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Methadone Uptake by L5178Y Mouse Leukemic Cells

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SUMMARY

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Mouse leukemic cells accumulate methadone at 37° while at 0° the drug associated with the cells is constant. After a suitable incubation period the accumulation at 37° ceases and the net drug associated with the cells remains constant. This steady-state level (37° uptake minus 0° uptake) increases in proportion to the free drug concentration up to at least 0.7 mM methadone where the cells contain 2.5 fmol/cell. The cells apparently concentrate methadone at 7.5–16 times the medium concentration. The rate of uptake appears to be a complex function of the free methadone concentration in the medium, however, no definite saturation was observed. The loss of [^{3}H]methadone from cells preloaded with drug is very rapid and the extent of loss is independent of the concentration of methadone in the medium. Methadone appears to be only weakly bound by the cells and is readily accessible to the medium. Various metabolic inhibitors do not reduce methadone uptake; further, the results of competition experiments with other opiates and the stereoisomers of methadone reveal little, if any, uptake specificity for methadone. We conclude that methadone is accumulated by passive diffusion which may involve extensive intracellular binding of the drug by macromolecules and/or partitioning of the drug into hydrophobic regions of the cell.

INTRODUCTION

Narcotics and narcotic antagonists have been shown to have many effects on cultured cells, such as the inhibition of protein synthesis and alterations of lipid synthesis (1, 2). Although these effects on cultured cells occurred at relatively high drug concentrations, similar effects have been reported *in vivo*, suggesting that the effects are of potential pharmacological relevance (2). One possible explanation for the requirement for high drug concentrations *in vitro* would be a lower drug uptake *in vitro* when compared to tissue concentrations *in vivo*. The objective of this study was to determine the characteristics of methadone uptake by mouse leukemic cells in culture.

Cells in culture are particularly useful for studies of drug interactions which can be followed on the cellular level. When compared with studies *in vivo* or with intact tissues *in vitro*, cell cultures have certain advantages; for example, there is free access of the drug to the cells, there are no changes in drug concentration due to excretion, the cells are of only one cell type, and the environment can be strictly controlled as needed (3). These advantages are particularly important for studies of drug uptake.

Mouse leukemic cells (L5178Y) were chosen for this study because they are a stable diploid cell line which is readily cultured on a large scale.

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Narcotics are accumulated *in vitro* by leukocytes, the choroid plexus, the cerebral cortex and the kidney. The characteristics of uptake by these cells or tissues suggest the presence of active transport systems (4–10). The results of this study[2] indicate that the uptake of methadone by mouse leukemic cells is by passive diffusion possibly facilitated by the partitioning of methadone into hydrophobic regions of the cell and or by extensive intracellular binding of the drug.

MATERIALS AND METHODS

Materials. The *l*-(−)[1-^{3}H]methadone hydrobromide was purchased from the New England Nuclear Corp. with a specific activity of either 92.3 or 135 Ci/mol. Thin-layer chromatography with three different solvent systems indicated that >98% of the radioactivity migrated the same as unlabelled methadone. Racemic methadone was obtained from the S.B. Penick Co. and *l*-(−)- and *d*-(+)-methadone hydrochloride were gifts of Eli Lilly and Co. Levorphanol tartarate and levallorphan tartarate were donated by Hoffman-La Roche Inc. [methoxy-^{3}H]-inulin was purchased from the New England Nuclear Corp. and α-[methyl-^{3}H]-aminoisobutyric acid was bought from ICN Pharmaceuticals Inc. Protoveratrin B and ouabain octahydrate were obtained from the Sigma Chemical Co. Powdered medium and serum were purchased from Grand Island Biological Co. and Pluronic (F68) was obtained from the Wyandotte Chemical Co. All other chemicals were reagent grade or better.

Cell culture. Mouse leukemic cells (L5178Y) were grown in suspension culture at 37° using Fischer's medium containing 10% (v/v) horse serum, 13 mM $NaHCO_3$, 1 mg/ml Pluronic F68, 50 μg/ml streptomycin sulfate, and 33 μg/ml penicillin "G" Na salt (11). The cells were diluted with fresh medium daily and the cultures were maintained at pH 7.4 in an atmosphere of 5% CO_2 in air. All experiments were done in growth medium with exponentially growing cells at ~7×10^5 cells/ml. Cell concentrations were determined with a hemocytometer (12), and cells were routinely tested to demonstrate freedom from contamination by fungi or bacteria (13).

Incubation of cells with methadone. Cells were preincubated to the appropriate temperature for at least 15 min. At the start of the incubation period [1-^{3}H]methadone was added with sufficient unlabelled methadone to achieve the desired concentration and at least 0.1 μCi/ml. Samples of cells were harvested periodically in 15 ml polystyrene centrifuge tubes (Spectrum) by centrifuging at 600 × *g* for 5 min in the cold. The supernatant medium was discarded, the tubes inverted, drained briefly, and the tips of the centrifuge tubes containing the cell pellets were cut off with a guillotine (Harvard Instruments) and placed in scintillation vials. The cells and the centrifuge tube tips were dissolved in a toluene-based scintillation solution (14) and radioactivity was measured with a Packard model 3310 scintillation counter. Corrections for variations in counting efficiency were performed using the automatic external standardization technique (15). The results of preliminary experiments indicate that harvesting cells in the hydrophobic polystyrene centrifuge tubes sufficiently reduces the extracellular contamination of the cell pellets to enable uptake measurements to be performed without washing (16).

Equilibrium dialysis. The binding of methadone to horse serum proteins was determined by equilibrium dialysis as described by Olsen (17) except that serum-free Fischer's medium was substituted for the phosphate buffer. The pH was maintained at 7.4 by performing the dialysis in an atmosphere of 5% CO_2 in air. Measurements were performed at a constant protein concentration (10% serum) of 8.2 mg/ml as determined by the Biuret technique (18). The free methadone concentration was determined by multiplying the total drug concentration by the fraction of methadone not bound to serum proteins (17).

