Polyvinylpyrrolidone-I^{131} as an Indicator of Net Intestinal Water Flux: Its Binding by Intestinal Mucus.* (27524)

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Intestinal absorption studies in man with test meals(1) or perfusion of the intestine in the approximately steady-state(2,3,4) depend upon unabsorbed indicators. The most used indicator, polyethylene glycol(PEG), has the disadvantage of a turbidometric method for analysis(5). Polyvinylpyrrolidone labelled with I^{131} ($PVPI^{131}$), because of its ease of analysis, would be a particularly desirable indicator if it could be shown to be unabsorbed under the conditions of absorption studies. This paper reports observations on $PVPI^{131}$ as a nonabsorbed indicator, and the binding of this polymer by intestinal mucus *in vivo* and *in vitro*.

Methods. Absorption was studied in man under conditions of brief gut contact time(3) by transintestinal intubation(6). The transintestinal tube enters at the nares, traverses the entire gastrointestinal tract, and emerges at the anus. Buffered isotonic solutions(7) containing test substances are pumped into the nasal end of the tube at 15 ml/min. The perfusion solution enters the gut through a hole in the side of the tube, perfuses the gut, and is sampled through an opening 15 cm distal to the entry site by siphonage from the anal end of the tube. Inlet and exit holes are separated by solid metal connectors. As the tube moves through the gastrointestinal tract (approximately 1M/day) absorption can be studied at any level. Absorption is calculated from the difference between initial concentration in the solution perfused and final concentration in the samples. Final concentrations are multiplied by the indicator ratio, initial indicator concentration/final indicator concentration, to correct for net water flux(7). A ratio of unity indicates no net flux; ratios less than one, net water absorption; greater than one, net water secretion. Solutions contained both PEG (1 mg/ml) and $PVPI^{131}$ (1 μc/liter). PEG concentrations were measured turbidometrically(8) and $PVPI^{131}$ by counting 5 ml aliquots in a scintillation well counter.

Results. In Table I are the ratios for PEG and $PVPI^{131}$ measured in the same samples. Since ratios depend on subject, solution, and site of perfusion, the data are divided so they are consistent with our previous findings on net water movement with PEG. Normal subjects H.E. and P.O. given glucose show net water absorption. Patients B.B. and M.J. with active sprue show net water secretion proximally. In the normal subjects the mean $PVPI^{131}$ ratios, except in one instance, were slightly larger than the PEG. Thus, there appeared to be loss of $PVPI^{131}$ during perfusion across the 15 cm segment of gut, but the difference between the ratios was small enough to suggest that $PVPI^{131}$ might be a useful indicator.

In sprue also, PEG and $PVPI^{131}$ ratios ran in parallel. Mucus was frequently recovered in the perfusates from sprue patient B.B. The samples were centrifuged and the supernatant counted. Samples from a study in B.B. not included in Table I contained much mucus. $PVPI^{131}$ ratios from these supernatant solutions were 3-4 times the PEG ratios. The apparent loss in radioactivity in the perfused samples could be accounted for by high counting rates in the centrifuged mucus. Data on binding of $PVPI^{131}$ by intestinal mucus during perfusions are in Table II. The perfusate samples containing mucus were thoroughly mixed and separated into an upper solution phase and a lower mucous suspension by centrifugation. A weighed aliquot of the supernatant solution in equilibrium

* Supported in part by research grant from Nat. Inst. of Arthritis and Metab. Dis., USPHS, and by Lederle Medical Faculty Award to senior author.

TABLE I. Indicator Ratios.

