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STUDIES OF THE DEOXYRIBONUCLEIC ACID FROM MESOPHILIC AND THERMOPHILIC BACTERIA

by

Bruce A. Roe

A Thesis
Submitted to the
Faculty of the School of Graduate
Studies in partial fulfillment
of the
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Bruce A. Roe

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INTRODUCTION

The first isolation of a thermophilic bacterium was done by Miquel in 1879.(1). Since this time much work has been done in studying these bacteria from many sources in nature. Thermophiles are bacteria which grow at much higher temperatures (about 55-80^{o}C) than mesophiles which grow at more moderate temperatures (about 20-45^{o}C).

Three major theories have been proposed to account for the phenomenon of thermophily. The first of these theories developed by Gaughran (1), considers thermophily to be due to the presence of heat stable lipids. The second theory, proposed by Allen (2), views thermophily as a system characterized by rapid rates of synthesis and degradation. The third theory ascribes thermophily to physical-chemical differences between the macromolecules of the thermophiles and those of the mesophiles.

The last theory has received the most support thus far. The evidence comes largely from recent comparative studies of proteins and ribonucleic acids (RNA) from thermophilic and mesophilic bacteria. Unusual thermal stability of cytoplasmic proteins from thermophilic organisms has been reported by Koffler (3). The α-amylase isolated

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from Bacillus coagulans grown at 55°C showed greater heat stability than the same enzyme isolated from B. coagulans grown at 37°C (4). A marked difference in the heat stability of flagella from thermophilic and mesophilic bacteria has been demonstrated by Stenesh and Koffler (5). More recently, Stenesh and Holazo (6) have shown that the ribosomal RNA from thermophilic strains of Bacillus was more heat stable and had a higher guanine plus cytosine (G+C) content than the ribosomal RNA from mesophilic strains of Bacillus. The ribosomes of the above strains of Bacillus differed likewise in their stability (7); the ribosomes from the thermophilic strains were more stable than the ribosomes from the mesophilic ones.

Studies of the deoxyribonucleic acid (DNA) from mesophilic and thermophilic organisms have been less numerous and have also been less conclusive in their support of the above theory of thermophily. One of the few papers is that by Marmur (8) in which he showed that there was no marked difference between the thermal stability of DNA from B. stearothermophilus and that of DNA from Escherichia coli. This study by Marmur as well as many of the other studies of DNA, proteins, and RNA have involved a comparison of thermophilic strains of B. stearothermophilus with mesophilic

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strains of E. coli. Such investigations do not rule out the possibility of intergeneric differences which may have affected the results. For this reason, our laboratory has been studying mesophilic and thermophilic strains from one genus, namely Bacillus. The present work deals with studies of the stability, physical parameters, and base composition of the DNA isolated from these strains.

DNA is found in all biological systems with the exception of certain viruses, and is largely confined to the cell nuclei where it is present in association with certain proteins. DNA is the fundamental carrier of genetic information for all living systems (excluding certain viruses), and as such it determines the primary structure of proteins. This is achieved by first transcribing the nucleotide sequences of the DNA into nucleotide sequences of messenger RNA's (a copying process). These messenger RNA's then direct the assembly of amino acids into proteins by a process whereby the nucleotide sequences of the messenger RNA's are translated into amino acid sequences of proteins.

The primary structure of DNA consists of a chain of deoxyribose molecules joined via 3'-5' phosphodiester linkages (9). Nitrogenous bases are attached at the

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number 1 position of the sugar. The major bases are adenine (A), guanine (G), cytosine (C), and thymine (T). Three minor bases are 5-methylcytosine (5-MC), 6-methylamino-purine (6-MAP), and 5-hydroxymethylcytosine (5-HMC). The complete DNA molecule is made up of two such chains which form a double alpha-helix. In this structure every A is paired to every T (and vice versa) by two hydrogen bonds and every C is paired to every G (and vice versa) by three hydrogen bonds. The two chains are therefore known as complementary strands.

This double helix has a radius of 10 A, and every turn in the helix corresponds to a distance of 34 A along the axis of the helix. The molecular weight of the DNA varies widely depending upon the isolation procedure, but it is generally of the order of several millions.

The DNA used in the present study was isolated by a modification of the procedure of Marmur (10). The base composition of this DNA was determined by four methods. These involved paper chromatography after acid hydrolysis, bromination, spectrophotometric measurements and calculations based on thermal denaturation profiles. In addition to the base composition of the DNA, its thermal stability, viscosity and molecular weight were determined.

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MATERIALS AND METHODS

Organisms

Three mesophilic and three thermophilic strains of the genus Bacillus were used in this study. The mesophilic strains included B. licheniformis (NRS 243), B. pumilus (NRS 236), and B. sp. (X-1). The thermophiles were strains of B. stearothermophilus (FJW, 10, 2184).

Isolation of DNA

The DNA used in this study had been isolated previously by other students. The procedure used was that of Marmur(10) except that the isopropyl alcohol step was omitted. The procedure was interrupted here and the DNA lyophilized and stored at -20°C. Subsequently, the DNA was further purified by dissolving it in 1M NaCl and subjecting it to a three-fold precipitation with 0.55M cetyl trimethylammonium bromide according to Jones (11). This removes polyribonucleotides and polysaccharides much as the isopropyl alcohol step in the procedure of Marmur. The final product was dialyzed for 24 hours versus water at 4°C, lyophilized and stored at -20°C. The purity of the DNA was essentially the

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same for all six strains; protein contamination amounted to about 2% and RNA contamination was about 3% in terms of ribose determined by the orcinol reaction. These analyses were performed by Dr. Stenesh.

Reagents

Paper chromatography:	70% $HClO_4$ (Mallinckrodt) Isopropyl alcohol (Baker) - redistilled HCL (Baker), 12N, 2N, and 0.1N
Estimation of denaturation:	0.01M NaCl 1M $MgCl_2$
Bromination:	N-bromoacetamide, 0.006M 1N H_2SO_4 (Baker)
Simultaneous spectral determinations:	0.01 M NaCl 12N $HClO_4$ Deoxynucleotide monophosphates of A, G, C, and T (Calbiochem)
Thermal denaturation profiles:	Standard saline citrate (0.15M NaCl, 0.015M Na-citrate, pH 7.0) Ethylene glycol: Distilled H_2O (1:1, v/v)
Viscosity:	Standard saline citrate
Minor Bases:	Isopropyl alcohol (Baker) - redistilled 2-Propyl alcohol (Baker) - redistilled n-Propyl alcohol (Baker) - redistilled n-Butyl alcohol (Baker) - redistilled 12N $HClO_4$ (Mallinckrodt) HCl (Baker) - 12N and 0..1N Polyethyleneimine cellulose (Cellex-PEI) Microcrystalline cellulose (Cellex-MX) Powdered cellulose (Cellex-N-1) All cellulose preparations were obtained from Calbiochem 5-MC and 6-MAP (Calbiochem)

Note: Three times distilled water was used for all solutions.

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Apparatus

All spectrophotometric measurements were made in a Zeiss model PMQ-II spectrophotometer except for the simultaneous spectral determinations and the minor base composition studies which were also carried out in a Cary model 14 recording spectrophotometer.

Viscosities were measured using a Cannon-Ubbelohde Four Bulb Dilution Viscometer (Cannon Inst. Co., State College, Pa.). The capillary of this specially designed viscometer had a diameter of 0.061 cm. Other specifications of the viscometer were as follows:

Bulb	Efflux Vol. (cm^3)	Driving Head (cm)	Shear Rate (at wall) (sec^{-1})	Viscometer Constant (centistokes/sec)
A	1.53	15.2	69,000/t	0.003429
B	0.83	9.5	37,000/t	0.003921
C	0.55	5.8	24,700/t	0.003592
D	0.30	3.4	13,500/t	0.003715

A "Mineralight" Model R-51 ultraviolet lamp (Ultraviolet Products, Inc., San Gabriel, Calif.) was used to view the

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paper chromatogram and thin layer chromatography (TLC) plates.

Paper chromatography was carried out in a square glass jar (60x30x30 cm). and TLC was carried out in rectangular glass jars (30x25x10 cm).

Estimation of Denaturation

The method of Shack (12) was used to determine the percentage of denatured DNA in the various samples. This involves absorbance measurements (at 260 mu) of "native" and heat denatured DNA in either a constant solvent (0.01M NaCl) and at two temperatures (0 and 22°C) or. alternatively, at a constant temperature (room temperature) and in two different solvents (0.01M NaCl and 0.01M NaCl plus 0.01M $MgCl_2$). The percentage of denatured DNA is then calculated from

$$\frac{(A_{22^o} - A_{0^o})_u}{(A_{22^o} - A_{0^o})_d} \times 100 \text{ and } \frac{(A_{Na} - A_{Mg})_u}{(A_{Na} - A_{Mg})_d} \times 100, \text{ respectively}$$

Here A, u, d, Na and Mg refer to absorbance, native sample, denatured sample, NaCl solvent, and the same solvent plus $MgCl_2$, respectively.

Base Composition

(i) Paper Chromatography

The major nucleic acid bases were determined by paper

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chromatography after hydrolysis with 12N $HClO_4$ following the method of Bendich (13). The percent recovery was determined by applying a known volume of calf thymus DNA hydrolysate to the paper, developing the chromatogram and eluting the spot in the usual manner. The recovery is then calculated by comparing the absorbance units (absorbance units = absorbance x volume in ml) at 260 mu applied to the paper with those eluted from the paper. Base composition results were calculated by the differential extinction technique (13).

(ii) Bromination

The base composition of the DNA in terms of (G+C) content was also determined by bromination using the method of Wang and Hashagen (14). The DNA was dissolved in 1N H_2SO_4 by shaking gently overnight at room temperature (Burrel shaker, setting - 1). The solution was then centrifuged in a clinical centrifuge to remove any undissolved DNA. Reaction mixtures were set up in test tubes and the cuvettes were rinsed and dried in a desiccator between determinations.

(iii) Simultaneous Spectral Determinations

Determination of the (G+C) content was also done according to the method of Skidmore and Duggan (15).

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using reference solutions prepared from standard deoxy-nucleotide monophosphates. These measurements were carried out using both the Cary model 14 recording spectrophotometer and the Zeiss model PMQ-II spectrophotometer. From the spectra of the reference solutions a constant can be calculated which in turn is used in conjunction with the spectrum of the DNA to yield a base composition in terms of (G+C) content. This constant, calculated as an average of several determinations, agreed with the value of 1.064 obtained by Skidmore and Duggan (15).

(iv) Minor Bases

The minor bases were determined by paper chromatography following the procedure outlined by Bendich (13) but modifying the second solvent according to Doskocil and Sormova (19). The DNA hydrolysate was streaked on Whatman no. 1 paper and the paper chromatogram was developed for 24 hours with isopropyl alcohol: HCl: water (65:16.7:18.3, v/v). The chamber was saturated with the same solvent. The cytosine streak (which also contained 6-MAP and 5-MC) was cut out and eluted with water by letting it hang from a trough in a closed chromatography tank, saturated with water vapor. The first ml (which contained more than 98%

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of the bases) was collected, evaporated to dryness under nitrogen, and redissolved on 0.04 ml of 0.1M HCl. This was then applied to a second sheet of Whatman no. 1 paper and the latter was developed for 24 hours in n-butyl alcohol: water:ammonium hydroxide (87:13:1, v/v). The chamber was saturated with ammonia vapor. Spots corresponding to the above three bases (as well as blanks) were cut out and eluted with 0.1N HCl (10 ml were used for cytosine and 5 ml were used for blanks and minor bases) by shaking for 2 hours at room temperature. By using standard bases it was shown that the above three bases could be separated by this procedure provided that the spot applied for the second chromatography was less than 5 mm in diameter.

After elution, the solutions were centrifuged in a clinical centrifuge. The supernatants (except for those containing cytosine) were evaporated to dryness under nitrogen and were redissolved in 1 ml of 0.1N HCl. The absorbance of these solutions was determined over the range 240-300 mu in the Cary recording spectrophotometer. The base composition was determined from the absorbance using the differential extinction method described by Bendich (13).

In order to calculate the amount of 6-MAP by this method, the absorbance of a standard solution (0.01 mg/ml

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in 0.1N HCl) was measured at 267 and 290 mu. The difference in the absorbance at these two wavelengths was 0.653 and this value was used for the calculations. Minor base concentrations were calculated as percent of cytosine and appropriate corrections were made for the different eluting volumes used.

Prior to deciding on the above paper chromatographic procedure, various thin layer chromatographic procedures were tried in an attempt to develop a workable separation of cytosine, 5-MC, and 6-MAP. This involved procedures described by Doskocil and Sormova (19), Pataki (20), and Unger and Venner (21).