Washout experiments. Cells were incubated with radioactive methadone as previously described; the cells were then har-

[2] Part of these data was included in a report delivered in October 1971 at the Regional Meeting of the American Chemical Society in St. Louis, Missouri.

vested by centrifuging in the cold at 200 × *g* for 10 min, the supernatant medium was discarded, and the centrifuge bottles were drained briefly in the cold. Excess medium was removed from the bottles by blotting with tissues. The cells were gently suspended in fresh medium which had been preincubated to the desired temperature.

pH experiments. Uptake measurements done at other than pH 7.4 were performed using Fischer's medium containing up to 27 mM $NaHCO_3$ and 1 mM Na_2HPO_4. The cell-free medium was adjusted to approximately the desired pH and temperature before adding the cells in a small volume of growth medium. After incubation with methadone, the cells were harvested and the final pH of the cell-free medium was again determined. The uptake results are reported at the final pH of the medium.

Cell mass determinations. The wet and dry weights of cells were determined by a modification of the method of Birch and Pirt (19) where the extracellular space in the wet cell pellets was determined using [methoxy-^{3}H]inulin. The results indicate that the cells weigh ~150 pg dry or ~910 pg wet.

RESULTS

Methadone accumulation by mouse leukemic cells. The amount of methadone associated with mouse leukemic cells at 92 μM methadone is shown in Fig. 1. These results demonstrate that methadone is accumulated at 37° while the drug associated with the cells at 0° is the same throughout the incubation. Since the amount of methadone associated with the cells at the first time point (2–5 min) is the same at both temperatures, it is thought that the drug present at 0° is a combination of nonspecific absorption and the methadone present in the extracellular space (16). The difference between the amount present at each temperature therefore represents the net drug accumulated by the cells and this amount increases until a steady-state level is reached.

The maximum net uptake of methadone by cells was examined over a wide range of drug concentrations. Since a substantial amount of methadone is bound by serum proteins, the free drug concentrations in the medium were calculated from the total drug concentration and the fraction bound by the serum proteins as measured by equilibrium dialysis (Fig. 2). In contrast to results with human serum proteins, the bind-

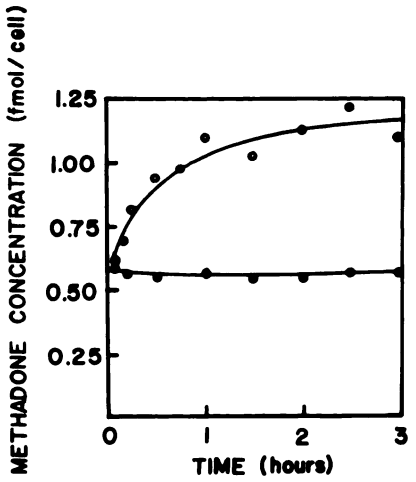

FIG. 1. *Time course of methadone uptake by mouse-leukemic cells*

Cells were incubated with [^{3}H]methadone and the radioactivity in the cells determined as described in MATERIALS AND METHODS. The data were obtained at 92 μM methadone with a specific activity of 0.51 Ci/mol. Open symbols represent results at 37° while data obtained at 0° are indicated by the filled symbols.

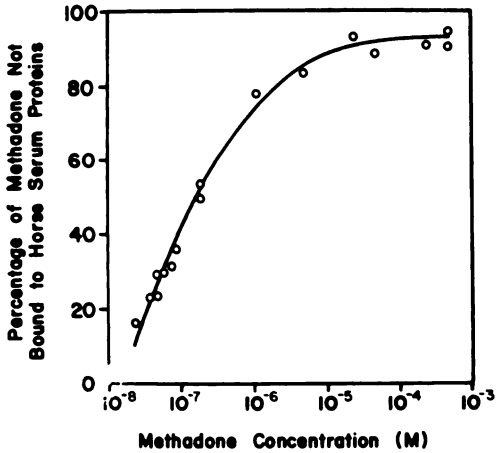

FIG. 2. *Binding of methadone to serum proteins at various methadone concentrations*

The binding of methadone to horse serum proteins was determined as described in MATERIALS AND METHODS. The protein concentration was 8.2 mg/ml (10% serum).

