ADSORPTION OF BACTERIA IN SALT LAKES

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INTRODUCTORY

The adsorption of bacteria by porous and powder-like substances has for a long time been the subject of research. It was shown by Krüger (1899) that the number of bacteria present in water may be reduced by addition of coke, sand, clay, brick-dust, magnesium oxide and other powder-like substances. The adsorbent action is much greater in the case of substances with a low specific gravity than with those whose specific gravity is high. The results were confirmed by a number of other investigators. According to Eisenberg (1918), the degree of adsorption of bacteria depends upon their lipoid contents which is higher in the cells of the gram-positive bacteria than in those of gram-negative forms. However, there is no sharp dividing line between these two groups, as regards adsorptive capacity: some gram-positive species (*Corynebacterium diphtheriae*) are only faintly adsorbed, while some gram-negative species (*Serratia marcescens*) are well adsorbed.

The problem of absorption of bacteria is of particular interest in soil investigation (Chudiakow, 1925–26; Dianowa and Woroshilowa, 1925). The degree of adsorption depends upon the size of the soil particles. The smaller their size the higher is the percentage of adsorbed bacteria. This holds true, however, only up to a certain limit beyond which there is a sharp decline in the degree of adsorption. Soil particles which do not exceed 0.27μ do not adsorb bacteria at all. On the contrary, Karpinska (1925) has shown that such particles may be adsorbed by large bacteria, as by the chains of *Bacillus mycoides*. The same phe-

nomenon of reversibility of adsorption was observed by Bechhold (1918) in experiments with white clay and other substances.

The results of our own work show that the adsorption of bacteria by liman mud exhibits a number of characteristic features; namely, dependence of the degree of adsorption not upon the concentration but upon the absolute number of bacteria; difference in adsorption of living and of dead bacteria; the presence in *Serratia marcescens* of a zone of maximum adsorption. However, it must be pointed out that, in general, Gibb's equation is only rarely fulfilled. In most cases it is accompanied by phenomena which complicate the picture and sometimes even disguise the adsorption itself. It is for this reason that, in the case of bacteria taken up by mud, we only provisionally employ the term adsorption. Later, however, after a more detailed study of the phenomena involved, it will be necessary to raise the question whether it really may be regarded as true adsorption.

EXPERIMENTAL

Methods

Various sediments are found at the bottom of salt lakes or limans, in the vicinity of Odessa, the most interesting of which may be considered the black mud. The latter is a complex product which owes its origin to various microbiological processes and contains a great quantity of microbes more than 3 billions in 1 gram of some samples. Nothing is known regarding the condition of the microbes in the mud, i.e., whether they are there in a free state or adsorbed by the mud. The capacity of black mud and other ground sediments of salt lakes for the adsorption of bacteria remained to be elucidated. There has been so far published only a brief statement (Sweshnikowa, 1926) to the effect that when mud of the Tembukan lake was shaken up with a bacterial suspension the number of bacteria in the latter decreased.

The method used in the following investigations can be outlined briefly as follows: Several loopfulls of a two- or three-day old agar slant culture of bacteria were introduced into a sterile flask containing sterilized tap-water. The bacterial mass was

then carefully distributed in the water. Since some small lumps of bacteria usually remained, it was necessary to filter the suspension through sterile filter paper. Two identical test-tubes received 10-cc. portions of this suspension. One test-tube received a definite amount of the adsorbent while the other remained as a control. Both tubes were shaken for the same length of time and the adsorbent mud allowed to settle. Usually the shaking lasted one minute while it took half an hour for the mud to settle. The number of bacteria was determined by diluting 10,000 or 100,000 times, and plating the final dilution on meat-peptone agar.

The accuracy of this method was determined in a preliminary experiment. To twenty-five test-tubes each containing 2 grams of mud were added 10-cc. portions of an aqueous suspension containing 160 millions of cells of *Serratia marcescens*. After one minute's shaking and thirty minutes settling of the mud the percentage of cells adsorbed in each test-tube was calculated. The data thus obtained were subjected to calculation by the method of variation, according to the equation (Sapehin, 1926):

$$M = \pm \sqrt{\frac{\Sigma v^2}{n} - M^2} \cdot \sqrt{\frac{1}{n-1}}$$

As a result the percentage of cells adsorbed was found to be: 96.4 ± 1.5; p (index of accuracy) = 1.55 per cent.

