steroid hormone solubilization is supported by the similarity between the thermodynamic parameters found in this study and those from octanol–water partitioning (8).

Although the flickering cluster hypothesis seems plausible, and the water ordering–disordering is important for the entropy change of solubilization, other factors may be significant. It was proposed (10) that the apolar solute molecule is held rigidly in a favored rotational configuration in the aqueous phase by the layer of water molecules surrounding it. In the micelle hydrocarbon core, its rotational oscillations are relatively unrestricted. This proposal is supported by the fact that there is no clearcut correlation between water solubility and ΔS_s°. Ethisterone has a much lower water solubility than testosterone [1.6 against 68.7 μmoles/liter at 20° (8)] and can be regarded as more hydrophobic. According to the flickering cluster hypothesis, ethisterone should have more structured water around it and should give a more positive ΔS_s° at the randomization of the water molecules. Thus, both the water and solute effects probably contribute to the effective ΔS_s°, but which is quantitatively the most important cannot be determined.

The contribution of the enthalpy change to the free energy change of solubilization is quantitatively minor for most steroids. Hence, there are interesting differences in their ΔH_s° values (Table III). The most notable difference is the rather large positive value of progesterone in polysorbate 40. Because similar ΔH_s° values can be expected for steroids solubilized by the same mechanism, the differences obtained indicate variations in that respect.

The simultaneous solubilization of steroids cannot be predicted by their free energies of solubilization (2). Testosterone and progesterone have less negative ΔH_s° values than ethinyl estradiol but are solubilized maximally in polysorbate 40, while the latter steroid has a lower solubility at simultaneous solubilization. These discrepancies may be explained by differences in the solubilization mechanism. If the solubilization loci of the steroids partly overlap, a steric hindrance to simultaneous solubilization will exist. When solubilized on its own in tetradecyltrimethylammonium bromide, ethinyl estradiol has a considerably larger ΔS_s° than progesterone, but the opposite is true at simultaneous solubilization (Tables III and IV).

On the contrary, progesterone and estradiol are solubilized independently of each other in polysorbate 40, and the ΔS_s° values of both decrease to about the same extent when the steroids are solubilized together. Also, the solubilizations of both testosterone and estradiol are changed at simultaneous solubilization in tetradecyltrimethylammonium bromide, and their ΔS_s° values are both lowered when the steroids are solubilized together. However, at this stage it is hard to rationalize these results in terms of the precise structure and ordering of the micelle and the solute in it.

Of course, the thermodynamic treatment in this study has limitations. Although the amount of solubilizate bound in a micellar system can be measured with accuracy, the calculation of C_s^m presents problems because it is a concentration term, and precise delineation of the micellar pseudophase boundary is difficult. Two other difficult aspects of the thermodynamic calculations regarding micellar binding are the selection of the standard state for the micellar cosolute and the incorporation of the appropriate activity corrections (11). For cosolutes with low water solubility such as steroid hormones, the C_s^{aq} term probably will approximate the activity value. However, the micellar solubilizate activity coefficients may differ significantly from unity. This deviation will introduce errors into the calculations of thermodynamic parameters derived from micellar binding equilibrium constants.

Although these limitations exist, data from studies of the temperature effect on solubilization processes can prove useful in expanding knowledge of this important branch of surface chemistry. Clearly, further studies of the effect of solutes on micelle structure are required before a theory rationalizing micellar solubilization can be formulated.

REFERENCES

(1) T. Lövgren, B. Heikius, B. Lundberg, and L. Sjöblom, *J. Pharm. Sci.*, **67**, 1419 (1978).

(2) B. Lundberg, T. Lövgren, and B. Heikius, *ibid.*, **68**, 542 (1979).

(3) D. Attwood and A. T. Florence, *Kolloid Z. Z. Polym.*, **246**, 580 (1971).

(4) C. Tanford, *J. Phys. Chem.*, **78**, 2469 (1974).

(5) D. Stigter, *J. Colloid Interface Sci.*, **47**, 473 (1974).

(6) K. J. Humphreys and C. T. Rhodes, *J. Pharm. Sci.*, **57**, 79 (1968).

(7) K. J. Simons and C. T. Rhodes, *Pharmazie*, **26**, 623 (1971).

