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CARRIER-MEDIATED TRANSPORT OF DAUNORUBICIN, ADRIAMYCIN, AND RUBIDAZONE IN EHRLICH ASCITES TUMOUR CELLS

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Abstract—The transport of DNR, ADR, and RBD was studied in Ehrlich ascites tumour cells *in vitro*. Within the first min intracellular drug binding, judged by measuring drug uptake in lysed cells considerably exceeded the uptake in whole cells, indicating that membrane transport is rate-limiting to drug uptake. Cellular net uptake showed a biphasic uptake pattern with a rapid phase within 5 sec and a subsequent linear phase of 1–4 min. The slope of the linear component was increased by sodium azide, suggesting an energy dependent efflux component for these drugs. To obtain near unidirectional influx the transport experiments were performed on cells inhibited by sodium azide. Several findings indicated a carrier mediated inward transport mechanism: (1) a high structural specificity for influx, (2) the influx exhibited simple saturation kinetics for each drug, (3) competition for transport was demonstrated by structural analogues, (4) an effect compatible with counter transport was demonstrated for DNR and (5) the influx of DNR was highly temperature-sensitive ($Q_{10} = 2.9$). Considerable variation in both K_m and V_{max} was noted between the three derivatives. K_m and V_{max} were highest for DNR and lowest for ADR. Discrepancies between K_i and K_m indicated that ADR and RBD were less efficient inhibitors of [^{3}H]DNR influx than would be expected from their K_m values. From the distribution of the drugs between chloroform/buffer and olive oil/buffer RBD appeared to be the most lipophilic drug; DNR proved nearly nine times more lipophilic than ADR.

Some characteristics of the uptake of the anthracyclines in Ehrlich ascites tumour cells were described in a previous study from this Laboratory [1]. Like others, we found a considerable difference in net uptake between DNR, ADR, and RBD [2–7]. This difference could not be explained by differences in the affinity for intracellular binding sites and therefore probably originates from a difference in the membrane transport of the drugs.

The purpose of the present study was to investigate the inward transport of the anthracyclines in Ehrlich ascites tumour cells and to elucidate the influence of substitution in the DNR molecule at C-14 (ADR) and at C-13 (RBD).

MATERIALS AND METHODS

Tumour cells and media. The Ehrlich ascites tumour cells were produced by standard methods as previously described [1]. Tumour cells were washed four times in ice-cold Ringer solution. Cell agglutination was prevented by addition of heparin (100 units/ml) to the initial washing fluid. Packed cell volume was determined by centrifugation of samples at 5500 *g* for 8 min in a hematocrit centrifuge. At pH 7.45 at 37° the medium contained 62.0 mM NaCl, 5.0 mM KCl, 1.3 mM $MgSO_4$ and 60 mM sodium phosphate.

Incubation and sampling procedure. In order to reduce distribution time, cells were added to the medium as follows: Medium containing the drug (and inhibitor, if any) and medium containing the cell suspension (10.0% by vol.) were incubated separately at 37° for about 10 min. Unless otherwise stated the experiment was started by transferring the cell suspension to the drug solution during rapid stirring, forming a final cell suspension of 0.5% by volume corresponding to $2.21 \pm 0.09 \times 10^6$ cells/ml (mean ± S.D.). Serial samples of 2000 μl were withdrawn at varying intervals (in experiments of initial rate the intervals were 5 sec). The flux reaction was terminated by rapid injection of the cell suspension into conical centrifuge tubes containing 8 ml ice-cold Ringer solution. Thereafter the cells were separated by centrifugation of 2350 *g* for 1 min (Labofugen 2, Heracus Crist, Germany), and the pellet was washed twice in ice-cold Ringer solution to remove extracellular drug. The supernatant liquid was discarded and the wash fluid was drained as far as possible from the pellet. Using [^{3}H]methoxyinulin as marker, the remaining extracellular wash fluid was determined as 23 ± 4 μl (S.D.) per test tube (six determinations), and the results were corrected on this basis.

Preparation of lysed cells. Washed cells in a suspension of 0.5% by volume were incubated for 5 min in a buffer of the following composition: Sucrose 250 mM, $CaCl_2$ 5 mM, Nonidet P 40 0.1% (v/v) and

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The abbreviations used are: DNR, daunorubicin: ADR, adriamycin; RBD, rubidazone.

Tris–HCl 25 mM (pH adjusted to 7.45). When observed under the microscope all cells were found to be lysed but the nuclei remained intact. The sampling procedure was performed as described for whole cells. Thereafter, the nuclei was separated by centrifugation of 6130 *g* for 1 min; no washing procedure was included in these experiments.