In those cases in which the plating method could not be employed (for instance, when the degree of adsorption of dead bacteria was to be determined), the direct microscopic method was used. For this purpose one drop of the above described diluted bacterial suspension was placed in a counting chamber (American standard Haemocytometer). The count was made in twenty-five large squares. It was, however, necessary to take into consideration the fact that the adsorbent, namely the mud or any other of the ground sediments, contains many bacteria, some of which may have been washed off while the adsorbent was shaken with water. As a control, the adsorbent was also shaken with 10 cc. of sterilized tap-water. Ten cubic centimeters of a suspension of *Serratia marcescens* contained a large enough number

of cells to give in one large square of the counting chamber, on the average, 100 bacteria. After shaking with 2 grams of mud for one minute and allowing the mud to settle for thirty minutes, the suspension contained on the average 14 bacteria in one square. Two grams of the same mud when the mud was shaken up for one minute with 10 cc. of sterilized water, and the mud allowed to settle for thirty minutes, gave 6 bacteria in one large square. It follows, therefore, that, of the remaining 14 bacteria, only 8 cells were *Serratia marcescens*. Hence the percentage of adsorption was 92. Table 1 illustrates the adsorption of 4 different bacteria by marine mud.

TABLE 1

Adsorption of different bacteria by the mud of the Kuyalnik liman

	NAME OF ORGANISM							
	S. marcescens		*B. vulgatus*		*B. mycoides*		*Sarcina lutea*	
	a	b	a	b	a	b	a	b
Number of bacteria in 10 cc. of the suspension, in millions:								
Before adsorption	125.4	301.4	31.4	150.0	59.5	210.0	25.1	130.0
After adsorption	1.0	14.2	3.4	25.5	0.4	14.7	0.24	7.5
Number of adsorbed bacteria, in millions	124.4	287.2	28.0	124.5	59.1	195.3	24.86	122.5
Percentage of adsorption	99.2	95.3	89.2	83.0	99.3	93.0	99.4	95.0

The next experiment (table 2) deals with the adsorption by the mud of different bacteria isolated from the liman water (nos. 1 and 2) and mud (nos. 3 to 7). The adsorption of the latter was much greater than of the former.

It was further found that bacteria isolated from the liman water are relatively little adsorbed and wash off easily while bacteria found in the mud are adsorbed in great quantity and few of them may be washed off with ease.

Besides black mud we have also investigated the adsorption capacity for bacteria of the other types of liman sediments. The following results were found to represent the percentage of adsorption of *S. marcescens:*

Non-plastic black-gray mud	68.0–79.1
Gray plastic mud	45.8–84.8
Black (young) mud	71.2
Black sand	39.5–61.9
Gray sand[1]	19.3–20.5
Grayish-black sand	21.0–22.4

It is evident from these data that the various types of ground sediments differ in their adsorption capacity for *S. marcescens*. The highest adsorption capacity took place with the plastic black mud and the lowest with the gray sand.

In all of the experiments referred to above the samples of the sediments investigated were taken at a depth of 10 to 15 cm. below the surface of the liman bottom. In one case only was the

TABLE 2

Adsorption of the bacteria of the liman water and mud

	NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	NO. 6	NO. 7
Number of bacteria in 10 cc. of the suspension (in millions):							
Before adsorption	135.5	34.0	133.3	78.5	86.3	109.9	78.5
After adsorption	45.6	7.2	0.2	0.7	0.9	0.25	0.5
Number of adsorbed bacteria (in millions)	89.9	26.8	133.1	77.8	85.4	109.65	78.0
Percentage of adsorption	66.6	79.8	99.8	99.2	99.0	99.8	99.4

adsorption capacity of deeper layers of the sediments also determined. The surface layer was black sand; at a depth of 1.25 m. gray non-plastic mud mixed with sand was found, while at a depth of 2.25 m. gray plastic mud was present. The percentage of adsorption of *S. marcescens* was found to be:

Surface layer	26.2
Layer at a depth of 1.25	39.8
Layer at a depth of 2.25	96.2

Desorption of S. marcescens from mud

Frei and Erismann (1922) reported that the number of bacteria in a suspension was greater after prolonged shaking of the latter

[1] The sand deposits of the liman contain some admixture of clay and of organic substances.

with sand than after short-time shaking; the difference in the results was explained by partial desorption. Dianowa and Woroshilowa (1925) have also observed partial desorption of bacteria inoculated in soil. In the case of *S. marcescens* we succeeded in washing off 13 millions of cells out of 219.8 millions inoculated in 2 grams of mud, i.e., 6 per cent.

The following experiment was performed in order to determine the influence of the length of time allowed for washing off on the degree of desorption. Into each of four test-tubes containing 2 grams of mud, 1-cc. portions of a suspension of *S. marcescens* containing 800.7 millions of bacterial cells, was added. After careful stirring, 9 cc. of sterilized water were added to each test-tube; one of the test-tubes was shaken 5 seconds, one 30

TABLE 3

800.7 millions of cells of S. marcescens in 2 grams of mud

TIME	NUMBER OF BACTERIA WASHED OFF	PERCENTAGE OF DESORPTION
seconds	*millons*	
5	5.0	0.6
30	23.2	2.9
60	46.2	5.8
300	49.6	6.2

seconds, one 60 seconds and one 300 seconds. The results presented in table 3 show that the percentage of bacteria desorbed increased from 5 to 60 seconds. However, if washing was continued for a longer period, the number of bacteria washed off remained unchanged, the difference being within the limits of the error of the experiment.

