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Correlation between Lipid Partition Coefficients and Surface Permeation in *Schistosoma japonicum*

Eain M. Cornford

Southwest Regional Veterans Administration Epilepsy Center, Veterans Administration Medical Center, Wadsworth, Research Service, Veterans Administration Medical Center, Brentwood, Los Angeles, California 90073, and
Department of Neurology, University of California, Los Angeles, Los Angeles, California 90024

Summary. Comparison of transintegumental membrane permeability and partition coefficients of selected nonelectrolytes was attempted to correlate the parameters of lipid solubility, hydrophilicity, and membrane permeation in male and female schistosomes (parasites of the portal venous tributaries of man). Surface permation (measured by the triple isotope technique) and octanol/water partition coefficients were determined for 17 compounds (acetamide, aminopyrine, antipyrine, benzyl alcohol, butanol, caffeine, ethanol, ethylene glycol, glycerol, inosine, mannitol, methanol, polyethylene glycol, propylene glycol, sucrose, thiourea, and urea).

Linear regression analyses comparing the logarithm of the partition coefficient to transintegumental uptakes indicate a positive correlation in both sexes: $R=0.76$ ($P<0.001$) for males, and $R=0.77$ ($P<0.001$) for females. Similarly, linear regression analyses comparing hydrogen bond number with the logarithm of tissue uptake index demonstrate a high (negative) correlation in both males ($R=-0.85$, $P<0.001$) and females ($R=-0.90$, $P<0.001$). The male and female schistosomes showed no statistically significant differences in correlation of these parameters. Surface permeation was the same in male and female schistosomes, suggesting that male-female variations in integumental uptake rates previously observed may be restricted to metabolites which enter by way of a selective carrier system.

Key words schistosomal membrane permeability · partition coefficients · hydrogen bond number · syncytial integument

Introduction

Partition coefficients, in systems such as octanol-water or olive oil-water, have been used frequently for characterization of lipophilic properties of drugs. The manner in which a solute equilibrates between aqueous and nonaqueous phases has been found useful in quantifying substituent effects for linear free energy relationships, which form the basis of quantitative structure-activity relationships employed widely in drug design. Partition coefficients for a large group of nonelectrolytes have been derived semiempirically from chemical structures and melting points in a recent study emphasizing the relationships between changing chemical structure (biotransformation) and concomitant alterations in solubility (Yalkowsky & Valvani, 1980). A discussion of the use of partition coefficients in this field may found in the recent review of Hansch and Leo (1979).

In the present study, transintegumental uptakes of some seventeen different molecules have been measured in *Schistosoma japonicum* males and females in an attempt to correlated partition coefficients with penetration of the living schistosomal surface membrane. The rationale for this specific investigation may be attributed to certain unique features of the membrane system. The integumentary surfaces of the male and female schistosome are characterized as a syncytial, rather than epithelial structure, and recent studies suggest male and female worms possess functionally different surfaces (Cornford & Huot, 1981). The external boundary may not be the typical bilaminate unit membrane, but a pentalaminate (Smith, Reynolds & von Lichtenberg, 1969) or heptalaminate (Hockley & McLaren 1973a) membrane. It has also been described as a continuous border of stacked unit membranes (Lumsden, 1975). This surface furthermore represents the host-parasite interface, and schistosomes escape recognition by the host immune system by maintaining host antigens on this surface by (nonspecific) passive adsorption (Smithers, Terry & Hockley, 1969), specific absorption (Kemp et al., 1977), and constitutive host-like antigens ("molecular mimicry"; Damian, 1979). Thus the barrier properties of the integument cannot be presumed to be identical to typical vertebrate unit membrane cell surfaces.

Ultrastructural studies of the surfaces of *S. japonicum* males and females (Sakamoto & Ishi, 1977; Voge, Price & Jansma, 1978) indicate sex-specific differences; the surface of the female is smoother and less irregular than that of the male (Sakamoto & Ishi, 1977). For this reason, male and female

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uptakes have been measured independently, employing a method which permits estimation of that proportion of test isotope which may be passively carried within the folds of the integumentary surface (Cornford & Oldendorf, 1979).

Materials and Methods

A Japanese strain of *Schistosoma japonicum* used for this study was obtained from the Lowell Research Foundation (Lowell, Mass.). Schistosomes were removed from the portal veins of Swiss-Webster mice (which had been exposed to about 40 cercariae) by perfusion of the liver with 37 °C Hanks balanced salt solution (20 mM Hepes buffer) using the method of Duvall and DeWitt (1967). The wet weight of male schistosomes ranged from 661–1394 µg and 392–698 µg for females. Schistosomes were washed with and maintained in RPMI # 1640 (Gibco, Santa Clara, Calif.), buffered with 20 mM Hepes (Sigma Chemical Company, St. Louis, Mo.), pH 7.4–7.5, at 37 °C until they were incubated in the isotope-containing media. The wet weight of male schistosomes ranged from 661–1394 µg and 392–738 µg for females.

