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IONTOPHORETICALLY INDUCED TRANSDERMAL DELIVERY OF SALBUTAMOL

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ABSTRACT

Passive and iontophoretically assisted transport of salbutamol from a hydrogel matrix has been studied *in vitro* across a model membrane and *in vivo* and *in vitro* across human stratum corneum.

In vitro experiments were conducted in specially designed glass diffusion cells and initial experiments using cellophane membranes showed that the passive release of salbutamol from the hydrogel across the membrane was matrix-controlled and that this transport could be significantly enhanced by the application of an iontophoretic current. Passive diffusion of salbutamol from a gel matrix containing the sulphate through stratum corneum membranes, was found to be negligible over a 24 hour period, but significant transport could be induced using current densities in the range 0.04-0.4mA cm^{-2}. The quantity of drug transported increased linearly with time, and was proportional to the current density used.

Preliminary *in vivo* trials in two subjects showed significant electrically assisted systemic delivery of the drug using iontophoretic currents of 0.1 - 0.2mA over a 4 hour period.

INTRODUCTION

The use of iontophoresis to enhance the transport of ions across the skin both *in vitro* and *in vivo* has been widely documented[1,2,3]. Although this technique has mainly been used for local administration it may have greater potential as a method for systemic delivery of drugs that normally do not penetrate the skin[4]. Iontophoresis has all the usual advantages of conventional transdermal delivery such as the avoidance of first pass metabolism and irregular absorption associated with oral administration. It also widens the range of drugs that may be delivered through the skin and offers more precise control over the rate of absorption. Furthermore, there is the possibility of using biosensors for direct feedback to the iontophoretic device for continuous monitoring of drug delivery and self-regulation.

We have previously reported the enhancement of drug transport through cellophane and stratum corneum membranes *in vitro* using iontophoresis[5,6].The present work demonstrates that transdermal transport of salbutamol sulphate may be induced *in vivo* as well as *in vitro*.

MATERIALS AND METHODS

Preparation of Membranes for *In Vitro* Studies

The Visking™ 18/32 cellophane membranes were pretreated by boiling several times in distilled water.[7]

The samples of human cadavar skin were taken from the mid-abdominal area within 48 hours post mortem. The method of Kligman and Christophers[8] was used to remove the stratum corneum and epidermal layers. The trypsinisation step to remove the epidermis was omitted. This was considered to be unnecessary as it is known that the stratum corneum is the rate limiting barrier to diffusion of drugs through the skin[9]. The membranes were dried overnight at 25% R.H. and stored in sealed packages at 0-2°C until required.

In Vitro Experiments

Passive and electrically assisted transport of salbutamol were carried out in specially modified glass diffusion cells as previously reported[5,6]. The receptor

compartment of the cells contained an isotontic phosphate buffer at pH=7.4, the temperature was kept at 37°C in a thermostatted water bath and the elution solution was stirred to maintain hydrodynamically uniform conditions. The passive transport of salbutamol sulphate from the hydrogel discs across both Visking and stratum corneum was first investigated. The release of the drug was then studied with an electrical current being used to assist the transport across the membrane and these results were compared with release profiles which were obtained from the passive experiments. The salbutamol sulphate was used as received.

The electrical circuit for iontophoresis consisted of two platinum electrodes with a galvanostatically controlled d.c. power supply and with the neccessary current and voltage monitoring equipment. Constant current densities in the range 0.04-0.4mA cm^{-2} were used.

Samples were withdrawn from the diffusion cells at predetermined time intervals and replaced with fresh drug-free solution. These samples were then analysed using an isocratic reverse phase HPLC method.

Chromatographic Conditions:

A μBONDAPAK C_{18} Radial-PAK™ 8mm x 10cm column was used and the mobile phase was water : acetonitrile (92:8) containing 12g/l NaH_2PO_4. The flow rate was set at 2ml/min and the samples were injected in 20μl aliquots by a Waters 712 WISP™ autosampler. Detection of the drug was by a Waters model 455-LC u.v. spectrophotometer at a wavelength of 276nm. An external standard method was used by a Waters 740 Data Module integrator to calculate the concentrations of salbutamol in the samples. A sharp peak was observed for salbutamol at a retention time of 6 mins.