Has the mud a toxic effect?

In connection with the plating method, used for the bacterial count, the possibility suggested itself that the decrease in the number of bacteria in the suspension after the latter had been shaken with mud, may have been due not to adsorption but to a toxic effect of the mud. To test the correctness of this assumption, the following experiment was performed. Into each of three

test-tubes containing 2 grams of mud, 1-cc. portions of the same suspension of *S. marcescens* were introduced and carefully mixed with the mud. Nine cubic centimeters of sterilized water were added to one test-tube after five minutes, to the second after thirty minutes and to the third after sixty minutes, and the test-tubes were shaken for one minute.

The following number of cells of *S. marcescens* was washed off:

After 5 minutes contact with the mud	3.3 millions
After 30 minutes contact with the mud	3.7 millions
After 60 minutes contact with the mud	3.1 millions

At the same time the number of cells of *S. marcescens* found in the mud was determined after they had been there for five, thirty and sixty minutes respectively. The number of cells was found to be in all cases the same, namely 60 millions per gram. It is thus evident that the mud did not show in sixty minutes any toxic action.

The influence of bacterial numbers on adsorption

Two grams of mud were shaken for one minute with 10 cc. of a suspension of *S. marcescens* which contained 4.8 million, 55 million, 110 million, 220 million and 395 million cells respectively. The length of time allowed for the settling of the mud was in all cases thirty minutes.

The results obtained (table 4) show that, beginning with 110 million cells the percentage of adsorption decreased with an increase in the number of bacteria in the suspension. When, however, the number of cells introduced was below 110 millions, the percentage of adsorption also decreased. In another experiment in which the number of bacteria in the suspension was 17.3 millions, 171.2 millions, 387.6 millions, 1,031 millions and 1,719 millions, respectively, the percentage of adsorption proved to be 88, 97.1, 90 and 71.

A characteristic peculiarity of adsorption is the fact that a relatively greater quantity of the substance in question is adsorbed from dilute solutions than from more concentrated ones. So far as our experiments are concerned, this rule proved to hold true, only when 10 cc. of the suspension contained not less than

110 to 171 millions of bacteria. In cases where the number of bacterial cells was smaller, the percentage of adsorption did not increase, as might have been expected according to this rule, but dropped. Thus, in the experiments with *S. marcescens*, a zone of maximum adsorption was evident within the limits between 110 millions and 171 millions of bacteria present in the suspension, while on both sides of this zone the degree of adsorption decreased.

TABLE 4

The influence of the number of bacterial cells

	A	B	C	D	E
Number of bacteria in 10 cc. of suspension (in millions):					
Before adsorption	4.8	55.0	110.0	220.0	385.0
After adsorption	1.0	2.7	1.2	9.4	46.2
Number of bacteria adsorbed (in millions)	3.8	52.3	108.8	110.6	338.8
Percentage of adsorption	81.0	95.0	98.9	95.7	88.0

The influence of the quantity of the adsorbent

In the following experiments, the quantity of adsorbent, i.e., of mud, was varied within the limits of 0.1 to 5 grams. Ten-cubic centimeter portions of a bacterial suspension containing 100 millions of cells of *S. marcescens* were added to test-tubes containing varying quantities of mud. All of the test-tubes were shaken for one minute and thirty minutes were allowed for the mud to settle. Table 5 shows that the percentage of adsorption was 80.5 when 0.1 gram of mud was used, 88.7 in the case of 0.5 gram, 94.7 with 2 grams and 99.2 with 5 grams.

Influence of acid treatment of mud

In order to show how a change in chemical composition of the mud influenced its adsorbing properties, mud was subjected to the action of a 10-per-cent solution of hydrochloric acid. Some of the colloids ($FeS \cdot H_2O$, $Al(OH)_3$) were dissolved. At the same time the usual alkaline reaction of the mud changed to

acid. To 2 grams of the altered, as well as of the normal mud, 10 cc. of a suspension of *S. marcescens* containing 135 million cells were added. The data given in table 6 show that the normal mud adsorbed 98 per cent of the bacteria added, while the one treated with HCl adsorbed only 20 per cent. It is evident from this result that the treatment of the mud with hydrochloric acid caused a sharp decline in its adsorption capacity.

