THE EFFECTS OF SUSPENDED SILTS AND CLAYS ON SELF-PURIFICATION IN NATURAL WATERS: PROTEIN ADSORPTION

The effects of suspended silts and clays on self-purification in natural waters: Protein adsorption

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ABSTRACT

The effects of the suspended sediments found in many natural waters on the microbial processes involved in the self-purification of those waters are not known. Clays and silts with their large surface area per unit weight have an immense capacity for adsorbing nutrient molecules from solution, but the extent to which such adsorption takes place is largely unknown. Adsorption of a major portion of a biodegradable substance from solution onto a solid surface would significantly alter its susceptibility to bacterial attack and, hence, also the rate at which it is decomposed.

In this paper are reported the results of adsorption experiments with soil materials found in some Alaskan waters which are typically heavily sediment-laden. The affinities of these soils for the protein bovine serum albumin were measured as a function of pH, temperature, and protein concentration. An empirical relationship was discovered, for a given soil material, between the equilibrium protein concentration and the initial protein-to-soil ratio. Temperature variations from 5 to 25°C had no detectable effect on adsorption, whereas variations in pH between 2 and 10 had dramatic effects on the extent of adsorption. The amount of protein adsorbed at the pH of the natural water system was so small as to lead one to predict that adsorption of this protein onto suspended sediments would have a negligible effect on the rate at which the protein would be decomposed by bacteria in the aqueous environment.

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INTRODUCTION

Little is known about the effects of suspended silts and clays on the self-purification process in streams, rivers, and estuaries. With their large surface area per unit weight, silts and clays have an enormous potential for adsorbing soluble nutrients and particulate matter from natural waters. To what extent such adsorption takes place is not known, nor is it clear how it affects the biological degradation of the adsorbed substances. One would expect, at first glance, that the degradation of particulate materials, such as sewage solids, would be enhanced by their adsorption, along with bacteria capable of attacking them, onto the surfaces of suspended soil particles. Sewage solids include such large, practically insoluble, molecules as proteins and polysaccharides whose biological breakdown requires the manufacture of extracellular enzymes on the part of the bacteria. Induction of enzyme synthesis requires some contact between the bacterium and the molecule to be degraded, and direct contact between the enzyme and its substrate is necessary for a reaction to occur. In free solution, the probability of achieving such intimate contact is small; diffusion through the large volume of water limits the frequency of collision. However, if the bacteria and the molecule to be degraded were localized on a silt or clay surface, the effective local concentrations of enzyme and substrate would be greatly increased, so that the collision frequency, and therefore the reaction rate, should be greater. In addition, the bacterium would be better able to benefit from the activities of its enzymes by being closer to the source of assimilable end-products.

But whether this adsorption of bacteria and substrate onto a surface has a significant effect on the overall rate of breakdown of the substrate in the natural water environment depends on the affinity of the soil surface for the species involved. If very little substrate is adsorbed, then the rate of its degradation is essentially that in free solution. However, if all of the substrate were adsorbed from solution, a dramatic effect on the rate of breakdown would be expected.

Consider the case of protein assimilation in a sediment-laden water. Assuming that the rate of hydrolysis of protein is initially a function of the collision frequency between the bacteria and the protein molecules and subsequently of the collision frequency between the enzyme and protein molecules, one can make approximate calculations of the protein hydrolysis rates in solution and on the soil surface. The collision frequencies can be calculated from a phenomenological equation based on Fick's laws of diffusion:

$$R = k\pi 4 \underline{a} (D_A + D_B) f C_{A_o} C_{B_o}$$

Where k is a symmetry number, equal to 1 for collisions between unlike molecules: $\underline{a}$ is the sum of the radii of the colliding molecules, in centimeters; D_A and D_B are the diffusion constants of molecules A and B; and C_{A_o} and C_{B_o} are the initial concentrations of these molecules, expressed in particles per ml. The factor f is a function of the electrostatic potential and is equal to 1 for neutral molecules. (Amdur and Hammes, 1966). In this case, however, the interacting species are not neutral in the pH range in question (6 to 9), but for the sake of argument, it is assumed that f has similar values for interaction between bacteria and protein molecules in solution, between bacteria and protein molecules adsorbed onto soil particles, and between bacteria and soil particles, although it undoubtedly differs in these situations.

For the system containing 1×10^6 proteolytic bacteria per ml. and 100 mg/liter (9.0×10^{14} molecules/ml) of serum albumin, the frequency of collision between the two species will be 2.0×10^{12} f' collisions/ml/sec. In this calculation, the effective radii of the bacteria and protein molecules were taken to be 3×10^{-4} cm and 8×10^{-7} cm, respectively, so that $\underline{a}$ essentially equals 3×10^{-4} cm. The diffusion constant of serum albumin is 5.94×10^{-7} cm^2/ sec. (Tanford, 1961a), and that for the bacteria is, by analogy with values for very high molecular weight molecules, 0.05×10^{-7} cm^2/sec.

Now consider the system of 100 mg/liter of albumin in equilibrium with 500

mg/liter of suspended sediment, (about 1×10^8 particles/ml), to which bacteria have been added to a final concentration of 1×10^6 per ml. The initial collision frequency between the bacteria and soil particles will be 5.0×10^3 f'' collisions/ml/sec., calculated using a value of 1×10^{-4} cm for the radius and 0.05×10^{-7} cm^2/sec. for the diffusion constant of the soil particle. If there is any aggregation of the soil particles and bacteria, the collision frequency will, of course, decrease. If one assumes that only a monolayer of protein has been adsorbed onto the soil surface, and that the soil surface area is $30 m^2/gm$, then 90% of the protein is in solution. The collision frequency between the bacteria and the protein molecules will now be the sum of the frequencies in solution and on the soil surface: $R = R_{soln} + R_{ads}$. The latter term can be estimated by multiplying the soil particle-bacteria collision frequency by the number of protein molecules occupying the impact cross-sectional area; namely, 3.8×10^4 molecules (calculated assuming a bacterium $1\mu \times 6\mu$ and an area of 1.57×10^4 $\AA^2$ per protein molecule in a monomolecular film). (Haurowitz, 1950). Thus, R_{ads} equals 1.9×10^8 f'' collisions/ml/sec. and R_{soln} equals 1.8×10^{12} f' collisions/ml/sec., so that the overall collision frequency is probably slightly lower than that calculated for the system without soil. If all of the protein molecules were adsorbed onto the soil surface, and the bacteria remained in free solution, then the overall collision frequency between the bacteria and the protein molecules would be about 2×10^8 f'' collisions/ml/sec.; much lower than if the protein were not adsorbed at all. It appears, therefore, that adsorption of the protein onto suspended soil particles tends to decrease the opportunity for the bacterial breakdown of the protein, at least initially. The rate-limiting factor seems to be the frequency with which the bacteria encounter the soil particles. If, however, the bacteria also become attached to the soil particles, eventually, the overall rate of protein hydrolysis would no longer depend primarily on the frequency of collision between bacteria and soil particles. Instead, it would be largely a function of the rate of enzyme-protein interaction. With the bacteria localized on the soil particle, the proteolytic enzyme concentration in the vicinity of the adsorbed protein is effectively much greater than that in solution. Thus, one would expect that under this con-

dition a protein molecule would be more readily hydrolyzed if it were adsorbed on the soil surface than in solution, except when the enzyme is inhibited by the soil itself.

So whether or not the presence of suspended sediments in natural waters enhances, decreases, or has no effect on the rate of decomposition of a molecule by bacteria cannot be predicted without knowing more about the many factors involved. What is the nature of the soil surface? What is its affinity for various biological substrates? What is its affinity for and effect on the activity of the bacteria and the bacterial enzymes? And how do these affinities vary with pH, temperature, and the ionic environment? For practical application of this information, it would be helpful to know what concentrations of the various substrate molecules might be encountered in the particular water under study as well as the numbers of bacteria with the biochemical capabilities necessary for decomposing those substrates. One might even need to know the oxygen requirements of the bacteria to accomplish the degradation in order to predict how much of the substrate could be added to the water without depleting its dissolved oxygen. In short, one is dealing here with a very complex system. What follows reports the partial answers to some of these questions for some Alaskan waters. The affinities of suspended sediment materials from various localities in the state for the protein bovine albumin and their variation with temperature, pH, and protein concentration have been determined. Although it is difficult to explain the information obtained in theoretical terms, its practical value is nevertheless obvious.

FACTORS INVOLVED IN ADSORPTION

By adsorption is meant the accumulation of a molecule from solution on the solid surface so that the molecule tends to remain attached unless the physical or chemical environment is changed. Thus, it indicates an attractive force great enough to withstand detachment of the molecule under the force of Brownian motion-type collisions. The extent of adsorption is therefore a measure of the affinity of the solid surface for the bound molecules. Adsorption processes are sometimes categorized into two classes depending on the strength of the attractive forces. When the forces are weak, that is, van der Waals type forces with low heats of adsorption ($\leq$ 10 kcal/mole), the adsorption is described as physical adsorption. When the attractive forces are strong, with heats of adsorption much greater than 10 kcal/mole, the adsorption is termed chemical adsorption, or chemisorption (Wayman, 1967).

The mechanisms involved in adsorption from solution by solids have been classified (Giles, 1959) as follows:

(a) Non-polar van der Waals attraction;

(b) Formation of hydrogen bonds or other polar non-ionic bonds;

(c) Ion exchange, or electrostatic interactions; and

(d) Formation of covalent bonds.

Since several or all of the above attractive forces may contribute to adsorption in any one system, identification of the adsorption mechanism can be difficult in practice.

The protein molecule used in the experiments which follow, bovine serum albumin (BSA), is a compact molecule with a shape described as similar to a prolate ellipsoid with dimensions 34 Å x 168 Å (Tanford, 1961b). BSA is

a polyelectrolyte as the result of the approximately 180 ionizable groups on the surface of the molecule. These include 99 β- and γ- carboxyl, 57 ε-amino, 22 guanidino, 19 phenolic, 16 imidazole, 1 α-carboxyl, 1 α-amino, and no free sulfhydryl groups. There are also approximately 80 aliphatic hydroxyl groups (Tanford, et al., 1955). Consequently, there is ample opportunity for hydrogen bonding and ion exchange with the soil surface. The isoelectric point of BSA is about pH 4.8 (Mahler and Cordes, 1966). Below this pH the protein molecule has a net positive charge and above it a net negative charge so that its adsorption can be expected to vary with pH. It should also vary with the ionic environment, since proteins adsorb ions, and water molecules, onto their surfaces and thus alter the charge density. The extent of protein adsorption onto the soil surface will also be a function of the isoelectric point of the soil particle and the manner in which its charge density varies with pH.

The suspended soil material encountered in natural water systems consists of oxides of silicon, aluminum, magnesium, iron, and other metals in various proportions and in complicated crystal structures which are not necessarily perfect or fixed. The surfaces of the simpler oxides, such as silica, are electrically charged, and in aqueous solution they become hydroxylated and the surface charge is diminished. (See Figure 1.) Through dissociations of these surface hydroxides, the surface may become charged again. Thus the surface is amphoteric and may have either a net negative or a net positive charge depending on the pH of the solution (Parks, 1967).

The clay minerals, such as kaolinite and montmorillonite, are sheet structures in which a negatively charged layer of $[(Si_2O_5)^{-2}]_n$ is balanced by a layer of aluminum or magnesium hydroxide (Marshall, 1964). Particles of these clay minerals have broken bonds only at the edges of the sheets. The broken bonds hydroxylate; they and the structural hydroxyl groups in the layer surfaces can dissociate to produce a pH-dependent charge in the same way described for the surfaces of the simpler oxides (Parks, 1967).

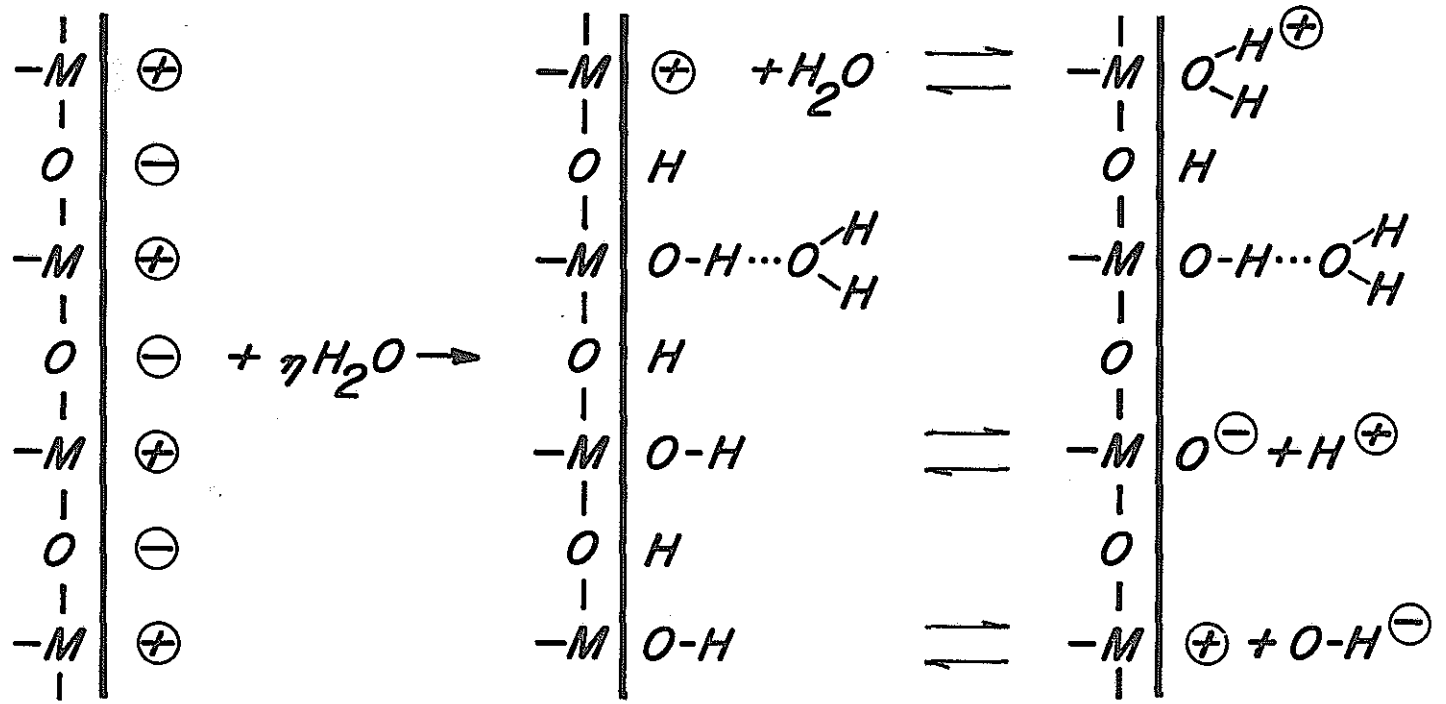

Figure 1. A Schematic Illustration of the Hydroxylation and Subsequent Reactions That Can Take Place at the Surface of a Simple Oxide in Water.

