PERMEABILITY OF THE DUODENUM OF THE TOAD TO NON-ELECTROLYTES

By D. K. C. TAY* and G. P. FINDLAY*

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Abstract

The reflection coefficients (σ) for the permeation, from serosa to mucosa, of 59 different non-electrolytes through the duodenum of the toad have been measured by the method used by Diamond and Wright (1969*a*) with the rabbit gall bladder. In this method σ for the solute is the ratio of the streaming potential across the epithelium, resulting from a water flow caused by an osmotic gradient of the test solute, to the streaming potential caused by water flow resulting from an equal osmotic gradient of impermeant solute.

The permeation of non-electrolytes through the toad duodenum is largely determined by the lipid solubility of the solutes, and apart from a few solutes is very similar to the pattern for the rabbit gall bladder.

I. INTRODUCTION

Recently, Wright and Diamond (1969*a*, 1969*b*) and Diamond and Wright (1969) have examined the permeation of non-electrolytes and the molecular forces governing this permeation through cell membranes of the gall bladder. In their comprehensive study they measured the reflection coefficients (σ) for 206 non-electrolytes, and concluded that, in general, σ decreased from 1·0 to zero as the lipid–water partition coefficients of the solutes increased, and that the intermolecular forces determining the permeation of the non-electrolytes through the cell membrane of the gall bladder were the same as those determining the partition of the solute between a water and a bulk lipid phase. Wright and Diamond also found two types of solute which deviated from this general pattern of permeation, and they concluded that the causes of these deviations were related to the structure of the cell membranes.

The determination of σ from the relative magnitudes of streaming potentials arising from water flows produced by equal osmotic gradients of test solute and totally impermeant solute (Smyth and Wright 1966) can also be made on the gut of the toad. This paper gives measurements of σ for the permeation of 59 non-electrolytes through the toad duodenum. Some preliminary investigations have also been made with the toad jejunum and ileum. The duodenum, in its electrical properties, is quite different from the ileum and jejunum, and from the rabbit gall bladder (Wright and Diamond 1969*a*, 1969*b*) and the rabbit intestine (Smyth and Wright 1966). In the duodenum, the routes for water flow appear to be positively charged (Tay and Findlay, unpublished data); in other tissues they are negatively charged. Nevertheless, our analysis shows that the patterns of permeation of non-electrolytes through the duodenum are similar to those in rabbit gall bladder.

* School of Biological Sciences, Flinders University, Bedford Park, S.A. 5042.

II. Materials and Methods

(a) *Materials and Ringer's Solution*

Queensland cane toads, *Bufo marinus*, of various sizes and weights were kept in water tanks in the laboratory, given plenty of water which was renewed occasionally, but were not given any food for several days before experimentation.

The toads were doubly pithed. An insertion was made in the wall of the abdomen to expose the stomach and the small intestine. Segments of small intestine, each about 2–3 cm in length, were isolated and transferred into normal toad Ringer's solution (NTR) where the excess connective tissues were removed with due care not to damage the intestinal wall. The first segment immediately distal to the pyloric end of the stomach was referred to as the duodenum, the second segment was called the jejunum, while the rest of the segments were designated segments of the ileum.

The NTR solution (pH 7·2–7·3) had the following composition: 114·0 mM NaCl, 3·0 mM KCl, 2·0 mM glucose, 1·0 mM $CaCl_2$, 1·0 mM Na_2HPO_4, and 1·0 mM NaH_2PO_4.

(b) *Eversion of the Intestinal Segment*

Segments of intestine were everted as follows. One end of the intestinal segment was securely tied with a piece of cotton thread by means of which the segment was pulled into a polyethylene cannula of about the same diameter as the intestine. The excess length of thread was cut off and the open end of the intestinal segment was turned over to cover the end of the cannula. After having securely tied this end to the cannula, a gentle jet of air was applied at the free end of the cannula to force the remaining part of the intestine out of the cannula, thus completing the eversion. Any leakages resulting from the procedure could be checked readily by applying a slight air pressure to the open end of the cannula while the other end was immersed in a beaker of NTR and looking for air bubbles.

