THE RELATION BETWEEN METHOCEL CONCENTRATION, FREQUENCY OF FOOD INGESTION, RATE OF LOCOMOTION, AND GEL/SOL RATIO IN AMOEBA PROTEUS

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Major professor

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THE RELATION BETWEEN METHOCEL CONCENTRATION, FREQUENCY OF FOOD INGESTION, RATE OF LOCOMOTION, AND GEL/SOL RATIO IN *AMOEBA PROTEUS*

By

NELLIE MARIE REUKEMA

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I. INTRODUCTION

Hopkins (1928) found a close correlation between the rate of locomotion and salt concentration of the culture medium. In buffered $CaCl_2$ or $SrCl_2$ solutions, the rate of locomotion increased from about zero in .0002 N $CaCl_2$ solution to a maximum in .0005 N solution, remained fairly constant until the concentration was increased to .005 N solution, and then rapidly decreased to zero in .05 N $CaCl_2$ solution, and to almost zero in $SrCl_2$ solution. Further, the nature of the substratum was an important factor in rate of locomotion. Rate of locomotion was highest in vessels coated with paraffin, lower in ordinary glass vessels, lower in quartz vessels, and lowest in Pyrex glass vessels. In monovalent (NaCl, KCl, LiCl or RbCl) buffered cation solutions, rate of locomotion reached a maximum in .0009 N solution, remained fairly constant until the concentration was .01 N, and then decreased to zero in .1 N solution.

Mast and Prosser (1932) studied the effects of temperature, salts, and hydrogen ion concentration on rupture of the plasmagel sheet, rate of locomotion and gel/sol ratio in Amoeba proteus. They demonstrated that the thickness of the plasmagel sheet was increased by decreasing the temperature and increasing the concentration of inorganic salts and hydrogen ions. As a consequence the frequency of rupture of the plasmalemma was decreased, but rate of locomotion was not specifically correlated with either the frequency of breaks in the plasmalemma, or the gel/sol ratio.

Mast and Pitts (1933, 1934a, 1934b) found that in a balanced salt solution the rate of locomotion increased from 0 at pH 5.0, to 257 microns per minute at pH 5.6, increased to a maximum of 281.8 microns at pH 6.2, decreased slightly to a median minimum at pH 7.0, and then decreased fairly rapidly to 148.0 microns at pH 8.0. Gel/sol ratio decreased as the hydrogen ion concentration decreased, but the rate of decrease was greater in the acid range than in the alkaline range.

In .001 N $NaCl_2$ solutions, rate of locomotion increased from 240 microns per minute to a maximum of 265 microns at pH 6.8, then decreased to 90 microns at pH 8.0. Gel/sol ratio rapidly decreased to a minimum as the hydrogen ion concentration decreased. In .001 N $CaCl_2$ solutions, rate of locomotion increased from 0 at pH 4.7 to 265 microns at pH 5.0, and then remained fairly constant to pH 8.0.

In solutions in which the concentration of K was .005 N, and the $CaCl_2$ concentration was .001 N, rate of locomotion increased from 1 at pH 4.1 to a maximum of 265 microns at pH 6.2, decreased to a minimum of 195 microns at pH 7.0, increased to a second maximum of 265 microns at pH 7.5, and then it remained fairly constant to pH 8.0.

Mast and Fennell (1938) found that frequency of ingestion by specimens of Amoeba proteus increased from zero in Chalkley solution to a maximum at 25 degrees, decreased to a median minimum at 30 degrees, increased to a second maximum at 35 degrees, and then rapidly decreased to zero at 40 degrees. These results strongly suggest that the factors

which control ingestion are essentially the same as those which control locomotion.

It is evident from the preceding review of the literature that the relation between inorganic salt concentration, hydrogen ion concentration, and frequency of ingestion of food by Amoeba proteus has been studied in considerable detail. Further, the relation between environmental factors and rate of locomotion and gel/sol ratio has been investigated extensively. However, there is no information concerning rate of locomotion, ingestion of food, and gel/sol ratio in balanced salt solutions with variable viscosities. Thus it is the objective of the present study to obtain additional information concerning these various physiological processes in Amoeba proteus.

II. METHODS AND MATERIALS

Dow Methocel was used to study the effects of viscosity on physiological processes in Amoeba proteus. Methocel is a cellulose ether manufactured in the form of white fibers. It is soluble in cold water, insoluble in hot water, saturated salt solutions, and most organic solvents, and is unaffected by oily or greasy materials of animal, vegetable or mineral origin. It is stable in alkalies and dilute acids. Aqueous solutions are stable and ordinarily do not require a preservative.

Methocel (15 centipoise) was prepared by thoroughly mixing 40 grams of the material with 500 cc. of Chalkley solution at a temperature of about 95°. It was left to soak for 30 minutes and then a 4 per cent solution was made by adding 500 cc. of Chalkley solution to the mixture. This solution was cooled to room temperature and stirred until smooth.

On analysis of the Methocel viscosity concentration chart, it can be seen that with a solution of 15 centipoise Methocel, the viscosity of the experimental solutions used varies considerably, reaching about 5.8 centipoise in a 1.0 per cent Methocel solution by weight. Since water has a viscosity of 1 centipoise, the addition of even a .1 per cent solution of Methocel will raise the viscosity considerably. As the concentration in percentage by weight increases, the viscosity increases markedly, so that in a 15 centipoise Methocel solution at a concentration of 4 per cent, the viscosity increases to 80 centipoise at a temperature of 20°.

The viscosity of Methocel solutions in centipoise is based on the viscosity of a 20 per cent aqueous solution at 20°. There are six viscosity types with varying centipoise values of 15, 25, 100, 400, 1,500 and 4,000. The 15 centipoise type was selected for experimentation.