In addition, negative charges in the clay framework can arise by isomorphic substitution, for example, aluminum for silicon, magnesium for aluminum. These are compensated for in the solid by the binding of cations such as Na^{+}, K^{+}, Mg^{++}, or Ca^{++} between the layers. In suspension, these cations are exchangeable and may diffuse into solution, leaving a net charge on the solid (Parks, 1967).

Thus, it can be readily seen that pH should be an important variable in the adsorption of a multivalent electrolyte such as protein onto an amphoteric soil surface. And since both the protein molecule and the soil may bind various ions onto their surfaces, and thereby alter the charge density, the ionic strength of the environment and the different types of ions present should also be important variables.

Temperature might also be expected to affect the adsorption process. By virtue of the increased kinetic energy at higher temperatures, it seems reasonable that the equilibrium amount of material adsorbed per unit surface area would be less at the higher temperature. On the other hand, equilibrium should be attained less rapidly at the lower temperature. However, the effects of temperature on adsorption probably diminish as the size of the molecule being adsorbed increases and, over the narrow temperature range of interest in natural water systems (0 - 25°C.), may even be negligible.

The surface of the bacterial cell would be expected to behave in a way similar to the protein molecule in its response to pH and the ionic environment. It, too, is polyelectrolytic but is not so homogeneous. The bacterial cell wall is made up of variable amounts of teichoic acids, protein, lipid, and polysaccharide as well as a heteropolymer consisting of acetyl-glucosamine, acetyl-muramic acid, alanine, glycine, and either diaminopimelic acid or lysine (Stanier, et al., 1963). The bacterial cell wall is therefore capable of interacting with the soil surface in many of the ways described above.

EXPERIMENTAL METHODS AND RESULTS

SAMPLE COLLECTION

Samples of Alaskan river and estuary waters were collected during the summer and fall of 1969 at the locations shown in Figures 2 and 3 and described in Table 1. Bottom soil samples were collected at each site at the same time. The Knik Arm water samples were taken on board ship by lowering weighted samplers to mid-depth and a few feet off bottom. Surface samples were scooped up with a polyethylene bucket. Most of the Interior Alaska and Valdez water samples were collected by wading into the water and collecting with a bucket. The Tanana River surface sample collected above Fairbanks was taken in midstream from a boat. The Valdez River #2 and Lowe River #2 waters were collected at midstream by lowering a sampler to mid-depth from a bridge.

SUSPENDED SEDIMENTS IN WATER SAMPLES

The amount of suspended sediment (SS) in each water sample was determined by filtering, under vacuum, a measured volume of the water, usually 50 or 100 mls, through washed, dried, and weighed membrane filters having a pore size of 0.45μ. The filters were then dried at 103°C. for 1 hour and weighed again. The difference in weights yielded the concentration of suspended sediment. Table 2 shows the results of duplicate measurements.

PREPARATION OF SOILS FOR ADSORPTION EXPERIMENTS

The bottom soil samples were first air-dried and then oven-dried at 103 - 105°C. for about 24 hours. They were initially ground by hand with a mortar and pestle to pass a No. 50 sieve, (U. S. Standard Sieve Series, A.S.T.M. specifications, opening = 297 microns), and then ground again until the particle size distribution of a distilled water suspension of each soil approximated that of the corresponding water sample. The soil samples treated in this manner were Lowe River, Stevens Village, Fairbanks, Delta River, Tanana, Minto, and Knik Arm #9. Three reference clay minerals (obtained from Ward's Natural Science Establishment, Rochester, N. Y.) were ground to a fine powder by passing the crushed mineral twice through a Weber pulverizer. These min-

erals were Kaolinite #9, Mesa Alta, New Mexico, 48W0290; Illite #35, Fithian, Illinois, 48W1535; and Montmorillonite #26, Clay Spur, Wyoming, 48W1260.

SURFACE AREA MEASUREMENTS OF SOILS

The surface area per gram of each soil thus prepared was assessed by measuring the amount of ethylene glycol retained by a known weight of soil in equilibrium with a free ethylene glycol surface at 25± 1°C. (after Bower and Gschwend, 1952, and Martin, 1955). A one gram sample of the ground, dry soil was weighed into tared aluminum dishes which were fitted with covers. The soil was spread out evenly over the bottom of the dish. The dish was placed over anhydrous P_2O_5 in a Pyrex desiccator, which was then evacuated for 10-15 minutes with a Duo-Seal Vacuum Pump. The dish was weighed every 2 hours until it reached a constant weight. Then the soil was saturated with re-distilled ethylene glycol. About 20-30 drops of glycol were added to each dish which was then covered and allowed to sit overnight. After at least 12 hours, the dish was weighed and then placed over anhydrous $CaCl_2$ in a Pyrex desiccator set in a water bath maintained at 25± 1°C. A free surface of ethylene glycol was provided by setting an open dish of the re-distilled glycol in the desiccator with the samples. The desiccator was evacuated for 10-15 minutes with the vacuum pump. The dish was weighed after 16-20 hours and approximately every 4 hours thereafter until the weight had reached an "equilibrium value", as evidenced by three successive weighings within ± 0.3 mg. The surface area, in square meters per gram of soil, was calculated by dividing the mg of ethylene glycol retained by one gram of soil by 0.31 mg ethylene glycol/square meter. (Bower and Gschwend, 1952). Each measurement was performed in triplicate and the values averaged. The results are shown in Table 3.

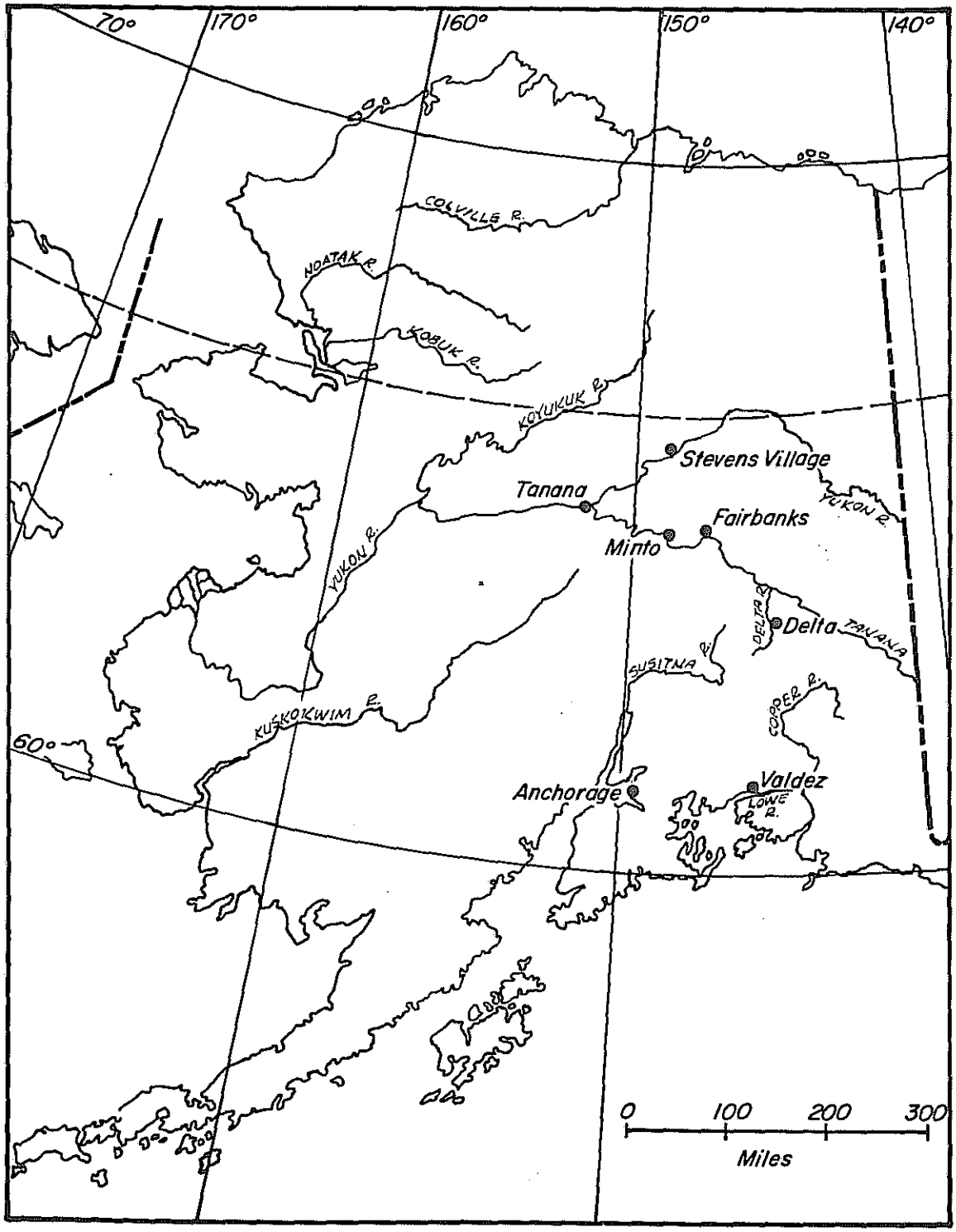

Figure 2. A Map of Alaska Showing Sampling Locations.

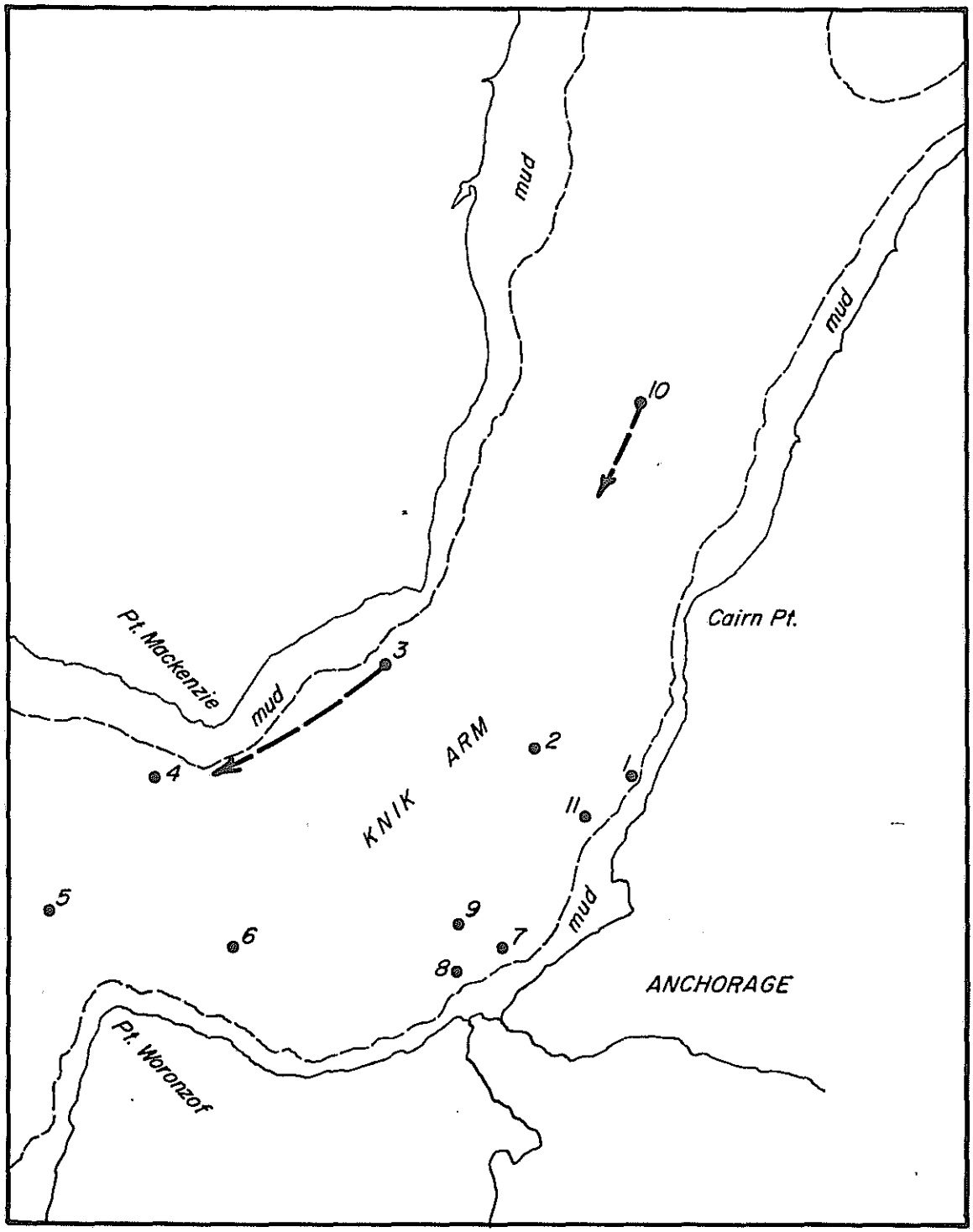

Figure 3. Locations of Samples Taken in Knik Arm.

TABLE 1

SAMPLE COLLECTION INFORMATION

Sample	Date	Location and Description
Interior Alaska		
Delta River	7/13/69	Near Richardson Highway about 30 miles North of Paxson.
Fairbanks	8/28/69	Tanana River from midstream about 5 miles above Fairbanks; surface sample.
Minto	9/03/69	Tanana River from bank at Minto; surface sample.
Tanana	9/03/69	From bank at Tanana just below the confluence of the Tanana and Yukon Rivers; surface sample.
Stevens Village	9/03/69	Yukon River from bank at Stevens Village; surface sample.
Valdez Area		
Lowe River #1	8/11/69	From bank at Valdez; surface sample.
Lowe River #2	10/16/69	From bridge at midstream and mid-depth.
Valdez River #2	10/16/69	From bridge at midstream and mid-depth.
Mineral Creek	10/17/69	At midstream and mid-depth.
Solomon Gulch	10/18/69	At midstream and mid-depth.