Thermal Denaturation Profiles

The DNA was dissolved in standard saline citrate to a concentration of about 80 ug/ml. The absorbance was measured at 260 mu in the Zeiss spectrophotometer equipped with a Haake circulating bath. The absorbance was measured after a 15 minute equilibration period at each temperature (the adequacy of this equilibration period was verified by absorbance measurements as a function of time), and was corrected for the thermal expansion of the sample. This was done by correcting for the volume expansion of water

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(Handbook of Chemistry and Physics, Chemical Rubber Co.). The temperature of the sample was obtained by calibrating the temperature in the cuvette versus the temperature of the bath. The temperature drop between the bath and the cuvette amounted to about 0.2°C at 25°C (bath) and to about 6.0°C at 100°C(bath). Care was taken to have the bath always filled to the same height and to have the thermometer immersed to the same depth. After the heating experiment, the solution was allowed to cool overnight while in the cell holder, and the absorbance was measured again the following day at the same wavelength. The (G+C) content of the DNA was calculated from these thermal denaturation profiles using the equation of Marmur and Doty (16), $T_m = 69.3 + 0.41$ (G+C). Here T_m refers to the midpoint of the transition between 25°C and 99.5°C, i.e. the temperature at which 50% of the hyperchromic effect is observed.

Viscosity

The DNA was dissolved in standard saline citrate to a concentration of about 80 ug/ml. Viscosities were measured in Cannon-Ubbelohde Four Bulb Shear Dilution viscometer in a constant temperature bath at 25°C. The temperature

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was constant to within $\pm$ 0.05°C. Nine determinations were made for the outflow time of the solvent for each bulb and the averages were used in the calculations. Outflow times were measured with a stopwatch to within $\pm$ 0.1 sec.

Minor errors due to variations in the vertical alignment of the viscometer were estimated by measuring outflow times with the viscometer tilted to a maximum in its holder. It could be concluded from these measurements that alignment errors were negligible in these studies.

The DNA solutions and the solvent were centrifuged to remove dust and fibers. After an experiment the viscometer was filled with cleaning solution and left that way overnight. It was then washed by suction with about 2 liters of three times distilled water and finally once with twice distilled acetone.

Ten ml of DNA solution were allowed to equilibrate in the viscometer for 15 minutes. Then the outflow time for each of the four bulbs was determined. The solution was then diluted with 5 ml of solvent, mixed by applying slight suction to the reservoir tube while the third tube (not the capillary) was closed, and the outflow times for the four bulbs were redetermined. Three additional dilutions, each time with 5 ml of solvent, followed by determination of

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outflow times, were performed in a similar manner.

Since the experiments involved only dilute aqueous solutions, the density was assumed constant and the relative viscosity (η_{rel}) was taken as the ratio of the outflow time of the solution over that of the solvent. The specific viscosity (η_{sp}) equals $\eta_{rel}-1$. The average rate of shear ($\bar{G}$, volume average) was calculated from the maximum rate of shear at the capillary wall, supplied by the manufacturer, and the outflow time of the solution by using the foll$\bar{G}$ = 2/3 xation:

$$\bar{G} = 2/3 \times \frac{\text{maximum rate of shear}}{\text{outflow time of solution}}$$

The intrinsic viscosity $[\eta]$ was determined by extrapolating a plot of $\eta_{sp/c}$, where c is the concentration in g/ml, versus concentration. The concentrations of the DNA solutions were obtained from the absorbance at 260 mu using the relationship

$$\text{mg/ml} = \text{absorbance}_{260} \times 0.04 \quad (17)$$

Molecular weights of the DNA were calculated from the intrinsic viscosity using the graph obtained by Eigner and Doty (18).

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RESULTS AND DISCUSSION

Estimation of Denaturation

The percent of denaturated DNA in the original samples was estimated using the method of Shack (12). The concentration of the DNA solutions used for these experiments was approximately 20 ug/ml, giving an absorbance of about 0.5 at 260 mu. Denatured DNA was obtained by heating the DNA for 20 minutes at 95°C in tightly covered tubes followed by rapid cooling in ice water. In the case of the NaCl-$MgCl_2$ solution, the absorbance was corrected for the change in volume due to the added $MgCl_2$ solution.

In addition to using matched cuvettes, all the samples were read in the same cuvette. The magnitude of the differential absorbance reading for the native samples was 0.001-0.002. Since the accuracy of an absorbance measurement in the Zeiss spectrophotometer is of the order of $\pm$ 0.001, the results are clearly within experimental error and the DNA could be considered to be fully native in all cases.

Base Composition

The base composition of the DNA's as determined by paper

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chromatography is given in Table I. The percent recovery was measured for a hydrolyzed sample of calf thymus DNA and was above 90%. It can be seen from Table I that the mole% of G equals the mole % of C within experimental error. This is expected on the basis of the Watson and Crick model for DNA. On the same basis one would expect the mole % of A to be equal, within experimental error, to that of T. However, it can be seen from Table I that that is not the case. The discrepancy between the amounts of A and T is too great to be accounted for by the small recovery loss or by the small amount of contaminating RNA. The latter would only amount to about $\pm$ .1 mole %. This loss of T has also been reported by Wyatt (22, 23) and Jordan (24). This is a specific loss of thymine which occurs in 12N perchloric acid solutions when the proportion of DNA to $HClO_4$ is 2.5 mg/0.05 ml. On the assumption that T had indeed been lost in this fashion and that originally the amount of T had been equal to that of A, the base composition was recalculated by setting the number of moles of T equal to the number of moles of A. The (G+C) content calculated in this manner is given in Table II. Because of this uncertainty in the amount of T, it was decided to determine the base composition, or at least the (G+C) content, by several other

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Table I. Base Composition of the DNA Obtained by Paper Chromatography

Mole Percent

Base	B. lich.	B. X-1	B. pum.	Average
Guanine	23.79±0.34	21.99±0.51	22.13±0.06	22.64
Adenine	28.85±0.24	31.47±0.48	30.01±0.75	30.11
Cytosine	23.55±0.21	21.32±0.22	24.02±1.02	22.96
Thymine	23.78±0.12	25.20±0.26	23.82±0.21	24.27

	B. FJW	B. 10	B. 2184	Average
Guanine	25.47±0.38	26.65±0.12	26.70±0.25	26.27
Adenine	26.86±0.16	23.97±0.11	25.02±0.06	25.28
Cytosine	25.77±0.22	27.82±0.98	27.70±0.27	27.10
Thymine	21.88±0.03	21.54±0.81	20.57±0.03	21.33

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Table II. Guanine plus Cytosine Content (mole%) of the DNA Obtained by Various Methods

Organism	Method						
	Paper chromatography		Simultaneous spectra		Bromination	Thermal denaturation profiles	Average
	uncorrected	corrected	Cary	Zeiss			
B. lich.	47.3	45.3	48.3	46.9	48.3	47.0	47.2
B. pum.	46.2	43.6	43.9	45.4	45.7	45.1	45.0
B. X-1	43.3	40.8	41.6	45.1	41.0	43.2	41.9
Average	45.6	43.2	44.6	44.6	45.0	45.1	44.7
B. FJW	51.2	48.9	52.9	56.0	56.3	50.9	52.7
B. 10	54.5	53.3	54.5	52.3	53.2	52.8	53.4
B. 2184	54.4	52.2	51.1	51.4	51.2	52.9	52.2
Average	53.4	51.5	52.8	53.2	53.6	52.2	52.8

methods. For this purpose the (G+C) content of the DNA was determined by bromination of the DNA, by simultaneous spectral measurements, and from the thermal denaturation profiles.

Bromination with N-bromoacetamide is based on the principle that N-bromoacetamide reacts quantitatively with all the bases except adenine (25, 26). This reaction leads to the disappearance of the absorbance in the 260 to 280 mu region. A correction for the slight amount of Rayleigh scattering is made by measuring the absorbance at 360 mu. The absorbancies at 270 and 360 mu were measured before and after bromination, and the percent absorbance remaining after bromination was calculated from the following relationship:

$$\text{Percent absorbance remaining} = \frac{(A_{270} - A_{360})_a}{(A_{270} - A_{360})_b} \times 100$$

where the subscript a and b refer to the solutions after and before bromination respectively.

The mole percent of (A+T) and, hence, of (G+C) was obtained from the percent absorbance remaining after bromination by referring to the standard curve constructed by Wang and Hashagen (14). These results are listed in Table II. The (G+C) content is generally in good agreement with that obtained from paper chromatography.

For the simultaneous spectral determinations, the DNA

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was hydrolyzed in 1N perchloric acid and the absorption spectrum determined with the Cary recording spectrophotometer over the wavelength range 240-310 mu. The absorbance at specific wavelengths was obtained from this absorption spectrum. These data, in conjunction with the constant (1.064) obtained from measurements of standard solutions of deoxyribonucleotide monophosphates, allowed a determination of the mole % of (A+T). The relationship is the following:

$$\text{mole \% (A+T)} = \frac{(A_{264} - A_{286})}{A_{273}} \times 1.064 \times 100$$

The major products of the hydrolysis with 1N $HClO_4$ are free purines and intact pyrimidine deoxynucleotides (14). However, the standard solutions and the samples were treated in an identical manner to compensate for the unequal hydrolysis of purine and pyrimidine nucleotides. The (G+C) content calculated from the above equation is listed in Table II. Because the tracings from the Cary spectrophotometer exhibited a fair amount of background noise (the instrument was set for average use rather than for maximum sensitivity, it was decided to perform the same absorbance measurements at the three specific wavelengths using the Zeiss spectrophotometer.

A final estimate of the (G+C) content of the DNA was

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obtained from the thermal denaturation profiles (see below). It has been stated earlier that Marmur and Doty (16) have shown that the T_m can be related to the (G+C) content of the DNA. The equation derived by them holds only for DNA solutions made up in standard saline citrate. In the present experiments, thermal denaturation profiles were obtained on DNA dissolved in standard saline citrate and hence the (G+C) content could be calculated from the equation of Marmur and Doty. These values are given in Table II and they again agree with those obtained by all the other methods.

It can be seen from Table II that the base composition is different for the DNA from the thermophiles and the DNA from the mesophiles. There is a definite shift in the (G+C) and the (A+T) content. For the thermophiles, the (G+C) content is higher and the (A+T) content is lower than is the case for the mesophiles. It should be noted that the variations in the (G+C) content from one method to another are relatively small and are much less than the differences between the mesophiles and the thermophiles. The variations between individual methods are similar to those reported for other DNA's (16). There is thus a significant difference in the base composition of the DNA from mesophilic and

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and thermophilic strains of Bacillus. Since in the Watson and Crick structure for DNA, a G and C pair contributes three hydrogen bonds while an A and T pair contributes two such bonds, it is apparent that the DNA of the thermophiles has a base composition that would allow it to form a much more stable structure because of more extensive hydrogen bonding. Further evidence for this conclusion comes from the thermal denaturation profiles.

The stability of the DNA could also be affected by the presence of methylated bases.It has been suggested, for example, that 5-MC increases the stability of DNA because of its ability to form three hydrogen bonds (26). On the other hand, 6-MAP would lead to a decrease in stability because of the strained structure that would result from its incorporation in the DNA molecule (26). Because of these reasons it was of interest to determine the minor bases in the above DNA samples.

Preliminary experiments involved attempts to separate C, 5-MC, and 6-MAP on paper. The R_f values were determined and are given in Table III. Because of the increased sensitivity of TLC, various procedures were used with cellulose as the stationary phase but the separations were not satisfactory even with 2 dimensional chromatography.

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Table III. R_f Values for Major and Minor Bases

Base	Whatman no.1			TLC (PEI - Cellulose)			
	A	B	C	D	E	F	G
Adenine	.36	---	---	.54	.44	.60	.32
Guanine	.25	---	---	.45	.13	.38	.42
Cytosine	.47	.35	.25	.71	.43	.63	.73
Thymine	.77	---	---	.93	.56	.73	.62
5-MC	.55	.43	.35	.78	.57	.72	.66
6-MAP	.49	.45	.55	.72	.62	.77	.30

Solvent Systems Used:

A. Isopropyl Alcohol:HCl:water (68:16.7:18.3, v/v)

B. n-Butyl Alcohol:0.1M NH_4OH (6:1, v/v)

C. n-Butyl Alcohol:water:concentrated NH_4OH (87:13:1, v/v)

D. Isopropyl Alcohol:HCl:water (60:20.5:19.5, v/v)

E. Isobutyl Alcohol:concentrated NH_4OH (90:15, v/v)

F. n-Propyl Alcohol:concentrated NH_4OH:water (6:3:1, v/v)

G. Isopropyl Alcohol:saturated Ammonium Sulfate: water (2:79:19, v/v)

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Moreover, the amount of minor base which might be recovered would be so small that it would have been very difficult to measure it accurately even in semimicro cuvettes. Because of these difficulties it was decided to use the paper chromatographic approach.

