The question whether differences in the ABC values of normal and pathological sera are caused by changes in the albumin molecules cannot be answered at the present time. The studies of Huggins and associates(6) are of interest in this respect. These authors, using equilibrium dialysis, found a decreased binding of phenolsulfonephthalein with the serum protein in 44.7% of the sera of 123 cancer patients (compare results listed in Groups 7 and 8, Table I, of the present paper). They correlated this change with a deficiency in the thermal coagulability of the albumin. Both of these abnormalities could be eliminated, however, on purification of the serum albumins by cold ethanol fractionation. The albumin fractions thus obtained from serum of cancer patients or normal serum, did not show significant differences in elementary composition, or in the content of thiol and amino groups.

In our own studies(13), the sera of 16 patients suffering from various diseases were used, showing normal as well as abnormal "observed" and "specific" ABC values (50.4 to 145.0 mg %; 3.2 to 8.2 moles x 10^{-5}). The albumin fractions were isolated, as in the studies of Huggins and co-workers, by the method of Cohn and associates(14). No proportionality was found between the specific ABC values of these albumin fractions and of the corresponding sera. No differences in the composition (arginine, histidine, tyrosine, tryptophane, and amino nitrogen) of the isolated albumin fractions could be found between sera giving normal and sera giving low ABC values. The specific ABC values for these separated albumins were found to be lower than in the original sera (2.6 to 5.6 moles x 10^{-5}). This decrease probably is due to a removal of salts by dialysis; diminishing the salt concentration has previously been shown to lead to a lowering of the ABC values in the test employed(7).

Summary. The relative capacity of normal and pathological sera to bind the acid dye, azorubin, was determined by means of a chromatographic method, using anionotropic aluminum oxide as absorbent. Subnormal azorubin-binding capacities (ABC) of serum albumins were demonstrated, in varying degrees, in nephrosis, diffuse damage of liver parenchyma, and severe toxic states of infectious diseases. In some cases of malignant neoplasms abnormally low ABC values were also observed.

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Effect of C-Reactive Protein on Normal Human Leukocytes.* (18650)

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Tillett and Francis described the occurrence of a reaction between the sera of patients acutely ill with pneumococcal pneumonia and the somatic C polysaccharide of the pneumococcus(1). This reaction was obtainable only with acute phase serum, and was generally not demonstrable after the onset of convalescence. In later studies by Avery and Abernethy, and Avery and MacLeod, the reactive substance was shown by chemical and immunological methods to be a specific abnormal serum protein which reacted selectively to form a pre-

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cipitate with the C polysaccharide of the pneumococcus(2-4). The reaction with the polysaccharide was shown to occur only in the presence of traces of ionized calcium and to be reversed by citrate, which rendered the calcium ions unavailable to the protein-carbohydrate complex. As a result of studies carried out over the past twenty years it has become evident that the C-reactive protein is present in the serum of patients ill with a wide variety of infectious and non-infectious diseases(5,6). The concentration of this protein in the serum as determined by a precipitin reaction with specific rabbit antiserum, or alternatively by reaction with the C polysaccharide, is being used as a measure of the activity of the disease process in rheumatic fever(7). Despite the knowledge of the wide variety of illnesses which are characterized by the appearance of the C-reactive protein in the serum, there have been as yet no indications of its site or mechanism of origin or of its biological significance. The present paper describes the effect of human C-reactive protein on normal human leukocytes. Quantitative studies using the slide cell technic of Martin, Pierce, Middlebrook and Dubos are described(8).

Materials and methods. 1. Acute phase sera from rheumatic fever patients were used as specimens containing high titers of C-reactive protein. Convalescent serum from one of these patients was used as a control. 2. Two preparations of human C-reactive protein, each isolated and purified in a different way, were used. In Preparation A, the isolation and purification of the protein from ascitic fluid were carried out with the use of pneumococcal C carbohydrate by a method essentially the same as that described by McCarty(9), except that crystallization was not carried out. The final solution of purified Preparation A had a concentration of C-protein of approximately 4%. In Preparation B, use was made of the property of calcium precipitability of C-protein as it occurs in blood serum. The procedure used in isolating and purifying this preparation is described in detail by MacLeod and Avery(3). This second preparation which had a concentration of approximately 0.2% C-protein was used in order to eliminate the possible effect on normal human leukocytes of the C carbohydrate and the citrate needed to keep the C-protein-C carbohydrate complex in solution. The concentration of C-protein contained in this and in the various other preparations used was determined by a quantitative spectrophotometric method(10).

3. The blood used for the determinations described in this study was that of one donor. The reproducibility of the observations reported was verified on blood from 4 additional donors. Immediately after being drawn, 6 cc of blood was put in a chilled tube containing 0.04 cc of a 200 mg % solution of heparin in physiological saline. The concentration of heparin used was at least 5 times less than the concentration toxic for human leukocytes. All work with the blood up to the time of incubation was done in a cold room at 4°C.

