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# Drug Targeting by Drug Entrapment into Ultrafine Compartments as Carriers

P. SPEISER

*School of Pharmacy, Swiss Federal Institut of Technology, ETH-Zürich, CH-8092 Zürich/Switzerland*

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## ABSTRACT

The incorporation of drugs into vesicles is one of several technological methods for the optimization of targeted drug delivery and controlled drug targeting. The main problems will always remain

- To design inert auxiliary accompanying materials in order to overcome side reactions
- To use body-friendly and biodegradable macromolecular carrier materials for the therapeutic system
- To miniaturize the dosage form dramatically in the submicroscopic size range in order to eliminate foreign body irritations
- To develop ultrafine solid and amorphous vesicular compartments (nanocapsules, nanopellets, nanoparticles) to get stable systems with good tissue transfer and organ targeting properties

The actual stand of the incorporation of drugs and biologic active material into ultrafine colloidal solid capsules is reviewed here as for instance:

- Immunoactive material
- Fluorescent indicators in body fluids
- Controlled and sustained release systems
- Nonspecific drug targeting of the first-order (passage through endothelial tissues)
- Second-order targeting (a specific transparenchymal migration), and a highly specific targeting of the third-order (transcellular passage, especially lysosomal transports)

Examples for some of these applications are given.

It can be shown that such ultrafine vesiculated capsules offer some advantages when applied parenterally, but also partly for oral applica-

tion. In the future, still more studies are necessary finally to clarify the importance and practical use of such ultrafine targeting carriers.

**Index Entries:** Drug targeting, by entrapment in carriers; entrapment, and targeting of drugs in carriers; targeting, and drug entrapment in carriers; vesicles, entrapment of drugs in; microencapsulation, of drugs;

## INTRODUCTION

The entrapment of drugs into ultrafine solid, colloidal carriers is one of the several methods for the optimization of drug transport and delivery. The solid carriers serve as reservoirs, vesicles, or compartments for drugs and can be used as dosage forms for application as transport, transfer, targeting, and delivery system.

## FUNCTION OF THE CARRIER

The carrier loaded with drug has the main function to crossing several hydrophilic and lipophilic body areas or membranes that generally represent barriers. Thus, after oral application the carrier in the solid state must permeate a lipophilic membrane system, the gut wall. After this passage the carrier arrives in the hydrophilic systemic transport system, the blood plasma. From here the distribution in the body takes place, until the carrier arrives finally at a lipophilic border area, the cell membrane of the organs of destination or the receptors. Here the carrier should release the drug under controlled conditions to fulfill its therapeutic task. An ideal carrier to reach the target organs has therefore to fulfill the following main requirements:

- The carrier material should not react with the drug and should be inert, i.e., no side reactions should occur.
- The carrier system should be physiologic or at least biocompatible and biodegradable. In other words, it should have low or no toxicity at all.
- The carrier must have a good loading capacity for the drug.
- The drug carrier system as dosage form should be miniaturized. Therefore the particle size should not exceed 0.35 μm (350 nm) to avoid foreign body reactions.
- Such an ultrafine, solid system in the nanometer size range guarantees a relatively good shelf stability.
- The amorphous, not the crystalline state of the carrier system, as well as its amphiphilic texture, guarantee a good tissual or cellular uptake with small or no intrinsic toxicity at all.

## PRACTICAL CARRIERS

The following carrier systems are pharmaceutically relevant:

- Liposomes (multilamellar or unilamellar).
- Hardened microemulsions or micellar systems leading to vesicular nanocapsules.
- Ultrafine coacervates, precipitates, or coated bioparticles leading to solid nanopellets or nanobeads.

Liposomes possess the advantage of being biocompatible. On the other hand they have low stability, a weak loading capacity for drugs, and no membrane transfer properties.

Nanoparticles are of ultrafine solid structure. Compared to other carrier systems, such nanocapsules or nanopellets are of spherical shape. Depending on the manufacturing procedure, the size may vary between 60 and 350 nm. The average specific surface is around 50 $m^2/g$ carrier. Nanoparticles and unilamellar liposomes possess similar size ranges, but liposomes have a liquid crystal structure, and nanoparticles an amorphous solid aggregate state and therefore differ in many properties (*1*).

