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BAROPHORESIS IN GELS

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During an investigation dealing with the diffusibility of certain bacteriological solutions through semi-solid media, which was undertaken by one of the authors (A. B. S.) together with Professor J. Klostermann, some fundamental points had to be elucidated. The diffusion of solutions into gels does not lend itself to as precise and simple experimentation as the diffusion of gases into one another. The influence of concentration and temperature, as well as of the nature of the solute, had to be studied. A remarkable influence of the direction of diffusion upon its rate was observed in these experiments; this phenomenon will be dealt with in the third section of this paper; its influence has already been taken into consideration in the preceding sections.

I

As the progress of colored solutions in gelatinous media may readily be followed, the distance and not the quantity diffused through the gel in a definite time was determined. This distance was measured from the vertex of the interface to the point within the gel where the slightest trace of the diffusing solution could still be visibly detected. "d," distance, could be determined, for example, for bromo cresol purple or sodium dichromate with an accuracy of ± 1 mm., whereas for methylene blue it could be measured with a precision of $\pm 1/4$ mm.

For diffusion in gels[1] where secondary disturbances by "convection currents" are apparently avoided, Auerbach[2] has shown experimentally that the amount diffused since the beginning of the experiment is proportional to the distance between the interface and a point within the gel at which the concentration of the diffusing substance is a definite fraction, for example, one-tenth of its concentration in the original solution. When diffusion is measured by the distance between interface and limit of detectable diffusion, as indicated before, this distance is found proportional to the square root of time.[3] The limit of detectability must correspond to a definite concentration of the dye; assume this concentration to be 10^{-8} and the concentration of the supernatant solution to be 0.1% or 10^{-3}. Then "d" may be termed $l_{1/10,000}$ in analogy to Auerbach's $l_{1/10}$ or $l_{1/100}$ designating the distance between the interface and the point where 1/10 or

[1] Voigtländer, *Z. physik. Chem.*, **3**, 316 (1889).

[2] Auerbach, *Kolloid Z.*, **35**, 202 (1924); **37**, 379 (1925).

[3] (a) Stefan, *Sitzber. Wiener Akad. Wiss.*, **79**, 215 (1879); (b) Chabry, *J. phys.*, [2] **7**, 115 (1888); (c) *Z. physik. Chem.*, **2**, 440 (1888).

1/100 the concentration of the supernatant solution prevails. Thus d can be expected to be proportional to the total amount diffused. This holds true since the distance of diffusion was experimentally found proportional to the square root of time and the square root of time is in turn proportional to the amount diffused as derived by Fick's law[4] and actually observed by Voigtländer.[1]

In our experiments diffusion followed the equation

$$d = k\sqrt{t} \qquad (1)$$

d is conveniently expressed in millimeters and t in hours from the beginning of the experiment.

k of equation (1) was found to be practically constant within the limits of error in the estimation of d under the condition of our experiments.[5]

k can be used as an index for the rate of diffusion for a given system and was thus employed for comparative purposes where this rate was involved.

Diffusion of methylene blue presents a different appearance from that of bromo cresol purple, picric acid or sodium dichromate. In the case of methylene blue the agar gel is deeply stained and the limit of diffusion is sharply demarcated. Beyond this zone of deeply stained gel there is another zone of lighter shade which in turn can be differentiated from the unstained agar. As long as the supernatant solution of methylene blue is not exhausted, this "fading zone" is narrower than or equal to 0.5 mm. in width at room temperature and 2.5 mm. at 37°. The dye is adsorbed up to the point of saturation of the agar and the progress of saturation is the controlling factor in the speed of methylene blue diffusion in its early stages. Since no unlimited supply of methylene blue was used it was exhausted before the entire agar column was stained.

With solutions of dichromate, picric acid or bromo cresol purple—substances which are not adsorbed by the agar—equilibrium is established when the concentration is uniform throughout the entire agar column and the supernatant. The diffusion of these substances is not hindered by adsorption; thus their progress in the gel is about three times faster than that of methylene blue (see Fig. 1 and Table I) and the limit of perceptible diffusion is not sharply demarcated.