Subject (category)	Perfusion solution	Small intestinal perfusion site, distance from nares, cm	Initial indicator conc. / Final indicator conc. PEG	PVPI^{131}
H.E. (normal)	D(+)-Glucose	90–155 (19)*	.96 ± .08†	.94 ± .01
		244–310 (18)	.99 ± .04	1.06 ± .08
	L-Methionine	78–333 (123)	.95 ± .07	1.03 ± .08
	Triamcinolone	66–123 (12)	1.07 ± .05	1.12 ± .09
P.O. (normal)	D(+)-Glucose	128–170 (10)	.75 ± .08	.80 ± .11
		238 (10)	.95 ± .04	.99 ± .02
R.L. (normal)	L-Methionine	74–148 (23)	1.06 ± .13	1.09 ± .22
B.B. (sprue, active)	D(+)-Glucose	75–197 (36)	1.16 ± .15	1.16 ± .13
		76–281‡(28)	1.18 ± .15	1.31 ± .24
		63–222§(39)	1.12 ± .08	1.22 ± .14
		332–412 (19)	.91 ± .11	.98 ± .11
		367–369‡(14)	1.03 ± .06	1.08 ± .10
M.J. (sprue, active)	D(+)-Glucose	80–154 (15)	1.07 ± .22	1.13 ± .24
		162–286 (48)	.97 ± .17	1.05 ± .20
A.T. (sprue, remission)	D(+)-Glucose	77 (12)	.81 ± .07	1.01 ± .21

* No. of samples. † Mean ± S.D. ‡ Different lot of PVPI^{131}. § Cortisol therapy with ‡ lot of PVPI^{131}.

with the mucus was counted, the water evaporated, and solution counts/g of water calculated (Table II, Column 2). A nearly equal weighed aliquot of mucous suspension was counted. The water was evaporated and mucous suspension counts/g of water calculated (Table II, Column 3). In column 4 are total mucous suspension counts in the approximately 5 ml sample. The amount of water in the mucus was used to correct for counts from the solution in the mucous fraction. Counts adsorbed on mucus (Table II, Column 5) are total counts of mucous suspension minus counts from solution in the suspension (*i.e.*, g H_2O in suspension × counting rate of solution/g H_2O). The weight of dried mucus (Table II, Column 6) is the residue after evaporating the water, corrected for the weight of other solutes determined separately on weighed aliquots of equilibrium solution free of mucus.

In Table II studies 2-6, the original solutions before perfusion counted about 500 counts/g H_2O (PVPI^{131}, 1 μc/liter). Solution counting rates after perfusion were lower (Table II, Column 2). The suspension counting rate was 4-40× that of the solution (Table II, Column 3). This increased counting rate was from adsorption of PVPI^{131} on the relatively small amounts of mucus (Table II, Column 6), contained in approximately 5 ml of centrifuged suspension. In study 6 the higher uptake of PVPI^{131} by suspension gave low concentration in the solution. The suspension contained an unknown white powder in addition to mucus. The powder predominated in solution 6a and mucus in 6b. In study 1 the original perfusion solution contained no PVPI^{131}. The mucus-containing perfusates were thoroughly mixed and divided into 2 portions. PVPI^{131} was added to 1a to give 1μc/liter final concentration and to 1b to give 10 μc/liter. After mixing and centrifugation the solutions and suspensions were treated as described and the data recorded in Table II.

Binding data are classically treated by the Freundlich adsorption isotherm(9). In Fig. 1 $\frac{\text{PVPI}^{131}\text{ adsorbed to mucus}}{\text{Weight of mucus (g)}}$ is plotted against PVPI^{131} concentration in the solution. In the Freundlich equation $\frac{\times}{m} = KC^n$, × = adsorbed PVPI^{131}; m = weight of mucus; C = conc. of PVPI^{131} in solution in equilibrium

TABLE II. PVPI^{131} Binding by Intestinal Mucus in Sprue.

