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Polybutylcyanoacrylate nanoparticles for the delivery of [^{75}Se]norcholesterol

J. Kreuter [1], S.N. Mills [2], S.S. Davis [2] and C.G. Wilson [3]

[1] *Institute of Pharmacy, Swiss Federal Institute of Technology, Zurich (Switzerland);* [2] *Department of Pharmacy, University of Nottingham; and* [3] *Department of Physiology and Pharmacology, The Medical School, University of Nottingham, Nottingham NG7 2RD (U.K.)*

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Summary

Polybutylcyanoacrylate nanoparticles have been used for the intravenous and intramuscular administration of a steroidal material ([^{75}Se]norcholestenol) to the rabbit. Whole body profiles of ^{75}Se, obtained using a gamma camera over a period of 30 days, show that the [^{75}Se]norcholestenol is retained for a longer period of time with the nanoparticle system than for the control system where the drug was dissolved in a micellar system. In vitro dialysis experiments indicate that the thermodynamic activity of the drug can be increased by its incorporation within nanoparticles. This increased activity may affect the distribution of the drug into body tissues.

Introduction

For optimal drug action it is necessary to deliver a drug to the desired site of action in the body in the most efficient way possible. Targeting the drug to the site of action either by using a prodrug or a specifically designed drug delivery system would not only improve the therapeutic efficiency but would also reduce the total drug dose necessary to achieve the desired therapeutic response, thus minimizing unwanted side-effects and adverse reactions.

One possible means of attaining this goal involves the use of colloidal drug delivery systems. Colloidal preparations lend themselves to parenteral administration and offer potential as drug carriers for the sustained release of an associated drug or for delivery of a drug to a specific organ or target site (Illum and Davis, 1982).

Colloidal systems which have been investigated previously include liposomes, albumin microspheres and nanoparticles (Gregoriadis, 1977; Gregoriadis and Neerunjun 1975; Torchilin et al., 1979; Oppenheim et al., 1978; Widder et al., 1978; Brasseur et al., 1980). Our particular investigations concern nanoparticles here defined as polymeric particles ranging in size from 10 to 1000 nm in which the active principle is entrapped, encapsulated and/or adsorbed.

In a previous study the fate of poly[methyl-2-^{14}C]methacrylate) nanoparticles in rats was investigated (Kreuter et al., 1979). This material had been shown to be a good adjuvant for certain vaccines (Kreuter and Speiser, 1976; Kreuter et al., 1976; Kreuter and Liehl, 1978). Polycyanoacrylates also have been studied instead of the methacrylates, because the former are much more rapidly degraded in vivo and have biomedical applications such as surgical adhesives (Mungui et al., 1979).

In these earlier studies the radiolabel ^{14}C was used with the major disadvantage that the experimental animals had to be killed in order to locate and quantify the radioactivity. The use of gamma-emitting radionuclide allows the detection and location of the radiation within the body of live animals by using the non-invasive technique of gamma-scintigraphy. Thus the periodic monitoring of the same animals over the whole time period of a study can be achieved. In the present investigation, [^{75}Se]norcholestenol, a lipophilic derivative of cholesterol, has been used as the labelling agent for polybutylcyanoacrylate nanoparticles.

Materials and Methods

Preparation and characterisation of nanoparticles

1 ml of a commercial micellar solution of [^{75}Se]norcholestenol (Scintadren, Radiochemical Centre, Amersham: 300 μCi (11.1 MBq) in 1.5 ml solution ($\pm$ 10%) containing Tween 20 (1.6%) was added to 10 ml of 0.1 N HCl containing 50 μl Tween 20 (Honeywill Stein, London). 100 μl butylcyanoacrylate (Sichel Werke, Hanover, F.R.G.) was added and the mixture stirred for 2 h resulting in the formation of polybutylcyanoacrylate nanoparticles. This mixture was neutralized with 1 ml of 1 N NaOH, stirred for 15 h and filtered (Whatman paper no. 4). The resultant nanoparticles had a diameter of 79.1 $\pm$ 6.5 nm, as measured by photon correlation spectroscopy (Malvern Instruments) compared to a value of 72.2 $\pm$ 3.3 nm for nanoparticles prepared without [^{75}Se]norcholestenol.

