brane-associated activity was evident. In cultures exposed to β-endorphin-(1-27) (30 nM) the relative predominance of the different forms of the enzyme was essentially the same as in controls (fig., E, cf A). The histological localisation of AChE was similar to that in the presence of β-endorphin but fewer nuclei were AChE-positive. However, β-endorphin-(1-27)-exposed cultures were unique in that dense reaction product was focally distributed in the cytoplasm as well as being associated with AChE-positive nuclei in these areas (fig., F). BuChE was barely detectable in control cultures and was seen mainly in association with myotube nuclei. Its distribution was unaffected by the peptides. These findings indicate that multiple effects of naturally-occurring β-endorphin derivatives can regulate the distribution of AChE, and the nature of the effect depends on the length of the C-terminus. β-endorphin alters the interconversion of low-molecular weight, globular (G_1, G_2, G_4) asymmetric and (A_{12}) AChE forms, stimulating the accumulation of the globular AChEs within the cell. β-endorphin-(1-27) stimulates the lateral localisation of the enzyme into active regions of the cell, reminiscent of those seen at sites of nerve contact. At the time of innervation, AChE loses its diffuse localisation in the rat muscle cell and becomes associated with specialised nuclei which become associated with the future motor endplate [10]. Our observations are consistent with the possibility that β-endorphin and related peptides, which may be released from motor nerve terminals in immature rats [11], exert a trophic control over muscle AChE.

1 Rieger, F., Koenig, J., and Vigney, M., Devl Biol. *76* (1980) 358.
2 Brockman, S. K., Younkin, L. H., and Younkin, S. G., J. Neurosci. *4* (1984) 131.
3 Bulger, J. E., Randall, W. R., Nieberg, P. S., Patterson, G. T., McNamee, M. G., and Wilson, B. W., Dev. Neurosci. *5* (1982) 474.
4 Fernandez, H. L., Patterson, M. R., and Duell, M. J., J. Neurobiol. *11* (1980) 557.
5 Haynes, L. W., Smyth, D. G., and Zakarian, S., Brain Res. *232* (1982) 115.
6 Haynes, L. W., and Smith, M. E., Neurosci Lett. *55* (1985) 125.
7 Haynes, L. W., Smith, M. E., and Smyth, D. G., J. Neurochem. *42* (1984) 1542.
8 El Badawi, A., and Schenk, E. A., J. Histochem. Cytochem. *15* (1976) 580.
9 Inestrosa, N. C., Silberstein, L., and Hall, Z. W., Cell *29* (1982) 71.
10 Kupfer, C., and Koelle, G. B., J. exp. Zool. *116* (1951) 397.
11 Haynes, L. W., and Smith, M. E., J. Physiol. (Lond.) *351* (1984) 22P.

0014-4754/90/020211-03$1.50 + 0.20/0

The effect of moderate hemodilution with Fluosol-DA or normal saline on acetaminophen disposition in the rat

R. P. Shrewsbury and L. G. White

Division of Pharmaceutics, School of Pharmacy, University of North Carolina, Chapel Hill (North Carolina 27599-7360, USA)
Received 14 November 1988; accepted 31 August 1989

Summary. Hemodilution with 40 ml/kg of Fluosol or saline reduced the acetaminophen V_d and the acetaminophen sulfate Cl_M at 48 or 72 h, respectively. Fluosol hemodilution increased the acetaminophen renal excretion at 24 and 72 h. But at 48 h, Fluosol hemodilution either inhibited the renal secretion of acetaminophen or enhanced its reabsorption.
Key words. Acetaminophen; Fluosol; sulfation; glucuronidation; hemodilution.

Perfluorochemical (PFC) emulsions are being evaluated as potential blood substitutes or acellular oxygen carrying substances because of their ability to dissolve oxygen. Numerous animal studies have demonstrated that severe hemodilution with PFC emulsions can sustain life. PFC emulsions have been used clinically in man for blood loss replacement, oxygen delivery to ischemic tissues, myocardial infarction, coronary angioplasty, spinal cord injury, sickle cell crisis, gangrene, chemotherapy of solid tumors and severe anemia [1].