TABLE 1, Continued:

Sample	Date	Location and Description			
		Time	Station	Sample depth feet	Bottom feet
Knik Arm					
1	11/6/69	3:00 pm	#1	surface	90
2				45	
3				90	
4		4:00 pm	#2	surface	92
5				42.5	
6				87	
7		5:00 pm	#3	surface	110
8				55	
9				105	
10	11/7/69	8:40 am	#4	surface	34
11				16	
12				32	
13		9:30 am	#5	surface	75
14				35	
15				70	
16		11:05 am	#6	surface	25
17				13	
18				20	
19		11:35 am	#7	surface	10
20		11:45 am	#8	surface	10
21		12:15 pm	#9	surface	11
22		12:50 pm	#10	surface	25
23				14	
24				22	

TABLE 2

SUSPENDED SEDIMENT IN NATURAL WATERS

Sample	Suspended Sediment mg/l
Interior Alaska	
Delta River	516.
Fairbanks	117.
Minto	150.
Tanana	42.
Stevens Village	24.
Valdez area	
Lowe River #1	64.
Lowe River #2	16.
Valdez River #2	87.
Mineral Creek	<1.
Solomon Gulch	<1.
Knik Arm	
1	330.
2	1600.
3	1800.
4	811.
5	1290.
6	1670.
7	2134.
8	1450.
9	1966.
10	1004.
11	1934.
12	1886.
13	992.
14	2094.
15	2230.
16	1260.
17	1640.
18	1907.
19	138.
20	202.
21	452.
22	1813.
23	2279.
24	2818.

TABLE 3

SURFACE AREAS OF SOILS
(as measured by Ethylene Glycol retention)

Soil	Surface Area m /gram
Minto	16.
Tanana	33.
Fairbanks	22.
Delta River	18.
Lowe River #1	8.
Stevens Village	63.
Knik Arm #9	40.
Illite	232.
Montmorillonite	853.
Kaolinite	32.

PARTICLE SIZE MEASUREMENTS

Because of the very small size of the majority of particles (less than 3μ), a microscopic method was used to determine the range of sizes of the particles in suspension. The presence of rapidly settling particles as well as a wide range of sizes of particles in the natural waters prevented the use of the more rapid electronic particle analyzer. A Zeiss phase-contract microscope at 1,250 X magnification was used in conjunction with a Petroff-Hausser bacteria counting chamber. This chamber had a grid of squares 50μ on a side over which the sample was laid to a depth of 1/50 mm. This allowed calculation of the volume of liquid observed. Another grid in the eyepiece of the microscope was calibrated against the Petroff-Hausser cell. The smallest division on this grid corresponds to 1.333μ in the plane of the slide. A particle was measured along its longest dimension against this grid and the size was expressed in units. Thus a particle of 1 unit size had a longest dimension between 0 and 1.33μ; one of 2 units between 1.33 and 2.67μ, and so on. For each sample, 1000 particles were measured to obtain a particle size distribution. In general, the particles were rectangular or square in shape.

INTERFERENCE FROM BACTERIA

A number of particles of 1 unit length or less were observed when the Interior Alaska water samples were first examined. One could not visually determine whether these were sediment particles or bacteria; hence, they were suspect. Since rod-shaped bacteria of 1 and 2 units length were definitely present, a way was sought to sterilize the samples without altering the sediment particles. Autoclaving and treatment with hydrogen peroxide were considered. Autoclaving has been found not to alter the x-ray characteristics of montmorillonite or kailinite (Stotzsky and Rem, 1966). One portion of the Minto water sample was treated with 30% hydrogen peroxide (1 part H_2O_2 solution to 4 parts sample); another was autoclaved at 15 psi for 15 minutes. The particle size distributions for these treated samples were then compared with that of the untreated Minto water. As Table 4 shows, treatment of the water with hydrogen peroxide reduced the particle concentration 66% and drastically altered the size distribution. Autoclaving re-

duced the particle concentration by 32% and did not significantly disturb the distribution. A cursory examination of the three samples revealed that the proportion of aggregates of particles in the sample treated with hydrogen peroxide had increased to 3% as compared with the 1% level in the untreated and autoclaved samples. Hence, it was assumed that autoclaving at 15 psi for 15 minutes was a suitable way of destroying the bacteria without altering the sediments, and thereafter all samples were treated in this manner prior to measuring the particle sizes.

The distributions of particle sizes in the bottom soils and reference clays were determined on autoclaved suspensions of known weights of the dried and ground soils in distilled water. These suspensions were approximately 500 mg/liter in suspended soils.

The results of the particle size distribution measurements are given in Tables 5 and 6.

METHOD OF PROTEIN ANALYSIS

The Folin-Ciocalteu colorimetric method of protein analysis (Lowry et al., 1951) was found to be more sensitive in the concentration range 0 to 100 mg/liter than ultraviolet adsorption at 280 mμ, (the wavelength of maximum adsorption found for BSA in distilled water). The procedure used is as follows: To 1.00 ml of sample containing 50 to 500 μg of protein in a 10-ml test tube is added 5.0 mls of Na_2CO_3-$CuSO_4$ reagent. (This reagent is prepared by mixing 1.00 ml of a solution containing 1% (w/v) cupric sulfate and 2% (w/v) potassium tartrate with 50.0 mls of a 2% (w/v) solution of Na_2CO_3 in 0.1 N NaOH.) The sample is mixed well with the reagent and then allowed to sit at room temperature for 10 minutes, at which time 0.5 ml of 1 N Folin Reagent is added and mixed in immediately. The test tubes are then incubated at room temperature and in the dark for 30 minutes. The adsorbance is measured at 750 mμ using 1 cm cells and a Beckman DB Spectrophotometer against a distilled water blank. It is then corrected by subtracting the adsorbance of a reagent blank prepared with the appropriate sample. When the protein concentration was less than 50 μg/ml, the amounts of sample and reagents were doubled, and the adsorbance was measured in 5 cm cells. Standards were always run at the same time as the samples.

TABLE 4

PARTICLE SIZE DISTRIBUTIONS
MINTO WATER SAMPLE

Units*	% Untreated N** = 1094	% Autoclaved N** = 1121	% Treated with H_2O_2 N** = 988
1	82.82	84.21	60.12
2	9.05	8.83	24.80
3	3.75	2.94	8.60
4	1.28	0.89	2.83
5	1.00	0.62	1.42
6	0.46	0.54	0.51
7	0.37	0.36	0.51
8	0.37	0.36	0.81
9	0.18	0.18	----
10	0.27	0.18	----
11	0.18	0.09	----
12	0.09	0.18	----
13	----	----	----
14	0.09	----	----
15	----	0.09	----
16	----	----	----
17	----	----	----
18	----	----	0.10
19	----	0.09	----
20	----	0.09	----
23	----	0.09	0.20
24	----	0.09	----
36	----	----	0.10
73	----	0.09	----
123	0.09	0.09	----
No. of particles per milliliter	1.46×10^8	9.96×10^7	4.94×10^7

* 1 unit = 1.333μ
** N = number of particles measured

TABLE 5

PARTICLE SIZE DISTRIBUTIONS OF SUSPENDED SEDIMENT IN NATURAL WATERS (%) - (autoclaved 15 mins @ 15 psi)

Units*	Tanana	Delta River	Minto	Fairbanks	Stevens Village
1	70.28	25.38	84.21	54.65	75.80
2	17.95	37.31	8.83	21.11	16.70
3	4.46	20.10	2.94	10.76	3.50
4	2.28	6.01	0.89	5.54	1.60
5	1.52	5.25	0.62	2.09	1.40
6	0.48	1.86	0.54	0.73	----
7	0.66	1.02	0.36	1.46	0.40
8	0.66	0.85	0.36	0.94	0.10
9	0.19	0.42	0.18	0.63	0.20
10	0.19	0.34	0.18	0.52	----
11	0.28	0.17	0.09	0.42	----
12	0.10	0.08	0.18	0.10	----
13	0.10	----	----	0.52	0.10
14	0.19	0.17	----	----	----
15	0.10	0.08	0.09	0.31	----
16	----	0.08	----	----	----
17	----	0.17	----	----	0.10
18	----	0.25	----	----	----
19	0.19	0.08	0.09	----	----
20	----	0.08	0.09	0.10	----
21	----	----	----	0.10	----
22	----	----	----	----	----
23	0.10	0.17	0.09	----	0.10
24	----	----	0.09	----	----
25	----	----	----	----	----
27	0.10	----	----	----	----
31	----	----	----	----	----
34	----	----	----	----	----
35	----	----	----	----	----
37	----	----	----	----	----
73	----	----	0.09	----	----
88	----	0.08	----	----	----
123	0.10	----	0.09	----	----
144	----	----	----	----	----
768	----	0.08	----	----	----
$\bar{d}$** =	1.79μ	3.84μ	1.46μ	2.22μ	1.25μ
N*** =	1053	1182	1121	957	1000

* 1 unit = 1.333μ
** $\bar{d}$ = arithmetic mean
*** N = number of particles measured

TABLE 5, Continued:

Units*	Lowe River #1	Knik Arm #14	Knik Arm #21	Knik Arm #23
1	83.38	23.06	23.13	60.82
2	9.73	30.01	40.66	22.89
3	3.15	20.11	18.77	7.94
4	1.38	7.29	5.43	2.37
5	0.59	5.63	4.36	1.44
6	0.39	3.41	2.22	0.52
7	0.39	2.86	0.71	0.93
8	0.30	1.85	1.69	1.03
9	----	1.66	0.62	0.72
10	0.10	0.83	0.89	0.21
11	0.10	0.37	0.27	0.10
12	----	0.46	0.27	0.21
13	0.20	0.74	0.09	0.10
14	----	0.09	0.18	0.10
15	----	0.28	0.09	0.31
16	----	0.09	----	----
17	----	0.18	0.18	0.10
18	----	0.09	0.27	----
19	----	0.28	----	----
20	----	0.18	----	----
21	----	0.09	----	----
22	----	----	----	----
23	----	0.09	0.09	0.21
24	----	0.09	----	----
25	----	0.09	----	----
31	0.10	----	----	----
34	0.10	----	----	----
35	----	----	0.09	----
37	----	0.09	----	----
144	0.10	----	----	----
$\bar{d}$** =	1.38μ	3.74μ	3.00μ	1.88μ
N*** =	1017	1084	1124	970

* 1 unit = 1.333μ
** $\bar{d}$ = arithmetic mean
*** N = number of particles measured

TABLE 6

PARTICLE SIZE DISTRIBUTIONS OF SOILS
SUSPENDED IN DISTILLED WATER (%)

Units*	Tanana	Delta River	Minto	Fairbanks	Stevens Village
1	77.06	55.54	81.64	77.50	60.21
2	16.71	27.38	10.07	13.40	21.81
3	3.70	10.01	2.17	5.00	7.87
4	0.63	2.26	0.99	0.90	2.10
5	0.45	1.28	1.38	0.60	2.01
6	0.27	0.39	0.69	0.20	0.91
7	0.27	0.20	0.49	0.10	0.82
8	0.27	0.39	0.49	0.40	0.73
9	0.27	0.20	0.20	0.20	0.54
10	----	0.29	0.40	0.20	0.36
11	0.09	0.20	0.10	0.10	0.45
12	----	0.20	0.20	0.10	0.27
13	----	0.39	0.10	0.10	0.64
14	----	----	----	0.20	----
15	0.27	----	----	0.10	----
16	----	0.10	0.20	----	----
17	----	0.49	----	0.10	0.09
18	----	0.20	0.20	0.30	0.18
19	----	0.10	0.10	----	0.09
20	----	0.10	----	----	0.18
21	----	----	----	----	0.09
23	----	0.10	----	0.20	----
24	----	0.10	----	0.10	0.09
25	----	----	----	0.10	0.09
26	----	----	----	----	0.09
29	----	----	----	----	0.09
30	----	0.10	----	----	----
31	----	----	----	----	0.09
36	----	----	----	0.10	----
37	----	----	0.10	----	----
48	----	----	0.10	----	----
50	----	----	0.10	----	----
53	----	----	0.10	----	----
55	----	----	0.10	----	----
65	----	----	0.10	----	----
$\bar{d}$** =	1.20μ	2.06μ	1.74μ	1.51μ	2.18μ
N*** =	1107	1019	1013	1000	1091

* 1 unit = 1.333μ
** $\bar{d}$ = arithmetic mean
*** N = number of particles measured

TABLE 6, Continued:

Units*	Lowe River	Knik Arm	Kaolinite	Illite
1	25.70	36.83	31.00	73.42
2	40.40	48.98	55.34	23.21
3	15.80	8.16	10.11	1.73
4	3.90	2.04	1.16	1.58
5	4.10	1.36	1.54	0.25
6	2.40	0.29	0.29	0.08
7	1.30	0.58	----	0.25
8	1.20	0.39	----	0.08
9	0.70	----	0.19	0.08
10	0.40	0.29	0.10	0.16
11	0.30	0.19	0.10	----
12	0.40	0.19	----	0.16
13	0.10	0.29	----	----
14	0.20	----	----	----
15	0.20	0.10	0.10	----
16	0.20	0.10	----	----
17	0.30	----	----	----
18	0.40	----	----	----
19	0.10	----	----	----
20	0.20	----	----	----
21	0.10	----	----	----
23	0.20	----	0.20	----
24	0.10	----	----	----
26	0.10	----	----	----
27	0.10	----	----	----
29	0.20	----	----	----
30	----	0.10	----	----
33	0.20	----	----	----
35	0.10	----	----	----
38	0.10	0.10	----	----
43	0.10	----	----	----
73	0.10	----	----	----
119	0.10	----	----	----
176	0.10	----	----	----
180	0.10	----	----	----
$\bar{d}$** =	4.18μ	2.05μ	1.96μ	1.15μ
N*** =	1000	1029	1039	1215

* 1 unit = 1.333μ
** $\bar{d}$ = arithmetic mean
*** N = number of particles measured

PROCEDURE USED IN ADSORPTION EXPERIMENTS

The dry, autoclaved soil preparations were used. Initially, the amount of soil used in preliminary experiments was such as to give a suspended solids concentration of approximately 500 mg/liter; a typical value found naturally. However, this concentration was not sufficient to permit a detectable amount of adsorption by the technique used. Consequently, the soil concentrations were increased to 5,000 - 9,000 mg/liter, values more typical of the peak concentrations of suspended sediments in the natural water systems under study.

The soil was weighed into a 250-ml Erlenmeyer flask, and to it was added 50.0 mls of a solution of the protein, in distilled water or buffer solution, which had been equilibrated to the temperature of incubation to be used. As a control, a 50.0 ml aliquot of the protein solution was added to an empty flask also. The control was incubated and otherwise treated in the same manner as the solution in which the soil had been mixed. The flasks were closed with rubber autoclavable caps and their contents were mixed. They were then set in a New Brunswick PsychroTherm incubator-shaker and shaken at the 200 rpm on a rotary motion shaker at the temperature desired for at least 24 hours. Experiment had shown that the solution protein concentration would remain the same after two weeks' incubation as it was after 24 hours. In fact, 80% of the equilibrium value was found to be reached within 6 to 8 hours at 5°C. Therefore, the 24-hour value could be considered the "equilibrium value".