TABLE 5

The influence of the quantity of mud

	QUANTITY OF MUD			
	0.1 gram	0.5 gram	2.0 grams	5.0 grams
Number of bacteria in 10 cc. of the suspension (in millions):				
Before adsorption	100	100	100	100
After adsorption	19.5	11.3	5.3	0.8
Number of bacteria (in millions)	80.5	88.7	94.7	99.2
Percentage of adsorption	80.5	88.7	94.7	99.2

TABLE 6

The influence of hydrochloric acid on the adsorption capacity of the mud

	UNTREATED MUD	MUD TREATED WITH HCl
Number of bacteria in 10 cc. of the suspension (in millions):		
Before adsorption	135.0	135
After adsorption	2.7	108
Number of bacteria adsorbed (in millions)	132.3	27
Percentage of adsorption	98.0	20

Exchange adsorption

It is well known that the liman mud contains a great number of diverse bacteria, some of which grow on meat-peptone agar. A part of these latter organisms are washed off (desorbed) from the mud when the latter is shaken with water. If now the water used for shaking contains some bacteria capable of being adsorbed by mud, then, a simultaneous adsorption of these bacteria and desorption of some bacteria contained in the mud should

occur. The following experiment shows the quantitative relation between the bacteria adsorbed and those desorbed. Mud (2 grams) was shaken up for one minute with 10 cc. of sterilized tap-water. As a result, it was found that 300,000 bacteria were washed off the mud. In another test-tube 2 grams of the same mud were shaken with 10 cc. of tap-water which contained 4.8 millions of cells of *S. marcescens*. In this latter case it was found that 4,175 millions of cells of *S. marcescens* had been adsorbed by the mud while 725,000 cells of other bacteria were washed off. Hence, it is clear that the adsorption of 4,175 millions of cells of *S. marcescens* caused an increase of the number of bacteria desorbed to 725,000 − 300,000 = 425,000, i.e., for every 10 cells of *S. marcescens* adsorbed there was washed off the mud one cell more than in the experiment with sterilized water. It is evident from these results that an exchange adsorption of bacteria is going on in the mud.

The survival of adsorbed bacteria in the mud

In order to determine the influence of adsorption on the viability of bacteria, the following experiment was carried out. Sterilized black mud (5 grams) was shaken with 10 cc. of a suspension containing 100 million of cells of one of the following species, taken from our laboratory collection: *Serratia marcescens*, *Bacillus mycoides*, *Bacillus vulgatus* and *Sarcina lutea*. As soon as the mud had settled, the suspension was poured off and the test-tubes with the mud closed with cotton plugs and placed in a dark moist-chamber at room temperature. Every five days, small lumps of the mud were taken from each test-tube with a platinum loop and smeared on the surface of solidified meat-peptone agar in Petri dishes. At the beginning of the experiment 50 to 75 mgm. of mud were taken for each sowing. Later, however, 1 gram of mud was always used. Whenever the bit of mud contained cells still capable of growth, colonies were found to have formed around it in a few days. As a result of these experiments, it was established that the adsorbed bacteria retained their viability for the following time periods:

	days
Ser. marcescens	40
S. lutea	55
B. vulgatus	>80
B. mycoides	

Influence of adsorption on the morphological, cultural and bio-chemical properties of bacteria

Serratia marcescens, *Bacillus vulgatus* and *Sarcina lutea* were used on this experiment. An aqueous suspension of these bacteria was shaken with sterilized mud for one minute, and after the latter had settled down, the suspension was poured off and the test-tubes closed with cotton plugs and kept in the dark at room temperature. The adsorbed bacteria remained in the mud for twenty-five days; they were then again isolated (by the plating method), inoculated on various culture media (meat-pepton broth, meat-pepton agar, meat-pepton gelatin, milk, potatoes) and their morphological, cultural and bio-chemical properties studied. Similar studies have been also made of cultures of normal, not-adsorbed bacteria which had been kept on meat-pepton agar for twenty-five days.

Only the most significant changes may be noted: *Sarcina lutea* and *Serratia marcescens* that had been adsorbed by mud were no longer capable of liquefying gelatin. *Serratia marcescens* did not coagulate milk and formed no pigment or very little of it. In the case of *Sarcina lutea* the size of the cells decreased somewhat and the majority of the cells were not arranged in packets but in twos or fours. These deviations proved to be temporary only, for after several re-inoculations under standard conditions the original properties of the cultures reappeared. Thus, the adsorption of saprophytic bacteria by mud for twenty-five days did not lead to any profound changes in their morphological, cultural and biochemical properties.

Similar results have also been obtained in experiments with pathogenic bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*) introduced into liman mud (Rubentschik, Hoicherman and Reich, 1932).

The influence of adsorption on the activity of microbiological processes in the mud

As already mentioned above, Dianowa and Woroshilowa have recorded a decrease of CO_2 production by bacteria adsorbed by the soil. Chudiakow (1926) assumed that this might be due to the absence of oxygen in the soil colloids, as established by Russel and Appleyard (1915).

The influence of adsorption on the processes of nitrification, oxidation of thiosulphate and sulphate reduction were determined in these experiments.

a. Nitrification. It is so far known (Rubentschik, 1929) that only the bacteria of the first phase of nitrification have been found in the Kuyalnik liman. Morphologically and culturally they appear to be identical with Winogradsky's *Nitrosomonas europea*, but differ from all known strains of the latter species in their obligatory halophily.