It was found that minor bases were present only to a very small extent amounting at the most to about 0.3% of C (Table IV). Moreover, only one minor base could be detected, namely 6-MAP and no 5-MC was detected. A hydrolysate of about 8-10 mg was required in order to see a spot of 6-MAP. In many cases, less DNA was available, and hence the spot corresponding to 6-MAP could not be visualized under UV light. However the "spots" were cut by reference to standard minor bases which were run along with the sample as markers. There was no significant difference between the 6-MAP content of the DNA from the mesophiles and the thermophiles. Besides, the amounts were so small that it appears that methylated bases are of little consequence for the phenomenon of thermophily.

Thermal Denaturation Profiles

Plots of the relative absorbance (the absorbance

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Table IV. Minor Base Content of the DNA

Organism	Base	Mole Percent
B. licheniformis	C	99.66
	6-MAP	0.34
B. pumilus	C	99.92
	6-MAP	0.08
B. X-1	C	99.90
	6-MAP	0.10
B. FJW	C	99.74
	6-MAP	0.26
B. 10	C	99.79
	6-MAP	0.21
B. 2184	C	99.98
	6-MAP	0.02

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at 260 mu of the heated sample, corrected for thermal expansion, divided by the absorbance of the sample at 25°C) versus the temperature after the sample has reached temperature equilibrium are shown in Figures 1, 2. In all cases a sigmoid type curve was obtained which showed a sharp rise as denaturation commenced. The steepness of this hyperchromic rise is further evidence for the essentially native state of the original DNA and for the absence of significant amounts of contaminating RNA. The hyperchromic change in absorbance was not due to spontaneous or enzymatic hydrolysis of the DNA since, upon cooling, the effect could be reversed in all cases. After 24 hours about 90% of the hypochromic effect had been restored. The T_m values compare well with other T_m values reported in the literature: 87.5-88°C for a strain of B. stearothermophilus (8, 16), 88.5°C for B. licheniformis (NRS-243) (16), 85.5°C for B. pumilus (NRS-236) (16), and the T_m value for other strains of Bacillus (16). All runs were performed in duplicate and the T_m values were reproducible to within 0.5°C.

The correlation between the melting out temperatures and the maximum growth temperatures of the organisms (5) is shown in Figure 3. Very similar results pertaining to

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Table V. Thermal Denaturation Profiles

Organism	T_m (°C)	Recovery of Hypochromic Effect (%)	Maximum Growth Temperature of the Organism (°C)
B. lich.	88.6±0.4	87.9	57
B. pum.	87.8±0.0	90.6	51
B. X-1	87.0±0.3	85.9	55
Average for Mesophiles	87.8	88.1	54
B. FJW	90.2±0.2	89.5	71
B. 10	91.0±0.05	88.5	75
B. 2184	91.0±0.02	91.1	78
Average for Thermophiles	90.7	89.7	75

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Figure 1. Thermal Denaturation Profiles of the Mesophilic DNA

a. *B. pumilus*

b. *B. sp.* X-1

c. *B. licheniformis*

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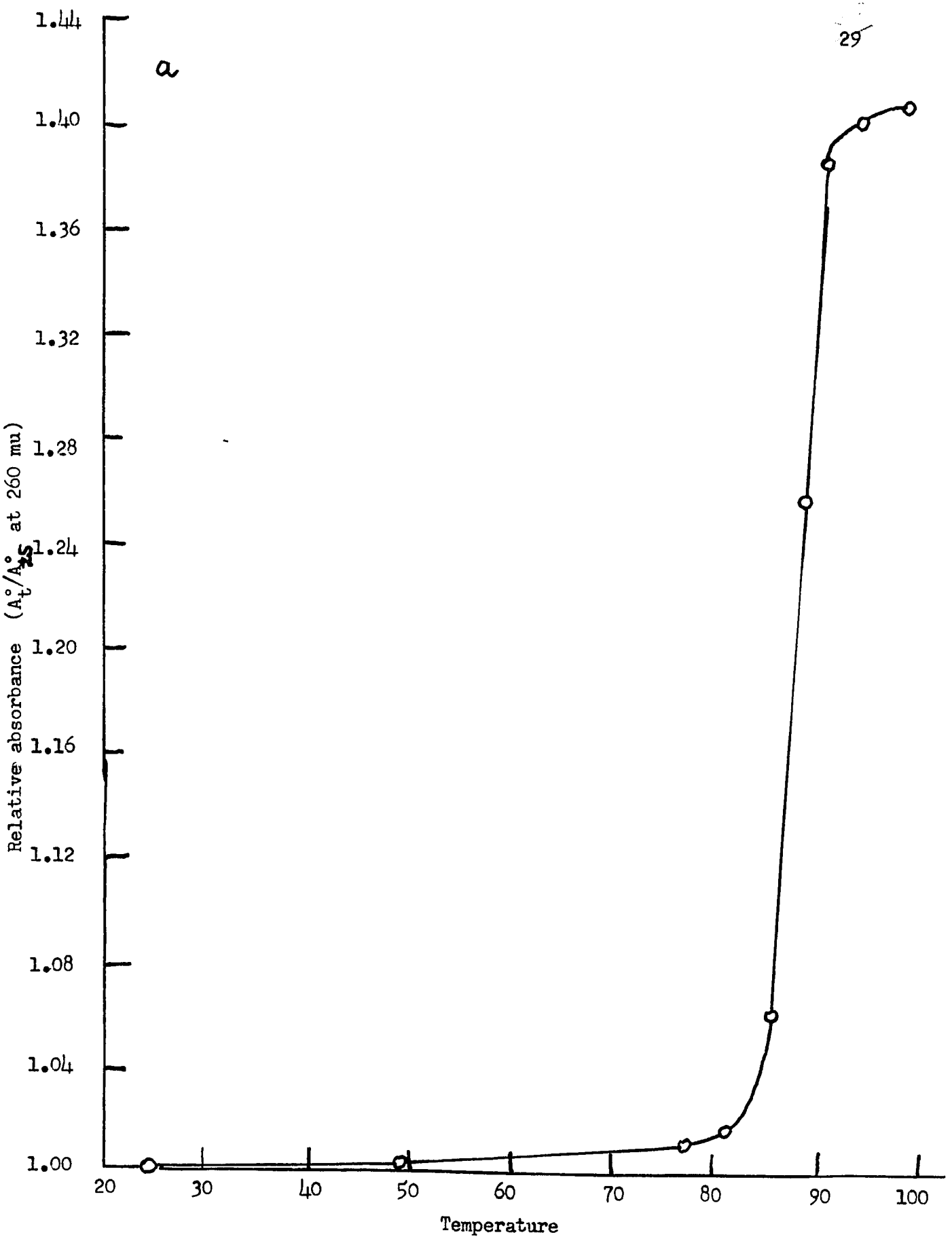

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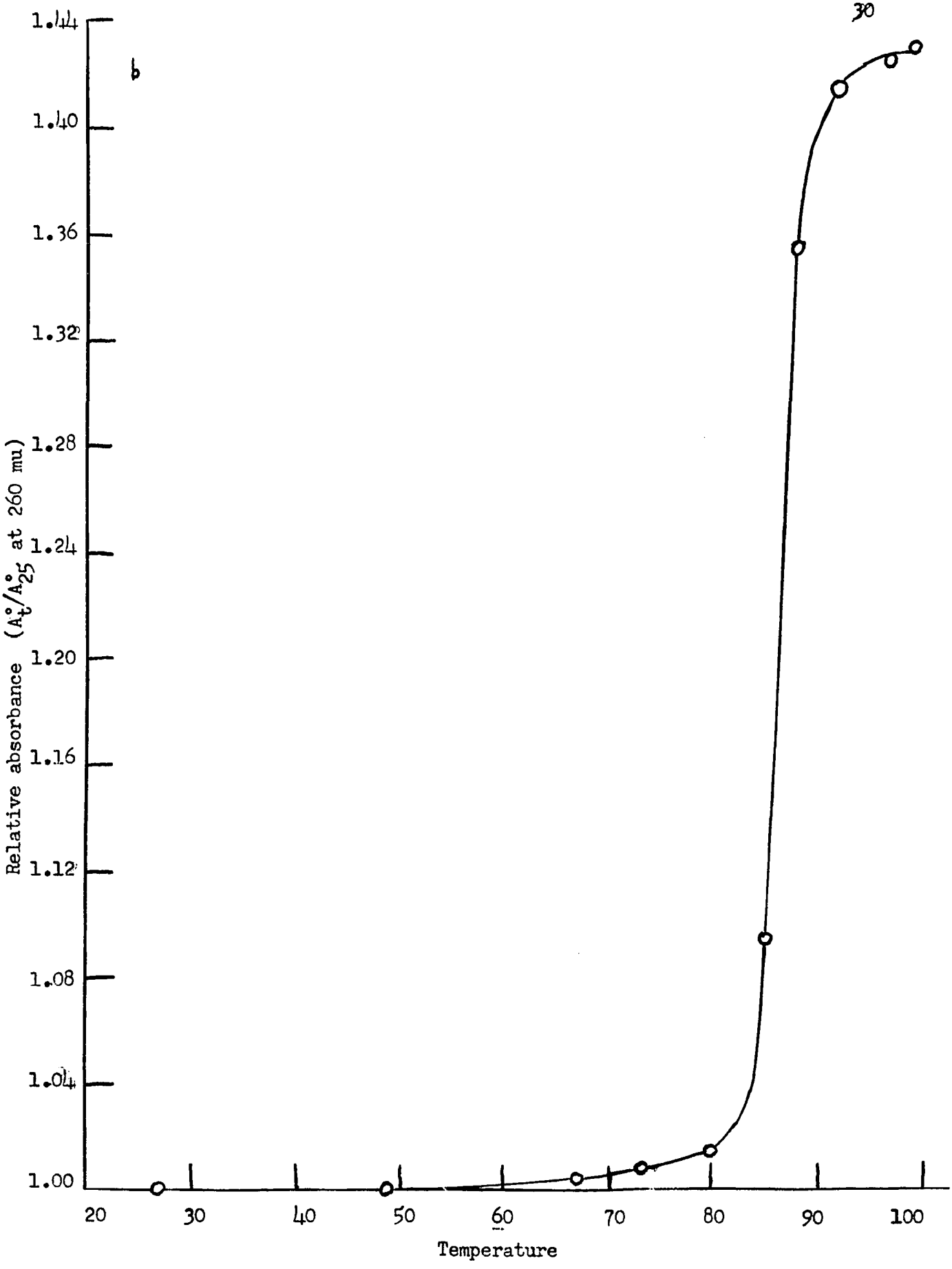

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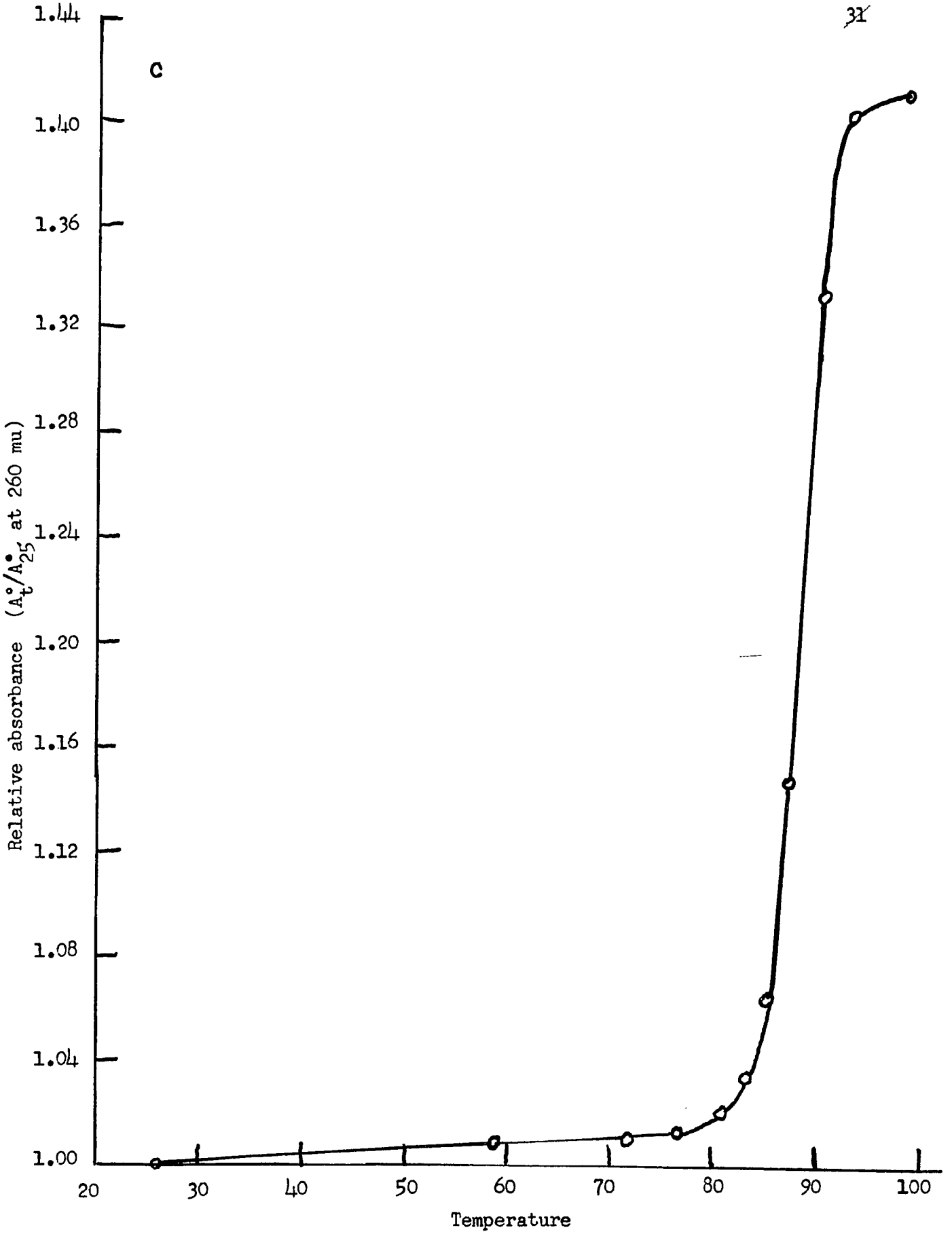