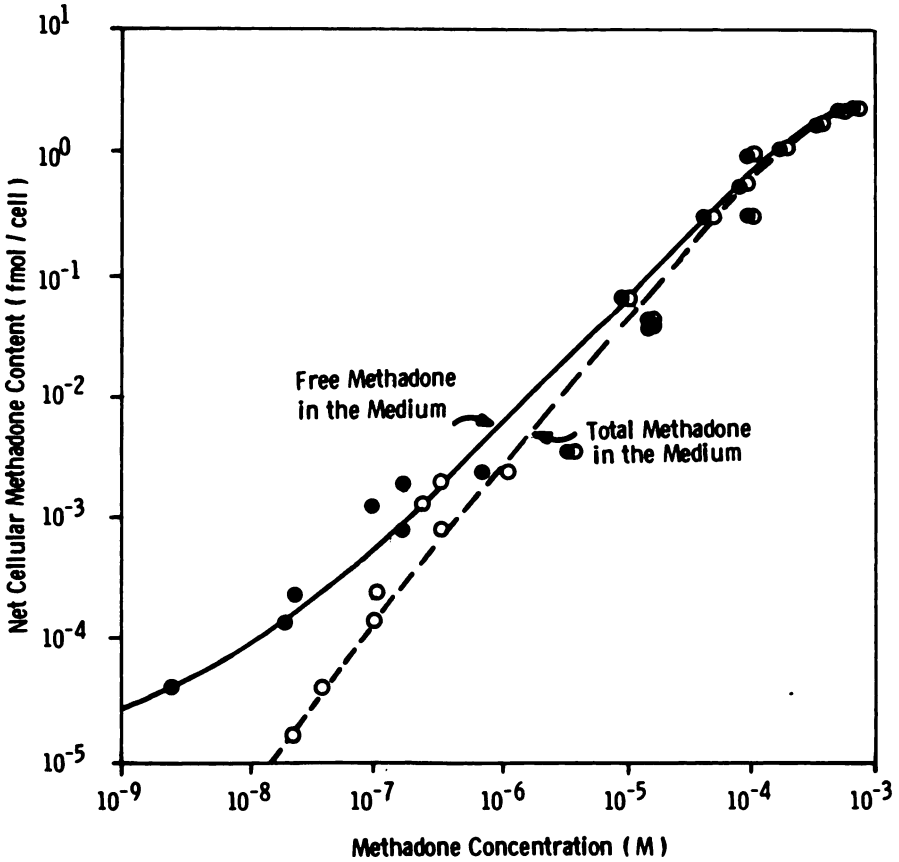

FIG. 3. *Effect of various methadone concentrations on the net methadone content of mouse leukemic cells* Experiments were performed as described in Fig. 1. The net uptake was determined as described in the text. The free (unbound) methadone concentration was measured as described in MATERIALS AND METHODS and the experimental data (filled circles) were replotted versus the appropriate free methadone concentration (open circles).

ing of methadone by horse serum is concentration dependent (17). The results in Fig. 3 indicate that the amount of methadone taken up by the cells is proportional to the free methadone concentration in the medium from 0.1 to 100 μM. When the data which were obtained at the highest drug concentrations were plotted on linear coordinates (not shown[3]) the results did not indicate any definite saturation. If it is assumed that all the accumulated methadone exists free in the cytoplasm, and none is bound to intracellular macromolecules, then the ratio of the concentration of drug in the intracellular water to the concentration in the medium would vary from 16 at 10 μM to 7.5 at 170 μM. If this assumption is valid, it suggests that methadone is accumulated by mouse leukemic cells against an apparent concentration gradient. Similar results have been reported for morphine uptake by rat fibroblasts (20).

A number of experiments similar to those in Fig. 1 were used to estimate the initial net rate of methadone uptake over a range of drug concentrations (Fig. 4). The data deviate substantially from a proportionality and seem to suggest that methadone facilitates its own uptake at the higher concentrations. The data obtained at the highest concentrations were plotted on linear coordinates (not shown[3]) and no definite saturation was observed. These results (Figs. 3 & 4) are generally consistent with meth-

[3] These data were available to the reviewers.

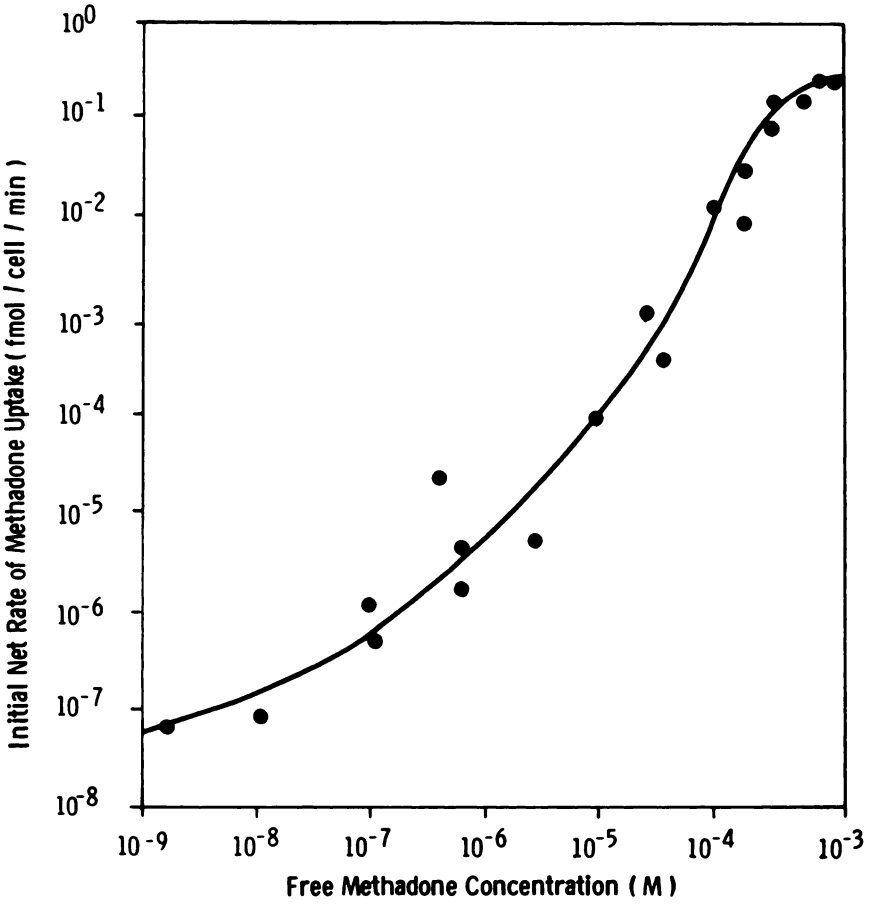

FIG. 4. *Effect of methadone concentration on the initial net rate of uptake by mouse leukemic cells*

Data from experiments as described in Fig. 1 were used to estimate the initial net rate of methadone uptake at various concentrations of methadone in the medium. The free methadone concentration in the medium was estimated from the results in Fig. 2.

adone being accumulated by mouse leukemic cells in a passive manner. The apparently high intracellular drug concentrations imply that other factors such as intracellular binding may be important for determining the total amount of methadone associated with the cells.

