broth. The mixture was additive for assay on K_1 and on 145 cells. The results were the same with phage mixed in dilute or in concentrated suspension. 4) Supernates from P_{14} (K_1) and P_{14}(145) were crossed after high speed centrifugation. Phage resuspended in these supernates was equal in titer to controls which had been centrifuged and resuspended in their own supernate. The above tests indicated that there was no interaction of phage lots passaged on different hosts. 5) Heat inactivation of P_{14}. Lots of $P_{14}(K_1)$ and P_{14}(145) were heated to 60°C. Dual titrations of samples removed at intervals revealed that activity for the K_1 cells decreased more rapidly than that for the 145 strain. Finally samples were obtained which had no phage active on K_1 but which exhibited residual activity for 145 cells. When fresh phage was diluted with these heated samples and exposed to 59°C, its activity decreased at rates similar to a control containing fresh phage diluted in broth, indicating that neither had anything heat stable been released nor had an inactivator been destroyed during heat treatment.

Examination of the last survivors of heat inactivation by plaque isolation from growth on 145 cells showed that all 47 isolated produced plaques both on K_1 and 145 cells. This occurred regardless of previous host passage. The ratio 145/K_1 obtained by plating aliquots of plaque suspensions again approached 40.

Summary. Analysis of phage activity by titration on two hosts has revealed the presence of variants hitherto undetected in stock phage P_1. One isolate, Phage 14, exhibits great changes in titration ratio on passage through the two hosts K_1 and WF 145. Produced on K_1 cells, the ratio of free phage assayed on two hosts is 1.3, whereas made on 145 cells, the free phage titrates in a ratio 145/K_1 of ca 40. This occurs regardless of the host employed in previous passage and is reproduced in the very first burst from infected cells. Attempts to isolate different strains from this phage by usual plaque isolation technics were not successful. The high ratio obtained with free phage made on 145 cells could not be explained on the basis of differences in adsorption onto or latent periods in the two hosts. The difference was traced to the production of a large number of particles from host 145 which adsorbed on K_1 but formed no plaques. No evidence was found for an inhibitor of K_1 activity associated with phage 14 production on 145 cells. Heat inactivation destroyed phage activity for K_1 cells much more rapidly than for 145 cells. There was no interaction of heat killed and active phage on exposure to 59°C.

Evidence has been accumulated which indicates that the phage P_{14} is altered on passage through host 145. The fact that phage particles surviving a heat treatment which destroyed all activity for K_1 cells produce on strain 145 a mixture of two phages (one active on K_1 and one—or both—active on strain 145) points to an unusual host effect on virus reproduction.

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Soluble Specific Leptospiral Complement-Fixing Antigens. (19576)

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Leptospiral complement-fixing antigens have been prepared from suspensions of the organisms by various methods in several laboratories (1-4). Carlinfanti(5) reported a "lipoidal" antigen extracted from washed, dried leptospirae. These antigens are characterized by relatively broad spectra of reactivity with antibodies against heterologous leptospiral strains.

This paper reports the demonstration of

soluble, highly strain specific antigens formed by leptospirae propagated on artificial mediums.

Materials and methods. Type strains of leptospirae, antigenically distinguishable by means of cross agglutination-lysis after Schüffner and Mochtar(6) were propagated in Stuart's(7) liquid medium. Cultures were incubated at 30°C for periods of 10 to 14 days. An aqueous solution of merthiolate, to a final concentration of 1:10,000, was added to the whole cultures to kill the leptospirae and to act as a preservative.

Leptospirae were removed from the culture liquor by centrifugation at 20000 g for 10 minutes at 5°C in a PR-1 International refrigerated centrifuge. The supernatant culture liquor was then filtered through a Seitz E-K pad. The organisms collected by centrifugation were resuspended in phosphate buffered saline solution at pH 7.2 to one-tenth the original culture volume. The organism suspension was exposed to vibration at 9400 cycles/second in a Raytheon Magnetostricton vibrator for 10 minutes, to prepare antigens of the sonic-vibrated type(4). In preliminary experiments, aliquots of the culture medium prior to inoculation, and of whole cultures prior to removal of leptospirae were employed as control antigens.