(8) B. Lundberg, *Acta Pharm. Suec.*, **16**, 151 (1979).

(9) G. Nemethy, H. A. Scheraga, and W. Kauzmann, *J. Phys. Chem.*, **72**, 1842 (1968).

(10) R. H. Aranow and L. Witten, *ibid.*, **64**, 1643 (1960)

(11) P. Molyneux and C. T. Rhodes, *Kolloid Z. Z. Polym.*, **250**, 886 (1972).

Tetrazolium Salts in Pharmaceutical Analysis II: Direct Assay of Diethylstilbestrol and Diethylstilbestrol Dipropionate

NABIL M. OMAR ˣ and NAWAL A. EL-RABBAT

Received November 24, 1978, from the *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Assiut, Assiut, Egypt.* Accepted for publication July 10, 1979.

Abstract □ A convenient spectrophotometric determination of diethylstilbestrol and diethylstilbestrol dipropionate was developed involving their interaction with triphenyltetrazolium chloride at 50° for 45 min and subsequent measurement of the formazan formed. The significance of extended conjugation within the 4,4′-stilbenediol molecule to induce the color reaction is documented. Ideal adherence of color absorption to Beer's law permitted accurate and precise determination of diethylstilbestrol and diethylstilbestrol dipropionate pure forms over the range of 2–22 μg of diethylstilbestrol/ml. Application of the tetrazolium color reaction to the analysis of diethylstilbestrol dipropionate dosage forms was achieved without prior hydrolysis or extraction.

Keyphrases □ Diethylstilbestrol and diethylstilbestrol dipropionate—analysis, triphenyltetrazolium chloride colorimetry □ Colorimetry—analysis of diethylstilbestrol and diethylstilbestrol dipropionate with triphenyltetrazolium chloride □ Tetrazolium salts—colorimetric analysis of diethylstilbestrol and diethylstilbestrol dipropionate

The usual relatively small doses of diethylstilbestrol [(*E*)-α,α′-diethyl-4,4′-stilbenediol] require especially sensitive and precise pharmaceutical analysis. The phenolic hydroxyl group reactivity of this stilbene derivative has been used to develop diverse estimation procedures based on acetylation (1, 2), nitrosation (3), polarography of the nitrosation product (4), bromination (5), and UV irradiation (6).

Of the chromogenic reagents reported for interaction with phenols, interest has focused on the utility of phosphomolybdotungstate (7), iron (8), antimony (9), and vanadium (10) salts for diethylstilbestrol colorimetry. Some

0022-3549/80/0100-0024$01.00/0

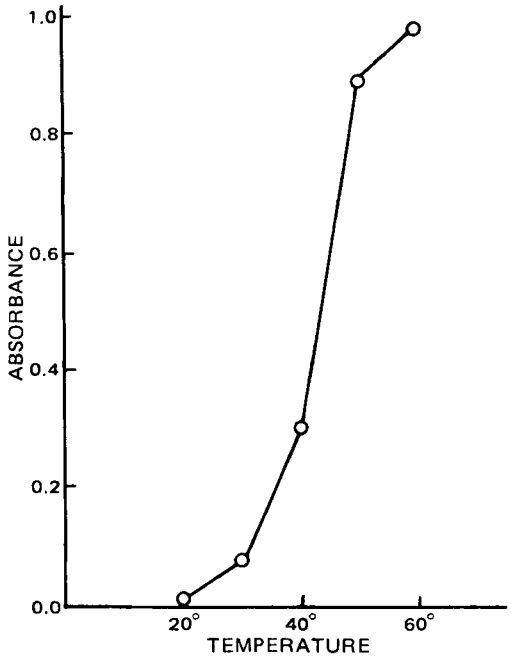

Figure 1—*Temperature effect on color development using 20 μg of diethylstilbestrol/ml, 45 min.*

of these methods lack specificity or sensitivity, and they often are subject to limitations. The compendial UV-irradiation procedures (11, 12), although apparently specific for diethylstilbestrol, may be tedious and time consuming, especially when oily formulations of diethylstilbestrol dipropionate are analyzed. Essential preliminary hydrolysis to diethylstilbestrol and its subsequent extraction might interfere with the accurate estimation of this ester.