Octanol/Saline Partition Coefficients

Although olive oil/water partition coefficients (*PC*) correlate well with the octanol/water system (Leo, Hansch & Elkins, 1971) according to the relationship $\log PC$ (octanol) $= 1.122 + 0.857 \log PC$ (olive oil), an octanol/buffered saline partition systems was chosen rather than olive/oil/water because of the variability in samples of olive oil. An ether/water partition system was not selected because a saturated water solution contains 6% ether at room temperature and the ether phase contains 1% water (Windholtz, 1976).

Partition coefficients were determined for each compound. About 1–2 µCi of the test compound was added to 6 ml of schistosome saline. The 6 ml of labeled saline was then added with a needle and syringe to an evacuated glass test tube with a rubber septum (Vacutainer, Becton-Dickinson, Rutherford, N.J.). An equal volume of *n*-octanol (octyl alcohol, J.T. Baker Chemical Company, Phillipsburg, N.J.) was added and the tube vortexed for one min at high speed and then centrifuged for 10 min at approximately $2000 \times g$ to separate the oil and saline phases. A sample of 100 µl was taken from the oil phase and the test tube gently inverted, centrifuged again, and the saline phase sampled (100 µl) and prepared for scintillation counting as described below. For each test compound, the first octanol/saline partition was discarded routinely as a wash because large variations tended to occur in the initial washings. The phase containing the greatest quantity (cpm) of the compound being partitioned was then removed and added to a fresh aliquot of the opposite phase, vortexed, centrifuged, and sampled as above. This procedure was performed on duplicate tubes and repeated until the partition coefficient had stabilized (typically two or three washes). The partition coefficient was then determined as follows:

$$\text{Partition coefficient} = \frac{{}^{14}\text{C dpm g}^{-1}\text{ octanol}}{{}^{14}\text{C dpm g}^{-1}\text{ saline}}.$$

It is laboratory it has been determined that, for chromatographically pure radioisotopes, there is no change in the calculated partition coefficient from the 2nd, 3rd, 4th, 5th or 6th washings, but the third washing is routinely reported.

Radiochemicals

The tritiated water and ^{14}C-labeled aminopyrine, antipyrine, caffeine, ethanol, mannitol, polyethylene glycol (mol wt = 4000) and thiourea were obtained from New England Nuclear (Boston, Mass.). The ^{14}C-labeled acetamide, benzyl alcohol, ethylene glycol, glycerol, propylene glycol, and urea were obtained from Roschem (Los Angeles, Calif.), and ^{14}C-labeled inosine and sucrose from Amersham (Arlington Heights, Ill.). The specific activities of these compounds (in $\text{mCi} \times \text{mmol}^{-1}$) were: acetamide, 20; aminopyrine, 93.9; antipyrine, 52.25; benzyl alcohol, 10; caffeine, 48.96; ethanol, 4.4; ethylene glycol, 57; glycerol, 60; inosine, 250; mannitol, 50; methanol, 3.4; polyethylene glycol, 0.16; propylene glycol, 20; sucrose, 600; thiourea, 45; urea, 57.5; and water, 0.18. The ^{113m}Indium generator was purchased from New England Nuclear, Radiopharmaceuticals Division (North Billerica, Mass.). Each 1.0 cc of Indium eluted from the generator was chelated by the addition of 10 µl of sterile disodium edetate ($150 \text{ mg} \times \text{ml}^{-1}$) solution (Endrate, Abbott Laboratories, North Chicago, Ill.). This Indium-EDTA chelate solution was then titrated with 0.5 N NaOH to neutrality (about 0.1 ml) and adjusted to pH = 7.55 by addition of 100 mM Hepes buffer. Both In-EDTA and In-transferrin were used for studies involving sucrose and polyethylene glycol (two compounds which are typically excluded by membranes). In the latter instance, the ^{113m}In eluate was mixed directly with an equal volume of dialyzed normal serum. The Indium then binds to (the large serum protein) transferrin, and this mixture was neutralized and used just as the In-EDTA chelate.

