monochloroacetic acid, and within 2 hours almost completely abolished. The effect of di- and trichloroacetic acids, while clearly defined, is much weaker and more delayed. It is important to note that here, as at all other concentrations studied, the order of decreasing effect on respiration is: monochloroacetic acid > dichloroacetic acid > trichloroacetic acid. Acetic acid in the range of 0.01M to 0.001M showed no effect.

Within the range of 0.001M to 0.03M, the depression of respiration by mono- and dichloroacetic acids is maximal; there is no indication of a progressively increasing effect with increasing concentration. Within the range of 0.001M to 0.00005M there is a gradually decreasing effect, which ceases below 0.00005M for both acids. This is a concentration of the order of 5 to 10 μg per milliliter of Ringer solution. At a concentration below 0.005M no effect of trichloroacetic acid has been noted, and at 0.01M evidences of tissue coagulation have been seen.

Summary. At the concentrations studied monochloroacetic acid produces a marked depression in the respiration of mouse liver slices. The order of decreasing effect of the 3 acids is: monochloroacetic > dichloroacetic > trichloroacetic. Acetic acid has no effect.

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Electrophoretic Analysis of Kala-azar Human Serum. Hypergammaglobulinemia Associated with Seronegative Reactions for Syphilis.*

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It is generally recognized that nonspecific positive reactions in tests for syphilis may occur with human sera from normal individuals[1,2] and from individuals affected with certain diseases[3-5] and conditions not associated with syphilis. The belief has arisen among serologists that nonspecific positive reactions are associated with hyperglobulinemia, and the presence of a high serum protein or globulin content has been suggested[6] as a criterion of the nonspecificity of a positive reaction.

The disease, kala-azar, is characterized in part by hyperglobulinemia[7] associated with the presence in high concentration of abnormal euglobulin, which gives rise to the formol-gel[8] reaction and a cold-precipitable pathological globulin.[9] In this disease, also, there has been reported[10] the occurrence of nonspecific positive reaction in tests for syphilis. Recently, in connection with a comparative study

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TABLE I.
Percentage Distribution, Concentration and Mobilities of the Components of Normal and Kala-azar Human Sera.

	Sample	Serum	Electrophoresis Solution	Components: Albumin A	Globulins: α_1	α_2	β	γ
%	477			21.7	3.0	3.4	7.8	64.1
Distribution	499			50.3	2.3	11.6	7.4	28.4
	Normal*			60.0	3.6	9.1	12.5	14.8
Protein	477	12.1	2.88	2.62	0.36	0.41	0.95	7.76
Concentration	499	8.65	2.58	4.35	0.20	1.00	0.64	2.46
gm per 100 ml	Normal*	7.23	—	4.34	0.26	0.66	0.90	1.07
Mobility	477			5.88	5.00	3.94	2.79	0.58
$-\mu \times 10^5$	499			5.98	5.04	3.91	3.08	0.62
$cm^2 sec^{-1} volt^{-1}$	Normal*			6.08	5.04	3.93	2.97	1.27

* Avg. values of 13 normal human sera.[11]

of syphilitic and nonspecific positive sera,[11] an opportunity was afforded for the examination of a serum from each of 2 patients with kala-azar. Electrophoretic analyses were made of the distribution, concentration and mobilities of the serum protein components, and the sera were thoroughly studied with a battery of serodiagnostic tests for syphilis. The results of these electrophoretic and serological studies are reported in the present paper.

Materials and Methods. The sera† were obtained from 2 patients in whom the diagnosis of kala-azar was established by the recovery of Leishman-Donovan bodies by puncture of the spleen and sternal marrow. One, Serum 477, was from a 23-year-old colored male, C.W.Y., who became ill February 28, 1944. The serum proteins were consistently elevated to levels reaching 12 g per 100 ml. The blood for electrophoretic analysis was drawn September 8, 1944, while the patient was still clinically ill. The second patient, Serum 499, was a 24-year-old white male, A.W.F., who had malaria in the summer of 1943 and typhoid in January, 1944. The onset of kala-azar occurred in April, 1944. The total serum protein and globulin contents were 6.7 and 3.35; 8.9 and 4.3; and 7.75 and 2.75 gm per 100 ml in July, August and October, respectively. The serum for the present studies was drawn on November 17, 1944, about 30 days after abatement of the disease.

Electrophoresis was carried out as previously described[11,12] at 1° in barbital buffer[13] at pH 8.6 and 0.1 ionic strength. The period of migration of Serum 477 was 234 minutes at 5.95 volts per cm and of Serum 499, 180 minutes at 6.42 volts per cm. The patterns representing the migrating boundaries were recorded by the method of crossed slits described by Svensson.[14]

The serum was prepared for electrophoresis by dilution with the barbital buffer to approximately 2% protein content and dialysis against frequently changed buffer at 2 to 8°. Protein concentrations were determined by the falling-drop procedure[15] standardized by Kjeldahl analyses.