## BIOLOGICAL PROPERTIES OF COLLOIDAL CARRIERS

The kinetic distribution, partition, biodegradation, and elimination of colloidal carriers have been studied in various animals with carbon-traced carriers of poly(methyl methacrylates). The distribution shows that shortly after intravenous injection, the major amount of carrier appears in the lung, the liver, the spleen, and the kidneys. Later, the distribution changes and after 1 wk these carriers appear also in the bone marrow and the vertebral column (important for immunology) (Fig. 1) (*2*). The metabolization of these carriers takes place by corrosion and hydrolysis, but mainly by oxidation, and usually leads to physiologic products. Thus the metabolic pathway of methacrylate, which is frequently used as carrier material, leads directly to physiologic products. In one pathway, methacrylate, after combination with coenzyme-A, is submitted to β-oxidation. After rearrangement it joins the citric acid cycle in the form of succinyl coenzyme-A (*3,4*). Another metabolic pathway is a simultaneous α- and β-oxidation leading to pyruvate and oxalacetate (*5*). In both cases, full oxidation of the carrier to products of the citric acid cycle occurs during metabolization (Fig. 2). In the urine no characteristic monomers, suspicious or toxic metabolites are found (*5*).

The excretion of the remaining monomers and oligomers, or short chain polymers, takes place very quickly in the first 24 h. The polymethacrylates however are excreted very slowly. A long chain poly(methyl methylacrylate) (MW, approx. 35,000) is excreted per week:

- 1% through the urine

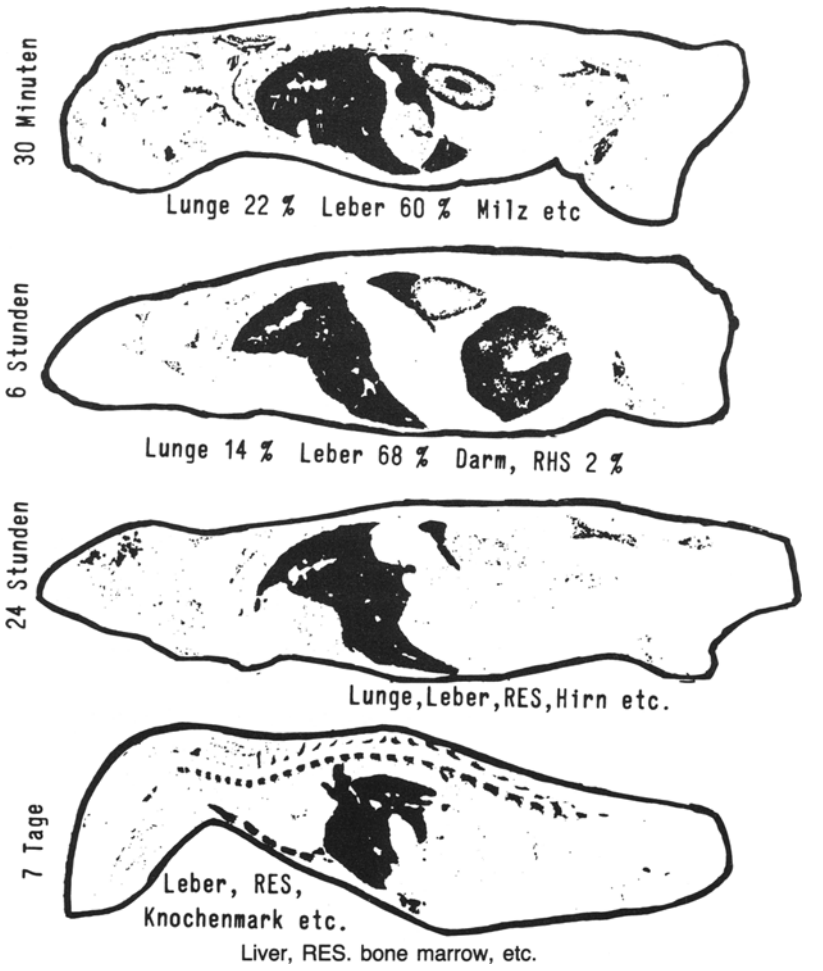


Fig. 1. Whole body autoradiography on rats: distribution of ultrafine $^{14}C$-tracered (17.4 μCi) placebo carriers of polymethyl methacrylate (PMMA), 30 min, 6 h, 24 h, and 7 d after intravenous application.

- 3.5% through the feces
- 1% by exhalation during respiration

So the first-order elimination kinetic is around 5.5–6% per week, which corresponds to a half-life of about 65 d. Now, by varying the kind and chain length of the carrier (MW, 3000–35,000), the desired stay period can be varied in between 12 h to 220 d (Fig. 3).