The reproducibility of the method was approximately 0.1% relative standard deviation. The integrator was programmed to recalibrate the standard after every five samples to provide a high degree of accuracy in the analysis.

In Vivo Electrically Assisted Transdermal Delivery

The iontophoretic transdermal patches complete with electrode assembly were applied to the volar aspect of the forearm of two subjects. For the first two hours

no current was applied. Then a galvanostatically controlled current of 0.1mA was applied for a further two hours. Immediately following this the current was increased to 0.2mA and again maintained at this value for two hours. The current application was then terminated but the transdermal preparation was left on the arm for three hours before being removed. Blood samples were taken regularly. These were analysed using a VG 12250 G.C. mass spectrometer[10]. The study was done using patches containing 2 and 4mg salbutamol sulphate and with the same volunteers. The volunteers' heart rate, blood flow and blood pressure were carefully monitored.

RESULTS

In Vitro Studies using Cellophane Membranes.

The release rates for passive diffusion of salbutamol sulphate from the moulded hydrogel matrix across a cellophane membrane were found to be matrix controlled with the quantity released per unit area, Q($mgcm^{-2}$), being proportional to the square root of time, t, according to the Higuchi equation[11],

$$Q'/A = Q = 2C_o(Dt/\pi)^{1/2} \qquad (1)$$

where C_o(mg/ml) is the initial concentration of drug in the gel having an area A(cm^2) and D (cm^2s^{-1})is its diffusion coefficient, and Q' (mg) is the quantity of drug released. This equation holds for up to 30% of the drug released and when the drug is dissolved in the gel matrix[11]. Plots of Q versus $t^{1/2}$ are shown in figure 1. The intercept of the lines with the x-axis indicates a boundary layer/membrane effect. The diffusion coefficient, D, was calculated from the slope of the line obtained from the plot C_o versus $Q/t^{1/2}$ as shown in figure 2 and D was estimated to be 9.11×10^{-7} cm^2s^{-1}.

Electrical assistance of the release using constant current values of 0.1-0.75mA, produced considerable enhancement and the release profiles were approximately linear with time as is evident in figure 3. As illustrated in figure 4, which is for a gel in which C_o was 11mg/ml, the rate of transport, R_i, was

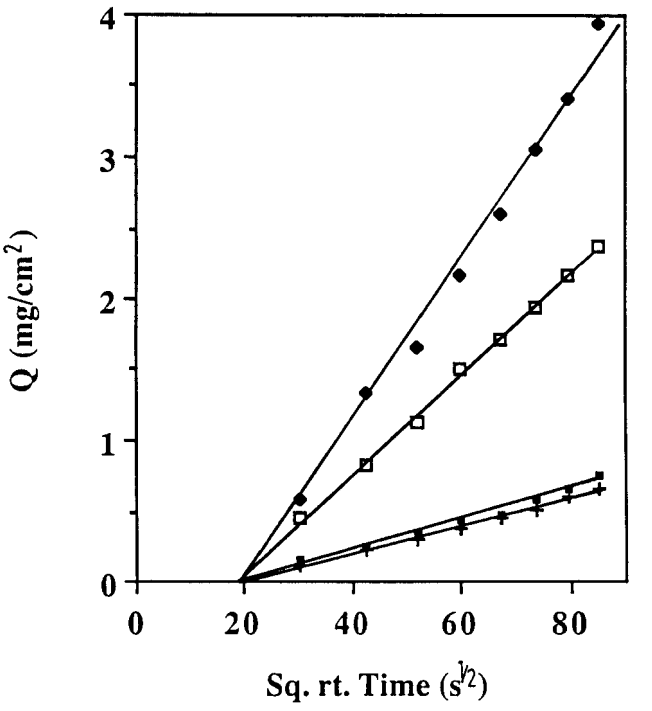


FIGURE 1

A plot of the quantity of drug transported, Q (mg cm^{-2}) vesus $\sqrt{t}$ ($s^{1/2}$) for passive transport of salbutamol through cellophane using various concentrations of drug in the vehicle.+8mg ml^{-1},■11mg ml^{-1},□27.5mg ml^{-1},◆55mg ml^{-1}.

