared from pools of normal human serum(3) and standardized by the Pillemer method(2). Serial 2-fold dilutions of test sera(4) were used. The amounts of zymosan* found optimal were 1 mg and 2.5 mg/ml of normal human serum in preparation of R3 and RP respectively, and 6 mg/ml of RP in the assay proper. Sensitivity and validity of the test were controlled by daily assays of 1 serum sample of known properdin content and 2 synthetic samples prepared by adding known

* Nutritional Biochemicals Corp., Cleveland, O.

amounts of properdin† to RP. The C'3 content of RP was always between 90 and 150 units/ml and degree of hemolysis in control tubes was always within permitted limits. Suitability of freshly prepared reagents was established by re-assaying 20 samples of known properdin content. The degree of surveillance required by this test is represented by the fact that our report contains results of 328 assays, whereas almost 200 additional assays were performed for control purposes only. The nature of our study necessitated certain departures from standard assay procedures. Blood specimens were not processed in accordance with the recommendation of Pillemer *et al.*(2), and serum samples assayed had been stored in frozen state for approximately 1 year. The method of reading the tests was modified as a consequence of experience with repeatability. The results of experiments designed to test the importance of these variables will be presented below.

Results. Method of reading. Pillemer *et al.*(2) defined the unit of properdin as the least amount of serum which, in presence of an optimal amount of zymosan, *completely* inactivates 120 ± 30 units of C'3 in 1 ml of RP during 1 hour at 37°C. According to this definition, the end-point tube must show no hemolysis after addition of R3 and sensitized sheep erythrocytes. This criterion for the end-point has been modified slightly(4,5) to permit interpolation when minimal hemolysis is noted. Our experience with repeatability of the assay (Table I), resulted in adoption of a method of reading in which % hemolysis figures were arbitrarily divided into 3 groups: 0-30%, 40-70%, and 80-100%. Table I, in which units of properdin are read directly in terms of reciprocal of serum dilution, further explains the method of reading. In samples which exhibited a zone (Patient Cr, Table I), the tube which showed 50% hemolysis or less was taken as the end-point. Only 7 samples (4 from normal and 3 from splenectomized) fell into this category.

Method of processing blood samples. Pillemer *et al.*(2) centrifuged blood specimens at 2°C for 30 minutes at 4000 rpm, and then recentrifuged the serum to remove residual red cells. Samples we used were centrifuged 5-7 minutes at 2000-2200 rpm at room temperature. The effect of this difference in technic was tested by assaying 24 blood samples divided and processed by the 2 methods. These samples were drawn from patients visiting the University Hospital Hematology Clinic and varied in properdin content from 0 to 16 units/ml. The method of centrifugation had no effect on properdin content, in that the differences noted were well within the limits of repeatability.

Effect of age of serum sample. All serum samples from splenectomized subjects had been stored at –22°C for 11-13 months before being assayed. Other workers(5,6) utilized sera stored 6-7 months, and reported no change in properdin titer during this time. To our knowledge, longer periods have not been

† Kindly supplied by Dr. B. E. Sanders of Merck Inst. of Therapeutic Research.

TABLE I. Repeatability of the Zymosan Assay for Properdin.

Sample	No. of assays	% hemolysis: Reciprocal of serum dilution U	2	4	8	16	Properdin units
Rb	6*	0	0	0	0	100	8
	1	0	0	0	0	90	8
Ru	4	0	0	0	0	100	8
	1	0	0	0	0	90	8
	1	0	0	0	50	100	6
He	3	0	0	0	100	"	4
	1	0	0	0	90	"	4
	1	0	0	0	80	"	4
	3	0	0	0	70	"	6
	1	0	0	0	60	"	6
	1	0	0	0	50	"	6
Co	1	0	0	0	75	"	4
	2	0	0	0	50	"	6
	1	0	0	0	40	"	6
	1	0	0	0	35	"	6
	4	0	0	0	25	"	8
	2	0	0	0	20	"	8
	2	0	0	0	15	"	8
Ro	1	0	60	100	100	"	1½
	1	0	30	"	"	"	2
	1	0	"	70	"	"	2
	1	0	20	60	"	"	2
	1	0	10	100	"	"	2
	1	0	0	40	"	"	3
Cr	1	80	50	90	"	"	2
	1	70	30	80	"	"	2
	1	60	0	25	90	"	2

* Indicates No. of times assay results were as indicated to the right.

TABLE II. Effect of Storage at −22°C on Properdin Content of Normal Human Serum Samples.