PFC particles are captured by the reticuloendothelial system and reach a maximum hepatic content in 2 days [2]. The PFC particles have been found in Kupffer cells, hepatocytes, mononuclear phagocytes, and 'foamy' macrophages [3]. Not unexpectedly, the disposition of several drugs is altered immediately after PFC administration [4]. Recent studies have indicated that Fluosol®-DA (Fluosol) hemodilution alters cytochrome P-450 mediated drug metabolism in a time-dependent and dose-dependent manner [5]. The current investigation determines if the conjugation pathways of sulfation and glucuronidation involved in acetaminophen disposition are altered by Fluosol or normal saline hemodilution in a time-dependent manner.

Materials

Fluosol was donated by Alpha Therapeutic Corporation (Los Angeles, California), and was prepared as directed within 0.5 h of use. Fluosol is a 20% (w/v) perfluorode-

calin and perfluorotripropylamine (14:6) emulsion containing Pluronic F-68 (2.7%), hydroxyethyl starch (3.0%) and smaller percentages of yolk phospholipids, potassium oleate, glucose, glycerol, and various inorganic salts. Acetaminophen, 2-acetamidophenol, and HPLC grade solvents and buffers were obtained from commercial sources and used without further purification. Beta-glucuronidase from *E. coli* (44,400,000 FU/ml) and beta-glucuronidase/arylsulfatase (100,000 FU and 800,000 RU/ml) were obtained from Boehringer Mannheim Biochemicals (Indianapolis, Indiana). Male Sprague-Dawley rats, 210–298 g, were used. They had free access to food and water and were synchronized to a 12-h light-dark cycle.

Methods

Acetaminophen disposition was determined in conscious, unrestrained, unexchanged rats and rats hemodiluted with 40 ml/kg of Fluosol or saline (0.9% NaCl). Saline hemodiluted rats were included in the study to differentiate between changes in acetaminophen disposition due to Fluosol itself and changes resulting from the loss of erythrocytes and other blood constituents. A silastic cannula was implanted in the right jugular vein under light ether anesthesia 24 h before any hemodilution or acetaminophen dosing. The study protocol and procedures for hemodilution have been previously reported[6]. Acetaminophen disposition was determined at 0.5, 24, 48 and 72 h after hemodilution by giving a single intravenous dose (30 mg/kg) through the implanted cannula. (Codes are: unexchanged, CONT; Fluosol groups, 0.5HF, 24HF, 48HF and 72HF; saline groups, 0.5HS, 24HS, 48HS and 72HS.)

Acetaminophen was administered between 08.30 and 09.30 h to minimize diurnal variation[7]. Blood samples (0.25 ml) were collected over 75 min and extracted as follows: to 0.1 ml of plasma 0.15 ml of internal standard (2-acetamidophenol, 10 mg/l), 0.1 ml of phosphate buffer, and 1 ml of ethyl acetate were added. The phosphate buffer was 0.3 ml of 1 M KH_2PO_4 and 0.2 ml of 85% phosphoric acid in 1800 ml of water (pH = 4.4). The samples were vortexed, centrifuged for 2 min, and the organic layer was collected and dried under nitrogen. Each sample was reconstituted with 0.12 ml of methanol. Plasma acetaminophen concentrations were determined by HPLC with a 10 µ C_{18} (250 × 4.6 mm i.d.) Alltech column, a mobile phase of phosphate buffer: methanol: acetonitrile (85:10:5), and a flow rate of 2.5 ml/min. Ultraviolet absorbency was monitored at 254 nm (0.005 AUFS) and the peak area ratio of acetaminophen to 2-acetamidophenol was determined. The standard curves were prepared using plasma from CONT animals or animals which had been hemodiluted with the appropriate hemodiluent.