## TOXICITY

Toxicity studies at the cellular level were done with isolated hepatocytes, macrophages. Furthermore, mutagenicity (Ames test), histotoxicity (necrosis tissue irritation), bacterial toxicity, and whole body ani-

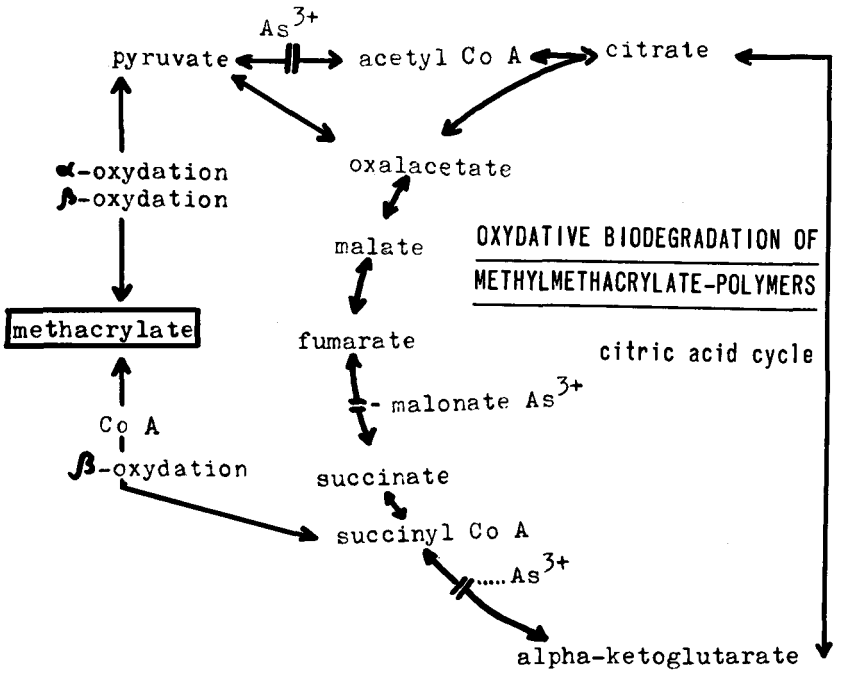


Fig. 2. Oxidative biodegradation of methacrylate polymers.

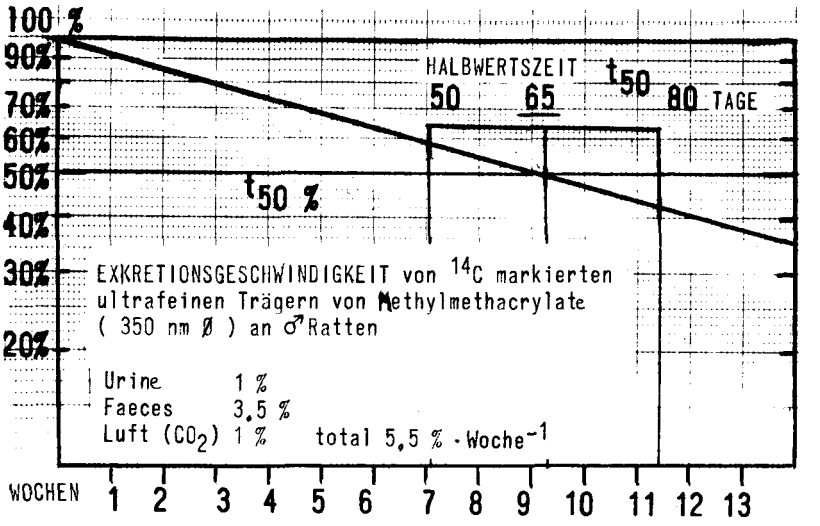


Fig. 3. Excretion velocity of $^{14}C$ tracered PMMA carriers (diameter, 350 nm) on male rats: urine 1%, feces 3.5%, air ($CO_2$) 1%, totally ~ 5.5%/wk.

mal studies do not demonstrate any acute or subacute toxicity of polyacrylate carriers that might hinder their use in human medicine. The toxicity given as $LD_{50}$ on mice after intravenous administration shows a slightly higher toxicity of the quickly biodegradable polyisobutylcyanoacrylate carrier (MW, around 3000) than the slowly biodegradable polybutylcyanoacrylate-carrier. Even polymerization medium containing only buffer and surfactants (without monomers and polymers) show a certain toxicity. In the purifed, cleaned state without any additives, the cumulative $LD_{50}$ would look even much better (Fig. 4) (*6*).

Such favorable biological properties offer a great many therapeutic possibilities, such as carrier systems with short activity or systems with sustained delivery, and a broad variation of release patterns can be synthesized.