found to depend on the magnitude of the applied current, i which may be represented by the equation [6]

$$R_i = f_i\, i \qquad (2)$$

where f_i is an iontophoretic constant. The constant, f_i is the slope of the line obtained from the plot R_i (mg s^{-1}) against the current, i, (mA) and was found to be 1.03 x 10^{-3}mg $s^{-1}mA^{-1}$. Using Faraday's laws the maximum rate of transport, R_F(mg $s^{-1}mA^{-1}$) due to the current, i, for a monovalent ion is given by ,

$$R_F = Mi/F \qquad (3)$$

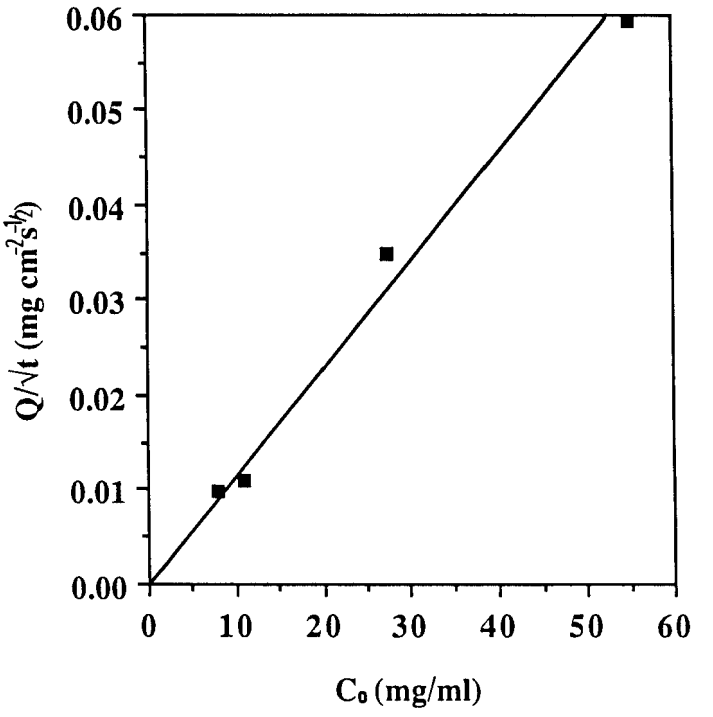


FIGURE 2

The relationship observed between the initial salbutamol sulphate concentration in the gel, C_o and $Q/\sqrt{t}$.

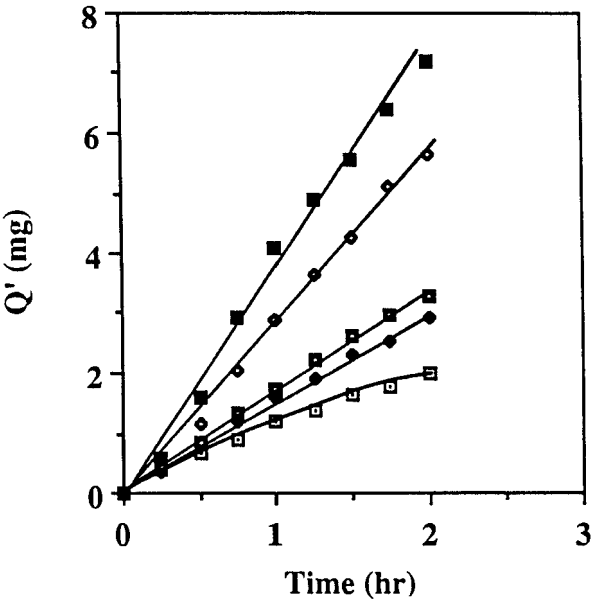


FIGURE 3

Release profiles for iontophoretic transport of salbutamol from a gel having an area of 2.76cm^2 through a cellophane membrane using current values of ⊡0.0mA,◆0.1mA, ◘ 0.25mA,◇0.5mA,■0.75mA.

