Cancer Chemother Pharmacol (1985) 14 : 238–242

Cancer Chemotherapy and Pharmacology

The distribution and elimination of methotrexate in mouse blood and brain after concurrent administration of polysorbate 80

M. Noor Azmin[1, 3], James F. B. Stuart[1, 2], Alexander T. Florence[1]

[1] Department of Pharmacy, University of Strathclyde, Glasgow Gl 1XW, Scotland
[2] Department of Clinical Oncology, University of Glasgow, Glasgow Gl 2, Scotland
[3] Present address: School of Pharmacy, Universiti Sains Malaysia, Penang, Malaysia

Summary. *This paper describes further exploration of the effect of polysorbate 80 on the absorption, distribution, and elimination of methotrexate (MTX). This study has confirmed the earlier finding that polysorbate 80 could increase the absorption of MTX from the mouse gastrointestinal tract and enhance the drugs uptake into the brain. The experiments reported here suggest that polysorbate 80 has a direct effect on the blood-brain barrier leading to the increased uptake of MTX, which is evident following IV administration. Measurements of MTX excreted in the urine and faeces confirmed the role of polysorbate 80 in facilitating the excretion of MTX into the bile and urine. Polysorbate 80 administered PO did not cause any reduction of plasma volume, thus excluding the possibility that the higher MTX concentrations measured in mice after concurrent administration of polysorbate PO might result from a reduction in blood volume due to osmotic effects. At the doses given, polysorbate 80 appeared not to have a damaging effect on the gastrointestinal mucosa.*

Introduction

There have been many reports highlighting the ability of the non-ionic surfactant polysorbate 80 to increase the absorption of drugs in animals and man [9, 12, 14, 15, 18]. Our previous results [4, 5] suggested that polysorbate 80 increased both the absorption of methotrexate (MTX) from the mouse gastrointestinal tract and the uptake of the drug into the brain. It was not confirmed that the observed increase in the brain MTX levels was due to increased permeability of the blood-brain barrier, since the possibility remained that the enhanced drug uptake was due to the increased plasma levels or to a reduction in drug elimination from the circulation.

Non-ionic surfactants may increase the rate or extent of absorption of a drug by increasing biomembrane permeability [3, 17], in some cases due to the solubilisation of membrane components [26], which eventually leads to the disruption of the mucosal membrane [7, 25]. Harrison et al. [10], on the other hand, have ascribed the higher concentrations of adriamycin measured in mouse plasma to the apparent reduction of plasma volume as a result of the osmotic effect of high-dose polysorbate 80 when injected IP togehter with the drug.

The aims of the present work were (a) to confirm that polysorbate 80 can increase the absorption of MTX from mouse gastrointestinal tract and enhance the uptake of the drug into the brain; and (b) to determine the mechanism of the latter effect. Whether this is due to a more specific effect of the surfactant in increasing the blood-brain barrier permeability may be shown by administering the drug preparations IV to ensure that each mouse receives the same systemic dose.

Experiments were also performed to investigate whether the surfactant caused disruption of the mouse gastrointestinal mucosal membrane, thus explaining the observed increased absorption, and to find out whether this surfactant reduces plasma volume when administered PO, resulting in an artificially higher MTX concentration in plasma or serum. Experiments to study the influence of polysorbate 80 on the excretion of MTX were also performed.

Materials and methods

Materials. Methotrexate injection (25 mg · ml^{-1}, Lederle) was diluted with sterile normal saline (Travenol) or normal saline containing polysorbate 80 so that 5 ml solution administered PO or IV/kg body weight delivered the intended MTX dose in milligrams per kilogram. Polysorbate 80 (Honeywill Atlas) was obtained from Sigma Chemical Company. The aspartic acid derivative used as an internal standard in high performance liquid chromatography (HPLC) was a gift from Drug Synthesis and Chemical Branch, NCI, USA. Female Porton mice (BKW) 10–13 weeks old and weighing 25–35 g were used in the experiments; they were obtained from Bantin and Kingman Ltd., England. All other chemicals and solvents were of HPLC grade.

Analysis of MTX in mouse serum, tissue, urine and faeces by high-performance liquid chromatography (HPLC). The HPLC instrument used comprised an ALTEX single-piston pump (Model 110A), an ALTEX-HITACHI variable wavelength detector (Model 155-00/100-00), an ALTEX 210 valve injector (loop volume 100 µl or 200 µl), and a KIPP and ZONEN recorder (Model BD8, 015 mV to 100 V). The stainless steel analytical column (0.46 cm I.d., 25 cm length) used contained (Hypersil-5-ODS HPLC Technology Ltd.) reverse-phase packing material with a mean particle size of 5 µm.