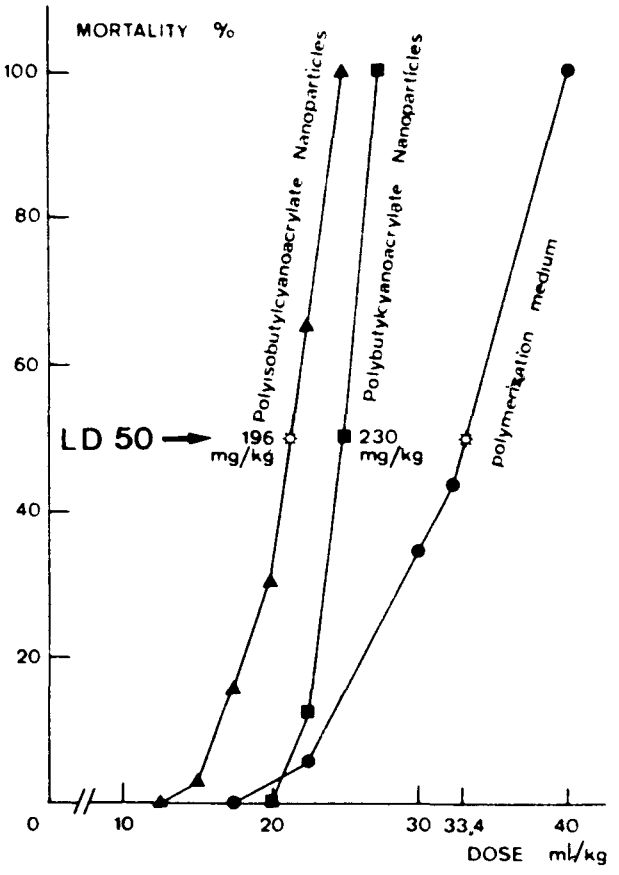


Fig. 4. Toxicity of ultrafine polycyanoacrylate carriers. *Cumulative mortality and toxicity ($LD_{50}$)*, of ultrafine carriers of polycyanoacrylate (average diameter, 400 nm) after intravenous administration to mice in doses of 12.5, 17.5, 22.5, 27.5, 32.5, and 40 mL/kg carrier suspension (six groups of 10 ♂ NMR mice of 20 g).

## PHARMACEUTICAL APPLICATION

Solid colloidal synthetic vesicles are studied for the following pharmaceutical purposes: as optical measurements of pH, *p*OH, $pO_2$, $pCO_2$, in body tissues (*7*); as oral or parenteral controlled or sustained drug delivery systems (*8*); as dosage forms for body cavities, and as a drug targeting system.

Such ultrafine solid carriers could also be used for various nonmedical purposes, such as dyes, textile products, photographic films, and so on.

## TARGETING

One further aim of carrier research is to contribute to the problem of transport and steering of the drug-loaded colloidal carrier to the target organs in the body. The first-order targeting is a nonspecific, nonselective transendothelial passage.

One example of a possible application of such carriers is in the RES system, i.e., its use as a stimulant or adjuvant in immunology.

Other examples include the use of carriers to pass material through the lung and the gut endothelium.

Second-order targeting is the use of a carrier as an unspecific, selective transparenchymal passage to diseased organs as, for instance, with a drug targeting to tumors or sarcomas.

Third-order targeting is a highly selective transcellular passage such as, for instance, the cellular uptake and passage of cell organelles (lysosomotropic transport).

## FIRST-ORDER TARGETING

One aim is to reach the RES system by a nonselective transendothelial passage and to stimulate there the antigen–antibody response in immunology. This can be done by embedding ultrafine, living or dead biologic material into ultrafine carriers. This encapsulation technique gives carriers strong immunostimulant properties.

### *The Example of Influenza Virus*

Because of the anatomy of a myxovirus, the inner parts of the virus are not important for immunoantigenity. It is mainly the outer phase, the glucoprotein-containing spikes, that are the carriers of the immunoantigenity. Thus, the entire influenza virus or fragments of the myxovirus (fluid vaccine) can be individually coated with the aid of special encapsulation processes. By this means, a stronger antigen complex

is obtained, which reacts as a true stimulant and improves the antigen–antibody response after subcutaneous application.

- Poly(methyl methacrylate) (PMMA) (squares) is compared with the
- Classical adjuvant 65 (stars)
- Aluminum phosphate (crosses) and the
- Control, untreated pure virus of the $A_2$-Hong Kong type X 31 (points).

The strength of the antibody formation measured with the aid of a hemagglutination-inhibition test is given on the ordinate as a function of time (Fig. 5). The results obtained with more than 200 guinea pigs show that the coated virus gives—after boostering 4 wk and more—clear antibody responses. After 21 wk, the antibody formation of encapsulated virus is significantly higher than in the case of $AlPO_4$ or of the classical adjuvant 65, (o) which is not used in human immunology (*9,10*).

Colloidal carriers containing ultrafine biologic material may, therefore, possibly be useful as immunologic stimulants or adjuvants for extremely small particulates, as in the case of viruses, spores, bacteria, allergens, etc.