4. Slide cells, as described by Martin, Pierce, Middlebrook and Dubos, were used in all determinations. These cells consist of a cover slip sealed to a microscopic slide by a U-shaped piece of filter paper impregnated with paraffin. This structure produces a small, wide, flat chamber, which is filled with blood by capillary action and which after centrifugation permits clear visualization of the buffy coat between a red cell and a plasma layer. Accurate microscopic observations can be made of the migration of leukocytes from the buffy coat into the plasma layer after incubation. All determinations described in this paper were carried out on a mixture of 10

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TABLE I. Effect of C-protein in Acute Phase Human Serum on Migration of Normal Human Leukocytes.

Rheumatic fever patient	Greatest distance of leukocytes from buffy coat: 1:2 dilution of untreated serum, mm	Greatest distance of leukocytes from buffy coat: Serum treated with C carbohydrate, mm
B	.50	.25
S	.65	.25

TABLE II. Effect of 3 Concentrations of Preparation A on Normal Human Leukocytes.

Final conc. of Preparation A, mg/cc	Leukocyte reaction
.04	Destruction of leukocytes
.015	Early migration (<1 hr) followed by destruction of cells
.004	Leukocytes migrated .50 mm
Citrate-saline control	Leukocytes migrated .25 mm

parts of blood to one part of test solution prepared immediately before centrifugation and incubation. The greatest distance of migration of leukocytes from the buffy coat was determined by both macroscopic and microscopic examination of each slide cell after incubation at 37°C for one hour.

Results. 1. *Effect of acute phase sera containing C-protein.* The effect of acute phase sera containing C-protein on the activity of normal human leukocytes was tested in the following way. To 0.4 cc of serum containing approximately 0.23 mg/cc of C-protein from 2 patients with acute rheumatic fever, S.B. and L.S., was added 0.4 cc of 0.1 mg/cc C carbohydrate. The C carbohydrate removed all but a trace of the C-reactive protein. The effect of these treated sera was compared in slide cells with that of the 1:2 dilutions of their corresponding untreated sera as controls. As will be seen in Table I, the sera containing C-protein stimulated the leukocytes to migrate twice as far as did the same sera after the C-protein had been removed. A comparable difference in degree of migration was observed when an acute phase serum containing 0.35 mg/cc of C-protein was compared with a convalescent serum containing no C-protein from the same patient. These 2 sera were tested in slide cells at the same time. There was twice as much leukocyte migration in the preparations containing acute phase serum as in the control preparations in which convalescent serum was used.

2. *Effect of C-protein isolated by use of the C carbohydrate.* Preparation A was set up in slide cells in final concentrations ranging from 0.04 to 0.004 mg/cc. The microscopic results obtained with this protein preparation are shown in Table II, where it is seen that the preparation containing approximately 0.004 mg/cc stimulated the leukocytes to 2 times the migration observed in the control cells. The higher concentrations of C-protein tested destroyed the leukocytes. To determine whether or not the C carbohydrate present in the protein preparation contributed to the increased leukocyte migration, slide cells were set up with 0.1 and 0.2 mg/cc C carbohydrate. Slide cells containing normal blood alone served as controls. No evidence of increased leukocyte migration was seen in the preparations containing carbohydrate alone.

3. *Effect of C-protein isolated and purified by calcium precipitation.* To demonstrate further that C carbohydrate, citrate, or calcium present in the first preparation tested were not causing the increased migration of the leukocytes, a serologically active solution containing approximately 0.2 mg/cc of purified C-protein in saline (Preparation B), with a trace of normal human serum protein as the only known contaminating substance or substances, was used. Solutions of Preparation B ranging from 0.2 to 0.04 mg/cc C-protein were set up in slide cells concurrently with cells filled with untreated blood. After incubation for one hour, the results were as seen in Table III. Again the higher concentrations caused destruction of cells, while at lower concentrations an increase in migration was observed. In order to determine whether the trace of normal human serum protein present in the preparation caused the increase in leukocyte migration, the C-reactive protein was removed by the addition of 3 parts of 0.2 mg/cc of C carbohydrate with a trace of calcium to one part of the undiluted pro-

TABLE III. Effect of C-protein Preparation B on Normal Human Leukocytes.

Final conc. of Preparation B, mg/cc	Greatest distance of leukocytes from buffy coat, mm
.02	.6 (with destruction of cells)
.009	.7
.004	.9
Normal blood control	.4

tein preparation. In comparison with control slides filled with normal blood, no difference in degree of migration was caused by the addition of this preparation which still contained the trace of normal human serum protein originally present.