All glassware used in the following experiments was thoroughly cleaned with cleaning solution (potassium-dichromate-sulfuric acid), rinsed 15 times with tap water and 3 times with distilled water. Distilled water used for preparation of Chalkley solutions and cultures was redistilled through Pyrex glass. C.P. chemicals were used exclusively.

Amoeba proteus was used exclusively in the following experiments. All organisms were cultured in Chalkley solution (100 mg. NaCl, 4 mg KCl, 6 mg. $CaCl_2$, and 1,000 cc. glass distilled water)[1]. Fifty amoebae were selected from a stock culture and transferred[2] to 200 cc. of Chalkley solution in a clean finger bowl. Three grains of rice were added to the culture and then the finger bowl was set aside until the amoebae were abundant. Essentially the same procedure was used for the preparation of all stock cultures used in these experiments.

Tetrahymeni geleii was used exclusively for study of the frequency of food ingestion by Amoeba proteus. A bacteria free stock culture was obtained from Dr. A. M. Elliot, Department of Zoology, University of Michigan. Subcultures of

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[1] Galtsoff: Culture Method For Invertebrate Animals

[2] Transferred by means of a pipette in order to reduce the amount of handling of the amoebae and thus the possibilities of mechanical injuries.

this organism were made in the following manner: About 50 cc. of culture solution (15 gms. Bacto-Tryptone, 1 gm. KH_2PO_4 and 1,000 cc. distilled water) were added to each of 5 sterile 125 cc. Erlenmeyer flasks. The flasks were autoclaved at 15 pounds steam pressure for 15 minutes, left to cool; then about 5 cc. of sterile stock culture were added to each of the flasks. They were then set aside and left at room temperature until the organisms were abundant. All subcultures used for experimentation were made by essentially the same methods.

Organisms were prepared for experimentation by the following method: An Erlenmeyer flask containing 50 cc. of Tryptone solution, in which specimens of Tetrahymeni geleii were abundant, was used for obtaining the necessary food for the study of ingestion by Amoeba proteus. To each of two 15 cc. centrifuge tubes 15 cc. of the culture solution were added, and these were then plugged with sterile cotton. The tubes were centrifuged at a moderate speed until the organisms were concentrated in the bottom of the tube. The tubes were removed from the centrifuge, 14 cc. of the supernatant fluid were poured off, and the volume of solution in each tube was again made up to 15 cc. by adding 14 cc. of sterile Chalkley solution. The tube was gently agitated until the specimens of Tetrahymeni geleii were uniformly distributed throughout the Chalkley solution. This procedure was repeated two additional times. Before transfer of the organism to the vessel in which ingestion was to be studied, the organisms were again concentrated by centrifuging and 14 cc. of the

solution were removed with a pipette. Thus the organisms were suspended in 1 cc. of solution. Essentially the same procedure was used for preparation of organisms that were to be used in experimental solutions, but the viscosity of the experimental solution was kept constant by a washing of the organisms in Chalkley solution to which the desired quantity of Methocel had been added.

The amoebae were prepared for experimentation by the following method: Two hundred amoebae were selected from a stock culture and transferred to 5 cc. of .1 per cent Methocel in Chalkley solution and left for 24 hours. After 4 cc. of the Methocel solution were removed, 4 cc. of fresh .1 per cent Methocel in Chalkley solution were added to the vessel containing the amoebae. This procedure was repeated two additional times. The amoebae were left for 20 minutes and then 4 cc. of the Methocel solution were removed with a pipette. Specimens of Tetrahymeni geleii suspended in 1 cc. of .1 per cent Methocel in Chalkley solution were added to the medium in which the amoebae were suspended. The volume of the solution in the vessel was made up to 5 cc. by the addition of 3 cc. of .1 per cent Methocel in Chalkley solution. After 20 minutes, 25 amoebae were removed from the vessel and put on glass slides. They were covered with a cover slip and sealed with vaseline, and then the organisms ingested by the amoebae were counted. The number of organisms ingested in 60 and 90 minute periods was ascertained in essentially the same manner.

Rate of locomotion and gel/sol ratio in Amoeba proteus was ascertained in various concentrations of Methocel in Chalkley solution. Organisms[3] were prepared by essentially the same procedure as described for study of ingestion, but in these experiments the addition of food to the experimental solution was omitted. Rate of locomotion was measured in microns per minute with a camera lucida and a stopwatch. Readings were made at 1 minute intervals for 5 minute periods. The average rate of locomotion for the 20 individuals will be presented in the following pages.

Amoeba proteus is characterized by a relatively rigid outer layer of plasmagel and a more fluid layer of plasmasol. Both layers exhibit granules of various sizes, but the concentration of granules is higher in the plasmagel. In monopodal specimens the plasmagel is tube-like and the plasmasol flows in the direction of locomotion. The width of the plasmasol and plasmagel was measured by means of a camera lucida. The gel/sol ratio was calculated by obtaining the average width of the plasmasol and plasmagel in 20 or more individuals.

In order to present all the experimental evidence in a form which could be easily read and compared, it seemed expedient to determine the mean control of each experiment, and

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[3] Monopodal amoebae were used exclusively. An animal with a single pseudopod, moving continually, is described as a monopodal amoeba. Rate of locomotion in such specimens is fairly constant and the variation between measurements on the same animal and measurements on different animals in the same medium is negligible.

to place in proportion to this average control the experimental results obtained in the various percentage concentrations of Methocel in Chalkley solution. The results placed in this proportion were then plotted on graphs.