Subject	Length of storage (mo) 13–14	11–12	8–10	2–6
	Units of properdin			
Fo	2	4	4	4
Do	12	16	12	16
Va	8	4	6	4
Up	6	6	8	8
Wh-1	6	3	4	4
Ra	16	8	16	8
Fe	8	8	4	8
Oc	8	12	16	16
Mo	0		0	0
Wh-2	0		2	1
Sa	16		12	12
Ca	8		12	12
Co	8		6	4
Lu	16		16	12
Wi	0		1	0
Sp	0		0	0
Re	8		12	8
Mu	0		0	0
	Mean = 6.78			Mean = 6.50

Difference in means not significant (t = .47).

reported. The effect of age of serum sample on properdin content was controlled by us by assaying normal serum samples which had been stored for varying periods, and by including in the control group of 113 normal serum samples, 70 which had been stored for 11-13 months. The results of these studies, Tables II and III, indicate that serum samples can be stored for approximately one year with no significant change in properdin titer.

Properdin levels in splenectomized persons. Table IV presents results of properdin assays on serum specimens from 105 splenectomized and 113 normal human subjects. Mean titers for the 2 groups were 3.71 units and 6.35 units, respectively, and the difference between the 2 means was highly significant (t = 4.89). Thirty-seven (35.2%) of the samples from splenectomized individuals and only 8 (7.1%) from normals were without properdin. Data on the 105 splenectomized subjects analyzed by the multiple regression method(7) showed no correlation between properdin level and age, age at splenectomy, years post-splenectomy and underlying disease (as grouped in Table IV) for which splenectomy was performed. There was, however, a correlation between properdin level and sex in that titers were significantly lower in females. This difference, significant at the 5% level but not at the 1% level, was a characteristic of the entire group of 105 patients and, to a varying extent, of each of the 5 sub-groups in Table IV. The greatest difference was noted in the congenital hemolytic anemia group. Normal males and females exhibited comparable properdin titers(8).

There was no correlation between level of properdin and ability to produce antibodies after subcutaneous inoculation of tularemia vaccine(1). Peak antibody titers were not significantly different in 53 subjects with low properdin (2 units or less) and in 52 subjects with normal amounts. Rate of formation of antibody was similar in the 2 groups in that mean antibody titers were not significantly different 1 week after immunization. Lack of correlation between properdin level and antibody production was obvious in 6 subjects splenectomized because of traumatic rupture of the spleen. All of these patients produced antibody in normal amounts(1) whereas 5 of 6 showed no properdin.

The above comparison of properdin titer and antibody production in the 105 splenectomized subjects does not take into consideration the slight but significant sex difference in properdin level. This difference takes on added importance in light of the fact that 11

TABLE III. Properdin Levels in "Fresh" and Stored Normal Human Serum Samples.

Storage (mo)	No. of samples	Properdin units 0	1	2	3	4	6	8	12	16
0- 2.5	41	1	2	4	3	5	4	15	5	2
11-13	72	7	3	5	3	10	8	28	5	3
			Low <3			Normal 3-12				High 16
0- 2.5	41		7-17%			32-78%				2-5%
11-13	72		15-21%			54-75%				3-4%

Mean properdin titer, "Fresh"—6.71. Mean properdin titer, Stored—6.14.
Difference in means not significant (t = .75).

TABLE IV. Properdin Levels in Splenectomized and Normal Persons.

Diagnosis	No. of subjects	Properdin units 0	1	2	3	4	6	8	12	16	Mean titer
Congenital hemolytic anemia	23	9	1	3	1	1	2	5	1		3.39
Idiopathic thrombocytopenic purpura	37	9	1	7	3	8	2	5	1	1	3.68
Acquired hemolytic anemia	13	3	1	2	2			4		1	4.54
Secondary thrombocytopenic purpura	10	3	1			3		3			3.70
Primary splenic neutropenia	6	2				1		2	1		
Secondary " "	2					1			1		
Primary splenic panhematopenia	2							1		1	
Secondary " "	3	3									3.64
" hemolytic anemia	2	2									
Aplastic anemia	1	1									
Traumatic rupture	6	5						1			
Total splenectomized	105	37	4	12	6	14	4	21	4	3	3.71
" normals	113	8	5	9	6	15	12	43	10	5	6.35

		Low <3 units	Normal 3-12 units	High 16 units
Total splenectomized	105	53-50%	49-47%	3-3%
" normals	113	22-19%	86-76%	5-4%

of 70 females and only 2 of 35 males did not produce antibodies after tularemia vaccination(1). These observations are not necessarily indicative of any immunologic weakness in splenectomized females but further studies are suggested.

Discussion. Our results demonstrate that properdin levels in splenectomized persons are lower than in normal persons. Although a minor sex difference was noted in the group of splenectomized patients studied, there was no correlation between properdin level and age of the individual, age at splenectomy, years post-splenectomy, or the underlying disease for which splenectomy was performed, leaving absence of the spleen the most probable common characteristic. Admittedly, the heterogeneous nature of some of the sub-groups in Table IV detracts somewhat from this contention. However, the fact that 5 of 6 subjects splenectomized because of traumatic rupture of the spleen showed no properdin is of significance.