Urinary concentrations of acetaminophen, acetaminophen sulfate, and acetaminophen glucuronide were determined as reported by Nakamura[8] since authentic standards of the two metabolites could not be obtained. Fourfold dilutions of 24-h collected urine were made with acetate buffer, and to 2 ml of the diluted urine, 0.5 ml of acetate buffer, beta-glucuronidase (1:99 in acetate buffer) or beta-glucuronidase/arylsulfatase (1:39 in acetate buffer) was added. The acetate buffer was 0.1 M acetic acid and sodium acetate (1:2), pH = 5.0. The samples were vortexed and incubated for 24 h at 37 °C. 2 ml of aqueous internal standard solution (2-acetamidophenol, 200 mg/l) was added before injection onto the column. Chromatographic separation for acetaminophen was achieved with the C_{18} column, a mobile phase of water:2-propanol (96:4, pH = 2.9 adjusted with 85% phosphoric acid), and a flow rate of 2.0 ml/min. Ultraviolet absorbency was monitored at 254 nm and the peak area ratios were determined. Standard curves were prepared using urine from CONT animals or animals hemodiluted with the appropriate hemodiluent.

Standard curves for both plasma and urinary samples were linear from 1 to 100 mg/l. Fitting of plasma acetaminophen concentrations to a monoexponential equation, calculation of disposition parameters, and statistical analysis (Wilcoxon rank sum test) were done with the non-linear regression program (NLIN) of the Statistical Analysis System (SAS) weighting the plasma concentrations by their squared reciprocal.

Results

Table 1 shows the acetaminophen pharmacokinetic parameters following hemodilution with 40 ml/kg of Fluosol or saline. The acetaminophen elimination rate constant (K_{el}) tended to be elevated in all groups but was significantly different at 48HS and 72HS. As expected, acetaminophen half-lives ($T_{1/2}$) inversely followed the K_{el} pattern, with all groups being less than CONT and significantly different in the 48HS and 72HS groups. The apparent volume of distribution (V_d) was significantly decreased 46% at 48HF and 34% at 72HS. Acetaminophen clearance (Cl) was significantly decreased 39% at 48HF. The significantly shorter $T_{1/2}$ and greater K_{el} at 48HS and 72HS resulted from the decrease V_d and not a Cl change as K_{el} is dependent upon the independent parameters V_d and Cl.

Table 2 shows the acetaminophen, acetaminophen glucuronide and acetaminophen sulfate urinary excretion percentages. The acetaminophen percentage was increased at both 24HF and 72HF, but the 48HF percentage was not different from CONT. None of the acetaminophen glucuronide or acetaminophen sulfate groups were significantly different from CONT, although the acetaminophen sulfate percentage was lowest at 72HF and 72HS.

Acetaminophen renal clearance (Cl_R) and the metabolic clearance(Cl_M) of acetaminophen relating to glucuronide and sulfate formation were calculated as the product of

Table 1. Averaged disposition parameters of acetaminophen after hemodilution with 40 ml/kg of Fluosol-DA or normal saline

	Treatment groups CONT	0.5HF	24HF	48HF	72HF	0.5HS	24HS	48HS	72HS
N	5	5	5	4	5	5	5	5	5
K_{el} (min^{-1})	0.050 +	0.053	0.057	0.056	0.059	0.054	0.052	0.064*	0.061*
	0.006	0.005	0.002	0.010	0.007	0.005	0.005	0.004	0.006
$T_{1/2}$ (min)	14.1	13.1	12.2	12.8	11.8	12.9	13.5	11.0*	11.4*
	1.6	1.3	0.5	2.2	1.6	1.3	1.3	0.7	1.2
V_d (ml/kg)	894	808	702	487*	737	727	978	652	593*
	162	258	107	56	104	195	128	193	154
Cl (ml/min/kg)	43.9	42.1	40.1	26.8*	43.7	38.8	50.2	41.4	35.7
	6.3	9.2	6.3	3.0	8.0	8.1	4.0	12.0	6.3

+ Mean with SD; * significantly different from CONT ($p \leq 0.05$).

