rabbits and rats (Shibazaki et al 1981).

Orally administered aspirin has been reported to be subject to first-pass metabolism both in the gut and liver of rats (Iwamoto et al 1982). In contrast to the results with aspirin, the hepatic effect was relatively greater than the gastrointestinal one in producing the first-pass effect of salicylamide in rats. Furthermore, it was demonstrated that the overall first-pass effect of salicylamide was more extensive than that of aspirin in both species, rats and dogs, even when considered on the same dose level basis.

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Influence of sodium ion-pair formation on transport kinetics of warfarin through octanol-impregnated membranes

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It is generally accepted that drugs in their ionized form cannot readily permeate through biological membranes, at least by means of a passive transport mechanism. This may be attributed to the fact that first, the solubility of ions in hydrophobic medium is much lower than the solubility of neutral uncharged analogues and second, an electromotive gradient opposing the diffusion gradient will be generated by the net transport of charged particles (at least in a closed system and in the absence of compensating mechanisms).

From recent research in our laboratory (Van Der Giesen & Janssen 1982) it appeared that the apparent partition coefficient of the anticoagulant warfarin at pH10·0 (warfarin having a pK of 5·0 being completely dissociated under these conditions), was increased by the presence of Na^+. A linear relationship was found between the logarithm of the apparent partition coefficient and the logarithm of the Na^+ concentration. This observation could be explained by assuming that ion pairs are formed between the anion of warfarin and Na^+. It also appeared that the lipophilicity of such an ion pair is comparable with the lipophilicity of the undissociated acid. In the present investigation we show that the presence of Na^+ stimulated the diffusion of warfarin at pH 11 through octanol-impregnated membranes.

* Correspondence.

Materials and methods

Sodium warfarin was purchased from ACF Chemiefarma (ACF, Maarssen, The Netherlands) and used as supplied. Octan-1-ol was purchased from Merck (Merck, Darmstadt, GFR) and purified by subsequent washings with an equal volume of 4 M NaOH, 2 M H_2SO_4 and 1 M Na_2CO_3. Finally the octanol was washed repeatedly with water until the water phase reacted neutral.

Membranes used in the transport studies were prepared by soaking Millipore ultrafiltration filters, type VSWP, pore size 0·025 μm, in water saturated octanol. Shortly before use the excess octanol was removed from the membranes by drying between filter paper.

To measure warfarin transport through these octanol-impregnated filters a two compartment cylinder assembly according to Kroon & Janssen (1982) was used. One compartment (A) was filled with 15 ml solution containing warfarin, NaOH and NaCl. The starting concentration warfarin was 1 mM and the pH was 11·0. The NaCl concentration was varied such that the total Na^+ concentration was between 0·01 and 5 M. The second compartment (B) was filled with 15 ml of the same solution as in A but without warfarin. The solutions in both compartments were presaturated with octanol. A spectrophotometric cell was placed on top of compartment B. Stirring was achieved by putting the whole on a roller-mixer such that the cylinder assembly

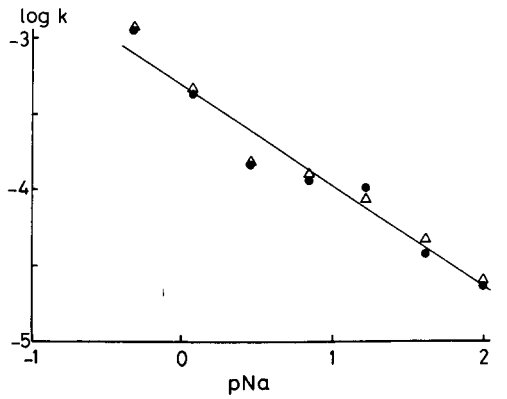

FIG. 1. Logarithm of the transport velocity constant k (min^{-1}) of warfarin as defined in equation 1, at pH 11, as a function of the negative logarithm of the Na^+ concentration (pNa). The symbols refer to duplicate experiments.

was turned around at 60 rev min^{-1}. Warfarin concentration was measured at 308 nm at distinct time intervals. This permitted the calculation of the concentration decrease in compartment A.

Experiments showing the pH-dependent transport of warfarin in the pH region 6–8 were performed using phosphate buffer in compartment A and borate buffer pH 9 in compartment B. These results were analysed as described earlier (Kroon & Janssen 1982).

All experiments were performed at room temperature. No detectable adsorption of warfarin to the walls of the cylinder assembly occurs under the present conditions.

In the regression equations the standard deviation of the regression coefficients is given in parentheses. Also the number of observations n, the correlation coefficient, r, and the standard error of estimate, s, are given.

Results and discussion

The model used here consists of two compartments, A and B, separated by an octanol-impregnated membrane. The composition of the solution in the two compartments is such that reversible kinetics have to be assumed. For the transfer of a single compound the following equation will hold

$$\ln \frac{[A]_t - [A]_\infty}{[A]_o - [A]_\infty} = -2\,kt \qquad (1)$$

In this equation $[A]_o$, $[A]_t$ and $[A]_\infty$ represent the warfarin concentration in compartment A at time zero, at time t and at equilibrium, respectively; k represents the transport rate constant (min^{-1}). It is assumed that $[A]_o$ and $[A]_\infty$ can be calculated from known initial concentrations and known volumes of the compartments, whereas $[A]_t$ is found experimentally. Equation 1, given in more detail by Albery et al (1976) was recently used by Guy & Hadgraft (1981). The latter authors when measuring the transfer of salicylic acid, corrected their concentrations for the degree of dissociation, which will yield a pH-independent transport rate constant. We used total concentrations not corrected for any secondary reaction. This means that the measured k will depend on experimental conditions. For example in the pH region 6 to 8 a strong pH dependence was observed given by

$$\log k = -0{\cdot}50\ (\pm\ 0{\cdot}04)\ pH + 0{\cdot}79\ (\pm\ 0{\cdot}25) \qquad (2)$$
$$(n = 4,\ r = 0{\cdot}995,\ s = 0{\cdot}06)$$

To investigate the influence of Na^+ we made experiments in a region where log k is pH-independent. It was found that in the pH region 10–11·8 log k did not show any pH dependence. Therefore the influence of Na^+ on the warfarin transport rate was studied at pH 11·0.

