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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room temperatures were obtained in an incubator which was placed in a refrigeration room.

Survival at reduced temperatures was checked by maintaining 15 to 20 inoculated cultures at the experimental temperature. At intervals duplicate cultures were removed to an incubator at 37° C and examined for evidence of multiplication after appropriate periods of incubation. Cultures showing no gross evidence of multiplication were incubated for a maximum of 9 days. The results of duplicate and triplicate tests are summarized in Table I.

TABLE I.
Survival of *Trichomonas vaginalis* at Reduced Temperatures.

Temp. °C	Hours survival
1 to 4	96-144
24	120-150
25	154
26	Multiplication occurs

Exposure to a temperature of approximately —72° C was accomplished by pipetting 0.2 ml of inoculated culture fluid into each of a series of sterile cotton-stoppered tubes, which were placed in a bath of solid carbon dioxide in 95% alcohol. Several tubes were transferred to a 37° C water bath as soon as the solid state was reached. The remainder were removed at one minute intervals. After melting the culture fluid and warming to 37° C, 0.1 ml was transferred to C.P.L.M. medium and incubated at 37° C for a minimum of 9 days with periodic examination for multiplying organisms. The remainder of the melted culture fluid was examined microscopically for motile trichomonads. The cultures exposed to —72° C revealed no motile organisms and no growth in the subcultures when the exposure had been for one minute or longer. Merely solidifying the fluid in the freezing mixture did not destroy the majority of the organisms though large vacuoles were observed in the cytoplasm.

The thermal death time was investigated by allowing 10 cotton-stoppered culture tubes containing 4.0 ml of culture fluid to reach the experimental temperature in a water bath. They were then inoculated at thirty-second intervals with 0.2 ml of a 2-day-old culture having a population of about 2000 organisms per cubic millimeter. Subcultures were made at intervals up to 30 minutes. In this way 10 duplicate subcultures were obtained containing organisms exposed for a given interval. The procedure was similar to that previously described for testing the action of antiseptics.[9] In order to investigate exposures of less than 5 minutes the number of culture tubes exposed was correspondingly reduced so that the allotted exposure time might elapse between the inoculation of the first tube and the first transfer to a subculture. For example, in an exposure time of 4 minutes, 8 tubes were inoculated at 30 second intervals. Thirty seconds following the inoculation of the eighth tube, the first tube inoculated contained organisms which had been exposed for 4 minutes. The subcultures were then incubated and examined at intervals of several days for evidence of multiplication. The death time at each temperature investigated is shown in Table II.

TABLE II.
Thermal Death Time of *Trichomonas vaginalis.*

	Minutes exposure							
Temp. °C	2	3	4	5	10	15	20	30
45				+	+	+	+	+
45.8				+	+	+	+	+
47				+	+	+	—	—
48				+	+	—	—	—
49				+	—	—	—	—
50	+	+	—	—	—	—	—	—

+ = Survival.
— = No survival.

Response to intermediate temperatures was measured in terms of generation time. In this investigation, 8 ml of sterile culture fluid were pipetted into each of a series of 10 sterile cotton-stoppered tubes and 2 ml of sterile, undiluted serum, adjusted to pH 6, were added. A 2-day-old culture supplied the inoculum. The population of this culture was determined by averaging 4 hemocytometer counts and was adjusted if necessary by dilution with sterile culture fluid. The number of organisms used in the inoculation was reduced in those cultures which were incubated at temperatures where the rate of multiplication was high. This was done in order that