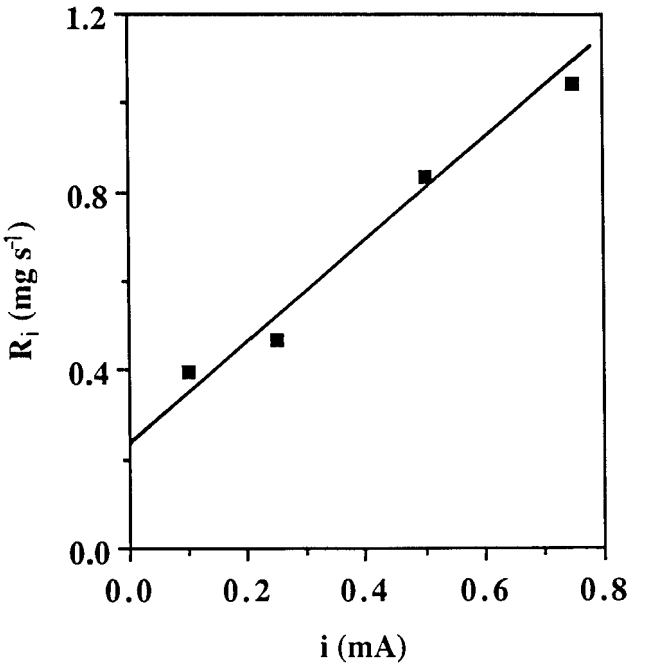


FIGURE 4

A plot of current, i (mA) against rate of transport, R_i (mg s^{-1}) for iontophoretic transport of salbutamol across a cellophane membrane.

where M is the molecular weight in grams and F is the Faraday constant. For salbutamol the proprtionality constant between R_F and i is 2.48 x 10^{-3} mg $s^{-1}mA^{-1}$. Therefore, an effective transport number of 0.41 was determined for the assisted transport of salbutamol when C_o was 11mg/ml. At the higher concentation of 27.5mg/ml an effective transport number of 0.82 was calculated in the same way. However, it should be emphasised that the transport of the drug is not entirely due to the iontophoretic current but that there is a contribution from passive diffusion. This passive contribution increases with an increase in the drug concentration in the gel. As a consequence, the largest relative enhancements were observed for the gels containing the lowest concentrations. Similar findings were reported earlier for nicotine[5] where an effective transport number of unity was obtained using an initial concentration of 33mg/ml.