Table 2. Averaged urinary excretion percentages of acetaminophen, acetaminophen glucuronide and acetaminophen sulfate after hemodilution with 40 ml/kg of Fluosol-DA or normal saline (percent of administered dose)

	Treatment groups CONT	0.5HF	24HF	48HF	72HF	0.5HS	24HS	48HS	72HS
Acetaminophen	7.5 +	7.6	13.8*	6.4	14.1*	4.7	8.3	9.2	8.4
	3.5	1.4	3.1	3.8	1.9	3.7	2.3	2.1	2.9
Acetaminophen glucuronide	1.8	2.0	2.6	3.4	3.6	5.3	2.4	2.6	3.0
	1.6	2.2	4.6	4.5	2.4	3.6	1.5	1.7	1.9
Acetaminophen sulfate	77.1	77.0	82.1	88.1	65.5	83.6	83.5	73.7	62.8
	12.3	12.3	3.6	3.7	5.0	9.4	4.6	7.0	5.6

+ Mean with SD; * significantly different from CONT ($p \leq 0.05$).

Table 3. Averaged renal clearance of acetaminophen and metabolic clearances of acetaminophen relating to glucuronide and sulfate formation after hemodilution with 40 ml/kg of Fluosol-DA or normal saline

	Treatment groups CONT	0.5HF	24HF	48HF	72HF	0.5HS	24HS	48HS	72HS
Acetaminophen (ml/min/kg)	3.3 +	3.3	5.5	1.7	6.1	1.8	4.1	3.6	3.0
	2.0	1.4	1.4	1.2	1.1	1.6	1.0	0.3	1.0
Acetaminophen glucuronide (ml/min/kg)	0.8	0.9	1.1	0.9	1.5	2.0	1.2	1.2	1.1
	0.7	1.2	2.0	1.2	0.9	1.2	0.8	0.8	0.6
Acetaminophen sulfate (ml/min/kg)	33.9	32.1	32.9	23.6*	28.7	32.2	41.8	30.2	22.2*
	8.0	10.0	5.3	2.9	5.9	4.3	2.5	8.1	2.8

+ Mean with SD; * significantly different from CONT ($p \leq 0.05$).

the percentage excreted times Cl, and are shown in table 3. The acetaminophen sulfate Cl_M was significantly decreased at 48HF and 72HS. The acetaminophen Cl_R was elevated at 24HF and 72HF, although not significantly different at $p \leq 0.05$.

Discussion

Acetaminophen, N-acetyl-p-aminophenol, is a commonly used analgesic and antipyretic substitute for aspirin. Acetaminophen disposition involves 4 pathways: two conjugation pathways forming a sulfate and glucuronide metabolite; the formation of a cytochrome P-450 mediated metabolite; and renal excretion of unchanged acetaminophen[9]. Toxicity has been shown to occur when the conjugation reactions become limited and acetaminophen is oxidized by the cytochrome P-450 enzymes to the chemically reactive metabolite, N-acetylbenzoquinoneimine. The acetaminophen sulfate conjugation pathway is the predominant pathway in rats and becomes limited due to the depletion of inorganic sulfate at dosages greater than 160 mg/kg[13]. The 30 mg/kg acetaminophen dose used in this study is clearly in the region where the cosubstrate availability is not rate limiting and where direct inhibition of the sulfotransferase would be the most likely explanation for a decline in drug clearance[10].