III. THE RELATION BETWEEN VISCOSITY OF THE CULTURE SOLUTION AND RATE OF LOCOMOTION IN *AMOEBA PROTEUS*

All organisms used in the experiments were prepared for experimentation by the methods described in the preceding pages. Analytical grade chemicals and glass distilled water were used exclusively for preparation of Chalkley solution. The various concentrations of Methocel were prepared by dilution of 4 per cent stock solution. Temperature was maintained at 22°. Rate of locomotion was adjusted by use of the following formula: $X = E \times (M/C)$. X, adjusted rate of locomotion; E, rate of locomotion in each experimental solution; M, arithmetic mean for locomotion in control solution; and C, rate of locomotion in individual control solution. The results obtained are presented in Table I, and Figure I.

It is evident in Table I, Figure I, that rate of locomotion increased from 337 microns per minute in a .1 per cent Methocel solution to 365 microns per minute in a .2 per cent solution, decreased to 274 microns per minute in a .3 per cent solution, again increased to reach 333 microns per minute in a .5 per cent solution, and then gradually decreased to 234 microns per minute in a 1.0 per cent Methocel solution. It is evident from this analysis of data that locomotion neither consistently decreased or increased in concentrations below .5 per cent but in concentrations above this figure rate of locomotion consistently decreased as the concentration of Methocel was increased to 1.0 per cent.

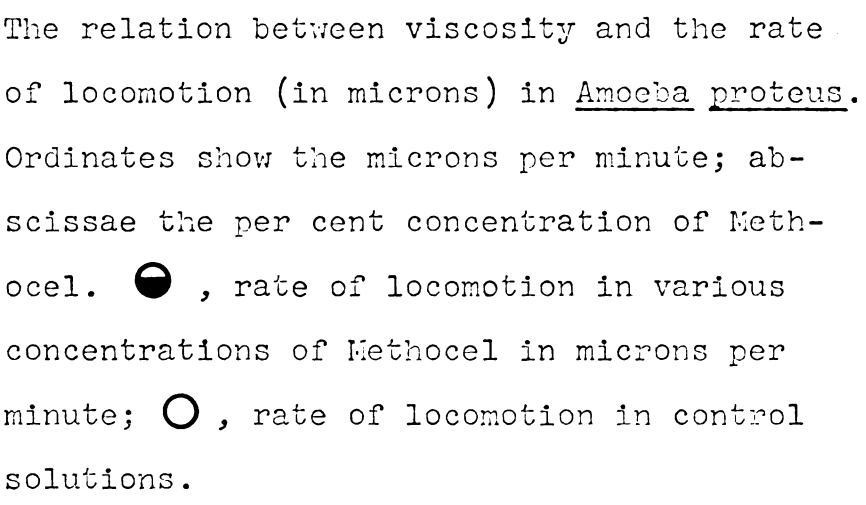
TABLE I

The Relation Between Viscosity and the Rate of Locomotion

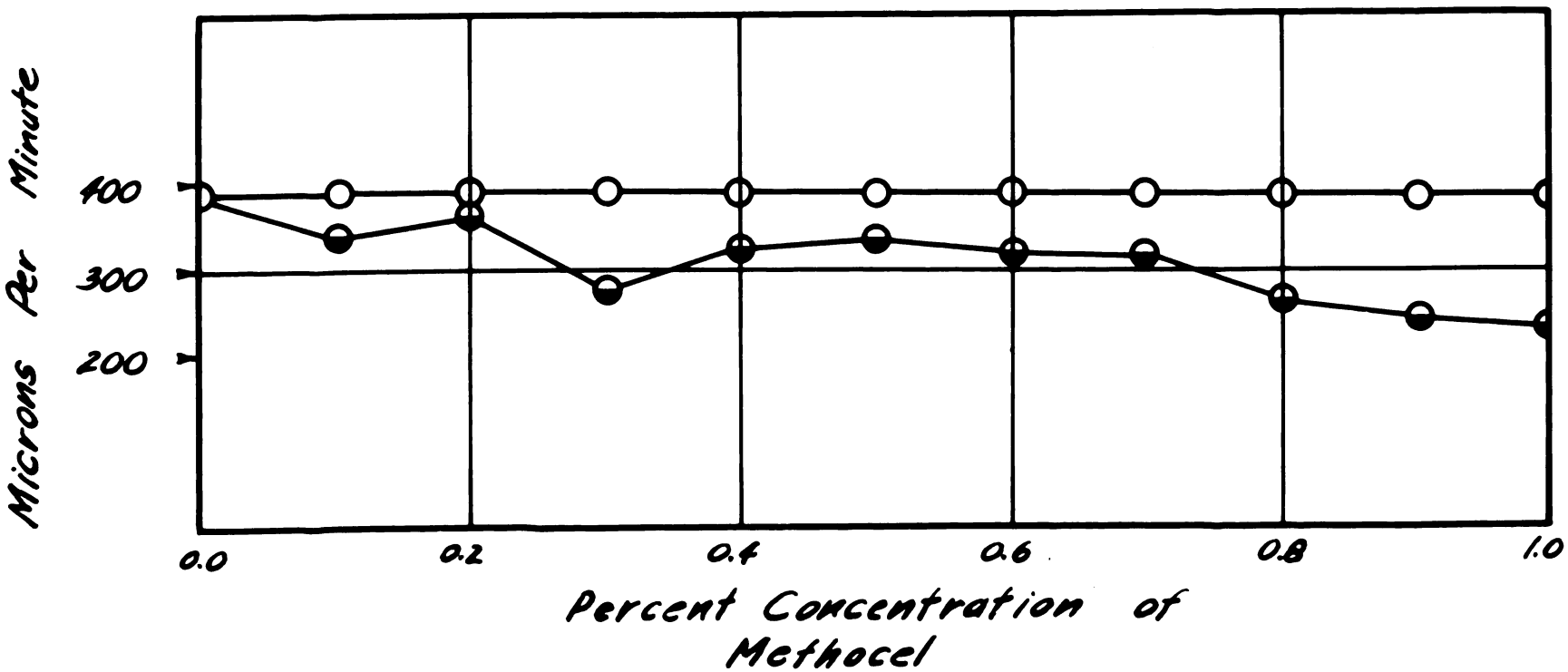
(in microns)