A culture of this liman species in Omelianski's medium made up with liman water (5° Baumé) has been used. After strong stirring of the liquid culture 4 cc. were transferred to a flask containing 16 cc. of sterilized liman water. After thorough mixing, the bacterial suspension was divided into two test-tubes. To one, 2 grams of oxidized sterilized mud[2] were added: the test-tube was shaken for one minute and left for thirty minutes to allow the mud to settle. The number of nitrifying bacteria in the suspensions of both test-tubes was then determined. The dilution method was employed, using Omelianski's medium for *Nitrosomonas*, made up with liman water. The flasks were placed in the thermostat at a temperature of 28° to 30°C. for thirty days. The bacteria were found to be adsorbed to the extent of 99 per cent.

The oxidation of ammonia was determined in the medium containing bacteria adsorbed by mud as well as in cultures which

[2] As was shown previously, neither oxidation nor sterilization of the mud has any notable effect on its adsorption capacity. Since the nitrifying bacteria are obligatory aerobes, their activity in the liman is possible only in the oxidized surface-layer of the mud. It was for this reason that in our experiment with *Nitrosomonas* we employed oxidized mud.

had not been adsorbed. For this purpose two flasks containing 28 cc. of sterile medium and 2 cc. of a *Nitrosomonas* culture were used. Two cubic centimeters of the same culture were placed in a test-tube containing 8 cc. of Omelianski's medium and 2 grams of oxidized and sterilized mud. After shaking the test-tube for one minute its contents were poured into the second flask, to which about 18 cc. of sterile medium were added, so that the surface level of the suspension appeared to be the same in both flasks. After the flasks had been in the thermostat for thirty days at a temperature of 28° to 39°C., 49 mgm. of nitrite were found to have formed in the first flask and only 18 mgm. in the second. In a later experiment in which the same method was employed, 57 and 21 mgm. respectively of nitrite have been obtained.

b. Oxidation of thiosulphate. It was shown by Saslawsky (1927) that the thiosulphate-oxidizing bacteria of the Kuyalnik liman are obligatory halophiles. In our experiments on adsorption we used a culture of these bacteria on Beijerinck's medium (with thiosulphate), made up with liman water. The methods employed for the determination of the percentage of adsorption of these bacteria by sterilized, oxidized mud as well as of the activity of thiosulphate oxidation by adsorbed and by not-adsorbed bacteria were exactly the same as in our experiment with *Nitrosomonas*.[3] As a result, it was found that, out of 100 million bacteria inoculated, 90 millions were adsorbed by 2 grams of mud. Accordingly the percentage of adsorption was 90. After twenty days, the medium containing bacteria that had not been adsorbed by mud had 165 mgm. of thiosulphate oxidized, while 163 mgm. were oxidized in the medium with bacteria which had not undergone adsorption. In another analogous experiment the quantity of oxidized thiosulphate amounted to 126 and 121 mgm. respectively. These results proved that adsorption has no effect on the activity of the thiosulphate-oxidizing bacteria of the liman.

[3] The only difference in the technique employed in the two cases was that, while the quantity of oxidized thiosulphate was determined by titration with 0.01 N I, the quantity of nitrite formed was found by titrating with 0.01 N $KMnO_4$.

c. Sulphate reduction. The sulphate-reducing bacteria of the Odessa limans are halophiles (Chait, 1924; Rubentschik, 1928). In the experiments described below, a culture of bacteria of the Kuyalnik liman in Beijerinck's medium made up with liman water was used. The degree of adsorption of these bacteria was determined in the following way. After active stirring of the culture in Beijerinck's medium, 2 cc. were transferred to a flask containing 18 cc. of sterilized liman water. The contents of the flask were thoroughly stirred and 10 cc. were poured into each of two test-tubes. To one of the latter, 2 grams of black mud were added and, after one minute's shaking and thirty minutes settling, the number of bacteria in the suspensions of both test-tubes determined by the dilution method. The material to be inoculated was poured into small wide-necked jars, 50 cc. in volume, filled with Beijerinck's medium prepared with liman water. The jars were closed with ground stoppers, care being taken to leave no air bubbles. Anaerobic conditions were thus obtained, indispensable for the growth of sulphate-reducing bacteria. The jars were kept in the thermostat at a temperature of 28° to 30°C. for a month and subsequently their contents were titrated with 0.01 NI. This experiment showed that out of 10 millions of cells 9 millions had been adsorbed by the mud. The percentage of adsorption, accordingly, was 90. For the comparative determination of the activity of sulphate reduction 2 cc. of a culture of the sulphate-reducing bacteria were inoculated in a flask containing 18 cc. of liman water and after thorough stirring the bacterial suspension was poured in equal parts (i.e., 10 cc. each) into two test-tubes. To one of the test-tubes 2 grams of black mud were added and after shaking for one minute the contents of both test-tubes were poured into two small jars. Since hydrogen sulphide (2 to 15 mgm.) was present in the mud placed in one of these jars, it was necessary for the sake of obtaining like conditions of anaerobiasis in the other jar without mud, to add the same quantity of hydrogen sulphide to the latter. Both jars were then filled to the top with Beijerinck's medium, closed with ground stoppers and kept in the thermostat at a temperature of 28° to 30°C. After twenty-five days it was found

that 20.3 mgm. of H_2S were formed in the jar containing bacteria adsorbed by mud and only 12.4 mgm. in the jar without mud. In another analogous experiment 23.8 and 13 mgm. respectively of H_2S were formed. These results show that the sulphate reduction was more active in the medium with adsorbed than in that with not-adsorbed bacteria.