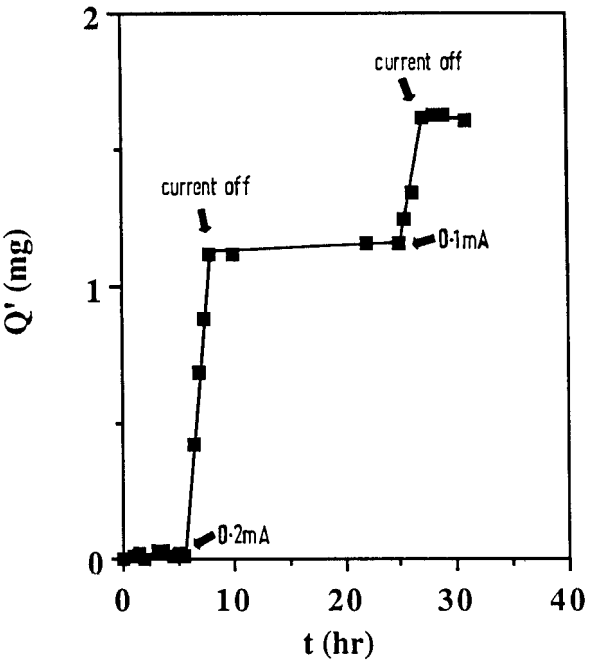


FIGURE 5

Alternate passive and iontophoretic transport of salbutamol sulphate through human stratum corneum *in vitro* using currents of 0.1 and 0.2 mA from a gel containing 8mg/ml. Application of the current is indicated by the arrows.

In Vitro experiments using Stratum Corneum Membranes

In vitro passive diffusion of salbutamol sulphate through stratum corneum was below the level of detection over a period of 24 hours. This contrasts with results obtained for nicotine[6] where passive transport of the drug through the skin was membrane controlled producing linear release profiles. However, the use of electrical assistance resulted in significant transport of the salbutamol. Release profiles from a gel matrix containing 8mg/ml were linear as shown by the example in figure 5 where currents of 0.1mA and 0.2mA were applied consecutively during the course of the experiment. On cessation of the current it was evident that transport of the drug again became negligible indicating that the barrier

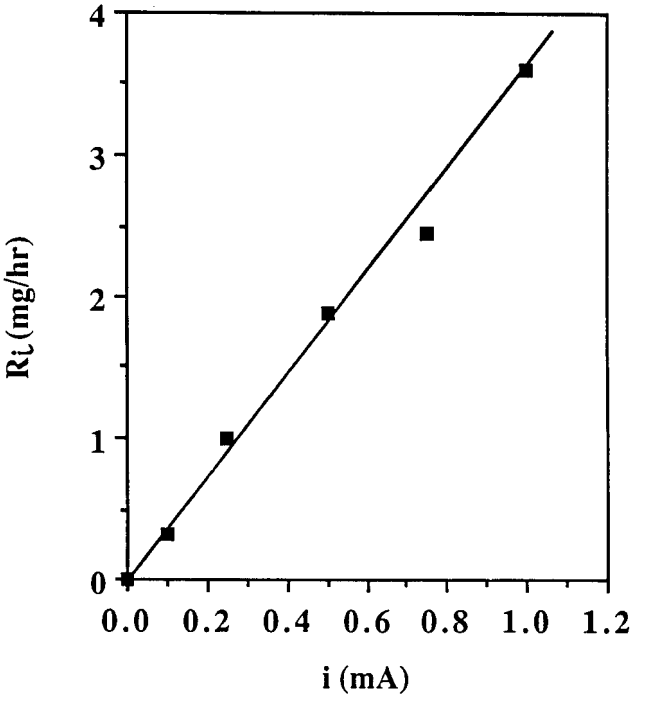
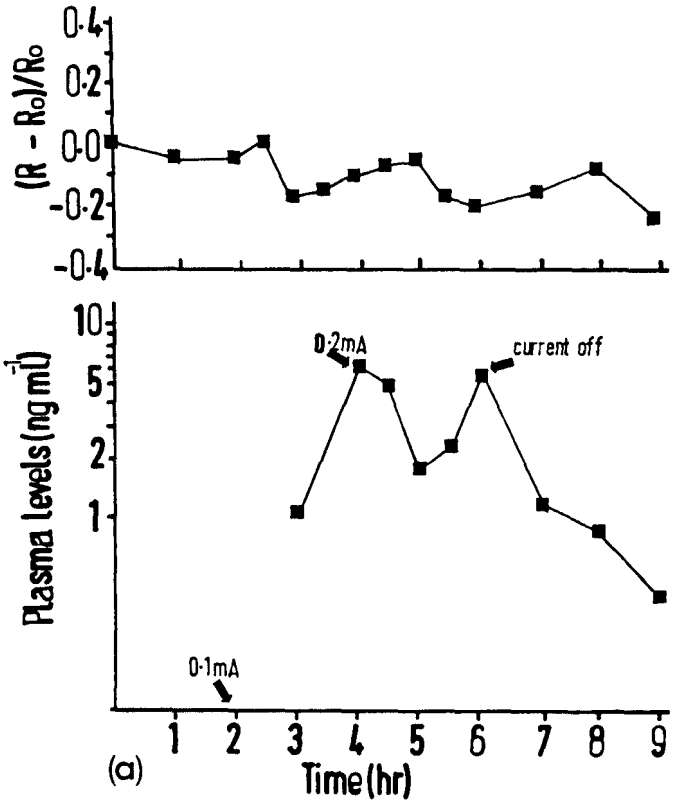