Determination of cellular drug content. Total drug was extracted from the drained cell pellet with 1500–3000 μl 0.3 N HCl 50% ethanol solution according to a method by Bachur *et al.* [8]. The fluorescence of the supernatant solution was determined in Aminco Bowmann spectrofluorometer (excitation 470 nm: fluorescence 585 nm), and drug concentration was determined by comparison with spectrophotometrically adjusted standards [1]. Fluorescence of samples extracted from untreated cells was subtracted from the experimental sample.

Determination of [^{3}H]DNR. In experiments with [^{3}H]DNR the radioactivity was determined in a Beckman LS-250 liquid scintillation spectrometer. In measurements of cellular drug content the drug was extracted as described above. 250 μl aliquots of each extract were placed in a scintillation counting vial and evaporated to dryness before addition of scintillation fluid. In measurements of [^{3}H]DNR in the medium 100 μl sample was added to 10 ml scintillation fluid (composition: POP 7 g; POPOP: 300 mg; naphthalene: 100 g; dioxane 1000 ml). In experiments employing [^{3}H]DNR and unlabelled drug simultaneously, the influence of quenching was negligible.

Calculation of initial rate. The rate of initial uptake was determined by multiplying the slope of the initial linear uptake phase by the average drug concentration in the medium during the sampling time (change in extracellular concentration during the flux period was approximately 10% of the initial value). The slope of the initial linear phase was determined by the method of least squares after correction for "adsorption component" (see below). The rate of uptake is stated as μg drug/10 μl packed cells/min. In replicate measurements (six determinations) the S.D. of rate of uptake was calculated as 3.6%. For comparison between the derivatives, the mol. wt are 564, 580, and 682 for DNR, ADR, and RBD.

Metabolism. The metabolism of DNR, ADR and RBD in Ehrlich ascites tumour cells has previously been evaluated in this Laboratory [1, 9]. By a thin-layer chromatography technique the metabolism was found to be below 10 per cent for the three derivatives during a 1 hr incubation. Our present studies were of such short duration that problems of metabolic modification could be disregarded.

Chemicals. DNR, ADR, both as hydrochlorides were obtained from Farmitalia Company, Milan, Italy. RBD (benzoyl-hydrazone DNR) and [^{3}H]DNR (0.5 mCi/mg) were kindly supplied by Rhone-Poulenc Research Laboratory, Paris, France. According to the analysis performed by the Rhone-Poulenc Laboratory, the RBD product contained 3–9% DNR. The radiochemical purity of [^{3}H]DNR was determined as 90 per cent. Sodium azide was obtained from Merck, Darmstadt, Germany, [^{3}H]methoxyinulin (55.5 μCi/mg) from New England Nuclear, Dreieichenhain, West Germany, and Nonidet P40 from Shell, Carrington, England.

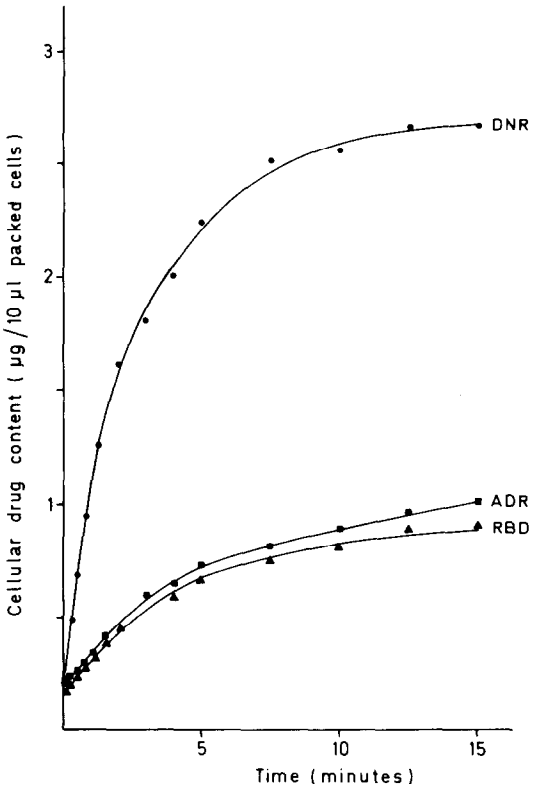

Fig. 1. Time-course of uptake of DNR, ADR, and RBD, by Ehrlich ascites tumour cells at 37°. Suspensions of cells (5 μl packed cells/ml) were incubated in the standard medium at 2.5 μg/ml drug, and serial samples were withdrawn at times indicated.