Serological demonstration of antigens was made by the quantitative complement fixation technic of Kolmer(8). Hyperimmune serums against each leptospiral strain studied were prepared in rabbits. An immunization schedule of 4 intravenous injections of living leptospirae at weekly intervals was employed.

TABLE I. Demonstration of Leptospiral Soluble Antigen by Quantitative Complement Fixation.

Antigen	Dilution of antiserum, 1: 10	20	40	80	160	320	640
Uninoculated culture medium	—	—	—	—	—	—	—
Whole culture	4+	4+	4+	4+	4+	3+	—
Leptospirae-free culture liquor	4+	4+	4+	4+	4+	3+	—
Sonic-vibrated* leptospiral suspension	4+	4+	1+	—	—	—	—

* Diluted to equivalent of original culture volume.

The initial dose was 0.5 ml of culture, followed by 1.0 ml, 2.0 ml, and 4.0 ml doses. Rabbits were bled on the fifth day after the last injection. Serums were separated from the clotted blood, filtered through Seitz E-K pads and stored at –20°C until used.

Results. Comparative quantitative complement-fixing antigen titrations of whole leptospiral culture, leptospirae-free culture liquor, sonic-vibrated leptospiral suspension, and uninoculated culture medium are shown in Table I. This titration clearly demonstrates the presence of a soluble complement-fixing antigen in the organism-free culture liquor. Similar antigens were likewise demonstrated in the leptospirae-free culture liquor of all other leptospiral strains studied.

Quantitative complement fixation studies with the soluble antigen on hyperimmune serums prepared against homologous and heterologous leptospiral strains, indicate that this antigen-antibody reaction is highly strain specific when critical antigen dilutions are employed. Table II illustrates the effect of

TABLE II. Effect of Antigen Concentration upon Strain Specificity.

Hyperimmune serum against	*L. canicola* soluble antigen, Diluted 1:2, Serum dilution, 1: 4	8	32	128	512	Diluted 1:4, Serum dilution, 1: 4	8	32	128	512
L. pomona	+	+	—	—	—	—	—	—	—	—
djasiman	+	+	—	—	—	—	—	—	—	—
sentot	+	+	—	—	—	—	—	—	—	—
autumnalis	+	+	—	—	—	—	—	—	—	—
ballico	+	+	—	—	—	—	—	—	—	—
ballum	+	+	—	—	—	—	—	—	—	—
canicola	+	+	+	+	+	+	+	+	+	+
icterohemorrhagiae	+	+	—	—	—	—	—	—	—	—
grippotyphosa	+	—	—	—	—	—	—	—	—	—
medanensis	+	+	—	—	—	—	—	—	—	—

TABLE III.
Quantitative Cross Complement Fixation.

Antiserum	Soluble Antigen											
	1	2	3	4	5	6	7	8	9	10	11	12
1.	[T]	T/4	T/16	T/64	T/128	T/128						
2.		[T]	T/32	T/16	T/16	T/128	T/16					
3.			[T]	T/64		T/64						
4.		T/4	T/4	[T]	T/4	T/128						
5.		T/8	T/32	T/16	[T]							
6.		T/8	T/16	T/64	T/128	[T]	T/16					
7.		T/8				T/64	[T]					
8.		[T]	T/16		T/16			[T]	T/32			
9.		T/8		T/128	T/128		T/32		[T]			
10.							T/16			[T]		
11.									T/32	T/32	[T]	
12.											T/64	[T]

T= Titer obtained with homologous antiserum

Leptospiral strains:

1. *L. pomona*
2. *L. autumnalis*, Akiyami A
3. *L. icterohemorrhagiae*, Wijnberg
4. *L. djasiman*
5. *L. sentot*
6. *L. canicola*
7. *L. ballum*
8. *L. autumnalis*, Fort Bragg
9. *L. grippotyphosa*, Moscau V
10. *L. pyrogenes*, alexi
11. *L. andaman*
12. *L. semerang*

antigen dilution upon strain specificity.

The strain specificity of these antigens was further evidenced in cross complement fixation studies. Results of these examinations are shown in Table III.