FIGURE 6

The relationship between current, i(mA) and the rate of transport, R_i (mA s^{-1}), for electrically assisted transport of salbutamol through human stratum corneum membranes.

properties of the skin had not been altered by the iontophoretic transport. The relationship between the current applied, i(mA), and the rate of transport R_i (mg s^{-1}) was again linear (figure 6) and the slope of this line, f_i, was calculated to be 9.71×10^{-4} (mg $s^{-1} mA^{-1}$). The effective transport number in this case was 0.39. Burnette and Ongpipattanakul (1987)[12] observed transport numbers of 0.61-0.63 for Na^+ and 0.26-0.30 for Cl^- using current densities in the range 0.078-0.23 mA cm^{-2}. In contrast to or studies using cellophane membranes there is not expected to be any passive transport of the drug across the skin and the transport number may therefore represent the true situation for iontophoretic transport of the salbutamol ion.



FIGURES 7a and 7b

Semilog plot of salbutamol plasma levels versus time for electrically assisted transport *in vivo* for a transdermal patch containing 2mg drug using currents of 0.1 and 0.2 mA as indicated by the arrows for subject number 1 (a) and number 2 (b). Heart rates are plotted above each figure as the fraction change ($R-R_o/R_o$) versus time(hr) where R_o (beats min^{-1})is the heart rate at time zero and R (beats min^{-1}) value at time, t.

On the basis of these experiments ethics committee approval was obtained to study the transdermal iontophoretic delivery of salbutamol in two normal human volunteers from patches containing 2mg and 4mg and in which the concentrations of drug were 4 and 8mg/ml respectively. From our *in vitro* data it was estimated that approximately 0.2 and 0.4mg/hr could be transported from gels containing these low concentrations using currents of 0.1 and 0.2mA, *c.f.* figure 5. The normal dose for salbutamol by slow i.v. injection is approximately 0.25mg[13].