RESULTS

Time-course of uptake. Figure 1 shows the typical time-course of DNR, ADR, and RBD uptake. The uptake is characterized by an early linear phase, whereupon the rate of net uptake gradually decreases. For DNR the linear phase was about 60 sec compared to 2–4 min for ADR and RBD. For each of the drugs the uptake, when extrapolated to zero time, showed an intercept considerably above the point of origin. The uptake component below the point of intersection is believed to be due to a rapid association of drug to sites on the surface of the membrane, and it will here be referred to as the "adsorption component", whereas the linear phase is believed to reflect the initial entry of drug.

In spite of a great similarity as regards the chemical structure, the initial net uptake of ADR and RBD was considerably slower than that of DNR, indicating a high structural specificity in the uptake process [1].

Initial uptake. Figure 2 shows the initial uptake for unwashed whole cells and for cells disrupted by lysis. For each of the drugs most of the binding to lysed cells took place within 5 sec, and within the incubation period the level of drug uptake in lysed cells was considerably above that of whole cells. This finding indicates that membrane transport is the

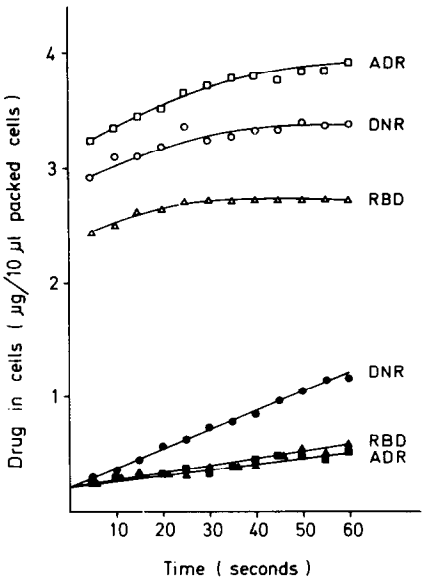

Fig. 2. Comparison of initial drug uptake in whole cells (closed symbols) and lysed cells (open symbols) in suspensions corresponding to 5 μl packed cells/ml. Drug, 2.5 μg/ml was added at time 0. Preparation of lysed cells and sampling procedure as described in Methods. No washing procedure was included in these experiments.

rate-limiting step in the initial cellular uptake of anthracyclines. The relationship between the affinity of the three derivatives for lysed cells was similar to that previously demonstrated for nuclear uptake at 1 hr [1]. Figure 3 illustrates the effect of the metabolic inhibitor sodium azide on initial cellular uptake of DNR at 37°. In repeated experiments this inhibitor significantly enhanced the initial rate of uptake. It can be seen also that the "adsorption component" took place within 5 sec and it was not significantly influenced by sodium azide. The "adsorption component" was nearly proportional to the extracellular concentration. This relationship applied at concentrations below 10 μg/ml, whereas saturation gradually set in at higher concentrations (data not shown). Previously it has been shown that sodium azide inhibits efflux of the anthracyclines from Ehrlich ascites tumour cells [1]. The effect of sodium azide on initial uptake suggests the presence of an energy-dependent efflux component which manifests its effect at a very early stage of the uptake. To avoid this component, subsequent experiments were performed on cells pretreated with sodium azide. To obtain the slope of the linear phase separately the calculations of initial rates are based on the uptake during 50 sec for DNR and during 120 sec for ADR and RBD.

Outward leak. To estimate the order of magnitude of passive efflux during measurements of initial uptake, initial unidirectional efflux of DNR was determined in the medium containing sodium azide (Fig. 4). After incubating the cells for 5 and 50 sec respectively at a concentration of 10 μg/ml, the influx was terminated. After cold-washing the cells were resuspended in a large DNR-free medium containing sodium azide, and the efflux was monitored. In the controls no significant efflux could be demonstrated at 0° within 2 min. At 37° the efflux was biphasic with an initial rapid loss of drug after which the rate of release became constant. The rapid phase probably represents the exit of osmotic free or

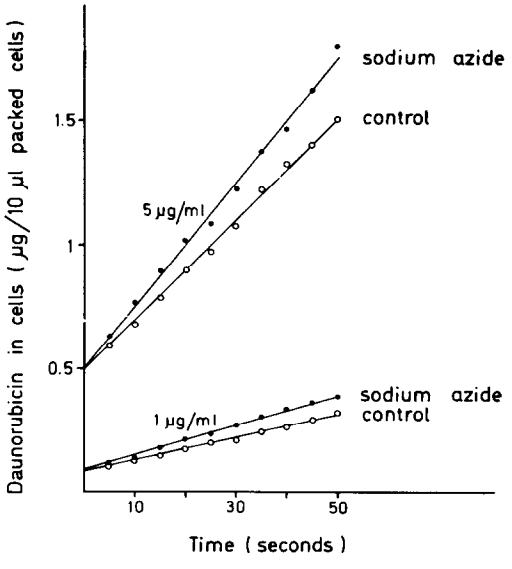

Fig. 3. Effect of sodium azide, upon initial uptake of DNR at 37°. Cells were exposed to 10 mM sodium azide for 10 min prior to exposure to DNR. The osmolarity of the incubation medium was maintained constant by equivalently decreasing the concentration of NaCl.