All acetaminophen CONT parameters in this study, both plasma and urinary excretion parameters, are comparable with numerous other reports[9,11-13]. Acetaminophen Cl was found to be significantly decreased only at 48HF compared to CONT. There is a corresponding reduction in the acetaminophen sulfate Cl_M (see table 3), but the excretion percentage of the conjugate did not

decrease, a phenomenon previously reported [10]. After saline hemodilution, there was a 35% decrease in the acetaminophen sulfate Cl_M at 72HS accompanied by a decrease in the mean total percentage excreted (acetaminophen, acetaminophen glucuronide, plus acetaminophen sulfate: CONT 86.4%, 72HS 73.9%). Neither of these changes caused a significant decrease in Cl, although Cl tended to be reduced.

Both Fluosol and saline hemodilution reduced the acetaminophen sulfate Cl_M at 48 and 72 h, respectively. Therefore, the effect appears to be inherent to the hemodilution process and independent of the hemodiluent used. Because of the dose of acetaminophen used in this study, a hemodilution mediated interference with sulfotransferase is the most likely explanation for the reduced acetaminophen sulfate Cl_M. However, the interference may be of a transitory nature as the acetaminophen sulfate Cl_M was not different from CONT at 72HF. Perhaps an endogenous substance released secondary to hemodilution is inhibiting the sulfotransferase. Studies in rats have shown that severe Fluosol hemodilution alters the concentrations of many plasma constituents, red and white cell counts, immunological competence, and coagulation response [14, 15]. Though detailed information of this nature is not available following moderate Fluosol or saline hemodilution, it is reasonable to assume that similar changes, perhaps abated, are occurring.

The acetaminophen excretion percentage and Cl_R were depressed at 48HF in marked contrast to their elevated values at 24HF and 72HF. This enhancing effect of Fluosol hemodilution is unique for the emulsion as saline hemodilution caused no such effect. The renal creatinine clearance, used to approximate glomerular filtration rate (GFR), was reported as 3.8–6.6 ml/min/kg in hemodiluted rats, and did not vary significantly from control animals [6]. In the present study's CONT group, the acetaminophen mean Cl_R was 3.3 ml/min/kg which suggests that acetaminophen undergoes significant reabsorption since the Cl_R is less than GFR. The increased acetaminophen Cl_R at 24HF and 72HF would imply that Fluosol hemodilution promotes renal secretion of acetaminophen or inhibits its reabsorption. The decreased Cl_R at 48HF could therefore suggest that Fluosol either inhibited the secretion or promoted the reabsorption of acetaminophen. A distinction between these mechanisms cannot be determined from this study's data alone. One or more of the non-PFC Fluosol components may play a role in the overall mechanism as it has been reported that Pluronic F-68 caused structural changes in the rat kidney tubules in vivo and caused increased protein loss in perfused rat kidneys [15].

V_d was significantly decreased at 48HF and 72HS and slightly reduced in all other groups except at 24HS. V_d is the quotient of the dose divided by the initial plasma concentration (i.e., at time zero, C_0), and was decreased because C_0 was significantly greater at 48HF and 72HS and slightly increased in all other groups except at 24HS (data not shown). Since both Fluosol and saline hemodilution influenced V_d and C_0 in the same manner, these changes appear to be dependent on the hemodilution process and independent of the hemodiluent used.

The increased C_0 would result from either a decreased blood volume or an increased plasma concentration of acetaminophen. The half-life of Pluronic F-68 and hydroxyethyl starch after Fluosol hemodilution is less than 40 h, and the blood volume will only be maintained if adequate albumin recovery has occurred by that time [16]. In severely Fluosol hemodiluted animals, plasma albumin concentrations were below control levels for 24 h and were not restored for 3 days [17]. Preliminary data from this laboratory showed that moderate Fluosol hemodilution reduced plasma albumin concentrations through 24 h, while saline hemodilution reduced the concentration only at 0.5 h after hemodilution. Thus the V_d may be reflecting blood volume changes as the Fluosol oncotic agents are eliminated and plasma albumin concentrations are restored.