Qualitative Characteristics of Uptake. Methadone uptake by mouse leukemic cells was examined at various temperatures and the results indicate (Fig. 5) that methadone accumulation is a maximum at 37°. As the amount of methadone accumulated by the cells at 0° and 46° is the same, it is concluded that nonspecific absorption does not change between these two temperatures (and was therefore subtracted to obtain the net drug uptake). Although these results may imply a requirement of cell metabolism for methadone uptake, the low Q_{10} of 1.4 suggests that other factors, such as protein binding or lipid solubility, may be important. The Arrhenius plot of the data between 0° and 37° (not shown) is linear with an apparent activation energy of 17 *j*/mol (4.1 cal/mol).

When the accumulation of methadone was examined at 37° following a preincubation with the drug at 0°, the uptake rate and the extent of uptake were not significantly different from the nonpreincubated controls (results not shown), and in another experiment the level of drug in the cells after uptake at 37° remained unaltered

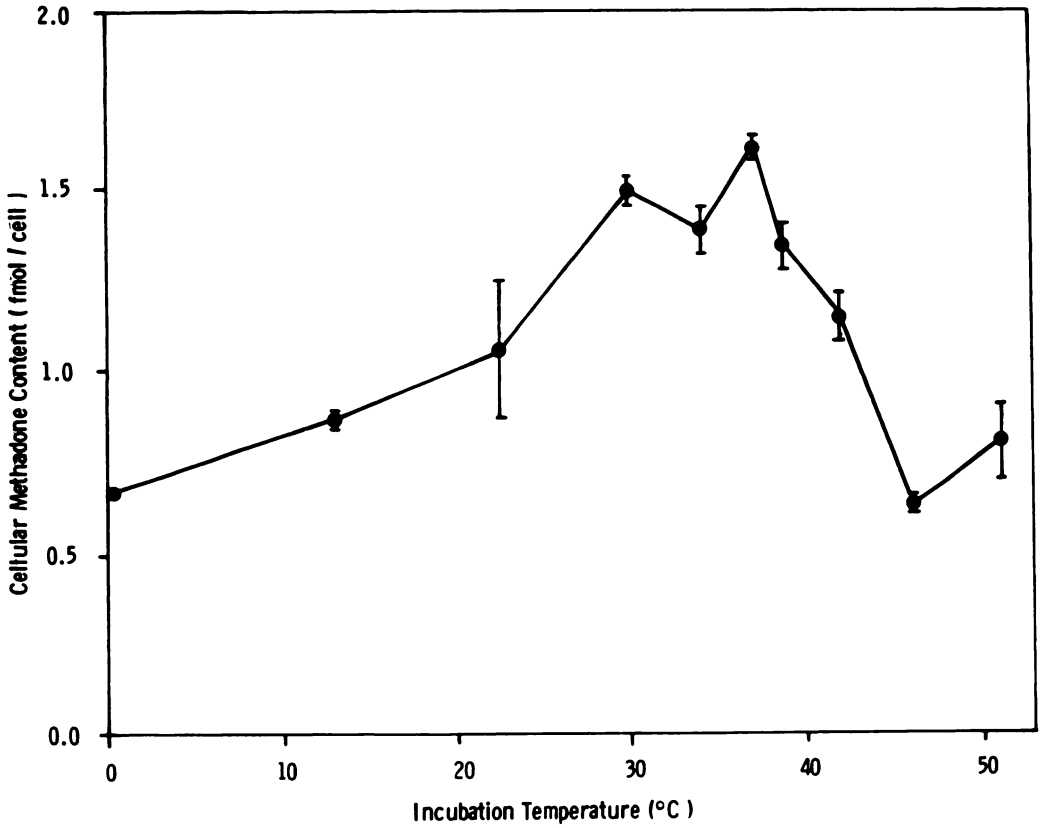

FIG. 5. *Effect of temperature on methadone uptake by mouse leukemic cells*

Cells were incubated for 2 hr at the appropriate temperature and uptake was measured as described at a methadone concentration of 0.1 mM with a specific activity of 0.23 Ci/mol. The uptake at each temperature was measured in triplicate and the results are shown as the mean ± SD.

when the cells were chilled to 0° for 2 hr (results also not shown). The methadone binding or transport sites evidently are only present or active in cells at ~37°, and there appears to be no temperature requirement for the forces which maintain the level of drug in the cell.

The pH of the medium has a significant effect on the net uptake of methadone (Fig. 6). Although most of the uptake experiments described in this report were done at pH 7.4 (to preserve cell viability), maximum drug accumulation was observed at approximately pH 7.7 The pH optima for net methadone uptake are quite narrow and differ significantly from the pK_a (8.62) of methadone (21), suggesting that the cellular constituents required for uptake and/or binding have well-defined ionization constants.