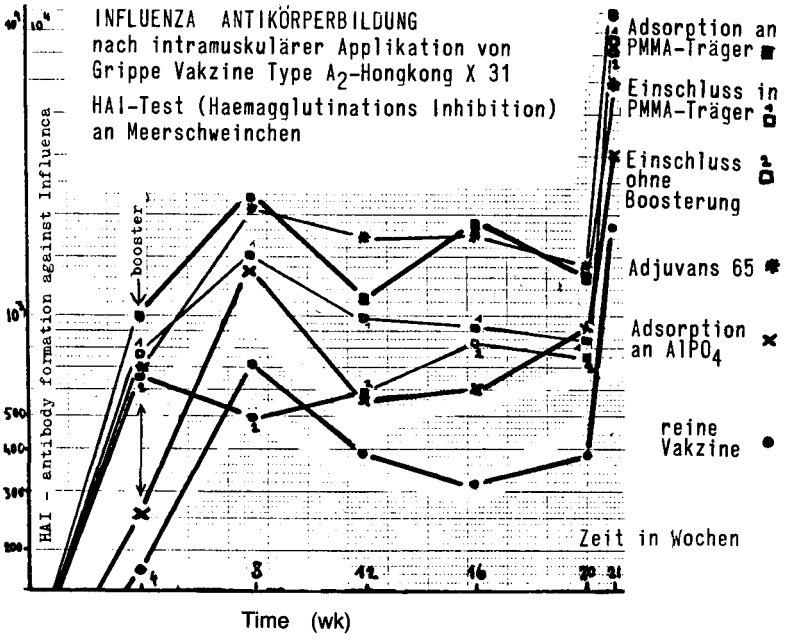


Fig. 5. Influenza antibody formulation after intramuscular application of influenza vaccine type $A_2$-Hong Kong A 31 incorporated in various adjuvant carriers: ■ adsorption on PMMA-carrier; $\overset{1}{\square}$ inclusion in PMMA-carrier with boostering; $\overset{2}{\square}$ inclusion in PMMA-carrier without boostering; *adjuvant *65*; × adsorption on $ALPO_4$; ● pure vaccine.

## ENTERAL CARRIER UPTAKE

The phenomenon of diffusion of ultrafine solid particles through the enteral endothelium is known for quite a long time (*11*). Insoluble fine powders, such as talcum, starch, aerosil, lycopodium powder, sulfur, etc., following oral ingestion, can appear after a short time in the blood, having crossed the enteral barrier by an endocytosis-like process. This cytopemsis is a cellular transport of foreign macromolecules or alien colloidal bodies by special active transfer processes. The mechanism of this persorption of "cell drinking" is very similar to endocytosis. The loaded carrier enters the intestinal epi- and endothelium by invagination processes, forming a phagosome. The phagosome migrates through the enteral tissue towards the capillary blood system or towards the lymphatic system, where—after exocytosis—it will be distributed systemically in the body (Fig. 6).

This carrier transfer process in the solid state can take place as a true transendothelial process (middle) or as extracellular transport phenomenon as well (right side). In the last case, no invagination process may occur. But the migration may take place as intercellular diffusion. Endocytosis is a very widespread cytophysiologic procedure, not only for enteral uptake, but for all kinds of transport in the RES system (phagocytosis), in the kidneys, the liver, the capillary endothelium, and for alveolar passage in the lung. The classical opinion that drugs would only diffuse through biologic membranes in the molecular or dissolved state, by ab-

