Tissue Distribution of Antitumor Drugs Associated with Polyalkylcyanoacrylate Nanoparticles

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Received July 26, 1979, from the **Laboratoire de Pharmacie Galénique, Faculté de Médecine, Université Catholique de Louvain, 1200 Brussels, Belgium*, and the ‡*School of Pharmacy, Federal Institute of Technology, 8006 Zürich, Switzerland.* Accepted for publication September 24, 1979.

Abstract □ Polymethylcyanoacrylate nanoparticles, polyethylcyanoacrylate nanoparticles, and free ^{3}H-dactinomycin and ^{3}H-vinblastine were studied with emphasis on their distribution pattern in rat tissues after intravenous administration. The adsorption of cytostatic drugs to polyalkylcyanoacrylate nanoparticles can modify drug distribution in tissues. Particularly with vinblastine, modification of drug disposition is important. Data are given concerning the formation and stability of nanoparticle–drug complexes. Polyalkylcyanoacrylate nanoparticles seem to be an interesting drug carrier owing to their size, structure, degradability, and drug sorptive properties.

Keyphrases □ Polymethylcyanoacrylate nanoparticles—with adsorbed dactinomycin and vinblastine, distribution patterns in rats, compared to polyethylcyanoacrylate nanoparticles □ Polyethylcyanoacrylate nanoparticles—with adsorbed dactinomycin and vinblastine, distribution patterns in rats, compared to polymethylcyanoacrylate nanoparticles □ Dactinomycin—on polymethylcyanoacrylate and polyethylcyanoacrylate nanoparticles, distribution patterns in rats □ Vinblastine—on polymethylcyanoacrylate and polyethylcyanoacrylate nanoparticles, distribution patterns in rats □ Drug carriers—polymethylcyanoacrylate and polyethylcyanoacrylate nanoparticles, distribution patterns of adsorbed dactinomycin and vinblastine in rats

The problem of directing a cytostatic agent to specific sites is a current topic of research. Specific targeting of drug carriers may improve the anticancer activity and lower the general toxicity.

The use of endocytizable and lysosomotropic carriers such as liposomes (1–3) and DNA complexes (4, 5) appears promising, and new lysosomotropic carriers like albumin microspherules (6) and acrylic nanocapsules (7, 8) have been developed. Entrapment of fluorescein in such polyacrylamide nanocapsules significantly increases dye uptake by cultured fibroblasts and allows its accumulation into lysosomes, which are cell compartments in which fluorescein does not spontaneously accumulate (9, 10). However, the polymer used to prepare nanocapsules is unlikely to be digested by lysosomal enzyme, which might restrict its clinical use.

It has been demonstrated that polyalkylcyanoacrylate nanoparticles are potential lysosomotropic carriers because of their size, structure, and sorptive properties (11, 12). Such degradable nanoparticles are able to adsorb various molecules, including antimitotic drugs, reproducibly (11, 13, 14). The hydrolysis rate is dependent on the alkyl chain length of the cyanoacrylic monomer, which allows the drug release kinetics to be selected (13).

This study compared the tissue distribution of free dactinomycin and vinblastine with that of the adsorbed drugs on polymethylcyanoacrylate and polyethylcyanoacrylate nanoparticles after intravenous administration to rats.

EXPERIMENTAL

Preparation of ^{3}H-Dactinomycin and ^{3}H-Vinblastine Nanoparticles—Either ^{3}H-dactinomycin[1] (40 μl) or ^{3}H-vinblastine[1] (75 μl) was added to 1.5 ml of an aqueous solution containing 0.5% polysorbate[2] 20 in 0.01 *M* HCl. Then 17 μl of monomer[3] (methyl- or ethylcyanoacrylate) was dispersed under mechanical stirring.

After polymerization (30 min), the milky suspensions obtained were buffered to pH 7 using 0.05 ml of 1.0 *M* NaOH and 2.0 ml of phosphate buffer [containing 25% (v/v) of 0.2 *M* KH_2PO_4 and 15% (v/v) of 0.2 *M* NaOH]. The colloidal suspension obtained after filtration through a 0.8-μm filter[4] was diluted with an equal volume of the phosphate buffer.

The solutions of free ^{3}H-dactinomycin and ^{3}H-vinblastine were prepared as described for the nanoparticles but without monomer addition.

Determination of Drug Adsorbed on Nanoparticles—*Dactinomycin*—Dactinomycin (3.3 mg) and ^{3}H-dactinomycin (20 μl) were dissolved in 50 ml of acid containing polysorbate 20. Monomers were then added and polymerized as already described.