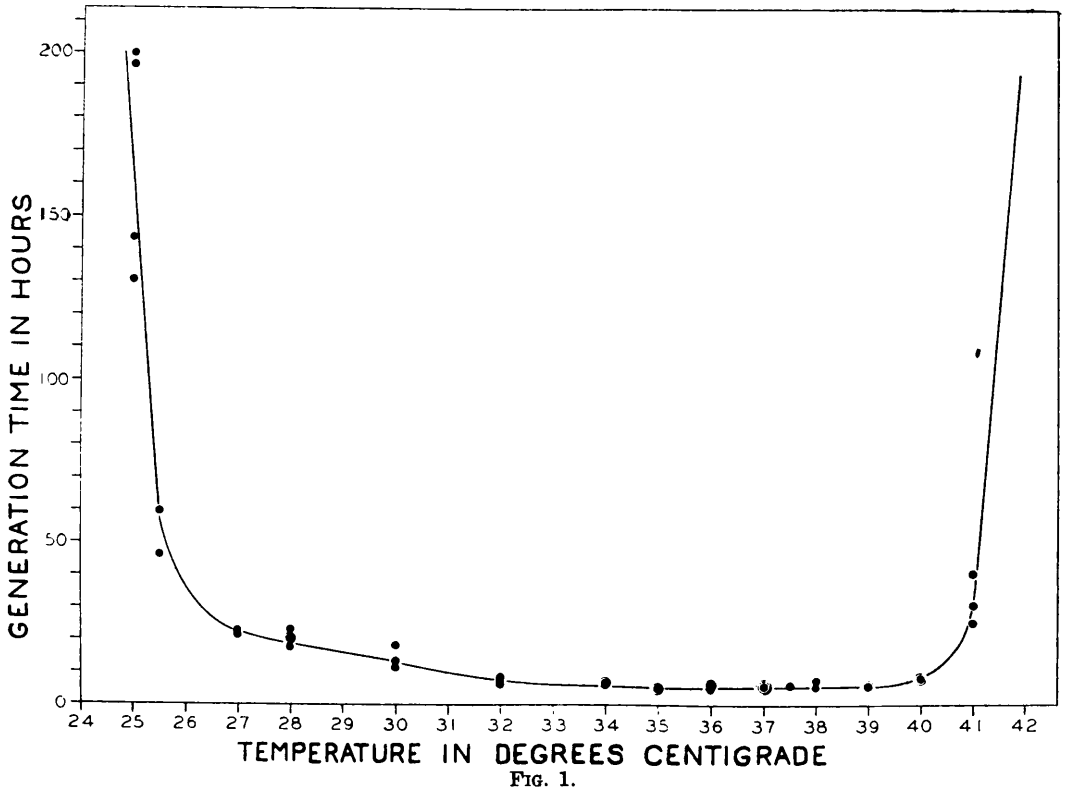

FIG. 1.

the final population might be determined before the cultures had reached the phase in which metabolic wastes limited further increase in population. The volume of culture fluid was maintained at a constant level during the period of incubation by replacing the cotton with sterile cork stoppers to prevent evaporation. After approximately 40 hours in the incubator, the cultures were brought to room temperature and the population determined by a hemocytometer. The generation time (g) was calculated from the equation, $g = \frac{t}{3.3 \log b/B}$, where t is the time in hours, b is the population in organisms per cubic millimeter at time t, and B is the original population as determined from the inoculation. This equation is derived from $g = \frac{t \log 2}{\log b - \log B}$.[10] The curve plotted in Fig. 1 summarizes the data from these experiments. It will be noted that at 25° C the generation time in half the experiments exceeded the maximum survival time for cultures exposed to this temperature. It is therefore concluded that 25° C is the lowest temperature at which the organisms multiply. The upper limit was 42° C and the minimum time required for optimal cell division, 5 to 7 hours, obtained over a broad range from 34° to 39°C.

Conclusions. 1. *Trichomonas vaginalis* survives exposure at temperatures near the freezing point for 96 to 144 hours and at 24-25°C for 120 to 154 hours. Exposure to −72°C for one minute destroys the organisms. 2. The thermal death time for temperatures above 43°C has been recorded. At 50°C it is 4 minutes. 3. The lowest temperature consistent with multiplication is 25°C; the upper limit 41°C. 4. The minimum time required for cell division is 5 to 8 hours and was observed over a broad range from 34° to 39°C.

[10] Buchanan, R. E., and Fulmer, E. I., *Physiol. and Biochem. of Bacteria*, Vol. I, 18-19, Williams and Wilkins, 1928.