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Figure 2. Thermal Denaturation Profiles of the Thermophilic DNA

a. B. stearothermophilus FJW

b. B. stearothermophilus 10

c. B. stearothermophilus 2184

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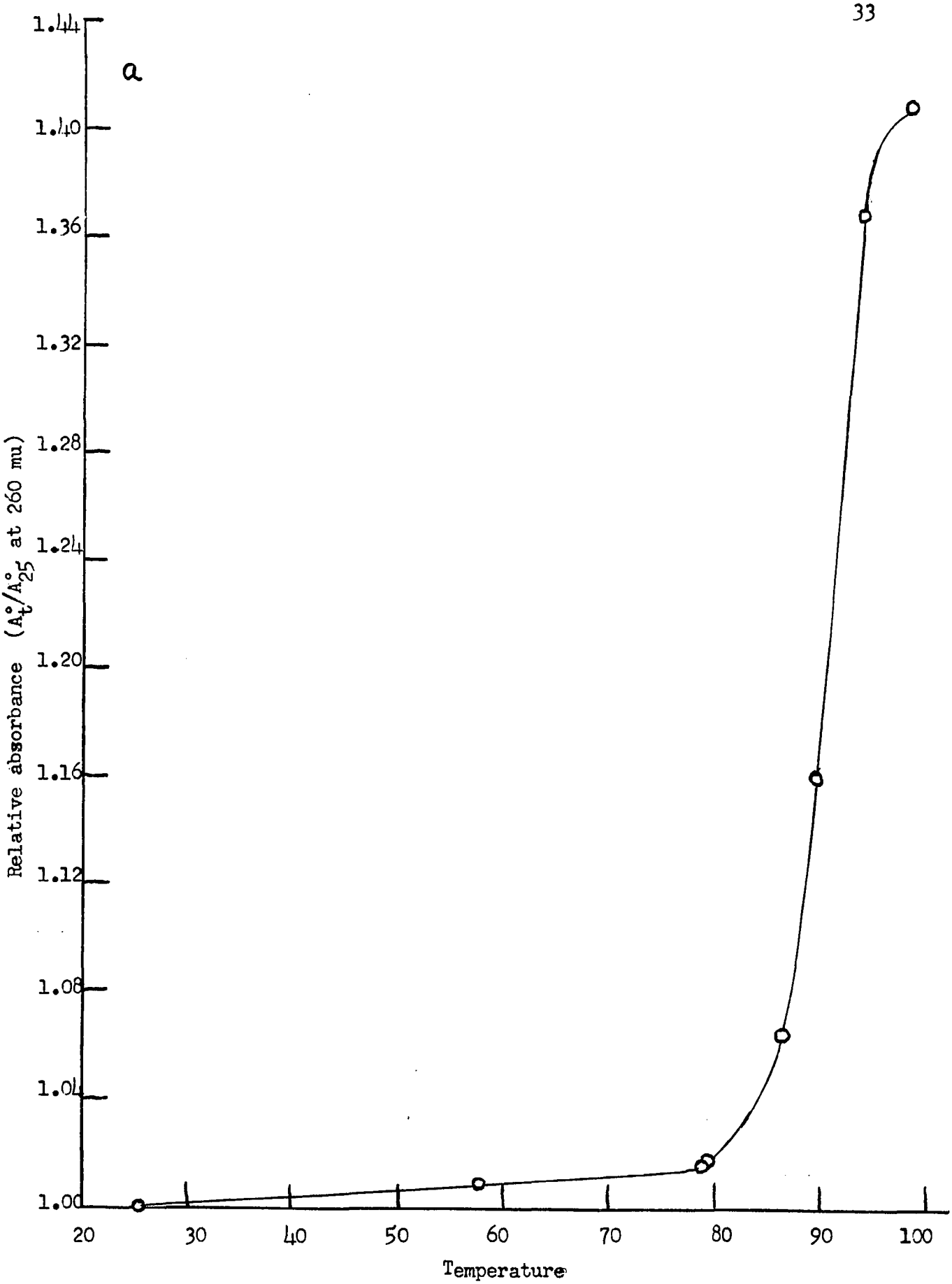

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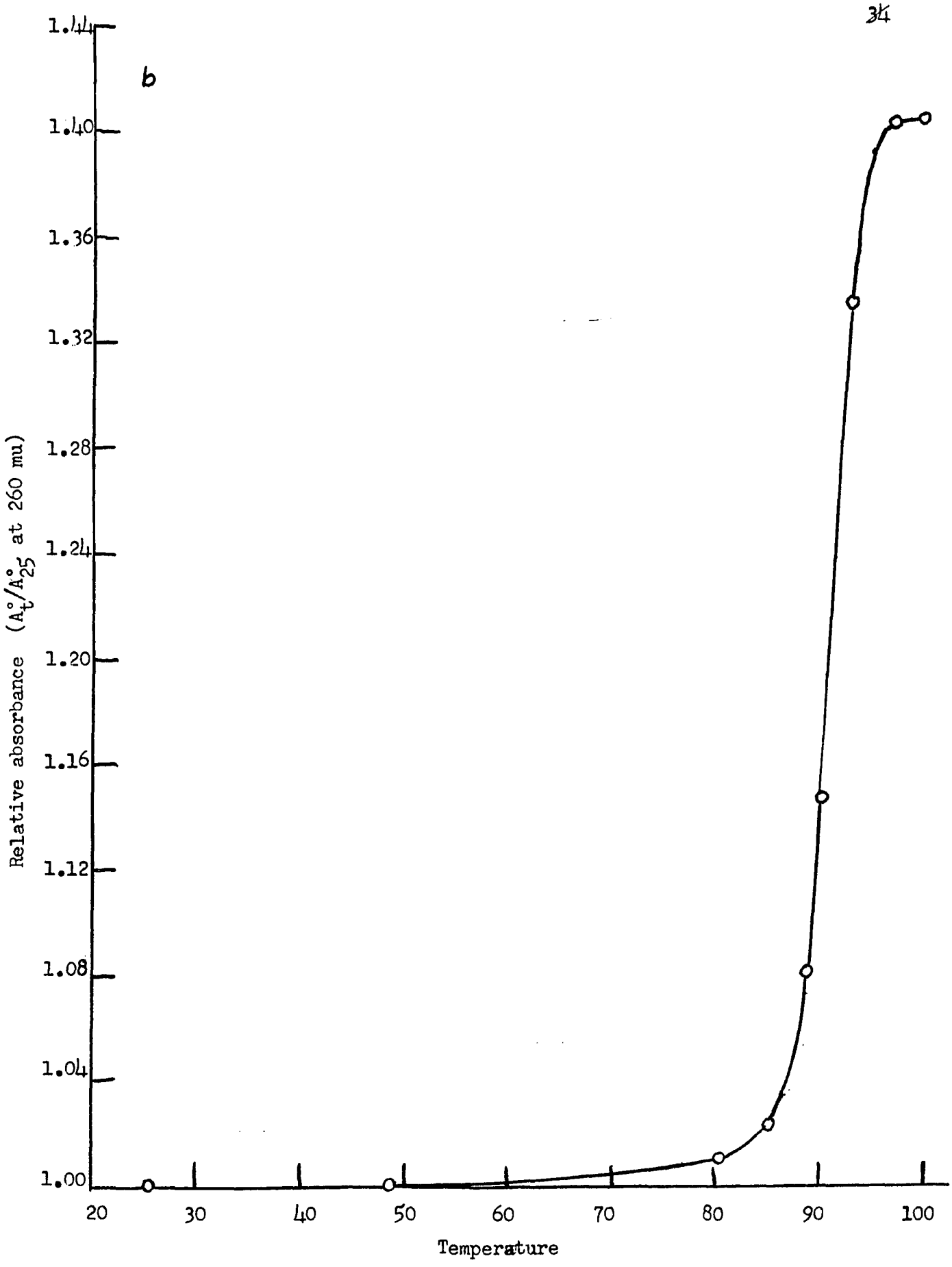

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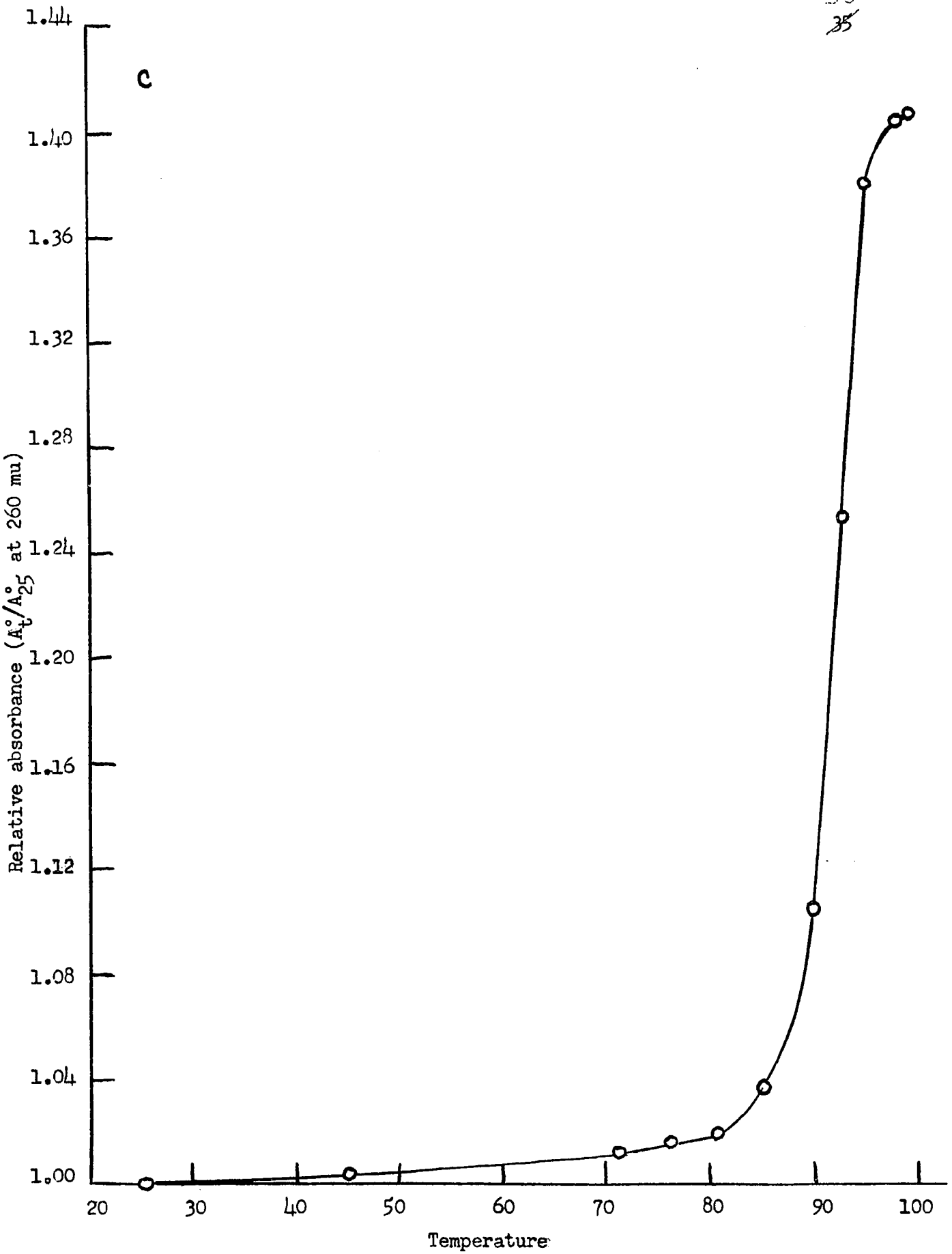

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Figure 3. Correlation between the T_m values and the maximum growth temperatures of the organisms. ●, mesophilic strains; ▲, thermophilic strains. See Table V.

