TABLE III.
Effect of Streptomycin upon Growth of Different Actinomycetes.

Organism	Units of streptomycin required to inhibit growth in 1 ml of medium	
	2 days	3 days
*A. albus**	0.4-1.25	0.4-1.25
A. asteroides	—	12.5
A. gypsoides	—	4.0-12.5
A. antibioticus	< 0.4	< 0.4
A. lavendulae	1.25	1.25
A. griseus	>12.5	>12.5
Actinomyces 3462	4-12.5	4-12.5

* Said to be causative agent of ear infection.

whereas *A. antibioticus*, the organism that produces the powerful actinomycin, was most sensitive to it.

Summary. *Mycobacterium tuberculosis* is subject to the bacteriostatic action of a variety of antibiotic substances. There is considerable variation in this respect, both in the sensitivity of the same organism to different substances and of different species or even strains of the same species of *Mycobacterium* to the same substance. Streptomycin is also highly effective against various related organisms, namely, *Erysipelothrix* and actinomycetes, comprising both saprophytic and parasitic strains, with considerable variation among different species.

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Identification of the Serum Fraction Carrying Syphilitic Reagin by Electrophoresis.*

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In earlier reports[1,2] it was shown that the serum proteins in all stages of syphilis show characteristic and significant alterations from the normal values. An increase in all of the globulin components was found with a decrease in the concentration of the albumin component which maintained the total protein concentration in the normal range. Since no correlation could be established between the concentration of any globulin component or components and the strength of the quantitative serological reactions, the fraction containing the reagin responsible for the positive serological reactions could not be determined. The present investigations were undertaken to establish the identity of the syphilitic reagin.

Soon after the Wassermann reaction was introduced and other flocculation and complement fixation tests appeared, a number of studies were made on syphilitic sera in an effort to determine in which fraction or fractions the reagin occurred.[3-7 and others] In general all workers were in agreement on the fact that the reagin was present in the globulin fraction and was not present in the albumin fraction. Further work on fractionation of the globulins, however, brought forth disagreement among the investigators on the globulin fraction carrying the reagin. The earlier fractionation technics involved separating the globulin into pseudoglobulin and euglobulin and the lack of exact characteriza-

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1 Cooper, J. A., and Atlas, D. H., in *Physical Biochemistry* by H. B. Bull, John Wiley and Sons, New York, 1943.

2 Cooper, J. A., *J. Invest. Derm.*, in press.

3 Mackie, T. J., *J. Hygiene*, 1923, **21**, 386.

4 Mackie, T. J., *J. Hygiene*, 1926, **25**, 176.

5 Gilmour, W., *Recent Methods in the Diagnosis and Treatment of Syphilis*, 2nd Ed., London, 1924, p. 346.

6 Noguchi, J., *J. Exp. Med.*, 1909, **11**, 84.

7 Eagle, H., *Laboratory Diagnosis of Syphilis*, C. V. Mosby Co., St. Louis, 1937, Chap. XVI.

TABLE I.
Effect of Inactivation and Flocculation by the Kahn Antigen on Syphilitic Sera.

Patient	pH	A / G	α / A	β / A	γ / A	Remarks
G.B.	7.81	1.26	0.11	0.25	0.43	Untreated
	7.80	1.28	0.11	0.25	0.41	Inactivated
	7.82	1.51	0.11	0.27	0.29	" and flocculated
J.M.	8.60	0.80	0.15	0.56	0.55	Untreated
	8.60	0.76	0.16	0.58	0.58	Inactivated
	8.60	0.90	0.14	0.52	0.48	" and flocculated

tion of the fractions led to divergent results.

In the work to be reported here, salt fractionation was employed, but the electrophoretic components present in the fractions thus obtained were determined and a correlation established between serological reactions and the electrophoretic globulin components present in the sample.