To every millilitre of serum or urine and every gram of brain or faeces, 1.5 ml of 9 : 1 acetonitrile : water was added. Brain and faecal samples were homogenised in the presence of acetonitrile : water by a Citenco variable speed homogeniser. The serum or urine-acetonitrile : water mixture and the homogenised tissue or faeces samples were shaken in a tabletop Buchler vortex (Buchler Instrument, USA) for

Offprint requests to: A. T. Florence

20 min. These samples were then centrifuged for 30 min at 2,000 rpm in a Minstrel centrifuge. An equal volume of chloroform was added to the supernatants and the mixtures were shaken for further 30 min. The samples were then recentrifuged for 30 min to separate the supernatants from the chloroform phase. A volume of 100 µl or 200 µl supernatant containing MTX was applied to the column and eluted with 15% methanol in 0.5 *M* Tris-phosphate buffer, pH 7, at a constant flow rate or 1 ml min^{-1}. Concentrations of drug were detected by UV at 303 nm, by comparison with a standard curve.

Absorption of MTX from mouse gastrointestinal tract. Two groups of 48 mice were given 2.7 mg MTX · kg^{-1} as a solution with or without 6% polysorbate 80. At intervals six mice from each group were sacrificed and blood samples were collected from the hearts. The brain samples were dissected and washed twice with ice-cold normal saline. Once the excess fluid had been blotted off the net weight of each brain samples was determined and the samples were stored at −20° C. Both serum and brain samples were assayed by HPLC for MTX content.

Blood and brain levels of MTX after IV injection. Groups of Porton mice received injections into the tail vein of MTX solutions equivalent to 0.5–8 mg MTX · kg^{-1} and 0–8 mg polysorbate 80 kg^{-1} body weight. The animals were sacrificed at 2 h after the injection, and the blood and brain samples collected and analysed for MTX.

In another experiment two groups of 48 mice were given 2.7 mg MTX · kg^{-1} IV in solution with or without 6% polysorbate 80. Six animals from each group were sacrificed at intervals to collect blood and brain samples to determine MTX content.

Plasma volume. Normal saline solution was administered PO to the control mice. The test animals were given 6 ml · kg^{-1} of 6%, 12%, or 24% polysorbate 80 solution. Six animals from each group were sacrificed at intervals, and blood samples were collected into heparinised capillary tubes. These samples were spun in a haematocrit centrifuge for 5 min and the percentage of plasma volume determined.

Gastrointestinal mucosa membrane. Control mice were given normal saline, while test animals were given 6 ml · kg^{-1} 6% or 12% polysorbate 80 with or without 8 mg MTX · kg^{-1} administered PO. At intervals, three animals from each group were sacrificed. About a 6-cm length of the stomach and upper intestine was dissected out, opened up, and washed in normal saline solution in each case. The specimens were fixed in 10% formal-saline solution, followed by routine histological preparation of paraffin-embedded tissue blocks. Approximately 5-µm-thick sections were cut and stained with haematoxylin and eosin. These specimens were examined by light microscopy for any histological abnormalities.

Excretion of methotrexate in mice. Groups of eight mice were given 2.7 mg MTX · kg^{-1} with or without 6% polysorbate 80 either PO or IV. After the administration the groups of mice was kept in separate Metabowls in which urine and faeces were collected separately. These were collected and analysed for MTX.

Results

The results schown in Fig. 1a and c confirm that polysorbate 80 increases the absorption of MTX from mouse gastrointestinal tract and enhances the uptake of the drug into the brain. The levels of MTX in serum and brain measured after PO administration are significantly higher in mice given the drug with 6% polysorbate 80 at 0.5, 1, 3, and 0.5, 1, 2, and 3 h. The increased uptake into the brain observed might have resulted from a direct effect of the increased levels in serum. The results shown in Table 1 and Fig. 1d, however, show higher brain