With the everted preparation, the bathing solution inside the cannula (serosal surface) was NTR while the mucosal surface was bathed with the appropriate solution at the time of any particular investigation. In the uneverted preparation the mucosal surface was bathed with NTR while the serosal surface was in contact with the test solution.

(c) *Electrical Measurements*

The intestinal preparation was mounted on the cannula that was clamped to a support and suspended in a beaker which usually contained 4 c.c. of bathing medium. The bathing solution in the beaker was stirred by means of a jet of air bubbles while the bathing solution in the cannula remained unstirred but was renewed regularly. The transepithelial potential difference was measured with polyethylene bridges filled with 3% agar in NTR. One bridge, the recording electrode, went down the cannula while the reference electrode remained in the outside bathing solution. Each bridge led to a container of NTR which was connected in turn by a saturated KCl bridge to a calomel half-cell. The potential difference was measured with an electrometer and recorded on a Rikadenki chart recorder.

The transepithelial potential difference was always measured as the potential of the mucosal surface with respect to the serosal surface, and denoted by ψ_{ms}. The potential of the circuit was measured before the commencement of each experiment by dipping both the NTR agar bridges into a beaker of solution bathing the outside of the organ. It was checked occasionally and was generally about 0·3–0·6 mV regardless of the solution into which they were placed. This potential across the bridges was subtracted from all the experimental potential differences. All experiments were carried out at ambient temperature (17–19°C) and during each individual experiment, the temperature was generally constant to within 0·5°C.

Experiments were done only after an initial equilibration period of about 30 min or until such time as the resting potential did not change appreciably over a 5-min period.

When the outside solution (i.e. solution in the beaker) was made hypertonic by the addition of a known concentration of non-electrolyte to the bathing medium, which otherwise had the same ionic composition, a change in ψ_{ms} was recorded. This change was the streaming potential.

(*d*) *Determination of Reflection Coefficient*

In this paper, a study of the permeability of the everted duodenum to 59 water-soluble non-electrolytes is described. The basic plan of the experiments was to measure the potential differences produced by a 0·15M osmotic gradient of the test solute, with the solute added to the mucosal side of the duodenum, preceded and followed by the same concentration gradient of the impermeant reference molecule, mannitol.

The time course of the transepithelial p.d. for a typical determination of σ is shown in Figure 1. In the example shown, ethyl urea was the test solute. Initially, when both the serosal

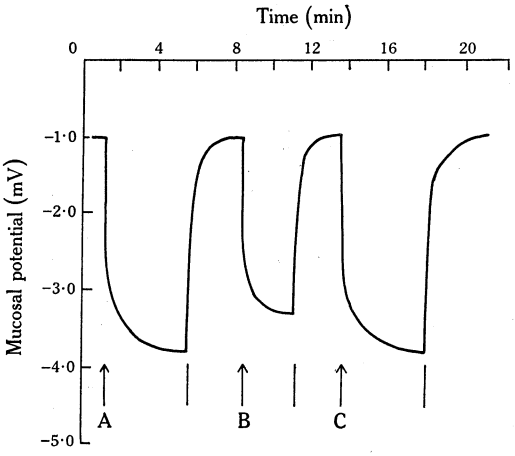

Fig. 1.—Time course of transepithelial potential difference for a typical determination of σ. Mannitol was the impermeant solute and at times A and C a 0·15M solution was added with the normal toad Ringer's solution to the mucosal side for 5 and 4 min, respectively. At time B, the test solute, in this case 0·15M ethyl urea was added to the normal toad Ringer's solution on the mucosal side.

and mucosal surfaces of the everted duodenal segment were bathed by identical NTR solutions, the resting potential was 1·00 mV; the mucosal side being negative with respect to the serosal surface. When the mucosal bathing solution was replaced by Ringer's solution made hypertonic by the addition of the impermeant reference molecule mannitol at 0·15M, the mucosal solution became more negative. A potential difference of −3·8 mV was measured. The streaming potential due to the mannitol was taken as the difference between the induced potential (after it had remained at a steady level for about 2 min) and the resting potential. Similarly, the streaming potential due to ethyl urea was −2·35 mV. Finally, the streaming potential caused by the mannitol gradient was again measured.