Per Cent Concentration of Methocel	No. of Organisms Tested	Avg. Rate of Locomotion in per min.	Range of Rate of Locomotion in per minute		Adjusted Rate of Locomotion
			Range		
.1%	23	283	123	598	337
Control	19	329	104	800	392
.2%	22	312	77	815	365
Control	18	335	143	800	392
.3%	23	241	51	500	274
Control	20	345	189	800	392
.4%	24	322	113	613	325
Control	23	388	113	800	392
.5%	21	324	143	780	333
Control	22	381	163	800	392
.6%	20	278	123	710	322
Control	23	338	120	800	392
.7%	23	333	165	633	317
Control	23	412	148	800	392
.8%	22	321	110	603	262
Control	24	480	310	800	392
.9%	26	292	92	563	248
Control	26	461	280	810	392
1.0%	25	276	50	550	234
Control	26	461	310	820	392

FIGURE I



The relation between viscosity and the rate of locomotion (in microns) in *Amoeba proteus*. Ordinates show the microns per minute; abscissae the per cent concentration of Methocel. ◒ , rate of locomotion in various concentrations of Methocel in microns per minute; ○ , rate of locomotion in control solutions.


Microns Per Minute
400
300
200
0.0
0.2
0.4
0.6
0.8
1.0
Percent Concentration of Methocel

The question immediately arises whether or not the observed fluctuations in rate of locomotion are significant in low concentrations of Methocel (.1 to .5 per cent). It was found during the course of experimentation that environmental factors such as abundance of food, age of culture, and methods of handling affected rate of locomotion. Thus it is possible and also probable that the observed fluctuations in rate of locomotion in low concentrations of Methocel can be attributed to environmental factors.

The results presented in the preceding paragraphs strongly suggest that in higher concentrations of Methocel (.6 to 1.0 per cent) an inverse relationship exists between rate of locomotion and viscosity, i.e., as the viscosity of the culture solution increases, rate of locomotion decreases. This view is consistent with that of Stiles (1947) who found an inverse relationship between viscosity and rate of locomotion in Paramecia.

IV. THE RELATION BETWEEN VISCOSITY AND INGESTION OF FOOD BY AMOEBA PROTEUS

Mast and Fennell (1938) found that ingestion of chilomonads by Amoeba proteus is not closely correlated with the number of organisms in the solution. Specimens of amoebae ingested an average of 80 organisms in 120 minutes in a solution containing 300,000 chilomonads per cc., and only 81 organisms in 120 minutes in a solution in which the number of chilomonads was 600,000 per cc.

Since there can be great variations in the number of organisms without appreciably altering rate of ingestion in the amoebae, the number of specimens of Tetrahymeni geleii added to the various solutions of Methocel was not ascertained. However, a special effort was made to have an abundance of food organisms in both the experimental and the control solutions.

Mast and Fennell (1938) found that as the temperature increased, frequency of ingestion increased to a maximum at 25°, and then decreased to zero at about 40°. Care was taken to maintain the temperature of the experimental solutions at about 22°. All amoebae and Tetrahymeni geleii used in these experiments were prepared for experimentation by the methods described in the preceding pages.

The results obtained in various concentrations of Methocel solutions are presented in Tables II and III. The raw results obtained in these experiments are presented in Table II, and the corrected or adjusted ingestion rates are presented

TABLE II

The Relation Between Viscosity and the Frequency of Ingestion of Food by *Amoeba proteus*

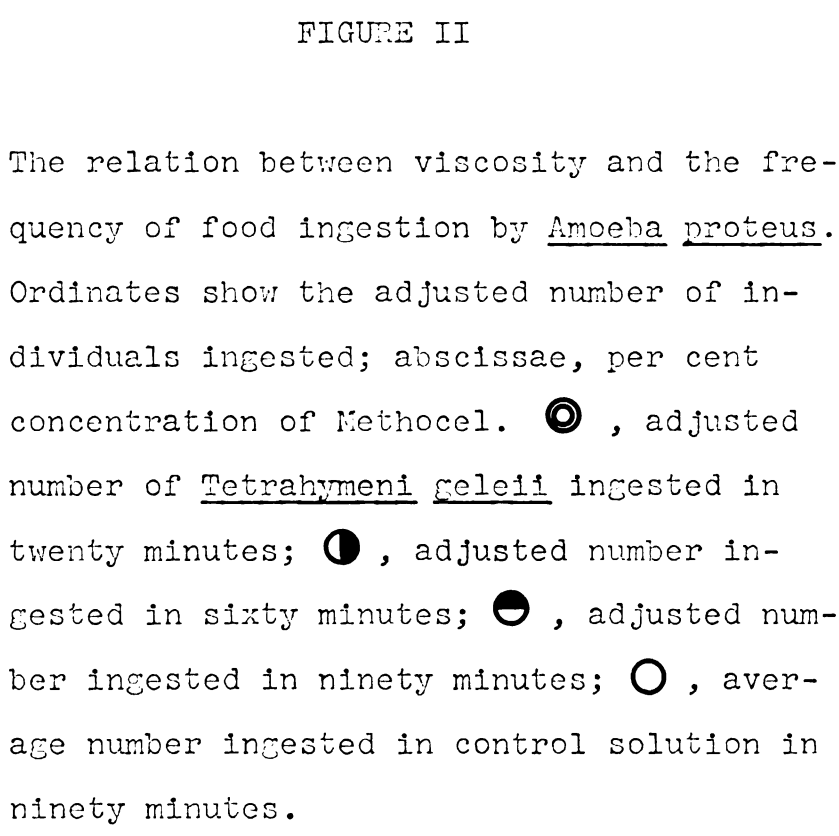
Per Cent Concentration of Methocel	No. of Organisms Tested	Average No. of *Tetrahymeni geleii* Ingested			No. Ingested per Individual in 90 minutes	
		20 Min	60 Min	90 Min	Range	
.1%	86	1.55	2.25	2.75	0	9
Control	77	2.12	4.24	5.16	0	15
.2%	82	1.56	2.35	2.56	0	8
Control	74	2.29	3.64	4.20	0	10
.3%	90	1.65	1.91	1.95	0	9
Control	93	2.65	3.95	4.25	0	16
.4%	81	2.19	2.94	2.35	0	8
Control	88	2.79	4.74	3.19	0	9
.5%	105	3.23	4.91	5.08	0	14
Control	110	3.85	5.41	7.88	0	17
.6%	99	2.67	3.50	4.18	0	18
Control	99	3.34	4.85	5.80	0	15
.7%	104	1.99	3.50	3.79	0	12
Control	102	3.65	5.72	6.25	0	15
.8%	102	2.44	3.74	5.41	0	15
Control	107	5.40	6.36	7.55	0	15
.9%	101	1.23	2.01	2.31	0	10
Control	113	3.27	4.34	4.98	0	19
1.0%	107	.91	1.58	2.25	0	11
Control	111	3.29	4.26	5.10	0	19