Methadone washout. The loss of methadone from cells previously loaded with the drug into medium without methadone (Fig. 7) indicates that the efflux at both 0° and 37° is very rapid. In a similar experiment with unlabelled methadone in the washout medium the results (not shown[3]) also indicate that the loss of [^{3}H]methadone at both 0° and 37° is very rapid. Since in the latter experiment there was no change in the medium drug concentration, the total *amount* of methadone bound by the cells (1.1 fmol/cell) was not changed by the washout (after correction of the specific activity). It would appear that the methadone associated with the cells is readily exchangeable with methadone in the medium, and that a concentration gradient of methadone

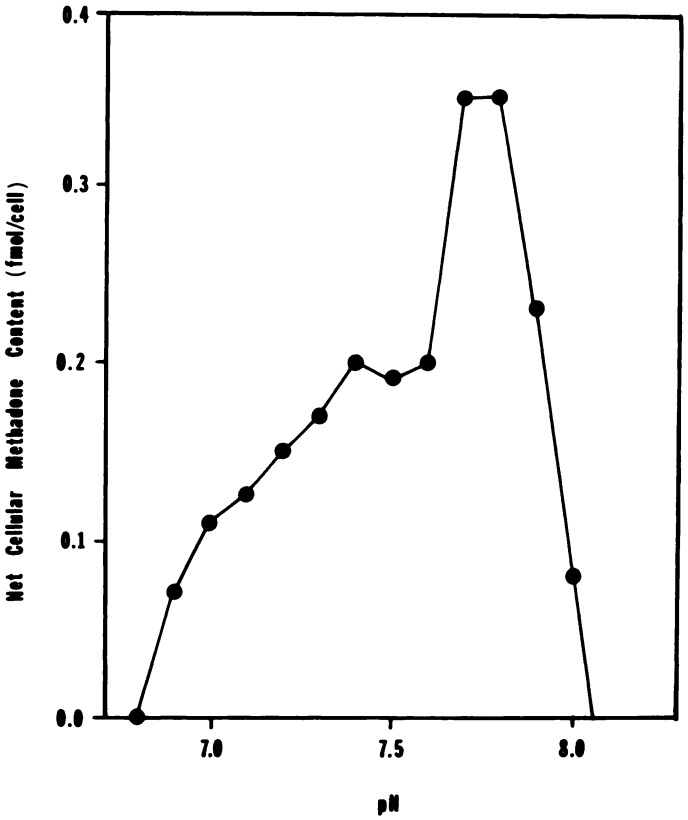

FIG. 6. *Effect of pH on net methadone uptake by mouse leukemic cells*

Cells were incubated at various pHs for 2 hr with 0.14 mM methadone at a specific activity of 0.46 Ci/mol. The net uptake at each pH was calculated as described in the text. Each point represents the differences between the means of triplicate measurements at 0° and 37°.

across the cell membrane is required for methadone transport and maintaining the intracellular methadone concentration.

Effect of metabolic inhibitors. Metabolic inhibitors have been shown to reduce the uptake of narcotics by several tissues (4, 5, 9, 10, 22). The uptake of α-aminoisobutyric acid which is actively transported (23), was reduced by all of the treatments shown in Table 1; however, the uptake of methadone was not affected in the same manner. The frequent stimulation of methadone uptake by the inhibitors appears to be real since the amount of stimulation was dependent upon the concentration of the inhibitors (e.g., iodoacetate or dinitrophenol) and since metabolic inhibitors have been found to increase the drug permeability of other cultured cells (24, 25) and the mouse kidney (10). This stimulation of drug intake may be due to an increase in the number of intracellular binding sites.

Competition by other narcotics and methadone racemates. The uptake of narcotic analgesics by the kidney (5, 10), the choroid plexus (5), and leukocytes (9, 22) is reduced in the presence of other opiates. These results suggest that the process responsible for the uptake of narcotics by these tissues has some structural specificity. Methadone uptake by mouse leukemic cells is inhibited by levallorphan and levorphanol (Table 2); therefore the transport or binding sites have some specificity for opiate-like molecules.

Opiate uptake has been found to be stereospecific in the choroid plexus and the kidney, and possibly in the cerebral cortex