The σ value for each test solute was then calculated by taking the ratio of the streaming potential produced by the test solute to the average of the two streaming potentials produced by both the preceding and the following mannitol solutions at the same concentration gradient. Thus, for ethyl urea, $\sigma = 2 \cdot 35/\frac{1}{2}(2 \cdot 80 + 2 \cdot 85) = 0 \cdot 832$. Where the test solute caused significant irreversible damage to the everted duodenum, as judged by the much lower subsequent value of the mannitol streaming potential, then only the preceding streaming potential was taken into the calculation of σ.

The variability in σ for various solutes and for successive determinations in the one duodenal preparation was examined in an experiment, lasting several hours, on the amide series. The values of σ for most amides measured at different times were the same within the limit of experimental errors indicating that the permeability characteristics of the duodenum had not been altered to any extent by the repeated exposure to the various amides.

III. Results

(*a*) *Reflection Coefficient*, σ

The values of σ for the permeation of 59 non-electrolytes through the everted duodenum have been measured, and the results are shown in Table 1. Related

TABLE 1

VALUES OF REFLECTION COEFFICIENT (σ) FOR PERMEATION OF NON-ELECTROLYTES THROUGH THE TOAD DUODENUM WITH CORRESPONDING VALUES FOR RABBIT GALL BLADDER