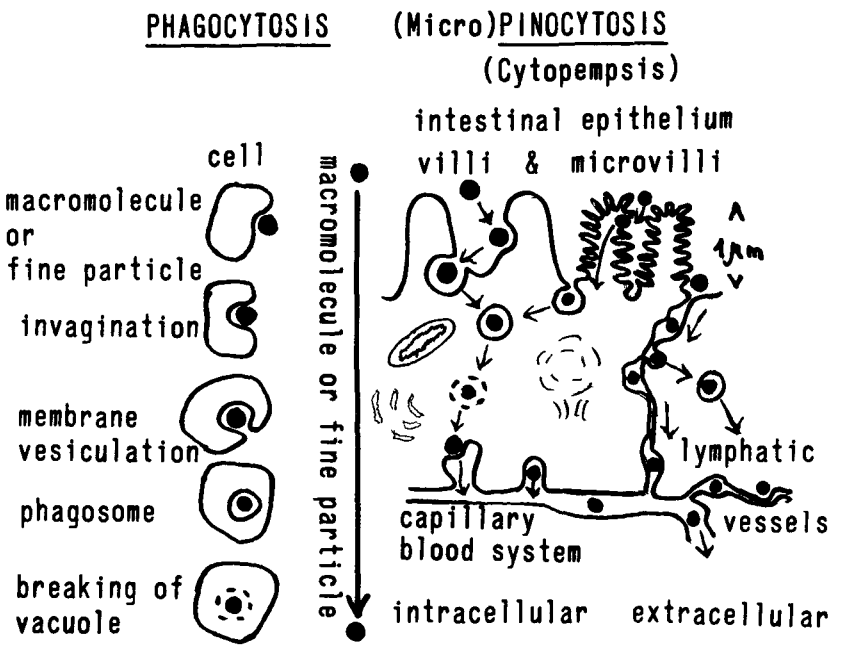


Fig. 6. Schematic procedure of (micro)pinocytosis.

sorption procedures, must probably be corrected in the near future, by also admitting that ultrafine particles can penetrate through cell layers. From preliminary studies it is known that ultrafine solid carriers loaded with drug molecules do promote cellular uptake via the enteral route. Five rats have been orally fed with radioactive tracered poly(methyl methacrylate) carrier of an average particle size of 350 nm (MW 35,000). After 48 h, 10–14% has been found in the bile and urine, whereas the rest stayed quantitatively in the feces (*12*). Carriers of polycyanoacrylate (MW 3000) do not show any significant uptake. Thus, not all ultrafine solid lipophilic carriers, loaded with drug, after oral application, can penetrate through the endothelium of the gut wall in the solid state into the body and give sustained drug delivery and bioavailability.

## EXAMPLES OF SECOND-ORDER TARGETING

Targeting of the second-order, a selective transparenchymal passage to diseased organs, is shown with the example of an antitumor drug actinomycin D. After application, whole body autoradiography shows an enrichment of tracered actinomycin D in a laboratory tumor S 250 on mice. The growth of the tumor goes on with a placebo treatment, stays constant with the drug treatment without carrier, but will be reduced and disappears with the same drug dose, but built into the carrier (Fig. 7) (*13*). So, by selecting the carrier properties exactly, we can in the future improve the therapy by specifically attacking the target organs. It is quite

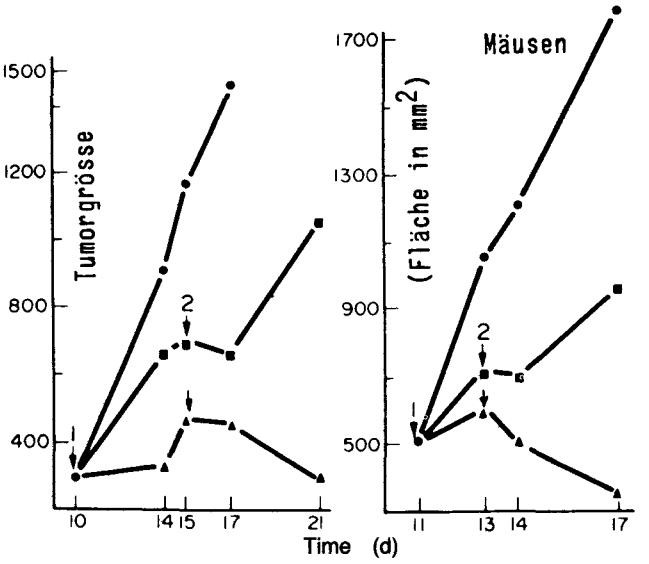


Fig. 7. Tumor growth with free and carrier-bound drug. Influence of free and carrier-bound actinomycin D after 2 iv injections of 222γg drug/kg mouse on tumor size (S 250): ● control (no treatment); ■ free actinomycin D; ▲ actinomycin in polycyanoacrylate carrier.

clear that this dream of the selective steering of the drug to the target organs, with the aid of colloidal carriers, must be studied in much more detail before a final positive or negative answer can be given.

## EXAMPLES OF THIRD-ORDER TARGETING

This kind of targeting involves a highly selective transcellular passage, for instance, to cell organelles such as the lysosomes. The lysosomotropic carrier hypothesis must still be studied in detail for this purpose. The active drug is incorporated into a biodegradable ultrafine-solid lipophilic carrier material. After this medication, the loaded carrier specifically adheres to the cell wall. Then the carrier is taken up in the cell by endocytotic invagination processes, forming a so-called endosome or phagosome. This endosome diffuses through the lysosomial membrane and fuses with the lysosomes. The lysosomes possess high enzymatic activities, biodegrade the carrier, and release the drug to act on the cell organelles. Principally, an intralysosomal effect is obtained, but when the lysosomes are breaking, then an extralysosomal effect or a cell nucleus activity might also arise. The use of such endocytosible and lysosomotropic carriers is promising in the therapy of intracellular infections, such as rheumatism, some tropical diseases, in other pathological processes, and mainly in the chemotherapy of cancer (*14–16*). Our actual knowledge shows that various cell types—healthy and ill cancer cells—possess different receptor systems for binding ultrasmall carriers. However, compared to healthy cells, the cancer cells possess the ability to take up molecules, molecule–complexes, or colloidal particles much more quickly than healthy cells.

