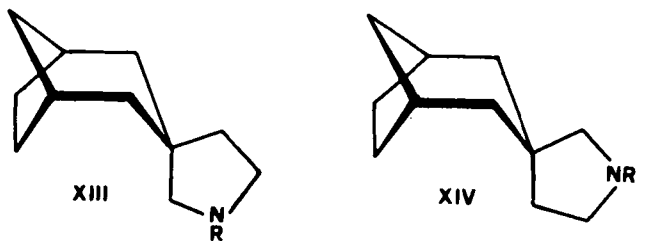
Table I—Inhibition of Growth of Influenza A Viruses by IX-XII and 1-Adamantanamine Hydrochloride

Inhibitor[a]	Titration of Infection[b], Plaque-Forming Units/mL	Inhibition[c]
None	2×10^7	—
IX	1×10^6	−1.30
X	7×10^5	−1.45
XI	1×10^7	−0.30
XII	7×10^6	−0.45
1-Adamantanamine hydrochloride	2×10^6	−1.00

[a] Inhibitors were administered at a concentration of 100 μM in an alcohol-water solution. [b] Plaque assay and primary isolation of influenza A viruses were carried out in an established line of canine kidney cells (MDCK), in the presence of trypsin (7). [c] Inhibition is expressed as the decimal logarithm of the quotient of plaque-forming units in the presence of inhibitor to that found in the absence of inhibitor: $\log_{10}$ [(pfu/mL of inhibitor)/(pfu/mL of control)].

appearance of two singlets, one each for the *endo* and *exo* $C_{4'}$-methylene groups. The same feature was observed in the NMR spectra of succinimides V-VIII.



Protons $H_AH_BH_{A'}H_{B'}H_X$ behave as two different ABX and A'B'X systems. The influence of the deshielding effect of the *endo* $C_{2'}$-carbonyl group on protons H_B and $H_{B'}$ is shown by the downfield chemical shift of these protons at $\delta H_B = 2.18$ ppm and $\delta H_{B'} = 1.96$ ppm relative to those of the less-affected protons H_A and $H_{A'}$, which appear upfield at δ 1.80 and 1.70 ppm, respectively. Assignation of H_B and $H_{B'}$ has been done on the basis of the observable geminal coupling constants $J_{BA} = -14.34$ Hz and $J_{B'A'} = -8.09$ Hz, which are in agreement with reported values for cyclohexane and cyclopentane, respectively (8).

X-ray diffraction data of succinimide VI (9) conclusively confirm that the $C_{2'}$-carbonyl group is attached on the *endo*-position. In this case the spirocyclohexane ring appears to adopt a boat conformation to avoid the steric interaction between the $C_{2'}$-carbonyl group and the $C_{6(7)}$-methylene groups.

Biology—According to results shown in Table I, IX-XII, especially the *N*-benzyl and *N*-cyclohexyl derivatives (IX and X), show antiviral activity against influenza A viruses similar to or greater than that shown by 1-adamantanamine hydrochloride.

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Distribution and Elimination of Polymethyl Methacrylate Nanoparticles After Peroral Administration to Rats

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Received March 7, 1983 from the **Sandoz Forschungsinstitut GmbH, Brunner Strasse 59, A 1235 Vienna, Austria*, the ‡*Department of Pharmaceutics, Swiss Federal Institute of Technology, Clausiusstrasse 25, CH 8092 Zurich, Switzerland*, and the §*Pharmaceutical Division, Sandoz Ltd., CH 4002 Basle, Switzerland.* Accepted for publication September 13, 1983.

Abstract □ Polymethyl [1-^{14}C]methacrylate nanoparticles were administered orally to bile cannulated rats. Ten to fifteen percent of the administered radioactivity was absorbed and found in the bile and urine. Within 48 h, 94 97% of the absorbed radioactivity had been eliminated from the body. After 8 d, the highest residual radioactivity was found in the bone marrow, fatty renal tissue, stomach, liver, and lymph nodes.