Triphenyltetrazolium chloride (I) can be reduced to the corresponding highly colored formazan derivative by various dihydroxybenzene derivatives (13–15). This reduction offered a basis for investigating possible interactions of I with a different aromatic diol system such as 4,4′-stilbenediol, with the hope of introducing a new formazan-based colorimetric analysis of diethylstilbestrol and its dipropionate ester.

EXPERIMENTAL

Instrumentation—A double-beam spectrophotometer[1], a pH meter[2] fitted with calomel and glass electrodes, and a suitable thermostated[3] water bath were used.

Materials—Pharmaceutical grade diethylstilbestrol and diethylstilbestrol dipropionate were the working standards. Ethyl oleate, peanut oil, and sesame oil were chemically pure; other chemicals were analytically pure. As dosage forms, commercially marketed tablets[4,5] and parenteral solutions[6–9] of diethylstilbestrol and diethylstilbestrol dipropionate were analyzed.

[1] Spektromon-203, MOM, Budapest, Hungary.
[2] Radelkis OP-401/2, Budapest, Hungary.
[3] T-606.MTA, Budapest, Hungary.
[4] Stilbestrol (Misr Co. for Pharmaceuticals, Cairo, Egypt) contains 5.0 mg of diethylstilbestrol/tablet.
[5] Stilbestrol Dipropionate (G. Richter, Budapest, Hungary) contains 1.0 mg of diethylstilbestrol dipropionate/tablet.
[6] Stilbestrol (Misr Co. for Pharmaceuticals, Cairo, Egypt) contains 1.0 mg of diethylstilbestrol/1-ml ampul.
[7] Stilbestrol Dipropionate (G. Richter, Budapest, Hungary) contains 5.0 mg of diethylstilbestrol dipropionate/1-ml ampul.
[8] Stilbestrol Dipropionate (Evans, Bristol, England) contains 10.0 mg of diethylstilbestrol dipropionate/1-ml solution in ethyl oleate.
[9] Stilbestrol Dipropionate (Bayer, Liverkühsen, Germany) contains 5.0 mg of diethylstilbestrol dipropionate/1-ml solution in sesame oil.

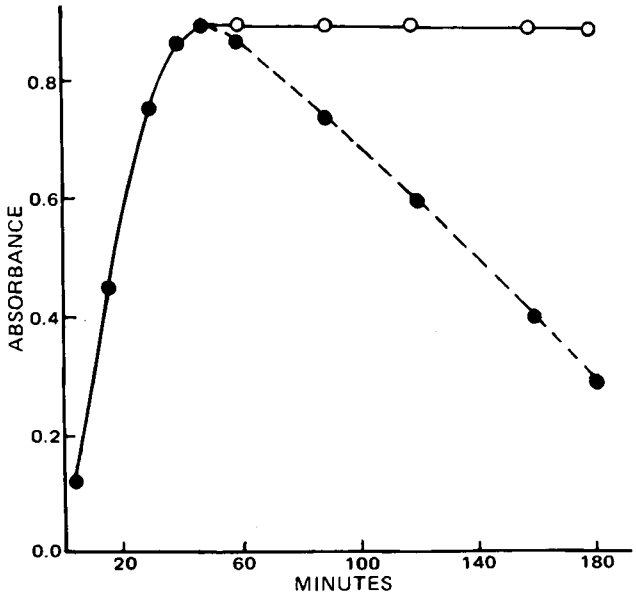

Figure 2—*Diethylstilbestrol–tetrazolium color–time curve using 20 μg of diethylstilbestrol/ml at 50° (●) and after cooling to room temperature (○).*

Reagents—*Tetrazolium Solution*—Compound I was diluted to 0.5% (w/v) in aldehyde-free ethanol[10]. This solution was kept in the dark.

Potassium Hydroxide Solution—Carbonate-free potassium hydroxide, 0.1 g, was dissolved in ~2 ml of distilled water and diluted to 100 ml with anhydrous ethanol. A fresh solution was prepared every 48 hr.

Standards—An accurately weighed amount of diethylstilbestrol or diethylstilbestrol dipropionate, previously dried at 80° *in vacuo* for 2 hr, was dissolved in anhydrous ethanol to a final concentration of 200 μg of diethylstilbestrol/ml.