The nanospherule suspensions (10 ml) were centrifuged at 20,000 rpm. After separation and dissolution of the sediment in dimethylformamide, the ^{3}H-dactinomycin was measured in both the sediment and the supernate by scintillation counting as described.

Vinblastine—Vinblastine (17.5 mg) and ^{3}H-vinblastine (20 μl) were dissolved in 50 ml of acid containing polysorbate 20. The determination of the amount of vinblastine adsorbed on polymethyl- and polyethylcyanoacrylate nanoparticles was made in the same manner as for ^{3}H-dactinomycin.

Sample Preparation for Scintillation Counting—One milliliter of the supernatant and sediment solutions was pretreated with 0.5 *N* quaternary ammonium hydroxide in toluene[5] (0.75 ml). Isopropanol (0.75 ml) and 30% H_2O_2 (0.5 ml) were added to decolorize the samples. After 60 min, xylol–0.5 *M* HCl[6] (9:1) (15 ml) was added, and counting was performed in a liquid scintillation counter[7]. Quench correction was obtained using a channels ratio method. The results are expressed in percent of the drug adsorbed on the nanospherules.

Injection of ^{3}H-Dactinomycin and ^{3}H-Vinblastine in Free Form and Nanoparticle-Associated Forms—Three groups of 20 male Wistar rats (180–220 g), anesthetized with ether, were injected by puncture in femoral vena. Each group received one of the following forms of ^{3}H-dactinomycin in a single injection of 0.80 ml: free drug and polymethyl- and polyethylcyanoacrylate nanoparticles. At 0.5, 3, and 24 hr, six, eight, and six rats, respectively, were killed by decapitation in each of the three groups. Samples of blood and various tissues were removed for ^{3}H-analysis.

The differential tissue distribution of ^{3}H-vinblastine was determined in the same manner on two groups of 18 rats. Each group received free or adsorbed ^{3}H-vinblastine, and six rats were killed in each of the two groups at the same time intervals.

Preparation of Rat Tissues for ^{3}H-Analysis—Samples of fresh tissues including spleen, small intestine, muscle, kidneys, liver, and lungs were taken. For each organ except the spleen, three samples weighing 45–55 mg were each solubilized in 1 ml of 0.5 *N* quaternary ammonium hydroxide. For the spleen, 20-mg samples were used. After solubilization (about 4 hr), samples were decolorized using 0.2 ml of 30% H_2O_2, followed by warming at 40° for 30 min.

For the blood analysis, 200 μl was solubilized in 1.5 ml of quaternary

[1] M.B.L.E., Radiochemical Centre, Amersham, England.
[2] Tween 20, I.C.I., Atlas International Division, Everberg, Belgium.
[3] Loctite, Dublin, Ireland.
[4] Millipore, Buc, France.
[5] Soluene, Packard Instruments S.A., Brussels, Belgium.
[6] Instagel, Packard Instruments S.A., Brussels, Belgium.
[7] Computer Philips PW 4510, M.B.L.E., Brussels, Belgium.

0022-3549/80/0200-0199$01.00/0

Table I—Efficiency of Drug Sorption on Nanoparticles

Drug	Concentration of Suspension, μg/ml	Sorption, % Polymethyl-cyanoacrylate	Sorption, % Polyethyl-cyanoacrylate
Dactinomycin	55	92.8	86.5
Vinblastine	350	35.8	80.0
Vinblastine	45	67.2	85.0

ammonium hydroxide in toluene–isopropanol (1:1) and decolorized with 0.5 ml of 30% H_2O_2.

After cooling at room temperature, 15 ml of the scintillation fluid was added to each sample, and counting was performed in the liquid scintillation analyzer.

For each form of ^{3}H-dactinomycin or ^{3}H-vinblastine used, the amount of radioactivity injected was calculated on the basis of analysis of triplicate samples of 0.2 ml measured with the same syringe used for injection. These samples were treated as described for the tissues.

Drug concentration in each tissue analyzed was expressed in microcuries × 10^{-3} per gram of tissue and per microcurie injected in the whole animal.

RESULTS AND DISCUSSION

Formation and Stability of Nanoparticle–Drug Complexes—Dactinomycin and vinblastine were adsorbed on the nanoparticles with high efficiency (Table I). Although the percentage of adsorbed drug declined with increasing amounts, substantial amounts of dactinomycin and vinblastine can be sorbed. The percentages of drug adsorbed to the nanoparticles were in all cases higher than those obtained with entrapment in liposomes (15, 16). Furthermore, the amounts of drug adsorbed on nanoparticles were reproducible.