Total number of determinations in each case given in parentheses

Name of compound	No. of carbon atoms	Mol. wt.	Partition coefficient, K		Mean σ ±S.E.M. for duodenum	Remarks†	Mean σ ±S.E.M. for gall bladder*
			Ether*	Olive oil*			
1. Formamide	1	45·0	$1·4\times10^{-3}$	$7·6\times10^{-4}$	0·33±0·01 (19)	2	0·57±0·13 (28)
2. Urea	1	60·1	$4·7\times10^{-4}$	$1·5\times10^{-4}$	1·02±0·01 (20)	2	0·53±0·16 (139)
3. Ethanol	2	46·1	$2·6\times10^{-1}$	$3·2\times10^{-2}$	0·02±0·01 (15)	2	0·05±0·02 (4)
4. Dimethyl sulphoxide	2	78·1	—	—	0·55±0·13 (3)	1	0·92±0·08 (6)
5. Acetonitrile	2	41·1	$6·0\times10^{-1}$	—	0·08 (2)	2,4	0·00±0·00 (4)
6. Acetamide	2	59·1	$2·5\times10^{-3}$	$8·3\times10^{-4}$	0·58±0·02 (31)		0·59±0·13 (51)
7. 2-Iodoacetamide	2	185·0	—	—	0·30±0·03 (9)	1,4	0·33±0·06 (5)
8. Glycine	2	75·1	$7·0\times10^{-7}$	—	1·02±0·02 (10)		1·02±0·05 (4)
9. Methyl urea	2	74·1	$1·2\times10^{-3}$	$4·4\times10^{-4}$	0·92±0·02 (21)	2	0·53±0·06 (7)
10. Acetone	3	58·1	$6·2\times10^{-1}$	$8·0\times10^{-2}$	0·03±0·01 (10)	2	0·01±0·02 (5)
11. 1,3-Dichloroacetone	3	127·0	—	—	0·05 (2)	1,4	0·25±0·09 (8)
12. 2-Propyn-1-ol	3	56·1	—	—	0·07±0·01 (6)		—
13. Ethyl formate	3	74·1	—	—	0·24 (2)	1,3	0·00±0·00 (2)
14. 1,2-Propanediol	3	76·1	$1·8\times10^{-2}$	$1·7\times10^{-3}$	0·39±0·02 (12)		0·84±0·06 (14)
15. 1,3-Propanediol	3	76·1	$1·2\times10^{-2}$	—	0·43±0·02 (10)		0·92±0·14 (7)
16. Propionitrile	3	55·1	2·4	—	0·05±0·00 (4)	4	0·00 (1)
17. Dimethyl formamide	3	73·1	$2·4\times10^{-3}$	$4·9\times10^{-3}$	0·14±0·02 (12)		0·24±0·07 (6)
18. Methyl acetamide	3	73·1	—	—	0·29±0·02 (32)	2	0·46±0·06 (9)
19. Propionamide	3	73·1	$1·3\times10^{-2}$	$3·6\times10^{-3}$	0·34±0·02 (29)	2	0·66±0·11 (26)
20. Acrylamide	3	71·1	—	—	0·28 (2)	4	0·55±0·11 (7)
21. 1-Amino-2-propanol	3	75·1	—	—	0·41 (1)	1,3	0·89±0·06 (3)
22. DL-Alanine	3	89·1	$1·4\times10^{-6}$	—	1·00±0·06 (17)		1·06±0·04 (4)
23. Ethyl carbamate	3	89·1	$6·4\times10^{-1}$	$7·4\times10^{-2}$	0·03±0·01 (9)		0·04±0·02 (4)
24. L-Serine	3	105·1	—	—	1·10±0·03 (8)		0·99±0·04 (4)
25. Malononitrile	3	66·1	—	—	0·03±0·01 (3)	4	0·07±0·02 (4)
26. 2-Cyanoacetamide	3	84·1	—	—	0·80 (1)	4	0·86±0·13 (4)
27. Ethyl urea	3	88·1	$4·1\times10^{-3}$	$1·7\times10^{-3}$	0·82±0·02 (30)	2	0·82±0·11 (5)
28. Isobutanol	4	74·1	6·2	$4·4\times10^{-1}$	0·01±0·00 (12)		0·05±0·03 (4)
29. t-Butanol	4	74·1	2·2	$2·3\times10^{-1}$	0·02±0·00 (10)		0·03 (1)
30. Ethyl acetate	4	88·1	8·5	2·5	0·02 (2)	4	0·03±0·02 (3)
31. 1,3-Butanediol	4	90·1	$4·2\times10^{-2}$	$4·3\times10^{-3}$	0·34±0·06 (7)		0·77±0·10 (8)
32. 2,3-Butanediol	4	90·1	$2·9\times10^{-2}$	$3·4\times10^{-3}$	0·46±0·01 (14)		0·74±0·07 (8)
33. 1,4-Butanediol	4	90·1	$1·9\times10^{-2}$	$2·1\times10^{-3}$	0·54±0·02 (11)	4	0·86±0·08 (17)
34. 2-Butyne-1,4-diol	4	86·1	—	—	0·58 (2)		0·69±0·11 (7)
35. Diethylene glycol	4	106·1	$4·0\times10^{-3}$	$5·0\times10^{-3}$	0·59±0·04 (7)		0·92±0·04 (8)
36. n-Butyramide	4	87·1	$5·8\times10^{-2}$	$9·5\times10^{-3}$	0·15±0·01 (20)	2	0·42±0·11 (103)
37. Dimethyl acetamide	4	87·1	—	—	0·04±0·01 (19)	2	0·32±0·13 (8)
38. Glycyl glycine	4	132·1	—	—	1·05±0·04 (12)		0·99±0·03 (4)
39. Piperazine hydrate	4	194·2	$5·2\times10^{-4}$	—	0·72 (1)	1,3,4	0·94±0·13 (3)
40. Creatinine	4	113·1	—	—	0·97±0·03 (13)		1·05±0·04 (8)
41. 3-Pentanol	5	88·1	—	—	0·06 (2)		—
42. Furfuryl alcohol	5	98·1	—	—	0·21±0·04 (3)	1	—
43. Tetrahydrofurfuryl alcohol	5	102·1	—	—	0·14 (2)	1	0·11±0·08 (6)
44. 1,5-Pentanediol	5	104·2	$5·5\times10^{-2}$	$6·1\times10^{-3}$	0·38±0·04 (13)		0·71±0·07 (9)
45. Pentaerythritol	5	136·2	$3·0\times10^{-4}$	—	0·89±0·02 (13)		1·04±0·05 (5)
46. L-Arabinose	5	150·0	$3·8\times10^{-5}$	—	1·03±0·02 (12)		1·03±0·05 (6)
47. L-Proline	5	115·1	—	—	1·02±0·03 (8)		1·01±0·02 (4)
48. L-Valine	5	117·2	—	—	1·03±0·03 (12)		1·01±0·02 (4)
49. Cyclohexanol	6	100·2	—	—	0·38±0·04 (3)		—
50. 2-Methyl-2,4-pentanediol	6	118·2	$5·1\times10^{-1}$	$2·4\times10^{-2}$	0·18±0·04 (10)	1	0·28±0·03 (4)
51. Pinacol	6	118·2	$4·3\times10^{-1}$	—	0·08 (2)		0·54±0·17 (4)
52. Ethyl acetoacetate	6	130·2	—	1·1	0·35±0·09 (3)		0·04±0·04 (3)
53. D-Galactose	6	180·2	—	—	0·96±0·02 (10)		1·02±0·03 (4)
54. D-Glucose	6	180·2	$4·5\times10^{-6}$	—	0·97±0·01 (23)	2	1·01±0·03 (4)
55. D-Sorbitol	6	182·2	—	—	0·98±0·02 (30)		—
56. Diethyl acetamide	6	115·2	—	—	0·09±0·02 (13)	2	0·13±0·03 (8)
57. Nicotinamide	6	122·1	—	—	0·46±0·03 (11)	4	0·84±0·07 (9)
58. Hexamide	6	140·2	$2·6\times10^{-4}$	$2·2\times10^{-4}$	0·98±0·02 (11)		—
59. Sucrose	12	324·3	—	—	0·95±0·01 (27)	2	1·0 (standard solute)