Assay Samples—*Tablets*—Not less than 20 tablets were ground to a fine powder. An accurately weighed powder sample, equivalent to ~5 mg of diethylstilbestrol, was transferred to a 50-ml volumetric flask. Then 25 ml of anhydrous ethanol was added, and the solution was allowed to stand for 30 min with frequent shaking. The solution was diluted to volume with anhydrous ethanol, mixed well, and filtered through a dry filter into a dry flask. The first portions of the filtrate were discarded.

Injections and Solutions—A 1-ml precision syringe was used to transfer 1.0 ml of the injection solution into a suitable volumetric flask. The syringe was rinsed with 20% (v/v) *n*-heptane in ethanol; the rinses were collected in the flask and diluted quantitatively and stepwise with heptane–alcohol to obtain ~100–140 μg of the claimed diethylstilbestrol/ml.

Procedure—A 1.0-ml sample of the standard or of the sample was pipetted into a 10-ml volumetric flask containing 3.0 ml of the tetrazolium solution and 1.0 ml of the potassium hydroxide assay solution. The sample was mixed well, stoppered, and allowed to stand in the dark in a thermostated water bath at 50 ± 0.1° for 45 min. The red-orange reaction mixture was cooled and brought to volume with ethanol. The absorbance of this solution was measured in a 1-cm glass cell at 485 nm *versus* a blank prepared from 1.0 ml of ethanol (for tablets) or from *n*-heptane–ethanol (for injections) and treated as for the assay solution (heated at 50° for 45 min).

RESULTS AND DISCUSSION

Tetrazolium–Stilbenediol Interaction—Tetrazolium salts were considered for use in pharmaceutical colorimetric analysis because they yield highly colored formazans upon reduction (14–21). The tetrazolium cation may oxidize the enediol function with appreciable selectivity since only the 1,2- and 1,4-dihydroxybenzene derivatives induce blue tetrazolium reduction (13).

Reaction of the following phenols with I in 0.1% KOH at 20° for 30 min also gave no color with resorcinol and phloroglucinol, while a moderate to intense color was observed with pyrocatechol, pyrogallol, and hydroquinone. The 4,4′-stilbenediol reaction mixture became faint orange-red, but the color was augmented by the application of heat. Neither dihy-

[10] Aldehyde-free spectrograde (Prolabo, France).

Table I—Effect of Relative Reagent Concentrations on the Diethylstilbestrol–Tetrazolium Reaction Rate

Milliliters Added per 10 ml of Assay Solution[a]		
0.5% Tetrazolium Chloride	0.1% KOH	Absorbance, 485 nm
1.0	0.50	0.210
2.0	0.50	0.315
3.0	0.50	0.385
1.0	1.00	0.125
2.0	1.00	0.375
3.0	1.00	0.475[b]
4.0	1.00	0.470[c]
2.0	1.50	0.280
3.0	1.50	0.330
4.0	1.50	0.425

[a] Containing 10 μg of diethylstilbestrol/ml. [b] pH 10.50. [c] pH 9.35.

Table II—Replicate Analyses of Diethylstilbestrol Standard Solutions

Replication[a]	Absorbance, 485 nm
1	0.471
2	0.473
3	0.474
4	0.474
5	0.472
6	0.474
7	0.475
8	0.472
Average	0.473
SD	$\pm 1.3562 \times 10^{-3}$
RSD	2.867×10^{-3}

[a] Containing 10.0 μg of diethylstilbestrol/ml.

drostilbestrol[11] nor *trans*-stilbene induced formazan development under these conditions. These findings, although mostly qualitative, underline the possible reduction of tetrazolium salts by aromatic diols with widely separated enediol functions when the latter are conjugated properly with the carrier aromatic nuclei. This possibility encouraged further investigation of I for use in the photometric analysis of pharmaceutical 4,4′-stilbenediol derivatives.

Assay—Interaction of diethylstilbestrol with I proceeded analogously to 4,4′-stilbenediol; heating of the reaction mixture gradually intensified the color. The formazan absorbed with minimum and maximum extinctions at 408 and 485 nm, respectively, consistent with the absorption of triphenylformazan (17, 19, 22).