In all experiments a lag time was observed ranging from 10 min at high Na^+ concentration to 30 min at low concentration. The transport was followed over a period ranging from 45 min to 4·5 h. This period was not long enough to reach equilibrium in all compartments as t½ ranges from 5 to 240 h. This follows from the results to be reported below. During this period no deviations from linearity were observed. No differences were observed when the initial warfarin concentration was lowered by a factor of 10.

The influence of Na^+ on the observed transport rate constant at pH 11 is given in Fig. 1. There is a significant influence of Na^+ which may be represented by

$$\log k = -0{\cdot}67\ (\pm\ 0{\cdot}04)\ pNa - 3{\cdot}31\ (\pm\ 0{\cdot}05) \qquad (3)$$
$$(n = 14,\ r = 0{\cdot}977,\ s = 0{\cdot}121)$$

in which pNa represents the negative logarithm of the Na^+ concentration. This Na^+ dependence is not unexpected considering the observed dependence between apparent partition coefficient (P_{app}) and pNa for warfarin in the water-octanol system (Van Der Giesen & Janssen 1982) as given in equation 4.

$$\log P_{app} = -1{\cdot}00\ pNa + 0{\cdot}62 \qquad (4)$$

Relationships between transfer rate constants and log P values have been observed (see for example Kubinyi 1979; Van De Waterbeemd et al 1980). The surprising fact in equation 3 is the slope value near 0·7, which contrasts with the value of 1·0 in equation 4. The question is which mechanism may cause such a slope value? First, for a single species the transport over a membrane already involves five steps, assuming that the membrane behaves isotropically (Albery et al 1976). When another equilibrium such as ion-pair formation is involved the situation becomes even more complex. In principle the transfer kinetics of Na^+, of the warfarin anion and of the ion pair should be considered. These rate constants are linked via the ion pair formation constants, but the number of rate constants to be considered remains large. (See, for example, Stehle & Higuchi 1972, who treated the problem of transport of acids and bases in the presence of buffers in a system as used here.) The problem of ion-pair formation may be

analogous. Simplification is possible when one or more of these steps are not rate-limiting. Nordgren & Sjöden (1978) and Kolstad & Nordgren (1980), for example, in their study on the extraction of ion pairs, concluded that ions are extracted rapidly from an aqueous to an organic phase, followed by a slow ion-pair formation step in the organic phase. Whether this is a general mechanism has to be proved. In our system the transport kinetics may depend on ion-pair formation constants in both phases. As only an approximate value of this constant in the aqueous phase is available (Van Der Giesen & Janssen 1982) we did not try to explain the observed pNa dependence.

A slope value near 0·5 was also observed in the pH-dependent experiments (see eqn 2). In view of the finding that warfarin may be transported as a sodium ion-pair, these results need to be reconsidered. At pH 8 the pH-dependent log k amounts to −3·21. This constant, however, contains a contribution from the sodium ion-pair transport. Correcting for this using equation 3 (pNa = 1) gives a value of −3·29. At pH 7 the observed log k from equation 1 amounts to −2·71, which becomes −2·73 after correction. The conclusion is that the observed slope is the pH profile in the pH region considered hardly depends on the contribution of the sodium ion-pair.

A further complicating factor in this system may be the variation in ionic strength during the experiment. The pNa values given in Fig. 1 have been calculated from the added amount of NaCl without activity effects being considered. However, linear relationships between Na^+ concentrations and the signal of a sodium-sensitive glass electrode was observed, indicating justification of the use of calculated pNa values instead of measured pNa activities (Van Der Giesen & Janssen 1982).

The lag time mentioned above may be explained by the observed pNa dependence. At higher Na^+ concentration, the higher concentration of ion-pairs will lead to shorter membrane saturation times, i.e. to shorter lag times. The exact relationship between lag time and pNa was not further investigated.

This phenomena of ion-pair formation may be related with in-vivo absorption observations. It is known that sodium warfarin is absorbed nearly completely and rapidly as has been observed for its direct instillation into the duodenum at pH 8 (Deckert 1974). At this pH warfarin is almost entirely in its anionic form. From our in-vitro experiments we find that the pH-dependent transport constant at pH 8 corrected for the contribution of the ion-pair is about −3·29 (or $k = 5{\cdot}1 \times 10^{-4}\ min^{-1}$). On the other hand, from equation 3 it follows that at pNa = 0·8 to 0·9 (correspond ing with the Na^+ concentration in the gut) log k is near −3·9 (or $k = 1{\cdot}3 \times 10^{-4}\ min^{-1}$). This means that in principle 20% (viz. 1·3/(1·3 + 5·1)) of the total warfarin may be transported as a sodium ion pair.

Ion-pair formation and its influence on absorption has been described earlier (see for example Tomlinson et al 1982 and references therein). Mostly only complex formation with large organic ions is considered. In view of the ubiquitous presence of Na^+, complex formation with this ion should be studied in more detail.

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