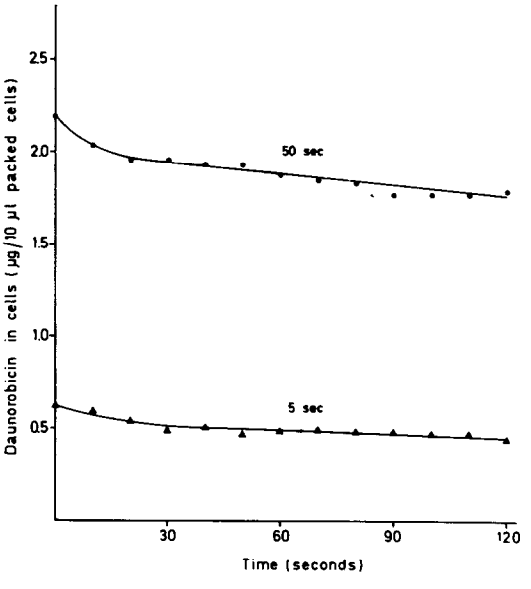

Fig. 4. Initial efflux of DNR. Cells were incubated with DNR at an extracellular concentration of 10 μg/ml. At time 5 sec (▲) and 50 sec (●) the influx process was terminated by rapid injection of icecold Ringer solution into the cell suspension. After being washed twice with 0° Ringer solution, the cells were collected and resuspended in a large volume of DNR-free standard medium including 10 mM sodium azide at 37°. Incubations were ended at time indicated as described above; thereafter the cells were separated by rapid centrifugation. The points at zero time were obtained from cells taken from the washed pellet immediately prior to resuspension at 37°. Cellular drug content was determined as described in Methods.

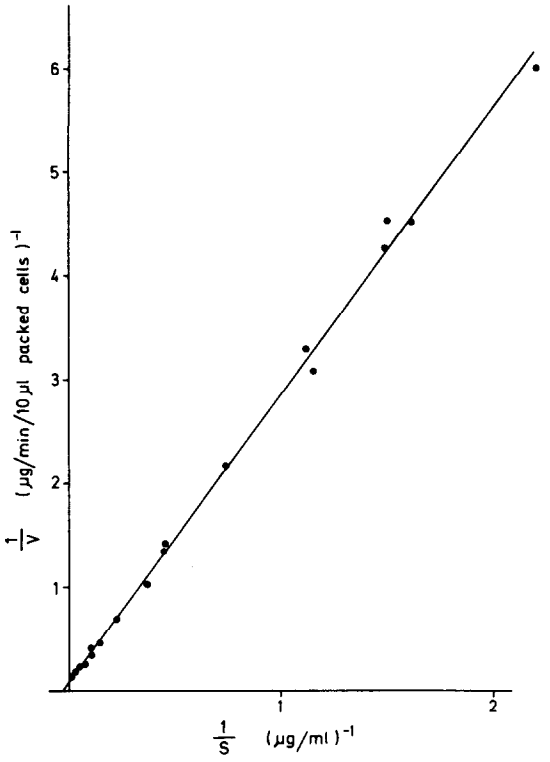

Fig. 5. Lineweaver–Burk plot of uptake of DNR at pH 7.45. Cells were exposed to 10 mM sodium azide for 10 min before being exposed to the drug. Ordinate: reciprocal uptake, abcissa: reciprocal substrate concentration. The line was fitted by the method of least squares.

loosely bound drug from the cell, whereas the slower linear phase probably represents the release of tightly bound drug mainly to DNA in the nucleus [10]. The mean efflux during the first minute is estimated as 0.12 and 0.3 µg/10 µl packed cells/min after loading for 5 and 50 sec respectively. In comparison, the influx was measured as 2.65 µg/10 µl packed cells/min at the same concentration in the same medium (see below). The slow efflux indicates that an almost unidirectional influx is obtained, if the initial entry is determined the medium including sodium azide.