Methods. Electrophoretic Methods. The 0.025 M lithium diethyl barbiturate, 0.025 M diethyl barbituric acid, 0.025 M lithium chloride buffer of Longsworth, Shedlovsky, and MacInnes[8] was used in all cases with the exception of the serum from J.M. shown in Table I. In this analysis the 0.1 M sodium diethyl barbiturate, 0.02 M diethyl barituric acid buffer[9] was used. The latter buffer has the advantage that the δ and ϵ boundaries separate from the γ boundaries.[9] The samples of serum and the protein fractions were diluted 1:2 with the buffer before dialysis. The cell was the conventional 3-piece cell of Tiselius[10] and the lens system was that of Philpot as modified by Svensson.[11]

Fractionation with ammonium sulfate. In order to identify the electrophoretic fraction which carries the Kahn and Wassermann reagin, samples of syphilitic serum giving strong Kahn titers, were combined to give a pooled sample of 1000 ml.

The pooled serum was diluted with an equal volume of distilled water and 2 liters of saturated ammonium sulfate solution were added slowly by a capillary pipette while stirring the solution. The precipitated globulins were filtered off and the filtrate was brought to 0.75 saturation with ammonium sulfate. The precipitate (A) was filtered off.

The precipitated globulins were dissolved in a liter of 0.9% sodium chloride and the resulting solution filtered. Saturated ammonium sulfate solution was added through the capillary pipette to 0.22 saturation and the precipitate filtered off (GI). The filtrate was brought to 0.34 saturated and the precipitate (GII) filtered off. The filtrate from GII was brought to 0.40 saturation with ammonium sulfate and the insoluble fraction (GIII) filtered off. The remainder of the protein (GIV) was precipitated at half saturation with ammonium sulfate. In the fractionation procedures, no attempt was made to control the pH.

All of the fractions were dissolved in 0.95 sodium chloride solution to give a final concentration around 5 g of protein/100 ml. These solutions were dialyzed in the ice box against 0.9% NaCl until free of sulfate ion. The protein concentrations were determined by Kjeldahl analyses.

Serological Reactions. The various fractions were submitted for serological examination by the standard Kahn test, the quantitative Kahn test, the Wassermann (Kolmer) test, the Kahn temperature verification test,[12] the Kahn salt verification test,[12] and the Hinton test. All of the tests with the exception of the Wassermann (Kolmer) reaction were done on both inactivated samples and uninactivated samples.†

[8] Longsworth, L. D., Shedlovsky, T., and MacInnes, D. A., *J. Exp. Med.*, 1939, **70**, 399.

[9] Longsworth, L. G., *Chem. Rev.*, 1942, **30**, 323.

[10] Svensson, H., *J. Biol. Chem.*, 1941, **139**, 805.

[11] Svensson, H., *Kolloid Ztschr.*, 1940, **90**, 141.

[12] Kahn, R. L., *J. Lab. and Clin. Med.*, 1943, **28**, 1170.

† The author wishes to express his thanks to Dr. Herman N. Bundesen, President of the Chicago Board of Health, for making serum from clinical

Results. In an attempt to determine the fraction or fractions of syphilitic serum which combine with the Kahn antigen to produce the flocculus a large sample of blood was obtained from each of two patients with strongly positive serological reactions and clinical manifestations of syphilis. The serum was prepared and divided into 3 portions. An electrophoretic analysis was made on one portion without further treatment. The second sample was inactivated according to the standard Kahn technic for 30 minutes at 56°C. After inactivation an electrophoretic analysis was obtained. The third sample was inactivated by heating 30 minutes at 56°C and Kahn antigen was added to the undiluted serum to obtain a copious floc. After standing overnight in the ice-box, the floc was centrifuged off and an electrophoretic analysis was made on the supernatant fluid.

The results obtained are given in Table I. No significant change in the distribution of electrophoretic fractions of the sera resulted from inactivation. The treatment of inactivated serum with the Kahn antigen produced a decrease in the γ/A ratio in both cases. The decrease in this fraction in the serum of J.M. was smaller. These experiments indicate that the reagin is present in the γ globulin fraction of syphilitic sera.