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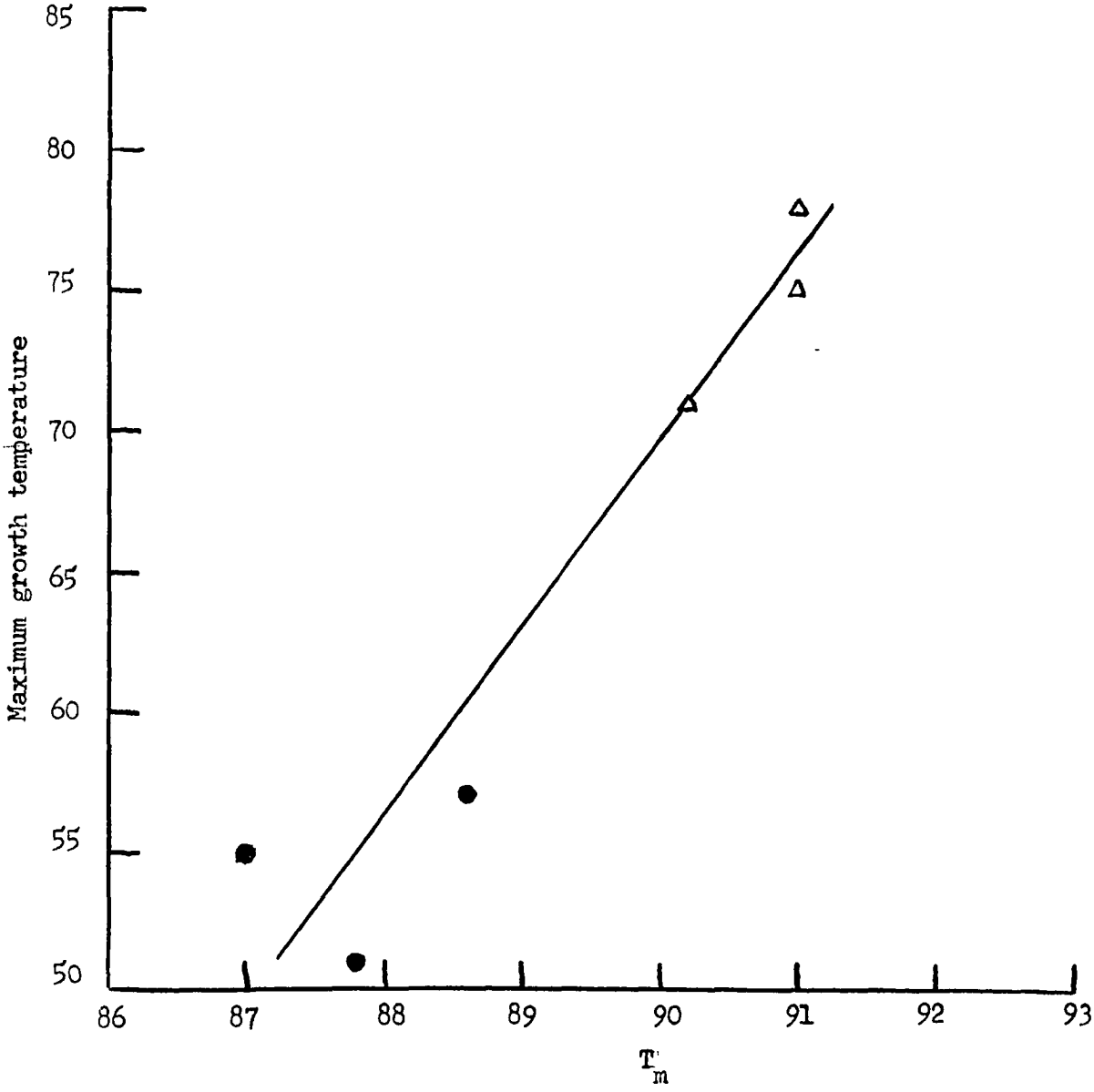

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base composition, T_m and correlation of T_m with growth temperature have been obtained in our laboratory for the ribosomal RNA from these same six strains of Bacillus (6).

Viscosity

Plots of $1/\eta_{sp}$ versus rate of shear are shown in Figures 4,5. There appears to be no significant difference between the results obtained for the mesophiles and those obtained for the thermophiles. Thus the dependence of viscosity on rate of shear is essentially the same for the DNA from all six preparations. This indicates that these DNA's do not differ greatly in the overall asymmetry of the molecule. This must be the case since generally the more asymmetric a molecule is, the greater would be the dependence of the viscosity on the rate of shear. The above conclusion is especially valid since the molecular weight of the DNA preparations were generally of the same order of magnitude (Table VI).

The extrapolation of the viscosities at zero rate of shear to infinite dilution is shown in Figures 6,7. The concentration dependence is fairly similar for the DNA from the three mesophiles but varies much more in the case of the DNA from the thermophiles. Both the maximum (B.

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Figure 4. Dependence of the Viscosity on the Rate of Shear for the Mesophilic DNA

a. *B. pumilus*

b. *B. sp.* X-1

c. *B. licheniformis*

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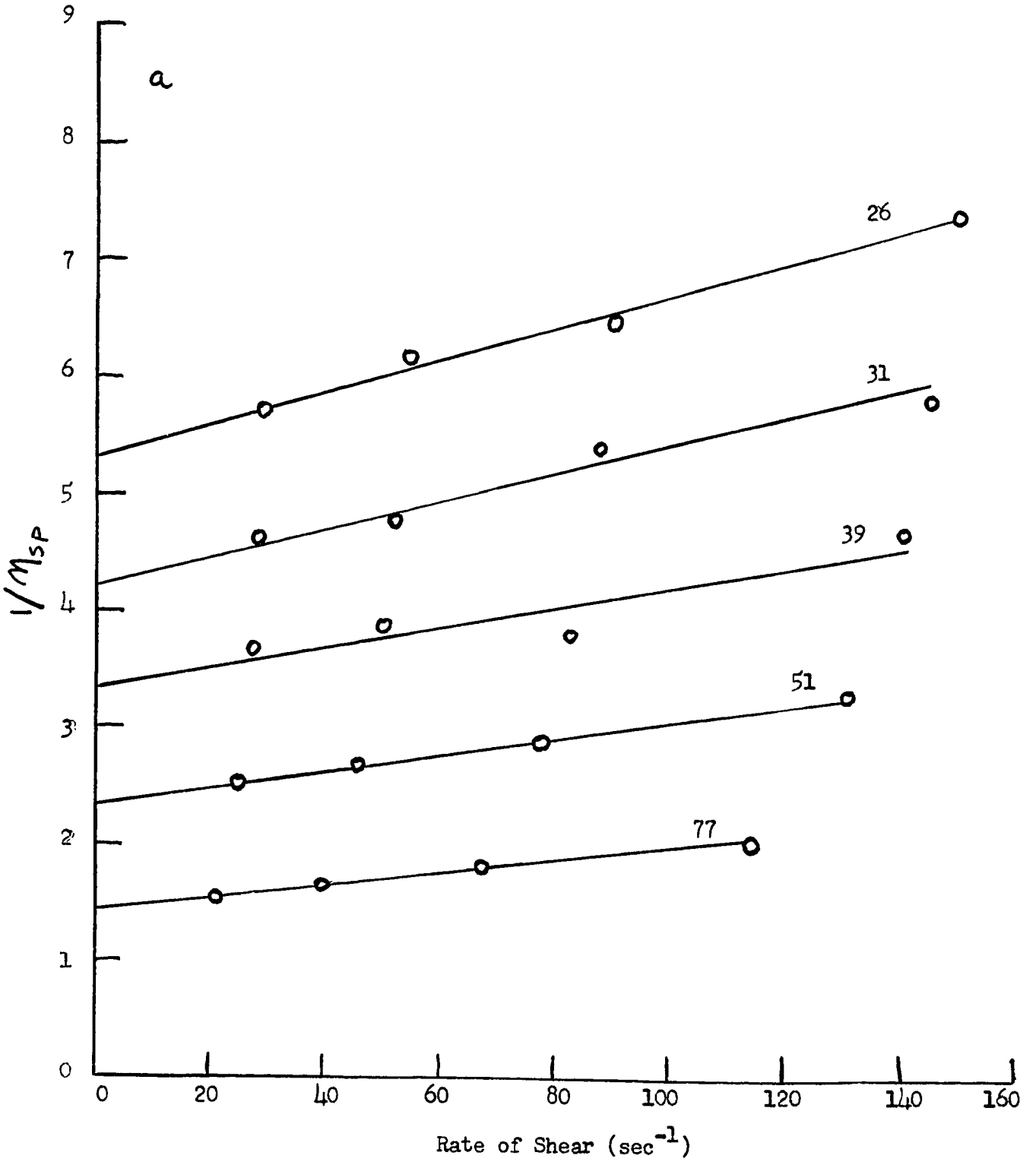

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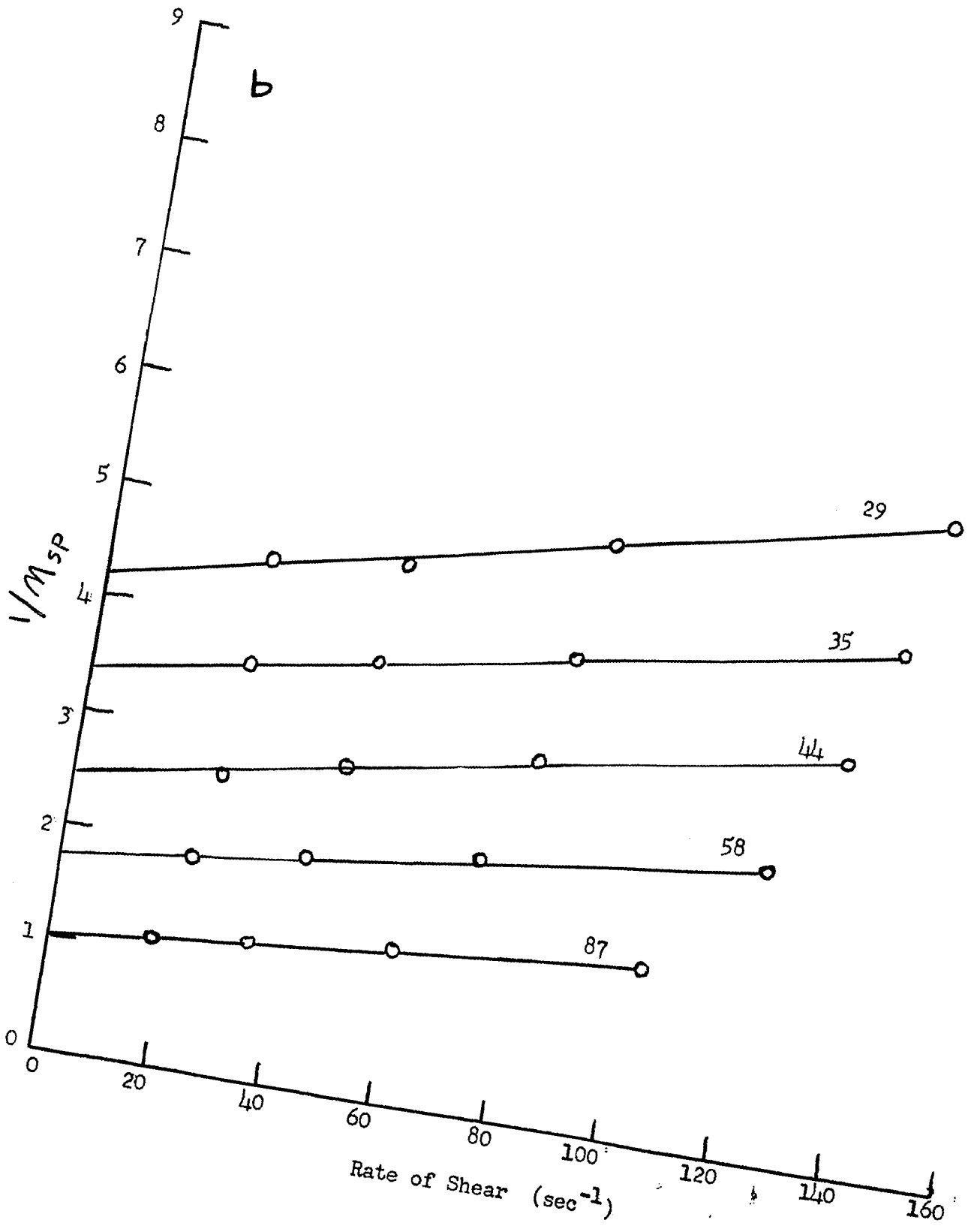