Two studies have suggested other mechanisms that may be causing blood volume changes. Fournier et al. reported that vascular concentrations of perfluorotributylamine (Fluosol 43) increased over 3 h after severe hemodilution [18]. The simultaneously determined ^{131}I-albumin volume suggested that the increased concentrations resulted from water transfer from the vascular to interstitial fluid. They proposed that the water extravasation was a response to histamine released secondary to hemodilution. Guidet et al. showed that even hypervolemic hemodilution with albumin plus gelatin or gelatin alone resulted in a reduced plasma volume, and suggested that vascular membrane permeability was altered [19].

The possibility that acetaminophen binds to Fluosol or one of its components to cause the change in V_d and C_0 is unlikely as acetaminophen binding to saline does not seem a reasonable interaction. But acetaminophen plasma concentrations would increase if acetaminophen binds to an endogenous substance released secondary to hemodilution. McCoy et al. studied the regeneration response of rats to severe Fluosol hemodilution for 21 days [17]. Several plasma constituents were found to rebound beyond control values 1–4 days after hemodilution. For example, reticulocyte levels peaked at 11-fold after 4 days, and leukocyte levels were doubled between 2 and 4 days. Gamma glutamyltranspeptidase and SGP and SGO transaminase concentrations were 2–5 times greater than control for 2–5 days after hemodilution. BUN and direct bilirubin concentrations were 2- and 3-fold greater than control at day 4 and days 2 and 3, respectively. Any of these constituents could be a potential binding site for acetaminophen.

Hemodilution with either Fluosol or saline has little influence on the acetaminophen glucuronide percentage excreted or the acetaminophen glucuronide Cl_M. Although microsomal glucuronidation is not the primary

route of acetaminophen disposition in the rat, it does compete with nonmicrosomal sulfotransferase for the substrate. One report indirectly suggested that Fluosol hemodilution enhanced glucuronidation since the elimination half-life of phenytoin's primary metabolite, HPPH, was decreased [20]. But it has been suggested that phenytoin disposition may not be an appropriate marker of hepatic microsomal activity [21].

Thus it appears that hemodilution with either Fluosol or saline does reduce the acetaminophen sulfate Cl_M and V_d at 48 or 72 h, respectively. In addition, Fluosol uniquely alters the renal excretion of acetaminophen. With such limited information regarding the physiological responses to moderate hemodilution for several days, the mechanisms responsible for the effects are necessarily speculation. Only as more detailed investigations are reported will the speculation yield to understanding of the complex effects of hemodilution on drug disposition.

Acknowledgement. Funded by the National Heart, Lung, and Blood Institute (HL 33227).