For the diethylstilbestrol concentration range studied, appropriate absorptivity readings were attained at 50–60° (Fig. 1). However, at >50°, blank solutions acquired a red tinge that could lower the sensitivity of the diethylstilbestrol–tetrazolium interaction. Accordingly, 50° was a convenient temperature because it combined a reasonable heating time with the production of only a faintly colored blank.

The color–time curve (Fig. 2) revealed maximum formazan formation when the reaction mixture was at 50° for 45–50 min; heating for longer periods seriously lowered color absorptivity. Interruption of the reaction after 45 min by efficient cooling to ambient temperature was sufficient to achieve good sensitivity. The formazan remained stable for >2 hr when kept in the dark. Under these conditions, maximum formazan development was effected by the addition of 1.0 ml of the working diethylstilbestrol solution to 3–4 ml of I in the presence of 1.0 ml of 0.1% KOH (Table I).

Quantitative Analysis—At fixed experimental conditions, the formazan absorption intensity was a function of the diethylstilbestrol concentration. Linear regression analysis of a Beer's plot at 485 nm revealed excellent adherence ($r = 0.9994$), with a slope (α) of 0.0465 ($\pm 1.08 \times 10^{-3}$) and an upper sensitivity limit of ~22 μg ($A = 1.02$) of diethylstilbestrol/ml. Replicate analyses of diethylstilbestrol working solutions (Table II) were fairly precise ($RSD = 2.811 \times 10^{-3}$). Recovery studies at different diethylstilbestrol concentrations (Table III) afforded a mean recovery of 99.45 ± 0.93%.

Since I could be used successfully for the photometric estimation of beclomethasone dipropionate[12] without prior hydrolysis (12, p. 44), a similar interaction for diethylstilbestrol dipropionate was considered.

[11] Hexestrol.
[12] Propaderm.

Table III—Recovery of Standard Diethylstilbestrol Solutions

Sample	Diethylstilbestrol, μg/ml Calculated[a]	Found[b]	Recovery, %
1	10.0	9.78	97.80
2	25.0	24.96	99.84
3	50.0	50.01	100.02
4	100.0	99.84	99.84
5	200.0	199.55	99.78
Mean			99.45
SD			±0.93
RSD			9.35×10^{-3}

[a] Initial concentration. [b] Average of five assays.

Table IV—Analysis of Diethylstilbestrol and Diethylstilbestrol Dipropionate Dosage Forms

Preparation[a]	Content, mg/unit Label Claim	Found[b]	Added	Recovered
Diethylstilbestrol				
Tablets	5.0	4.96	10.0	14.94
Injection	1.0	0.98	25.0	26.00
Diethylstilbestrol dipropionate				
Tablets	1.0	1.10	5.0	6.11
Injection[c]	5.0	4.98	5.0	9.95
	10.0	10.05	10.0	20.00
	10.0	9.75	10.0	19.80

[a] See *Experimental* for composition. [b] Average of three determinations. [c] Found 4.75, 9.89, and 9.64, respectively, as analyzed by the NF XIV procedure.

A direct estimation of this ester has not been reported. When reacted under the standard diethylstilbestrol assay, diethylstilbestrol dipropionate produced formazan with Beer's plot r and α values of 0.9992 and 0.0328 ($\pm 1.05 \times 10^{-3}$), respectively. Comparison of the diethylstilbestrol–diethylstilbestrol dipropionate molecular weight ratio to the respective Beer's plot slopes at 485 nm confirmed quantitative *in situ* diethylstilbestrol generation during the base-catalyzed interaction of I with diethylstilbestrol dipropionate.

The color reaction was applied successfully to the analysis of diethylstilbestrol and diethylstilbestrol dipropionate tablets (Table IV). Potential interference by reducing sugars in the tablets was eliminated by alcohol extraction prior to analysis. Interference studies of some common ingredients in diethylstilbestrol dipropionate formulations revealed no reduction of I. The compounds studied included phenobarbital, tetracaine, benzocaine, sulfathiazole, sulfathiourea, and nitroglycerin. Pharmaceutical catecholamines oxidized by I are not likely to be in diethylstilbestrol dosage forms.