The sex difference in properdin level in the splenectomized group was minimal in comparison to the difference between properdin levels in the splenectomized and control groups. Properdin titers were lower in females, however, and this observation is of interest in that 11 of 70 females and only 2 of 35 males did not produce antibodies after tularemia vaccination(1). In general, however, there was no correlation between properdin level and ability to produce antibodies. All but 13 of 105 splenectomized subjects produced antibodies(1), whereas about half (53 of 105) showed little or no properdin. An attractive but purely speculative interpretation of these facts would be that the spleen is one of the principal sites of properdin formation and, after splenectomy, other organs take over this function of the spleen to a lesser and more variable degree than they do the antibody-forming function of the spleen. The data in the present report do not warrant such an interpretation, however, since they do not prove conclusively that the low properdin levels were due to absence of the spleen rather than to the underlying disease process. Our present knowledge of the properdin system and the *in vivo* activities which seem to affect properdin levels as determined by the zymosan assay make it reasonable to believe that subclinical conditions or altered physiologic states could be responsible for the observed low properdin values. However, if this were the case, these factors would have to be operative to the same degree in all of the disease sub-groups in Table IV, since the means of the different sub-groups were not significantly different. Further studies are being carried out in this laboratory in an attempt to clarify this point.

Summary. Properdin titers were definitely lower in 105 splenectomized persons than in 113 normal, healthy subjects. Within the

splenectomized group, there was no correlation between properdin level and age of individual, age at splenectomy, and years post-splenectomy. Properdin titers were significantly lower in splenectomized females, but the sex difference was minimal in comparison to the difference between total splenectomized and control groups. Suggestive evidence is presented that low properdin levels in splenectomized persons are related in part to absence of spleen.

The authors are indebted to Joann Sparks for technical assistance and to Dr. D. R. Whitney, Director of Statistics Research Lab., for the more complex statistical analyses.

1. Saslaw, S., Bouroncle, B. A., Wall, R. L., Doan, C. A., *N. Eng. J. Med.*, 1959, v261, 120.

2. Pillemer, L., Blum, L., Lepow, I. H., Wurz, L., Todd, E. W., *J. Exp. Med.*, 1956, v103, 1.

3. Finklestein, R. A., Allen, R., Sulkin, S. E., *J. Inf. Dis.*, 1959, v104, 184.

4. Kent, J. F., Toussaint, A. J., Hook, W. A., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 676.

5. Rottino, A., Levy, L. L., *Cancer*, 1957, v10, 877.

6. Southam, C. M., Pillemer, L., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 596.

7. Snedecor, G. W., *Statistical Methods*, Iowa State College Press, Ames, 1956.

8. Hinz, C. F., *Ann. N. Y. Acad. Sci.*, 1956, v66, 268.

Received July 16, 1959. P.S.E.B.M., 1959, v102.

Trypsin, Invertase and Amylase Content of Feces of Germfree Rats.* (25174)

B. BORGSTRÖM, A. DAHLQVIST, B. E. GUSTAFSSON, G. LUNDH AND J. MALMQUIST
Depts. of Histology and Physiological Chemistry, University of Lund, Sweden

As measured with specific methods the trypsin and chymotrypsin concentration of intestinal content decreases with length of the intestine, decreasing to zero in feces(1). This fact indicates that intestinal enzymes are autodigested or are inactivated by intestinal microorganisms. To elucidate this problem the enzyme content in feces of germfree and conventional animals was measured.

Material and methods. Germfree rats reared according to Gustafsson(2-3) were used in these studies. Two germfree male adult rats of the 4th generation were fed the semisynthetic diet D_6(4) and kept individually in metabolism cages. Feces and urine were collected each 24 hours. Two conventional rats were fed the same autoclaved diet and kept in metabolism cages in the animal room. The feces were collected for 8 consecutive days. On the 8th day the germfree rats were infected with cecal content of a conventional animal heated to 90°C for 5 min. Thereafter the feces were collected for another 12 days during which time sporeforming organisms were cultivated from the feces. Then one of the animals was killed for assessment of the effect of the sporeformers on the cecum enlargement. The remaining germfree animal was on the same day infected in the apparatus with a suspension of feces from the conventional rats. The feces were homogenized with 10 ml ice cold saline and centrifuged. Aliquots of the clear supernatant solution were used for enzyme determinations. Trypsin was determined spectrofotometrically using benzoyl-arginine ethyl ester as substrate(5). Invertase activity was assayed according to a modification of the method of Sumner(6), and expressed as mg of invert sugar produced in one hour by 1 ml of the sample at 25°C. Amylase activity was determined by the method of Meyer *et al.*(7) and expressed as mg of maltose liberated in 3 minutes by 1 ml of the sample at 25°C.

Results. The feces of the conventional animals did not contain measurable amounts of trypsin or invertase but held 40-100 units of amylase (Fig. 1). On the other hand, feces of the germfree rats contained 1-6 mg trypsin daily and 12-25 units of invertase per day.

* This investigation supported by grants from Swedish Medical Research Council, Wallenberg Fn., Stockholm, Sweden, and Nat. Inst. Health, Bethesda, Md.