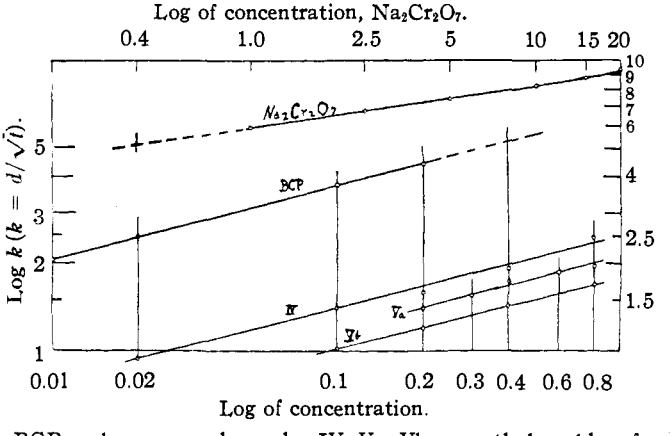
With methylene blue, after equilibrium is practically reached, the further progress of diffusion is controlled by the establishment of secondary equilibria between stained and unstained agar. At this point the "fading zone" grows considerably wider and by the further diffusion of methylene blue

[4] Fick, *Pogg. Ann.*, 94, 59 (1855).

[5] When, as in the case of methylene blue, the experiment had to be extended over a period of many days the k's of the lower concentrations slightly decreased (see Table IV). Lower k's invariably obtained at the beginning of diffusion, as also observed by other workers, are usually explained by the greater proportional error in the determination of d.

new zones of diminishing concentration are developed. After a 0.1% solution has been diffusing for three weeks, two distinct zones besides the fading zone could be differentiated, the ratio being 5:2:1 at room temperature and 4:2:2 at 37°; the first figure corresponds to the darkest band, the second to the following one and the third to the extent of the fading zone.[6]

When a drop of melted agar was spread on a slide to form a film and by means of a capillary tube a round hole was scooped from the center and filled with methylene blue solution, two distinct concentric zones of deep and lighter color and a third exterior "fading" ring were observed under the microscope.



BCP = bromo cresol purple; IV, Va, Vb = methylene blue, for details see tables. Notice the use of different abscissas in the case of $Na_2Cr_2O_7$.

Fig. 1.—Relation between concentration and diffusion.

II

The influence of the concentration of the diffusing substance on its diffusion can be expressed by the mathematical relation between the quotients of two concentrations and of the two corresponding constants, k.[7]

TABLE I

AVERAGE VALUES FOR $k = d/\sqrt{t}$ FOR VARIOUS SUBSTANCES AND CONCENTRATIONS

All experiments except No. 4 at 22°. Numbers of experiments refer to Tables where complete data are given.

		Concentration, %													
No.	Subs.	0.01	0.02	0.1	0.2	0.3	0.4	0.6	0.8	1.0	2.5	5.0	10.0	15.0	20.0
4	Methylene blue (37°)	..	0.96	1.41	1.56	..	1.90	..	2.45	..	..	..	..	..	..
5a	*Methylene blue*	..	..	..	1.36	1.57	1.75	1.83	1.91	..	..	..	..	..	..
5b	Methylene blue	..	..	1.02	1.18	..	1.45	..	1.68	..	..	..	..	..	..
—	Bromo cresol purple	2.04	2.47	3.72	4.39	..	5.22[a]	..	..	..	..	..	..	..	..
—	$Na_2Cr_2O_7$	..	..	..	..	..	5.07[a]	..	..	5.91	6.56	7.19	8.25	8.64	9.25

[a] Values extrapolated from equation (4).

[6] Traube and Kohler, *Intern. Z. physik. chem. Biol.*, **2**, 205 (1915).

[7] Auerbach's (see ref. 2) $l_{1/10}$ and $l_{1/100}$ is independent of the concentration.