4. *Protective action of serum.* Although both acute phase serum containing C-protein and the purified isolated protein caused increased migration of the leukocytes in the test system, it was apparent that a far lower concentration of the purified preparation than of serum C-protein was required to obtain the effect, and that the purified protein was more toxic to normal leukocytes than was acute phase serum. Whereas as much as 0.1 cc of an 0.3 mg/cc solution of C-protein in serum stimulated the leukocytes in the test system without destroying them, 0.1 cc of an 0.054 mg/cc solution of C-protein isolated from serum destroyed them. In order to clarify this apparent contradiction, the following experiments were done. A sample of serum containing approximately 0.3 mg/cc of C-protein was employed. The C-protein was removed from an 0.5 cc portion by the addition of 0.5 cc of 0.1 mg/cc C carbohydrate. The protein-carbohydrate precipitate was washed in 0.01% calcium chloride. The untreated serum, the isolated C-reactive protein, and the supernatant from the carbohydrate-treated serum were tested for their effect on leukocytes. It was found that the C-protein isolated from the serum caused destruction of leukocytes, while the same amount of C-protein contained in serum enhanced migration. The supernate from the carbohydrate-treated serum had no visible effect on the leukocytes. The apparent protective action of serum against the effect of C-protein on leukocytes seen in this experiment was further tested by incubating normal human serum with Preparation B to give a C-protein concentration of approximately 0.23 mg/cc of serum. The serum C-protein mixture was then tested in comparison with an equivalent amount of C-protein in saline. The protective effect of serum was also seen in the results of this experiment, where the C-protein in serum enhanced leukocyte migration, while the same concentration of the protein in citrate and saline destroyed them.

5. *Effect of differences in the rates of migration of leukocytes from different normal donors.* In the course of the studies described in this paper, confirmation was obtained that the rate of leukocyte migration of the blood varies with each normal donor(11). In the one hour period of observation employed, the greatest distance of migration of normal unstimulated leukocytes varied between 0.1 mm for the cells of one donor to 1.0 mm for those of another. Moreover, the rate of migration exhibited by normal leukocytes has been shown by other workers to be both characteristic and constant for any individual over a period of years. The work described in this paper was done on cells which consistently showed good motility in the lower range of normal leukocyte migration. Determinations, not described in detail here, were done also on blood from donors whose leukocytes normally showed maximal migration. It was found that the cells from such individuals were stimulated to increased migratory activity by a lower final concentration (0.002 mg/cc) of purified C-reactive protein than were those of the standard blood described above. It was found also that a final concentration of purified C-protein which was stimulatory to the standard test system blood (approximately 0.004 mg/cc) destroyed the leukocytes with a normally high rate of migration. In view of these facts, it is evident that the toxic and the stimulating concentrations of purified C-protein must be determined for the leukocytes of each different normal blood to be used in the test system.

Conclusions and summary. On the basis of

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studies using the slide cell technic to determine the effect of human C-reactive protein on normal human leukocytes, the following conclusions are indicated. 1. Purified human C-reactive protein in final concentrations higher than 0.005 mg/cc destroys normal human leukocytes. 2. Purified human C-reactive protein in lower concentrations stimulates the migration of normal human leukocytes. 3. C carbohydrate, sodium citrate, and normal human serum protein do not stimulate normal human leukocytes in the concentrations employed in this study. 4. The acute phase human sera tested caused increased migration of normal human leukocytes by virtue of their content of C-reactive protein. 5. Human serum diminishes at least in part the toxic effect of the C-reactive protein on normal human leukocytes.

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Acute Toxicity of Veratrum Derivatives.* (18651)

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Interest in veratrum preparations has varied considerably over a period of years. Fruitful clinical application of the blood pressure lowering powers of veratrum derivatives depends on the use of materials of constant hypotensive power in the form of pure alkaloids or of mixtures standardized by their ability to lower mammalian blood pressure. The interrelationship between lethality of these substances and their hypotensive property has attracted interest as a possible method of assay(1). The results detailed below should do much to eliminate from further consideration lethality as a method of assay. While some data of the acute toxicity of veratrum derivatives have been published (1-3), strictly comparable toxicity data for the various substances have not been found. This study of the acute toxicity of veratrum preparations has been divided into (a) comparison of the acute toxicity of pure ester alkaloids or of alkaloidal mixtures from veratrum with their hypotensive potency(4). (b) Elucidation of influence of species of animal and of route of administration on the acute toxicity of a clinical veratrum preparation (Veriloid†).

Method. Albino Swiss mice (15-25 g) and albino Wistar rats (175-245 g) were weighed to the nearest 0.1 g. The solutions to be injected were prepared so as to hydrate the mouse at a rate of 0.01 ml and the rat at 0.005 ml per g of body weight. The injected animals were observed for 24 hours (actually all deaths occurred within the first hour). The New Zealand albino rabbits (2-3 kg), and the adult mongrel dogs (3-12 kg) were hydrated at the rate of 0.5 ml per kg and observed for 24 hours. The solutions of the pure alkaloids‡ were prepared by dissolving the alkaloids in a minimum amount of 5% acetic acid and diluting to the desired concen-

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† *Veriloid* (Trademark of Riker Laboratories) is a biologically standardized alkaloidal extract of *Veratrum viride* of uniform hypotensive potency.

‡ The substances utilized were kindly provided by the following sources: Eli Lilly and Company, Ind., Protoveratrine; Merck and Company, Rahway, N. J., Veratrine; S. B. Penick and Company, Detroit, Mich., Veratrine; Dr. S. J. Ringel, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Beltsville, Md., Cevadine; Riker Laboratories, Los Angeles, Calif., Protoveratrine, Veriloid and Veratridine; Squibb Institute for Medical Research, New Brunswick, N. J., Germitrine, Neogermitrine, and Germidine.