DISCUSSION

Rawitsh (1930) found that the fraction of the mud which is not soluble in a 10-per-cent solution of HCl takes up cations (K, Na, Mg) as well as basic dyes, while neither anions (Cl, SO_4) nor acid dyes are adsorbed. The fact that this fraction of mud which contains mainly compounds bearing the negative charge (silicic acid) adsorbs substances that bear the positive charge would seem to indicate that the adsorption capacity of the mud may depend upon the magnitude and the sign of the electric charge of its component parts. It has been shown that when mud is treated with a 10-per-cent solution of HCl the result is a sharp decline in its adsorption capacity for bacteria. In this case the hydrochloric acid dissolves these ingredients of the mud which have the positive charge ($FeS \cdot H_2O$, $Fe(OH)_2$, $Fe(OH_3)$, $Al(OH)_3$). On the other hand it is known that living bacteria usually have a negative charge (Kraus and Uhlenhuth, 1923; Buchanan and Fulmer, 1928; Rubinstein, 1932). The conclusion thus seems to be justified that the adsorption of bacteria by mud depends upon electric phenomena taking place at the boundary between the surfaces of mud particles and those of the bacteria cells. It is possible, however, that besides electric forces some other factors are here at work, for instance the magnitude of the surface tension, the degree of the moistening capacity of the surface of the bacterial cell, its adhesion capacity to the mud, etc. Here further work is necessary.

There are some contradictory statements in the literature regarding the influence of adsorption on bacteria. According to Karpinska (1925), bacteria adsorbed by the soil are in a condition of depression and even perish in part. This is the reason why no bacterial growth is observed when soil containing adsorbed

bacteria is inoculated in a culture medium. Chudiakow and Karpinska assume that the forces underlying bacterial adsorption suffice to destroy the bacterial cell. Frei and Erismann (1922) also find that adsorption of bacteria by sand filters leads to a depression and even to partial dying off of the bacteria. Bechhold and Schlossberger (1923), on the contrary, assume that adsorption as such has no injurious action on bacteria. Friedberger and Kumagai (1918) found that when some bacteria die as a result of adsorption, this is due to the mechanical injury which they sustain from the sharp points of the adsorbent. But whenever bacteria, previously adsorbed by animal coal, kaolin and other indifferent adsorbents are plated out, the authors could in each case record growth on suitable culture media. Our own experiments show that adsorbed bacteria retain their vitality in the mud for a long time, and when subsequently transferred into suitable culture media, they grow there and call forth certain processes. That is why we are inclined to think that adsorption alone, as such, has no influence on bacteria. It might, however, result in some collateral phenomena which affect the life processes of bacteria.

In this connection the following possibilities may now be considered. In case the metabolic products of the bacteria are not taken up by a certain adsorbent, adsorption may, *ceteris paribus*, unfavorably affect the bacteria. For it must be kept in mind that while bacteria, when free, may occupy all of the space of the culture, they are, when adsorbed, localized only in that part of the medium which is occupied by the adsorbent. In the latter case, however, around every adsorbed cell there should be a zone of more highly concentrated toxic metabolic products than around free cells, especially, if the latter are motile. If, however, the metabolic products are adsorbed, the effect on the adsorbed bacteria may be a favorable one.

This assumption is supported by some data recorded in the literature. Thus, according to Söhngen (1913), the addition of various colloids (ferric oxide, animal coal a. o.) to a culture of Urobacteria stimulates the decomposition of urea, as these colloids adsorb the ammonia formed. The growth of yeast and the

formation of alcohol is also increased by colloidal substances, which possibly adsorb the noxious products of metabolism (Reinhard and Obrastzowa, 1935; Plewako and Kunsburgsky, 1932). Just how nitrogen-fixing bacteria are activated by colloids is not yet clear; it is possible, however, that in this case the adsorption of metabolic products may play its part; adsorption may change the conditions of the nutrition of bacteria. Since the latter when adsorbed occupy a part only of the medium, the nutritive substances of the latter may at some time become exhausted and the normal metabolism will depend upon the rapidity of diffusion of nutritive substances from the remaining part of the medium. Some nutrients may, besides, be taken up by the adsorbent employed and thus be unavailable for the bacteria. Furthermore, what has been said regarding the conditions of nutrition of adsorbed and free bacteria applies also to their respiration, for the access of oxygen to every bacterial cell may be altered by adsorption. The action of adsorption is accordingly, an indirect one and its effect, while favorable in one way may be detrimental in other respects. The final result depends upon the total environmental conditions of the case and upon the bacterial species. It is evident from these considerations that no generalizations can be made regarding the influence of adsorption on bacteria and that a special study is needed in each particular instance to determine this influence.