Incubations

Schistosomal permeability to the various isotopes was studied in a mansonian schistosome saline (E.E. Bueding, *personal communication*) containing 92 mM NaCl, 4 mM KCl, 1.1 mM $CaCl_2$, 0.8 mM $MgCl_2$, and 20 mM Hepes (Sigma Chemical Company, St. Louis, Mo.). The incubations were carried out at a pH = 7.5 and a temperature of about 38 °C. The isotopically labeled incubation media typically contained about 1–2 µCi of ^{14}C-labeled test isotope, a tenfold greater amount of tritiated water, and 0.2 mCi of ^{113m}Indium-EDTA (prepared as described above) in a total volume of about 1.5 ml of schistosome saline.

Groups of five or six male and female schistosomes were removed from the RPMI # 1640 maintenance medium in polystyrene baskets (approximately 1 cm diameter) with fine (70 micron mesh) nylon screen (Tetko, Elmsford, N.Y.), bottoms of which allow free movement of medium in and out of the baskets. the worms and baskets were rinsed in unlabeled schistosome saline and incubated in the labeled media for 5 sec. At the end of the incubation, the worms (in the basket) were blotted quickly on an absorbent pad to remove excess medium and then covered with ice cold silicone oil, density = 0.96 (Aldrich Chemical Company, Milwaukee, Wisc.), to minimize possible evaporation of tritiated water and/or other volatile isotopes. The oil rinse additionally removes excess isotopic medium from the worm surface by a sheeting action. The schistosomes were then rapidly transferred to scintillation vials, solubilized at room temperature with an organic base (Soluene-100, Packard Instruments, Downers Grove, Ill.) and quickly prepared for scintillation counting as described below. Triplicate 20 µl aliquots of each incubation medium were taken to determine relative isotopic content.

Liquid Scintillation Counting

The digested schistosome, or the sample dissolved for partition coefficient determination, was mixed with 5 cc of scintillation fluid (Instagel, Packard Instruments, Downers Grove, Ill.), and the pH of the resulting solution was brought to near neutrality by addition of 37.5 µl of glacial acetic acid to the scintillation vial. The Indium was then counted (1-min counts) in a Packard Tri-carb 3390 scintillation counter with the windows set as described by Oldendorf and Szabo (1976). Days later, after most of the Indium ($T_{1/2} = 100$ min) had decayed, the vials were recounted for determination of ^{3}H and ^{14}C content. The net Indium counts were obtained by subtraction and appropriate decay correction (each successive vial is decay-corrected for the elapsed counting time; i.e., 1.0 min plus the time required for the machine to change samples, 0.375 min. The ^{14}C and tritium counts per minute (cpm) were then converted to disintegrations per minute (dpm) by cubic regression analysis and correction for quench as described previously (Cornford & Oldendorf, 1979).

Tissue Uptake Indices

The schistosome transintegumental influx, or Tissue Uptake Index (TUI), of the nonelectrolytes examined was determined by the equation:

$$\mathrm{TUI}(\%) = \left[\frac{^{14}\mathrm{C}/^{3}\mathrm{H\ worm}}{^{14}\mathrm{C}/^{3}\mathrm{H\ media}} - \frac{^{113m}\mathrm{In}/^{3}\mathrm{H\ worm}}{^{113m}\mathrm{In}/^{3}\mathrm{H\ media}}\right] \times 100\,\%.$$

In this equation the minuend, or the uncorrected TUI, represents the total radioactivity present both within the worm and carried on the external integument. The subtrahend, or IUI (Indium-EDTA Uptake Index), is an estimate of the test substance which is carried on the outer surface of the integument, since Indium-EDTA is excluded by membranes. The resulting subtraction yields a TUI value which has been corrected for extra-integumental isotope (Cornford & Oldendorf, 1979).

Analysis of Data

All data are presented in the form of a mean (x) and standard deviation (SD) unless otherwise specified. Linear regression analyses and other calculations were performed on a Hewlitt-Packard model 9820 programmable calculator.

Results

Partition Coefficients

Octanol/saline partition coefficients determined for 17 test compounds are presented in Table 1. The range of these partition coefficients extends from a low of 0.00003 for polyethylene glycol (the most hydrophilic compound tested) to a high of 12.53 for benzyl alcohol (the most lipophilic of the group). These compounds additionally represent a variety of molecular weights and possess variable hydrogen bonding potential. The partition coefficients could be serially ranked in the following sequence: benzyl alcohol > butanol > aminopyrine > antipyrine > caffeine > ethanol > methanol > propylene > glycol > thiourea > acetamide > ethylene glycol > urea > glycerol > inosine > mannitol > sucrose > polyethylene glycol.