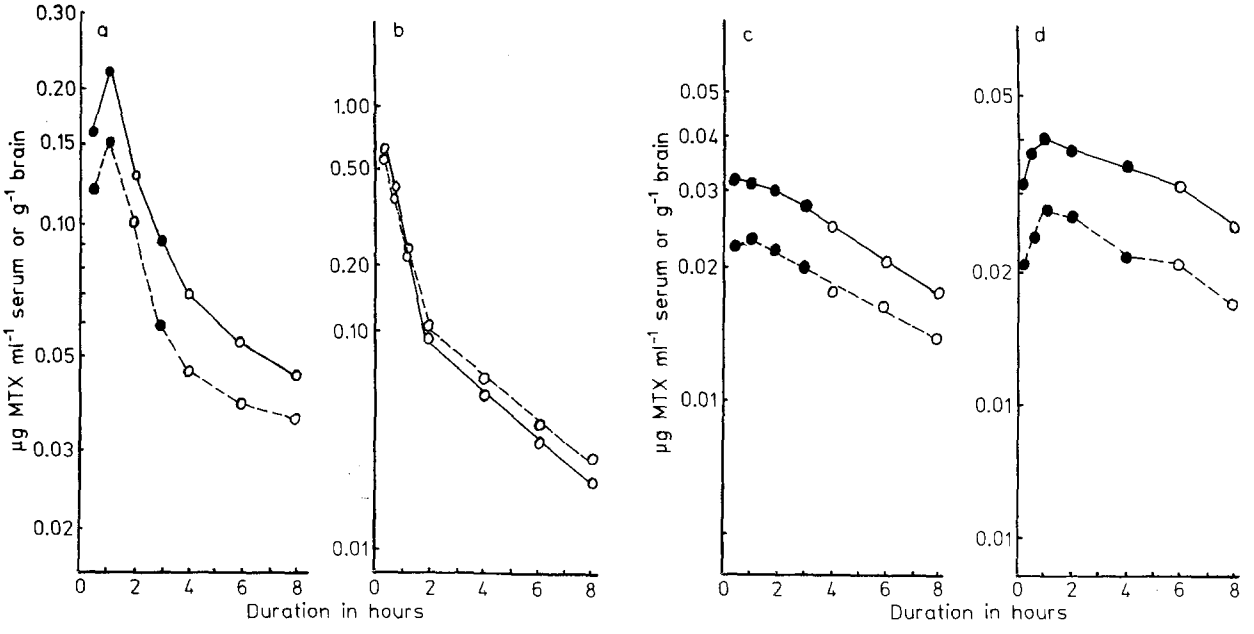

Fig. 1 a–d. Levels of MTX measured after administration of MTX solution without surfactant (– – –) and MTX in 6% polysorbate 80 solution (———). **a, b** Levels in mouse serum after PO and IV administration, respectively; **c** levels in brain after PO administration; levels in brain after IV injection. *Open circles,* no significant difference between points; *closed circles,* significant difference ($P \leq 0.05$)

levels of MTX in mice given MTX together with polysorbate 80 than in mice given the drug without the surfactant after IV injection, but the reverse is true for serum levels (Table 1 and Fig. 1b). This indicates that polysorbate 80 has a direct effect on the blood-brain barrier, resulting in an increase in MTX uptake into the brain. Although the differences were not significant, Fig. 1b indicates the generally lower serum levels of MTX in mice given the drug with polysorbate 80 IV than in mice given the drug without the surfactant, which suggest that polysorbate 80 might have an effect on elimination of the drug.

There is no significant difference in the plasma volume of the control and test mice (Table 2), indicating that polysorbate 80 at the doses administered PO did not cause any reduction in plasma volume; therefore, enhanced levels [10] are not osmotically induced artefacts. Histological studies on mucosal tissue of stomach and intestine suggested that polysorbate 80 did not cause significant damage to either tissue. There was a slight loss of stomach mucosal border caused by 12% polysorbate 80 solution at 4 h after administration, but the mucosa was found to have recovered at 24 h.

Figure 2a and b shows the average amount of MTX excreted in urine murine during the first 24 h after PO administration and IV injection of MTX solutions. The results

Table 1. Levels of serum and brain methotrexate in Porton mice 2 h after IV injection of methotrexate solutions with or without polysorbate 80

Dosage mg · kg^{-1}		Methotrexate concentration	
Methotrexate	Polysorbate 80	Serum (μg · ml^{-1})	Brain (μg · g^{-1})
0.5	0	0.20	0.0
	3.2	0.13	0.06
	8.0	0.14	0.13
	32.0	0.16	0.21
4.0	0	0.19	0.07
	3.2	0.14	0.08
	8.0	0.11	0.14
8.0	0	0.42	0.10
	3.2	0.23	0.15
	8.0	0.17	0.18

Table 3. Total amount of methotrexate (μg) excreted in the faeces after PO and IV administration

