virtue of this capture, thyroidal accumulation of iodide and tissue content of iodide is reduced to a level much lower than normal. When the drug is administered daily, "a steady state" of drug concentration is achieved in the body. The presence of the drug in the tissues of the body increases their iodine concentration to values which are greater than normal. When the iodine affinity of the drug remaining in the body is satisfied, then thyroidal accumulation of iodine returns to normal levels.

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Diffusibility of Dyes in Buffered and Unbuffered Solutions.* (20050)

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In aqueous solutions the rate of diffusion of a substance depends primarily on its concentration gradient. In biological fluids the presence of buffers and other cell constituents may have a significant influence on the rate of diffusion. The purpose of this investigation was to compare the diffusion of basic dyes in aqueous medium, and in acid buffer. The acetate buffer was chosen because most dyes are relatively soluble in this buffer.

Method. The two half-cells were made from centrifuge tubes. Dimensions: 6.0 cm high and 2.5 cm in diameter with a side arm 1.0 cm in diameter and 1.0 cm long at a distance of 1.0 cm from the bottom of the main tube. The cellophane membrane (Eimer and Amend 8-667) was clamped between 2 rubber stoppers of 1.0 cm thickness with a bore of 0.9 cm. The side arms of the half-cells were then inserted into the rubber stoppers. Each half-cell contained either 15 ml of dye (1×10^{-4} M) in acetate buffer (pH 5.0) or water. The intensity of the diffused dyes was measured at appropriate intervals in a Beckman Spectrophotometer (Model DU). For each dye there was determined a standard concentration curve in order to transform the observed optical densities into micrograms. The *cell constant* was determined by allowing a 0.1 N HCl solution to diffuse into water. The diffused acid was measured with a Beckman pH-meter, or titrated with 0.01 N NaOH using phenolphthalein as an indicator. Ohölm(1) found that the diffusion coefficient "D" for 0.1 N HCl at 5° = 1.85 cm^2/day. The cell constant $K = D \times t/Q$. Q equals the number of ml of diffused acid expressed in units of the original concentration (the latter having a unit value of 1), and t equals time. It was found that 0.238 ml of 0.1 N HCl diffused during 30 minutes. Therefore, $K = 1.85 \times 0.0208/0.238 = 0.162$. K represents the ratio h/A, h is the effective distance through which diffusion takes place. A is the effective area of the surface available for diffusion. The diffusion coefficient $D = K \times Q/t$. The values for D are given in Tables I and II, t = 1 day. Q = micromoles (1×10^{-6} M) of diffused dye in 24 hours. In the first group of experiments the dyes were dissolved in water and allowed to diffuse into water. In the second group, the dyes were dissolved in acetate buffer (pH 5.0) and allowed to diffuse into the same buffer. In the third group there were four sections. Table III: a) The buffer in the half-cell (A) which contained the dye was diluted while that in half-cell (B) remained constant; b) the buffer in both half-cells was diluted simultaneously; c) the buffer in half-cell (A) was diluted with 10% water; and d) the concentration of the dye was varied in both water and buffer. The results are reproducible within 2-5% depending on the tinctorial power of the dye.

Results. In Table I we find the dyes arranged in decreasing order of their diffusibility "D" in water. "Stomach/blood" is the ratio of the concentration of the dye found in the

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TABLE I. Diffusion in Water through a Cellophane Membrane.

Compound conc., 1×10^{-4}M	Color index	Diffusion coeff.*	Stomach/ blood(2)	Membrane delay (hr)	pH
Thionin	920	.60	36.8	.75	5.4
Pyronine B	741	.43	5.7	.5	4.55
Acridine red	740	.34	8.4	.3	4.9
Safranin O	841	.29	27.3	2.4	6.02
Methylene blue	922	.27	15.6	.5	5.76
Methylene green	924	.26	13.6	2.6	5.7
Rhodamine B	749	.23	6.1		4.38
Toluidine blue	925	.21	36.4	2	5.02
Brill. cresyl blue	877	.18	11.3		4.27
Chrysoidin Y	20	.13	75	.5	4.9
Neutral violet	826	.13	27.2	.5	5.5
Bismarck brown	331	.12	26.5	6.8	4.1
Neutral red	825	.07	26.4	1.6	5.5

* cm²/day.

TABLE II. Diffusion in Acetate Buffer (pH 5)* through a Cellophane Membrane.