Transintegumental Uptakes

The transintegumental uptakes for *S. japonicum* male and female schistosomes are indicated in Fig. 1. For both sexes the compound which showed the highest TUI was benzyl alcohol (86.5 ± 6.9 % for males, 80.5 ± 12.2 % for females). Butanol, aminopyrine, and caffeine were also high uptake (ca. 70 %) compounds,

Table 1. Octanol/saline partition coefficients

Compound	Molecular weight	Hydrogen bond number	Partition coefficient	Logarithm of partition coefficient	Octanol/ water *PC*
Benzyl alcohol	108.13	2	12.53	1.096	1.1
Butanol	74.12	2	8.50	0.929	0.89
Aminopyrine	231.29	1	7.34	0.865	0.8, 1.0
Antipyrine	188.22	1	2.003	0.302	0.28
Caffeine	194.19	2	1.036	0.0154	–
Ethanol	48.07	2	0.664	−0.1778	−0.31
Methanol	32.04	2	0.300	−0.523	−0.77
Propylene glycol	76.09	4	0.119	−0.924	−0.92, −1.7
Thiourea	76.12	5	0.112	−0.951	−1.14
Acetamide	59.07	3	0.082	−1.086	−1.15, −1.26
Ethylene glycol	62.07	4	0.046	−1.337	−1.93
Urea	26.64	5	0.029	−1.538	−2.11
Glycerol	92.09	6	0.017	−1.770	−1.76
Inosine	268.23	8	0.0083	−2.081	−2.08
Mannitol	182.17	12	0.0078	−2.108	–
Sucrose	342.3	16	0.00058	−3.24	−3.7
Polyethylene glycol	4000 (ca.)	–	0.00003	−4.5	–

Hydrogen bond numbers were determined according to Stein (1967).
Octanol/water partition coefficients (*PC*) are log values from Hansch and Leo (1979) or estimates derived from other solvent systems obtained from Dr. Corwin Hansch (*personal communication*). These constants are in good agreement with values obtained for octanol/schistosome saline partitions in the present study.

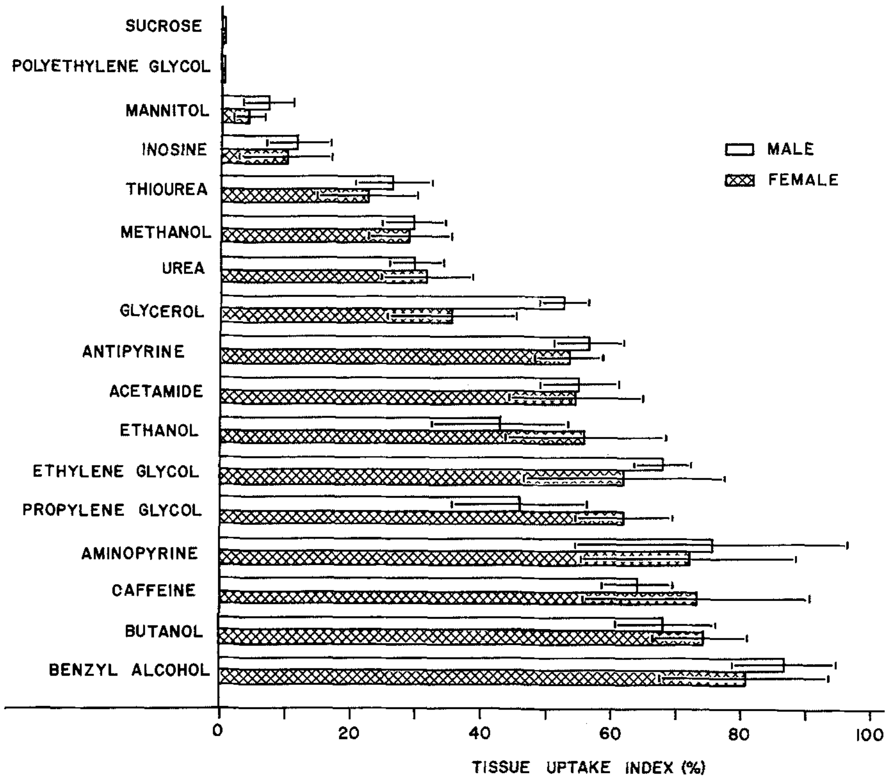

Fig. 1. Transintegumental uptakes (TUI) for the seventeen measured compounds in *Schistosoma japonicum*. Bars indicate means and lines indicate standard deviations of each compound. Male values are indicated with open bars, and female values are cross-hatched. The sample size ranged from 5-16 schistosomes of each sex per compound and schistosomes were studied 37-71 days post-infection