In our investigation only three microbiological processes which take place in salt lakes have been considered, and yet it was possible to ascertain that the adsorption of bacteria by ground sediments has a marked influence on the degree of activity of some bacteria, lowering it in some cases (nitrification) and increasing it in other cases (sulphate reduction). *On this basis adsorption may be regarded as an ecological factor which should be taken into account in any study of the reciprocal action between microbes and the environment in salt lakes.* Our experiments have shown that the various types of ground sediments differ in their adsorption capacity. The idea thus suggests itself of employing the method of bacterial adsorption for the study of the ground sediments in salt lakes. In the classification of these sediments,

paleontological, petrographic, physicochemical and other data have so far been considered. It is possible that the study of the bacterial adsorption capacity might contribute new and interesting material that could be made use of for the same purpose.

It is a well known fact that microbes play an important part in the formation of black mud in salt lakes. There are various microbiological processes going on in the limans and other salt lakes, as a result of which simple decomposition products of animal and plant bodies as well as more complex compounds are formed. These substances impregnate the clayish-sandy skeleton, giving rise to the black, plastic, greasy mass—black mud. One of the factors which influences the microbiological process is, as was shown in the preceding pages, the adsorption of bacteria. It follows from this that the latter deserves the attention of balneologists in connection with the problem of the formation and conservation of therapeutic mud.

Little is known so far, concerning the distribution of bacteria in salt lakes. The question regarding the factors which may control the formation of bacterial benthos and bacterial plankton is entirely in the dark. Data regarding the bacterial species inhabiting the bottom and the water of the limans are very meagre and a mere beginning has so far been made. Yet the data presented above in regard to bacterial adsorption would seem to point the way of approach to the problem regarding the factors that influence the distribution of bacteria in these lakes. It was shown by our experiments that liman mud contains bacteria, the degree of adsorption of which is higher and of desorption lower than that found in bacteria isolated from liman water. Hence the conclusion that *the bacterial benthos in salt lakes should consist of easily adsorbable species while the bacterial plankton should contain species that have a low degree of adsorption by the ground sediments.* While it is clear that the local distribution of the bacteria on the bottom and in the body of the water depends not on one but on many factors, it appears from this investigation that in adsorption we have found one of these factors.

It is, however, clear that the bacterial population of the upper layer of the ground sediments and also of the water of the limans cannot be invariably the same. Every year, especially when the

snow is thawing, small rivers and ravines carry into the limans a great number of various species of bacteria differing not only with regard to their systematic position but also in the degree of their capacity for adsorption. Some of them perish under the specific conditions of life in the limans while others adapt themselves. Owing to reciprocal adsorption which we have noted, the upper layer of the sediments adsorbs some and at the same time releases other species. The bacterial flora of this layer should thus be subject to periodic changes in regard to both quantity and species.

Some of the drainage water from the sewage fields of Odessa flows into the Chadjibey liman. The Coli-titer of this water usually varies within the limits from 0.1 to 0.01. A great number of *Escherichia coli* are thus constantly carried into the liman. Analyses, however, have shown that with increasing distance from the sewage fields a very marked decrease of the number of this bacterial species in the liman water is noted. Thus at a distance of about 100 m. from the sewage fields the Coli-titer appears to be the same (from 50 to 100) as in various other parts of the liman. The question now arises, what becomes of these colon bacilli? The possibility of their rapid death may be left out of consideration, for there is experimental evidence for the long survival of this species in liman water, (Rubentschik, Hoichermann and Reich 1932). The adsorption capacity of the ground sediments of the Chadjibey liman for this species was found to be high. The conclusion is thus fully justified that the disappearance of *E. coli*, brought into the liman by the drainage water is due to its being adsorbed. Owing to shallowness of the shore water and to frequent tides in this lake the various layers of the water and also the upper layer of the ground sediments easily intermix. And this should result in the adsorption of *E. coli*. It is thus evident that adsorption plays an important part in the processes of self-purification of the water in salt-lakes.

SUMMARY

1. The ground sediments of the limans (salt lakes) are capable of adsorbing bacteria.

2. The various types of sediments differ in their adsorption

capacity for the same bacterial species. The adsorption capacity of the black, plastic mud is especially high.

3. The degree of adsorption of various species of bacteria by a certain type of sediment differs according to the species.

4. Bacteria adsorbed by mud may be partly desorbed.

5. Bacteria isolated from mud are more readily adsorbed and less readily desorbed than bacteria isolated from liman water.

6. The percentage of adsorption decreases when the quantity of bacteria in the suspension is increased. In the case of *Serratia marcescens*, however, a zone of maximum adsorption has been observed when 10 cc. of the suspension contain from 110 million to 171 millions of cells. The degree of adsorption declines on both sides of this maximum zone.