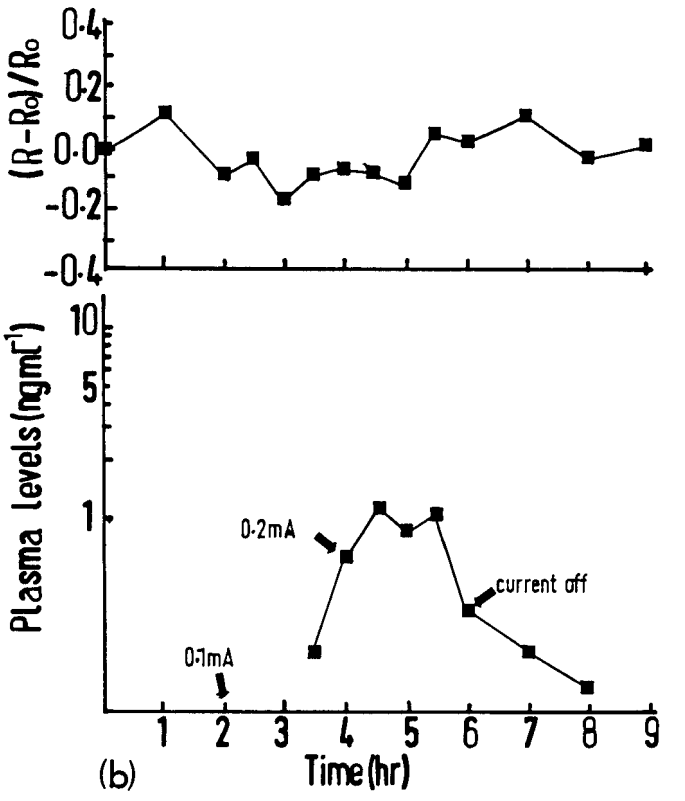
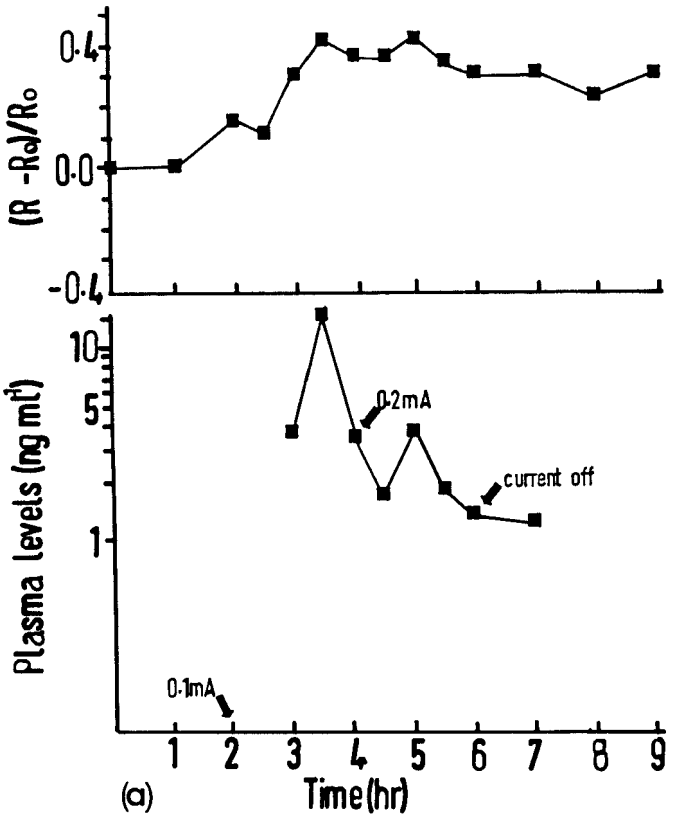


FIG. 7 CONTINUED

In Vivo Trials

Semilog plots of plasma levels versus time for both subjects following application of the 2mg patch and current application for 2 hours at 0.1mA and 2 hours at 0.2mA are shown in figures 7a and 7b. Although the measured plasma levels were somewhat erratic, significant amounts of salbutamol were evidently absorbed by both subjects two hours after current application. Plasma levels were considerably higher and drug was detected earlier in subject number 1. In the absence of current, low plasma levels were detected in this subject. In contrast, application of the patch without current to subject 2, gave no detectable plasma



FIGURES 8a and 8b

Semilog plot of salbutamol plasma levels versus time for electrically assisted transport *in-vivo* for a transdermal patch containing 4mg drug using currents of 0.1 and 0.2 mA as indicated by the arrows for subject number 1 (a) and number 2 (b). Heart rates are plotted above each figure as in figures 7a and 7b.

levels and much lower levels than those observed in subject 1 when current was applied. On cessation of the current, plasma levels of both subjects declined indicating elimination half-lives in the range 1-2 hours. These rates are faster than the published range of 2-6 hrs following i/v and oral administration[14]. When the iontophoretic experiment was repeated using patches containing 4mg of drug, higher overall plasma levels were obtained, absorption again being better in subject 1, (figures 8a and 8b). Areas under the plasma concentration versus time curve were calculated using the trapezoidal rule and are summarised in table 1.