For a 55-μg/ml suspension of dactinomycin, 92.8 and 86.5% of the drug were adsorbed on polymethyl- and polyethylcyanoacrylate nanoparticles, respectively, when the drug was incorporated before polymerization. In contrast, when a suspension of prepared nanoparticles was mixed with an aqueous solution of free dactinomycin, the percentage of the drug adsorbed on the particles decreased to 66.0 and 64.5% for polymethyl- and polyethylcyanoacrylate nanoparticle forms, respectively. These results are in agreement with the porous structure of particles observed after cryofracture by electron microscopy (11). Indeed, when the drug is added after the nanoparticles are formed, the inner surface of the carrier probably is no longer available for adsorption.

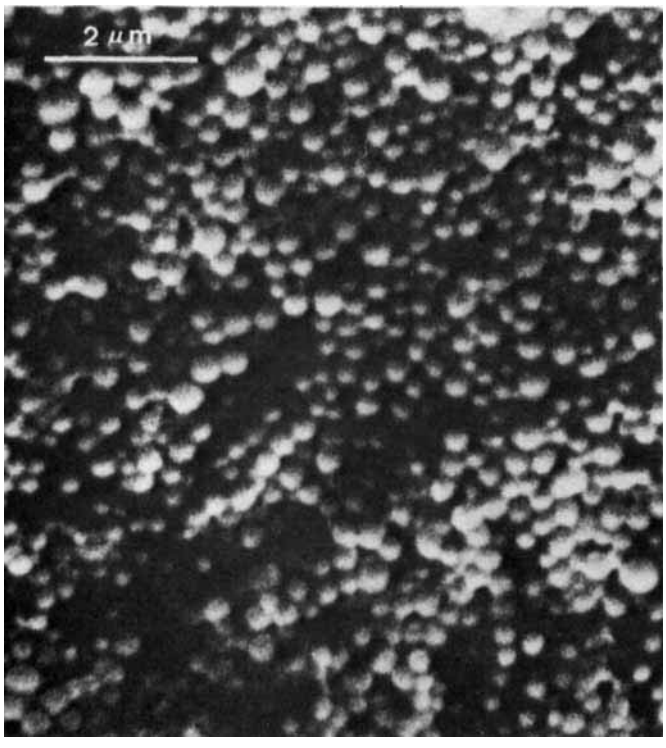

Figure 1—*Morphological appearance of dactinomycin associated to polymethylcyanoacrylate nanoparticles at the scanning electron microscope. Thickness of the gold layer was 100 nm.*

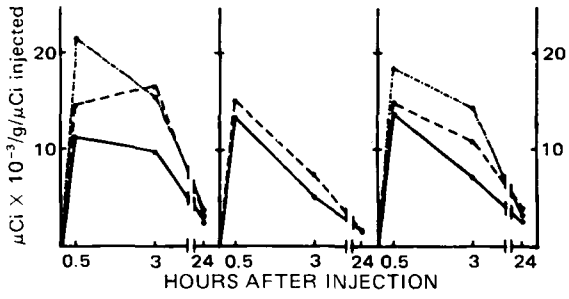

Figure 2—*Tissue concentrations [small intestine (left), liver (center), and lungs (right)] in rats at various times after administration of ^{3}H-dactinomycin. A single intravenous injection of polymethyl- (·–·) or polyethyl- (– –) cyanoacrylate nanoparticles or free (—) dactinomycin was given to each rat. Each point is the mean value from at least six animals.*

A scanning electron microscopic[8] study showed that drug adsorption at the surface of the particles induces no morphological modification of these nanoparticles. Figure 1 shows spherical dactinomycin–polymethylcyanoacrylate nanoparticles with a diameter of ~200–300 nm, considering the thickness of the gold layer (100 nm) used for sample preparation.

Dactinomycin Distribution in Rat Tissues—Table II shows the concentrations of ^{3}H-dactinomycin found in the blood, spleen, small intestine, muscle, kidney, liver, and lung samples after administration of either free dactinomycin or nanoparticle-adsorbed forms. Each value at various times after the injection represents a mean result obtained with six or eight animals and with three pretreatment levels from each tissue analyzed. A statistical analysis (Student t test) was carried out to determine, with a probability of 0.975, the significant differences in the tissue distributions induced by adsorption.