To determine the amount of protein remaining in solution after equilibrium had been reached between the soil surface and the protein molecules, a 5-ml aliquot of the suspension was centrifuged at 2160 x G in a Sorvall SP/X clinical centrifuge. Then the one- or two-ml aliquot needed for protein analysis was carefully pipetted from the upper layer of supernatant. Preliminary experiments had shown that 15 minutes at 2160 x G was sufficient to clarify the soil suspensions without affecting the soluble protein concentration. Filtering the suspension through 0.45μ membrane filters to remove the soil particles had been considered, but it was found that some of the soluble

protein was also removed in the process, and the amount removed varied with the initial amount present.

ADSORPTION OF BOVINE SERUM ALBUMIN AS A FUNCTION OF pH

The adsorption of BSA by the seven soil samples and the three reference clay minerals was measured as a function of pH. Solutions of 25 mg/liter of BSA buffered at pH values from 2 to 10 and an incubation temperature of 5°C. were used. The buffers and their ionic strengths (μ) are shown in Table 7. The BSA solutions were of low ionic strengths although not as low as the natural waters themselves, where μ equals 0.003-0.004 according to calculations based on the chemical compositions of the waters. The results of these experiments are given in Table 8 and are shown graphically in Figures 4 through 13.

ADSORPTION OF BSA AS A FUNCTION OF CONCENTRATION AND TEMPERATURE

In order to obtain additional information on the nature of the adsorption mechanism, the variation in adsorption with initial solution BSA concentration was examined at four temperatures. The Knik Arm, Lowe River, and Fairbanks soils were suspended in distilled water BSA solutions which ranged in concentration from 4 to 800 mg/liter. Incubation temperatures of 5, 10, 15, and 25°C. were used. The results of these experiments are presented in Table 9.

TABLE 7

BUFFERS USED IN pH EXPERIMENTS

Buffer System	pH of BSA - Buffer Solution @ 20°C.	Ionic Strength of Buffer $\mu = \frac{1}{2} \sum C_i Z_i^2$
KCl - HCl	2.02	0.0666
KHP - HCl	2.98	0.0670
KHP - HCl	3.84	0.0526
KHP - NaOH	4.60	0.0592
KHP - NaOH	5.00	0.0750
KHP - NaOH	5.58	0.0885
KH_2PO_4 - Na_2HPO_4	6.57	0.1630
KH_2PO_4 - Na_2HPO_4	7.70	0.2608
$NaHCO_3$ - Na_2CO_3	8.87	0.1581
$NaHCO_3$ - Na_2CO_3	10.03	0.1100

TABLE 8

ADSORPTION OF BOVINE SERUM ALBUMIN AS A FUNCTION OF pH

BSA = 25 mg/liter Temperature = 5°C.

Soil Sample	Soil gm/liter	pH	% of Available Protein Adsorbed	Protein Adsorbed mg/gm Soil
				(27.6)*
Knik Arm	5.0-5.3	2.02	87.7	4.69
		4.60	84.6	3.82
		5.04	81.4	4.01
		5.58	58.7	2.70
		6.59	35.1	1.72
		7.70	44.1	2.07
		8.87	45.8	2.34
		10.03	38.6	2.11
				(5.58)*
Lowe River	7.2-7.4	2.00	41.4	1.59
		3.00	77.9	2.13
		4.60	81.4	2.66
		5.03	62.1	2.18
		5.58	30.0	1.02
		6.58	14.0	0.49
		7.70	20.9	0.68
		8.87	23.3	0.80
		10.03	14.7	0.58
				(15.0)*
Fairbanks	6.8-7.1	2.05	35.7	1.47
		3.00	100.0	2.90
		4.60	69.1	2.34
		5.08	41.6	1.57
		5.58	18.5	0.65
		6.58	9.61	0.36
		7.70	15.9	0.55
		8.87	15.4	0.58
		9.99	4.25	0.18
				(10.7)*
Minto	7.2-8.0	2.98	83.4	2.30
		4.60	81.4	2.43
		5.58	17.4	0.52
		7.70	4.6	0.14
		8.87	17.2	0.54

*Values in parentheses represent the mg BSA/gm soil required to form a monolayer.

TABLE 8, Continued:

BSA = mg/liter			Temperature = 5°C.	
Soil Sample	Soil gm/liter	pH	% of Available Protein Adsorbed	Protein Adsorbed mg/gm Soil
				(43.5)*
Stevens Village	6.3-6.9	2.98	46.5	1.48
		4.60	37.6	1.32
		5.58	9.3	0.32
		7.70	0.0	0.00
		8.87	17.2	0.64
Delta River	8.8-9.1	2.98	100.0	2.27
		4.60	77.2	2.07
		5.58	35.3	0.92
		7.70	26.5	0.73
		8.87	32.1	0.94
				(588.)*
Montmorillonite	6.4-6.9	2.98	100.0	3.10
		4.60	100.0	3.52
		5.58	77.5	2.71
		7.70	73.1	2.66
		8.87	84.8	3.14
				(22.3)*
Kaolinite	6.3-6.7	2.98	100.0	3.06
		4.60	89.4	3.14
		5.58	80.5	3.02
		7.70	72.5	2.60
		8.87	73.0	2.84
				(160.)*
Illite	5.9-6.2	2.98	64.8	2.07
		4.60	67.2	2.64
		5.58	76.3	2.99
		7.70	100.0	4.06
		8.87	78.3	3.34

*Values in parentheses represent the mg BSA/gm soil required to form a monolayer.

TABLE 9

ADSORPTION OF BSA FROM DISTILLED WATER SOLUTIONS: VARIATION WITH TEMPERATURE AND INITIAL PROTEIN CONCENTRATION

KNIK ARM

Temperature ° C.	Initial Protein Added mg/gm Soil	Equilibrium Protein Concentration mg/liter	Protein Adsorbed mg/gm Soil	Surface Area Covered Percentage
5	0.68	3.3	0.06	0.22
	2.35	7.8	0.87	3.16
	4.47	17.2	1.20	4.36
	10.20	35.4	3.29	11.94
	13.50	58.0	2.78	10.09
	24.10	115.0	2.94	10.67
	27.10	128.0	3.11	11.29
	50.60	245.0	5.84	21.20
	52.10	263.0	2.73	9.91
	89.60	460.0	4.80	17.42
	111.00	627.0	0.00	0.00
	150.00	696.0	20.80	75.50
10	1.46	0.0	1.46	5.30
	3.26	11.4	1.10	3.99
	4.93	26.0	0.00	0.00
	9.92	46.7	1.13	4.10
	30.10	138.0	3.98	14.45
	40.70	208.0	1.33	4.83
15	1.04	3.8	0.33	1.20
	3.03	10.7	1.02	3.70
	5.01	24.6	0.36	1.31
	9.65	42.1	1.72	6.24
	23.80	106.0	3.77	13.69
	39.30	186.0	4.16	15.10
	72.00	338.0	8.29	30.09
25	1.38	7.0	0.06	0.22
	3.29	13.5	0.74	2.69
	6.38	23.9	1.86	6.75
	10.50	42.6	2.48	9.00
	26.80	116.0	4.91	17.82
	42.10	170.0	10.00	36.30
	73.80	338.0	10.20	37.03

TABLE 9, Continued:

FAIRBANKS

Temperature ° C.	Initial Protein Added mg/gm Soil	Equilibrium Protein Concentration mg/liter	Protein Adsorbed mg/gm Soil	Surface Area Covered Percentage
5	0.52	3.6	0.00	0.00
	1.78	10.2	0.32	2.11
	3.40	20.8	0.39	2.57
	7.80	44.2	1.41	9.29
	10.60	75.0	0.00	0.00
	20.80	128.0	2.38	15.70
	40.00	268.0	1.38	9.09
	40.20	291.0	0.00	0.00
	70.80	502.0	0.00	0.00
	87.00	604.0	0.00	0.00
	117.00	784.0	3.48	22.90
10	1.11	4.5	0.46	3.03
	2.48	14.0	0.46	3.03
	4.18	28.9	0.00	0.00
	7.62	51.5	0.17	1.12
	23.00	147.0	1.74	11.50
	31.10	227.0	0.00	0.00
15	0.84	5.8	0.00	0.00
	2.32	10.7	0.78	5.15
	3.84	26.5	0.00	0.00
	7.39	49.7	0.22	1.45
	18.20	123.0	0.43	2.84
	30.00	189.0	2.74	18.10
	55.20	368.0	2.02	13.30
25	1.13	7.8	0.00	0.00
	2.54	12.8	0.67	4.42
	4.88	25.7	1.16	7.64
	8.07	50.8	0.71	4.68
	20.60	120.0	3.20	21.10
	32.40	213.0	1.45	9.56
	57.00	351.0	5.96	39.30

TABLE 9, Continued:

LOWE RIVER

Temperature ° C.	Initial Protein Added mg/gm Soil	Equilibrium Protein Concentration mg/liter	Protein Adsorbed mg/gm Soil	Surface Area Covered Percentage
5	0.48	3.6	0.00	0.00
	1.67	7.6	0.64	11.60
	3.16	20.2	0.44	7.98
	7.26	49.3	0.63	11.42
	9.78	69.0	0.54	9.79
	17.50	118.0	1.73	31.36
	19.30	129.0	2.01	36.44
	37.20	289.0	0.00	0.00
	37.20	269.0	1.07	19.40
	65.00	484.0	0.27	4.90
	80.30	642.0	0.00	0.00
	108.00	744.0	8.55	155.00
10	1.03	0.0	1.03	18.67
	2.31	10.2	0.94	17.04
	3.47	22.2	0.48	8.70
	7.09	45.8	0.93	16.86
	21.40	134.0	3.36	60.92
	28.90	209.0	0.81	14.69
15	0.74	4.2	0.18	3.26
	2.17	11.8	0.58	10.52
	3.56	25.6	0.12	2.18
	6.90	49.1	0.28	5.08
	17.00	115.0	1.48	26.83
	29.50	220.0	0.00	0.00
	51.40	342.0	5.38	97.54
25	0.98	3.2	0.55	9.97
	2.33	11.5	0.79	14.32
	4.54	23.6	1.36	24.66
	7.48	51.7	0.54	9.79
	19.10	144.0	0.00	0.00
	29.90	192.0	4.16	75.42
	52.80	355.0	4.98	90.29

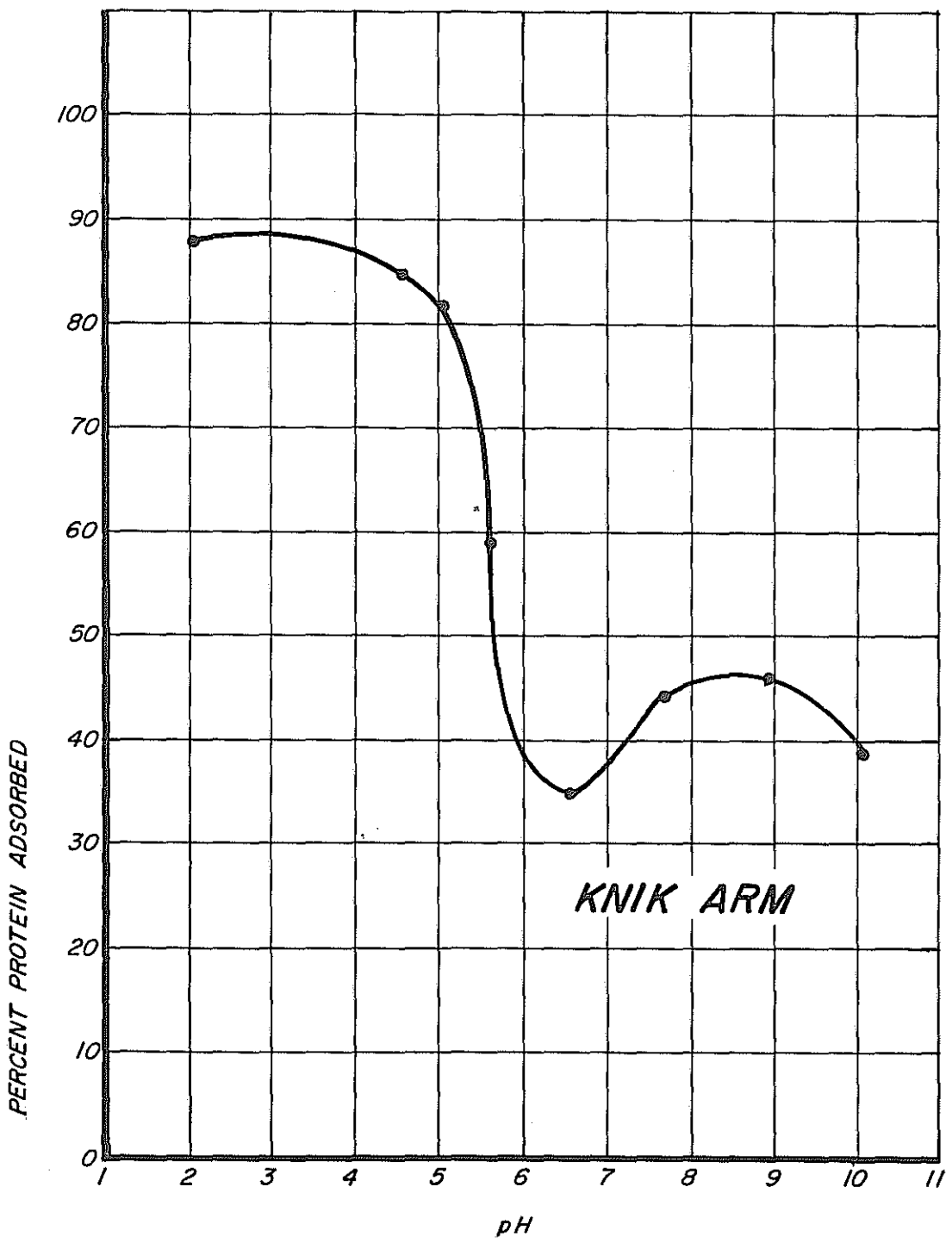

Figure 4. Adsorption of Bovine Serum Albumin as a Function of pH: Knik Arm (T = 5°C.).