Keyphrases □ Polymethyl methacrylate—peroral administration, distribution, elimination, rats □ Peroral administration—polymethyl methacrylate, elimination and distribution, rats

A possible pathway of absorption is the uptake of colloidal particulate materials by the GI tract in liquid (pinocytosis) or solid (endocytosis) form. This uptake pathway was suggested for fat absorption by Frazer (1, 2). The uptake of corn starch and some other particulate materials by endocytosis was extensively studied by Volkheimer (3). No uptake of polymethyl methacrylate particles (labeled with a fluorescent dye and ranging in size from 10 nm–1.2 μm) was observed by Juhlin (4). This may, however, be due to leakage of the label or to the small size of the particles, which prevented optical observation. For this reason, ^{14}C-labeled polymethyl methacrylate nanoparticles of a mean size of 130 nm were employed in this study. These particles were shown to be taken up by the reticuloendothelial system after intravenous administration (5). After subcutaneous administration, the particles stayed at the injection site for about 200 d. After this time, the beginning of redistribution and elimination was observed (6).

Oral administration of these nanoparticles seems to be of

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Table I—Amount of Radioactivity Excreted after Peroral Administration (Percent of the Administered Dose)

Animal No.	1017	1018	1020	1024	1025	
Sex	F	F	M	M	M	
Body Weight, g	294	302	370	275	181	Mean, ±*SD*
Bile	7.0	5.4	6.6	8.4	6.4	6.8 ± 1.1
Urine	5.2	5.5	4.2	6.2	4.0	5.0 ± 0.9
Feces	89.3	86.5	86.0	116.1	72.8	83.7 ± 7.3[a]
Total	101.5	97.4	96.8	130.6	83.2	94.7 ± 8.0[a]
Amount Absorbed[b]	12.2	10.9	10.8	14.5	10.4	11.8 ± 1.7
Excreted within 24 h[c]	86	90	90	65	83	82.8 ± 10.4
Excreted within 48 h[c]	94	96	97	94	96	95.4 ± 1.3

[a] Calculation without rat 1024. [b] Bile + urine = amount absorbed. [c] Percentage of the total amount absorbed excreted within 24 or 48 h.

interest for the administration of certain vaccines. For example, good protection was observed with an inactivated rabies vaccine adsorbed to polymethyl methacrylate nanoparticles, whereas no protection was obtained with different control vaccines[1]. These particles might also be useful as cytostatic carriers against tumors of the GI tract.

EXPERIMENTAL SECTION

Preparation of Polymethyl [1-^{14}C]methylacrylate nanoparticles—Polymethyl [1-^{14}C]methacrylate was produced as described by Kreuter *et al.* (6). The monomer was dissolved in phosphate buffered saline, with or without influenza virions, to yield 0.5% polymethyl [1-^{14}C]methacrylate solutions. Nanoparticles were produced by polymerization of these monomer solutions using γ-irradiation with 500 krad (2.2 krad/min) in a cobalt-60 source. The resulting suspension had an activity of (5.22 ± 0.46) × 10^8 dpm/mL. The physicochemical characteristics of similar nonradioactive nanoparticles were described by Bentele *et al.* (7) and Kreuter (8).

Excretion and Tissue Distribution after Peroral Administration—Two female and three male bile-cannulated Sprague–Dawley rats weighing 181–370 g were administered 0.5 mL of the well-stirred virus containing nanoparticle suspension with a stomach tube. The animals were kept in metabolism cages with free access to food and a salt solution containing 0.9% sodium chloride, 0.04% potassium chloride, 0.017% calcium dichloride dihydrate, and 0.02% sodium hydrogen carbonate.

Urine was collected in dry ice. The storage container contained 100 μL of diethylamine in order to keep the urine at a pH ≥ 9, thus assuring the binding of the carbon-14 activity excreted as carbonate. After each collection period, the cage was rinsed with water (30–40 mL) to wash out dried urine residues. Samples of 400–500 μL were assayed for radioactivity. The collected feces was homogenized with about the same amount of water. Three samples (200–400 mg) of each homogenate were analyzed. The bile was collected in ice-cooled containers. Samples of 40 (0–8 h) and 100 μL (24–192 h) were assayed for radioactivity.

Eight days after administration, the activity in the organs was measured after sacrificing the animals with an intraperitoneal injection of ~0.5 mL of a euthanasia solution[2]. From the adrenal glands, epididymis, ovary, thyroid gland, lymph nodes, and bone marrow, one 7–130 mg sample was taken; from the other organs, two 50–400 mg samples were taken.

The radioactivity of the excreta, blood, plasma, and organs was determined after combustion in a sample oxidizer[3] (recovery of standard radioactivity: 92–100%). The resulting carbon dioxide was absorbed in a mixture of absorber solution and scintillation liquid[4]. The samples were then counted in a liquid scintillation counter for 20 min. The counting efficiency was determined by an automatic external standardization and previously prepared quench curves. The detection limit was 4 ng/g for the bone marrow, lymph nodes, and thyroid gland and 2 ng/g for all other organs and tissues.