Component analyses were made from measurements in photographic enlargements of the electrophoretic diagrams in which the components were separated by vertical lines

[11] Cooper, G. R., Craig, H. W., and Beard, J. W., in press.

† The sera and histories of the patients were provided through the generosity of Capt. James Liebmann, M. C., A.U.S., Walter Reed General Hospital, Washington, D.C.

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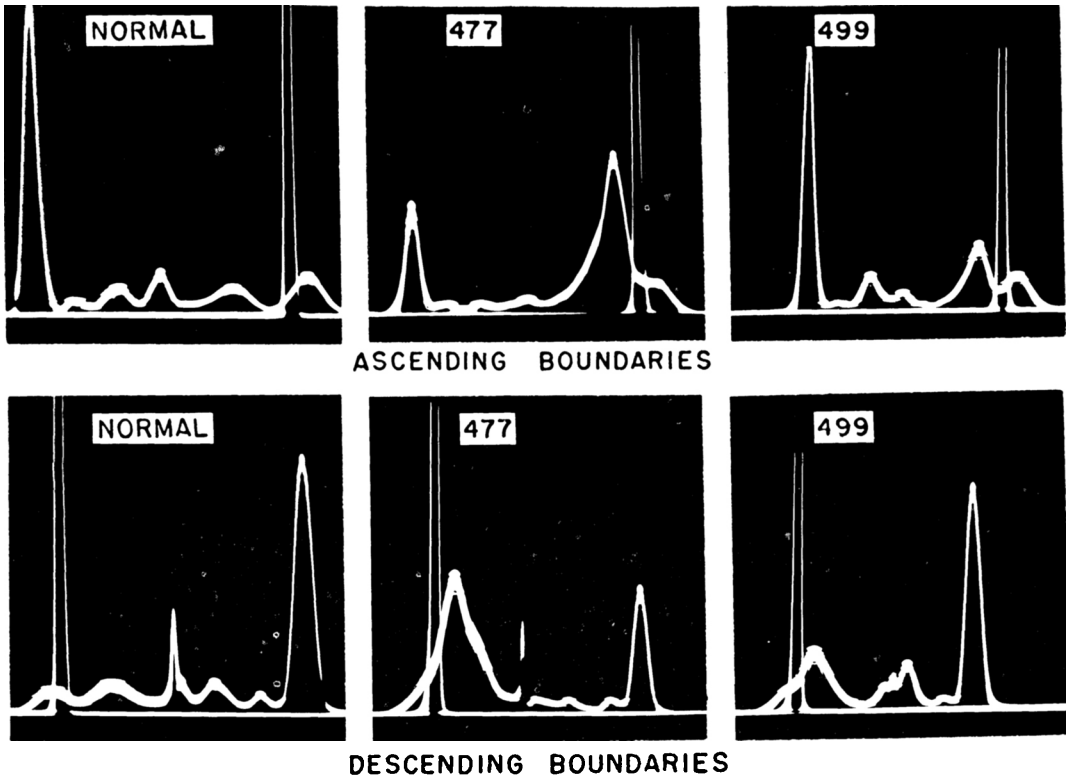

Fig. 1.

Electrophoretic patterns of human serum from a normal individual and from 2 patients diseased with kala-azar.

to the base at the minima between the peaks.[16] The respective areas were bounded by the mid-line of the curve above and the mid-line of the base below. The mobilities of the various components were calculated from the distances measured from the starting point of migration to the maximum ordinates of the respective peaks.[13] Calculations were made for both ascending and descending sides and the values given in Table I are the average of the 2 sets of data.

The sera of both patients were subjected to serologic examinations with a battery of 6 serodiagnostic tests, including the Kline diagnostic, Kline exclusion, Boerner-Jones-Lukens, Mazzini, Kahn and Kolmer procedures. The tests were made on other sera drawn in August, September and October of 1944 in addition to the 2 sera on which the electrophoretic studies were made. Further, the blood specimen, Serum 477, drawn from patient C.W.F. on September 8, 1944, was also subjected to chemical fractionation by Dr. Hans Neurath, Duke University School of Medicine, and each fraction, the albumin and the GI, GII and GIII globulins, was tested with the 5 flocculation procedures enumerated above. Formol-gel tests[8] were made on both of the sera.

Results. Electrophoretic patterns of the ascending and descending boundaries of the kala-azar sera and of a normal[11] serum for comparison are reproduced in Fig. 1. The pattern of Serum 477 reveals a γ globulin peak much greater in height and area than the albumin peak. In addition, there is pronounced skewing of the γ globulin peak to the slow side and diffuseness of the curve on the fast side. The percentage distribution and actual concentration of the components of Serum 477 are given in Table I. It is seen that the γ globulin fraction was

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about 7 times the normal value,[11] while the albumin was less than one-half the normal. The other electrophoretic fractions showed insignificant deviation from the concentrations observed in normal serum.