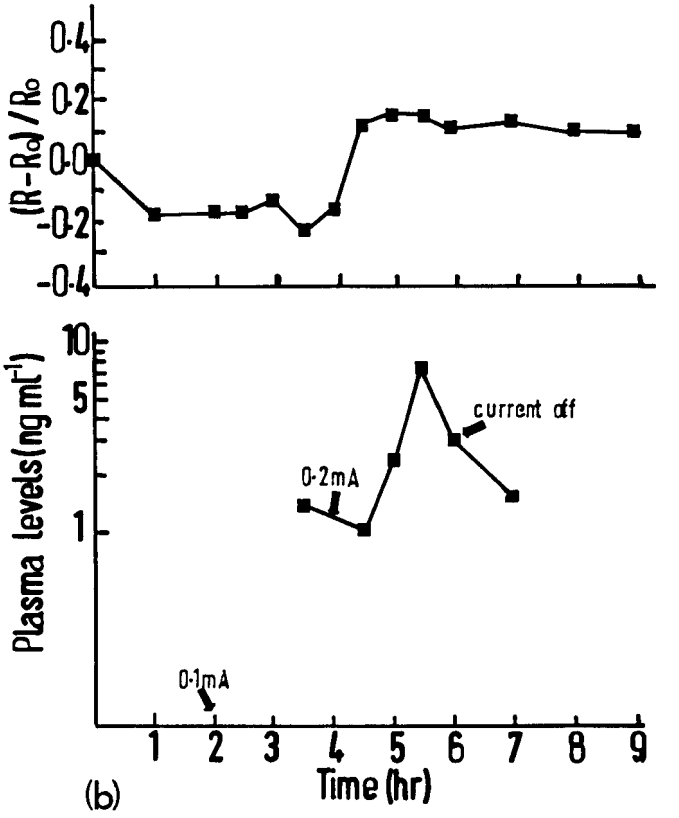


FIG. 8 CONTINUED

TABLE 1

AUCs for Plasma Levels vs Time for *In Vivo* Passive and Active Transport of Salbutamol.

Subject Number	AUC using Passive patch. (ng $ml^{-1}hr$)	AUC using 2mg patch (ng $ml^{-1}hr$)	AUC using 4mg patch (ng $ml^{-1}hr$)
1	2.56	13.978	17.460
2	0.0	2.573	9.710

Changes in heart rate are also illustrated in figures 7a, 7b, 8a and 8b. When the 2mg patch was applied there was very little change in heart rate during the course of the experiment. However, when the larger dose of 4mg was used the heart rate for both subjects was raised after the application of the iontophoretic current.

When these results are taken in conjunction with the published pharmacokinetic parameters for salbutamol[14], following i/v and oral administration, they suggest that over the four hour period during which the iontophoretic current was applied, of the order of 10% of the dose applied in the transdermal patch reached the systemic circulation.

CONCLUSIONS

In the gel matrix system used the rate of transport through cellophane membranes was matrix controlled and the diffusion coefficient of salbutamol sulphate in the gel was $9.11 \times 10^{-7} cm^2 s^{-1}$. Application of an iontophoretic current resulted in enhanced transport of the drug and the rate of transport became proportional to the current intensity.

In vitro experiments indicated that salbutamol did not penetrate the stratum corneum passively in detectable amounts but that significant transport could be obtained when the process was assisted iontophoretically.An effective transport number for this process was calculated to be 0.39. Preliminary *in vivo* trials confirmed that passive transport of salbutamol was negligible and that iontophoresis could induce systemic delivery of the drug. Using current values of 0.1 and 0.2mA, of the order of 10% of an applied dose of 2mg and 4mg reached the systemic circulation with an elimination half-life in the range 1-2 hours.

These investigations demonstrate, through a logical progression of *in vitro* experiments using model membranes and stratum corneum to *in vivo* testing in human volunteers, that iontophoresis maybe an effective and safe method for systemic delivery of salbutamol.

ACKNOWLEDGEMENTS

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FOOTNOTE

TM - Trade Mark

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