Significant increases of ^{3}H-dactinomycin uptake were obtained for the small intestine and lungs of polymethylcyanoacrylate nanoparticle-injected rats at 0.5 hr (Fig. 2). Three hours after the injection, the ^{3}H-dactinomycin content in both of these two tissues decreased, but the radioactivity levels remained higher than those found in the small in-

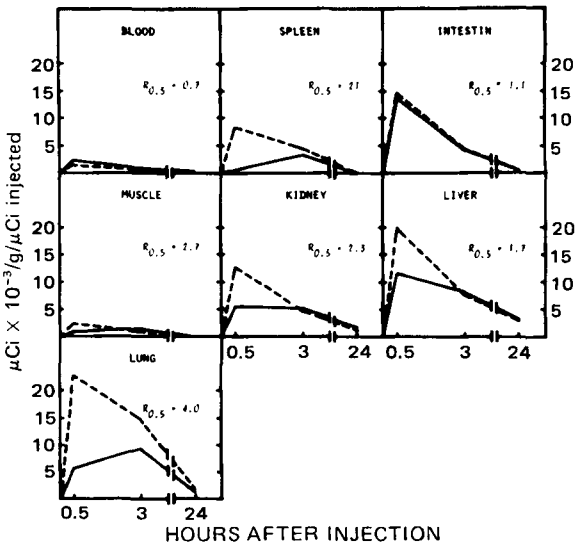

Figure 3—*Tissue concentrations (blood, spleen, intestine, muscle, kidneys, liver, and lungs) of free (—) and polyethylcyanoacrylate nanoparticle (– –) ^{3}H-vinblastine forms at various times after intravenous administration. Each point is the mean value from a minimum of six animals. The $R_{0.5}$ represents the relation between vinblastine adsorbed on polyethylcyanoacrylate nanoparticles and free vinblastine tissue concentration at 30 min after injection ($R_{0.5} = PEN_{0.5}/F_{0.5}$).*

[8] "Super Mini SM," International Scientific Instruments, Mountain View, Calif.

Table II—Tissue Distribution of Drug at 3 hr Postinjection

Organ	F^a	PMN[a]	PEN[a]	t_{stud} ($p = 0.975$) PMN	t_{stud} ($p = 0.975$) PEN	PNM or PEN / F PMN	PNM or PEN / F PEN
Blood	1.2	0.5	1.2	4.17	0.37	0.42	1.00
Spleen	15.0	15.3	17.5	0.16	1.51	1.02	1.17
Small intestine	9.8	15.3	16.8	2.70	3.76	1.56	1.71
Muscle	2.2	3.1	2.2	1.33	0.06	1.41	1.00
Kidneys	9.6	14.1	12.8	1.79	3.29	1.47	1.33
Liver	4.9	5.6	6.9	0.93	3.69	1.14	1.41
Lungs	6.8	14.3	10.5	3.85	3.80	2.10	1.54

[a] Units are microcuries × 10^{-3} per gram per microcurie injected. t_{stud} ($p = 0.975$) represents the results of the statistical analysis (Student test) for a probability of 0.975. Underlined numbers correspond to a significant difference between free (F), polymethyl- (PMN), and polyethyl- (PEN) cyanoacrylate nanoparticle forms for the chosen probability.

testine and lungs of rats injected with the free drug. No significant difference was found 24 hr after injection.

Similar observations were made for polyethylcyanoacrylate nanoparticles. Thus, the small intestine and lungs of the injected rats showed significantly higher contents of ^{3}H-dactinomycin at 3 hr in comparison with free drug. Furthermore, the ^{3}H-dactinomycin concentration in the liver was higher for the polyethylcyanoacrylate nanoparticle form; this observed difference was slight but significant (Table II).

In both the spleen and kidneys, the decrease of radioactivity remained similar for all three forms of ^{3}H-dactinomycin during the experiment. Except for the lungs and small intestine, the tissue distribution of ^{3}H-dactinomycin was poorly modified by adsorbing it on nanoparticles, in spite of generally higher drug levels in tissues of rats injected with nanoparticles. Previous studies on dactinomycin tissue distribution in mice showed that the small intestine retains high dactinomycin concentrations (17, 18). The present results are similar to these observations, but it was surprising to find still higher concentrations of radioactivity in the intestinal wall of rats injected with ^{3}H-dactinomycin adsorbed on nanoparticles. Likewise, the poor accumulation of the nanoparticle forms in the liver and the spleen is difficult to explain. The tissue distribution of dactinomycin nanoparticles was comparable to that observed when the drug was incorporated into the aqueous phase of multilamellar liposomes (18).