Direct spectrophotometric determination of oily diethylstilbestrol dipropionate dosage forms is unreliable because of the high absorptivity of the oily vehicles (1, p. 475). However, neither sesame and olive oils nor ethyl oleate showed measurable absorptivity at 485 nm when tested as 0.8% (w/v) in heptane–alcohol. In addition, no formazan was observed when such solutions were reacted with I under the standard assay conditions. These results encouraged direct estimation of diethylstilbestrol dipropionate dosage forms by the proposed tetrazolium reaction (Table IV). Concordant results were obtained when the oily dipropionate formulations were analyzed directly by I or were preliminarily freed from their vehicles by the NF XIV procedure (10). However, relatively higher values than the claimed diethylstilbestrol dipropionate contents were shown by some intense yellow batches. Correction for intrinsic absorption of such samples was made by determining their initial absorptivity at 485 nm.

REFERENCES

(1) "The Qualitative Analysis of Drugs," D. C. Garrat, Chapman-Hall, London, England, 1964, p. 470.

(2) "The Egyptian Pharmacopoeia," University Press, Cairo, Egypt, 1953, p. 257.

(3) S. Gottleib, *J. Am. Pharm. Assoc., Sci. Ed.*, **37**, 147 (1948).

(4) D. Gry, *Dan. Tidsskr. Farm.*, **23**, 139 (1949).

(5) T. T. Cocking, *Analyst*, **68**, 144 (1943).

(6) D. Banes, *J. Assoc. Off. Agr. Chem.*, **44**, 323 (1961).

(7) M. Tubis and A. Bloom, *Ind. Eng. Chem., Anal. Ed.*, **14**, 309 (1942).

(8) J. D. Duerr and B. A. Pappas, *J. Am. Pharm. Assoc., Sci. Ed.*, **48**,

13 (1959).
(9) F. L. Warren, F. Goulden, and A. M. Robinson, *Biochem. J.*, **42**, 151 (1948).
(10) "The National Formulary," 14th ed., Mack Publishing Co., Easton, Pa., 1975, p. 205.
(11) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, p. 142.
(12) "British Pharmacopoeia 1973," University Printing House, Cambridge, England, 1973, p. 443.
(13) J. E. Sinsheimer and E. F. Salim, *J. Anal. Chem.*, **37**, 566 (1965).
(14) E. F. Salim, P. E. Manni, and J. E. Sinsheimer, *J. Pharm. Sci.*, **53**, 391 (1964).
(15) N. M. Omar and N. A. El-Rabbat, *ibid.*, **67**, 779 (1978).
(16) R. Kuhn and D. Jerchel, *Ber.*, **74**, 949 (1941).
(17) W. J. Mader and R. R. Buck, *Anal. Chem.*, **24**, 666 (1952).
(18) D. Banes, *J. Am. Pharm. Assoc., Sci. Ed.*, **42**, 669 (1953).
(19) A. M. Mattson and C. O. Jensen, *Anal. Chem.*, **22**, 182 (1950).
(20) H. M. Hashmi, A. S. Adel, A. V. Viegas, and I. A. Ahmad, *Mikrochim. Acta*, **3**, 457 (1970).
(21) P. S. Vassileva-Alexandrova and P. S. Shismanor, *Anal. Chem.*, **47**, 1432 (1975).
(22) R. E. Graham, E. R. Biehl, and C. T. Kenner, *J. Pharm. Sci.*, **66**, 965 (1977).

Tissue Distribution and Metabolism of Drugs V: Effect of Secretin and Pancreozymin on Drug Transport in Rabbit Pancreas

MASATO ARAKAWA *§, KATSUHIKO OKUMURA *, and RYOHEI HORI‡x

Received April 10, 1979, from the **Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, Hiroshima 734, Japan,* and the ‡*Department of Pharmacy, Kyoto University Hospital, Sakyo-ku, Kyoto 606, Japan.* Accepted for publication July 16, 1979. §Present address: General Research Center, Wakunaga Pharmaceutical Co. Ltd., Kotachi, Hiroshima, Japan.

