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The passage of drugs across the rat intestine *in vitro*

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The *in vitro* methods of Smyth & Taylor (1957) and everted sac technique of Crane & Wilson (1958) have been evaluated for studying the passage of ferrioxamine-B hydrochloride and its derivatives, glutethimide, thalidomide, a polypeptide, salicylic acid, aniline and phenol red from the mucosal to serosal side of the rat intestinal barrier *in vitro*. The ferrioxamines were not significantly absorbed, glutethimide was more rapidly absorbed than thalidomide, the polypeptide was broken down rapidly and appeared as amino-acids in the serosal fluid, salicylic acid and aniline were found in the serosal fluid but phenol red was little absorbed. Although allowance must be made for differences in the transit pathways of *in vivo* and *in vitro* systems because of the non-functional nature of the capillary network in *in vitro* studies, our results suggest that these methods can supply useful information on the permeability characteristics of newly developed drugs through the intestinal barrier.

MUCH evidence (Schanker, 1960, 1961; Brodie, 1964) has been found to support the role of pH gradients, partition coefficients and lipid solubility in the gastrointestinal absorption of drugs. The concept of a lipid-intestinal barrier preferentially permeable to lipid-soluble molecules in undissociated form does not, however, satisfy all the requirements for the absorption of a drug. The passage of some drugs in their dissociated forms must undoubtedly be governed by other special transport mechanisms. Hogben (1960a, b) and Smyth (1960, 1964) have emphasised the complex nature of this intestinal barrier and Wilson (1962) has pointed out the difficulties which the membrane thickness and available surface area impose on the application of Fick's diffusion equation for passage of drugs across intestinal epithelium.

As the diffusion involved movement across three permeability-barriers (two cell membranes and a basement membrane), Wilson (1962) conceives one membrane to be the effective permeability-barrier for water-soluble substances and another for lipid-soluble substances. In spite of an incomplete understanding of the intestinal barrier, there has been a growing interest in intestinal absorption in recent years and Nelson (1961), Wagner (1961) and Nogami & Matsuzawa (1961, 1962, 1963) have given excellent treatments of the kinetic and physico-chemical factors underlying absorption processes.

In the present report two recent methods, those of Smyth & Taylor (1957) and Crane & Wilson (1958), have been evaluated for studying the passage of drugs from the mucosal to serosal side of rat intestinal preparations *in vitro* using compounds of current pharmaceutical interest. These included ferrioxamine-B hydrochloride* (Bickel, Hall, Keller-Schierlein, Prelog, Vischer & Wettstein, 1960) and its derivatives, deferrioxamine

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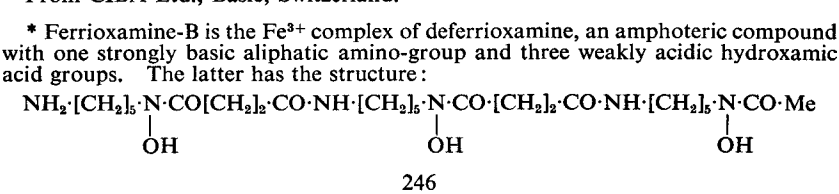
* Ferrioxamine-B is the Fe^{3+} complex of deferrioxamine, an amphoteric compound with one strongly basic aliphatic amino-group and three weakly acidic hydroxamic acid groups. The latter has the structure:



(Tripod & Keberle, 1962), a metabolite of a species of *Streptomyces* which specifically binds Fe^{3+} to give ferrioxamine-B, glutethimide (Tagmann, Sury & Hoffman, 1952), thalidomide and Val^5-angiotensin-Asp-β-amide. For the sake of comparison salicylic acid, aniline and phenol red have also been investigated.

Experimental

METHODS

Male and female rats weighing between 200–240 g had their food withheld 24 hr before use and were given plain water and 5% glucose solution (100 ml) to clear the intestine of solid contents.

