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A COMPARATIVE STUDY ON THE ACCUMULATION OF PROBENECID AND ANALOGUES IN RABBIT KIDNEY TUBULES *IN VITRO*

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Abstract—The characteristics of renal accumulation of probenecid (di-n-propylsulfamylbenzoic acid) and di-methyl $[CH_3]_2$-R,di-ethyl $[C_2H_5]_2$-R, and di-butyl $[C_4H_9]_2$-R analogues by cortical slices suspended in an electrolyte medium have been compared. The compounds were accumulated both under aerobic and anaerobic conditions. Rate of initial active uptake increased in the order: $[CH_3]_2$-R < $[C_2H_5]_2$-R = $[C_3H_7]_2$-R > $[C_4H_9]_2$-R. The compounds inhibited the aerobic uptake of PAH and phenol red with the following order of effectiveness: $[CH_3]_2$-R < $[C_2H_5]_2$-R < $[C_3H_7]_2$-R < $[C_4H_9]_2$-R. PAH affected the accumulation of probenecid and analogues in the reverse order. The accumulation of probenecid and analogues under anaerobic conditions could be accounted for by binding to various tissue constituents and exhibited close similarity to binding to human serum albumin and liposomes. Phenol red, in contrast to PAH, inhibited anaerobic binding of the compounds to various extents. Active accumulation of probenecid and analogues was markedly stimulated by acetate (10 mM), while fumarate and octanoate had a biphasic effect, except on the accumulation of dibutyl analogue which was little influenced by these metabolites. It is concluded that probenecid and analogues are actively transported by the organic anion system. The inhibitory potency is correlated with the hydrophobicity of these compounds as in the case of phenolsulphonphthalein dyes.

Probenecid (di-n-propyl sulfamyl-benzoic acid) is widely used as a therapeutic agent and as a test substance in experimental nephrology, because it is an effective inhibitor of renal transport of organic anions and at the same time is slowly eliminated from blood plasma [1, 2]. Probenecid is one member of a series of homologous compounds of sulfamylbenzoic acid containing hydrocarbon substitution of various length, originally synthesized by Miller *et al.* [3]. Beyer and coworkers [4, 5] found that the renal clearance of probenecid analogues decreased as the hydrocarbon chain length was increased (clearance of di-n-methyl sulfamyl-benzoic acid $[CH_3]_2$-R > di-n-ethyl sulfamyl-benzoic acid $[C_2H_5]_2$-R > di-n-propyl sulfamyl-benzoic acid $[C_3H_7]_2$-R). Furthermore, these authors noted an inverse relationship between the inhibition of the transport organic anions like *p*-aminohippurate (PAH), phenol red and penicillins by probenecid analogues and excretion of these drugs by the dog kidney. Thus, the inhibitory potency of these substances decreased in the following order: $[C_3H_7]_2$-R > $[C_2H_5]_2$-R > $[CH_3]_2$-R.

On the basis of above mentioned experimental data, Beyer [5] suggested that the effective inhibitors of tubular excretion of organic anions, were "refractory" substrates of the transport system, i.e. they combine with the carrier in a reversible manner, but without being themselves transported. This view was challenged by Weiner *et al.* [2], who showed that net secretion of probenecid $[C_3H_7]_2$-R was demonstrable during infusion of $NaHCO_3$, which produces alkalinization of luminal fluid. These authors suggested that the decisive difference in the renal excretion of probenecid and analogues resided in the extent to which these compounds were reabsorbed by the renal tubules by non-ionic diffusion [2].

The existence of tubular secretion of probenecid is supported by the demonstration of accumulation of this compound in kidney slices *in vitro* [6]. In a recent report, using [^{14}C] probenecid, we showed that this drug is taken up by kidney tubules *in vitro*, at low medium concentrations by an energy requiring system, susceptible to metabolic inhibition [7]. Moreover, demonstration of competitive inhibition of probenecid uptake by other organic anions, like PAH, phenol red and other substituted phenolsulphonpthlaein (PSP) dyes, established that the renal transport of this drug occurs by the common organic anions transport system [7]. The results indicate that the kidney slice system may be used for evaluation of parameters of active transport of this compound without introducing undue complications from the reabsorptive process, which is the case in *in vivo* experiments.

Accordingly, in this communication, we have taken the opportunity to compare the uptake of various [^{35}S]probenecid analogues with that of [^{14}C]probenecid by kidney slices. The inhibitory potency of these compounds with increasing lipophilic properties is evaluated by examining their influence on PAH and phenol red accumulation. Furthermore, the binding characteristics of these compounds to kidney homogenate are discussed in relation to the interaction of these drugs with model systems (human serum albumin and liposomes).