1	2	3	4	5	6
Study	Supernatant solution, counts/g H_2O	Mucous suspension, counts/g H_2O	Total mucous suspension counts	Column 4 minus counts from solution in suspension	Wt of mucus, mg
1a	389*	3,276	13,160	11,597	93
1b	2,753	16,430	63,871	53,169	84
2	459	5,380	26,194	23,960	121
	459	5,185	24,835	22,636	101
3	308	1,286	6,144	4,672	122
	308	1,236	5,924	4,449	129
	308	1,200	5,879	4,369	115
4	303	1,580	6,233	5,028	198
5	318	1,210	5,537	4,081	90
6a	72	2,995	13,746	13,416	49
6b	215	12,000	61,952	60,842	89

* No PVPI^{131} in original solution; added to mucous suspension after perfusion to give a final conc. of 1 μc/l to "a" and 10 μc/l to "b." The other studies had PVPI^{131} 1 μc/l in the original solution before perfusion.

with mucus; and K and n are constants for the particular system. The data from study 1, where the 2 different concentrations of PVPI^{131} were added *in vitro*, have been connected by a solid line which has the form of an adsorption isotherm. All PVPI^{131} concentrations in the solutions from studies 2-5 were about equal (300-450). The amount of bound PVPI^{131} at this concentration varied and is shown by the circles above and below the line. The data suggest heterogeneity in PVPI^{131} affinity of different mucous samples. In study 6 samples showed higher affinity, and consequently lower concentrations of PVPI^{131} were present in solutions in equilibrium. These data are connected by the broken line. The logarithms of these data are plotted in Fig. 2, and the corresponding points are connected by solid and broken lines. The binding constant K is the antilogarithm of the intercept at unit PVPI^{131} concentration (log c = o) and the constant n is the slope of the line. K is 1000 for study 1 and 7943 for study 6 and n is 0.81 in both. This means that at unit PVPI^{131} concentration (1 count/g water) 1000 and 7943 counts of PVPI^{131} are adsorbed on one gram of mucus.

Discussion. PVPI^{131} is absorbed if it is in contact with the gut mucosa for several hours (10), but there are no data on absorption during short contact periods. During studies of PVPI^{131} solutions which were rapidly perfusing the intestine in normal subjects and patients with non-tropical sprue, we noted discrepancies in PVPI^{131} and PEG ratios in some patients with sprue. These differences were most pronounced in specimens containing mucus, and binding of PVPI^{131} by mucus has been demonstrated. Since patients with sprue characteristically have excessive intestinal mucus, PVPI^{131} is not a suitable nonabsorb-

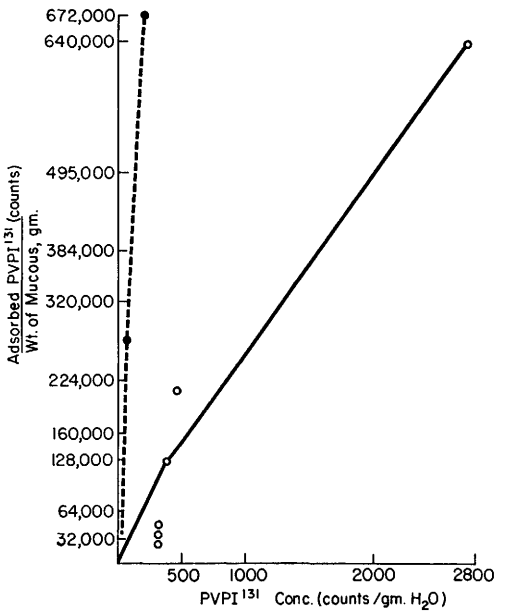

FIG. 1. Adsorbed PVPI^{131} per g of mucus in relation to equilibrium PVPI^{131} concentrations in solution (counts/g water).

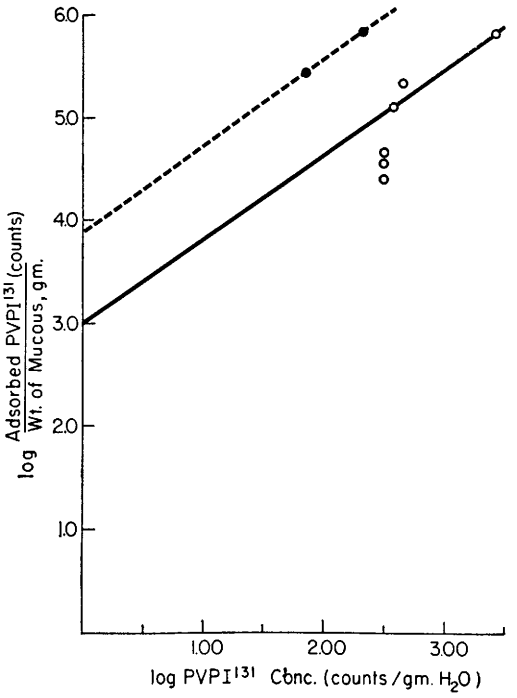

FIG. 2. Log plot of data in Fig. 1. The intercepts show that at unit PVPI^{131} concentration (1 count/g water) 1000 and 7943 counts are bound by one g of mucus.