information concerning the non-electrolytes and the corresponding σ values for rabbit gall bladder has also been included.

(*b*) σ *Values for the Ileum*

In one experiment, the reflection coefficients of nine solutes for a segment of everted ileum were determined. The following tabulation shows the results (each value the average of two determinations) and compares them with corresponding ones for the duodenum (from Table 1). Except for glycine and 2-methyl-2,4-pentanediol, the values of σ for the ileal segment are higher than those for the duodenum indicating that the ileum is more impermeable to the range of solutes tested:

Compound	σ value for duodenum (from Table 1)	σ value for ileum	Compound	σ value for duodenum (from Table 1)	σ value for ileum
Glycine	1·02	0·56	Diethylene glycol	0·59	0·77
Glucose	0·97	1·02	Propionitrile	0·05	0·71
Arabinose	1·03	1·10	Isobutanol	0·01	0·22
Ethyl urea	0·82	0·96	2-Methyl-2,4-pentanediol	0·18	0·04
1,3-Butanediol	0·34	0·66			

It should be noted here that the polarity of the streaming potential (positive on mucosal side, to which water is flowing) shows that the routes of water flow are negatively charged (Tay and Findlay, unpublished data).

IV. Discussion

(*a*) *Analysis of* σ *Values for the Duodenum*

(i) *Permeation of the Solute as a Function of Its Lipid Solubility*

If the permeability of the epithelium of the duodenum to non-electrolytes is governed solely by their lipid solubility, a regular increase in permeability coefficients and decrease in σ values would be expected with increasing lipid:water partition coefficients (K). Figure 2 shows σ plotted against $K_{\text{ether}}\ M^{-1/2}$, where M is the molecular weight of the solute. Most of the points lie within the arbitrarily drawn envelope. Similar permeation patterns are observed when σ is plotted against K_{ether} or $K_{\text{olive oil}}$. However, the points are more scattered when plotted against the olive oil partition coefficients.

(ii) *Permeation as a Function of Chemical Structure of the Solute*

According to the empirical rules formulated by Overton (1896, 1899) the permeation of a solute is increased by alkylation, by increase of chain length, and

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* Values from Wright and Diamond (1969*a*).

† Remarks are as follows:

1. Caused significant irreversible damage to the everted duodenum as judged by the lower subsequent values of mannitol streaming potential.
2. Includes some determinations at pH 5·4.
3. The pH of the Ringer's solution with added 0·15M non-electrolyte became greater than 8.
4. At least one estimation of σ was made within a few minutes of the solute being dissolved, to check against changes in σ with time due to possibility of instability of solute in aqueous solution.

halogenation The same modifications of the chemical structure also describe the partition of non-electrolytes between a lipid solvent and water (Collander 1949). Most of the σ values for gall bladder also follow these predictions (Wright and Diamond 1969*b*).