METHODS

Experimental procedure

The general experimental procedures were similar to those previously reported from this laboratory

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[8, 9], and they are only briefly summarized here. The experiments were performed on both male and female rabbits, weighing 2.5–4 kg. Thin slices and homogenates of cortex were prepared from kidneys which had been perfused with approximately 50 ml of the Krebs–Ringer phsophate solution via a needle inserted in the renal artery. This procedure was performed to removed plasma albumin from the renal tissue.

Incubation of kidney slices. Slices (200 mg) were incubated in Warburg cups containing 3 ml 145 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 10 mM Sodium phosphate buffer (pH 7.40) and 0.04 mM radioactive [^{35}S]probenecid analogues (di-n-methyl, di-n-ethyl, di-n-butyl-sulfamyl benzoic acid) or 0.04 mM [^{14}C]probenecid (di-n-propyl sulfamyl benzoic acid). Addition of other organic anions which interfere with the renal transport of probenecid [7] to the incubation medium is mentioned in the description of the particular individual experiment. In a series of experiments the effect of cyanide (8 mM) and glycolytic inhibitors such as fluoride (20 mM) and iodoacetamide (0.5 mM) was examined. Incubation was carried out in a Warburg respirometer under the following conditions unless otherwise specified: shaker speed, 100 c/min; gas phase, either 100% O_2 or 100% N_2; temp. 25°; pH 7.4; incubation period, 2 hr. The centre well contained KOH for CO_2 absorption. After incubation, slices were weighed following a brief blotting on a piece of filter paper and transferred to conical flasks, each containing 4 ml of NaOH (1N). The renal tissue was dissolved by placing the flask in an oven at 80° for 20 min. A sample of medium (1 ml) was deproteinized by adding 4 ml 5% (w/v) trichloroacetic acid.

Homogenate experiments. Binding of probenecid analogues to different fractions of kidney cortical homogenate in the presence or absence of another organic anion was studied by ultrafiltration and differential centrifugation techniques as described in detail elsewhere [8]. A 20% (wet w/v) homogenate was prepared in a solution containing 0.25 M sucrose, 0.033 M phosphate buffer (pH 7.4) and one of the probenecid analogues used in this study. The homogenate was then divided into two equal parts, to one of which was added a small volume of phosphate buffer containing one of the following substances: PAH or phenol red. The resulting mixture was called test homogenate. The other part, denoted as reference homogenate, was diluted with the same volume of phosphate buffer.

Binding of probenecid analogues to the whole homogenate was investigated by ultrafiltration [10]. Binding of these substances to different fractions of homogenate was studied by differential centrifugation. After each centrifugation, duplicate samples (0.2 ml) of the supernatant were pipetted and the radioactivity was measured as described below.

The purity of the fractions of the homogenate was examined by microscopic observation and by estimation of the various enzymatic activities [8].

Interaction of probenecid analogues with human serum albumin. Binding of probenecid analogues to human serum albumin was investigated by ultrafiltration as recently described [7, 11]. A tracer amount of [^{35}S]probenecid analogue together with non-labelled compound in the desired concentrations was used, whereas most of the experiments were carried out with a fixed concentration of albumin (2% w/v). Reference solutions i.e. solutions having the same concentration of probenecid analogue, but no albumin, were made in 0.033 M phosphate buffer. Binding of these organic anions to albumin was measured by comparing the ultrafilterability of the test and reference solutions.

Binding of probenecid analogues to liposomes. Liposomes were prepared essentially according to Bangham *et al.* [12], except that the swelling salt solution, in addition to KCl (145 mM), contained 0.033 M phosphate buffer, a trace amount of radioactive probenecid analogue and various concentrations of non-labelled compounds. Samples (1 ml) of thoroughly mixed dispersion of phospholipids (15 μmoles licithin/ml swelling salt solution) were centrifuged at 27,000 *g* for 1 hr. Duplicate samples (0.2 ml) of the supernatant were subjected to radioactivity measurements. Binding of probenecid analogues to liposomes was

Table 1. Effect of phenol red on binding of probenecid and analogues to various fractions of rabbit kidney cortex homogenate