When plotting the logarithms of the concentrations as abscissas and the values of log k as ordinates, approximately straight lines were obtained. Thus the quotient of two k's in an experiment should be a definite power of the quotient of the two concentrations.

$$\left(\frac{c_1}{c_2}\right)^n = \frac{k_1}{k_2} \tag{2}$$

The power n was calculated for all possible combinations in each experiment according to

$$\frac{\log k_2 - \log k_1}{\log c_2 - \log c_1} = n \tag{3}$$

n was found with methylene blue between 0.02% and 0.8 to be: 0.249 ± 0.050 (Expt. 4); 0.241 ± 0.076 (Expt. 5a); 0.246 ± 0.024 (Expt. 5b); for bromo cresol purple (0.01–0.2%), 0.255 ± 0.008; and for sodium dichromate (1–20%), 0.157 ± 0.023. When $n' = 2n$ is calculated from

$$\frac{k_1^2}{k_2^2} \text{ or } \frac{d_1^2/t}{d_2^2/t} = \left(\frac{c_1}{c_2}\right)^{n'} \tag{4}$$

it is approximately $1/2$ for methylene blue and bromo cresol purple and $1/3$ for sodium dichromate; thus k^2 is proportional to the square root of the concentration in the former and to the cube root in the latter.

The influence of the concentration on the diffusion is the same at 5, 22 and 37°, as can be seen from Table II where the diffusion of 5 and 15% sodium dichromate is compared at these temperatures. The influence of the temperature is in turn independent of the concentration.

TABLE II

MUTUAL INFLUENCE OF TEMPERATURE AND CONCENTRATION UPON DIFFUSION OF SODIUM DICHROMATE IN AGAR-AGAR

Each value for $k = d/\sqrt{t}$, as given in this table, is the average of 5 observations on 12 test-tubes.

	Values for $k_{t,c}$			Quotients		
	$t = 5°$	$t = 22°$	$t = 37°$	$\frac{k_{22,c}}{k_{5,c}}$	$\frac{k_{37,c}}{k_{5,c}}$	$\frac{k_{37,c}}{k_{22,c}}$
$c = 5\%$	6.06	8.06	8.88	1.33	1.47	1.10
$c = 15\%$	7.22	9.64	10.52	1.33	1.46	1.09
Quotient $\frac{k_{t,15}}{k_{t,5}}$	1.19	1.19	1.18	..	..	..

Influence of Temperature.—Both in 5 and 15% solutions, dichromate was found to diffuse 1.33 times faster at 22° than at 5° and 1.10 times faster at 37° than at 22°. The figure 1.10 for the interval 22–37° is remarkably lower than the analogous figure for methylene blue, as recorded in Table III and Fig. 2; the diffusion of methylene blue at 37° is proceeding at doubled speed compared with 22°.[8] This may be explained by the fact that agar adsorbs the methylene blue and thus impedes its diffusion; with increasing

[8] The experiments 4 and 5a, b of Table I cannot be compared with regard to temperature since different gels were used.

temperature, however, adsorption is diminished and diffusion may increase.

TABLE III

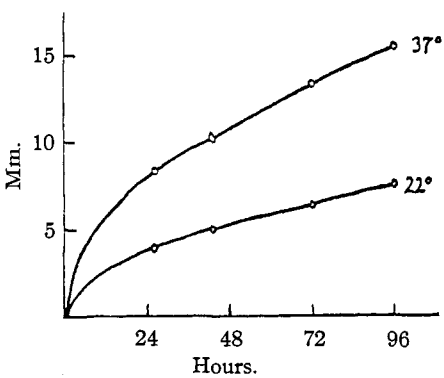
INFLUENCE OF TEMPERATURE ON DIFFUSION OF METHYLENE BLUE

Each figure is the average of 5 similar tubes. "U" means mouth of tube upward; "I" tube inverted (c = 0.1%).

Position of tubes	Temp., °C.	Distance d in millimeters after 26 hours	43 hours	72 hours	96 hours
U	37	7.5	8.7	12.3	15.4
U	22	3.8	4.2	5.9	7.6
I	37	9.1	11.5	14.3	15.4
I	22	4.0	5.8	7.0	7.7

III

Peskoff[9] observed that potassium permanganate in small concentration contained in a ferrihydroxide sol will diffuse faster into a supernatant layer of urea solution than into a layer of distilled water. He termed this and similar phenomena "barophoresis;" it is due to the greater specific gravity of the urea solution as compared with the lesser sp. gr. of the lower layer in so far as only the diffusible permanganate is considered. His observations were confined to sols.