Compound conc., 1×10^{-4}M	Color index	Diffusion coeff.†	Stomach/ blood(2)	Membrane delay (hr)
Methylene blue	922	.48	15.6	
Methylene green	924	.42	13.6	
Neutral red	825	.39	26.4	.8
Safranin O	841	.36	26.3	
Neutral violet	826	.36	27.2	
Brill. cresyl blue	877	.35	11.3	
Pyronine B	741	.33	5.7	
Chrysoidin Y	20	.29	75	
Acridine red	740	.24	8.4	
Bismarck brown	331	.24	26.5	3.4
Thionin	920	.22	36.8	
Rhodamine B	749	.21	6.1	
Toluidine blue	925	.18	36.4	

* The buffer was prepared by mixing 590 ml of .2M acetic acid with 141 ml of .2M sodium acetate (3).
† cm²/day.

stomach to the concentration in the blood as determined by Ingraham and Visscher(2). In column 5 is listed the lag in diffusion caused by the membrane. We see that it takes different times for the membrane pores to become saturated with the dyes. In column 6 the pH values of the aqueous solutions are given. There seems to be no obvious relation between diffusibility and the pH of the solution.

In Table II the dyes are arranged in decreasing order of their diffusibility in acetate buffer (pH 5.0). Most of the dyes diffuse faster in buffer than in water. However, Acridine red, Rhodamine B, Pyronine B, Toluidine blue and Thionin diffuse faster in water than in buffer. It invariably takes more time for the membrane to become saturated with the dye in water than in buffer. Whereas a lag in diffusion by the membrane is observed for most dyes in aqueous medium, in acetate buffer only Bismarck Brown and Neutral Red are delayed. Their delay in buffer is only 44% and 50% respectively of the values in water. The spread between the fastest diffusing compound and the slowest is greater in water than in buffer, the comparative ratios being 8.6 and 2.7. Thionin is the fastest in water but one of the slowest in buffer. The probable explanation of these differences involves both hydration (or hydrolysis) and

TABLE III. Effect of Dilution of Buffer on Rate of Diffusion.

	Half-cell A, dye + % buffer*	Half-cell B, % buffer	Diffusion† Methylene blue	Diffusion† Methylene green
a.	100	100	.48	.42
	75	100	.82	.77
	50	100	1.02	.89
	25	100	1.28	1.18
	0	100	1.51	1.51
b.	100	100	.48	.42
	75	75	.43	.39
	50	50	.42	.36
	25	25	.32	.36
	0	0	.27	.26
			Acridine red	Pyronine B
c.	100	100	.24	.33
	90	100	.36	.50
			Methylene blue	
d.		Conc.	D (H_2O)	D (buffer)
		$.5 \times 10^{-4}$M	.15	.48
		1×10^{-4}M	.27	.48

* Conc. of 100% buffer, .2M; 75% buffer, .15M; 50% buffer, .1M; 25% buffer, .05M; 90% buffer, .18M.
† cm²/day.

ionization. Those substances that are highly ionized and which do not react with water should diffuse better in water. In buffer their ionization is repressed and diffusion is slower. On the other hand, if a substance is readily hydrated it might be expected to diffuse more slowly in water. A third possibility stems from the fact that in water the amines are in the form of their hydrochlorides while in acetate buffer they are, for all practical purposes, acetates. Certain acetates are known to dissociate to a lesser extent than the corresponding chlorides. In such cases an inhibition of diffusion would occur in buffer. Thionin, Toluidine Blue and Pyronine B are among the stronger bases(4) so that the increased ionization in water is a dilution phenomenon. Rhodamine B and Acridine Red, on the other hand, are weak bases and hydrolysis should be the predominant factor. That it is not, seems to point to the greatly reduced dissociation of their acetates. Probably the most important finding from the biological viewpoint is that the *order of diffusibility* is different in acid buffer than in water, Tables I and II. There seems to be little relation between the rates of diffusion and the ability of the stomach to secrete the dyes. The influence of dilution was studied for Methylene Blue, Methylene Green, Acridine Red and Pyronine B, Table III. The observed results show that the rate of diffusion depends not only upon the concentration gradient (Fick's Law) but also upon the concentration of buffer present in both half-cells.

If solutions of Methylene Blue and Methylene Green (1×10^{-4} M) are compared and only the buffer in the dye compartment (Half-cell A) is diluted by 25%, 50%, and 75% respectively and allowed to diffuse into the full strength buffer (Half-cell B), one notices that the diffusion increases almost linearly, Table III, a. However, if both half-cells A and B are diluted by 25%, 50%, and 75%, there is little effect, except possibly for the 25% buffer with Methylene Blue, until pure water is reached. The pH is unchanged by dilution.

Since dilution increases the ionization of the dye in half-cell A, the rate of diffusion should be increased. Now, if half-cell B contains the concentrated buffer it represses the ionization and thus decreases the back diffusion. If, however, half-cell B is diluted in the same proportion as half-cell A, it no longer can repress the ionization and the dye diffuses back into half-cell A. The ultimate result is a decrease in diffusion rate because of increased hydration. The latter effect is less striking than the former.