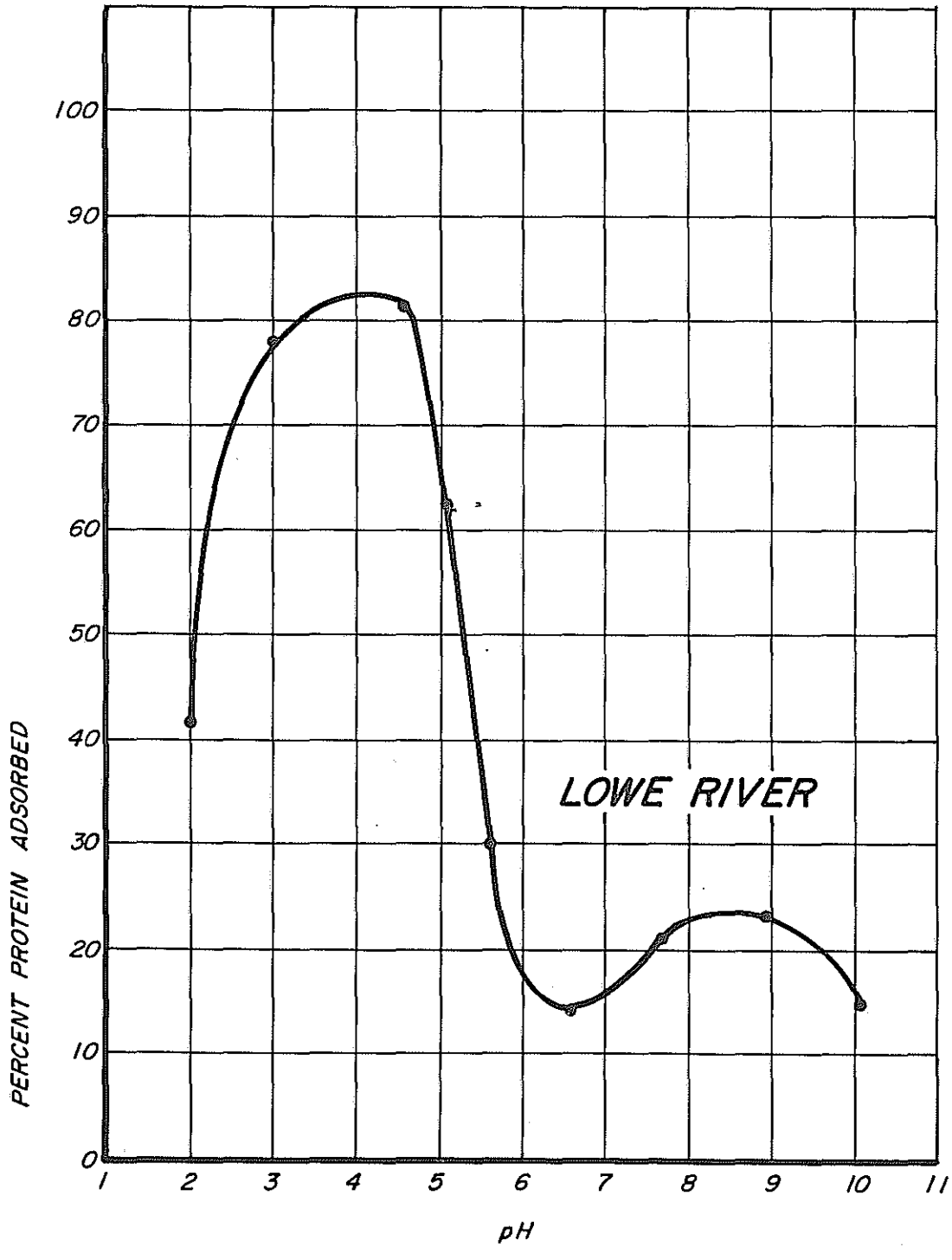

Figure 5. Adsorption of Bovine Serum Albumin as a Function of pH: Lowe River (T = 5°C.).

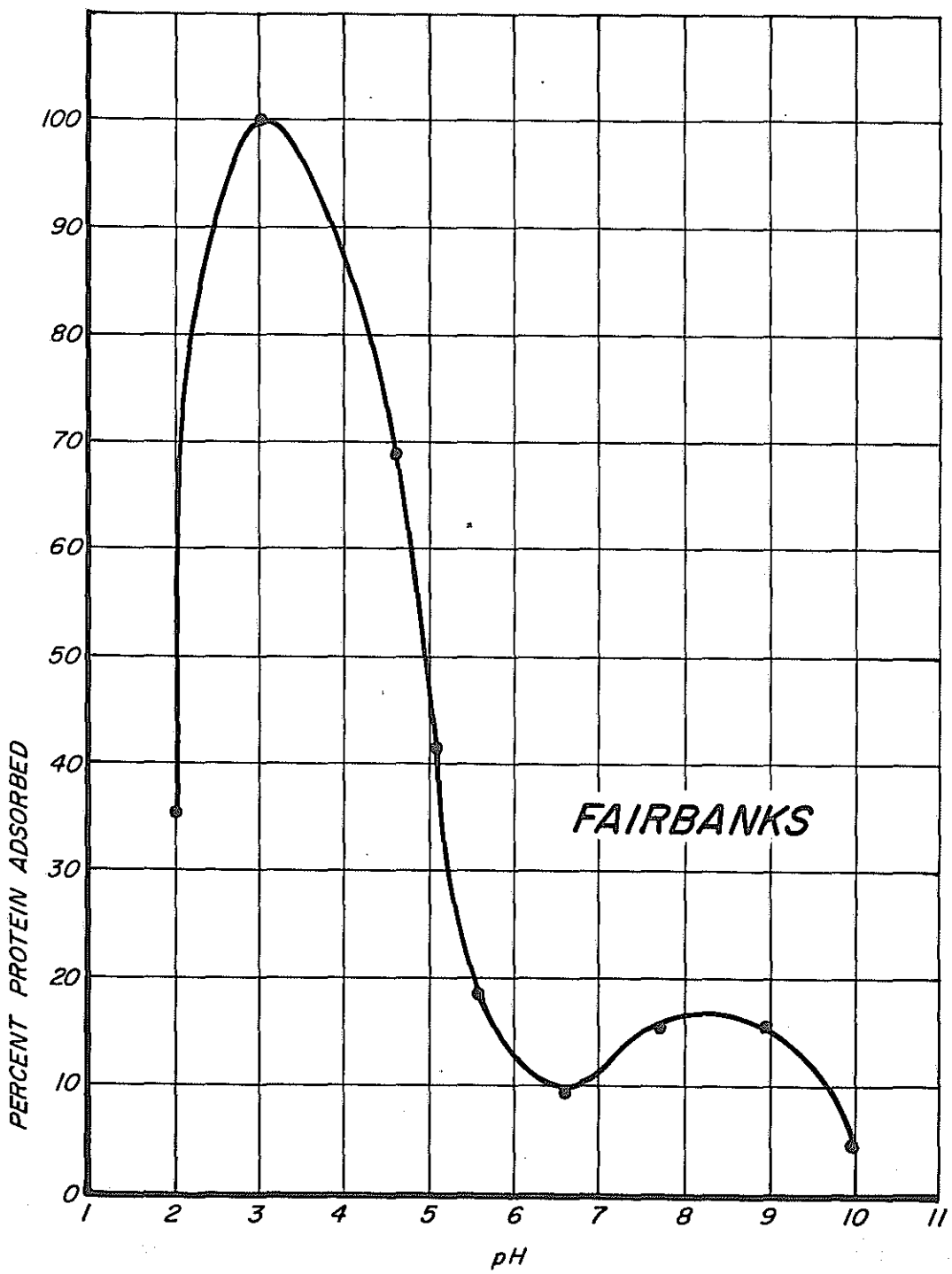

Figure 6. Adsorption of Bovine Serum Albumin as a Function of pH: Fairbanks (T = 5°C.).

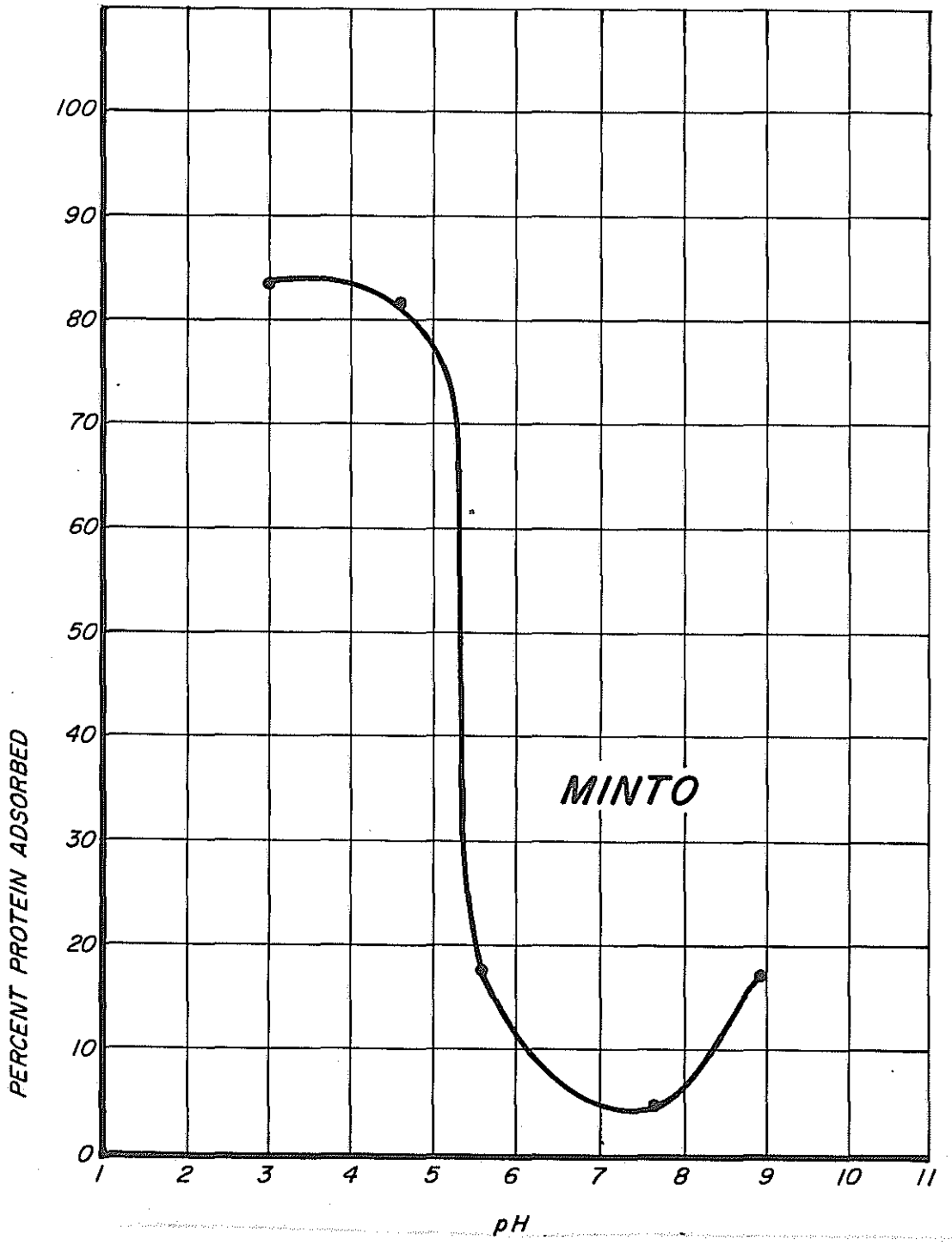

Figure 7. Adsorption of Bovine Serum Albumin as a Function of pH: Minto (T = 5°C.).

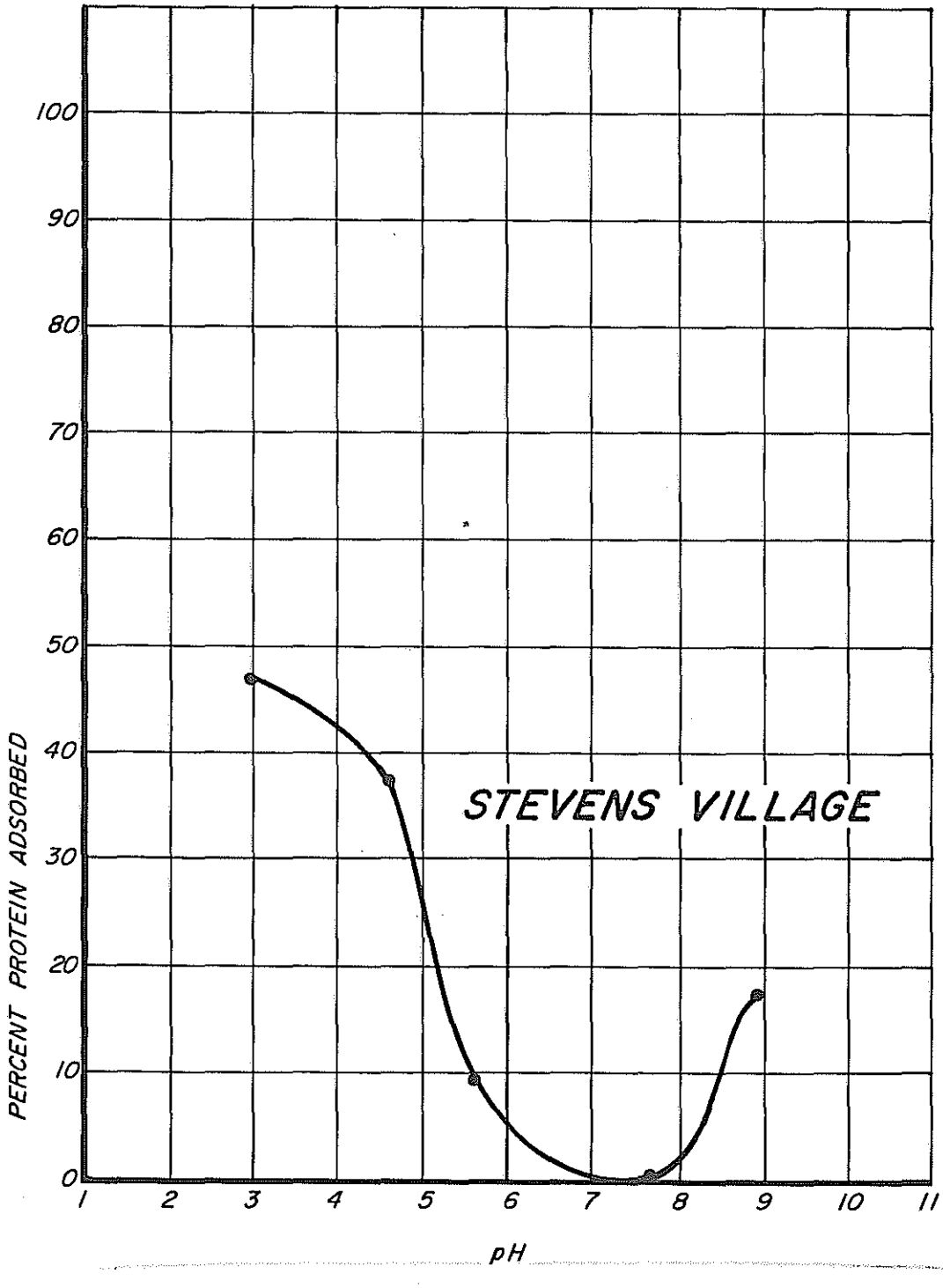

Figure 8. Adsorption of Bovine Serum Albumin as a Function of pH: Stevens Village (T = 5°C.).