TABLE III

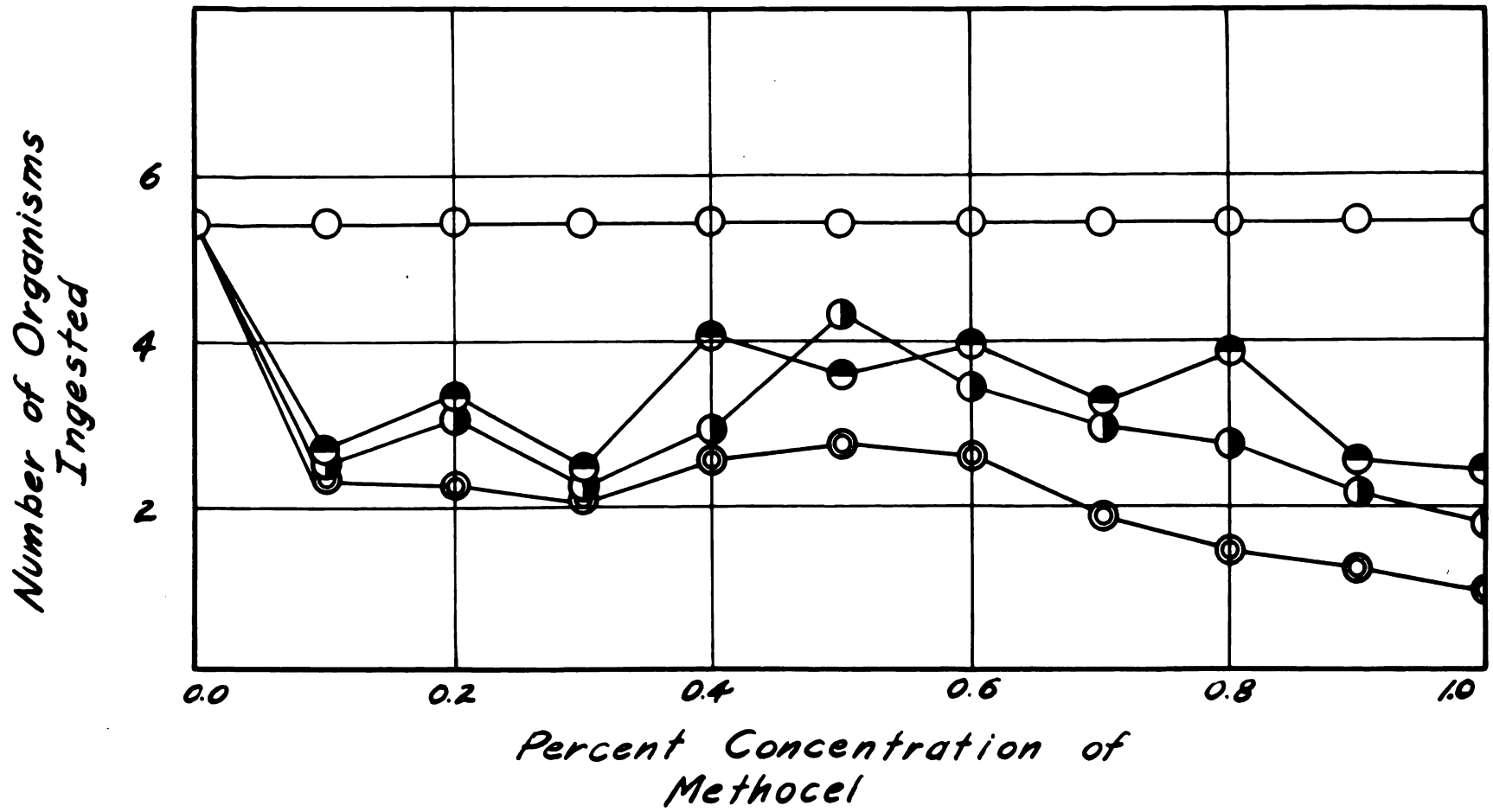
The Relation Between Viscosity and the Frequency of Ingestion of Food by *Amoeba proteus*

Per Cent Concentration of Methocel	Adjusted No. of *Tetrahymeni geleii* ingested		
	Time in Minutes		
	20	60	90
.1%	2.39	2.52	2.71
.2%	2.23	3.07	3.32
.3%	2.04	2.29	2.49
.4%	2.57	2.94	4.01
.5%	2.74	4.31	3.57
.6%	2.61	3.43	3.92
.7%	1.78	2.91	3.29
.8%	1.48	2.79	3.89
.9%	1.23	2.19	2.52
1.0%	.91	1.74	2.40
Control	3.27	4.75	5.44

FIGURE II



The relation between viscosity and the frequency of food ingestion by Amoeba proteus. Ordinates show the adjusted number of individuals ingested; abscissae, per cent concentration of Methocel. ◎ , adjusted number of Tetrahymeni geleii ingested in twenty minutes; ◑ , adjusted number ingested in sixty minutes; ◓ , adjusted number ingested in ninety minutes; ○ , average number ingested in control solution in ninety minutes.