Centrifugation of samples of the nanoparticle suspension (100,000 *g* for 1 h, Airfuge, Beckman Instruments) revealed that 87% of the [^{75}Se]-activity was bound to the nanoparticles.

Dialysis experiment

150 μl of nanoparticle suspension or commercial solubilized [^{75}Se]norcholestenol (~ 3 μCi, 0.11 MBq) was placed in one compartment of a two-compartment perspex dialysis cell (half-cell volume of 14 ml) containing either phosphate-buffered saline (pH 7.4) or horse serum at 37°C separated by a cellulose acetate dialysis membrane (Spectropor) in both compartments. Periodically 250 μl samples were recovered from

the receiver compartment and the [^{75}Se]-activity measured using a gamma-counter (Intertechnique). The derived values were corrected for dilution and radioactive decay. As each sample was taken, it was replaced by an equal volume of the relevant dialysis medium.

Imaging procedure

(i) Intravenous study. A dose of 40 μCi (1.48 MBq) [^{75}Se]-labelled nanoparticle suspension (2 ml) or solubilized norcholestenol followed by 0.3 ml sodium citrate buffer (29.4%, pH 7.0) was administered via the marginal ear vein of 3 rabbits (New Zealand White) using a two-way valve. Anterior images were taken with the rabbits loosely restrained within a Perspex box placed on the face of a gamma camera (General Electric Maxi Camera) fitted with a high energy parallel hole collimator.

Images were taken at suitable intervals over a period of 30 days post-injection. Data from the gamma camera were recorded using a computer-based data processor (Link Systems). Regions of interest (ROIs) were defined (liver, whole body) and the radionuclide distribution quantified, the values being corrected for circulating activity (liver ROI), background activity and radioactive decay.

(ii) Intramuscular study. A dose of 10 μCi (0.37 MBq) [^{75}Se]-labelled nanoparticle suspension or solubilized norcholestenol (0.5 ml) mixed with 0.1 ml sodium citrate buffer (29.4%, pH 7.0) was injected into the thigh muscle of 3 rabbits (injection depth 1 cm, 25-gauge needle). Anterior images were taken and analyzed as before, including an additional ROI; the injection site, and a corresponding ROI on the contralateral thigh. This last ROI was analyzed so that injection site data could be corrected for circulating activity.

Results and Discussion

The results of the dialysis experiments are expressed in Figs. 1 and 2, as the [^{75}Se]-activity appearing in the receiver chamber. In both cases, using phosphate-buffered saline or horse serum as dialysis medium, the rate of transfer of [^{75}Se]norcholestenol to the receiver compartment was greater when the norcholestenol was associated with the nanoparticles than when solubilized. This increased transport rate when the norcholestenol was associated with the nanoparticles can be explained by a higher thermodynamic activity of the nanoparticle-bound material as compared to the micellar system. The findings of Higuchi and co-workers (Bikhazi and Higuchi, 1970, 1971; Surpuriya and Higuchi 1972a and b) support this suggestion: in their transport studies with cholesterol they demonstrated that surfactants such as the polysorbates can cause considerable interfacial barriers, retarding by orders of magnitude the transport of solutes solubilized in micelles. The faster delivery from phosphate-buffered saline as compared to serum can be explained by the interaction of norcholestenol with serum components retarding its transport (Meyer and Guttman, 1968).