The method of Smith & Taylor (1957). The experiment was set up according to Smyth & Taylor (1957). The intestine was removed as described by Wiseman (1953) except for the use of light anaesthesia with chloroform. The compounds were dissolved in Krebs Ringer bicarbonate saline (pH 7·4) containing glucose (5 mg/ml) in concentrations given in Table 1. Before circulating the drug solution, Ringer saline containing glucose was circulated for about 3 min through the intestinal segments and then taken out and replaced by 25 ml of a solution of drug. The head of pressure at the upper end of the intestinal segment was 7 cm and at the lower end was 21 cm of water. All experiments lasted 75 min. At the end of each experiment, volumes of mucosal and serosal fluids were noted and suitable aliquots analysed for content of drugs.

Everted sac method of Crane & Wilson (1958). These authors used a simple everted sac technique incorporating features of several earlier methods, for the study of intestinal absorption of sugars. Because of its simplicity, we have examined its suitability for studying the permeability characteristics of the drug.

The experiment was similar to that of Crane & Wilson (1958). The sac was everted according to Wilson & Wiseman (1954). 10 ml of Krebs Ringer bicarbonate saline with a glucose concentration of 5 mg/ml was placed in the outer glass jacket and 1 ml of Ringer solution in the everted sac. After 5–10 min of oxygenation in a thermostat at 37°, the sac relaxed and was filled with Ringer. The volume of fluid in the cannula was sufficient to give about 1–2 cm of fluid above that in the outer jacket. The sac was then transferred to another glass jacket containing a known concentration of drug dissolved in 10 ml of Krebs Ringer bicarbonate saline containing glucose. The gas mixture was allowed to bubble at a moderate rate. The duration of the experiment was 40 min. With glutethimide-^{14}C and thalidomide-^{14}C, aliquots of 0·1 ml fluid from serosal side were withdrawn at various time intervals for counting the radioactivity. In other instances, the serosal and mucosal fluids were removed, their volumes recorded and suitable aliquots analysed for drug content.

ESTIMATIONS

Phenol red and salicylic acid were estimated by the method of Schanker, Shore, Brodie & Hogben (1957), aniline by Bratton & Marshall's method

(1939), ferrioxamines and deferrioxamine by Tripod & Keberle's method (1962), non-labelled glutethimide by the method of Goldbaum & Williams (1960) and labelled glutethimide and thalidomide by liquid scintillation counting. Due to low solubility of thalidomide in Ringer solution, only a small concentration was used. For the estimation of Val^5-angiotensin-Asp-β-amide, the mucosal and serosal fluids from the experiments were immediately immersed in hot water at 95° for about 3 min to check enzymatic cleavage; suitable aliquots of the fluid were then freeze-dried and the residue extracted repeatedly with n-butanol saturated with water. The residue from butanol extracts was dissolved in a known volume of distilled water for paper chromatography. Aliquots of angiotensin amide solution containing known concentrations were extracted concurrently and worked up by the above procedure to serve as standard references for paper chromatography.

Results and discussion

The results of investigations using Smyth & Taylor's method are presented in Tables 1 and 2. The data on diffusion of phenol red, salicylic acid and aniline show a parallel with *in vivo* absorption data of Schanker & others (1957). Phenol red is known not to be absorbed

TABLE 1. TRANSPORT OF DRUGS BY *in vitro* RAT INTESTINAL PREPARATION (SMITH & TAYLOR, 1957)