Cell fraction	Binding (%)							
	$[CH_3]_2$-R		$[C_3H_5]_2$-R		$[C_3H_7]_2$-R*		$[C_4H_9]_2$-R	
	Control	PR	Control	PR	Control	PR	Control	PR
Whole homogenate	17.5 ± 2.3	3.7 ± 0.7	19.6 ± 2.8	4.5 ± 0.5	39.2 ± 3.5	28.8 ± 4.5	48.2 ± 4.9	33.6 ± 2.9
700 *g* fraction (I)	9.8 ± 1.1	1.9 ± 0.4	11.3 ± 1.6	2.3 ± 0.3	10.4 ± 2.7	8.2 ± 2.2	16.7 ± 2.8	10.6 ± 1.2
Mitochondrial (II) fraction	1.7 ± 0.2	0.8 ± 0.2	2.3 ± 0.3	1.1 ± 0.2	7.6 ± 1.1	4.6 ± 1.7	3.6 ± 0.3	1.8 ± 0.2
Microsomal (III) fraction	2.3 ± 0.3	0.4 ± 0.2	1.8 ± 0.4	0.5 ± 0.2	3.2 ± 0.8	2.3 ± 0.4	4.7 ± 0.3	2.5 ± 0.3
Soluble protein fraction (IV)	3.6 ± 0.3	0.8 ± 0.3	3.3 ± 0.3	0.8 ± 0.3	19.5 ± 0.8	13.7 ± 0.8	23.6 ± 1.9	16.8 ± 1.4
I + II + III + IV	17.4	3.9	18.7	4.7	40.7	28.8	48.6	31.7

The experiments were carried out as described in the Methods section. The final concentrations in the homogenate preparations were as follows: probenecid and analogues, 0.15 mM; phenol red (PR), 1.7 mM.

* Probenecid $[C_3H_7]_2$-R figures are taken from a previous paper [7].

determined by measuring the decrement of the concentration of these compounds in the sample after centrifugation.

Gel electrophoresis. In order to examine whether the cytosol fraction of kidney cortex homogenate was contaminated with extravascular albumin, the supernatant obtained after the final centrifugation (see Table 1) was subjected to gel electrophoresis. The results of this analysis showed that the supernant was completely free of albumin.

Preparation of ^{35}S-labelled probenecid analogues

[^{35}S]4-*chlorosulfonyl benzoic acid.* This substance was prepared by a modification of the procedure described by Meerwein *et al.* [13]. 4-aminobenzoic acid (206 mg, 1.5 mmol) in methanol was suspended in concentrated (12 N) hydrochloric acid (800 μl). Sodium nitrite (114 mg, 1.65 mmol) was dissolved in 600 μl water, and this solution was added dropwise to the 4-aminobenzoic acid solution during the course of 1 hr and with continuous stirring. The ring temperature was maintained at 0°–6°. The cuprous chloride (50 mg) was added to the solution and immediately after this 1000 μl acetic acid in which was dissolved 100 μl (2.2 mmol) liquid sulfur dioxide containing 5–10 mCi $^{35}SO_2$. The temperature was maintained at 0°, and stirring was continued until the evolution of nitrogen gas ceased. The solution was diluted with 3 ml ice cold water which resulted in precipitation of the 4-chlorosulfonyl benzoic acid. This was further sedimented by centrifugation. The product was washed 2–3 times with ice cold water, each time followed by centrifugation. Finally the product was dried in a desiccator. The crude yield was 230 mg corresponding to 72 per cent and it was used without further purification.

4-(*di-n-alkyl*) *sulfamyl benzoic acid.* [^{35}S]4-chlorosulfonyl benzoic acid (50 mg, 0.227 mmol) was added to a continuously stirred mixture of the di-n-alkylamin (0.227 mmol) in 0.35 ml 10% NaOH. After stirring for 1 hr at 10° the solution was acidified with 3 ml 2 N HCl. The precipitated product was collected and washed twice with water before drying *in vacuo*. The products were further purified by thin layer chromatography using silica gel (60/kieselguhr F_{254}) pre-coated aluminium sheets, 20 × 20 cm. The material was dissolved in 3 ml methanol and applied in a band of 1 × 17 cm and the plates were developed with light petroleum (boiling point 40°–60°)–diethyl ether–acetic acid (50:50:2). As reference and for identification of the radioactive compounds the corresponding "cold" compounds were used. These were in advance synthesized in larger scales, applying the same procedure but purified by recrystallization in methanol or diluted methanol. Their purity was controlled by melting point, i.r. spectroscopy and in some cases by nuclear magnetic resonance. The following melting points were found: values in parentheses are melting points taken from Itaya *et al.* [14]: 4-dimethyl, 252 (252.5°); 4-diethyl, 195 (194–195°), and 4-dibutylsulfamyl benzoic acid, 163° (163°). The i.r. spectra were in agreement with that expected by comparing with i.r. spectra of commercial dipropylsulfamyl benzoic acid (probenecid). The bands containing the radioactive compounds were cut out after development and eluted with methanol. The eluate was centrifuged and finally the methanol was evaporated by flushing the solution with oxygen-free nitrogen. The yields were approximately 20 per cent, calculated on the basis of the starting amount of 4-aminobenzoic acid.

Analytical methods and calculations

Duplicated sample (0.2 ml) of digested solution of renal tissue were added to counting vials containing 7 ml of the toluene scintillation fluid described in the previous report [7]. Trichloroacetic acid filtrates of the medium (1 ml) were added to 10 ml of the scintillation fluid originally reported by Bray [15].