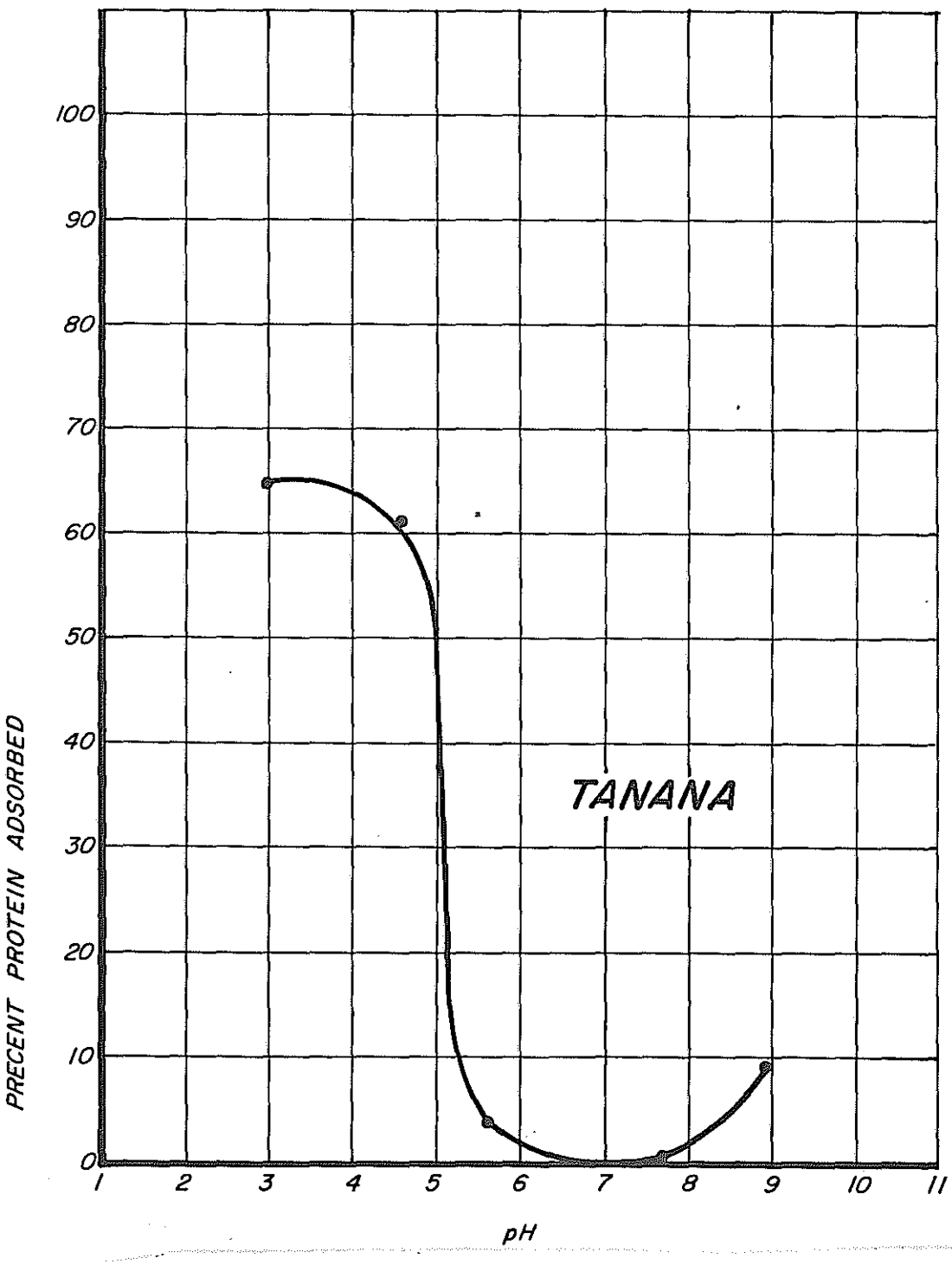

Figure 9. Adsorption of Bovine Serum Albumin as a Function of pH: Tanana (T = 5°C.).

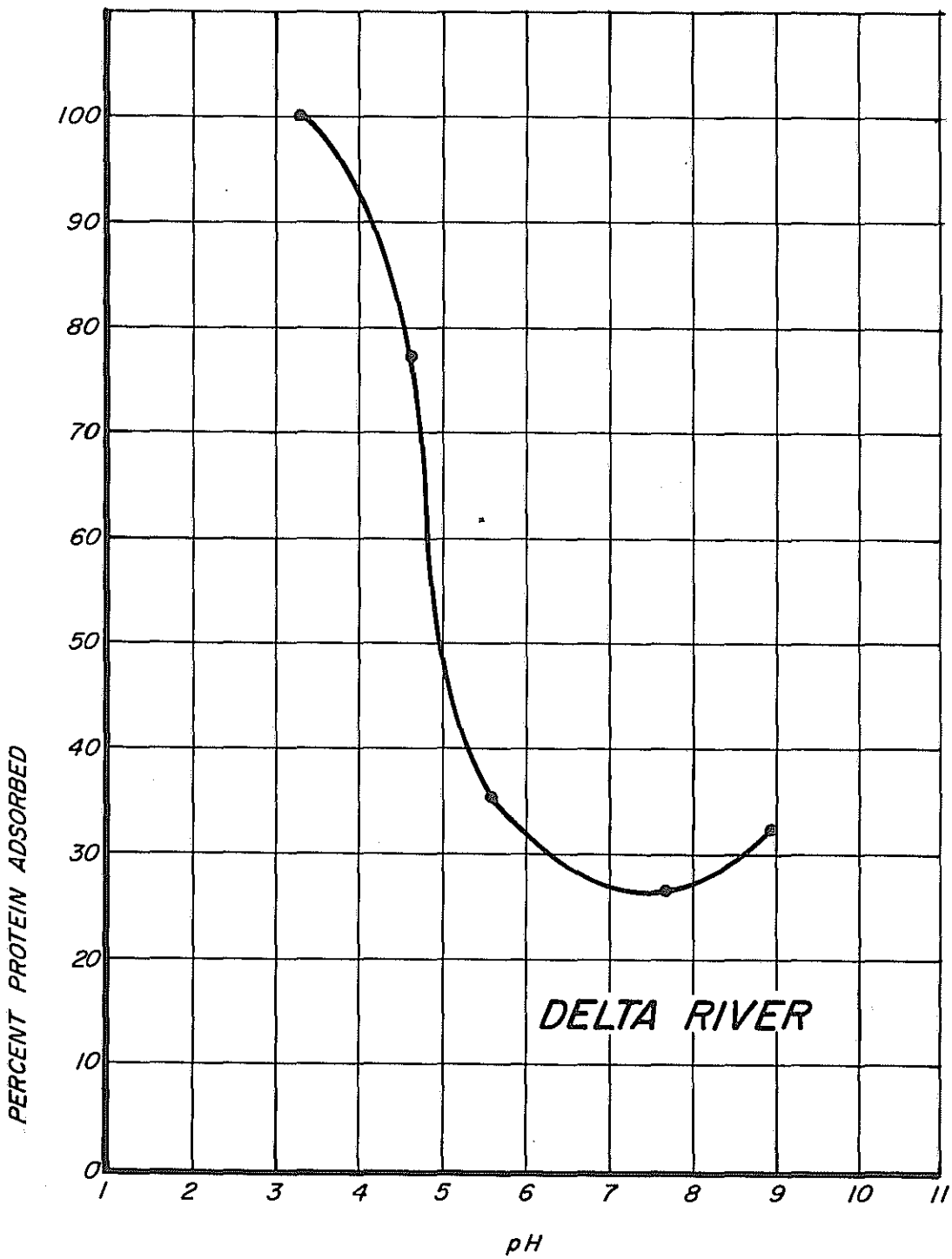

Figure 10. Adsorption of Bovine Serum Albumin as a Function of pH: Delta River (T = 5°C.).

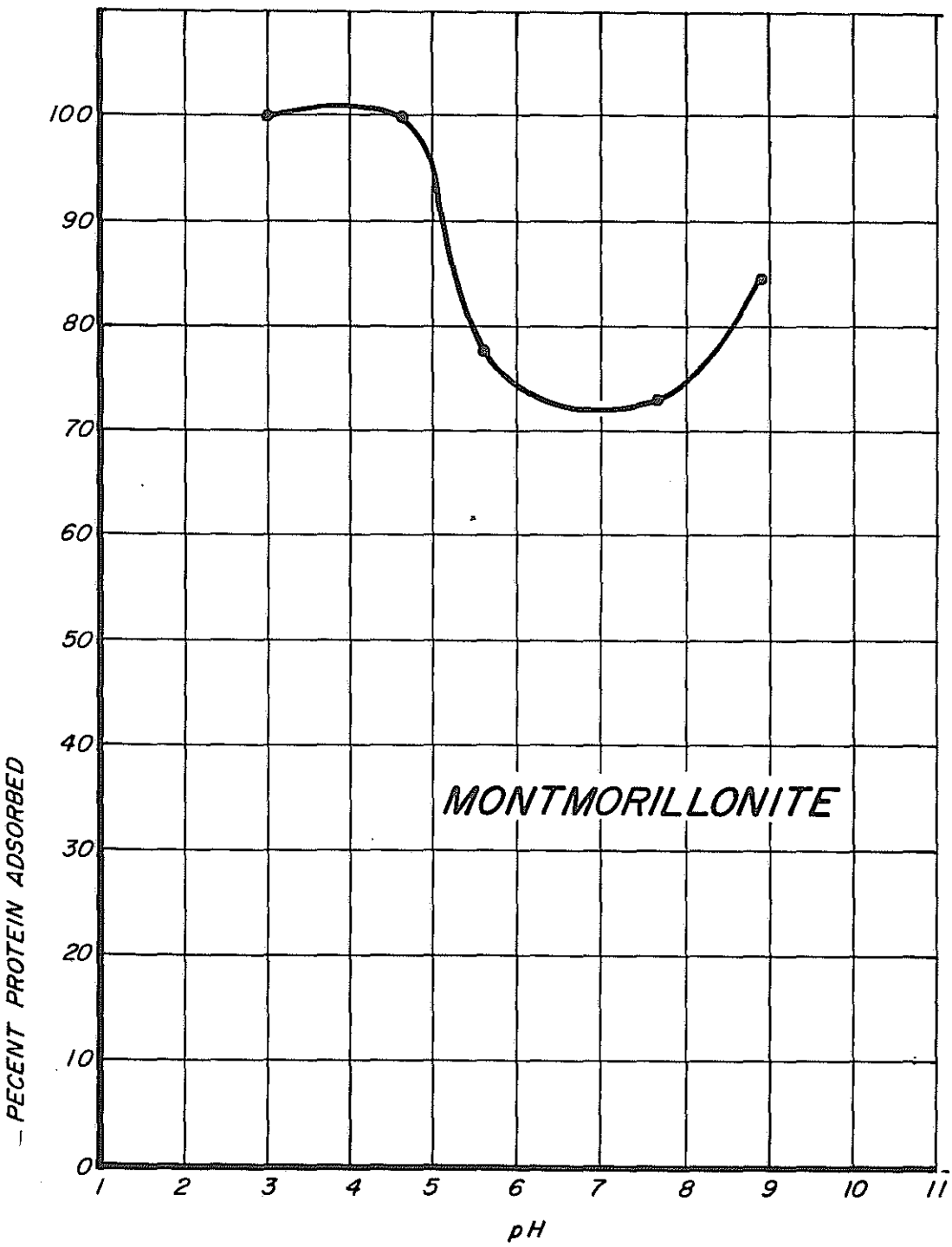

Figure 11. Adsorption of Bovine Serum Albumin as a Function of pH: Montmorillonite (T = 5°C.).

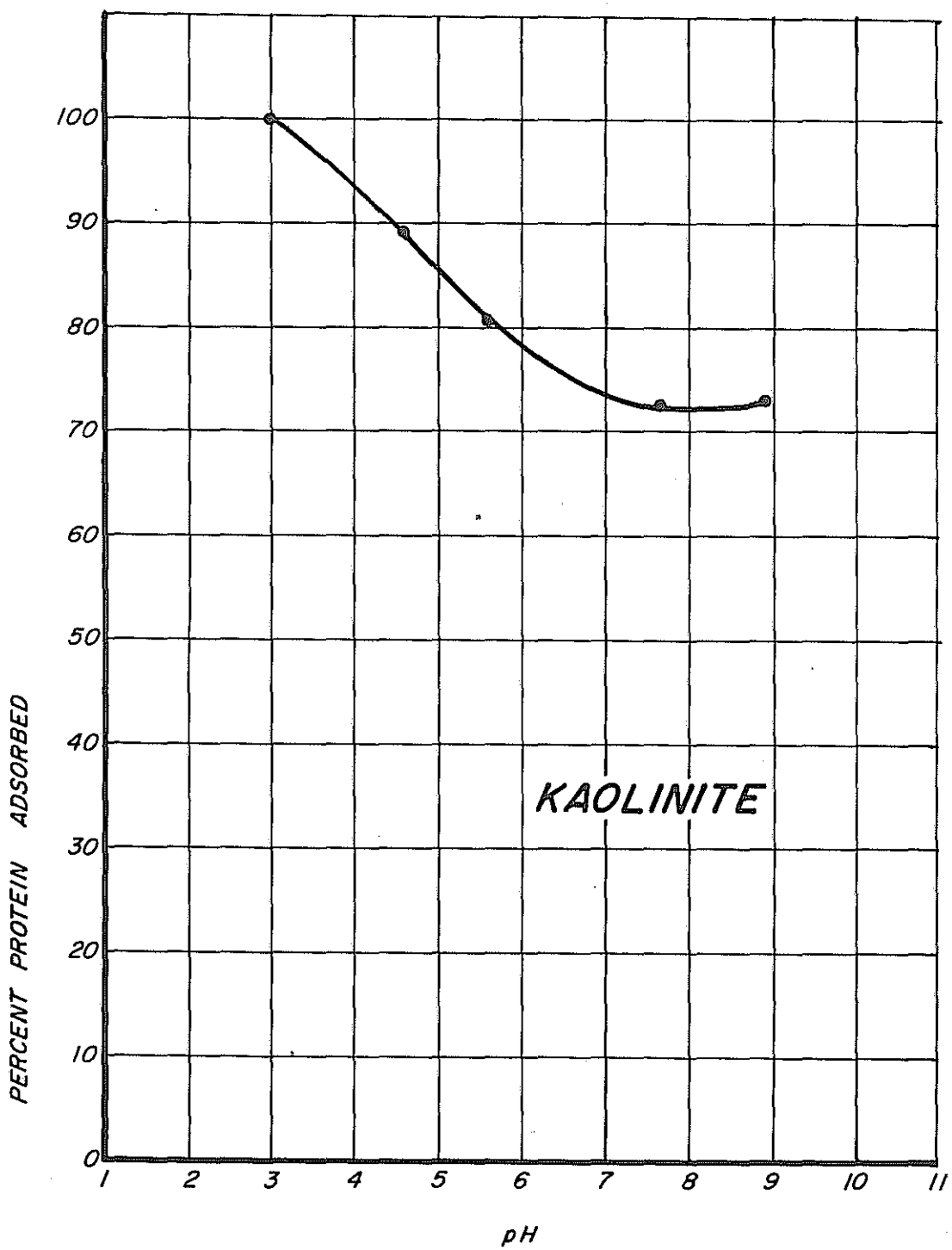

Figure 12. Adsorption of Bovine Serum Albumin as a Function of pH: Kaolinite (T = 5°C.).