^{3}H-Vinblastine in Rat Tissues—Table III lists the ^{3}H-concentration after the injection of free drug or polyethylcyanoacrylate nanoparticle–^{3}H-vinblastine. Concentrations of ^{3}H-vinblastine found in tissues after administration of nanoparticles were generally higher than those obtained with the free form. This result was particularly remarkable at 0.5 hr after the injection and for tissues rich in reticuloendothelial macrophage cells such as the liver, spleen, and lungs (Fig. 3). Thus, 0.5 hr after injection, the spleen accumulated 21-fold more nanoparticles with adsorbed ^{3}H-vinblastine than free drug. Likewise, after the same time, the drug concentrations observed with nanoparticles were 1.7-fold higher in the liver, fourfold higher in the lungs, and 2.3-fold higher in the kidneys compared to levels with free vinblastine.

More surprising was the higher concentration of the nanoparticles compared to the free form in the muscle. Indeed, the muscle retained 2.7-fold more vinblastine when the drug was adsorbed on polyethylcyanoacrylate nanoparticles. At 30 min after injection, the tissue concentrations observed with nanoparticles were generally two- to 20-fold higher than with the free drug (Table III). Thus, the kinetics of tissue uptake of the nanoparticles were quite different compared to those of the free form (Fig. 3). Maximum levels were observed after 0.5 hr for the nanoparticle formulation and after 3 hr for the free form, except for the liver and blood. Differences in the tissue drug concentrations were more marked a short time after injection.

Table III—Tissue Accumulation of Drug at 0.5 hr Postinjection

Organ	F^a	PEN[a]	t_{stud} ($p = 0.975$)	PEN / F
Blood	2.2	1.5	1.54	0.7
Spleen	0.4	8.4	6.17	21.0
Small intestine	13.8	14.9	0.17	1.1
Muscle	0.8	2.2	4.69	2.7
Kidneys	5.5	12.8	8.34	2.3
Liver	11.6	20.0	3.84	1.7
Lungs	5.6	22.7	8.97	4.0

[a] Units are microcuries × 10^{-3} per gram per microcurie injected. t_{stud} ($p = 0.975$) represents the results of the statistical analysis (Student test). Underlined numbers correspond to a significant difference between free (F) and polyethylcyanoacrylate nanoparticle (PEN) forms for a probability of 0.975.

Earlier studies with liposomes (19, 20) showed preferential cellular capture by the liver and spleen. The present results show that the ^{3}H-vinblastine nanoparticles have a broader tissue distribution than liposomes and provide evidence that nanoparticles can be used to modify tissue distribution of vinblastine. The significant difference in the accumulation in the muscle between free and nanoparticle-adsorbed vinblastine cannot now be explained, but it is interesting to note that cultured fibroblasts, which have a moderate endocytic capacity, accumulate polyacrylamide nanoparticles (9).

These results agree qualitatively with previous reports in that the favored sites for free vinblastine include the spleen, intestine, lungs, and liver (19, 21).

CONCLUSIONS

The present study provides evidence that the adsorption of cytotoxic drugs to polyalkylcyanoacrylate nanoparticles can modify the drug distribution pattern in tissues. This effect was particularly marked for the vinblastine nanoparticles. Furthermore, the results obtained with dactinomycin and vinblastine show that the differential tissue distribution (between free and adsorbed forms) was not only related to the carrier but also depended on the active molecule. For example, both free vinblastine and dactinomycin had a marked affinity for the small intestine, which was enhanced with dactinomycin adsorbed on nanoparticles but unchanged with vinblastine nanoparticles. In contrast, the increase of the uptake of nanoparticle-adsorbed drugs by the liver and spleen was more marked, comparatively, for vinblastine than for dactinomycin.

Such results are in agreement with the observations reported for liposomes (22, 23). Indeed, 3 hr after injection of radiolabeled free drug or the liposome-encapsulated form, the tissue distribution was quite different for dactinomycin, cytosine arabinose, vinblastine, $^{86}Rb^+$, or ethylenediaminetetraacetate.

All modifications of distribution patterns of the examined drugs were observed at the earlier times after administration (0.5 or 3 hr). Thus, the effect of the carrier seems rapid, especially with vinblastine nanoparticles. Such observations are compatible with the rapidity of endocytosis, which may be carried out in minutes (24).