Values are averages of those given for "U" and "I" in Table III.

Fig. 2.—Influence of temperature on diffusion of methylene blue.

Concerning the diffusion in gels Freundlich[10] states that "the motion of the dissolved substance takes place by diffusion alone; convection is almost entirely absent, since the molecules of the solvent as elementary parts of the adsorption envelope and in the amicronic capillaries have to a great extent lost their free mobility." Liesegang[11] asserts even more positively that "diffusion in jellies is free from those convection disturbances which are hardly to be avoided in liquids." Ruhland[12] in his diffusion experiments placed test-tubes half filled with the gel mouth downward into the colored solution; thus he intended to exclude what he thought might be the accelerating effect of gravity, but he does not offer any observation of such influences when omitting this precaution.

[9] Peskoff, *Kolloid-Z.*, **33**, 215 (1924).

[10] Freundlich, "Colloid and Capillary Chemistry," Methuen and Co., London, 1926, p. 729.

[11] Liesegang in Alexander, "Colloid Chemistry," Vol. I, Chemical Catalog Co., New York, 1926, p. 783.

[12] Ruhland, *Jahrb. f. wissensch. Botanik*, **51**, 376, 402 (1912).

We shall now describe the phenomenon of barophoresis in gels. The suspicion that the direction of diffusion might affect its rate was justified when considerable differences between diffusion in the direction of gravity and against it were actually observed. To several tubes containing "nutrient agar"[13] a few cc. of a 0.2% solution of methylene blue was added, the tubes were covered with vaseline and half of them were turned mouth downward. After twenty-four hours the dye had diffused farther in the inverted than in the other tubes.

If any variation in the rate of diffusion with direction were to be expected at all it would be in favor of faster diffusion in the direction of gravity. To account for the paradoxical phenomenon it was assumed that the density of the intermicellular liquid in the agar might be greater than that of the diffusing solution, thus establishing convection currents.

TABLE IV

DIFFUSION OF METHYLENE BLUE INTO A 2% AGAR GEL AT 37°

Every figure represents the average value for three test-tubes; U = test-tubes with mouth turned upward, I = inverted, H = horizontal.

Concn., %	Dir.		Diffusion in mm. and $k = d/\sqrt{t}$ after t hours: t = 12	39	63	87	129	158	229	277	Av. for k	$k_I - k_U$
0.02	U	d	2.8	4.3	7.0	8.2	8.8	9.3	10.7	...	0.79	
		k	0.80	0.69	0.89	0.88	0.77	0.74	0.71	...	±0.06	+0.29
	I	d	3.66	...	10.0	10.5	12.0	12.7	15.0	...	1.08	
		k	1.05	...	1.27	1.13	1.05	1.00	1.00	...	±0.06	
	H	d	3.33	4.8	9.7	10.2	11.5	11.5	13.8	...	0.99	
		k	0.95	0.77	1.23	1.10	1.00	0.91	0.91	...	±0.09	
0.1	U	d	5.0	7.2	12.5	13.0	15.6	15.6	21.0	...	1.37	
		k	1.43	1.16	1.58	1.40	1.37	1.24	1.39	...	±0.09	+0.07
	I	d	4.6	7.2	13.7	14.5	16.3	17.5	22.5	...	1.44	
		k	1.31	1.16	1.73	1.56	1.43	1.39	1.49	...	±0.13	
	H	d	5.33	8.4	14.5	15.3	17.8	19.0	22.5	...	1.56	
		k	1.52	1.35	1.83	1.65	1.56	1.51	1.49	...	±0.10	
0.2	U	d	6.0	9.5	14.2	15.5	18.0	19.3	22.0	23.2	1.59	
		k	1.71	1.53	1.80	1.67	1.58	1.53	1.46	1.40	±0.10	−0.11
	I	d	5.0	8.7	13.5	15.0	17.2	18.5	20.7	22.5	1.48	
		k	1.43	1.40	1.71	1.60	1.51	1.47	1.37	1.35	±0.09	
	H	d	6.16	10.0	14.7	15.7	18.3	19.3	22.5	23.5	1.62	
		k	1.76	1.61	1.86	1.69	1.61	1.53	1.49	1.42	±0.10	
0.4	U	d	7.16	12.0	17.5	19.3	22.0	23.5	27.5	29.0	1.96	
		k	2.05	1.94	2.22	2.07	1.93	1.87	1.82	1.75	±0.12	−0.20
	I	d	5.5	10.0	16.0	17.7	20.7	22.0	26.0	28.0	1.76	
		k	1.57	1.61	2.02	1.90	1.82	1.75	1.72	1.70	±0.11	
	H	d	7.5	12.2	17.3	19.7	22.3	23.8	27.8	29.3	1.99	
		k	2.14	1.96	2.20	2.12	1.96	1.89	1.84	1.80	±0.12	
0.8	U	d	9.16	15.2	23.2	25.2	29.0	31.5	36.5	38.8	2.57	
		k	2.62	2.45	2.94	2.71	2.54	2.50	2.42	2.34	±0.15	−0.24
	I	d	7.66	13.4	20.7	22.7	27.2	29.2	34.0	37.5	2.33	
		k	2.19	2.16	2.62	2.44	2.39	2.32	2.25	2.25	±0.09	
	H	d	8.88	14.3	21.5	23.8	28.0	30.0	35.8	37.5	2.45	
		k	2.54	2.31	2.72	2.56	2.46	2.38	2.37	2.25	±0.12	