The radioactivity of the samples (0.2 ml) obtained from different fractions of homogenates, ultrafiltrates or supernatants was determined by adding 10 ml of insta-Gel (Packard) to the counting vials, which were counted in a Packard scintillation counter. The counts per minute were converted to disintegrations per minute by determining the efficiency of the samples by the addition of standard solution of [^{35}S]dioctyl sulphate, or [^{14}C]toluene in the case of [^{14}C]probenecid.

In a series of experiments, the steady-state accumulation of both radioactive and non-radioactive probenecid analogues was determined by chemical and radioactive measurements. The results of these experiments established that there was no significant differences between the uptake of labelled and non-labelled compound.

In a few experiments in which the effect of increasing concentrations of probenecid analogues on the uptake of PAH or phenol red in cortical slices was examined, PAH or phenol red was extracted from the tissue and medium [8, 16]. PAH was analyzed by the diazotization method of Bratton and Marshall [17] as modified by Smith *et al.* [18]. The extracellular space (inulin space) and dry weight of kidney slices were determined as previously reported [9].

The amount of probenecid analogues, PAH or phenol red in the intratubular part of the renal tissue was calculated as the difference between content of these compounds in the whole slices and that of the inulin space. This difference was then divided by tubule water content (wet wt of tissue-inulin space-dry wt) to obtain the tubular concentration of these organic anions from which the accumulation of probenecid analogues (T/M_{PBS}), PAH (T/M_{PAH}) or phenol red (T/M_{PR}) was calculated.

RESULTS

Renal uptake of probenecid and analogues

The uptakes pattern of [^{14}C]probenecid and its radioactive ^{35}S analogues in renal cortical slices as a function of time was studied in a series of experiments. Figure 1A shows the results of experiments in which the influence of acetate on the renal uptake of probenecid and analogues under aerobic conditions were examined. During the first 10 min of incubation, a rapid rate of uptake of all these compounds occurs, followed by a slower rise in the concentration of these substances as time proceeds. The aerobic T/M_{PBS} is distinctly higher in the presence of acetate than in the absence of this metabolite in the incubation medium. After 1 hr of incubation, a steady-state accumulation is

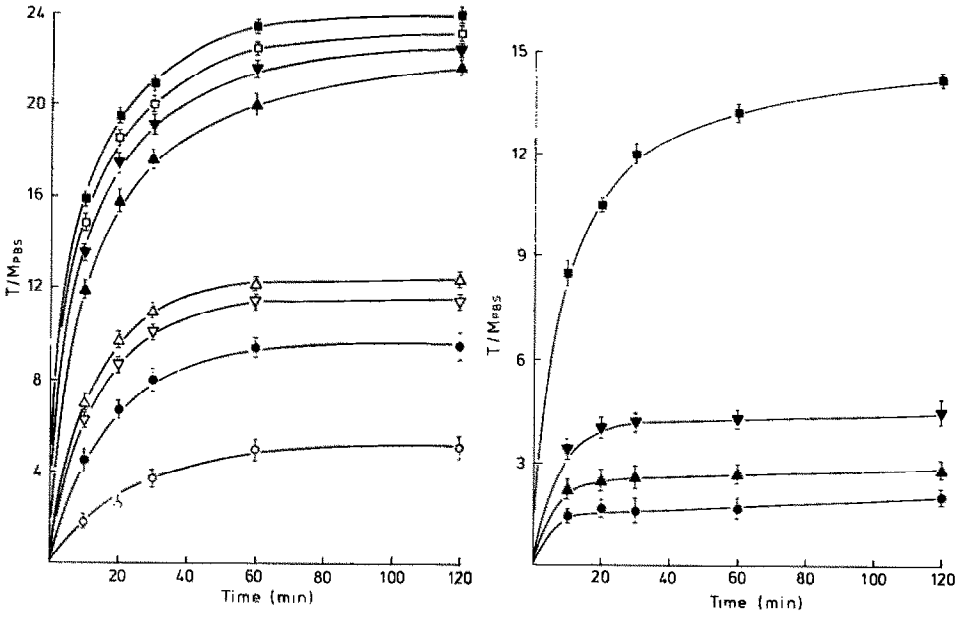


Fig. 1. Left (A) and right (B) panels demonstrate the uptake of $[CH_3]_2$-R (●—●), $[C_2H_5]_2$-R (▲—▲), $[C_3H_7]_2$-R (▼-▼) and $[C_4H_9]_2$-R (■—■) in the presence of acetate (10 mM), under aerobic and anaerobic conditions, respectively. Corresponding open symbols in 1A represent accumulation of these compounds in the absence of acetate. R denotes sulfamylbenzoic acid moiety. The initial concentrations of probenecid and analogues were 0.04 mM. Ordinate: T/M_{PBS}, ratio between the concentration of probenecid and analogues in the tubule water and medium. Abscissa: time of incubation. Note: the same symbols have been used throughout in this paper to indicate the renal accumulation or binding of these substances to liposomes and human serum albumin (see Fig. 7A, 7B). Figure 1 and subsequent figures in this paper show mean values of five to eight experiments. Vertical bars indicate ±SE. In the case of Fig. 1 statistical tests were performed on the closest lying points. The values obtained were: $P < 0.01$.