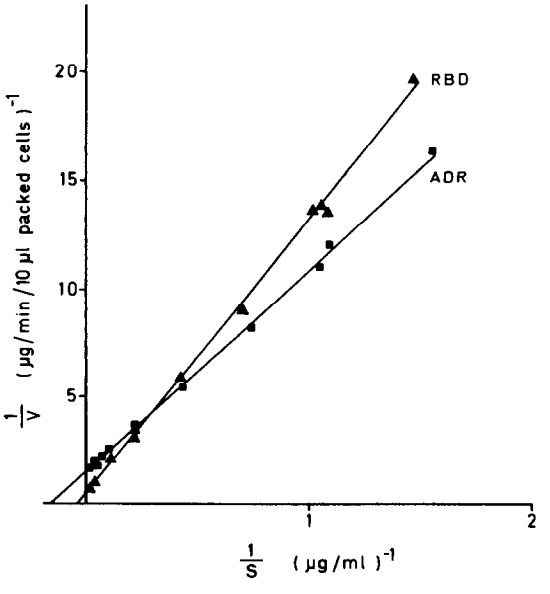

Fig. 6. Lineweaver–Burk plot of uptake of ADR and RBD at pH 7.45. Method as described in Fig. 5.

Table 1. Kinetic constants for the transport of DNR, ADR, and RBD

Drug	K_m (µg/ml)	V_{max} (µg/10 µl packed cells/min)
DNR	35.0 ± 1.4 (29)	12.7 ± 0.5 (29)
ADR	5.72 ± 0.23 (20)	0.64 ± 0.03 (20)
RBD	29.5 ± 1.5 (18)	2.64 ± 0.13 (18)

The data were derived from linear regression equations of Lineweaver–Burk plots of drug uptake. The x intercept $= -1/K_m$, the y intercept $= 1/V_{max}$. The values shown are the mean ± S.E. Figures in brackets indicate the number of transport experiments.

Kinetics of influx. Figures 5 and 6 show the initial rate of uptake of the three anthracycline derivatives over a range of 0.75–50 µg/ml. Presented as a Lineweaver–Burk plot, a straight line with a positive intercept on the ordinate is obtained for each of the drugs, indicating simple saturation kinetics. For DNR the intercept approached the origin, but the deviation was significant ($P < 0.001$). The kinetic constants obtained by analysis of the approximate initial rates of uptake are summarized in Table 1. Considerable variation in both K_m and V_{max} was noted between the three derivatives.

Effect of temperature on influx. Figure 7 and Table 2 illustrate the temperature sensitivity of the influx process for the three anthracycline derivatives. By lowering the temperature from 37° to 27° the Q_{10} for influx was calculated as 2.9, 1.22, and 1.63 for DNR, ADR and RBD respectively (Table 2). A surprising finding is the great variation in Q_{10} between these closely related drugs. The lower Q_{10} for ADR compared to DNR is, furthermore, demonstrable by measuring the influx at 0° (Fig. 7). It appears that now ADR penetrates the membrane significantly faster than DNR.

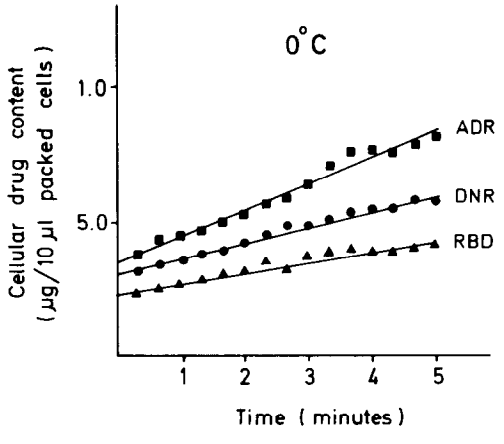

Fig. 7. Time-course of uptake of 5 µg/ml DNR, ADR, and RBD at 0°. Experimental conditions as described in Fig. 5.

Table 2. Effect of temperature on influx of anthracyclines

Drug	Temperature 37° (μg/10 μl packed cells/min)	27° (μg/10 μl packed cells/min)
DNR	1.499 ± 0.025	0.523 ± 0.021
ADR	0.271 ± 0.007	0.222 ± 0.003
RBD	0.305 ± 0.012	0.187 ± 0.004

The experimental conditions were identical to those described in Fig. 5. In all experiments the concentration of drug was 5.0 μg/ml. The values shown are the mean ± S.E. of three experiments done on different days.