[13] 1.5% agar, 0.3% beef extract, 1.0% peptone and 0.5% NaCl.

TABLE V

DIFFUSION OF METHYLENE BLUE (a) INTO A 2% AGAR GEL CONTAINING 0.2% SODIUM CHLORIDE; (b) INTO "NUTRIENT AGAR" AT 22°

For U and I see Table IV. Each k given in this table is the average from four tubes.

Concn. of methylene blue, %	(a) Agar plus 0.2% NaCl k_I	k_U	$k_I - k_U$	(b) Nutrient agar k_I	k_U	$k_I - k_U$
0.1	.....	.....	....	1.13±0.06	0.92±0.04	*+0.21*
.2	1.51±0.01	1.23±0.02	*+0.27*	1.30±0.04	1.07±0.05	*+0.23*
.3	1.68±0.05	1.47±0.02	*+0.21*	.....	.....	....
.4	1.72±0.04	1.79±0.05	*−0.07*	1.59±0.04	1.30±0.04	*+0.29*
.6	1.77±0.02	1.89±0.07	*−0.12*	.....	.....	....
.8	1.83±0.01	2.00±0.02	*−0.17*	1.77±0.04	1.59±0.02	*+0.18*

In order to test this assumption the densities of both the diffusing solution and the intermicellular fluid had to be varied. The "nutrient agar" was replaced by a plain agar gel. Up to a methylene blue concentration of 0.1%, the same effect was observed as with nutrient agar; above 0.1% diffusion in the inverted tubes was slower than in the upright ones. When using a 2% agar containing 0.2% sodium chloride, the "critical concentration" of the methylene blue was raised to 0.4%, which is higher than in plain agar, but lower than in nutrient agar. Even a concentration as high as 0.8% failed to reverse the effect with nutrient agar, where the diffusion was invariably ahead in the inverted tubes with 0.8% as well as with the lower concentrations.

The diffusion of sodium dichromate in the concentrations used is so much faster that any accelerating effect due to convection currents in the intermicellular fluid, if present, is below the range of detectability. With bromo cresol purple, too, the acceleration is but very slight; none the less and despite the lesser accuracy in the reading, the critical concentration of this dye toward plain agar can be placed approximately at 0.1% in the range of that of methylene blue.