In general, the σ values for the duodenum agree with Overton's rules. For instance, the addition of hydroxyl groups decreases the permeating power of the molecule. Also the position of the substituted hydroxyl groups affects the reduction of the permeability. If a molecule has two hydroxyl groups, a lower σ is observed when they are far apart than when they are close together. The introduction of oxygen functions (such as hydroxyl, ketone, aldehyde, ether, and carboxylic groups) or amino

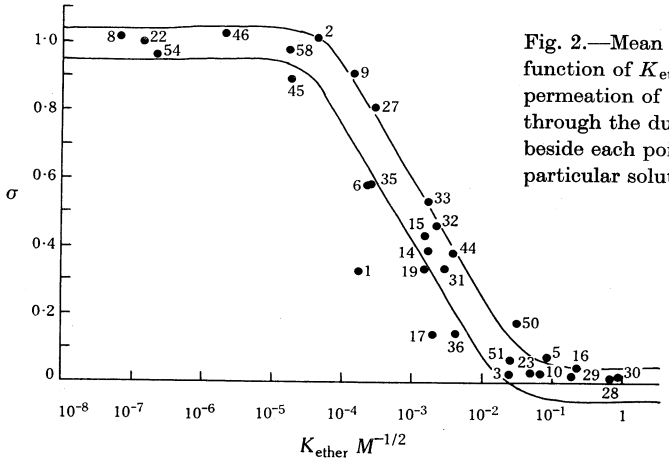

Fig. 2.—Mean value of σ plotted as a function of $K_{ether}\ M^{-1/2}$ for the permeation of 31 different solutes through the duodenum. The number beside each point identifies the particular solute as shown in Table 1.

groups would decrease membrane permeability to these solutes. The way in which such chemical modification affect the values of σ in the duodenum are shown in the following tabulation:

Solute	σ	Solute	
Addition of –OH Group		Addition of –O–	
3-Pentanol	0·06	1,4-Butanediol	0·54
1,5-Pentanediol	0·38	Diethylene glycol	0·59
Pentaerythritol	0·89		
Arabinose	1·03		
Position of –OH Group		Replacement of $-CH_3$ by $-NH_2$	
1,2-Propanediol	0·39	Acetone	0·03
1,3-Propanediol	0·59	Acetamide	0·58
2,3-Butanediol	0·46	Urea	1·03
1,3-Butanediol	0·34*	Ethyl acetate	0·02
1,4-Butanediol	0·54	Ethyl carbamate	0·03

* Lower than expected.

Replacement of a methyl group by an amino group also decreases the permeability as indicated by the acetone, acetamide, urea series.

The relationship between σ and the lipid : water partition coefficients for solutes of varying shapes and sizes indicates that for most solutes the intermolecular forces governing the selective non-electrolyte permeation through the duodenum are the same as the forces governing the non-electrolytes partition between the bulk lipid phase and water. The relation between the chemical structure and the permeation pattern are in general agreement with the set of rules which were first formulated empirically by Overton (1899). These observations imply that the regions of the tissue controlling permeation are predominantly lipoidal in composition and that the intermolecular forces which govern the distribution of the substance between a bulk solvent phase and water also govern the substance's entrance into and passage through the tissue.