Moreover, these results show that there is a general increased uptake of cytostatic drugs when they are adsorbed on nanoparticles. But this increase is not uniform for all tissues. Differential and selective increases in tissue uptake of cytotoxic drugs will be of interest in cancer chemotherapy. Polyalkylcyanoacrylate nanoparticles could be useful in this area. The action of nanoparticles associated with cytostatic agents on tumor-bearing animals and the selective uptake by different cell types will be investigated.

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ACKNOWLEDGMENTS

Supported in part by the Belgian "Fonds National de la Recherche Scientifique" and by the "Fonds de Developpement Scientifique" of the University of Louvain, Belgium. P. Couvreur is "Chargé de Recherches" to the Belgian "Fonds National de la Recherche Scientifique."

The authors thank Dr. Dunn, Loctite Co., Ireland, for a generous gift of the monomer samples and Dr. Kramp, Merck Sharp & Dohme, Brussels, Belgium, for a generous gift of dactinomycin.

Effect of Carnitine Analogs on Carnitine Acetyltransferase

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Abstract □ Carnitine analogs with various substituents on the nitrogen were tested for their effect on carnitine acetyltransferase from rat sperm and pigeon breast. A radiometric assay was used to measure the formation of acetylcarnitine in the presence of other enzymes that competed for acetyl coenzyme A in the sperm preparation. The apparent enzyme inhibition caused by the analogs was explained by the analogs serving as alternative substrates with higher K_m and lower V_{max} values. The analogs had no effect on whole sperm.

Keyphrases □ Carnitine analogs—effect on carnitine acetyltransferase, spectrophotometric and radiometric measurements, kinetic parameters, *in vitro* □ Kinetics—analysis of carnitine analogs on carnitine acetyltransferase activity, spectrophotometric and radiometric measurements □ Spectrophotometry—analysis of carnitine analogs on carnitine acetyltransferase activity, kinetics

The primary importance of carnitine acetyltransferase (EC 2.3.1.7) in mitochondria is not known. Since the enzyme is found in high levels in cells that derive much of their energy requirements from lipid metabolism, it was thought that its major function was in lipid metabolism (1). For example, pigeon breast and heart muscle are common sources of the enzyme. The enzyme also is abundant in sperm (2), where the acetylation of carnitine may serve as an important regulator of both lipid and carbohydrate metabolism (3, 4).

Carnitine analogs that inhibit the enzyme may alter the path of energy metabolism (5). Rat and mouse sperm offer a convenient means of testing the biological activity of carnitine analogs. Carnitine appears to be important for sperm metabolism. Not only are carnitine acetyltransferase levels high in sperm, but carnitine is also in high concentration in epididymal fluid (6). The sperm can be isolated easily with little contamination by other cells, and they can be checked easily for viability by examining their motility under a microscope.

Unfortunately, the most commonly used spectrophotometric assay for carnitine acetyltransferase cannot be used with crude sperm enzyme; this assay measures the disappearance of the thiol ester bond of acetyl coenzyme A, and competing reactions using acetyl coenzyme A interfere with this measurement unless the enzyme is purified. However, crude enzyme can be assayed by measuring the transfer of labeled acetate to acetylcarnitine, which can be precipitated as the periodide (7). Since carnitine acetyltransferase probably is essential for sperm function, any inhibitor also should be detected with whole sperm by changes in the fertilization rate.

In this study, carnitine analogs were examined for their effect on the enzyme in both crude sperm preparations and crystalline pigeon breast enzyme. In addition, selected analogs were examined in various test systems to determine the cellular effects of the analogs.

EXPERIMENTAL

Carnitine Analogs—The *dl*-carnitine analogs (I–VII) were prepared as described previously (5).

Spectrophotometric Assay—The spectrophotometric assay of carnitine acetyltransferase activity measured the decrease in absorbance[1] of the thiol ester bond of acetyl coenzyme A at 232 nm (8). The reaction mixture in each cell contained 1.0 m*M* L-carnitine[2], 0.1 m*M* acetyl coenzyme A[2], and the carnitine analog of the desired concentration. The reaction was carried out at 25° in 100 m*M* tromethamine buffer, pH 8.0,

[1] Gilford spectrophotometer.
[2] Acetyl coenzyme A, PL Biochemicals.

0022-3549/80/0200-0202$01.00/0