Another approach to the problem was made. A large volume of syphilitic serum (1000 ml) was obtained by pooling small quantities of serums which gave strongly positive Kahn reactions. This serum was fractionated as outlined above with ammonium sulfate and five fractions were obtained.

The original pooled serum and the 5 fractions were analyzed electrophoretically. The number of components present in each fraction was determined and the concentration of each fraction obtained. The electrophoretic

TABLE II.
Electrophoretic Analyses of Protein Fractions Obtained from Syphilitic Serum by Ammonium Sulfate Precipitation.

Fraction	Fractional sat. with $(NH_4)_2SO_4$ causing precipitation	pH	Potential gradient volts/cm in tube	Total protein conc. g/100 cc	Concentration of Electrophoretic fractions, g/100 cc				Mobilities of electrophoretic components, cm/sec volt $\times 10^5$			
					Albumin	α Glob.	β Glob.	γ Glob.	Albumin	α Glob.	β Glob.	γ Glob.
GI	0.22	7.80	7.31	6.00	—	—	1.64	4.36	—	—	3.1	0.4
GII	0.34	7.81	7.31	6.21	—	0.59	2.24	3.38	—	4.9	3.5	0.3
GIII	0.40	7.80	7.31	4.61	—	2.66	1.02	0.93	—	4.6	3.4	0.3
GIV	0.50	7.80	7.31	2.59	—	2.59	—	—	—	4.9	—	—
A	0.75	7.80	7.31	5.50	4.30	0.60	0.60	—	6.1	4.6	3.1	—
Original serum		7.80	7.31	7.20	3.60	0.76	1.12	1.63	5.9	4.1	2.9	0.2

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TABLE III.
Serological Reactions of Protein Fractions from Syphilitic Sera.

Fraction	Electrophoretic fractions present	Standard Kahn		Quant. Kahn	Temperature verification		Salt verification		Wassermann	Hinton	
		Uninact.	Inact.	Inact.	Uninact.	Inact.	Uninact.	Inact.	Inact.	Uninact.	Inact.
Original sera	A, α, β and γ	pos.	pos.	40	—	—	—	—	—	—	—
GI	β and γ	,,	,,	120	syph.	syph.	syph.	syph.		neg.	neg.
GII	α, β and γ	,,	,,	80	,,	,,	,,	,,		,,	,,
GIII	α, β and γ	neg.	,,	—	,,	,,	,,	,,	—	,,	doubt.
GIV	α	,,	neg.	—	neg.	neg.	—	—	—	,,	neg.
A	A, α and β	,,	,,	—	,,	,,	—	—	—	,,	,,

components were identified by their mobilities.

The results of the electrophoretic analyses are given in Table II.

Samples of the 5 fractions were submitted for serological examination. The results are given in Table III.

Consideration of the results of the electrophoretic analyses and the results of the standard Kahn test shows that all of the fractions which give positive serological reactions contained β and γ globulins. Since fraction GI, which gave the highest titer in the quantitative Kahn reaction contains no α globulin, the latter fraction apparently carries none of the reagin. These findings indicate that the Kahn reagin is carried in the β or γ globulin fraction or both. The Wassermann (Kolmer) reaction was strongly positive in the fractions having positive Kahn reactions. The reagin for the complement fixation test is carried in the same fractions as the Kahn reagin.

Coburn and Moore[13] report that the Wassermann reagin present in the sera of patients with lupus erythematosus who were apparently not syphilitic, is found in the β_2 and γ fractions. The present work would then indicate that the true reagin and the reagin in falsely positive blood are present in the same fractions. The fact that their electrophoretic mobilities are the same, however, does not mean that they are alike in other respects.