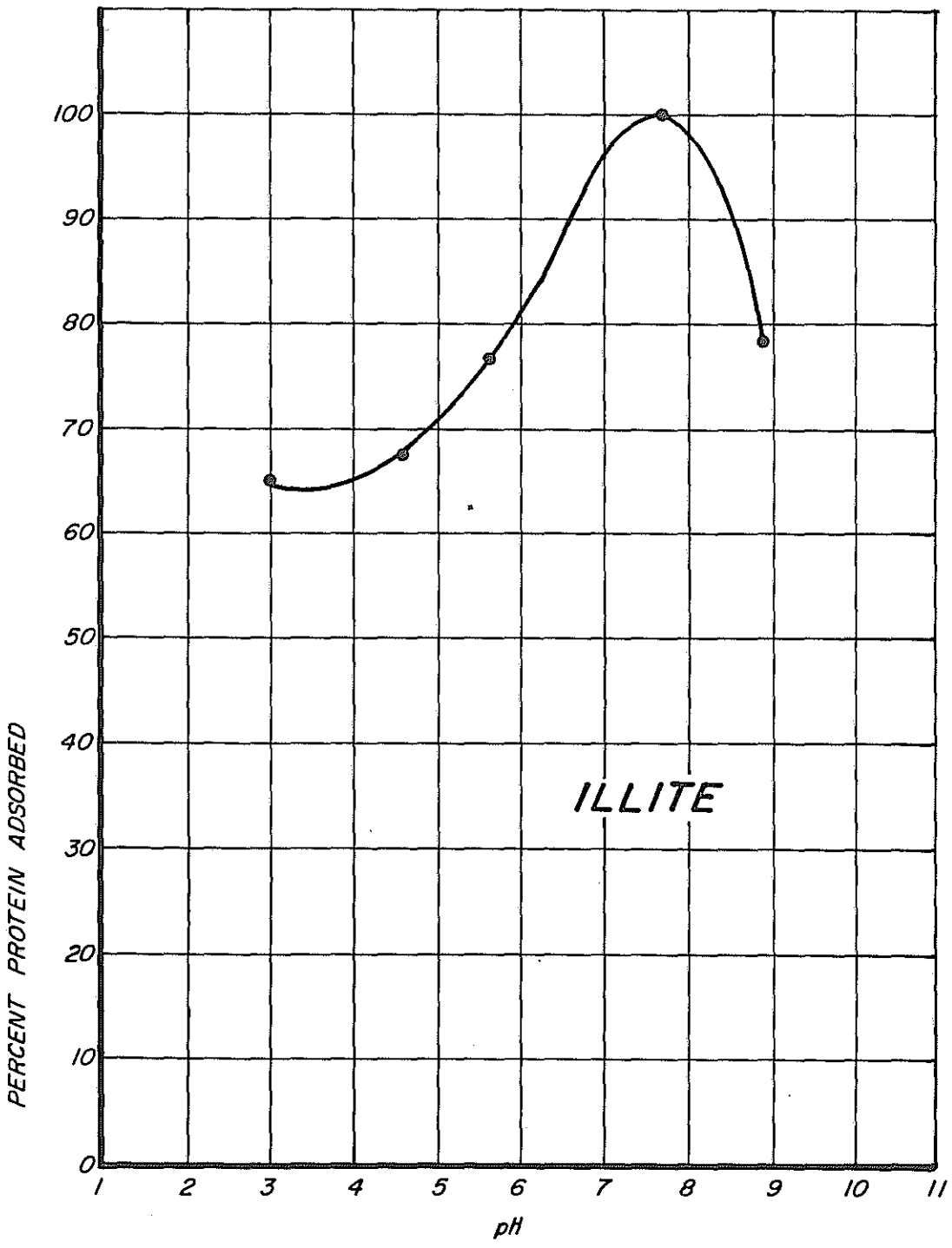

Figure 13. Adsorption of Bovine Serum Albumin as a Function of pH: Illite (T = 5°C.).

DISCUSSION

The fluctuations in the extent of adsorption with pH are consistent with a system in which electrostatic forces play a predominant role in the interactions between the soil surface and the protein molecules. An adsorption maximum occurs near pH 3. At this pH the protein molecule is extensively positively charged and below pH 3 the positive charge increases but slightly (Tanford et al., 1955). The soil particles are apparently negatively charged at pH 3 and above, but somewhere in the vicinity of pH 2 - 3 they appear to undergo a charge reversal and acquire a net positive charge, as evidenced by the fact that the attraction of the soil surface for the protein molecule decreases considerably. (See Figures 5 and 6).

At pH values greater than 3, the net positive charge on the protein molecule diminishes. The protein undergoes a charge reversal near pH 5. As the pH is increased, both the soil surface and the protein become more and more negatively charged. The adsorption data indicate that a minimum in electrostatic attraction occurs between pH 6.5 and 8, the pH region of the natural waters. Above this pH of minimum adsorption, the affinity of the soil surface for the protein molecules apparently increases again, although only slightly. Between pH 5 and 10 the protein molecule loses protons less readily than between pH 2 and 5; an average of 7 H^+ ions dissociate per unit increase in pH from 5 to 10 versus 32 per unit increase from 2 to 5. (Tanford et al., 1955). It is difficult to explain the apparent decrease in negative charge on the soil as the pH is increased from 6.5 to 9 unless it can be attributed to the presence of divalent ionic species in the buffers used and the concomitant higher ionic strengths and/or to solubilization and subsequent rearrangement in crystal structure of the soil.

The behavior of the soils with respect to adsorption as a function of pH parallels that of montmorillonite (Figure 11) and kaolinite (Figure 12) but not that of illite (Figure 13), although the amounts of protein adsorbed per unit weight of soil above pH 5.6 are considerably less than the amounts adsorbed by the reference clays. The Knik Arm soil is an exception. The

Knik Arm soil and the reference clays adsorbed 2 to 4 mg BSA/gm soil, under the conditions of the experiment, compared with a fraction of 1 mg/gm soil adsorbed by the other soils. This observation is most likely a reflection of a variety of soil materials in the natural samples.

The usual method of plotting the data obtained from experiments in which the amount of material adsorbed is measured as a function of its initial concentration in solution is to graph the adsorption density against the equilibrium solution concentration of the adsorbate to yield a Langmuir-type adsorption isotherm. When the data for the adsorption of BSA as a function of concentration were treated in this manner, no simple relationship between these two variables could be ascertained. (Figure 14). A Freundlich-type log-log plot of these same variables gave only a scatter diagram. However, when the equilibrium protein concentration is plotted against the initial amount of protein per unit weight of soil, a smooth curve is obtained, which on a log-log plot becomes a straight line of the form:

$$\log c = n \log \left(\frac{P_o}{m}\right) + \log k$$

where c is the equilibrium protein concentration in mg/liter, P_o/m is the initial protein added in mg BSA/gm soil, and k and n are constants. (Figures 15, 16 and 17). The value of n is 1.06 for the Lowe River and Knik Arm samples and 1.04 for the Fairbanks sample. The constant k equals 5.46 for Lowe River, 3.60 for Knik Arm, and 5.50 for Fairbanks. Thus, for any one of these samples, the amount of protein remaining in solution after equilibrium has been reached is related to the initial amount of protein by a constant factor:

$$c = k\left(\frac{P_o}{m}\right)^n$$

Since n is approximately 1, the value of the equilibrium solution protein can readily be predicted for any given ratio of initial protein concentration to soil concentration from about 1 to 100. For the Fairbanks and Knik

Arm soils it will be about five and a half times P_o/m and for the Lowe River soil about three and a half times P_o/m, in mg/liter. Thus, although the data do not lend themselves to the usual type of adsorption isotherms, perhaps because of the relatively very low concentrations used, an empirical relationship has been observed which allows one to predict the amount of protein that will be adsorbed by these three soils for various protein-soil ratios.

As can be seen in Table 9, the amounts of protein adsorbed by these soils in the concentration range studied is really quite small, being a few mg BSA/gm soil or less in most instances. This represents a sparse coverage of the surface area available. Only at the higher concentrations is a monolayer even approached. Since one would not expect protein to be present in the natural waters at more than a few hundred mg/liter, in the natural system then only a small portion of the soil surface would be covered with protein. Thus, according to the model described here, adsorption would not be expected to alter the rate at which protein is decomposed by bacteria in those waters. This statement, however, requires experimental verification, for there may be other factors involved in the decomposition process that are more important than the proximity of the bacterium to its substrate and which outweigh it.

As can be seen in Figures 15, 16 and 17, the adsorption isotherms for 5, 10, 15, and 25°C. all coincide for any one soil. Thus, temperature variations in this range have no effect on the amount of protein adsorbed by a given soil.

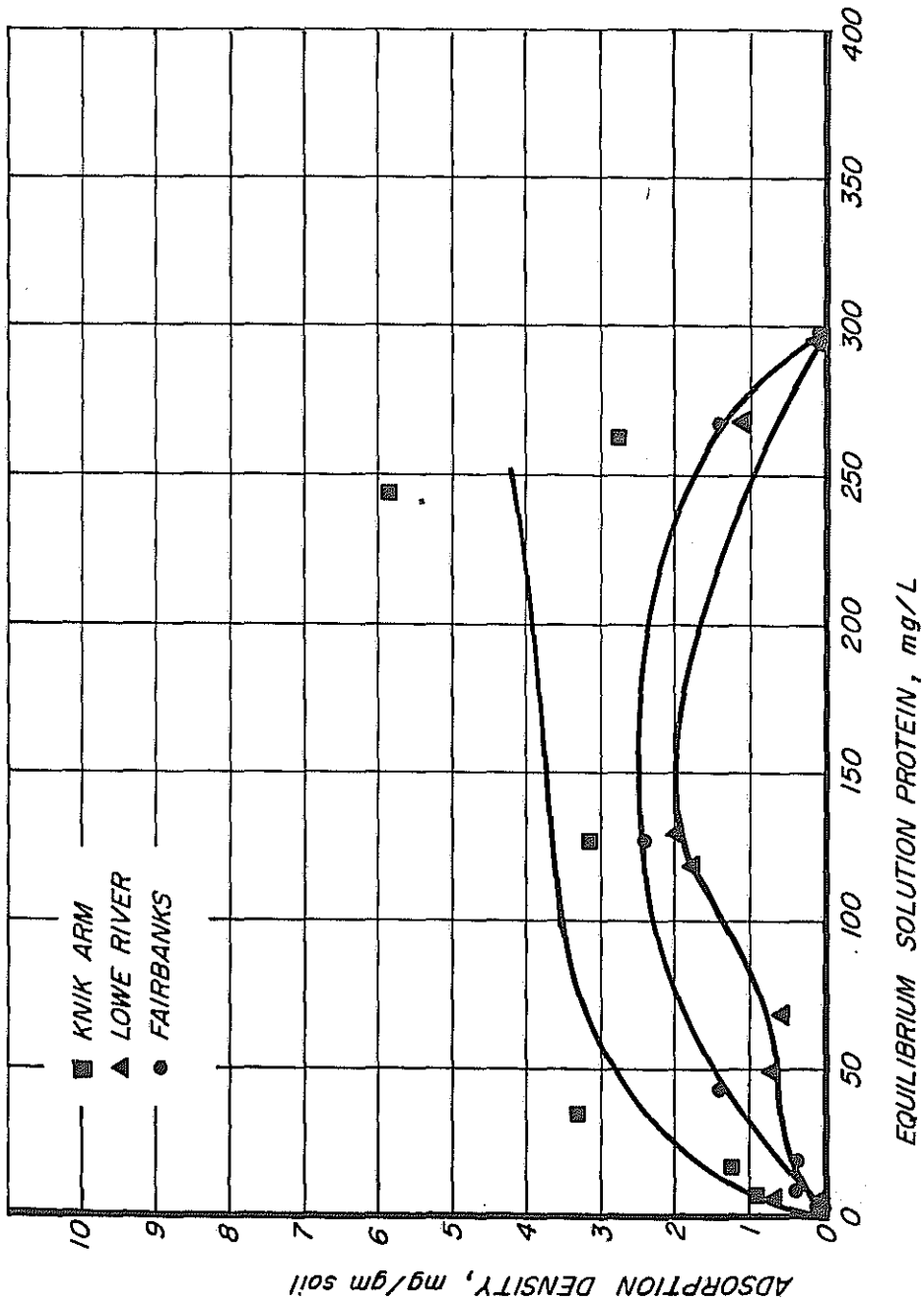

Figure 14. Typical Adsorption Isotherms Obtained When Data Are Treated According to the Langmuir Convention, (T = 5°C.).