The diffusion of sodium dichromate can only be observed in relatively concentrated solution. A difference in favor of the upright tubes was only observed during the later stages and this can be explained by the fact that in the inverted tubes the layer adjacent to the interface decreases in concentration by the rapid diffusion of the dichromate. Since this exhausted layer, because of its lesser density, does not mix readily with the denser strata below, a lower actual concentration will prevail at the interface. The inhomogeneity of the dichromate solution in inverted tubes is manifested by the stratification taking place when these tubes are moved. In the upright and in horizontal tubes the contact layer is situated beneath or alongside of the rest of the solution and as it becomes exhausted homogeneity is maintained by convection currents in the solution. This effect can be made responsible only for a delay of diffusion in the inverted tubes, but not for an acceleration in these tubes nor for any effect upon upright or horizontal tubes.

Method.—Ten grams of large shreds of agar-agar are thoroughly cleaned by dialysis in a cheese-cloth against running water for twenty-four to forty-eight hours. The agar is heated with 500 cc. of distilled water

to make a 2% solution. Portions of 10 cc. each of this melted gel are put into a series of test-tubes of uniform diameter. If the gel in the tubes is not covered within four to five hours after it has set, it is remelted and allowed to cool for an hour to insure an even meniscus and to prevent leakage between the shrinking agar and the glass. Each tube receives 3 cc. of whatever solution is used in the experiment and a layer of vaseline is put on top to prevent evaporation and to render possible the inversion of the tubes; "d" may conveniently be measured by means of a compass.

IV

The density of the intermicellular liquid of a gel cannot be directly determined and indirect methods for its estimation had to be resorted to. At first a definite amount of agar gel was extracted with a definite amount of distilled water for several days and the density of this extract determined. The density of the intermicellular fluid, however, may be greater than that of the aqueous extract; for not only may the agar form a very dilute saturated solution but it may also contain small amounts of diffusible substances which finally would be equally divided between the gel and the extract, provided that they are not adsorbed by the gel. For this reason the amount of dissolved and diffusible substances in the gel had to be determined.

Diffusible substances, for example, electrolytes, present to a limited amount per unit weight of agar and dissolved in the intermicellular fluid, will cause varying density of the extract depending on the volume of water used. The agar itself or any slightly soluble substance forming a considerable portion of it will on extraction yield a saturated solution and the density of the extract should be independent from the volume used, since increasing amounts of water will dissolve additional amounts. Whether or not both these factors are present and in what proportion was determined by the following method.

Three series of several test-tubes were set up (a) with 8 cc. of 2% agar gel, prepared as previously described, and 8 cc. of sterile distilled water above the gel; (b) with 4 cc. of agar and 12 cc. of distilled water and (c) with 2 cc. of agar and 14 cc. of distilled water. All these tubes were covered with sterile vaseline and kept at room temperature. The total solids in the filtered supernatants were determined after the intervals recorded in Table VI.[14]

The amount of extractible and diffusible material per cc. of extract should be equal to that contained in 1 cc. of the intermicellular fluid after equilibrium is attained. Let x equal the amount of diffusible material in 1 cc. of the original agar gel and y the amount of agar or of a slightly soluble portion of the agar dissolved in 1 cc. Then at equilibrium each tube

[14] These experiments were carried out under sterile conditions to exclude interference by bacterial contamination. Also, controls showed neither the glass tubes nor the vaseline contained extractible matter nor the distilled water any ponderable residue.

will contain dissolved in series (a) $8x + 16y$, in series (b) $4x + 16y$ and in series (c) $2x + 16y$. One cc. of series (a), (b) and (c) will contain:

$$A = x/2 + y \quad (5)$$
$$B = x/4 + y \quad (6)$$
$$C = x/8 + y \quad (7)$$

Hence the values for x and y can be computed

$$x = \frac{8}{3}(A - C) \qquad x = 4(A - B) \qquad x = 8(B - C) \qquad (8)\ (9)\ (10)$$

$$y = \frac{1}{3}(4C - A) \qquad y = 2B - A \qquad y = 2C - B \qquad (11)\ (12)\ (13)$$

The introduction of a third independent equation (three series) permits of checking x and y by computation from these three pairs of equations.

TABLE VI

EXTRACTION OF TWO TYPES OF SOLUBLE MATTER FROM 2% AGAR-AGAR GEL

The table gives mg. per cc.; the total solids were actually determined in samples of 20 or 10 cc.