Number of Organisms Ingested
6
4
2
0.0
0.2
0.4
0.6
0.8
1.0
Percent Concentration of Methocel

in Table III and Figure II. Adjusted rate of ingestion was obtained by the formula presented in Section III.

It is evident in Table III that the number of organisms ingested by Amoeba proteus in 90 minutes increased from 2.71 in a .1 per cent solution of Methocel to 4.01 in a .4 per cent solution, remained fairly constant until the concentration reached .8 per cent, and then decreased to 2.40 in a 1.0 per cent solution. A maximum number of 2.74 and 4.31 organisms was ingested in a .5 per cent solution in 20 and 60 minute periods respectively. As the concentration of Methocel increased from .5 per cent to 1.0 per cent solutions, ingestion decreased from 2.74 to .91 in 20 minutes, and from 4.31 to 1.74 in 60 minutes.

It is evident in Table II, that the number of organisms ingested by the amoebae reached a maximum of 5.08 organisms in .5 per cent Methocel solution. These results strongly suggest that this concentration of Methocel increased the availability of Tetrahymeni geleii for ingestion by decreasing ciliary activity, i.e., by retardation of locomotion. The availability of food particles was found to be an important factor in ingestion by Fennell (1944). He demonstrated that low concentrations of strychnine sulphate in NaCl solution (.0029 M NaCl plus .000069 M strychnine sulphate) inactivated specimens of Chilomonas paramecium and increased adhesiveness of the plasmalemma. Because of this existential relationship between inactivation and adhesiveness, large numbers of chilomonads adhered to the cell surface. The number of chilomonads ingested increased from 29 in 10 minutes

to 46 in 20 minutes. These results were considered highly significant since the t[1] value in the former was 10.45 and in the latter it was 7.45.

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[1] By t value is meant the difference between the means divided by the standard error of that difference. When the t value is over 2.6 the results are considered significant.

V. THE RELATION BETWEEN VISCOSITY AND GEL/SOL RATIO IN AMOEBA PROTEUS

The gel/sol ratio was measured by methods essentially the same as those used by Mast and Prosser (1932). All measurements were made on monopodal specimens. The diameter of the plasmasol and the widths of the plasmagel were measured after projection on a sheet of black paper of the image of the organisms under observation. An average for the regions was obtained by marking the projected image at the posterior, middle and anterior ends. Then the mean diameter of the plasmasol and the mean widths of the plasmagel were carefully measured with the projected image of a micrometer slide which was calibrated in .01 mm. units. The mean diameter divided by the sum of the mean widths is presented as gel/sol ratios. Each figure denoting gel/sol ratio is an average obtained by measuring the plasmasol and plasmagel in 24 to 28 specimens. The results obtained are presented in Tables IV and V, and in Figure III, along with the corrected or adjusted ratios obtained by the formula presented in Section III.

It is evident in Table IV, Figure III, that the gel/sol ratio decreased from 1.21 in a .1 per cent Methocel to a minimum of 1.09 in a .3 per cent Methocel, increased slightly to reach 1.45 in a .5 per cent Methocel, decreased to 1.35 in a .7 per cent Methocel, increased to a maximum of 1.76 in a .9 per cent Methocel, and then decreased to 1.42 in a 1.0 per cent Methocel solution.

TABLE IV

The Relation Between Viscosity and the Gel/sol Ratio in *Amoeba proteus*

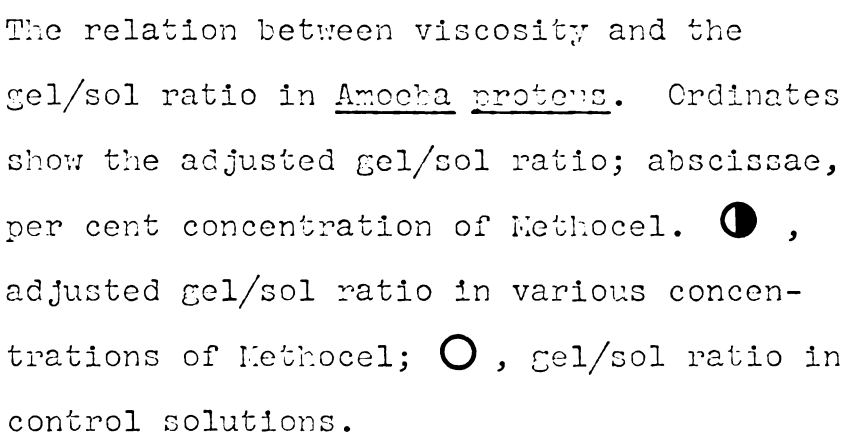
Per Cent Concentration of Methocel	Gel/Sol Ratios in the Various Experiments				Arithmetic mean	Adjusted Gel/Sol Ratio
	No. 1	No. 2	No. 3	No. 4		
.1%	1.28	1.56	1.28	1.11	1.31	1.21
Control	1.11	1.38	1.36	1.16	1.25	1.15
.2%	1.31	1.34	1.41	1.20	1.32	1.22
Control	1.11	1.50	1.50	1.16	1.24	1.15
.3%	1.21	1.21	1.26	1.16	1.21	1.09
Control	1.51	1.36	1.10	1.16	1.28	1.15
.4%	1.49	1.28	1.19	1.10	1.27	1.28
Control	1.51	.82	1.05	1.16	1.14	1.15
.5%	1.56	1.25	1.22	1.22	1.31	1.45
Control	1.08	1.03	.87	1.16	1.04	1.15
.6%	1.34	1.19	.86	1.29	1.17	1.45
Control	1.08	.81	.76	1.16	.95	1.15
.7%	1.58	1.00	1.03	1.53	1.29	1.35
Control	1.45	.95	.83	1.16	1.10	1.15
.8%	1.67	1.21	1.06	1.15	1.02	1.04
Control	1.45	1.13	.83	1.16	1.13	1.15
.9%	1.36	1.99	1.91	1.24	1.82	1.76
Control	1.04	1.29	1.27	1.16	1.19	1.15
1.0%	1.43	1.43	1.55	1.45	1.47	1.42
Control	1.04	1.29	1.27	1.16	1.19	1.15