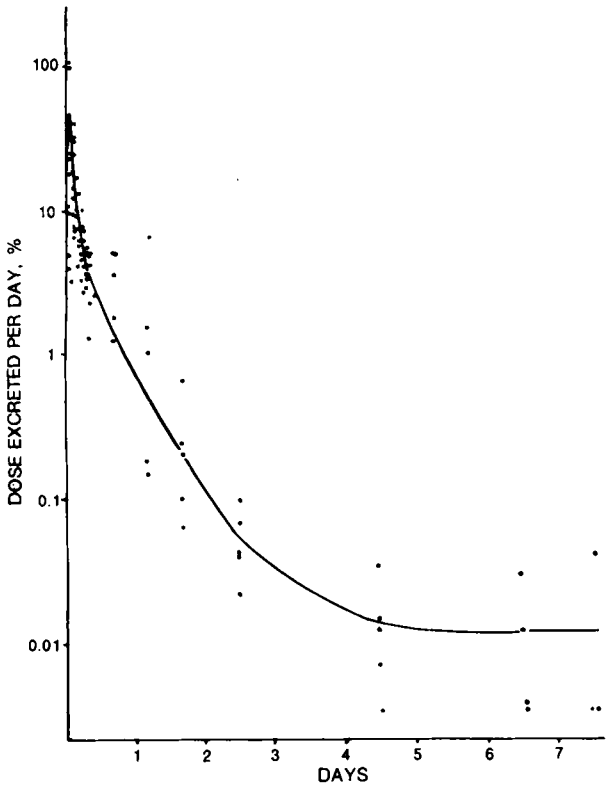

Figure 1—*Rate of biliary excretion of carbon-14 after peroral administration of ^{14}C-labeled polymethyl methacrylate nanoparticles to rats (n = 5).*

RESULTS AND DISCUSSION

Bile-cannulated rats were used so that the amount of radioactivity excreted *via* the bile could be determined. Within 8 d, between 5 and 8% of the administered dose was excreted *via* the bile and 4–6% *via* the urine (Table I).

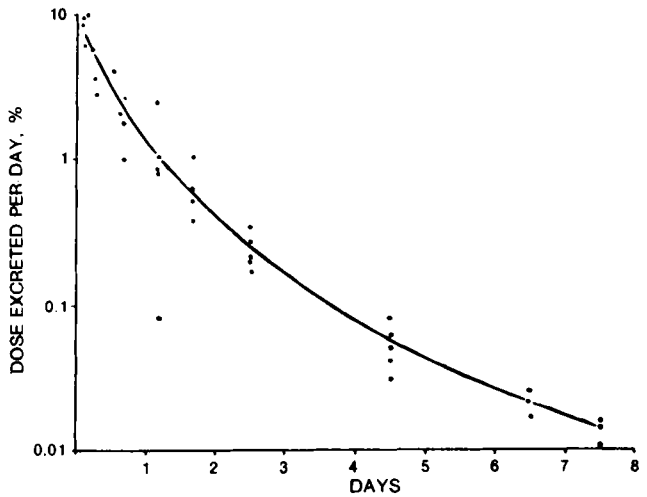

Figure 2—*Rate of urinary excretion of carbon-14 after peroral administration of ^{14}C-labeled polymethyl methacrylate nanoparticles to rats (n = 5).*

[1] Unpublished results.

[2] T-61 (each mL contains: 200 mg *N*-[2-(*m*-methoxy-phenyl)-2-ethyl-butyl-(1)]-gamma-hydroxybutyramide, 50 mg 4,4′-methylene-*bis*(cyclohexyl-trimethyl-ammonium)iodide, and 5 mg tetracaine hydrochloride with 0.6 mL dimethylformamide in distilled water). Farbwerke Hoechst, Frankfurt/Main, West Germany.

[3] Sample oxidizer; Packard Instruments, Downers Grove, Ill.

[4] Carbosorb and Permafluor; Packard Instruments.