		First experiment		Second experiment 5 days	
	Series	2 days	4½ days	"U"	"I"
Total solids,	(a)	0.66	0.80	0.77	1.06
mg. per cc.	(b)	.40	.58	.545	.655
	(c)	.365	.50	.47	.525
Values for	(8)	.81	.80	.80	1.425
x from	(9)	1.04	.88	.90	1.62
equation	(10)	.28	.64	.60	1.04
x (average)			.77	.77	1.36
Values for	(11)	.23	.40	.37	.35
y from	(12)	.14	.36	.32	.25
equation	(13)	.33	.42	.395	.395
y (average)			.39	.36	.33
$x + y$			1.16	1.13	1.69

Consistent values for x and y were obtained after four and one-half days, as shown by the first experiment in Table VI; y reached constant values by this time ranging around 0.35 mg. per cc. When inverting the tubes the diffusion and extraction of x from within the agar column was enhanced. Further increases of x when extraction was prolonged over weeks cannot be due to matter originally present in free solution. To show the probable identity of the extracted substance, or its portion y, with agar, a little water was added to dried extracts (for example, those recorded in the next paragraph); the dry material swelled, dissolved upon heating, and after cooling the solution set into a gel.

The bearing of these results upon constitutional problems of agar-agar will be made the subject of a separate investigation. For our present scope it is safe to assume that the sum $x + y$ found after five days repre-

sents a maximum for the dissolved matter in the intermicellular fluid. The average of the three values for $x + y$ given in Table VI is $(1.16 + 1.13 + 1.69)/3 = 1.33$ mg. per cc.

The relation between specific gravity and total solids can be derived from experiments like these.

One hundred and twenty-three cc. of mixed extracts from the above experiments had a specific gravity of 1.000185 and contained 42.6 mg. of solid matter, that is, 0.345 mg. per cc. when dried at 100°.

Ten cc. of agar spread over one side wall of a square bottle was covered with 115 cc. of water in a horizontal position. The resulting extract, of specific gravity = 1.00021, contained 4.3 mg. per 10 cc. The latter figure incidentally yields, from $x = 12.5\ (0.43 - y)$, $x = 0.87$ mg. per cc., when assuming $y = 0.36$ mg., in good agreement with the previous results.

The density of the undiluted intermicellular fluid containing three to four times more total solids than these extracts should range near 1.0006 and 1.0007.

Assuming that the increment of density of such dilute solutions can be evaluated by simple addition, the intermicellular fluid in agar containing 0.2% of sodium chloride should be near 1.002 (from 1.00145 for aqueous 0.2% of sodium chloride solution plus the above estimation for plain agar).

For "nutrient agar" the density of a six-day extract, using 25 cc. of distilled water above 50 cc. of nutrient agar, was 1.0044. The dissolved matter belongs for the most part to the readily soluble group "x." Thus the increment of density for the intermicellular fluid must be at least $0.0044 = (50 + 25)/(50)$; thus specific gravity ≥ 1.0066.

The specific gravities of methylene blue and bromo cresol purple solutions,

TABLE VII

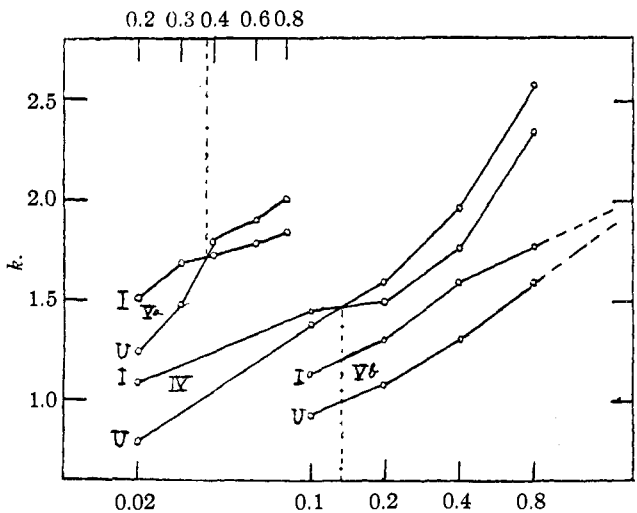
SPECIFIC GRAVITIES OF DIFFUSING SOLUTIONS AND INTERMICELLULAR FLUIDS

Values $d_{22°}^{22°}$ for diffusing solutions pycnometrically, for intermicellular fluids indirectly