Furthermore, the inclusion of an anticancer drug into the solid colloidal vesicles is a genuine alternative to other carrier principles such as the complexation of antimitotic drugs with DNA (desoxynucleinic acid) (*17*), or to the complexation with various proteins (*18*), or to the entrapment of drugs into liposomes (*19*). Colloidal carriers containing a cell tracer such as fluorescein might readily confirm this hypothesis of DeDuve, (*20–22*). Cultured tumor cells of a rat embryonal fibroplast tissue in cell cultures have been incubated with such colloidal carriers. The carriers with the cell tracer are then checked for endocytosis by spectrophotometric fluorescence measurement. After 12 h, the cultivated cells had accumulated six times more of the carrier-bound fluorescent material per milligram of cell protein than the free fluorescein-sodium. The free cell tracer shows very poor cellular uptake (Fig. 8) (*23*).

## INTRACELLULAR LOCALIZATION OF CARRIER

The exact localization of the colloidal carriers in the cell interior is done by fluorescence fibroblast gradient measurement with tissue cul-

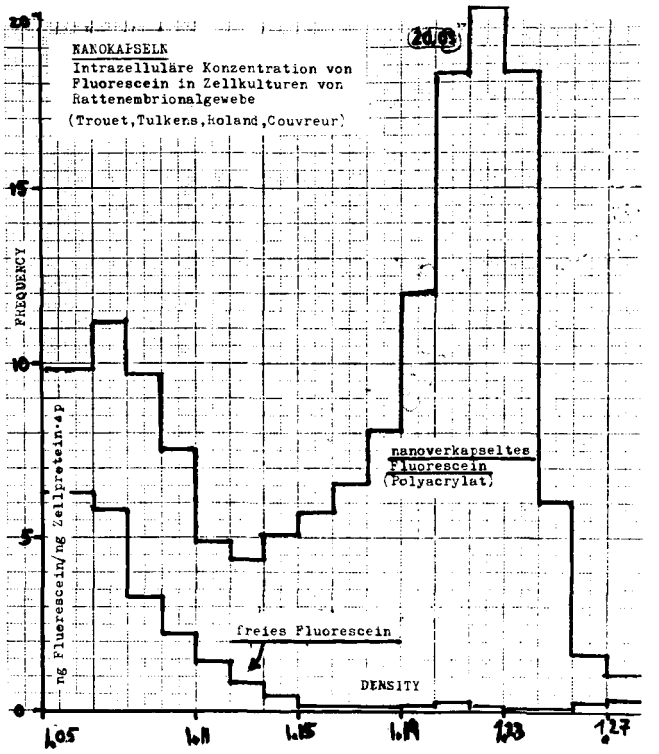


Fig. 8. Intracellular enrichment of a cell tracer (fluorescein) in cell cultures of rat embryonal tissue. Right side: Fluorescein built into polyacrylate carrier. Left side below: Free fluorescein.

tures. The spectrum of the vesiculated fluorescein in a carrier (Fig. 9, right side) and the free fluorescein-sodium in the cell (Fig. 9, left side) is quite different. The isopycnic centrifugation of a cytoplasmatic extract shows that ⅔ of the entrapped, vesiculated fluorescent material displayed a distribution pattern identical to that of the two lysosomal enzymes: *N*-acetyl-β-glucosaminidase (middle left) and of catepsin-B (middle right) (Fig. 9). The remaining one-third of entrapped fluorescein was bound in the top fractions of the gradient. Complete dissociation was obtained from protein (above, left side), and from S′-nucleotidase (above right side) a marker enzyme of plasma membranes and related structures. The enrichment of fluorescein carriers can be calculated as 15 times higher in the lysosomes than in the culture medium under the condition that its distribution is uniform within those organelles.

On the other hand, cell incubation with free fluorescein-sodium shows a very weak and diffuse uptake by the cell protein: after isopycnic centrifugation, the fluorescein salt was found exclusively in the top fractions, where neutral pyrophosphatase, a soluble enzyme of the cytoplasma, is present (24).