Table II—Distribution of Carbon-14 in Organs 8 d after Peroral Administration of ^{14}C-Labeled Polymethyl Methacrylate Nanoparticles to Rats

	ng/g[a]
Blood	5.9 ± 1.6
Plasma	3.6 ± 1.1
Liver	12.7 ± 3.7
Spleen	5.1 ± 1.3
Pancreas	11.9 ± 4.6
Kidneys	9.6 ± 2.3
Adrenals	11.4 ± 6.2
Fatty renal tissue	24.5 ± 14.4
Testicles[b]	4.5
Uterus[c]	7.2
Epididymis[b]	9.0
Ovary[c]	7.0
Colon	5.0 ± 1.34
Stomach	14.5 ± 11.2
Small intestine	4.2 ± 0.6
Salivary glands	4.2 ± 1.4
Lymph nodes	13.3 ± 4.2
Thyroid glands	10.0 ± 4.8
Lungs	3.9 ± 1.4
Heart	4.1 ± 1.5
Muscles	2.6 ± 1.9
Bone marrow	66.5 ± 30.1
Skin	12.5 ± 7.1
Brain	4.3 ± 1.8
Stomach content	4.9 ± 2.3
Small intestine content	4.3 ± 1.6
Colon content	4.6 ± 2.7

[a] $n = 3$; mean, ±*SD*. [b] $n = 1$. [c] $n = 2$.

Since little radioactivity remained in the body after 8 d (Table II), the total absorption amounted to ~10–15% of the administered dose.

The absorption and excretion occurred rather rapidly. The biliary excretion rate reached a maximum after 1 h (Fig. 1) and then declined in a hyperbolic fashion. The maximal urinary excretion rate probably was reached within 2 h (Fig. 2) followed by a hyperbolic decline. Within 1 d ~83% and within 2 d ~95% of the total amount absorbed were eliminated from the body.

After 8 d, <0.5% of the administered dose remained in the body. The highest radioactivity (10 times more than in blood) was found in bone marrow. Higher levels of radioactivity were observed in renal fatty tissue, liver, pancreas, skin, adrenals, and the lymph nodes than in the blood. The values from the lymph nodes, however, give only an estimate of the actual value, due to the very small amounts of material available for the determination of the radioactivity in these organs.

The small amount of radioactivity absorbed from the GI tract probably consisted of low molecular weight components in the polymer. The figure of 10–15% is very similar to the 13% that was rapidly excreted after subcutaneous injection (6) and was, as mentioned above, attributed to low molecular weight components of the nanoparticle material. Whether these components can be used as carriers for nonabsorbable drugs is still an open question. The main part of the nanoparticle material, though capable of a carrier function, is not absorbable by rats. Because of the enhanced endocytotic activity of tumor cells (9), nanoparticles loaded with cytostatic carriers may be useful for the treatment of carcinomas of the GI tract.

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High-Performance Liquid Chromatographic Determination of Acetylcholine in a Pharmaceutical Preparation

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Received September 7, 1982, from the *Analytical Development Department, CooperVision Pharmaceutical, Inc., Mountain View, CA 94043.* Accepted for publication September 22, 1983.

Abstract □ A simple and rapid method for quantitating acetylcholine in a lyophilized preparation by high-performance liquid chromatography (HPLC) is described. A reverse-phase column with a refractive index detector was utilized for the assay. The HPLC system was able to separate acetylcholine from choline, a major degradation product, which was verified by running a degraded sample of a commercial preparation. The HPLC results were compared with the results obtained by a spectrophotometric procedure.

Keyphrases □ Acetylcholine—high-performance liquid chromatographic determination, lyophilized preparation □ High-performance liquid chromatography—determination of acetylcholine □ Lyophilized preparation—acetylcholine, high-performance liquid chromatographic determination

Acetylcholine is an endogenous parasympathomimetic agent thought to play an important role in the transmission of nerve impulses at synapses and myoneural junctions. Because the action of this parasympathetic stimulation is abrupt and fleeting, acetylcholine has limited therapeutic use. However, this unique property is presently being used to advantage in an intraocular irrigating fluid to obtain complete miosis instantaneously after delivery of the lens in cataract surgery. The product[1] is in a vial of two compartments: the lower chamber contains a lyophilized preparation of acetylcholine chloride with mannitol as a tonicity adjusting agent. The upper chamber contains sterile water for injection, and the two chambers are separated by a rubber plug. Since aqueous solutions of acetylcholine are unstable, the drug is reconstituted in sterile water just prior to use.

There have been several reports on chemical assays for acetylcholine. An excellent review of the current chemical methods has been published (1). The classical colorimetric method (2) suffers from lack of specificity.

Several gas chromatographic procedures have been reported. Primary means of detection have been either flame ionization (3) or mass spectrometry (4). In all cases, they involve volatilizing acetylcholine either by demethylation (5)

[1] Miochol, CooperVision, Inc.

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