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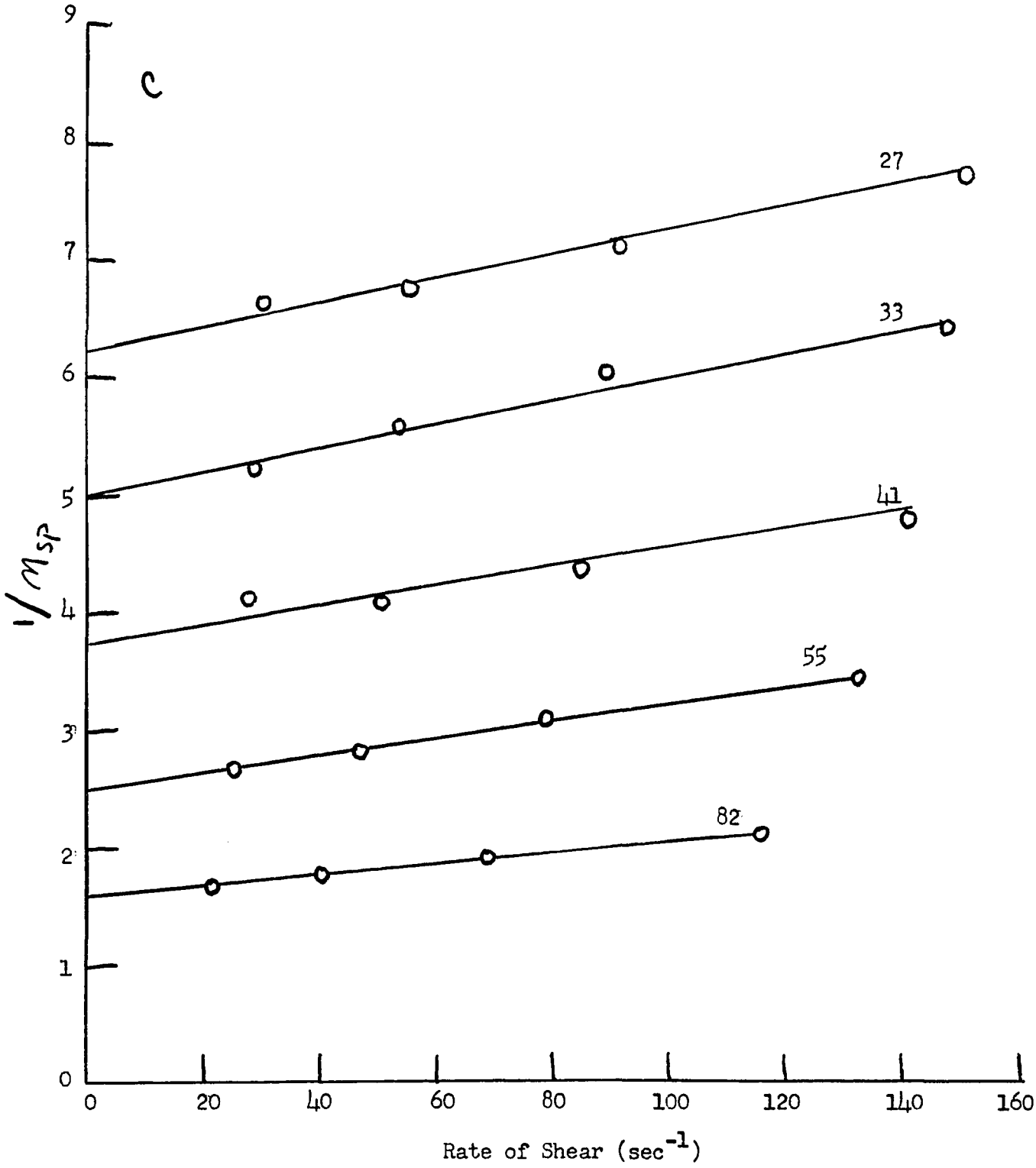

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Figure 5. Dependence of the Viscosity on the Rate of Shear for the Thermophilic DNA

a. B. stearothermophilus FJW

b. B. stearothermophilus 10

c. B. stearothermophilus 2184

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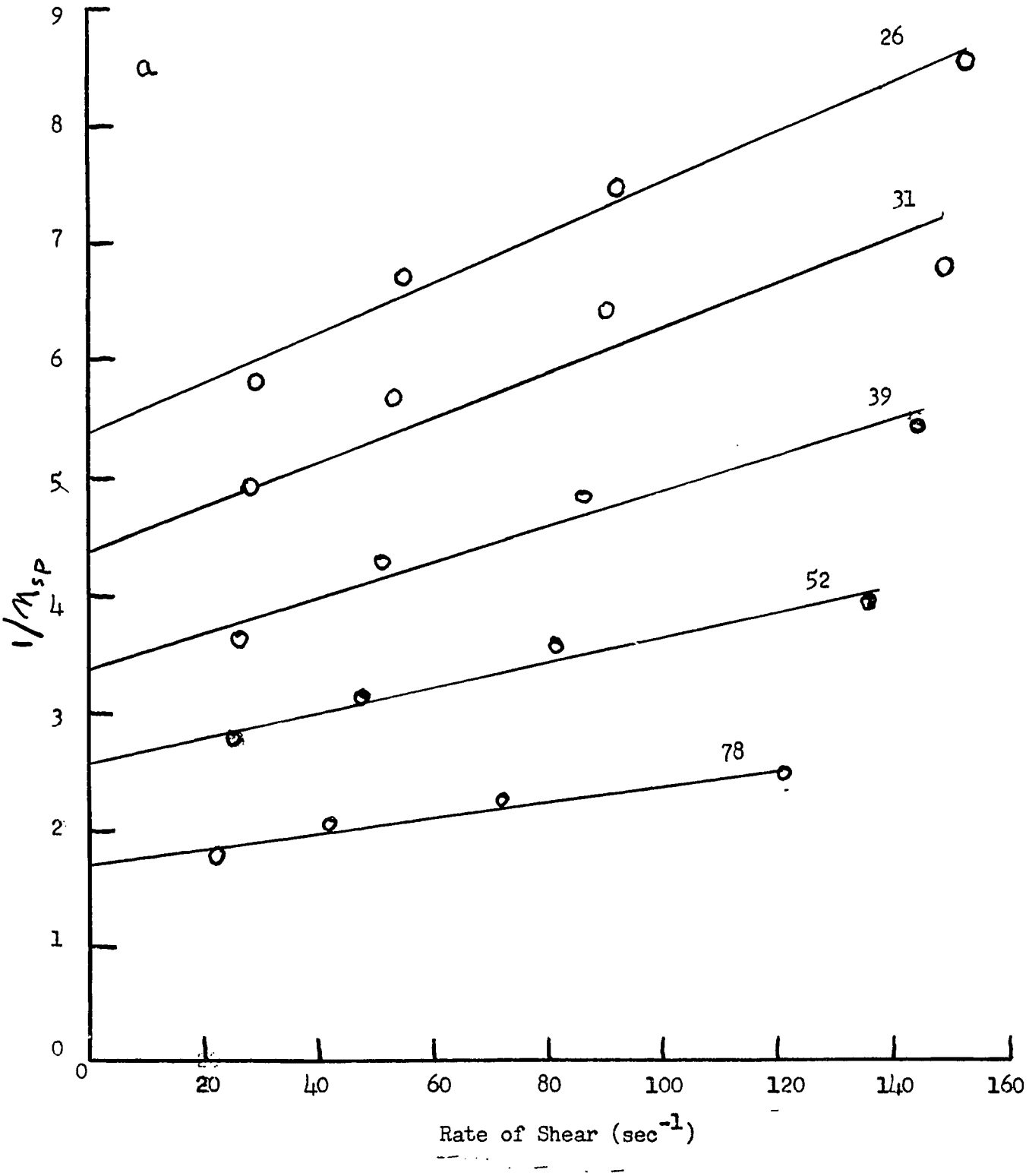

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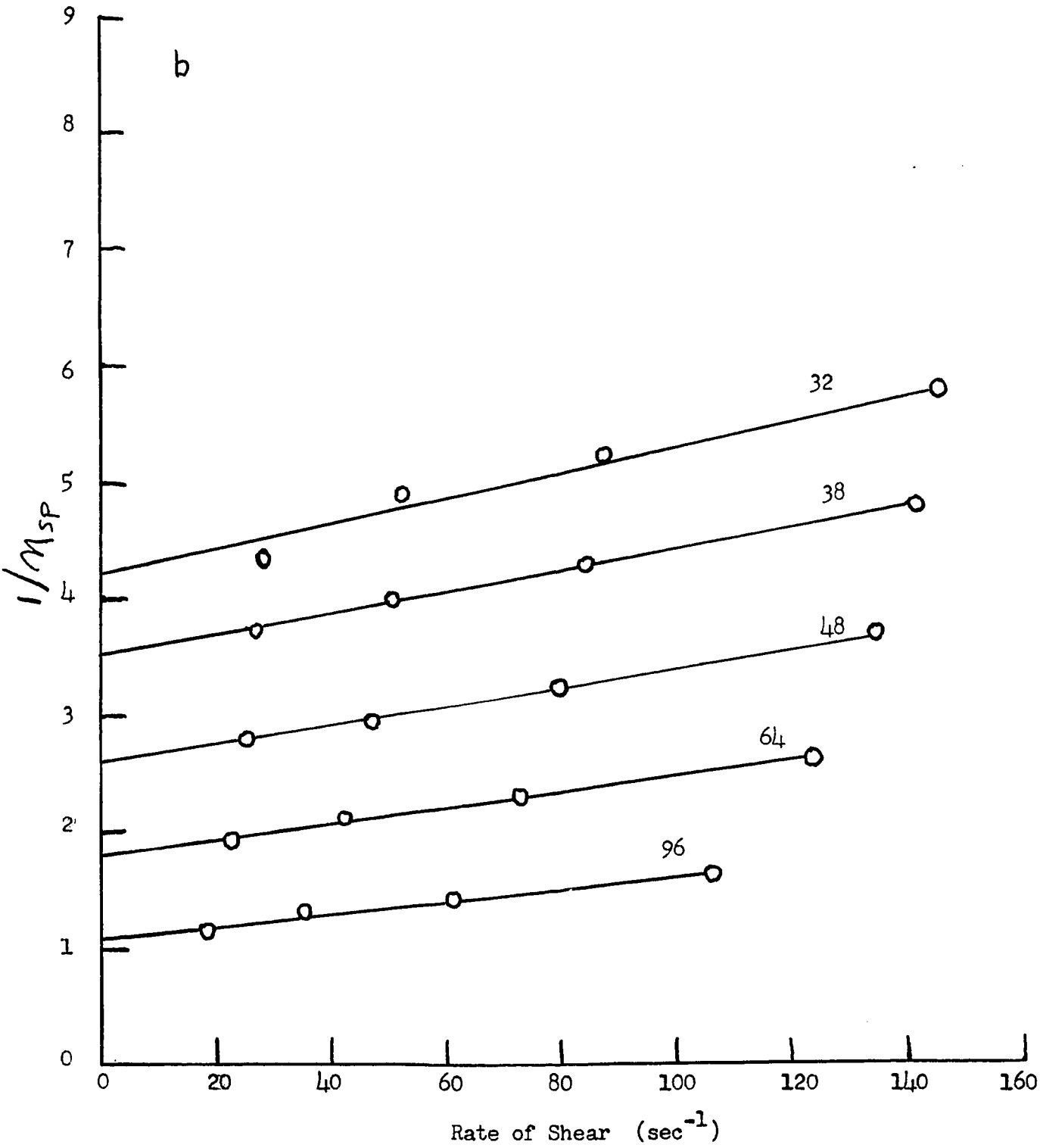