able indicator for them because it does not follow the same intralumenal distribution pattern as test substances. The data show that different mucous samples vary considerably in their PVPI^{131} binding. In terms of the Freundlich adsorption isotherm, affinity and capacity vary, but both are high.

Binding by intestinal mucus may be important in the physiological distribution of PVPI^{131} in the normal and disease states. Fecal excretion of intravenous PVPI^{131} reflects equilibrium of other body compartments with the gut. This equilibrium would be shifted toward increased PVPI^{131} in the gut compartment by binding in the gut lumen, and the unabsorbed complex would be excreted in the feces. Thus the "leak" of PVPI^{131} into the gut in patients with sprue(11-14) may be displacement of equilibrium toward the gut compartment. Binding by intestinal mucus could also be important in fecal PVPI^{131} excretion in protein losing gastroenteropathy. During perfusion of normal subject P.O. a small amount of mucus (<3 mg) showed binding of PVPI^{131} qualitatively similar to Table II, hence binding also occurs in normal subjects.

Summary. Polyvinylpyrrolidone labelled with I^{131} was compared with polyethylene glycol as an indicator of net water absorption in intestinal perfusion studies. Small losses of PVPI^{131} in normal subjects and large losses in patients with sprue could be explained by binding of the polymer to intestinal mucus. The role of this factor in fecal PVPI^{131} excretion is discussed. PVPI^{131} is not a useful indicator of net water flux in patients with sprue.

We thank Abbott Laboratories for PVPI^{131} used in these studies and the Radiation Research Laboratories, State University of Iowa, for providing facilities for measurement of radioactivity.

1. Borgström, B., Dahlquist, A., Lundh, G., Sjövall, J., *J. Clin. Invest.*, 1957, v36, 1521.

2. Hendrix, T. R., *Am. J. Dig. Dis.*, 1957, v2, 643.

3. Schedl, H. P., Clifton, J. A., *J. Clin. Invest.*, 1961, v40, 1069.

4. Fordtran, J. S., Levitan, R., Bikerman, V., Burrows, B. A., Ingelfinger, F. J., *Trans. Assn. Am. Phys.*, 1961, v74, 195.

5. Lundh, G., *Acta Chir. Scand.*, 1958, Sup. 231.

6. Blankenhorn, D. H., Hirsch, J., Ahrens, E. H., Jr., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 356.

7. Schedl, H. P., Clifton, J. A., *Gastroenterology*, 1961, v41, 491.

8. Hyden, S., *Kgl. Lantbruks-Högsk Ann.*, 1955, v22, 139.

9. Getman, F. H., Daniels, F., *Outlines of Theoretical Chemistry*, 6th Edition, John Wiley & Sons, Inc., 1937, 221.

10. French, A. B., Ragins, A. I., Pollard, H. M., Dickason, B., *Fed. Proc.*, 1961, v20, 242.

11. Vesin, P., Troupel, S., Acar, J., Renault, H., Desbuquois, G., Cotton, R., *Bull. et mem. Soc. med. d hop. de Paris*, 1960, v76, 261.

12. Parkins, R. A., *Lancet*, 1960, vii, 1366.

13. London, D. R., Bamforth, J., Creamer, B., *ibid.*, 1961, vii, 18.

14. Dawson, A. M., Williams, R., Williams, H. S., *Brit. Med. J.*, 1961, vii, 667.

Received April 12, 1962. P.S.E.B.M., 1962, v110.