TABLE II. Percentage of Deaths and Average Survival Time (Hours ± S.D.) of Normal Crayfish Injected with Autoclaved-dialyzed Control Solutions or Autoclaved-dialyzed Toxic Substance.

	Control		Toxin	
	Autoclaved-dialyzed (22)	Untreated (16)	Autoclaved-dialyzed (21)	Untreated (15)
% deaths	35.0	12.5	69.0	100.0
Survival time in hr (± S.D.)	39.2 ± 23.8	38.7 ± 12.4	36.7 ± 20.3	24.4 ± 13.1

Discussion. These experiments show that a toxic factor (or factors) was present in the blood of the scalded crayfish. Normal crayfish injected with blood of normal animals lived longer than those injected with blood of scalded crayfish.

Summary. Crayfish (*Orconectes virilus* and *Procambarus clarkii*) were scalded at 76°C for one to 3 minutes. A toxin was found in the blood of scalded crayfish, which, upon injection in normal crayfish, killed 91.1% of the recipients. The toxin taken from a scalded crayfish is a heat stable, nondialyzable substance.

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Amino Acid Analyses of Rheumatoid Factors and Normal Gammaglobulins.* (27562)

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Recently available methods for preparing highly purified rheumatoid factors(1,2) facilitated separation of rheumatoid factors of different serological behavior in individual sera. These factors were found essentially to be of two types. One reacted with indicator particles coated with human as well as rabbit gammaglobulin, and the other reacted with indicator particles coated with human but not with rabbit gammaglobulin. In the ultracentrifuge both types were mainly 19.5 S, although moieties sedimenting at slightly lower as well as somewhat higher sedimentation constants were also encountered.

To obtain further evidence for differences in rheumatoid factors, we compared the amino acid composition of rheumatoid factor preparations of both serological types and again compared it to that of normal 7 S gammaglobulin as well as highly purified 19 S gammaglobulin. The amino acid compositions of normal 19 S gammaglobulin and rheumatoid factors have not been reported previously.

Materials and methods. Preparation of 19.5 S rheumatoid factors. Three samples of serum, approximately 80 to 100 ml each, obtained from patients with rheumatoid arthritis, and stored in a frozen state for varying periods of time, were used in the preparation of rheumatoid factors A, B, and C. The sera were absorbed with sheep erythrocyte stroma previously exposed to homologous rabbit antiserum. The rheumatoid factors so absorbed onto the immune complex were eluted from it by suspension in pH 5.5 phosphate buffer (0.15 M). The eluate was further purified by density gradient ultracentri-

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fugation in a sucrose-saline medium. Details of the procedure have been reported(1).

Preparation of the 17.5 S rheumatoid factor. Rheumatoid factor C′, sedimenting at 17.5 S, and rheumatoid factor C, sedimenting at 19.5 S, occurred in the same serum. Rheumatoid factor C′ differed from rheumatoid factor C in that it failed to react with indicator particles coated with rabbit gamma globulin. To separate this macroglobulin from the other rheumatoid factor, the serum was exhaustively absorbed with a complex prepared from sheep erythrocyte stroma and its homologous rabbit antiserum. The absorbed serum was diluted with 10 volumes of water, and adjusted to pH 6.1. After 2 hours in the refrigerator, the precipitate was collected and redissolved in pH 7 phosphate buffer (0.03 M) and then purified by chromatography on DEAE cellulose. The chromatographic column (20 × 1.5 cm) was developed by stepwise elution with phosphate buffers of decreasing pH and increasing ionic strength. Material which eluted at pH 5.5, ionic strength 0.45, was pooled and concentrated by ultrafiltration. The concentrate was 17.5 S macroglobulin predominantly, but contained approximately 10% 7 S material which was removed by density gradient ultracentrifugation. Details of these procedures have been reported(3).

Preparation of normal 19.5 S gammaglobulin. Fresh sera of 2 ostensibly healthy donors, totalling 250 ml, were pooled, and precipitated with 91 g $(NH_4)_2SO_4$. The precipitate was redissolved and dialyzed against 0.005 M tris (hydroxymethyl) aminomethane and 0.05 M phosphate buffer at pH 8.6, and purified by chromatography on DEAE cellulose as described by Peterson *et al.*(4). The fractions containing macroglobulin were further purified by starch block electrophoresis in 0.05 M veronal buffer at pH 8.6. The gammaglobulin fractions were pooled, concentrated and subjected to density gradient ultracentrifugation (Sample D).