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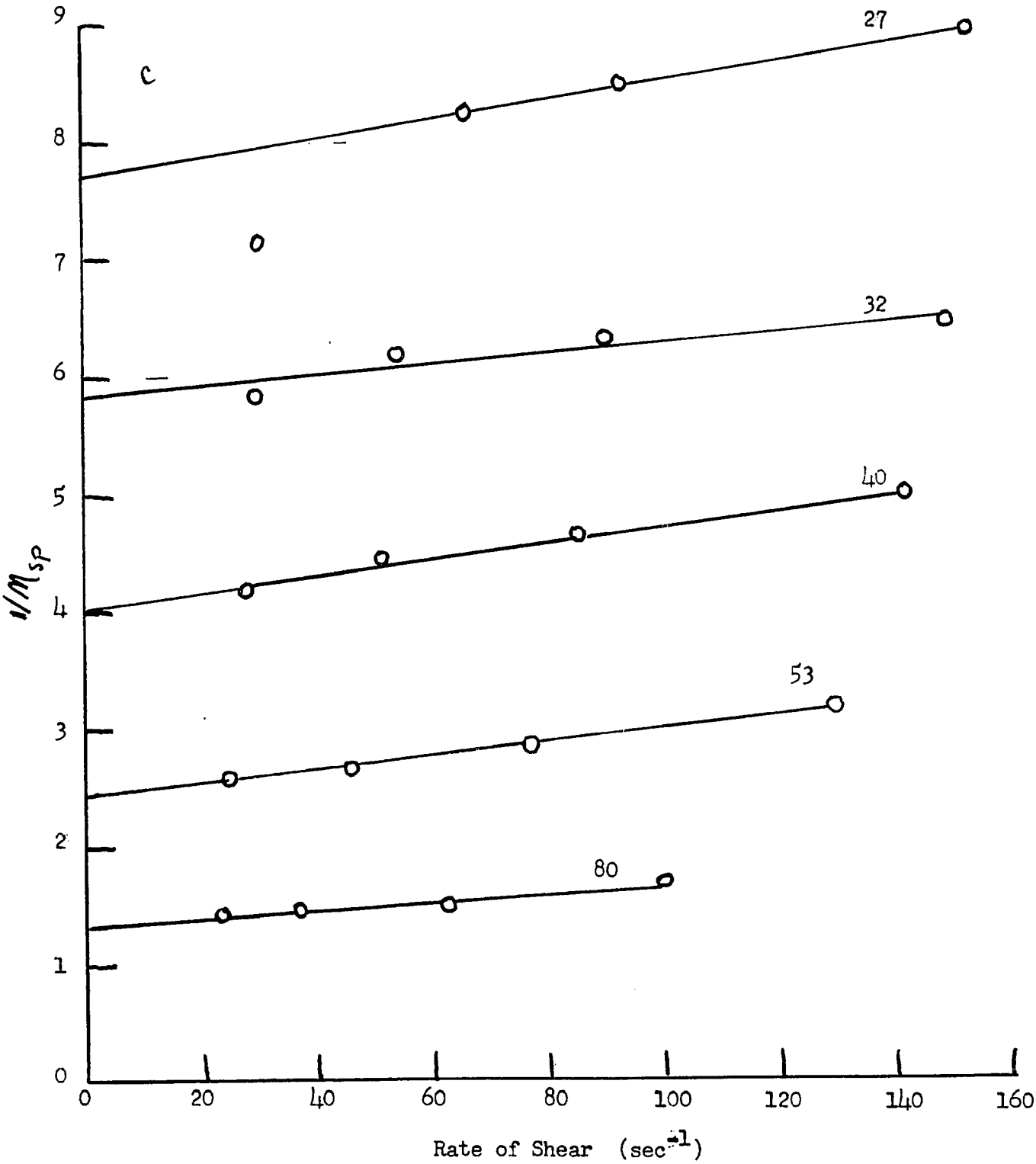

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Table VI. Viscosity

Organism	$[\eta]$ ml/g	Molecular Weight ($\times 10^{-6}$)	$(\overline{R}^2)^{1/2}$ (A)
B. lich.	4.70	8.0	5650
B. pum.	5.94	12.0	6800
B. X-1	6.60	15.0	7800
B. FJW	6.31	13.0	7320
B. 10	6.15	12.5	7150
B. 2184	2.56	3.7	3560

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Figure 6. Dependence of Viscosity on Concentration for the Mesophilic DNA

a. *B. pumilus*

b. *B. sp.* X-1

c. *B. licheniformis*

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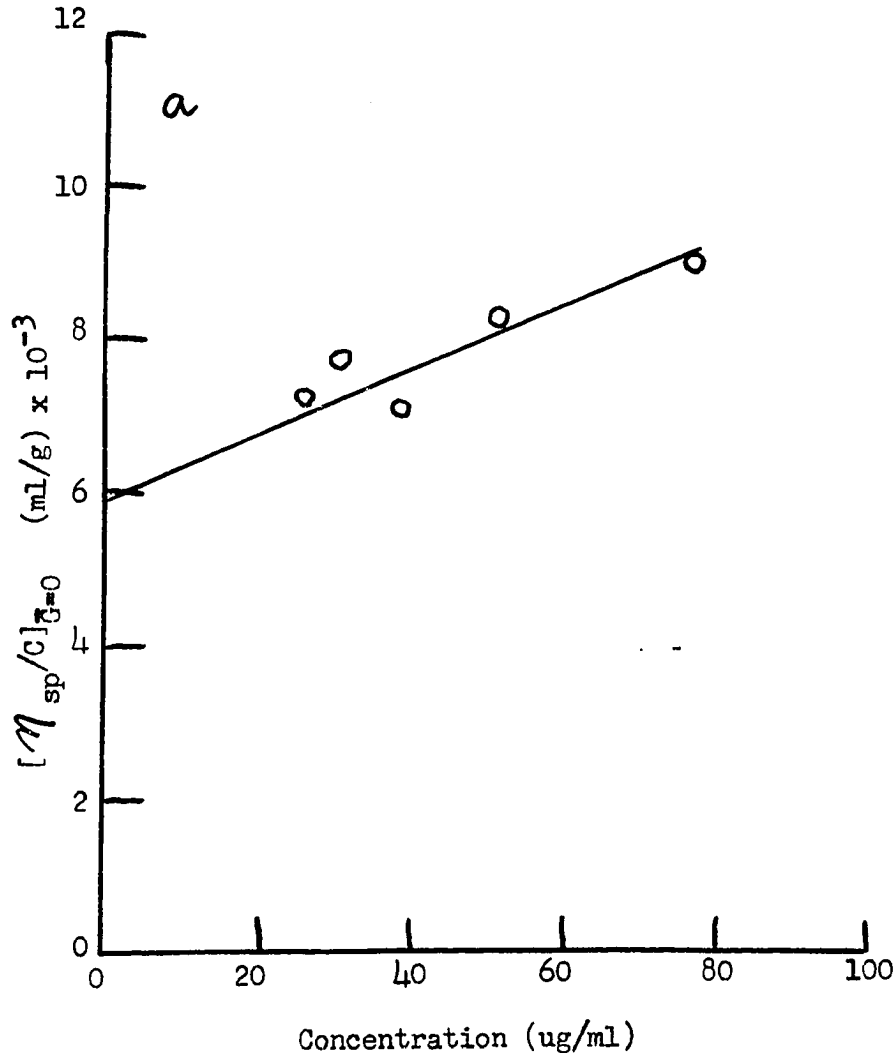

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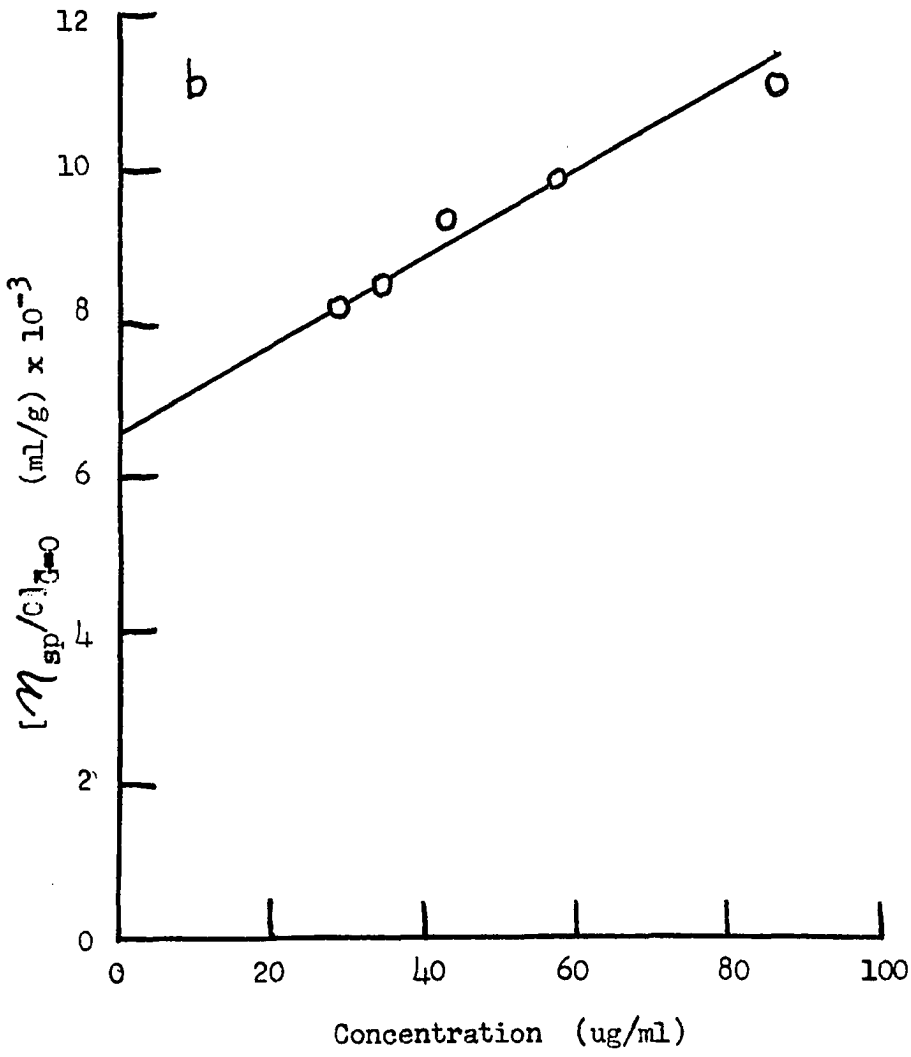

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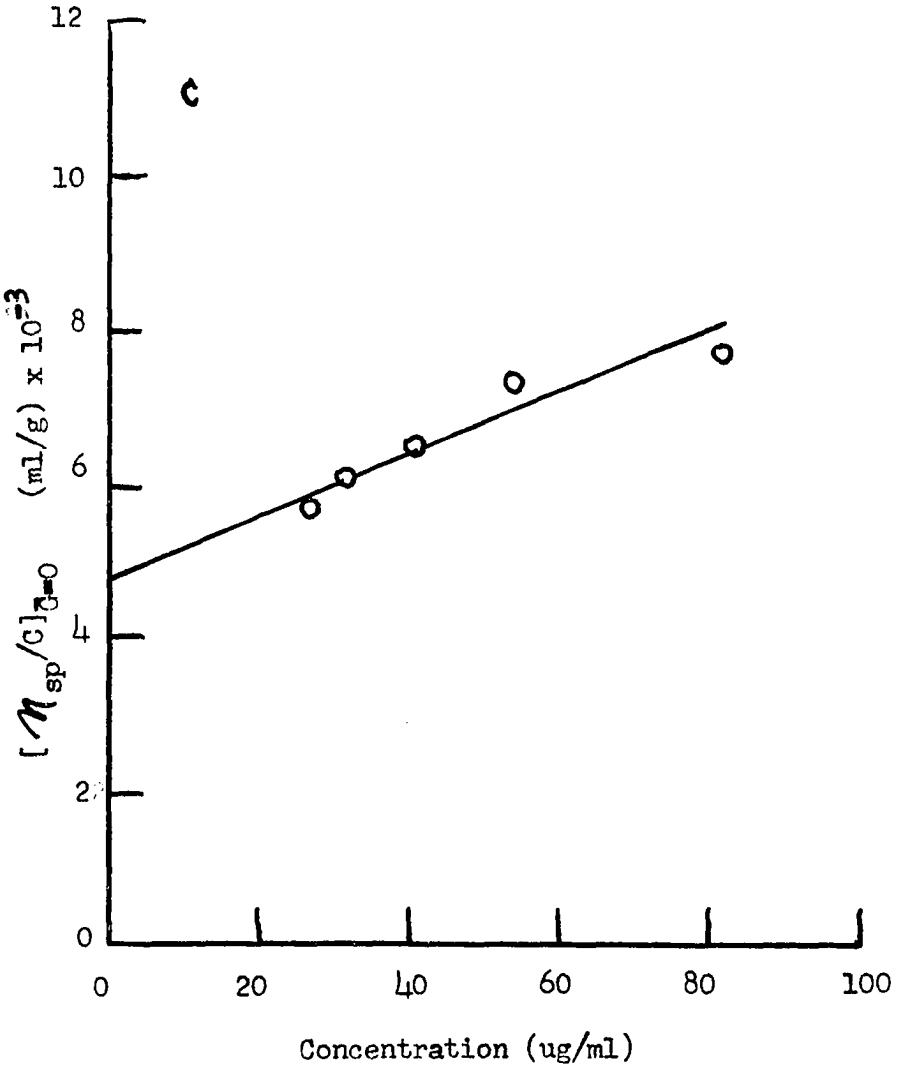