MTX preparation given	μg MTX collected in the faeces after	
	PO administration	IV injection
Solution of free MTX	14.6	2.3
MTX in 6% polysorbate 80 solution	16.6	4.0

Table 2. The effect of polysorbate 80 on the apparent plasma volume after oral administration

% w/v Polysorbate 80 in solutions administered: dose = 6 ml · kg^{-1}	% Plasma volume			
	Duration (h)	1	2	4
Control (normal saline)		57.6 ± 3.9	59.3 ± 4.1	–
6		60.8 ± 6.9	56.7 ± 2.4	58.1 ± 2.9
12		58.4 ± 4.5	57.3 ± 3.0	–
24		60.7 ± 2.3	58.0 ± 4.5	58.5 ± 3.2

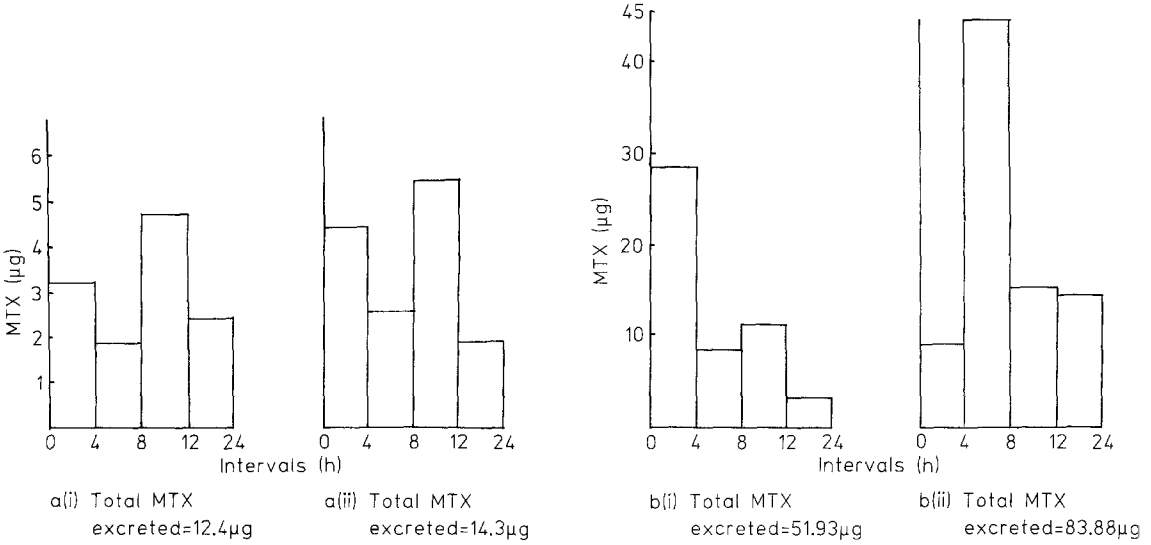

Fig. 2 a, b. The amounts of methotrexate excreted in mouse urine during the first 24 h after PO administration of **a(i)** MTX solution without surfactant; **a(ii)** MTX in 6% polysorbate 80 solution and after IV injection of **b(i)** MTX solution with no polysorbate 80; **b(ii)** MTX in 6% polysorbate 80

suggest that polysorbate 80 enhances urinary excretion of MTX. Table 3 shows the amount of MTX excreted in the faeces after PO or IV administration. If the amount measured in the faeces of mice given the drug IV could be taken as the amount excreted through biliary excretion, the results show that polysorbate 80 increases the excretion of MTX into bile. These results confirm the suggestion (Fig. 1b) that polysorbate 80 might influence the elimination of systemic MTX.

Discussion

It is generally apparent that chemotherapy against malignant brain tumours is usually ineffective because of the lack of penetration across the blood-brain and blood-cerebrospinal fluid barriers. Levin et al. [16] generalised that drugs which cross the blood-brain barrier and produce appreciable brain levels are either hydrophilic, with molecular weights of less than 160, or lipophilic, with molecular weights of less than 400. Thus MTX, a drug with molecular weight of 454, is unable to penetrate the blood-brain barrier readily in appreciable amounts. Shapiro et al. [23] have shown that administration of a dose of 50 mg IV is followed by a peak level of only 10^{-8} *M* MTX in the ventricular cerebrospinal fluid, but even less is detected after PO administration.