Compound	Conc. used (µg/ml)	Vol. of serosal fluid (ml)	Vol. of mucosal fluid (ml)	Ratio of serosal to mucosal conc. per ml
Phenol red	200	4·2	19·6	0·164
Salicylic acid	400	3·0	19·4	1·086
		2·2	21·2	1·081
Aniline	408·8	2·2	19·8	1·070
		1·6	21·0	0·980
Ferrioxamine-B hydrochloride[1]	1600	2·4	20·0	0·200
		3·5	19·5	0·100
		2·3	19·0	0·310
N-Acetylferrioxamine-B[2]	1600	3·6	20·0	0·177
		2·5	20·5	0·242
N-Valerylferrioxamine-B[3]	1600	2·7	20·6	0·200
		5·1	17·2	0·070
N-Benzoylferrioxamine-B[4]	1600	3·3	20·8	0·157
		2·3	19·6	0·384
		2·9	20·6	0·150
N-[2-(*p*-Ethoxyphenyl)-acetyl] ferrioxamine-B[5]	1600	3·3	19·8	0·184
		3·2	20·2	0·256
Deferrioxamine	1600	2·3	20·2	0·180
		1·7	20·0	0·460
		3·8	19·5	0·110
Glutethimide	133·3	2·5	19·0	0·830
	95	2·5	17·4	0·846
Val^5-angiotensin-Asp-β-amide	2000	3·0	20·0	—
		2·5	17·5	

Water/chloroform ratios: [1] >99 : <1; [2] 98 : 2; [3] 58 : 42; [4] 36 : 64; [5] 7 : 93.

TABLE 2. DIFFUSION OF GLUTETHIMIDE-^{14}C AND THALIDOMIDE-^{14}C THROUGH RAT *in vitro* INTESTINAL PREPARATION (SMITH & TAYLOR, 1957)

Compound	Time of sampling of serosal fluid (min)	Vol. of serosal fluid (ml)	Vol. of mucosal fluid at end of experiment (ml)	Ratio of radioactivity in serosal to that in mucosal fluid
Glutethimide-^{14}C ..	20	0·40	18·5	0·62
	40	0·75		0·83
	60	0·90		0·70
	75	0·80		0·99
Thalidomide-^{14}C ..	25	0·65	18·5	0·14
	40	0·75		0·64
	60	0·90		0·58
	75	0·75		0·64

through the intestine *in vivo*. Ferrioxamines of increasing lipid solubility and deferrioxamine showed a ratio of serosal to mucosal concentration in the range 0·1–0·3 and would not therefore be taken to be significantly absorbed. The pharmacological and clinical *in vivo* data with ferrioxamine-B and deferrioxamine bear out this point. A striking contrast is noticeable with salicylic acid and aniline, both of which are known to be well absorbed through rat intestine *in vivo*. In these cases the ratio of concentration in serosal to that in mucosal fluid approached 1·0 to 1·1. These values are comparable with the ratio of 0·85 obtained with non-labelled glutethimide which is also known to be well absorbed *in vivo*. From the comparative data on rate of diffusion of glutethimide-^{14}C and thalidomide-^{14}C across the intestinal barrier, it is evident that glutethimide-^{14}C crosses the barrier faster than thalidomide-^{14}C for the duration of the experiment. The greater lipid-solubility of glutethimide compared to thalidomide may be a factor contributing to this effect. The effect of probable degradation of thalidomide to other products by intestinal mucosal cells has not been assessed in this study. The increasing lipid solubility with ferrioxamine derivatives did not seem to have any significant effect in promoting their passage through intestinal barrier *in vitro*. With Val^5-angiotensin-Asp-β-amide after 2 min circulation of Ringer saline containing drug, a marked degradation could be seen from the paper chromatography of an aliquot of mucosal fluid worked up according to the stated procedure, but angiotensin amide could still be detected, though in a much reduced concentration. However, the circulation of drug solution through intestinal segments for 1 hr led to disappearance of all angiotensin amide from the mucosal fluid, only degraded peptides or amino-acids being detected by paper chromatography. This was also the case with serosal fluid. No attempt was made to identify the degradation products. Newey & Smyth (1959a) found that when dipeptides are present in the lumen of intestine, it was the constituent amino-acids which appeared in the blood stream. Newey & Smyth (1959b) further concluded that dipeptides entered the mucosal cells as peptides, were hydrolysed intracellularly and emerged as amino acids. According to Wiggans & Johnston (1959) tri- and tetra-glycyl peptides, when present on the mucosal side of the intestine, also emerged as amino-acids on the serosal side.