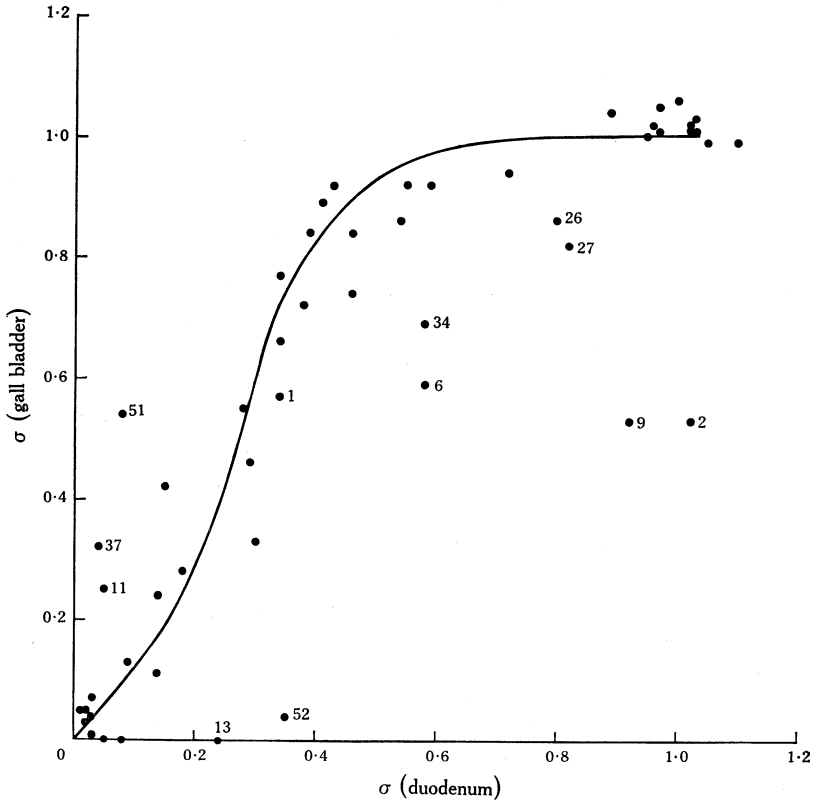

Fig. 3.—Values of σ for rabbit gall bladder (Wright and Diamond 1969*b*) plotted against σ values for toad duodenum for 53 solutes. The curve is a theoretical one as described in the text. The numbers against some of the points identify the solutes as shown in Table 1.

Some small molecules permeate more rapidly than expected for the main pattern. The most conspicuous examples are the points for formamide and dimethyl formamide in Figure 2. The value of σ for the first and smallest members of the amide series, formamide, suggests that its permeation is not governed by the lipid : water partition coefficient but rather by its size. Such exceptions to the main pattern have been observed in *Chara ceratophylla* (Collander and Barlund 1933), *Nitella mucronata*

(Collander 1954), and the rabbit gall bladder (Wright and Diamond 1969*b*). However, no such effect was detected in the urea series in the duodenum, although it occurs in the rabbit gall bladder (Wright and Diamond 1969*b*) and in giant algal cells (Collander 1954).

Another class of anomalous molecules deviating from the general patterns are those which are highly branched. The most markedly deviant points in the graph of σ versus K_{ether} are 2-methyl-2,4-pentanediol and ethyl acetoacetate. Although they are not as highly branched as pinacol, they are too impermeant for their lipid partition coefficients.

(*b*) *Comparison of* σ *Data for Toad Duodenum and Rabbit Gall Bladder*

Figure 3 shows a comparison of our σ data for 59 solutes for toad duodenum with those of Wright and Diamond (1969*b*) for rabbit gall bladder. The curve in this figure was obtained by eliminating $K_{\text{ether}}\ M^{-1/2}$ between the functions of σ versus $K_{\text{ether}}\ M^{-1/2}$ for the gall bladder and for the duodenum (taking the line midway between the extremes of the pattern in Figure 2 and in Figure 2 of Diamond and Wright as an estimate of σ).

The general trend of the points in Figure 3 is, of course, to follow this curve. The figure shows that for most of the solutes σ for the gall bladder is greater than σ for the duodenum. A few points lie considerably off the line, some above and some below. The two points most distant below the line are for 2 (urea) and 9 (methyl urea). The deviation from the line arises in the deviation of these two points from the general relationship for σ and $K_{\text{ether}}\ M^{-1/2}$ for the gall bladder. The same observation applies to 51 (pinacol).

On the other hand, a solute like 6 (acetamide) lies away from the curve in Figure 3 because of its deviant behaviour in both gall bladder and duodenum. A few points which actually lie near the curve, e.g. 1 (formamide) are, however, deviant in both gall bladder and duodenum but in the same direction.

The general conclusion from our results nevertheless is that apart from a few particular solute molecules the permeation of most solutes through the epithelium of the duodenum is controlled by their lipid solubility and is very similar to the permeation of solutes through the rabbit gall bladder as described by Wright and Diamond (1969*a*, 1969*b*).

V. Acknowledgments

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