To increase the penetration of MTX into the central nervous system, the drug has to be given in high doses IV to create a high concentration gradient between blood and the brain [6, 22]. It is necessary to achieve a brain MTX level of more than 10^{-7} *M* for the drug to be effective in the treatment of brain tumours and meningeal leukaemia. Brain levels of greater than 10^{-7} *M* can be achieved with an IV dose of more than 500 mg · m^{-2} infused over 1 h or 24–42 h [23]. Methotrexate is also administered by other routes to achieve the required therapeutic levels. Albelson et al. [1, 2] used intraventricular injections to achieve cerebrospinal fluid levels of 5×10^{-6} to 2×10^{-5} *M* MTX. Intrathecal and lumbar injections [23] have also been used. Only about 0.007% of capillary surface needs to be breeched to produce a 10-fold increase in permeability [16]. Many workers have exploited this to increase MTX uptake into the brain. Neuwelt et al. [19] and Hasegawa et al. [11] used hyperosmolar mannitol administered via the artery to cause reversible disruption of the blood-brain barrier; MTX injected IV soon afterwards was thus able to enter the brain readily. The procedure also works for adriamycin [20].

The major problem of high-dose MTX, however, remains to be solved. Renal failure results from a direct toxic effect of MTX on renal tubules [8] and from precipitation of MTX and its 7-hydroxy metabolite in renal tubules [13].

The results presented show that polysorbate 80 increases the absorption of and enhances the uptake of MTX into the brain. Higher levels of MTX in the serum do not necessarily lead to higher brain levels (Table 1). The results suggest that polysorbate 80 might have a direct effect on altering the blood-brain barrier. The lower serum levels of MTX in mice given the drug with polysorbate 80 seem to strengthen the earlier suggestion [5] that polysorbate 80 also enhances the excretion of MTX by the kidneys and liver. This is confirmed by the measurement of MTX excreted in urine and faeces, as shown in Fig. 2a and b and Table 3. The ability to increase the elimination of MTX might be a useful means of reducing the toxicity observed with high-dose MTX.

Oral polysorbate 80 at the dose levels used did not cause any reduction in plasma volume. The increase in MTX absorption from the gastrointestinal tract observed and reported here was therefore not due to the artefactual levels resulting from the reduced blood volume, as noted by Harrison et al. [10]. Experiments with mice have shown a minimal loss of stomach mucosal border caused by 12% polysorbate 80, but no significant disruption of intestinal mucosal membrane was observed. These results are in agreement with the observation by Nissim [21] and Yamada and Tamamoto [27]. The lack of polysorbate 80 effect on intestinal mucosa may be due to the fact that this surfactant is metabolised in the intestine by pancreatic lipase. Our results [4] suggested that only about 3.2% of the total ^{131}I-labelled polysorbate 80 dose administered PO is excreted unchanged into the urine. Treon et al. [24] observed that polysorbate 80 was extensively metabolised in the rat gastrointestinal tract into oleic acid and polyoxyethylene sorbitol.

In conclusion, the animal experiments confirmed the ability of polysorbate 80 to increase the absorption of MTX from the mouse gastrointestinal tract. Polysorbate 80 was also shown to have a direct effect on the blood-brain barrier, resulting in an increase in MTX uptake into the brain. Polysorbate 80 also has an additional effect, in that it enhances the excretion of MTX into both bile and urine.

Acknowledgements. We thank the Cancer Research Campaign for the award of a grant, SP 1429, towards this work and the Universiti Sains Malaysia for support to M. N. Azmin. Our thanks also to Dr Ian Brown from the Department of Pathology, University of Glasgow, for the assessment of the histological specimens to and Professor Calman for his encouragement and support.