Barry, Mathews & Smyth (1961) have shown that energy for glucose-dependent transfer of water through the intestines came from glycolysis or the hexose monophosphate shunt and energy for glucose-independent transfer of water came from the citric acid cycle. Since the drugs passed through the intestinal barrier into water in varying proportions, there may be some kind of coupled absorption of water and drug molecules as a basis of absorption of most drugs (Fisher, 1955). In such a process the solubility of drug in the lipid material of which the membrane is composed, partition coefficients and pH gradients, would all seem to play a dominant role.

The results with the everted sac technique are in Tables 3 and 4. In this preparation phenol red and ferrioxamine-B and its progressively more

TABLE 3. PASSAGE OF SUBSTANCES FROM MUCOSAL TO SEROSAL SIDE BY EVERTED SAC TECHNIQUE

Compound	Conc. (μg/ml)	Initial and final vol. of mucosal fluid (ml)	Initial and final vol. of serosal fluid (ml)	Ratio of serosal to mucosal conc.
Phenol red	375	10, 9·75	1, 0·85	0·016
	250	10, 10	1·5, 1·5	0·050
Salicylic acid	138	10, 9·75	1, 1	0·28
	138	10, 9·75	1, 0·90	0·29
	1000	10, 9·5	1·5, 1·5	0·49
				0·38
				0·45
				0·30
Aniline	102·2	10, 9·8	1, 0·8	0·77
	670·0	10, 9·8	1·5, —	0·77
Ferrioxamine-B	650	10, 9·9	1, 0·75	0·011
N-[2-(*p*-Ethoxyphenyl)-acetyl]ferrioxamine-B	812	10, 10	1, 0·9	0·06
	2960	10, 10	1·5, 1·4	0·025
Glutethimide	217	10, 9·3	1, 1	0·60
	217	10, 9·3		0·56

TABLE 4. DIFFUSION OF GLUTETHIMIDE-^{14}C AND THALIDOMIDE-^{14}C THROUGH EVERTED INTESTINAL SAC PREPARATION

Compound	Time of sampling of serosal fluid (min)	Vol. of serosal fluid taken (ml)	Vol. of mucosal fluid after expt. (ml)	Ratio of radioactivity in serosal to that in mucosal fluid	
Glutethimide-^{14}C ..	10	0·1	9·85	0·26	
	20	0·1		0·31	
	30	0·1		0·72	
	40	0·1		0·58	
Thalidomide-^{14}C ..	10	0·1	9·0	0·11	0·31
	20	0·1		0·24	0·20
	30	0·1		0·31	0·38
	40	0·1		0·67	0·53

lipid-soluble derivatives did not pass from mucosal to serosal side of intestinal barrier and therefore would not be expected to be significantly absorbed. Salicylic acid permeated in amounts much less than those found using Smyth & Taylor's method (1957). Aniline, however, was transported well, giving a ratio of 0·77 of serosal to mucosal concentration.

Glutethimide crossed the barrier easily giving a ratio of 0·6. This observation was further strengthened by studies on the comparative rates of transfer of glutethimide-^{14}C and thalidomide-^{14}C, the results of which are given in Table 4. Glutethimide crossed the barrier faster than thalidomide. The mechanism of transfer of fluids and solutes in the everted sac preparations has been investigated by Barry & Smyth (1960) who pointed out that what appeared in serosal fluid *in vitro* using everted sac preparations was only a fraction of that transported by epithelial cells. This concept should be taken into account in the interpretation of results by such a technique. The parallelism in results, however, between this method and that of Smyth & Taylor (1957), suggests that these methods can supply useful information on the permeability characteristics of newly-developed drugs.

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