Isotope experiments. To investigate the effect of a structural analogue on the influx process, the initial rate of uptake of [^{3}H]DNR was measured in the presence of increasing concentrations of ADR (Fig. 8). It appears that even though ADR exerts a marked substrate-dependent inhibitory effect on the influx of [^{3}H]DNR, the inhibition was significantly lower than predicted, if the kinetic constants for the two drugs were used (Table 1). Thus, at 20 μg/ml ADR (corresponding to 3.5 × K_m), the initial rate of [^{3}H]DNR at 0.25 μg/ml was reduced by 47 per cent, whereas a reduction of 78 per cent was expected. The insufficient inhibition of ADR on [^{3}H]DNR influx might be expressive of a nonsaturable transport of [^{3}H]DNR. To evaluate this point, the influence of DNR on the influx of [^{3}H]DNR was investigated (Fig. 9). As the concentration of [^{3}H]DNR was low compared to that of unlabelled DNR, the rate-concentration relationship was considered linear within the range of the labelled substrate concentration; that is the slope of the linear function reflects the rate-concentration relationship at the level of unlabelled drug concentration. It appears that 50 μg/ml DNR (corresponding to 1.43 K_m) reduced the initial rate of uptake of [^{3}H]DNR by 55.7 per cent, and 100 μg/ml DNR (corresponding to 2.86 K_m) reduced the rate by 70.0 per cent. Using the value of K_m for DNR previously determined as K_i, the reduction was predicted as 58.1 and 73.6 per cent at 50 μg/ml and 100 μg/ml respectively (Table

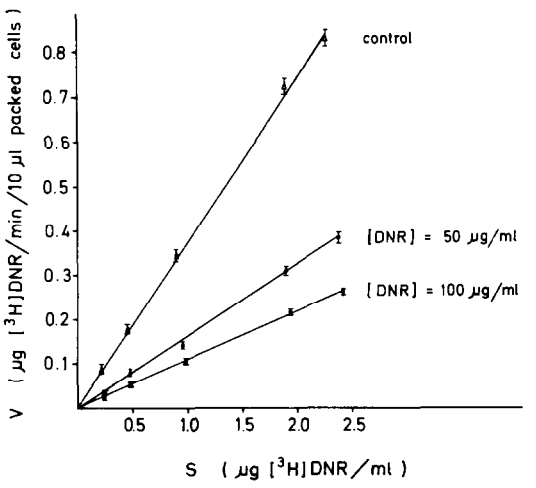

Fig. 9. Self-inhibition of DNR. Initial rate of uptake of [^{3}H]DNR at 0.25–2.5 μg/ml at 37° in the absence (△) and in presence of 50 μg/ml (●) and 100 μg/ml (○) DNR. Experimental conditions as described in Fig. 5. The confidence intervals shown are the S.E.

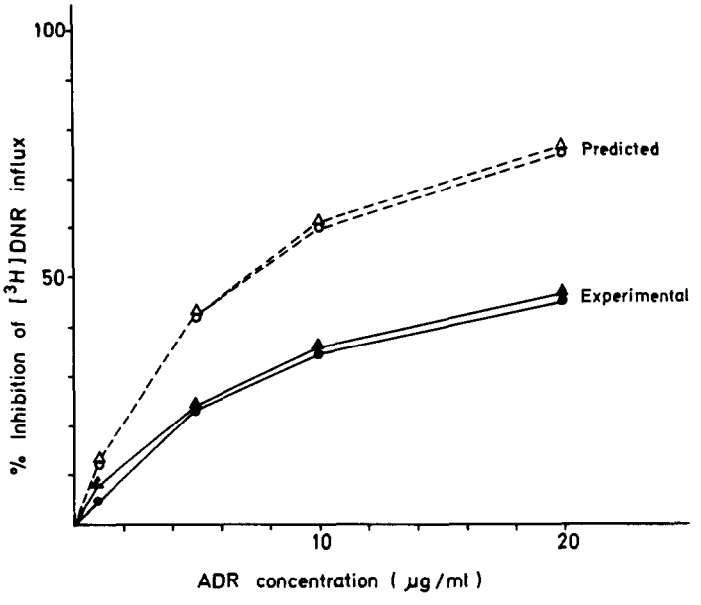

Fig. 8. Effect of ADR on the initial rate of uptake of [^{3}H]DNR corresponding to 1 μg/ml. Experimental conditions were identical to those described in Fig. 5. Interrupted lines represent values predicted from the kinetic constants calculated for DNR and ADR (Table 1), assuming simple competitive inhibition

$$(v = \frac{V_{max} \cdot S}{S + K_m(1 + I/K_i)},$$

where S was the concentration of [^{3}H]DNR and I the concentration of inhibitor).