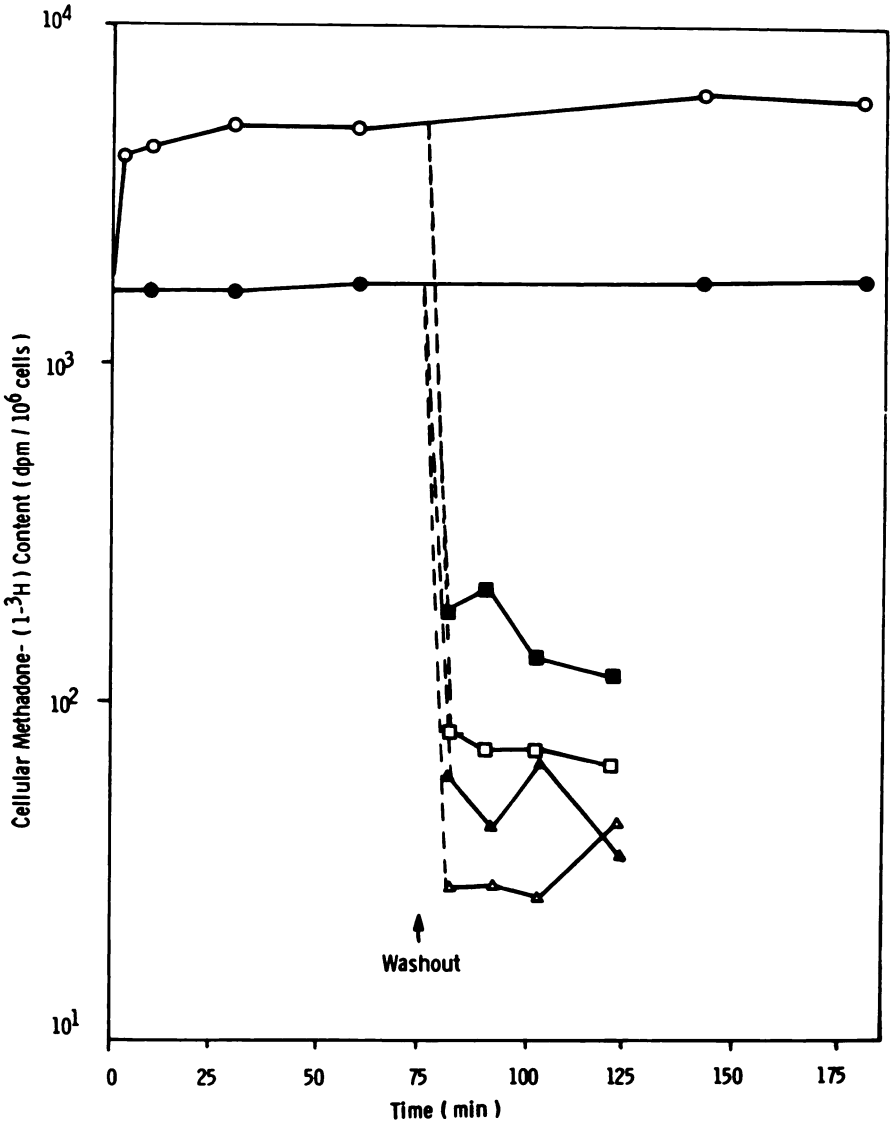

FIG. 7. *Efflux of methadone -[1-^{3}H]from mouse leukemic cells into medium without methadone*

Cells were incubated at 37° (open circles) or 0° (filled circles) in 0.1 mM methadone with a specific activity of 0.78 Ci/mol. The radioactivity in the cells was determined periodically as described in the MATERIALS AND METHODS. After 77 min the cells at 37° were harvested and suspended in drug-free medium at either 37° (open squares) or 0° (filled squares). The cells labelled with [^{3}H]methadone at 0° were also suspended in drug-free medium after 77 min at 37° (open triangles) or 0° (filled triangles).

(5, 7, 10). To examine the stereospecificity of the process responsible for methadone uptake by mouse leukemic cells, the uptake of 0.4 μM *l*-(−)-[1−^{3}H]methadone was measured in the presence of 6 to 240 μM unlabeled *l*-(−)- or *d*-(+)-methadone. The results (not shown) indicate that both stereoisomers are equally inhibitory. The process responsible for methadone uptake by eoisomers are equally inhibitory. The process responsible for methadone uptake by mouse leukemic cells has no substrate ste-

TABLE 1

The effect of various inhibitors on the uptake of α-aminoisobutyric acid and methadone by mouse leukemic cells

Concentrated solutions of the inhibitors were adjusted to pH 7.4 before use. Glucose and Ca plus Mg concentrations are given when appropriate as a percentage of the normal concentrations in Fischer's medium with 10% horse serum (‡). Cells were incubated with the inhibitors and 5.2 nM α-aminoisobutyric acid for 1 hr or 16 μM methadone for 2 hr. Control cells contained 0.82 ± 0.04 amol/cell α-aminoisobutyric acid or 144 ± 15 amol/cell methadone. All measurements were performed in triplicate and differences from the controls are considered significant if $p < 0.05$ and are indicated by the (*).

Treatment	Concentration mM	α-Aminoisobutyric acid Uptake	Methadone Uptake
	mM	*% Control*	*% Control*
None	—	100	100
0°	—	5*	56*
52°	—	7*	—
Low Glucose	17‡	82*	168*
Low Ca & Mg	4‡	22*	158*
N_2 Atmosphere	—	62*	250*
Minus Serum	—	59*	186*
Dinitrophenol	0.30	74*	148*
Dinitrophenol	0.48	44*	211*
Dinitrophenol	2.10	17*	217*
Edta	1.40	93	79*
Edta	5.60	38*	95
p-Hydroxymercurobenzoate	0.15	9*	108*
Iodoacetate	0.12	111	—
Iodoacetate	0.46	67*	107
Iodoacetate	0.55	24*	254*
Levallorphan	2.20	12*	50*
Methadone	0.10	56*	86
N-ethylmaleimide	0.13	11*	87*
Ouabain	0.05	25*	188*
Protoveratrin B	0.61	42*	141*
Sodium Azide	2.60	89*	164*
Sodium Cyanide	2.40	9*	82
Sodium Flouride	4.90	71*	249*
Tris	39.00	62*	115

reospecificity and has a large capacity for opiate-like molecules (see Fig. 3 and Table 2). Results similar to these would be expected if the opiate drugs were transported across the plasma membrane and localized intracellularly as a function of their organic-solvent/water partition coefficients (7).

TABLE 2

The effect of other narcotic drugs on methadone uptake by mouse leukemic cells

Cells were incubated with 1.6 μM *l*-(−)-[1-^{3}H]methadone and the following additions for 60 min at 37°. Each treatment was performed in triplicate and the results are shown as the mean ± SD.

Additions	Radioactivity	% Control
	(dpm/10^6 cells)	
None	2412 ± 88	100
530 μM Levorphanol	1207 ± 49	50
130 μM Levallorphan	1658 ± 111	69
550 μM Methadone	1355 ± 12	56

DISCUSSION

Methadone has been shown to be accumulated by mouse leukemic cells. The net accumulation is roughly proportional to the free methadone concentration up to at least 0.7 mM where the cells contain ~2.5 nmol/mg wet cells. Although there is no apparent saturation of the cells with methadone, the extent of uptake is comparable to the results of dihydromorphine (5) and morphine (4) uptake by the rabbit choroid plexus *in vitro*. The uptake by mouse leukemic cells is also similar to methadone accumulation by various parenchymous tissues of the rat *in vivo* (26). Narcotic accumulation *in vitro* does not appear to be greater than the accumulation *in vivo* although much higher drug concentrations are generally required (21, 26).