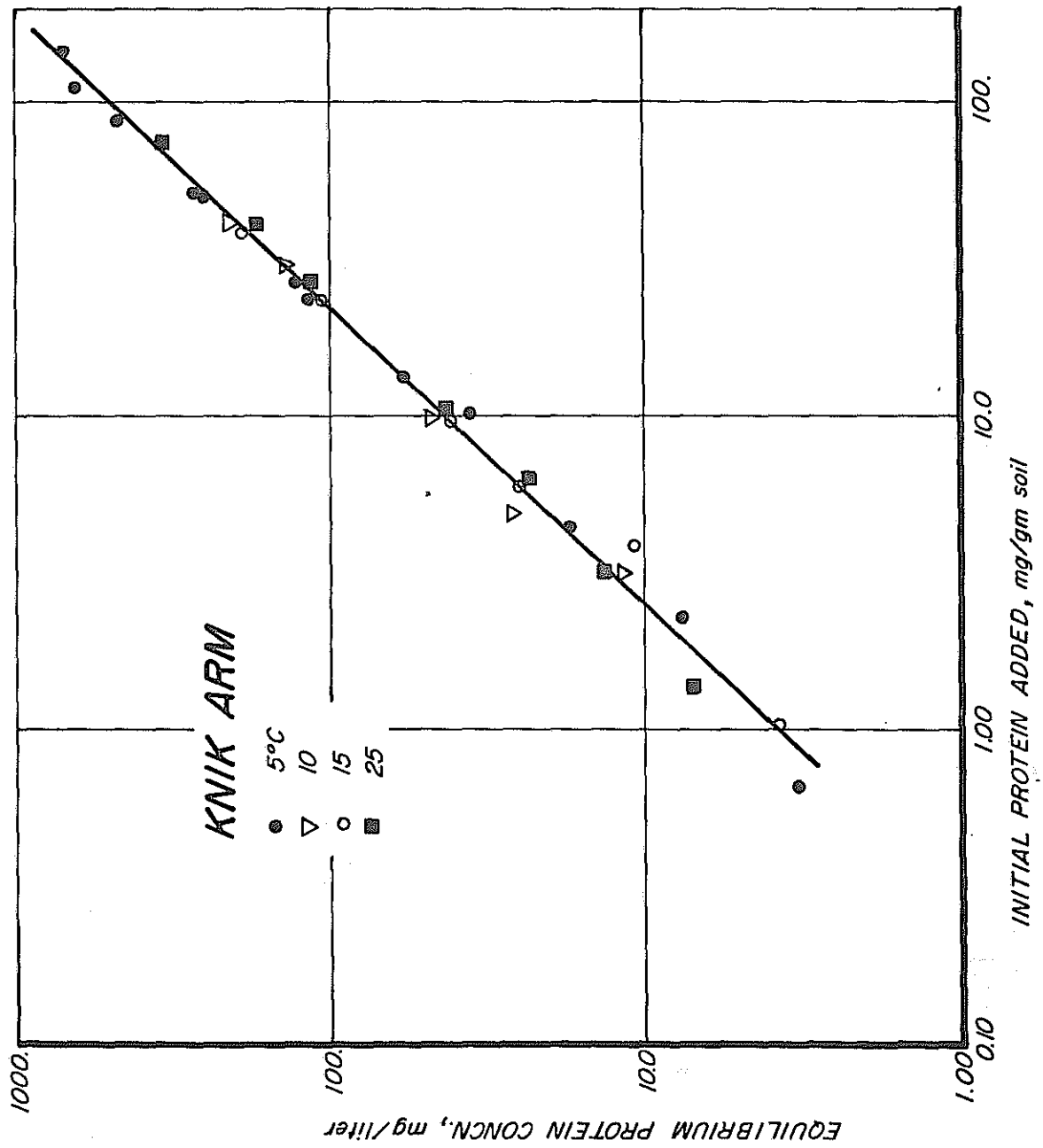

Figure 15. The Relationship Between the Equilibrium Protein Concentration and the Initial Amount of Protein Added: Knik Arm (T = 5°, 10°, 15°, 25°C.) Log-log-plot.

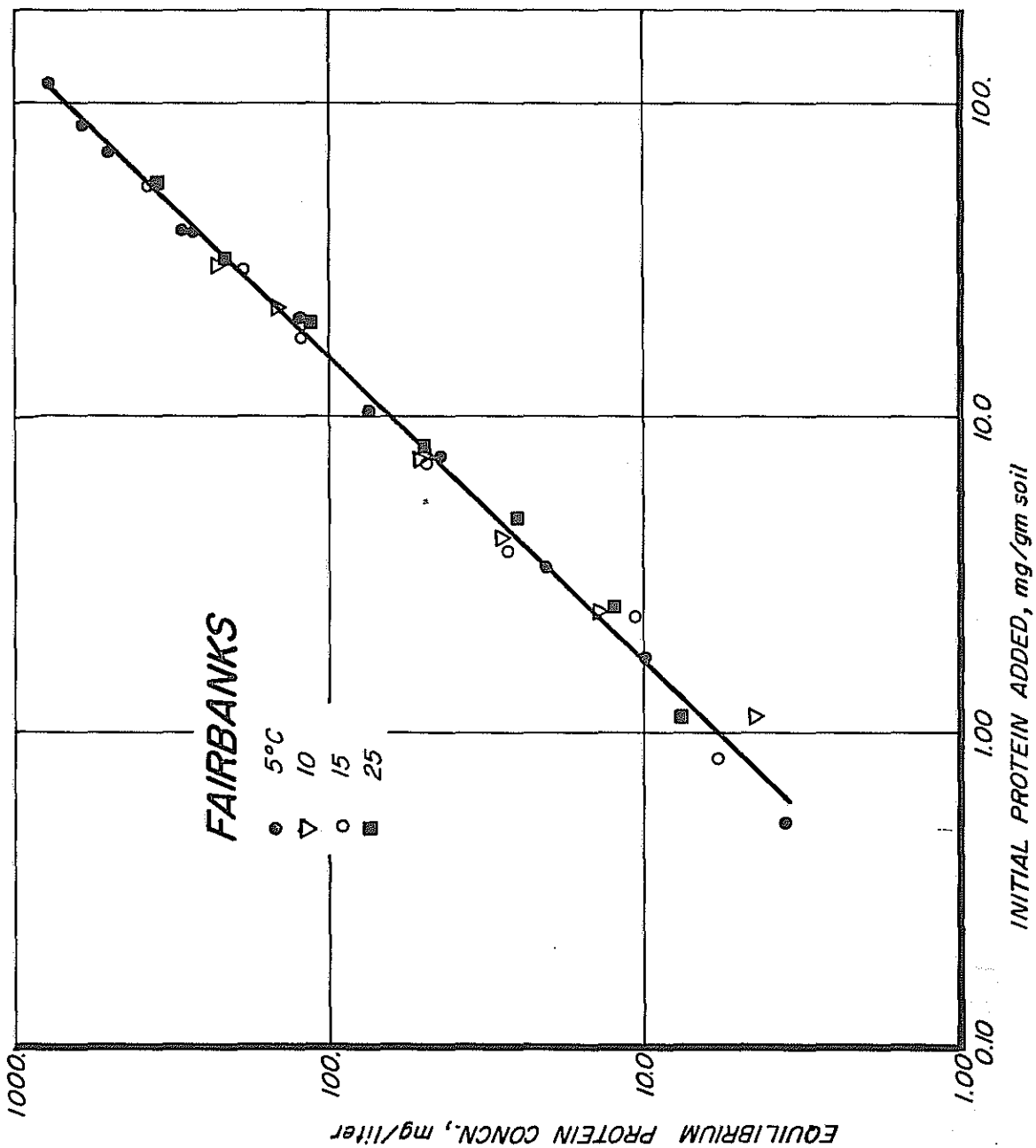

Figure 16. The Relationship Between The Equilibrium Protein Concentration and the Initial Amount of Protein Added: Fairbanks (T = 5°, 10°, 15°, 25°C) Log-log-plot.

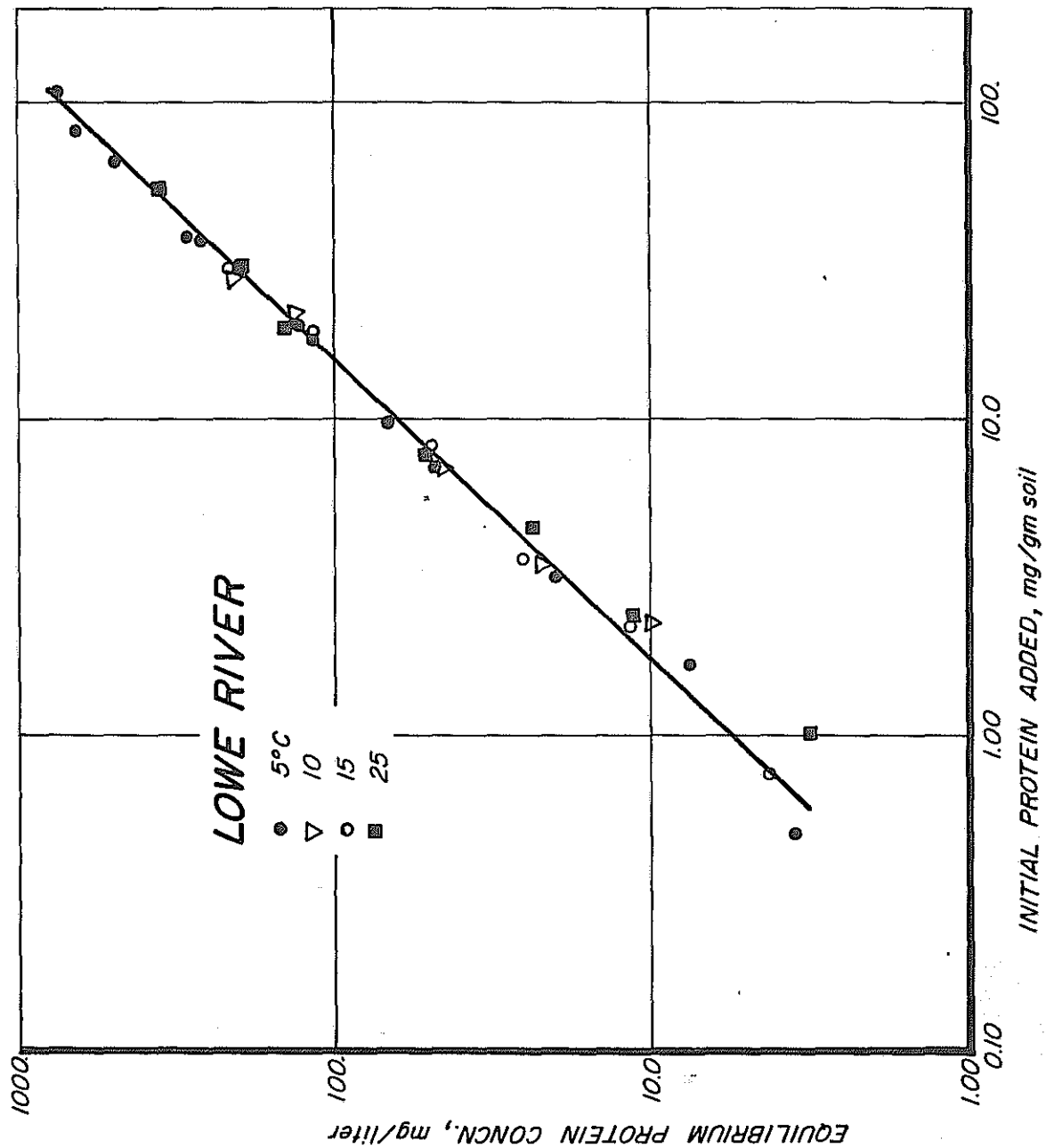

Figure 17. The Relationship Between the Equilibrium Protein Concentration and the Initial Amount of Protein Added: Lowe River (T = 5°, 10°, 15°, 25°C.) Log-log-plot.

SUMMARY

Water samples collected from Alaskan rivers and estuaries were characterized in terms of their suspended sediment loads and the particle size distributions of the suspended material. Samples were taken from the Yukon-Tanana River drainage in Interior Alaska and from Knik Arm at Anchorage and from the Lowe River in Valdez. Soils collected from the same sampling sites were dried, screened, and ground to have particle size distributions similar to those of the corresponding waters. The specific surface of each soil was determined by ethylene glycol retention. These soils, plus the three reference clay minerals, kaolinite, montmorillonite, and illite, were used in adsorption experiments designed to measure the affinities of the soils for protein. It was necessary to learn the capacities of the soils for protein. It was necessary to learn the capacities of the soils to adsorb protein from solution in order to predict the fate of proteinaceous waste submitted to sediment-laden natural waters.

From experiments in which the affinities of the soils for the protein bovine serum albumin (BSA) were measured at various pH values from 2 to 10, it was concluded that electrostatic interactions play the most important role in the adsorption mechanism. It was also observed that the pH of minimum adsorption of the protein by the soils was in the pH range of the natural waters, namely 6.5 to 8. The pH of maximum adsorption was 2 or 3. The behavior of the Alaskan samples in this respect was similar to that of montmorillonite and kaolinite but unlike that of illite.

From experiments in which the adsorptive capacities of the Knik Arm, Lowe River, and Fairbanks soils were measured as a function of the initial amount of protein, it was found that the equilibrium concentration of protein remaining in solution could be related by a constant to the initial protein added per gram of suspended sediment:

$$c = k\left(\frac{P_o}{m}\right)^n$$

In the case of the Knik Arm and Fairbanks samples, this constant is 5.5; for the Lowe River sample it is 3.6. The exponent n is approximately 1 in each case. At the concentration of protein used in these experiments, 4 to 800 mg/liter, most of the protein remains in solution once equilibrium is reached. Only a few mg BSA is adsorbed per gram of soil in most instances; the fraction of the available surface area covered is low. Therefore, it would be expected that the adsorption of this protein by suspended soil material would have a negligible effect on the rate at which the protein is decomposed by bacteria in the natural water systems.

In addition, it was learned that variations in temperature from 5 to 25° C. had no detectable effect on the amount of protein adsorbed by a given soil.

LITERATURE CITED

Amdur, I. and Hammes, G. G. (1966) Chemical Kinetics: Principles and Selected Topics p. 50. McGraw-Hill, New York.

Bower, C. A. and Gschwend, F. B. (1952) Ethylene Glycol Retention by Soils as a Measure of Surface Area and Interlayer Swelling. Proc. Soil Sci. Soc. Am. 16, 342-345.

Giles, C. H. (1959) Hydrogen Bonding in Adsorption Processes. IN Hydrogen Bonding, D. Hadzi, ed., p. 449-460. Pergamon Press, New York.

Haurowitz, F. (1950) Chemistry and Biology of Proteins p. 101. Academic Press, New York.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) Protein Measurement with the Folin Phenol Reagent. J. Biol. Chem., 193, 265-275.

Mahler, H. R. and Cordes, E. H. (1966) Biologic Chemistry p. 54. Harper & Row, New York.

Marshall, C. E. (1964) The Physical Chemistry and Mineralogy of Soils p. 71. John Wiley, New York.

Martin, R. T. (1955) Ethylene Glycol Retention by Clays. Proc. Soil Sci. Soc. Am. 19, 160-163.

Parks, G. (1967) Aqueous Surface Chemistry of Oxides and Complex Oxide Minerals. IN Equilibrium Concepts in Natural Water Systems, pp. 121-160. American Chemical Society, Washington, D. C.

Stanier, R. Y. Doudoroff, M., and Adelberg, E. A. (1963) The Microbial World p. 371. Prentice-Hall, Englewood Cliffs, N. J.

Stotzsky, G. and Rem, L. T. (1966) Influence of Clay Minerals on Micro-Organisms. I. Montmorillonite and kaolinite on bacteria. Canad. J. Microbiol. 12, 547-563.

Tanford, C. (1961a) Physical Chemistry of Macromolecules p. 358. John Wiley, New York.

Tanford, C. (1961b) Physical Chemistry of Macromolecules p. 445. John Wiley, New York.

Tanford, C., Swanson, S. A., and Shore, W. S. (1955) Hydrogen Ion Equilibria of Bovine Serum Albumin, J. Am. Chem. Soc. 77, 6414-6421.

Wayman, C. H. (1967) Adsorption on Clay Mineral Surfaces. IN Principles and Applications of Water Chemistry pp. 127-167. John Wiley, New York.