approached. The rapidity of initial uptake and the steady-state accumulation of those drugs increase in the following order: $[CH_3]_2$-R < $[C_2H_5]_2$-R < $[C_3H_7]_2$-R < $[C_4H_9]_2$-R. Figure 1B records the anaerobic accumulation of these compounds. Except for $[C_4H_9]_2$-R the anaerobic T/M_{PBS} is markedly lower than that attained under aerobic conditions (compare Fig. 1A and 1B). Furthermore, it can be seen that the steady-state accumulation ratios of these compounds under anaerobic conditions increase in the same order as observed under aerobic conditions.

The active uptake of probenecid and analogues by cortical slices, calculated as the difference between aerobic and anaerobic uptake in the presence of acetate, is shown in Fig. 2. The steady-state active accumulation of probenecid and its di-ethyl analogue is similar and much greater than that of di-methyl and di-butyl derivatives. Furthermore, it is seen that the rate of active uptake of these compounds varies in the following order:

$$[CH_3]_2\text{-R} < [C_2H_5]_2\text{-R} = [C_3H_7]_2\text{-R} > [C_4H_9]_2\text{-R}.$$

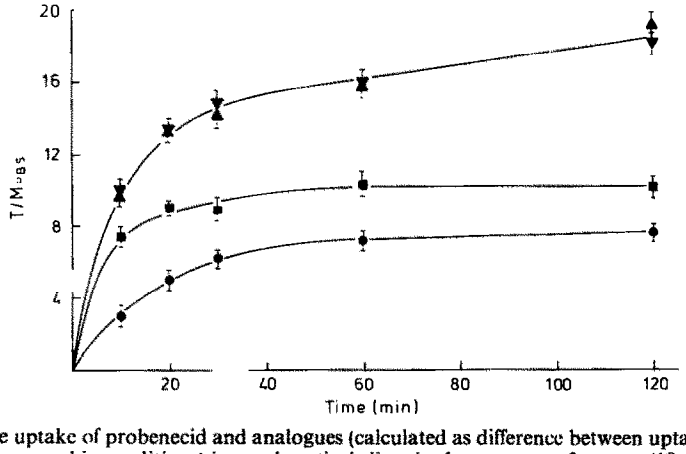


Fig. 2. Active uptake of probenecid and analogues (calculated as difference between uptake in aerobic and anaerobic conditions) in renal cortical slices in the presence of acetate (10 mM).

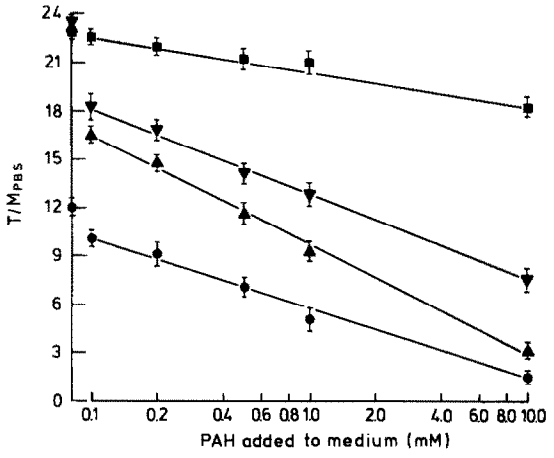


Fig. 3. Effect of PAH on the aerobic accumulation of probenecid and analogues. Acetate (10 mM) was added as metabolite to the incubation medium. The initial concentration of probenecid and analogues was 0.04 mM.