All fractions which gave positive Kahn reactions also gave positive verification reactions in both the temperature and salt dispersability tests, and hence no indication of a non-syphilitic reagin was found. Fraction GIII which showed a negative Kahn reaction when inactivated, nevertheless gave a positive verification test after inactivation. The inactivated samples of this fraction gave positive Kahn reactions and verification tests. These phenomena are probably due to the small amounts of γ globulin present in this fraction.

The Hinton test was negative for all fractions except GIII. In the test of this fraction without inactivation the result was doubtful. The presence of 0.9% NaCl in the samples may have interfered with the technic of the examination with this reaction. The possi-

[13] Coburn, A. F., and Moore, D. H., *Bull. Johns Hopkins Hosp.*, 1943, **73**, 196.

bility that the mechanism for this reaction differs from that of the Kahn and Wassermann reactions also exists.

Summary. 1. Electrophoretic analyses are reported for syphilitic sera, before inactivation by heating to 56°C for 30 minutes, after inactivation, and after inactivation and flocculation with the Kahn antigen. No significant change occurred on inactivation but flocculation with the antigen apparently removes some γ globulin from solution. 2. Electrophoretic analyses and serological reactions of protein fractions obtained by salting out with ammonium sulfate are reported. From the data obtained, the β or γ globulin fractions or both have been identified as carriers of the Kahn and Wassermann reagins.

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Physiology of Bacteria-free *Trichomonas vaginalis.* VII: Temperature in Relation to Survival and Generation Time.*

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Information regarding the response of *Trichomonas vaginalis* to various temperatures should be useful in studies of the epidemiology of the infection and may indicate the limitations of heat in therapy.

The effect of temperature extremes upon *Trichomonas vaginalis* in contaminated cultures has been described. Leuckardt[1] and Dock[2] reported a sensitivity to cold. Fukushima[3] subcultured the organisms successfully after 3 days in an ice box. Fischer[4] reported growth following 21 days storage at 3-5° C. Mohr[5] described multiplication of trichomonads that had been frozen for 24 hours after being kept for 8 days at room temperature. Survival at room temperature for 7 days was reported by Fukushima,[3] and for 8 days by Mohr.[5] Weiler[6] failed to subculture from cultures kept at 18-20° C for 36 to 48 hours. The thermal death time was described as 10 minutes at 46°C by Davis[7] and 5 minutes at 47° C by Matsuda.[8]

In the present studies, a culture-medium containing cysteine, peptone, liver infusion, maltose and human serum was used.[9] Test organisms were obtained from 2-day cultures, demonstrated to be bacteria-free with thioglycollate medium. The numbers of organisms in the inocula were determined by hemocytometer count. Fluctuations in temperature were minimized by immersing the culture tubes in water. Temperatures were maintained within less than ± 0.5° C. Thermometers were standardized against a Bureau of Standards certified instrument under conditions comparable to the test involved. Experimental temperatures below the prevailing

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1 Leuckardt, R., *Die Parasiten des Menchen und die von ihnen herruhrenden krankheiten,* 2 aulf. Bd. 1, pp. 311-317, Leipzig, E. F. Winter, 1879.

2 Dock, G., *Tr. Path. Soc. Phila.,* 1896, **17**, 287.

3 Fukushima, K., *Nihon Fujinka Gk. Z.,* 1934, **29**, 8 (*Jap. J. M. Sc., Gnec.,* Feb., 1936).

4 Fischer, I., *Prensa med. argent.,* 1935, **22**, 340.

5 Mohr, H., *Z. f. Geburtsh. u. Gynak.,* 1937, **115**, 115.

6 Weiler, P., *Z. f. Hyg. u. Infektionsk.,* 1938, **121**, 27.

7 Davis, C. H., *Am. J. Obst. and Gynec.,* 1929, **18**, 575.

8 Matsuda, K., *J. Orient. Med.* (Abstr. Sect.), 1935, **23**, 47.

9 Johnson, G., and Trussell, R. E., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 245.