TABLE V

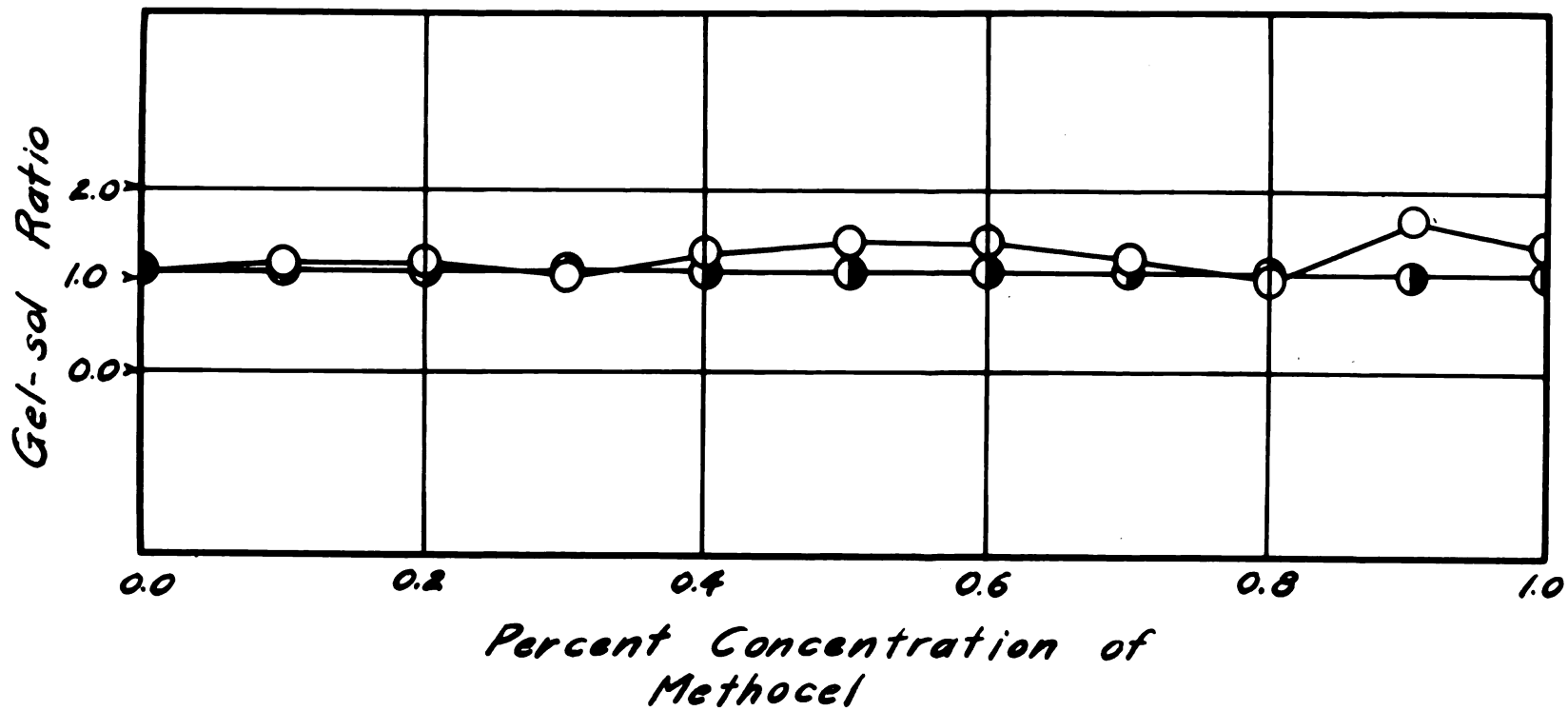
Showing the Width (in microns) of the Plasmagel, Plasmasol and the Gel-Sol Ratio