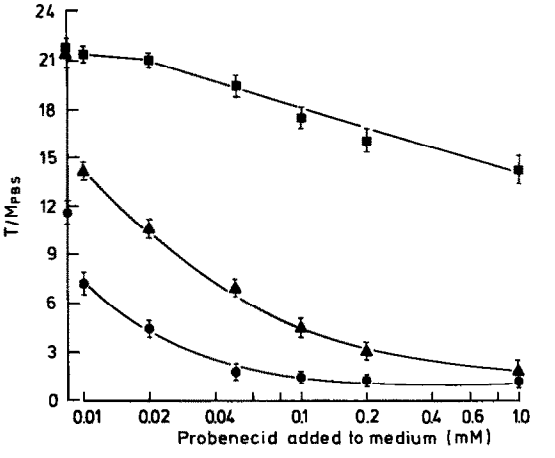


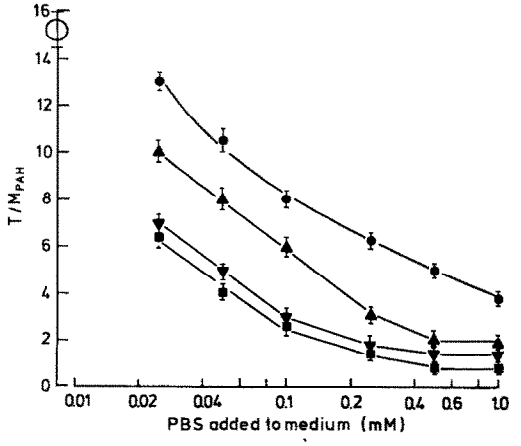
Fig. 4. Effect of probenecid on the accumulation of $[CH_3]_2$-R (●—●), $[C_2H_5]_2$-R (▲—▲), and $[C_4H_9]_2$-R (■—■) substitutes under aerobic conditions. Acetate (10 mM) was added to the incubation medium.

It has recently been reported that anaerobic accumulation of probenecid in kidney slices is due to binding of this drug to various cellular constituents [7]. Therefore, we have studied the effect of CN^- and glycolytic inhibitors such as F^- and iodoacetamide on the anaerobic accumulation of probenecid analogues in a manner similar to that previously described [8]. The results of these experiments showed that the anaerobic T/M_{PBS} of all these compounds was unaffected by these metabolic inhibitors. Thus, the anaerobic uptake of probenecid analogues, like that of probenecid, cannot be due to active transport, but presumably is caused by binding of these compounds to renal tissue.

The effect of PAH on the uptake of probenecid and analogues is illustrated in Fig. 3. The steady-state accumulation of these compounds is reduced to various extent by PAH. Thus, the aerobic accumulation of di-methyl and di-ethyl derivatives of probenecid is nearly abolished, while little inhibition of the di-butyl substitute's accumulations is observed.

The effect of probenecid on the accumulation of di-methyl, di-ethyl and di-butyl analogues is shown in Fig. 4. Probenecid inhibits the T/M_{PBS} in an expected order. The uptake of both $[CH_3]_2$-R and $[C_2H_5]_2$-R analogues is almost abolished, whereas a moderate inhibition of the dibutyl derivative's accumulation is observed.

Since probenecid and various analogues of this drug presumably are excreted by renal tubule by the same transport system as that of other organic anions like PAH and phenol red [2, 5], we have attempted to compare the affinity of these compounds for the transport system by examining their effectiveness as inhibitors of PAH and phenol red uptake in slices. Figure 5A and 5B describe the influence of probenecid and analogues on the renal accumulation of PAH and phenol red. It appears from the figure that all these



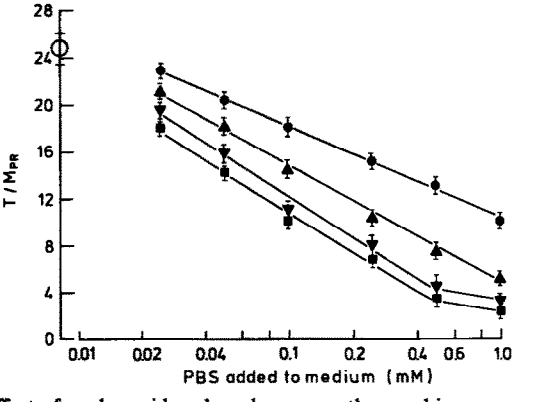


Fig. 5. Left (A) and right (B) panels demonstrate the effect of probenecid and analogues on the aerobic accumulation of PAH (T/M_{PAH}) and phenol red (T/M_{PR}), respectively. Acetate was added to the incubation medium. The open symbols (○) on the ordinate indicates the accumulation ratio of PAH and phenol red in the absence of inhibitors.