Table 2. Comparison of integumental permeability in copulating and separated male and female *Schistosoma japonicum*

Compound	Tissue uptake index (%)			
	Unpaired male	Paired male	Paired female	Unpaired female
Antipyrine	55.4±3.8	53.7±6.8	51.7±4.3	52.8±5.5
Acetamide	52.6±3.7	55.8±4.0	52.8±6.1	57.0±7.3
Ethylene glycol	69.1±7.4	67.4±9.2	60.1±6.7	62.9±8.3
Urea	30.1±4.3	31.7±5.7	33.7±8.8	31.5±6.6

Sample size = 3–7 for each mean ± SD.

and slight male-female differences were not statistically significant. The two compounds showing the lowest uptakes in both sexes were polyethylene glycol and sucrose, both compounds being well below background level (<2%) for the method. (A mean negative TUI of −3.2% was obtained for polyethylene glycol, presumably because greater quantities of ^{113m}In than ^{14}C were washed off the surface with the oil rinse. A numerical zero uptake is reported.) When the mean of the male and female uptakes are considered. TUI's can be serially ranked: benzyl alcohol > aminopyrine > butanol > caffeine > ethylene glycol > antipyrine > propylene glycol > acetamide > ethanol > glycerol > urea > methanol > thiourea > inosine > mannitol > sucrose > polyethylene glycol. This sequence is similar, but not identical, to the serial ranking of partition coefficients.

Uptakes were compared in copulating and unpaired (apart) males and females in a small group of metabolites (Table 2). These studies confirm the similar permeabilities of the male and female integument for these compounds and indicate that no changes in transintegumental permeability to these compounds can be attributed to the copulating state. In contrast, preliminary data from this laboratory suggests that glucose uptake (at a 20 μM concentration) is lower in unpaired *S. japonicum* females (TUI = 18.2 ± 3.9%) than paired females (22.2 ± 3.5%), and relatively higher in copulating males (TUI = 26.1 ± 2.8%) than in separated males (24.1 ± 4.5; $n = 6$ for each mean).

Correlation between Partition Coefficients and Transintegumental Uptakes

The semi-logarithmic correlation between partition coefficients and transintegumental uptakes in male schistosomes can be seen in Fig. 2. Linear regression analysis of these parameters indicated a high correlation ($R = 0.76$) which was statistically significant ($P < 0.001$). The same parameters were analyzed in *S. japonicum* females (Fig. 3). These data similarly demonstrate positive, statistically significant corre-

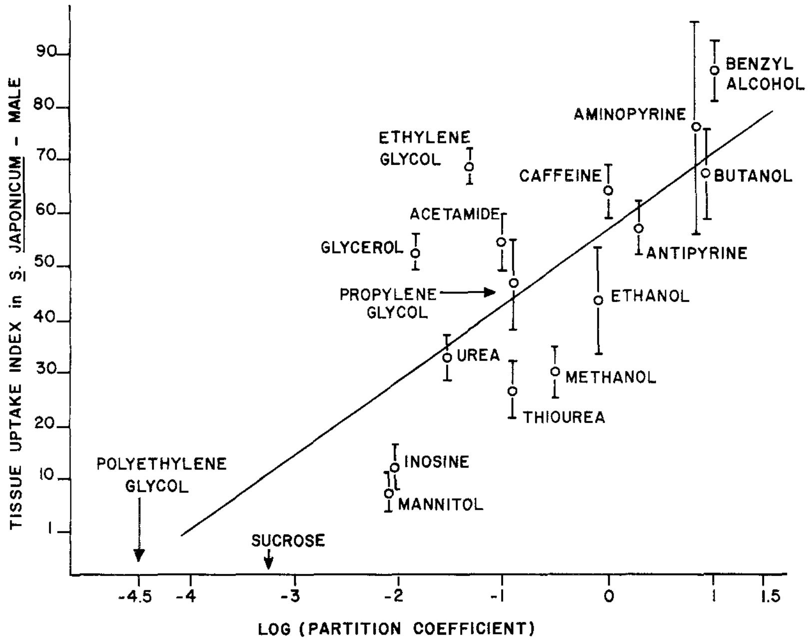

Fig. 2. Correlation between the TUI and the logarithm of the partition coefficient for male *Schistosoma japonicum*. Points indicate mean values and bars indicate standard deviations of the TUI's for the compounds studied. Males displayed a high correlation between these parameters ($R=0.76$), which was statistically significant ($P<0.001$). The slope ($\pm$SE) of the regression line is 14.1 ± 1.3, the intercept$=57.1$. A lesser correlation ($R=0.63$, $P<0.01$) was observed in comparing TUI *vs.* log (partition coefficient)/(square root of mol wt) as employed by Bissonette et al. (1979) and Levin (1980), perhaps suggesting that, for the compounds herein studied, molecular weights do not make a major contribution to schistosomal uptake