Per Cent Concentration of Methocel	No. of Organisms Tested	Average Diameter in Microns				Gel-Sol Ratio
		Plasmagel		Plasmasol		
		Range	Average	Range	Average	
.1%	26	60 - 158	92	44 - 115	81	1.31
Control	25	43 - 143	74	53 - 103	68	1.25
.2%	24	55 - 118	84	40 - 103	64	1.32
Control	20	43 - 118	82	57 - 90	67	1.28
.3%	23	25 - 115	76	25 - 103	67	1.21
Control	25	30 - 113	77	28 - 83	62	1.28
.4%	25	58 - 115	91	50 - 95	72	1.27
Control	24	53 - 113	81	53 - 92	72	1.14
.5%	27	60 - 113	84	45 - 93	65	1.31
Control	28	40 - 150	73	50 - 110	76	1.04
.6%	23	37 - 112	79	35 - 88	68	1.17
Control	27	15 - 135	72	35 - 128	75	.95
.7%	24	42 - 135	88	45 - 103	70	1.29
Control	23	50 - 138	82	50 - 107	73	1.10
.8%	24	58 - 123	85	50 - 85	66	1.02
Control	25	51 - 138	84	53 - 107	72	1.13
.9%	24	50 - 125	90	20 - 85	58	1.82
Control	27	65 - 117	83	60 - 105	72	1.19
1.0%	24	60 - 117	87	38 - 78	61	1.47
Control	27	65 - 117	86	60 - 105	72	1.19

FIGURE III



The relation between viscosity and the gel/sol ratio in *Amoeba proteus*. Ordinates show the adjusted gel/sol ratio; abscissae, per cent concentration of Methocel. ◑, adjusted gel/sol ratio in various concentrations of Methocel; ○, gel/sol ratio in control solutions.


Gel-sol Ratio
2.0
1.0
0.0
0.0
0.2
0.4
0.6
0.8
1.0
Percent Concentration of Methocel

A review of the results given in Table V shows that the mean diameters of the plasmagel in all concentrations of Methocel was consistently higher than it was in the control solution. Further, the mean diameter of the plasmasol neither consistently decreased or increased as Methocel concentration increased from .1 to .5 per cent, but in solutions in which Methocel was varied from .5 to 1.0 per cent, the mean diameter of the plasmasol was consistently lower than it was in control solutions. These results demonstrate a direct relationship between plasmagel width and viscosity, and an inverse relationship between plasmasol width and viscosity.

VI. DISCUSSION

It was shown in Table II and III, that the number of Tetrahymeni geleii ingested by Amoeba proteus reached a maximum in .5 per cent Methocel solution. Facilitation of ingestion in this concentration of Methocel seems to be closely correlated with decreased rate of locomotion in Tetrahymeni geleii. This view is consistent with the views of Fennell (1944) who studied amoeboid ingestion in strychnine sulphate solutions. He found that low concentrations of this drug inhibited flagellar activity and locomotion in Chilomonas paramaecium. As a consequence the number of organisms made available to Amoeba proteus was increased by decreasing the frequency of escape of Chilomonas paramaecium from food vacuoles during the ingestion process. Stiles (1947) likewise found that Methocel decreased ciliary activity and rate of locomotion in paramaecia.

Mast and Fennell (1938) suggested that there was a close correlation between amoeboid locomotion and amoeboid ingestion. It is evident in Table I that rate of locomotion is fairly uniform in concentrations of Methocel varying from .1 to .5 per cent and that ingestion of food reaches a maximum of 5.08 organisms in .5 per cent Methocel. If this increase in ingestion was determined by another factor, i.e., an interaction between Methocel and cellular substances, it is possible that this condition would be manifest by observable changes in gel/sol ratio and morphological alterations in amoebae. The results presented in Tables II and III and also repeated

observations made on amoebae revealed a constancy in both gel/sol ratio and morphological features in low concentrations of Methocel (.1 to .5 per cent). For these reasons a correlation between this factor and ingestion is not tenable.

It was demonstrated in Table V that the diameter of the plasmagel increased and the width of the plasmasol decreased as the concentration of Methocel increased from .5 to 1.0 per cent. Obviously several explanations could be offered for these observed cytoplasmic variations: (1) It is possible that the width of the plasmagel may depend on entrance and union of inorganic salts with some cytoplasmic constituent, and that rate of entrance may vary with concentration of Methocel; (2) increased width of the plasmagel may depend on entrance of Methocel into the cell and rate of entrance may vary with concentration of the Methocel; or (3) increased width may be due to loss of water from the cell and the rate of loss may be dependent on Methocel concentration. There is little evidence to support the first two hypotheses, but it is well established that plasmasol can be transformed into plasmagel or vice versa by loss or gain of water. Mast (1926) demonstrated that during amoeboid locomotion there was a solation of the plasmagel at the posterior end and a gelation at the anterior end. These reversible cytoplasmic changes were correlated with intake of water at the posterior end and loss of water at the anterior end. These results suggest, but do not demonstrate, that increased width of the plasmagel and decreased width of the plasmasol are closely correlated with loss of water, and that this loss of water is dependent on Methocel concentration.

VII. SUMMARY

1. An inverse relationship exists between rate of locomotion and viscosity of the culture solution.
2. A direct relationship exists between viscosity and ingestion in low concentrations of Methocel, but an inverse relationship exists between viscosity and ingestion in higher concentrations.
3. A direct relationship exists between plasmagel width and viscosity, while an inverse relationship exists between plasmasol width and viscosity. Increased width of the plasmagel and decreased width of the plasmasol seems closely correlated with loss of water from the cytoplasm.

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CURRICULUM VITAE

Nellie Marie Beukema was born March the 11th, 1927, in Hollywood, California. She attended Grammar School, Junior High School, and Culter Academy in that city, and graduated from the latter in 1944. She entered Calvin College at Grand Rapids, Michigan, in the fall of 1944 and received the A.B. degree in Education from this institution in 1948. She was a graduate assistant in Zoology at Michigan State College in 1948-1949, and in the fall of 1949 and the winter of 1950 she returned to Michigan State College to complete work on her Master's degree. She majored in Zoology and minored in Chemistry and Nutrition.

During the summer of 1949 she was employed as a Dietician at Blodgett Memorial Hospital in Grand Rapids, Michigan. At the present time she is a member of the faculty at Calvin College in Grand Rapids where she is serving as Assistant in Biology.