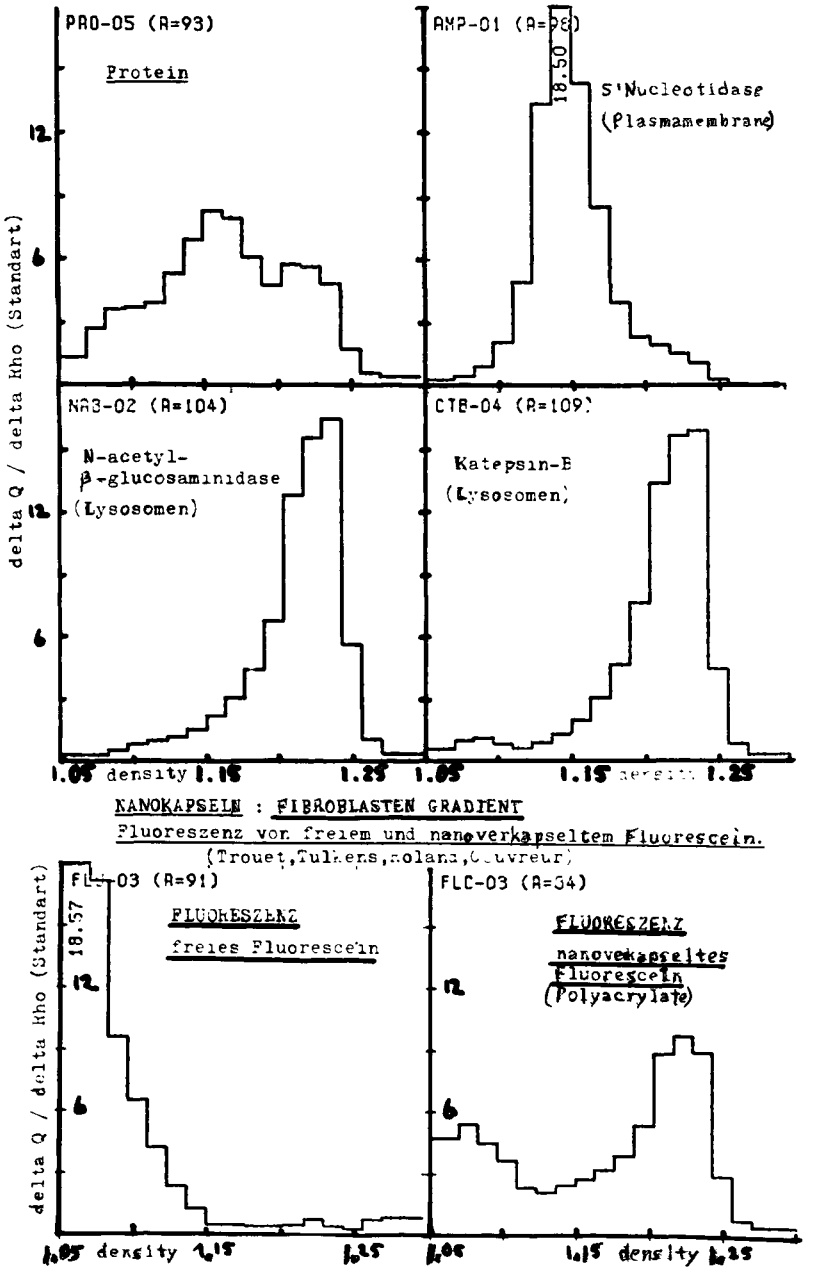


Fig. 9. Intracellular localization of cell tracer (fluorescein) with and without carrier. Left side below: free fluorescein. Right side below: fluorescein built into polyacrylate carrier.

These preliminary results indicate that colloid polyacrylate carriers, loaded with fluorescein-sodium, show a significant increase of uptake by cultured fibroplasts and allow its association and accumulation into lysosomes, a cell compartment, where free fluorescein-sodium does not spontaneously accumulate. It is known that the primary cell organelles possess strong hydrolytic enzyme systems that later break up the carrier and specifically release the cell tracer at the lysosomes. It can therefore be admitted that ultrafine solid carriers, loaded with active component, may be used as lysosomotropic carriers for compounds that have no access to lysosomes. Such a lysosomotropic release procedure would be highly interesting, but still a great deal of work has to be done until a final answer to this problem can be obtained.

## CONCLUSIONS

The examples mentioned for drug targeting with the aid of solid ultrafine carriers do not claim to cover all the areas of application for such carriers. This kind of controlled drug delivery is still open for future research.

The further development of such colloidal carriers must be directed toward furnishing the biologist "tailor-made" carrier systems with defined physical, chemical, and biological properties. Emphasis must lie, in the future, on size, surface properties, texture, and structure of the carrier, its compatibility, controlled release, steered bioavailability, and correct therapeutic effect at the desired location, for a defined period of time, and in the desired body organ. The aim is to obtrain a specific, systemic transport system that can be exactly directed and steered to the diseased body organ or cell compound.

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