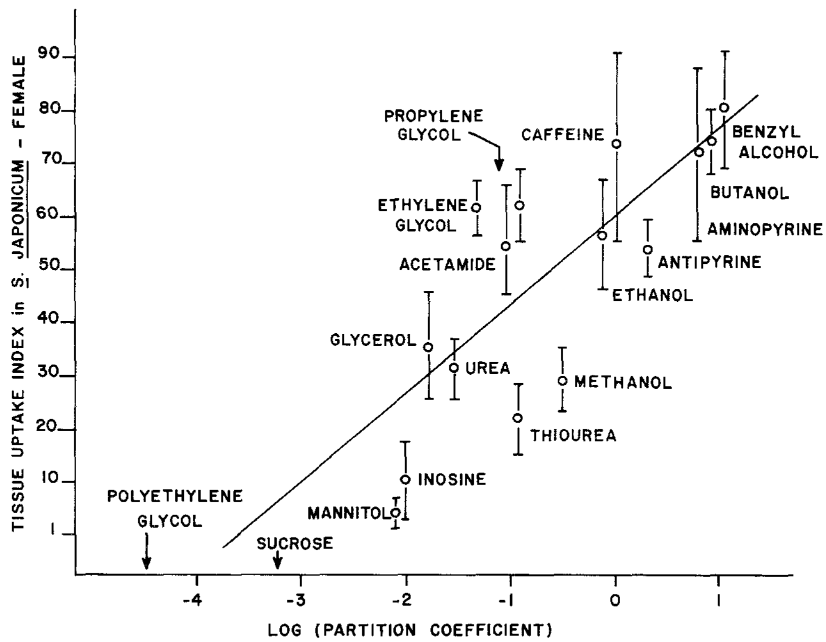

Fig. 3. Linear regression analysis of TUI *vs.* the logarithm of the partition coefficient for female schistosomes. As in Fig. 2, points indicate means and bars indicate standard deviations of the female transintegumental uptakes. The correlation between these parameters in *Schistosoma japonicum* females was also positive and quite high ($R=0.77$) and showed statistical significance ($P<0.001$). The transintegumental uptakes and the slope ($\pm$SE) of the regression line (15.8 ± 1.4, intercept$=59.4$) are not significantly different from the values seen in the male worms despite rather marked sexual differences in ultrastructure. As with the males, a lesser correlation ($R=0.48$) was observed contrasting TUI with log(PC)/(square root mol wt), but this was not statistically significant ($P<0.1$). Thus for the range of molecular weights examined (30–350) this parameter does not appear to make a major contribution to membrane permeability as indicated by the TUI method

lation ($R=0.77$; $P<0.001$) between log partition coefficient and transintegumental uptake.

Since the uptakes of the nonelectrolytes tested were similar in male and female schistosomes, with the possible exception of glycerol (Fig. 1), it follows that the slopes of the regression lines for males (14.06 ± 1.28; y intercept$=57.1$) and females (15.81 ± 1.4; y intercept$=59.4$) do not differ significantly.

Data in Table 1 demonstrate that the hydrogen bond number, an estimator of hydrophilic proper-