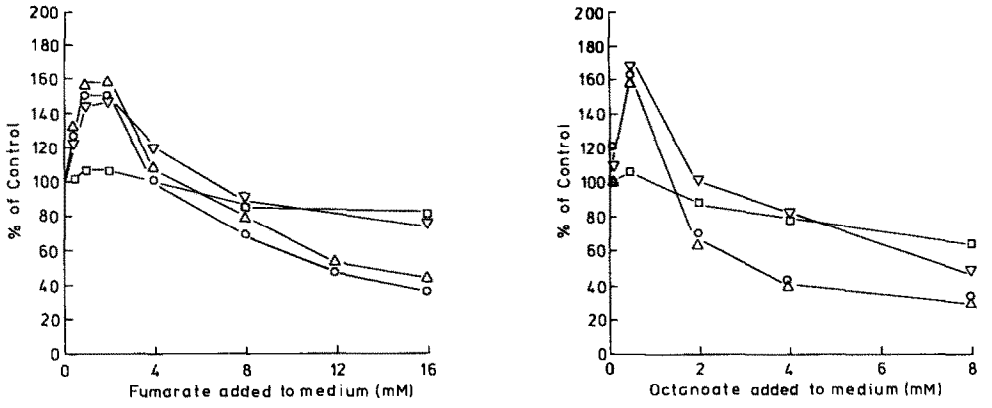


Fig. 6. Left (A) and right (B) panels demonstrate the effect of various concentrations of fumarate and octanoate on the renal aerobic uptake of probenecid and analogues, respectively. Acetate was not added to the incubation medium in these experiments. Accumulation ratios of probenecid and analogues, in the absence of fumarate and octanoate has been taken as 100 per cent. The initial concentration of probenecid and analogues was 0.04 mM. Each point denotes the mean value of 3 experiments.

compounds inhibit T/M_{PAH} and T/M_{PR} to various extents, but the accumulation of phenol red is less susceptible to inhibition than that of PAH. The effectiveness of probenecid and its substitutes as competitive inhibitors of PAH and phenol red transport increases in the order: $[CH_3]_2$-R < $[C_2H_5]_2$-R < $[C_3H_7]_2$-R < $[C_4H_9]_2$-R.

Figure 6A and 6B demonstrate the effect of increasing concentrations of fumarate and octanoate on the aerobic T/M_{PBS}. It is seen that both fumarate and octanoate at low medium concentrations markedly enhance the uptake of di-methyl, di-ethyl and di-propyl compounds, whereas little stimulation of the di-butyl analogue's accumulation is detected. However, at higher concentrations both fumarate and octanoate strongly inhibit the uptake of di-methyl and di-ethyl derivatives, while less pronounced inhibition of the accumulation of the di-butyl analogue is noted.

Binding characteristics of probenecid and analogues to various materials

The results shown in the foregoing section indicate that probenecid analogues interact with the cellular constituents of kidney tissue. The binding of these substances to different fractions of kidney homogenate (see Methods) is shown in Table 1. The upper column

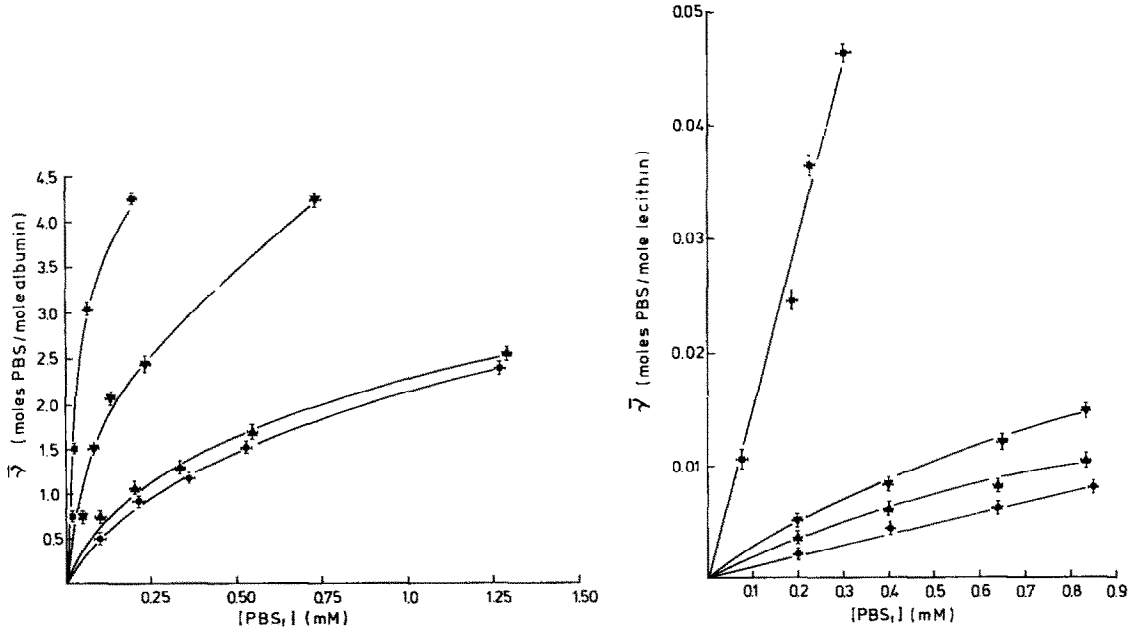


Fig. 7. Left (A) and right (B) panels demonstrate the binding of probenecid and analogues to human serum albumin and lipsomes, respectively. Ordinate: ($\bar{\nu}$) denotes average number of probenecid and analogues molecules bound per molecule of human serum albumin (A) or lecithin (B). Abscissa: (PBS_f) indicates concentration of free probenecid and analogues.