Methylene blue	$d_{22°}^{22°}$	Nutrient agar
0.8	1.00297	>1.0066
.4	1.00148	
.2	1.00070	0.2% NaCl agar
.1	1.00037	1.0020
.02	1.00007	
.01	1.00003	
Bromo Cresol Purple		Plain 2% agar
.2	1.00108	1.0006–
.1	1.00057	1.0007
.02	1.00011	
.01	1.00006	
$Na_2Cr_2O_7$		
1.0	1.007	
20.0	1.141	

used in the diffusion experiments, were determined pycnometrically and compared with those of the intermicellular fluids in Table VII.

This comparison reveals that the "critical concentration" of methylene blue for nutrient agar is far above 0.8%; for 0.2% sodium chloride agar near 0.5% and for plain agar below 0.2%. Graphical extrapolation and intrapolation of the experiments recorded in Tables IV–V yields empirical "critical concentrations" of nearly 2% for nutrient agar, a little below 0.4% for sodium chloride agar and 0.15% for plain agar, in good accordance with the above deductions.



U = upright, I = inverted test-tubes. IV plain agar; Va (for clearness' sake these two curves are moved to the left, as indicated by values for abscissas in left upper corner, ordinates are the same as for the other four curves) agar containing 0.2 % of NaCl; Vb nutrient agar.

Fig. 3.—Influence of direction upon rate of diffusion.

Although a more satisfactory explanation for the influence of gravity upon diffusion in gels is hard to conceive, we ought to mention the observation that diffusion in horizontal tubes proceeded as fast as or even faster than in those tubes that were the faster ones among the two vertical directions. (Table IV, series "H"). This corroborates the explanation of the phenomenon by convection currents in the intermicellular fluid, since swift convection will take place across a vertical plane between two liquids differing in specific gravity.

Summary

1. Various methods of measuring diffusion in semi-solid media lead to identical conclusions, both experimentally and theoretically. Two types

of diffusion of colored solutions into gels, with and without adsorption, are described. The rate of diffusion follows a simple mathematical law.

2. The rate of diffusion is a simple function of the concentration of the solution. The influence of temperature upon both types of diffusion is discussed.

3. Considerable differences between the rates of diffusion in upward and in downward direction can be observed under certain conditions. The application of the term "barophoresis" is suggested for this phenomenon. For a given system consisting of a gel and a diffusing substance, a critical concentration of the latter is found at which the sense of barophoresis is reversed.

4. The specific gravities of the intermicellular fluids of various gels are indirectly determined. They coincide with the specific gravities of the corresponding critical concentrations.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF CALIFORNIA]

SOLUBILITY OF LEAD MONOXIDE AND BASIC LEAD CARBONATE IN ALKALINE SOLUTIONS

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Heretofore no attempt has been made to prepare a comprehensive table of the free energies of formation of the compounds of the metallic elements. From a cursory search of the literature it was seen that data were available from which the free energies of formation of a large number of compounds of lead could be calculated. These values are particularly interesting because of the technical importance of lead, its amphoteric character and its marked tendency to form "basic" compounds. We have therefore undertaken a systematic investigation of the free energy of the lead compounds. The new equilibria measured are those between the basic carbonate, the hydrated monoxide, the red and yellow monoxides and the plumbite ion. The results of these measurements will be given in the present paper.

Berl and Austerweil[1] measured the solubility in sodium hydroxide solutions of lead monoxide prepared by heating pure basic lead carbonate. Their results appeared untrustworthy since the value of the solubility in pure water was much higher than that obtained by other investigators. Their oxide was evidently neither the most stable form[2] nor the most important. Only the dissolved lead was determined, and the amount of sodium hydroxide in the solutions was calculated according to assumptions which we shall show to be erroneous.

[1] Berl and Austerweil, *Z. Elektrochem.*, 13, 165 (1907).

[2] A discussion of the allotropy of lead monoxides will be given in a later paper.