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Figure 7. Dependence of Viscosity on Concentration for the Thermophilic DNA

a. *B. stearothermophilus* FJW

b. *B. stearothermophilus* 10

c. *B. stearothermophilus* 2184

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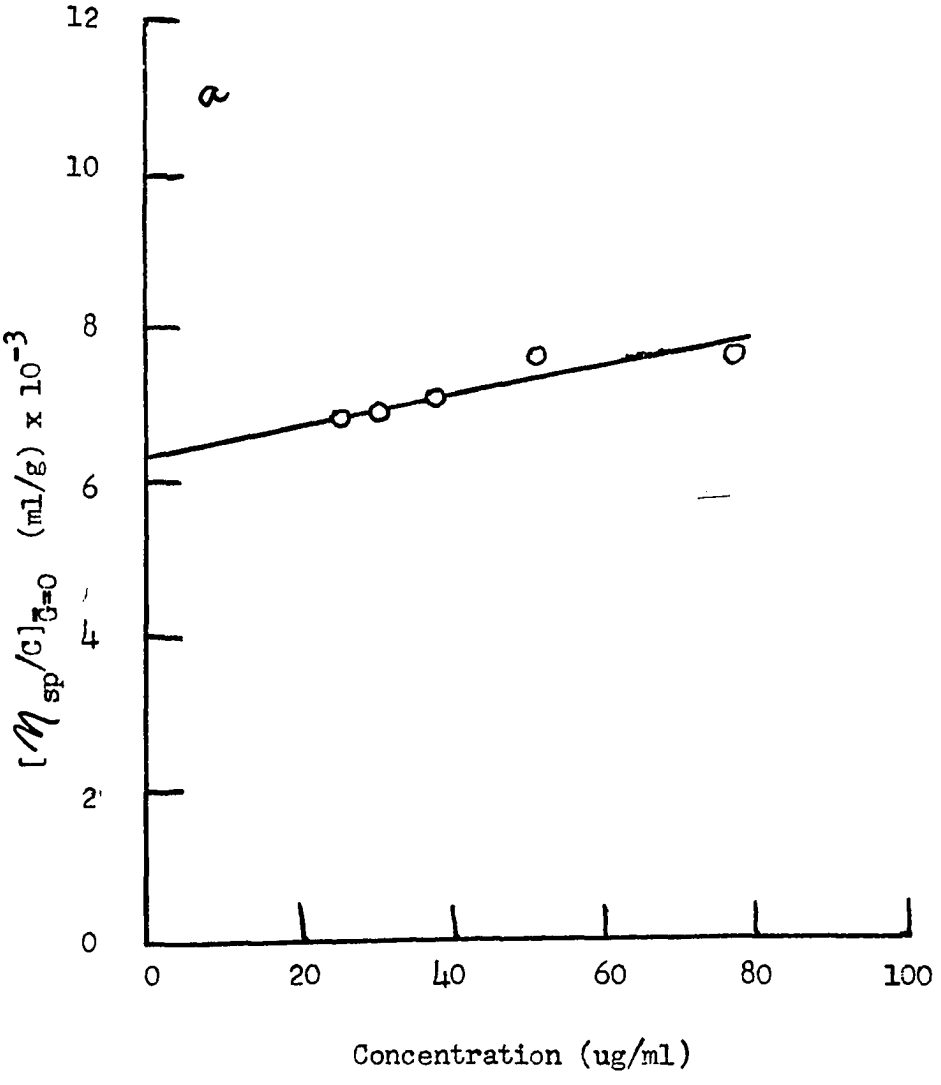

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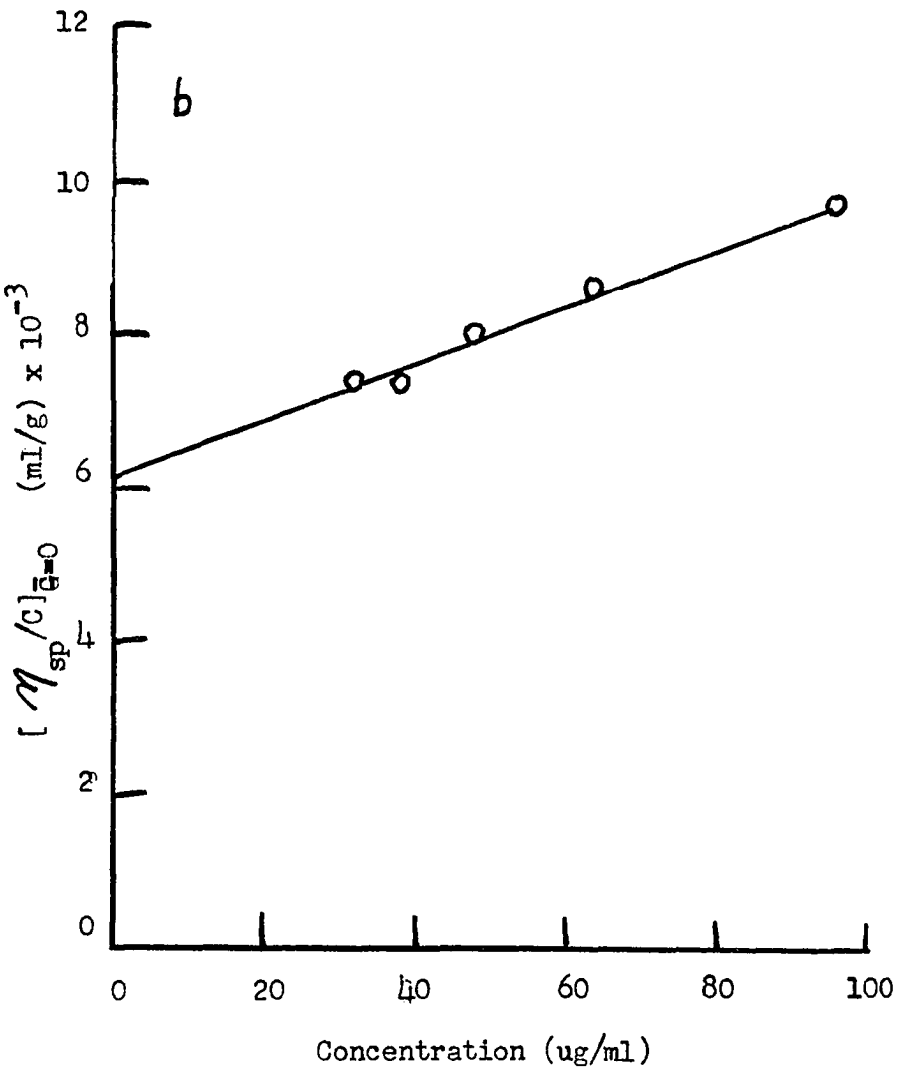

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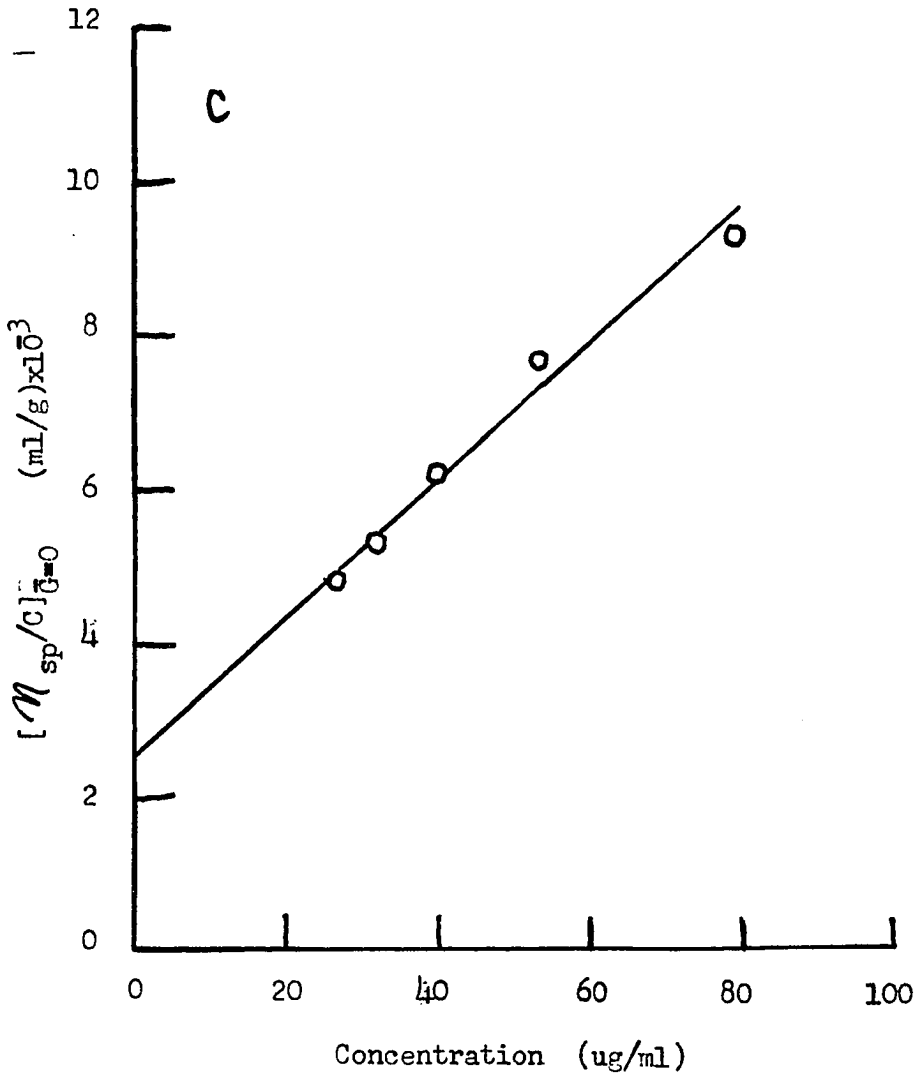

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stearothermophilus 2184) and the minimum (B. stearothermophilus FJW) dependence on concentration were exhibited by DNA preparations from thermophiles. This indicates a greater variability in the interactions between DNA molecules from thermophiles than between those from mesophiles.

The extrapolated value of the intrinsic viscosity (at zero rate of shear and zero concentration) is about 5-7 ml/g (Table VI) which is well within the range found by other workers for bacterial DNA (16). The molecular weights obtained from these values are very similar with the exception of the DNA of B. stearothermophilus 2184. For some reason this DNA had apparently been more degraded during the isolation. The molecular weights are of the order of 10×10^6 (Table VI) which is in agreement with molecular weights of other DNA's (16). Thus the method of isolation developed in this laboratory by Stenesh and Snyder (27) yields high molecular weight DNA which is essentially fully native. There was no correlation between the molecular weight of the DNA isolated and the thermal stability of the organism.

It has been pointed out (29) that a system of stiff coils probably best approximates the shape of the DNA molecule in solution. The root mean square end to end distance of such a molecule can be calculated from the Flory-Fox equation (28) and is in turn another measure

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of the asymmetry of the molecule. The root mean square end to end distance $(\overline{R}^2)^{1/2}$ has been calculated for the six DNA preparations and is shown in Table VI. With the exception of B. lichenformis and B. stearothermophilus 2184, the values are rather comparable. This again shows that in overall size and shape these DNA molecules are very similar.

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SUMMARY

The deoxyribonucleic acid from six strains of Bacillus has been studied. Three of these were mesophilic strains: B. licheniformis (NRS 243), B. pumilus (NRS 236), and B. sp. (X-1); and three were thermophilic strains: B. stearothermophilus 2184, B. stearothermophilus 10, and B. stearothermophilus FJW. Within experimental error, the DNA's were fully native.

Determination of the base composition in terms of the four major bases was carried out by paper chromatography, simultaneous spectral determinations, bromination with N-bromoacetamide, and from thermal denaturation profiles. These studies showed that the DNA from the thermophiles had a higher guanine plus cytosine content than the DNA from the mesophiles.

The thermal denaturation profiles showed a greater thermal stability for the DNA of the thermophiles compared to the DNA from the mesophiles.

The molecular weights and the root mean square end to end distances of the DNA's were determined from viscosity measurements and no significant differences were found between the DNA's from the two types of bacteria.

A determination of minor bases showed that 6-methylaminopurine was present in amounts less than 0.3% of

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the cytosine in all the strains and that 5-methylcytosine could not be detected in any of the strains.

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VITA

The author was born on January 1, 1942, in New York City, New York. He received his primary and secondary education in Hasbrouck Heights, New Jersey, and New Hyde Park, New York. He then studied at Hope College in Holland, Michigan, graduating with a degree of Bachelor of Arts in chemistry in June 1963. He is now employed as a chemistry, physics, and general science teacher in the Marshall Public Schools.

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