References

1. Albelson HT, Ensminger W, et al. (1979) High-dose methotrexate-carboxypeptidase. A selective approach to the therapy of central nervous tumours. In: Kisliuk RL, Brown GW (eds) Chemistry and biology of pteridines. Vol 4, North Holland, Amsterdam, pp 629–633
2. Albelson HT, Kufe DW, Skarin AT, Major P, et al (1981) The treatment of central nervous system tumours with methotrexate. Cancer Treat Rep [Suppl] 65: 137
3. Attwood D, Florence AT (1983) Surfactant systems. Their chemistry, pharmacy and biology. Chapman and Hall, London
4. Azmin MN (1983) Polysorbate 80 and the absorption and distribution of methotrexate in the mouse. PhD thesis, University of Strathclyde, Glasgow
5. Azmin MN, Stuart JFB, Calman KC, Florence AT (1982) Effects of polysorbate 80 on the absorption and distribution of oral methotrexate (MTX) in mice. Cancer Chemother Pharmacol 9: 161
6. Bertino JR (1981) Clinical use of methotrexate, with emphasis on use of high-dose. Cancer Treat Rep [Suppl] 65: 131
7. Bryan AJ, Kaur R, Robinson G, Thomas NW, Wilson CG (1980) Histological and physiological studies on intestine of rat exposed to solutions of Myrij 52 and PEG 200. Int J Pharm 7: 145
8. Condit PT, Chanes RE, Joel W (1969) Renal toxicity of methotrexate. Cancer 23: 126
9. Gantt L, Crochman N, Dyniewicz JM (1961) Effect of a detergent on gastro-intestinal absorption of a steroid. Lancet I: 486
10. Harrison SD, Cusic AM, McAfee SM (1981) Tween 80 increase plasma adriamycin concentration in mice by an apparent reduction of plasma volume. Eur J Cancer 17: 387
11. Hasegawa H, Allen JC, Mehta BM, Shapiro WR, Posner JB (1979) Enhancement of CNS penetration of methotrexate by hyperosmolar intracarotid mannitol on carcinomatous meningitis. Neurology 29: 1280

12. Hikal AH (1981) Effect of polysorbate 80 on the apparent partition coefficient of drugs on their intestinal absorption in the rat: II. Phenobarbital. Int J Pharm 7: 205
13. Jacobs SA, Stroller RG, Chabner BA, et al. (1976) 7-hydroxy-methotrexate as a urinary metabolite in human subjects and rhesus monkeys receiving high-dose methotrexate. J Clin Invest 57: 534
14. Kaneda A, Nishimura K, Muranishi S, Sezaki H (1974) Mechanism of drug absorption from micellar solution: II. Effect of polysorbate 80 on the absorption of micelle-free drug. Chem Pharm Bull 23: 523
15. Katzemi K, Arita T, Muranishi S (1965) Absorption and excretion of drugs: XXVI. Effect of non-ionic surface-active agents on rectal absorption of sulphonamides. Chem Pharm Bull 13: 976
16. Levin VA, Patlack CS, Landahl HD (1980) Heuristic modelling of drug delivery to malignant brain tumour. J Pharmacokinet Biopharm 8: 257
17. Levy G, Anello JA (1968) Effect of complex formation on drug absorption V. Studies on the mechanism on the secobarbital absorption enhancing of polysorbate 80 in goldfish. J Pharm Sci 57: 101
18. Matsuzawa T, Fujisawa H, Aoki H, Mima H (1969) Effect of some non-ionic surfactants on the absorption of enduricidin from the muscles. Chem Pharm Bull 17: 999
19. Neuwelt EA, Diehl JT, Vu LH, Hill SA, Michael AJ, Frenkel EP (1981a) Monitoring of methotrexate delivery in patients with malignant brain tumours after osmotic blood-brain barrier disruption. Ann Intern Med 94: 449
20. Neuwelt EA, Pagel M, Barnett P, Glassberg M, Frenkel EP (1981b) Pharmacology of toxicity of intracarotid adriamycin administration following osmotic blood-brain barrier modification. Cancer Res 41: 4466
21. Nissim JA (1960) Action of some surface-active compounds on the gastro-intestinal mucosa. Nature 137–805
22. Rosen GA, Ghavimi F, Nirenberg A, et al. (1977) High-dose methotrexate with citrovorum-factor rescue for treatment of central nervous system tumours in children. Cancer Treat Rep 61: 681
23. Shapiro WR, Young DF, Mehta BM (1975) Methotrexate distribution in cerebrospinal fluid after intravenous, ventricular and lumbar injections. N Engl J Med 293: 161
24. Treon JF, Gongwer LE, Nelson MF, Kirschman JC (1967) Physiology and metabolic patterns of non-ionic surfactants. In: Paquot P (ed) Chemistry, physics and application of surface-active substances, vol 3. Gordon and Breach, London, p 381
25. Walters KA, Dugard PH, Florence AT (1981) Nonionic surfactants and gastric-mucosa transport of paraquat. J Pharm Pharmacol 33: 207
26. Whitemore DA, Brookes LG, Wheeler KP (1979) Relative effects of different surfactants on intestinal absorption and release of proteins and phospholipids from tissue. J Pharm Pharmacol 31: 277
27. Yamada H, Yamamoto R (1965) Biopharmaceutical studies on factors affecting rate of absorption of drug: I. Absorption of salicylamide in micellar solution. Chem Pharm Bull 13: 1279

Received June 1, 1984/Accepted November 8, 1984