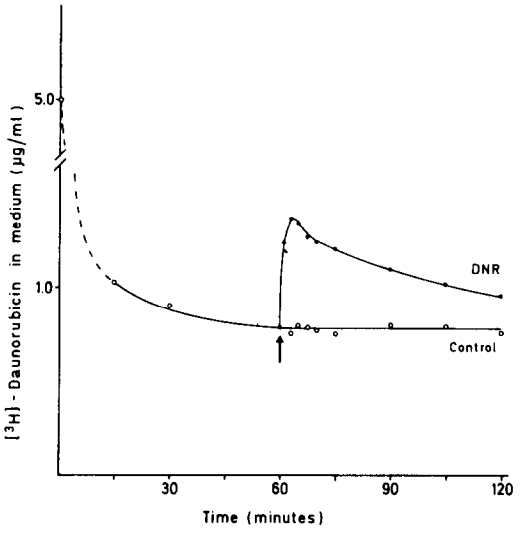

Fig. 10. Counterflow of DNR in Ehrlich ascites tumour cells. Suspensions of cells 1.0 per cent by volume were incubated at 37° in standard medium containing 10 mM sodium azide. At zero time [^{3}H]DNR was added to obtain an initial external concentration of 5 μg/ml, and serial samples were withdrawn at times indicated. At the arrow unlabelled DNR was added to obtain an external concentration of 15 μg/ml. In controls a corresponding volume of isotonic NaCl was added. [^{3}H]DNR in the medium was determined by liquid scintillation spectrometry.

1). This finding indicates that the unspecific component of influx for DNR in negligible.

To compare the inhibition of each of the drugs on influx of [^{3}H]DNR, initial rate of uptake of [^{3}H]DNR was determined in the presence of equimolar concentrations (0.1 mM) of unlabelled drug. At 1 μg/ml [^{3}H]DNR the mean (three experiments) of the inhibition was 60.2 ± 1.9 per cent (S.E.), 65.6 ± 1.3 per cent (S.E.), and 57.3 ± 1.7 per cent (S.E.) for DNR, ADR and RBD respectively. The observed inhibition corresponds to the following K_i values: 36.1 μg/ml for DNR, 29.4 μg/ml for ADR, and 49.6 μg/ml for RBD.

Figure 10 demonstrates that a *trans*-effect of [^{3}H]DNR could be induced by the unlabelled form of the drug. The cells were preloaded with 5 μg/ml [^{3}H]DNR and the concentration in the medium was followed. Due to pronounced intracellular accumulation the extracellular concentration dropped to 0.78 μg/ml at steady state. At 60 min a high concentration of DNR was added to the medium. In serial samples cells were separated by centrifugation, and the exit of [^{3}H]DNR to the external medium was measured. Addition of DNR induced a transitory movement of labelled drug from the cells into the external medium. At the peak the external concentration of [^{3}H]DNR was 1.37 μg/ml which corresponds to an efflux of 14 per cent of the cellular drug content. The intracellular concentration of free drug at time 60 min was estimated by measuring the equilibrium concentration of DNR in the supernatant of a suspension of homogenized cells (1 per cent by vol.) as previously described [1]. In four experiments the average concentration was 0.69 ± 0.03 μg/ml (S.D.). This finding indicates that the efflux of [^{3}H]DNR induced by DNR took place against the concentration gradient; the flux thus probably reflects counter transport.

Table 3. Partition coefficient for the anthracyclines

Drug	Chloroform/buffer	Olive oil/buffer
DNR	28.8	11.4
ADR	3.2	1.3
RBD	41.8	16.2

Lipid solubility was determined by measuring the partition of the drugs between an aqueous phase and two different lipophilic solvents, chloroform and olive oil. 10 μg drug/ml in phosphate buffer at pH 7.0 was shaken with an equal volume of chloroform and olive oil respectively at 25°. The concentration in the buffer and in chloroform was determined spectrofluorometrically. The concentration in olive oil was calculated by subtraction of drug in buffer from the amount added to the solvents. The values indicate mean of duplicate determinations.

Lipid solubility of the anthracyclines. The distribution of the three drugs between chloroform/buffer and between olive oil/buffer was measured (Table 3). The results show that the compounds are most lipophilic. In spite of a great similarity with regard to the chemical structure there was an appreciable difference in the partition coefficient. Thus, DNR proved nearly nine times more lipophilic than ADR. Of the three derivatives RBD was most lipophilic. Comparison of these data with the data of initial rate of uptake revealed that the influx (at 0° as well as at 37°) is not correlated to the lipophilic characteristics as would be expected if the transport were a simple diffusion through a lipid membrane.