The significant uptake of methadone at 0° by mouse leukemic cells shown in Figs. 1 and 5 is similar to the uptake in the cold of other opiates by the rabbit choroid plexus (4, 5), the rat cerebral cortex (7, 27), and the dog renal cortex (4). These contrast, however, with the minimal accumulation in the cold of pentazocine and other drugs by rat leukocytes (9, 22) and of the alkaloid vincristine by cultured L1210 mouse leukemic cells (28). It is likely that tissues which accumulate opiates in the cold do so by mechanisms which are not energy-dependent.

The binding of methadone to horse se-

rum proteins (results not shown[4]), and the uptake of methadone by mouse leukemic cells (Fig. 5) are both optimal at 37°, therefore it seems plausible that the binding of the drug to macromolecules of the cells could be important in the uptake process. Furthermore, when the uptake of two other lipophilic molecules, benzo(a)pyrene and cholesterol, were examined at various temperatures, it was found that the partitioning was not optimal at any temperature but merely increased roughly in proportion to the increase in temperature (29, 30). Consequently, we feel that since methadone uptake by mouse leukemic cells is optimal at 37° and the uptake at 0° and 46° is the same, then methadone is not likely to be accumulated by a simple partitioning into hydrophobic regions but probably is taken up by some process which involves extensive binding to macromolecules. We realize, however, that the localization of methadone in hydrophobic regions cannot be completely discounted because of the high partition coefficient of the drug (21, 31).

The pH optima for methadone uptake by mouse leukemia cells is similar to the pH optima for morphine uptake by the rabbit choroid plexus (4) and the rat cerebral cortex (32); however, it is quite different from that of pentazocine accumulation by rat leukocytes where the drug was speculated to have been taken up as the unprotonated amine (9). Although the transport of narcotics across the plasma membrane is generally believed to be due to their high lipid solubility (4, 33–36), the uptake of dihydromorphine by the choroid plexus was inhibited by decamethonium and other organic bases (5) which are transported as cations (37). Furthermore, mepiperphenidol, an inhibitor of base transport in the kidney, reduced dihydromorphine uptake *in vitro* (6) and excretion *in vivo* (6, 38), and certain characteristics of methadone accumulation by the perfused rabbit lung also suggest active transport of the amine (39). It seems likely that narcotics are transported across *certain* membranes in an ionized form. However, the correlation of activity and lipid solibility by Seeman and others (33–36, 40) suggests that lipid permeability may be an important characteristic of the uptake of these drugs at their sites of pharmacological action *in vivo*.

Methadone accumulation by mouse leukemic cells probably involves both transport and binding. The transport aspect is undoubtedly very rapid because of the results in Fig. 7 and probably is by simple diffusion of the free base across the plasma membrane. The net accumulation may be due to extensive intracellular drug binding with the relative slowness of the binding caused by a slow acquisition of intracellular binding sites. The existence of discrete pH and temperature optima for uptake is consistent with the presence of such sites.

A variety of drugs have been found to be accumulated by cells in culture by endocytosis with resulting concentration of the drug in lysosomes (20, 41, 42). Although the time course of methadone uptake by mouse leukemic cells is consistent with such a mechanism, the rapid loss of methadone when the cells are placed in drug free medium (Fig. 7) and the ineffectiveness of metabolic inhibitors at preventing drug uptake (Table 1) (43), argue that such a process does not contribute to methadone uptake by mouse leukemic cells.

Previous reports describing the uptake of narcotics by the choroid plexus (4, 5), rat leukocytes (9, 22), and the kidney (5, 6, 10) have presented convincing evidence that these tissues accumulate narcotics by active transport systems. The uptake of narcotics by the cerebral cortex has also been examined in some detail and conflicting results concerning the role of metabolic energy have been reported (27, 32). Furthermore, several groups have indicated that the uptake of dihydromorphine or methadone by the cerebral cortex could not be saturated (7, 8, 27), although it is possible that the concentrations studied could have been too low. The uptake of methadone by mouse leukemic cells is not likely to involve a mediated process as it does not appear to be saturable, is not inhibited by metabolic inhibitors, and the accumulation process appears to have little stereospecificity. The net accumulation of drugs and other lipo-

[4] P. C. Will and W. D. Noteboom, manuscript in preparation.

philic compounds by tissues may be due to transport across the plasma membrane by simple diffusion in which the net uptake rate may be limited by the rate of intracellular partitioning into hydrophobic regions or by the rate of binding to intracellular macromolecules (31, 33–36, 44–47). Since methadone is extensively bound by macromolecules (17, 48) and there exists very discrete pH and temperature optima for uptake, we conclude that the transport of methadone through the plasma membrane of L5178Y mouse leukemic cells is by simple diffusion with the total uptake determined by the availability of intracellular binding sites and/or by the partitioning of the drug into hydrophobic regions of the cell.