Normal 7 S gammaglobulin. Human gammaglobulin, containing apropximately 95% 7 S material, was a product of Pentex, Inc. It was not further purified (Sample E).

Sedimentation analyses. A Spinco ultracentrifuge, Model E, was used. Analyses were performed at 20°C, and corrections were made for viscosity. The sedimentation coefficients reported herein were obtained from graphs of observed sedimentation constants plotted against protein concentration.

Amino acid analyses. The samples were hydrolyzed with 6 N hydrochloric acid for 20 hours at 110°C in Pyrex tubes sealed under nitrogen. After hydrolysis the contents of the tubes were dried in a flash evaporator. The residue was taken up with 2 ml of water and the pH adjusted to 2.2 by addition of 1 N NaOH. The solutions were quantitatively transferred to 10 ml volumetric flasks and diluted to volume with 0.2 N citrate buffer, pH 2.2. Aliquots of 4.5 ml were used for amino acid analyses by the recording method of Spackman, Stein and Moore(5). In computing the results, losses of serine and threonine during hydrolysis were considered by applying zero time correction factors calculated from the data of Smith *et al.*(6) for the change in composition of hydrolysates of rabbit gamma globulin. A 7.3% loss was assumed for serine, and a 4.2% loss for threonine. In computing cystine contents, the first chromatographic peak eluted was assumed to be cysteic acid and its value was added to that of cystine and mesocystine. Methionine sulfoxides appeared in the chromatograms just before aspartic acid, and their values were added to those of methionine. Only one determination was performed on each sample. Previous analyses of human gammaglobulin had indicated that a 20-hour hydrolysis was sufficient for nearly complete recovery of the amino acids. As only small quantities of purified materials were available, nitrogen determinations had to be omitted. The data are here presented in grams per cent amino acid residues analyzed. Hexosamines, constituting approximately 2.5% of the protein after hydrolysis, were also not included in the data. A more detailed analysis of the samples was precluded by lack of material.

Results. Tracings of the sedimentation patterns of the 5 macroglobulins appear in Fig. 1. Rheumatoid factors A, B and C, reacting with indicator particles coated with

TABLE I. Amino Acid Composition of Samples.*

Samples	Rheumatoid factors A	B	C	C′	Normal D	Normal E
$S^0_{20,w}$	19.5	19.5	19.7	17.5	19.5 S	7 S
Residues						
Lysine	6.27	6.83	6.09	6.88	6.24	7.4
Histidine	2.63	2.07	2.38	2.21	2.31	1.9
Arginine	5.93	5.43	6.57	5.92	6.07	4.3
Aspartic acid	8.95	9.43	8.98	9.08	9.14	8.6
Threonine	8.65	8.73	8.62	8.41	8.82	6.3
Serine	8.63	10.33	9.45	8.77	9.40	10.4
Glutamic acid	12.39	13.15	12.81	13.16	12.16	11.7
Proline	6.26	5.85	5.93	5.89	6.40	6.8
Glycine	4.03	4.49	4.54	3.78	3.99	3.9
Alanine	4.58	5.26	4.81	4.89	4.35	3.8
½ Cystine	2.34	3.65†	.43†	2.58†	1.87	2.2
Valine	7.01	6.85	7.77	7.62	7.65	8.5
Methionine	1.42	1.28	.46	.59‡	1.46	1.1
Isoleucine	3.44	3.36	3.44	3.74	2.73	2.3
Leucine	8.16	8.20	8.16	8.56	8.05	8.3
Tyrosine	4.65	.79	4.28	1.99	4.42	6.3
Phenylalanine	4.64	4.49	5.24	5.96	4.85	4.8
Total	100.00	99.95	99.96	100.00	100.02	100.1
Amide ammonia§	7.03	5.66	7.24	3.76	2.75	—

* Results reported in gram per cent amino acid residues analyzed. Tryptophan was not determined.