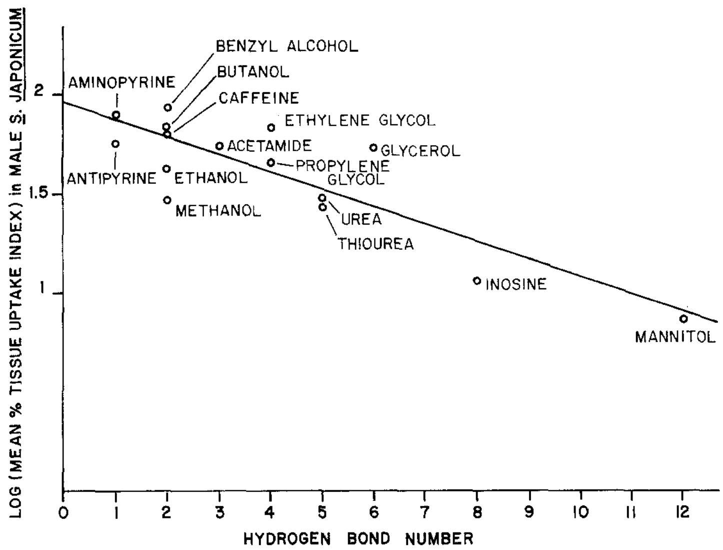

Fig. 4. Correlation between the logarithm of Tissue Uptake Index and hydrogen bond number in male *Schistosoma japonicum*. Hydrogen bond numbers, an indicator of relative hydrophilic properties, are indicated in Table 1. The high (negative) correlation ($R = -0.85$, $P < 0.001$) indicates a greater permeability of hydrophobic molecules and is consistent with the concept illustrated in Fig. 1. The relationship (slope = -0.086, intercept = 1.95) is a higher correlation than seen in contrasting log (TUI) × (square root of mol wt) *vs.* hydrogen bond number ($R = 0.71$, $P < 0.01$; slope = -0.076, intercept = 2.89)

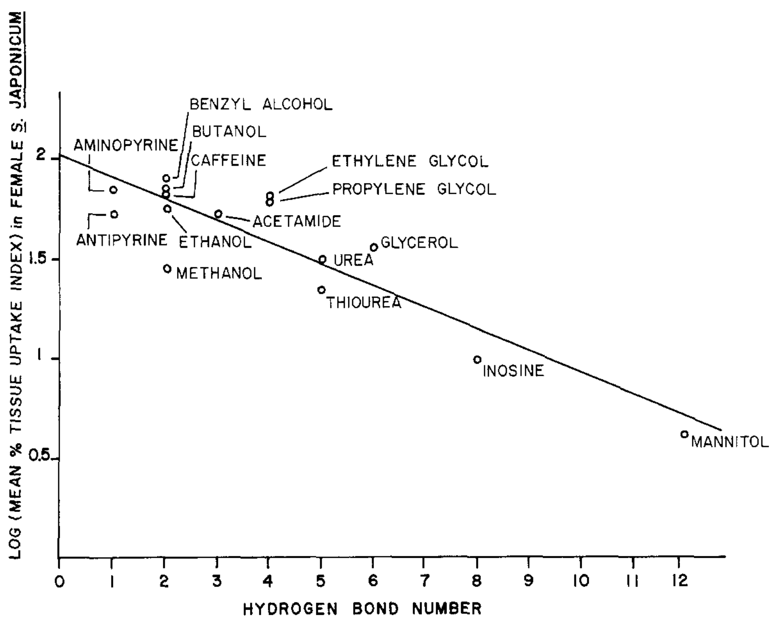

Fig. 5. Hydrogen bond number compared with the logarithm of Tissue Uptake Index in female *Schistosoma japonicum*. As demonstrated previously for males, a similarly high negative correlation ($R = -0.90$, $P < 0.001$) is observed (slope = -0.11, intercept = 2.02) in females. This relationship represents a higher degree of correlation than seen in comparing log (TUI) × (square root of mol wt) *vs.* hydrogen bond number ($R = -0.80$, $P < 0.001$: slope = -0.097, intercept = 2.95), just as has been demonstrated for males (Fig. 3)

ties, varies inversely with the measured octanol/-saline partition coefficient (which increases in lipophilic compounds). A high (negative) correlation is apparent when hydrogen bond number is plotted as a function of the log TUI in both males ($R = -0.85$, $P < 0.001$; Fig. 4) and females ($R = -0.90$, $P < 0.001$; Fig. 5). This confirms the concept that lipophilic properties greatly influence physiological exchanges at the schistosome surface (Figs. 2 and 3) and indicates a distinct similarity between the male and female integuments with respect to penetration of nonelectrolytes.

Attempts were made to correct measured TUI's for the varying size of the molecules studied. Previous workers have, for example, multiplied permeability (Stein, 1967, p. 77) or divided the partition coefficient (Levin, 1980) by the square root of the molecular weight. In the present study a higher correlation was always obtained when data were not expressed as a function of molecular weight (*see* Figs. 2–5). This suggests that for the compounds herein studied, and the range of molecular weights (30–350) examined, penetration of the schistosome surface is not greatly influenced by molecular weight.