DISCUSSION

Several findings presented in this paper indicate that transport of the anthracycline antibiotics in Ehrlich ascites tumour cells was carrier-mediated: (1) a high structural specificity for the initial rate of uptake, (2) the influx exhibited simple saturation kinetics for each of the drugs, (3) competition for transport was demonstrated by structural analogues, (4) an effect compatible with the phenomenon of counter transport was shown and (5) the influx of DNR was highly temperature-sensitive ($Q_{10} = 2.9$).

The anthracycline drugs investigated in this study are chemically closely related. ADR differs from DNR merely in a hydrogen atom at position 14 being replaced by a hydroxyl group. RBD is a semisynthetic derivative of DNR. Compared to DNR, a benzoyl-hydrazone-group is substituted at position 13 in the aglycone component. Nevertheless, these structural modifications of the DNR molecule result in considerable changes in both kinetic constants and in temperature sensitivity for transport.

Some unexpected findings might suggest a high additional component of unspecific transport especially for DNR. Thus V_{max} of DNR was considerably higher than that of ADR and RBD, and the competitive inhibition of ADR on the influx of [^{3}H]DNR was significantly lower than predicted from the saturation kinetics of ADR. However, this explanation is not compatible with the inhibitory

effect of DNR on [^{3}H]DNR transport which agreed well with K_m of DNR serving as substrate. The straight-line double reciprocal plot of the rate-concentration relationship for DNR is also against this hypothesis. Finally, according to the lipophilic characteristics, RBD is expected to diffuse at least as easily as DNR, that is V_{max} of DNR and RBD would be expected to be in the same order of magnitude (Table 3).

Considering DNR separately, all the data are in fair agreement with the classical carrier model [11, 12]. The demonstration of counter flow is strong evidence for mobile carrier involvement [12]. However, the interpretation of counter transport which does not involve a mobile carrier but interaction with sites in the membrane which undergo a transition is also compatible with our findings [13, 14].

A remarkable finding was the discrepancy for ADR and RBD between K_i obtained by competitive inhibition of [^{3}H]DNR and K_m based on saturation kinetics. The findings indicate that these drugs are less efficient inhibitors of [^{3}H]DNR influx than would be expected from their K_m. This discrepancy explains the difference between the observed and predicted inhibition of ADR on the influx of [^{3}H]DNR. Assuming a single common carrier for the anthracycline transport the K_i/K_m ratio reflects that the number of sites which can adsorb ADR and RBD does not correspond to the number of sites which can translocate these drugs; possibly because a fraction of the carrier molecules which form complexes with ADR or RBD do not (or very slowly) carry out the subsequent movement across the membrane and/or the dissociation at the inner surface. On the other hand the discrepancies between K_i and K_m for some of the analogues together with the differences observed in V_{max} and Q_{10} values may also be an expression of involvement of more than one carrier.

None of our findings supported the existence of an active mechanism of the inward transport. Even though, during the uptake phase, the mean intracellular drug concentration very rapidly exceeded the extracellular concentration, this observation is probably not expressive of an influx against the electrochemical gradient (Fig. 1). Thus it was previously demonstrated that the free intracellular drug concentration at steady state is below the extracellular concentration [1]. The effect of sodium azide on the initial rate of uptake supports our previous findings of a mechanism of active efflux for these drugs in the Ehrlich ascites tumour cells (Fig. 3) [1].

In our experiments the "adsorption component" constituted a considerable part of the initial uptake. As this component was present in spite of two cold washes it probably represents a fairly tight binding. Our findings do not permit any conclusion regarding the nature of binding of this component. However, other studies may suggest that phospholipids in the membrane are operative. During transport of amino acids by Ehrlich ascites tumour cells the author has observed complexes between the amino acids and phospholipids which remained intact on silicic acid columns [15]. Complexes between sugars and phospholipids have also been reported and recent findings suggest the formation of complexes between ADR and phospholipids [16–18].

Only a few studies have presented kinetic data concerning cellular anthracycline uptake. A previous study from this Laboratory showed a linear rate-concentration relationship for DNR by a filter disc method [9]. In that study, however, the highest concentration used was 10 μg/ml. Considering the high value of K_m for DNR, this finding is not in conflict with our conclusion. Other studies have demonstrated Michaelis–Menten kinetics for DNR as well as ADR in L1210 cells, suggesting carrier-mediated transport in this cell-type too [4, 19]. Like our study, it revealed considerable differences in K_m as well as in V_{max} for the two drugs. Unfortunately, neither study presented evidence indicating that measurements of the rate of uptake reflected a unidirectional influx.

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