† Cystine was not detected, but a peak appearing in chromatogram at column volume was considered to be cysteic acid. Result for ½ cystine is based on this value.

‡ Methionine was not detected. Peaks in chromatogram just preceding aspartic acid were considered to be methionine sulfoxide and reported as methionine .

§ % of total nitrogen. Results not included in totals.

human and/or rabbit gamma globulin, were 19.5 S. Rheumatoid factor A contained a small quantity of material sedimenting above 19.5 S. Rheumatoid factor B appeared to be homogeneous, and rheumatoid factor C contained approximately 15% of a substance sedimenting in the ultracentrifuge at 27 S. Rheumatoid factor C′, reacting with indicator particles coated with human gamma globulin but not rabbit gamma globulin, was 17.5 S and showed some skewing owing to small quantities of higher sedimenting material.

Control experiments performed with serum samples from healthy subjects yielded fractions devoid of nitrogenous substances. Therefore, rheumatoid factors described herein were essentially free of other macroglobular impurities and contained no low molecular weight contaminants. The normal macrogammaglobulin, lacking the serological characteristics of rheumatoid factor, was also 19.5 S and appeared to be homogeneous on ultracentrifugation.

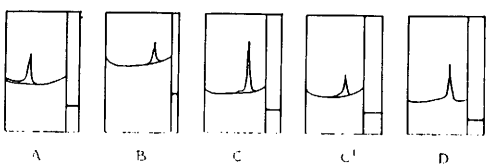

FIG. 1. Ultracentrifuge patterns of the macroglobulins analyzed for amino acid content.

The results of the amino acid analyses appear in Table I. It is apparent that there are differences in the amino acid composition of rheumatoid factors A, B and C. The most pronounced of these was the tyrosine content. Lesser differences were found in the content of lysine, histidine, arginine, serine, glycine and phenylalanine.

When rheumatoid factor C was compared to rheumatoid factor C′, the most pronounced difference was the tyrosine content, and lesser ones were found in the content of lysine, arginine, serine, glycine and phenylalanine.

Comparison of the normal 19.5 S macroglobulin with the individual rheumatoid factors showed good agreement with rheumatoid factor A. Only histidine, serine, valine and isoleucine seemed to be slightly different.

Less striking was the similarity between the normal 19.5 S macroglobulin and the other 3 rheumatoid factors.

We also compared the amino acid content of highly purified 19.5 S gammaglobulin and fraction II gammaglobulin, containing 95% 7 S. The amino acid content of these proteins was dissimilar, indicating that the high molecular weight antibodies have a primary structure different from that of the 7 S antibodies.

The results for cysteine and methionine were disregarded in the above comparisons, as these amino acids are not present in sufficient quantity to estimate accurately their original content, and are furthermore subject to considerable hydrolytic destruction.

Discussion. Owing to the difficulty of obtaining adequate samples for extensive studies, we did not study the physico-chemical properties of the rheumatoid factors and the normal 19.5 S macroglobulin other than by ultracentrifugation. However, these macroglobulins appeared to be free of significant quantities of low molecular weight material, and the rheumatoid factors were free of other contaminating macroglobulins.

The differences in amino acid composition of rheumatoid factors A, B and C suggest that these proteins are not identical. Non-identity may be due to group or even individual specificity. Group specificity for rheumatoid factors has been established through serological technics by several investigators(7,8). Such group specificity appears to derive from the allotypic nature of gammaglobulin, referred to as the Gm system. In the framework of this system, the rheumatoid factors are considered to be anti-Gm substances. Unfortunately, we were unable to assign Gm types to the sera used in this study. It is, however, also possible that individual specificity, over and above group specificity, may also play a role in the varying amino acid composition of the rheumatoid factors.

Of particular interest is the large difference in tyrosine content found in rheumatoid factors C and C′ coming from the same individual. This difference may also be explained in terms of Gm specificity. Individual sera are known to be complex in their Gm make-up, and Gm subgroups are acknowledged to co-exist(7). Rheumatoid factors C and C′ might therefore represent different types of anti-Gm substances. The observed difference in behavior of the 2 rheumatoid factors toward serological reagents might in itself constitute sufficient reason for the varying amino acid content.