Discussion

Overton's rule, which states that in a homologous series of molecules increasing the number of CH_3

groups increases membrane permeability, suggested a relationship between lipophilicity and membrane permeation (*see* Naccache & Sha'afi, 1973). Beginning with the classical studies (*see* Collander, 1954; Stein, 1967; and the review of Diamond & Wright, 1969), several investigators have corroborated the relationship between lipophilicity and membrane permeability in various systems. Wright and Diamond (1969) demonstrated a correlation between partition coefficients, in both an ether/water and an olive oil/water system, and the permeabilities to several molecules of the gallbladder membrane in rabbits. Smulders and Wright (1971) confirmed the relationship in rabbit gallbladder epithelium using an isobutanol/water partitioning system. Both Savitz and Solomon (1971) and Naccache and Sha'afi (1973) were able to show that partition coefficients of compounds in an ether/water system are proportional to their permeabilities in human red blood cell membranes. Oldendorf (1974) showed that lipid solubility is important in determining a molecule's permeability to the blood-brain barrier *in vivo*, and this concept was recently corroborated in a study of several brain tumor chemotherapeutic agents (Levin, 1980). Bindslev and Wright (1976) confirmed this relationship for the toad urinary bladder, and Bissonette et al. (1979) showed that the relationship holds for both guinea pig and sheep placental membranes. Partitioning characteristics and penetration of alcohols have also been correlated in the rabbit intestinal mucosa (Sallee & Dietschy, 1973) and rat adipocytes (Sherrill & Dietschy, 1975). Thus the correlation between these parameters has been established in the (above mentioned) vertebrate and plant (Collander, 1954; Stein, 1967) cell systems. The distinct similarity in hydrophobic surface properties herein demonstrated for male and female schistosomes is interesting because their integuments are so different ultrastructurally (Sakamoto & Ishi, 1977), and the glycogen/protein ratio in males (0.5) is considerably larger than that of females (0.2) in this species (Cornford & Huot, 1981). These data suggest the external unit membranes of males and females possess functionally similar permeability properties, and the great differences in surface topography (visualized by scanning electron microscopy) are related to some surface function other than lipid mediated uptake. The unusual accumulation of host antigens (Smithers et al., 1969; Kemp et al., 1977) and constitutive host-like antigens (Damian, 1979), which have been demonstrated in *S. mansoni* and are presumably also present in *S. japonicum*, do not modify lipid mediated membrane permeability in the *in vitro* system utilized in this study. If these absorbed host antigens do not inhibit membrane penetration of compounds such as antischistosomal drugs, then the suggestion that antibody to specific schistosome antigens might be useful in selectively delivering a drug to the schistosomal surface, may be worthy of further study.

The present studies also indicate that for antischistosomal drugs which gain access to the blood fluke via a lipid mediated mechanism, relatively equal amounts would be expected to be delivered to the male and female schistosome. Thus if such a lipophilic drug had a differential effect upon one sex it seems probable that some biochemical difference in the male and female schistosome would best explain differential drug sensitivity. Presumably such a drug would either have a greater binding affinity for the protein or lipid constituents of one sex, or the drug would affect a biochemical pathway which operated differently in that one sex. However, identification and characterization of any male or female specific proteins which possess specific affinity for antischistosomal agents has not been achieved to date. In situations where the administered drug might be quite lipophilic, such antischistosomal metabolites may show logarithmic reductions in lipid solubility (e.g., through systemic biotransformation) before any measurable decrease in schistosomal uptake would be observed.

Certain compounds (e.g., ethylene glycol and glycerol, Fig. 2) have disproportionately high uptakes in relation to their measured partition coefficients. These deviations from Overton's rule have been observed for ethylene glycol in studies of rabbit gallbladder (Wright & Diamond, 1969), red cells (Naccache & Sha'afi, 1973), dog lung (Perl, Silverman, Delea & Chinard, 1976), and the placenta (Bissonette et al., 1979). Selective carrier-mediated uptake of glycerol has also been described in tapeworm integument (Uglem, Pappas & Read, 1974), so there are previous data suggesting that glycerol and glycol uptakes might not be easily predicted from their lipid solubilities. Traditionally, the apparent "sieving" of small hydrophilic solutes has been ascribed to the presence of aqueous pores, which select among the nonelectrolytes according to size, and size is a function of molecular weight. Lieb and Stein (1971) challenged the pore theory, alternatively suggesting the increased permeability of smaller hydrophilic molecules was a natural consequence of the bilayer structure. Their suggestion that membrane properties should be considered more to a rubber-like polymer than an oil layer was not corroborated by Finkelstein (1976). It has been further indicated that recent attempts to address this controversy have diverted attention from the physiologically relevant correlations of lipid/water partitioning and permeability, and "obscured the simplicity and usefulness of Overton's rule" (Orbach & Finkelstein, 1980). With respect to the present study, the data do not provide any insight pertinent to the theory of water movement across this membrane system, but do suggest the practical use of water as a reference molecule *in vitro* studies of this type, as previously demonstrated *in vivo* (Oldendorf, 1974; Oldendorf & Szabo, 1976).

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