of the table shows that the binding of these substances to the whole homogenate increases in the following order: $[CH_3]_2$-R < $[C_2H_5]_2$-R < $[C_3H_7]_2$-R < $[C_4H_9]_2$-R. Furthermore, the table shows that the binding of these substances to various fractions of kidney homogenate is reduced by the addition of phenol red. PAH did not have any influence on the interaction (not shown). In the case of di-methyl and di-ethyl derivatives, binding is nearly abolished by phenol red, whereas moderate inhibition of the binding of probenecid and di-butyl analogues to renal tissue is obtained.

Finally, Fig. 7A and 7B reveal the binding characteristics of probenecid and analogues to liposomes and human serum albumin, respectively. It appears from the figure that the di-butyl derivative of probenecid binds very strongly to both liposomes and albumin. The binding of di-methyl and di-ethyl substitutes to these preparations are not significantly different; whereas moderate binding of probenecid is recorded.

DISCUSSION

The present data illustrate the usefulness of the kidney slice system in studying tubular secretion of probenecid and analogues of this compound. The active accumulation of these compounds under aerobic conditions has many features in common with that of PAH and phenol red, viz.: (1) stimulation by acetate as metabolic substrate, (2) biphasic response (except for the di-butyl analogue) to addition of fumarate and octanoate to the incubation medium and (3) mutual inhibition of transport. On the basis of these observations the aerobic accumulation may be attributed to active uptake across the peritubular membrane of proximal tubule cells as in the case of other organic anions [19–21].

The results obtained with the present series of compounds provide an interesting analogy to that of various PSP dyes, the tubular secretions of which have been studied in detail [22–26]. The affinity of PSP dyes for the transport system and binding by cellular constituents increase in parallel and this correlation seems to be largely determined by the hydrophobicity of the compounds [11]. This is also the case for probenecid and analogues where inhibition studies indicated that the affinity of the compounds and passive binding increased in the order: $[CH_3]_2$-R < $[C_2H_5]_2$-R < $[C_3H_7]_2$-R < $[C_4H_9]_2$-R. Binding of these compounds to tissue constituents, liposomes and serum albumin also increases in the same order. For PSP dyes the transport capability is inversely related to their lipid solubility, and it has been proposed that PSP dyes by virtue of hydrophobic interactions with the membrane either retard the movements of the mobile part of the transport system or impede dislodgement of bound dye from the "carrier" [8, 11]. In the present study such a simple relationship between transport rate and hydrophobicity was not obtained, since relative transport rates were $[CH_3]_2$-R < $[C_2H_5]_2$-R = $[C_3H_7]_2$-R > $[C_4H_9]_2$-R. However, it should be noted that at sufficiently low medium concentrations transport rates are a function both of the turnover number and affinity of the compound for the carrier system. In the present investigation transport rates were only studied at one medium concentration, because of limited supply of the radioactively labelled compounds. From our previous and more extensive experiments on the uptake of probenecid [7] this medium concentration suffices to sustain nearly maximal rates of transport of this compound. However, the same medium concentration may not have been sufficient to approach saturation of the analogues with smaller hydrocarbon substituents, since the inhibitor studies indicated lower affinity to the transport system. Hence we cannot exclude the possibility that relatively larger transport rates of these compounds would have been obtained at higher medium concentrations. On the other hand the transport rate of the di-butyl analogue should be nearly maximal at 0.04 mM and in this case we observe the expected decrease in transport relative to that of probenecid.

Another prominent feature of this investigation was the pronounced lack of metabolic effect of acetate, fumarate and octanoate (at low concentrations) on the active transport of di-butyl sulfamyl benzoic acid. We have previously reported that the most lipid-soluble dye, bromothymol blue, uptake was independent of the addition of metabolites and metabolic inhibitors. We were unable to demonstrate active uptake of bromothymol blue *in vitro* [11], but a recent report from our laboratory indicated that bromothymol blue is secreted and reabsorbed by the renal tubules, just like probenecid [25]. The conclusion to be drawn from these investigations is that bromothymol blue and di-butyl analogue behave in a similar way, by either inhibiting the transport of fumarate and octanoate or influencing the intracellular metabolism of these substances. It is preferable to avoid further discussion until a detailed study is provided on this subject.

In conclusion the *in vitro* data presented in this paper affirm previous data *in vivo*, which clearly show that probenecid is secreted [2]. However, effective inhibitors with hydrophobic characteristics appear to be transported to a lesser extent and are less susceptible to environmental factors which influence the transport of organic anions.

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