Comparison of the amino acid content of the rheumatoid factors with that of 7 S and 19 S gammaglobulin is also of interest. While there are certain common features in amino acid content, consistent discrepancies are also apparent. When compared to 7 S gamma globulin, the rheumatoid factors have a significantly higher content of arginine, alanine and isoleucine, but a lower content of valine and tyrosine.

Differences between the rheumatoid factors and normal 19.5 S gammaglobulin are less pronounced.

Particularly interesting is the discrepancy in amino acid content of 7 S and 19.5 S gammaglobulin. These differences support evidence previously obtained by other technics that, although macroglobulins on reduction depolymerize to monomers sedimenting at 7 S, the similarity between the products obtained on reduction and the 7 S gammaglobulins is confined to ultracentrifugation only(9).

Summary. Four rheumatoid factors obtained from the sera of 3 patients with rheumatoid arthritis, a sample of 19.5 S gammaglobulin and a sample of 7 S gammaglobulin from healthy donors were analyzed for their qualitative and quantitative amino acid content.

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Effect of Westphal Lipid A on Viral Activities in Mice and Hamsters. (27563)

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Endotoxins of diverse microbial origin have long been recognized to exhibit a striking array of pathophysiologic effects(1-6) in animals, including enhanced resistance to infection with a variety of microbial agents. Chemically, the endotoxins are protein-lipid-polysaccharide complexes of high molecular weight. It is generally agreed that biologic activity of the endotoxin resides in the lipopolysaccharide part of the endotoxic complex. There is currently disagreement, however, as to whether biologic activity is resident in the lipid or in the polysaccharide component. Westphal(5), by acid hydrolysis, obtained a fraction from endotoxin called "Lipid A" which he believes is the active principle of the molecule even though it represents but a small fraction of initial total activity. By contrast, Ribi *et al.*(7) have obtained a fraction of endotoxin of undiminished activity which is mainly carbohydrate and which contains only a small residuum of lipid.

Endotoxins present in whole bacteria, in bacterial filtrates, or in bacterial extracts have been shown (8-15) to possess antiviral activities which include suppression of neurotoxicity of concentrated influenza virus (not due to viral proliferation), and of inhibition of pathogenicity or proliferation of ectromelia, encephalomyocarditis group or eastern equine encephalomyocarditis viruses. There have been no reports, however, of the activity of lipid A against viruses. Findings in the present study show that lipid A derived from *E. coli*, like whole endotoxin, induces resistance in animal hosts against viral toxicity and affords a slight protection against clinical consequences of certain viral infections.

Materials and methods. Lipid A and endotoxin of Escherichia coli were prepared* according to the general procedures of Westphal(5,16,17). The materials were dispensed in an aqueous solution of 1% Emulphor.† Endotoxin and lipid A fractions used in the studies were active in promoting host resistance in mice against *Klebsiella pneumoniae*. The Emulphor was inert in all tests. The *cholera filtrate* was prepared according to procedures described earlier(18) and was found to be active in destroying receptors of red blood cells and non-specific inhibitors in mammalian sera. *Viruses.* All viruses employed were from the inventory of documented strains maintained in this laboratory. Influenza A virus strain NWS (neurotropic) was propagated in embryonated hen's eggs. Pneumonia virus of mice (PVM) was propagated in lungs of weanling mice. Encephalomyocarditis strain Columbia SK, herpes simplex and poliovirus II (Lansing, mouse adapted), viruses were propagated in mice inoculated intracerebrally. The hamster adapted Philadelphia 26 strain of measles was grown in suckling hamsters inoculated intracerebrally, Coxsackie B3 was propagated in HeLa cell tissue cultures, and influenza A strain PR8 was prepared in lungs of mice.

Influenza neurotoxin. Influenza A strain WS was propagated in the allantoic sac of 10-day-old embryonated hen's eggs. The virus was concentrated 10-fold by high speed

* Kindly supplied by Dr. K. Folkers, Merck Sharp & Dohme Research Laboratories.

† Obtained from Antara Chemical Co., New York.