7. The percentage of bacteria adsorbed increases with the increase (up to a certain limit) of the quantity of the adsorbent (mud).

8. The adsorption capacity of black mud is changed neither by oxidation nor by sterilization in the autoclave. A sharp decline of the adsorption capacity, however, results when the mud is treated with a 10-per-cent solution of HCl.

9. The degree of adsorption depends not upon the quantity of the liquid medium of the concentration of bacteria but upon their absolute number.

10. The same quantity of bacteria are adsorbed from a suspension made up with liman water as from a suspension prepared with tap-water.

11. The degree of adsorption is higher for living bacteria than for those killed by high temperature.

12. When two bacterial species are adsorbed simultaneously the one may affect the degree of adsorption of the other.

13. An exchange adsorption takes place in the liman mud, the adsorption of some species being accompanied by desorption of other species from the mud.

14. Adsorbed bacteria may survive in the mud for a long time without any profound changes in their morphological, cultural and bio-chemical properties being noted.

15. Adsorption may affect the life processes of the bacteria adsorbed by the mud, the activity of some (nitrification) being

lowered while that of others, on the contrary, is increased (sulphate reduction).

16. The differences in the degree of adsorption and of desorption of bacteria inhabiting the ground sediments and the liman water suggest the assumption that adsorption may play some part in the differentiation between bacterial plankton and benthos forms of the limans.

17. The adsorption of bacteria plays a part in the self-purification of the water in salt-lakes.

REFERENCES

BECHHOLD, H. 1918 Kolloid-Zeitschr., **23**, 35.

BECHHOLD, H., UND SCHLOSSBERGER, H. 1923 Handbuch der mikrobiol. Technik, Herausgeg. von Kraus und Uhlenhuth, **2**, 1413.

BUCHANAN, R. E., UND FULMER, E. I. 1928 Physiology and Biochemistry of Bacteria, **1**, 312.

CHAIT, S. 1924 Journal nautsch.-issled. Kafedr w Odesse (Russian), **1**, Nr. 10–11, 32.

CHUDIAKOW, N. N. 1925–1926 Nautschno-agron. Journal, **2**, 742; Centrbl. f. Bakt., Abt. 2, **68**, 345; Pedology, **2**, 98; Selsko-Chosaistwenaja Microbiologia, p. 215.

DIANOWA, E. W., AND WOROSHILOWA, A. A. 1925 Nautschno-agron. Journal, **2**, 520.

EISENBERG, P. 1918 Centrlbl. f. Bakt., Abt. 1, Orig., **81**, 72.

FREI, W., UND ERISMANN, H. 1922 Centrlbl. f. Bakt., Abt. 1, Orig., **88**, 306.

FRIEDBERGER, E., UND KUMAGAI, T. 1912. Zeitsch. f. Immunitätsforsch., **13**, 127.

KARPINSKA, N. S. 1925. Nautschno-agron. Journal, **3**, 587.

KRAUS, R., UND UHLENHUTH, P. 1923 Handbuch der mikrob. Technik, **2**, 1419.

KRÜGER, W. 1889. Zeitsch. f. Hyg., **7**, 135.

PLEWAKO, E. A., AND KUNSBURGSKY, F. M. 1932 Brodilnaja Promishlenost (Russian), Nr. 7, 17.

RAWITSH, M. 1930. Iswestia Inst. Phys.-Chim. Analisa (Russian), **4**, 384.

REINHARD, A., ET OBRASTZOWA, V. 1935 Bolletino dela sezione italiana Soc. Inter. di Microbiol., **7**, Fasc. VIII–IX, 331.

RUBENTSCHIK, L. 1928 Centrlbl. f. Bakt., Abt. 2, **73**, 483.

RUBENTSCHIK, L. 1929 Centrlbl. f. Bakt., Abt. 2, **77**, 1.

RUBENTSCHIK, L., HOICHERMANN, D., AND REICH, G. 1932 Trudi Wseukr. Balneo-Physio-Therapewt. Int., **1**, 12.

RUBENTSCHIK, L., AND HOICHERMANN, D. 1935. Microbiology (Russian), **4**, 403.

RUBINSTEIN, D. L. 1932 Phis.-Chim. Osnowi Biologii (Russian), p. 229.

RUSSEL, E. J., AND APPLEYARD, A. 1915 Jour. Agric. Sci., **7**, 1.

SAPEHIN, A. A. 1926 Variatsina Statistika (Ukrain), p. 89.

SASLAWSKY, A. S. 1927 Centrlbl. f. Bakt., Abt. 2, **72**, 236.

SÖHNGEN, N. L. 1913 Centrlbl. f. Bakt., Abt. 2, **38**, 636.

SWESHNIKOWA, I. A. 1926 Trudi Balneol. Instit. Kawkas. Mineral. Wod (Russian), **3**, 165.