Concentration, purification, and characterization of these antigens is currently in progress. Preliminary results obtained in this laboratory(9) indicate that the antigens are alcohol soluble materials which withstand heating at 100°C for 30 minutes with no appreciable loss of serological reactivity. The materials fail to illustrate biuret or ninhydrin reactions, but react positively in the Molisch test.

Discussion. Previously prepared leptospiral complement-fixing antigens have been prepared from the organisms or from extracts of washed organisms. One may reasonably infer from their method of preparation, and from their broad antigenic spectra, that they are somatic, and neither surface nor soluble antigens. The substances herein described are clearly soluble antigens exhibiting a high degree of strain specificity.

The use of complement-fixing antigens in serological studies on the leptospiroses has, in the past, been limited by the method of preparation of the antigens. The slow growth rate and low maximum population density of leptospirae in culture, as well as the expensive mediums required for their propagation, makes preparation from the organisms laborious and costly. In addition, the limited availability of equipment such as sonic vibrators and freeze-drying apparatus has limited the number of laboratories capable of preparing such antigens. In marked contrast, is the ease and low cost of preparation of the soluble antigens. Yager and his associates(10) have reported studies demonstrating the many advantages of the use of sonic-vibrated complement-fixing antigens as diagnostic agents for acute leptospirosis *per se*. However, as Collier and Mochtar(11) and Gsell(12) point out, identification of the infecting strain may often be desirable. It appears, that in these instances, the specific soluble complement-fixing antigens might advantageously supplement the broader screening antigens.

Summary. A soluble, specific complement-fixing antigen, or antigen complex, was demonstrated in the organism-free culture liquor of each leptospiral strain studied. The antigenic material is heat stable and nonprotein in nature. The antigens are present in amounts which make feasible their employment in serological investigations of the leptospiroses.

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Presence of a Leukotaxine-like Substance in Extract of Severely Injured Muscles. (19577)

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Recently(1) the writer expressed the view that leukotaxine, the substance primarily responsible for the increased capillary permeability and the migration of leukocytes in inflammation, is formed whenever cells are severely injured; consequently one cannot really speak any longer of "normal tissue" as is done by Moon and Tershakovec(2,3) when one macerates tissues and prepares an extract from them. One would expect to recover either leukotaxine or a substance closely resembling leukotaxine in such extracts of severely injured cells. In 1937, (unpublished data) the writer has obtained such a leukotaxine-like substance from beef heart extract. The reason for not continuing and publishing these studies was neither to go on a tangent nor to reason by analogy, but only to center on his main theme which was inflammation and its natural product, namely the exudate. As stated in his discussion(1) leukotaxine and its polypeptide nature, has been frequently confirmed.

In the present communication, observations are reported on the presence of a leukotaxine-like substance in the saline extracts obtained from muscles of rabbits as a result of grinding such tissues in a mortar. Such injured cells yield essentially the same factors as obtained from inflammatory exudates(4,5).

Method. Rabbits were killed by injecting air intravenously. The muscles of the thigh were immediately removed and weighed. The tissue was cut up in small fragments and treated with physiological saline. The amount of saline utilized was approximately equal to a volume in cubic centimeters of twice the weight of the muscle tissue as measured in grams. Some of this whole saline extract was tested on the skin of normal rabbits by the intracutaneous injection of 0.5 cc of the material on the abdominal surface. Five to 6 cc of 1% trypan blue in saline was then injected intravenously. Readings on the accumulation of the dye in the treated area were recorded periodically for about 1 to 2 hours. The rest of the saline preparation was extracted for the presence of a leukotaxine-like substance by only slightly modifying the method described previously for the extraction of leukotaxine from inflammatory exudates (6). The scheme of extraction utilized may be stated as follows: the saline extract of the thigh muscles was treated with dioxan (1:1) and stirred for about two hours. The mixture was then centrifuged and the supernatant phase treated with an equal volume of acetone. A precipitate resulted. This was centrifuged off and the supernatant evaporated to dryness *in vacuo* at $\pm 50°C$. To the dried material an amount of butyl alcohol equal to ½ the volume of the acetone supernatant-treated fraction was added, and the mixture was stirred for about one hour. This was then concentrated *in vacuo* at $\pm 55°C$ to about 1/10th volume. At that point the butyl alcohol concentrate was divided into two parts. One part contained a granular-like precipitate

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