The mobilities of the proteins of Serum 477 (Table I) were the same as those of the components of normal serum,[11] except in the instance of the γ globulin fraction. The mobility of this fraction was outside the limits of the mobility usually associated with normal human γ globulin.[11] The diffuseness in the curve in the early part of the γ peak indicates the presence of the normal γ component.

Similar abnormalities but of less degree were observed in the proteins of Serum 499. The γ globulin peak was sharply heightened and skewed to the slow side of the γ globulin region. There was little suggestion of disturbance of the curve corresponding to the region of the normally behaving γ globulin. In percentage composition (Table I) the γ globulin fraction was double the normal, and the albumin and β globulin fractions were slightly less than normal. The actual concentrations of the components (Table I) showed that no significant changes from the normal had occurred except in the γ globulin fraction. The β anomaly peak[13] was absent. Mobilities of the components were within the normal range except that of the γ globulin fraction, which was found to be the same as in Serum 477.

The serodiagnostic tests on all of the serum samples investigated were uniformly negative and so, too, were the tests of the albumin and GI, GII and GIII globulins obtained by chemical fractionation. The sera, likewise, were negative to the formol-gel test.

Discussion. The abnormalities of the γ globulin component of kala-azar sera differ greatly from those of the serum of patients with other diseases involving the liver and spleen.[17,18] In syphilis,[11] rheumatic fever,[19] sarcoidosis,[20,21] tuberculosis,[22] as well as other pathological states,[23-25] the rise in γ globulin is associated with the entire normal γ globulin region. The findings indicate the presence in human kala-azar serum of a γ globulin component not previously observed in human serum. A γ globulin[26] component of low mobility has been observed, however, in the serum of rabbits hyperimmunized with Type 1 pneumococci.

The possible relation between the abnormal γ globulin and the globulins giving the formol-gel reaction[8] or precipitating in the cold[9] is subject only to surmise. Since the formol-gel test was negative, the abnormal γ globulin could not have been representative of the whole of the material responsible for this reaction. The cold-precipitable euglobulin, which may persist after recovery, may have contributed to the γ globulin. The electrophoretic abnormalities, pronounced during the course of the disease and still present in the convalescent serum, may prove on further investigation to be unique with kala-azar and of greater diagnostic significance than the formol-gel test or cold precipitation.

The nonspecific positive reactions in tests for syphilis seen in a variety of nonsyphilitic diseases and conditions may be due: to the presence of antibody-like substances similar to the antibodies produced in syphilitic disease; to an increase or alteration of some chemical substance or substances in the blood; or to an increase or alteration of the seroglobulin fraction. Cardon and Atlas[6] investigated a series of 34 patients with hyperproteinemia, of which 8 gave nonspecific positive reactions for syphilis. These au-

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thors believe that blood protein studies should be instituted when a nonspecific positive reaction is suspected and that care should be taken in the interpretation of a positive serologic reaction for syphilis in the presence of conditions with hyperproteinemia and hyperglobulinemia. Proven syphilitics may also show[11] a hypergammaglobulinemia and patients with kala-azar, as shown in the present work, and sarcoidosis[27] may have hyperglobulinemia associated with negative serologic reactions for syphilis. The mere presence or absence of hyperproteinemia, hyperglobulinemia or hypergammaglobulinemia is not sufficient to prove or disprove the specificity of positive serologic reactions for syphilis.

Summary. The electrophoretic patterns of 2 human kala-azar sera revealed the presence in high concentration of a unique, abnormal component migrating with the γ globulin of the slowest mobility. The kala-azar sera, negative to serological tests for syphilis, provide instances which show that the presence of hyperproteinemia, hyperglobulinemia or hypergammaglobulinemia cannot be used to prove or disprove the specificity of positive serologic reactions for syphilis.

[27] Cooper, G. R., Rein, C. R., and Beard, J. W., unpublished observations.

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Inactivation of Glutamic-Aspartic Transaminase by Irradiation.*

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Glutamic-aspartic transaminase is an enzyme which catalyzes the reaction: 1 (+) glutamic acid + oxaloacetic acid $\rightleftarrows$ α-ketoglutaric acid + 1 (—) aspartic acid. The prosthetic group of this enzyme is a derivative of vitamin B_6,[1,2] probably identical with codecarboxylase.[3,4] Since pyridoxine, pyridoxal and pyridoxamine are very sensitive to light,[5,6,7] it was expected that transaminase preparations would also be inactivated by irradiation.

It was found that sunlight destroys the enzymatic activity at about the same rate as it inactivates the vitamin B_6 present in the preparations (Table I). The destruction by ultraviolet light is much more rapid (Table II). The effect of X-rays on transaminase was also investigated. Vitamin B_6 has been considered as a remedy for X-ray sickness.[8,9] It was found that X-ray irradiation in therapeutic and higher doses has no effect on transaminase activity. Therefore, the possible therapeutic value of vitamin B_6 in this condition cannot be explained in terms of a restoration of inactivated transaminase.

Experimental Procedures. For the determination of transaminase activity the procedures developed by Cohen[10] were used. The enzyme was purified according to the

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