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REFERENCES

1. Noteboom, W. D. & Mueller, G. C. (1966) *Mol. Pharmacol.*, **2**, 534–542.
2. Clouet, D. H. (1968) *Int. Rev. Neurobiol.* **11**, 99–128.
3. Schindler, R. (1969) *Ann. Rev. Pharmacol.* **9**, 393–406.
4. Takemori, A. E. & Stenwick, M. W. (1966) *J. Pharmacol. Exp. Ther.*, **154**, 586–594.
5. Hug, C. C. (1967) *Biochem. Pharmacol.*, **16**, 345–359.
6. Hug, C. C., Mellett, L. B. & Cafruny, E. J. (1965) *J. Pharmacol. Exp. Ther.*, **150**, 259–269.
7. Scrafani, J. T. & Hug, C. C. (1968) *Biochem. Pharmacol.*, **17**, 1557–1566.
8. Miller, J. W., & Elliott, H. W. (1954) *J. Pharmacol. Exp. Ther.*, **110**, 106–114.
9. Marks, M. J. & Medzihradsky, F. (1974) *Mol. Pharmacol.*, **10**, 837–848.
10. Teller, D. N., DeGuzman, T. & Lajtha, A. (1976) *Biochem. Pharmacol.*, **25**, 1271–1284.
11. Fischer, G. A. (1958) *Ann. N.Y. Acad. Sci.*, **76**, 673–680.
12. Tolnai, S. (1975) *TCA Manual*, **1**, 37–38.
13. Coriell, L. L. (1973) in *Tissue Culture Methods and Applications* (Kruse, P. F. & Patterson, M. K., eds.), pp. 718–721, Academic Press, New York.
14. Carter, G. W. & Van Dyke, K. (1971) *Clin. Chem.*, **17**, 576–580.
15. Hash, J. H. (1972) in *Methods in Microbiology* (Norris, J. R. and Ribbons, D. W., eds.), Vol. 6B, pp. 109–155, Academic Press, New York.
16. Will, P. C. & Noteboom, W. D. (1977) *Cancer Biochem. Biophys.*, **2**, 131–135.
17. Olsen, G. D. (1972) *Science* **176**, 525–526.
18. Faulkner, W. R. & King, J. W. (eds.) (1967) *Manual of Clinical Laboratory Procedures*, pp. 78–79, Chemical Rubber Co., Cleveland, Ohio.
19. Birch, J. R. & Pirt, S. J. (1971) *J. Cell Sci.*, **8**, 693–700.
20. Liesse, M., Lhoest, G., Trouet, A. & Tulkens, P. (1976) *Arch. Int. Physiol. Biochim.*, **84**, 638–639.
21. Baselt, R. C. & Casarett, L. J. (1972) *Clin. Pharmacol. Ther.*, **13**, 64–70.
22. Marks, M. J. & Medzihradsky, F. (1974) *Biochem. Pharmacol.*, **23**, 2951–2962.
23. Oxender, D. L. (1972) *Ann. Rev. Biochem.*, **41**, 777–814.
24. See, Y. P., Carlsen, S. A., Till, J. E. & Ling, V. (1974) *Biochim. Biophys. Acta*, **373**, 242–252.
25. Goldman, I. D. (1969) *J. Biol. Chem.*, **244**, 3779–3785.
26. Elliott, H. W., Chang, F.N-H., Abdou, I. A. & Anderson, H. H. (1949) *J. Pharmacol. Exp. Ther.*, **95**, 494–501.
27. Kayan, S., Misra, A. L. & Woods, L. A. (1970) *J. Pharm. Pharmac.*, **22**, 941–943.
28. Bleyer, W. A., Frisby, S. A. & Oliverio, V. T. (1975) *Biochem. Pharmacol.*, **24**, 633–639.
29. Brunette, D. M. & Katz, M. (1975) *Chem. Biol. Interactions*, **11**, 1–14.
30. Rothblat, G. H., Hartzell, R. W., Mialhe, H. & Kritchevsky, D. (1966) *Biochim. Biophys. Acta*, **116**, 133–145.
31. Seeman, P. (1972) *Pharmacol. Rev.*, **24**, 583–655.
32. Bell, J. L. (1958) *J. Neurochem.*, **2**, 265–282.
33. Schanker, L. S. (1964) in *Advances in Drug Research* (Harper, N. J. & Simmonds, A. B., eds.) Vol. 1, pp. 71–106, Academic Press, New York.
34. Von Cube, B., Teschemacher, H. J., Herz, A. & Hess, R. (1970) *Naunyn Schmiedelbergs Arch. Pharmacol.*, **265**, 455–473.
35. Kosterlitz, H. W., Lork, J. A. H. & Watt, A. J. (1973) in *Agonist and Antagonist Actions of Narcotic Analgesic Drugs* (Kosterlitz, H. W., Collier, H. O. J. & Villarreal, J. E., eds), pp. 45–61, University Park Press, Baltimore.
36. Brodie, B. B. & Hogben, C. A. M. (1957) *J. Pharm. Pharmac.*, **9**, 345–380.
37. Tochino, Y. & Schanker, L. S. (1965) *Am. J. Physiol.*, **208**, 666–673.
38. Watrous, W. M., May, D. G. & Fujimoto, J. M. (1970) *J. Pharmacol. Exp. Ther.*, **172**, 224–229.
39. Anderson, M. W., Orton, T. C., Pickett, R. D. & Eling, T. E. (1974) *J. Pharmacol. Exp. Ther.*, **189**, 456–466.
40. Roth, S. & Seeman, P. (1972) *Biochim. Biophys. Acta*, **255**, 207–219.

41. Will, P. C., Longworth, J. W., Brake, E. T. & Cook, J. S. (1977) *Mol. Pharmacol.*, **13**, 161–171.
42. Noronha-Blob, L., Vengris, V. E., Pitha, P. M. & Pitha, J. (1977) *J. Med. Chem.*, **20**, 356–359.
43. Cook, J. S., Will, P. C., Proctor, W. R. & Brake, E. T. (1976) in *Biogenesis and Turnover of Membrane Macromolecules* (Cook, J. S., ed.) pp. 15–36, Raven Press, New York.
44. Wolpert, M. K. & Ruddon, R. W. (1969) *Cancer Res.*, **29**, 873–879.
45. Faust, R. G., Shearin, S. J. & Misch, D. W. (1972) *Biochim. Biophys. Acta*, **255**, 685–690.
46. Ichibagase, H., Imamura, Y. & Nakagami, H. (1976) *Chem. Pharm. Bull.*, **24**, 204–210, Japan.
47. Begleiter, A., Lam, H-Y.P. & Goldenberg, G. J. (1977) *Cancer Res.*, **37**, 1022–1027.
48. Olsen, G. D. (1973) *Clin. Pharmacol. Ther.*, **14**, 338–343.