Acridine Red and Pyronine B are difficultly soluble, and their diffusion was studied in full strength buffer (Half-cell A) and then compared with the diffusion after diluting half-cell A by 10% water and allowing to diffuse into full strength buffer (Half-cell B), Table III, c. Acridine Red showed in 10% dilution an increase in concentration of 6.35%, whereas the rate of diffusion was increased by 50%. In Pyronine B the concentration is increased by 9.1%, whereas the rate of diffusion is increased by 50%. Even 10% dilution thus shows the same effect as was observed for Methylene Blue and Methylene Green, Table III, a.

We also noticed that the membrane has a different capacity for the same dye in different media, Table III, d. Methylene Blue in water for a concentration of 0.5×10^{-4} M has a value of D = .15. If we double the concentration the value for D increases to .27. The same dye in acetate buffer does not change its value of D = .48 on doubling its concentration. One would be apt to conclude that .48 represented the capacity of the membrane, but Table III, part a, shows that diffusion values above 1.5 may be obtained with the same membrane by using a different concentration in each half-cell.

Summary. The rate of diffusion of basic dyes depends not only on the concentration gradient but also the medium. The diffusion of dyes into a concentrated buffer is faster than into a diluted one. The concentrated buffer represses the ionization, and thus retards the back diffusion. If the buffer compartment is diluted in the same proportion as the dye compartment, the rate of diffusion of the dyes is only slightly affected, if at all, by a 4-fold dilution. In diffusion studies with Methylene Blue it was found that the mem-

brane has, in different media, a different diffusion capacity. This work suggests that conclusions based on diffusion measurements made on simple aqueous solutions may not be equally valid for biological systems.

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Effect of Butazoldin[R] on I^{131} Uptake by the Thyroid Gland—II. (20051)

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Butazolidin[R]* is a pyrazolon derivative which has been found to be useful in the treatment of various rheumatic diseases. In the course of a clinical investigation of effectiveness of this compound a radioactive iodine uptake test for thyroid function was performed and uptake measured 6% at 24 hours, about one-third of normal average and inconsistent with the patients' appearance. This led to the studies reported here.

Methods. The I^{131} uptake of the thyroid gland was determined prior to and after the administration of butazolidin[R] for 2 or 3 days. Each of eleven euthyroid patients and one hyperthyroid patient was given 5-15 μc of I^{131} and after 24 and 48 hours, the radioactivity over the thyroid gland, expressed as percent of the administered dose, was measured using a scintillation counter. After the last measurement a daily dose of 1.0 g of butazolidin[R] was given intramuscularly to each of the patients for 2 or 3 days. A second I^{131} uptake test, using 30-50 μc was performed on 4 of the euthyroid patients 24 hours after the last of 2 daily injections. The remaining 8 patients, including the hyperthyroid case, were tested concomitantly with the administration of the third daily injection of butazolidin[R]. Studies in this laboratory have shown, as might be expected, that the thyroid uptake of a dose of I^{131} is not related to the amount given in μc. The number of iodine atoms which are present in a test or therapy dose of I^{131} represents a quantity which is less than 0.1 μg of iodine.

TABLE I. % I^{131} Uptake by Thyroid at 24 Hr in 14 Patients Receiving Repeat but Variable Amounts Expressed in Microcuries.

Patient	Diagnosis	Dose I^{131} (orally), μc	Date	% uptake
L.	Hyperthyroid	15	8/16/52	66
		125	17	68
		3000	19	65
J.	"	25	9/14/51	65
		6700	20	68
C.	"	66	3/27/52	66
		9100	31	64
W.	"	9	8/ 8/52	75
		8800	13	76
R.W.	"	100	5/ 9/51	52
		7500	12	53
Q.W.	"	120	5/ 1/52	67
		10000	9	64
C.W.	"	20	10/27/51	60
		1000	11/ 1	65
"	"	100	9/27/52	73
		4530	10/ 3	73
K.	Cardiac	15	4/20/52	44
		10000	5/26	45
B.	Euthyroid	46	11/ 4/52	24
		100	12/16	21
V.W.	Thyroid block	5	12/ 3/52	1–2
		50	4	1–2
G.B.	Euthyroid	5	5/28/52	10
		140	6/24	11
H.	"	2	4/16/52	50
		30	17	48
G.W.	"	2	4/16/52	20
		30	17	18

* Butazolidin[R] is 3,5-dioxo-1,2-diphenyl-4n-butyl-pyrazolidin and is marketed as *Butazolidin[R]*. We wish to thank the Geigy Co. for a generous supply of this drug.