Abstract □ The effect of secretin and pancreozymin on the tissue distribution and penetration of drugs in the rabbit pancreas was studied to clarify hormonally regulated drug distribution. Drugs with high liquid solubility were distributed easily within the pancreas even during secretin or pancreozymin treatment, and these hormones had little effect on drug distribution from the blood to the pancreas. However, secretin increased the concentration ratio of dimethadione in the pancreatic juice (J) to plasma unbound dimethadione (Pf), probably because the pancreatic juice during secretin infusion is alkaline relative to the control. Secretin had no effect on the J/Pf of isonicotinamide and sulfanilamide. Secretin decreased the J/Pf of drugs with low lipophilicity or large molecular weight because the penetration rates of these drugs from cell water to pancreatic juice were not rapid enough to reach equilibrium. Pancreozymin was unable to change the J/Pf of any drug tested. These results suggest that the barrier between the blood and the pancreas or the barrier between the pancreas and the pancreatic juice is unchanged by secretin or pancreozymin.

Keyphrases □ Pancreas—drug transport, various drugs, effect of secretin, pancreozymin, pancreatic juice flow □ Pancreatic juice—effect of flow rate on pancreatic drug transport, various drugs □ Drug transport—pancreas, effect of secretin, pancreozymin, pancreatic juice flow, various drugs

Drug distribution has received increasing attention during recent years because such knowledge concerning the blood, organs, and tissues is needed to provide optimal treatment or protection from adverse reactions. Previous papers reported tissue distribution and penetration of drugs in the pancreas (1), lungs, (2, 3), and testes (4).

It was suggested that the lipid barrier in the pancreas plays a dominant role in drug distribution from the blood to the pancreas and that the lipid barrier and the molecular sieve barrier have independent roles in transport from the pancreas to the pancreatic juice. In these experiments, pancreatic juice secretion was stimulated by secretin infusion to maintain a constant juice flow. However, the pancreatic barriers could be changed by endogenous hormones that regulate pancreatic secretion during food digestion.

The purpose of this study was to determine experimentally whether secretin and pancreozymin, typical peptide hormones that stimulate pancreatic juice flow and bicarbonate or enzyme secretion, affect drug distribution and penetration in the pancreas. Some model drugs that exhibit remarkable lipophilicity, molecular size, and pKa were selected as discussed previously (1).

EXPERIMENTAL

Materials—Secretin[1], 3110 CHR U[2]/mg, was used. Pancreozymin[3], dimethadione, isonicotinamide, isonicotinic acid, sulfanilamide, sulfanilic acid, sulfisoxazole, and procainamide hydrochloride were obtained commercially. All other chemicals were analytical grade.

Animals—Male white rabbits, 2.0–3.0 kg, were housed in constant environment rooms and allowed free access to water and food.

Drug Permeation from Blood to Pancreatic Juice—The experimental procedures were almost identical to those described previously (1). Rabbits were anesthetized with pentobarbital sodium (27 mg/kg iv). Pancreatic juice was collected by cannulation into the pancreatic duct as described in the literature (5). Plasma drug concentrations were established and maintained by a suitable combination of priming injections and continuous intravenous infusion.

As the control, pancreatic juice was collected over 75 min prior to the administration of secretin or pancreozymin. The secretin effect was studied by a priming injection (1 CHR U/kg) and continuous intravenous infusion (2 CHR U/kg/hr), after which the pancreatic juice flow was stimulated from 14 (7–24 μl/min) to 39 (15–66 μl/min) μl/min. Pancreozymin also was studied using a priming injection (1 CHR U/kg) and continuous infusion (4 CHR U/kg/hr)[4]. In this experiment, secretin also was infused simultaneously to minimize the effect of secretin contamination in the pancreozymin preparation.

Drug Distribution—The pancreas was removed and homogenized at the end of each permeation experiment, and drug concentrations in the pancreas and blood were measured to determine the distribution ratio.

Analytical Methods—Drug concentrations in the plasma, plasma ultrafiltrate, pancreatic juice, and pancreas homogenate were determined

[1] Supplied by Eisai Co. Ltd., Tokyo, Japan.
[2] Crick, Harper and Raper Unit.
[3] Boots Pure Drug Co. Ltd., Nottingham, England.
[4] Since pancreozymin is less stable than secretin, a higher dose was used.

0022-3549/80/0100-0027$01.00/0