1 Vercellotti, G. M., and Hammerschmidt, D. E., in: Blood Substitutes, pp. 173–183. Ed. K. C. Lowe. VCH Publishers, New York 1988.
2 Lutz, J., Metzenauer, P., Kunz, E., and Heine, W. D., in: Oxygen Carrying Colloidal Blood Substitutes: Fifth Symposium on Perfluorochemical Blood Substitutes, pp. 73–81. Eds R. Frey, H. Beisbarth and K. Stosseck. W. Zuckschwerdt Verlag, München 1982.
3 Lowe, K. C., and Bollands, A. D., Med. Lab. Sci. *42* (1985) 367.
4 Kemner, J. M., Snodgrass, W. R., Worley, S. E., Hodges, G. R., Melethil, S., Hignite, C. E., and Tschanz, C., Res. Commun. Chem. Path. Pharmac. *46* (1984) 381.
5 Shrewsbury, R. P., Oliver, S. R., Anderson, W. T., Lewis, L. M., and White, L. G., J. Pharm. Pharmac. *40* (1988) 392.
6 Shrewsbury, R. P., J. Pharm. Pharmac. *38* (1986) 647.
7 Belanger, P. M., Lalande, M., Dore, F., and Labrecque, G., J. Pharmacokinet. Biopharm. *15* (1987) 133.
8 Nakamura, J., Baba, S., Nakamura, T., Sasaki, H., and Shibasaki, J., J. Pharmacobio-Dyn. *10* (1987) 673.
9 Galinsky, R. E., and Levy, G., J. Pharmac. exp. Ther. *219* (1981) 14.
10 Galinsky, R. E., and Corcoran, G. B., Drug Metab. Disp. *16* (1988) 348.
11 Galinsky, R. E., J. Pharmac. exp. Ther. *236* (1986) 133.
12 Lin, J. H., and Levy, G., J. Pharmac. exp. Ther. *239* (1986) 94.
13 Watari, N., Masuaki, I., and Kaneniwa, N., J. Pharmacokinet. Biopharm. *11* (1983) 245.
14 Lowe, K. C., Comp. Biochem. Physiol. *87A* (1987) 825.
15 Lowe, K. C., in: Blood Substitutes, pp. 149–172. Ed. K. C. Lowe. VCH Publishers, New York 1988.
16 Biro, G. P., and Blais, P., CRC Crit. Rev. Oncol. Hemat. *6* (1987) 311.
17 McCoy, L.E., Elliott, L. A., Lucas, C. E., and Ledgerwood, A. M., in: Blood Substitutes, pp. 575–583. Eds T. M. S. Chang and R. P. Geyer. Marcel Dekker, New York 1989.
18 Fournier, G., Barrois, V., Girard, C., Robert, D., and Foulletier, L., Experientia *36* (1980) 568.
19 Guidet, B., Luquel, L., De Labriolle Vaylet, C., and Offenstadt, G., Int. J. artif. Organs *10* (1987) 325.
20 Shrewsbury, R. P., Lewis, L. M., and Oliver, S. R., J. Pharm. Pharmac. *39* (1987) 349.
21 Bachmann, K. A., Jahn, D., Yang, C., and Schwartz, J., Xenobiotica *18* (1988) 161.

0014-4754/90/020213-05$1.50 + 0.20/0

Failure of (+)-naloxone to accelerate feline colonic transit

B. Krevsky, R. S. Fisher and A. Cowan

Departments of Medicine and Pharmacology, Temple University School of Medicine, Philadelphia (Pennsylvania 19140, USA)

Received 12 April 1989; accepted 15 September 1989

Summary. To determine whether the colonic transit accelerating effect of (−)-naloxone (0.3 mg/kg, i.m.) is due to an action at opioid receptors or a direct pharmacologic effect, its enantiomer, (+)-naloxone (0.3 mg/kg, i.m.), was administered to cats and compared to saline control using colonic transit scintigraphy. Transit was not accelerated by (+)-naloxone. The effects of naloxone on colonic transit are thus stereospecific, and are probably mediated by opioid receptors.

Key words. (−)-Naloxone; (+)-naloxone; cat; transit; scintigraphy; colon.

The discovery of opioid immunoreactivity in the colon has led to the suggestion that colonic function is controlled, at least in part, by endogeneous opioids. Consistent with this theory, several studies have demonstrated altered colonic motility or accelerated transit after the administration of the narcotic antagonist (−)-naloxone to humans [1], cats [2, 3], and rats [4]. The improvement of chronic idiopathic constipation in 2 patients by (−)-naloxone further suggests that this antagonist may have clinical usefulness in certain colonic disorders [5]. However, since biological effects of (−)-naloxone not involving opioid receptors have been reported [6], it is necessary to demonstrate more conclusively that the accelerating effect of naloxone is an opioid receptor-mediated phenomenon. The (+)-enantiomer of naloxone has only $^1/_{1000}$ to $^1/_{10000}$ the opioid antagonist activity of (−)-naloxone [7], and has been used successfully to differentiate endogenous opioid receptor effects from the possible pharmacological actions of (−)-naloxone on its own [8]. In this study the effect of (+)-naloxone on colonic transit was compared to saline control. The dose, route of administration, and animal model were identical to a previous study from our laboratory which demonstrated accelerated transit with (−)-naloxone [2].