Figs. 3 and 4 show typical images for the distribution of radioactivity after intravenous administration of [^{75}Se]norcholestenol associated with nanoparticles or

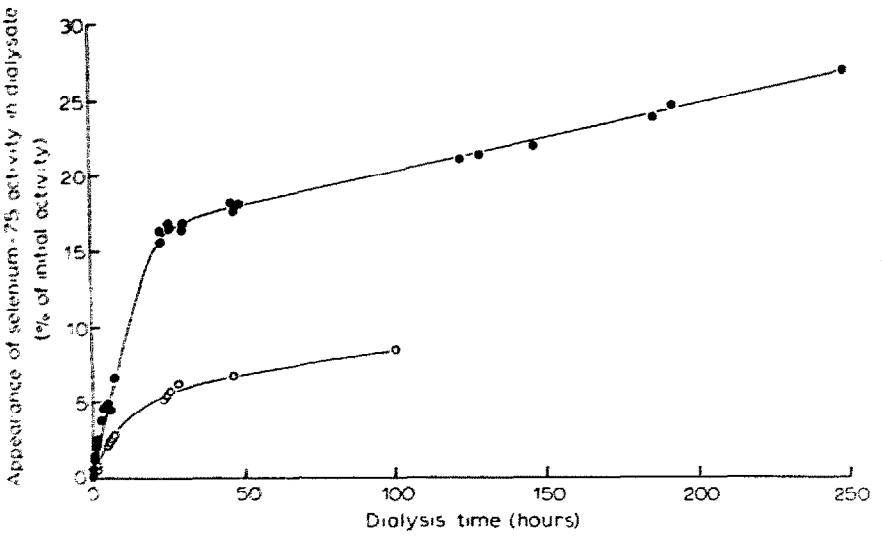


Fig. 1. Dialysis in phosphate-buffered saline of [^{75}Se]norcholestenol in a micelle system (○) or incorporated within polybutylcyanoacrylate nanoparticles (●).

as the diluted micellar preparation, respectively. The resulting whole body and liver profiles after intravenous injection of the two above preparations are shown in Fig. 5. The results for the corresponding intramuscular study are given in Figs. 6–8.

After injection of nanoparticles by both routes, a significantly higher retention of [^{75}Se]-activity in the whole body of the rabbit was found as compared to the micellar system. The activity in the liver was lower after intravenous injection of nanopar-

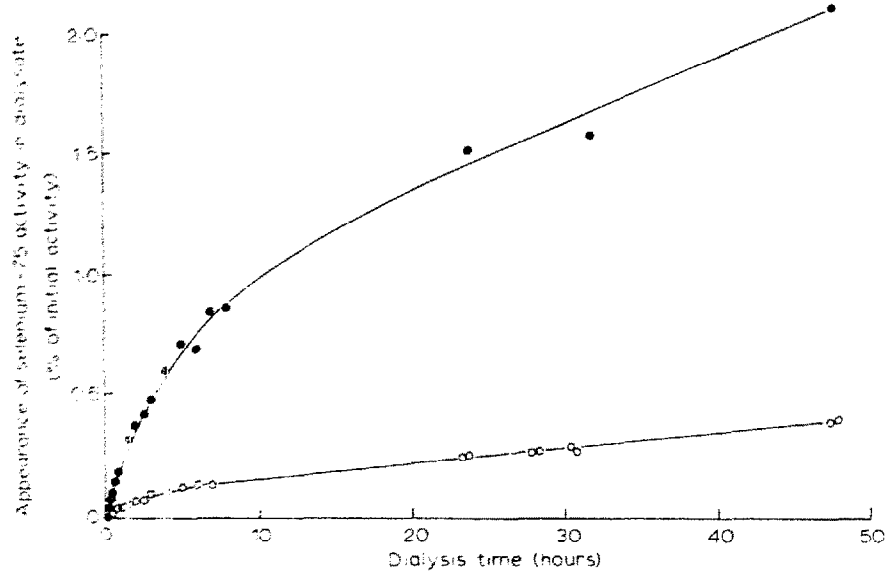
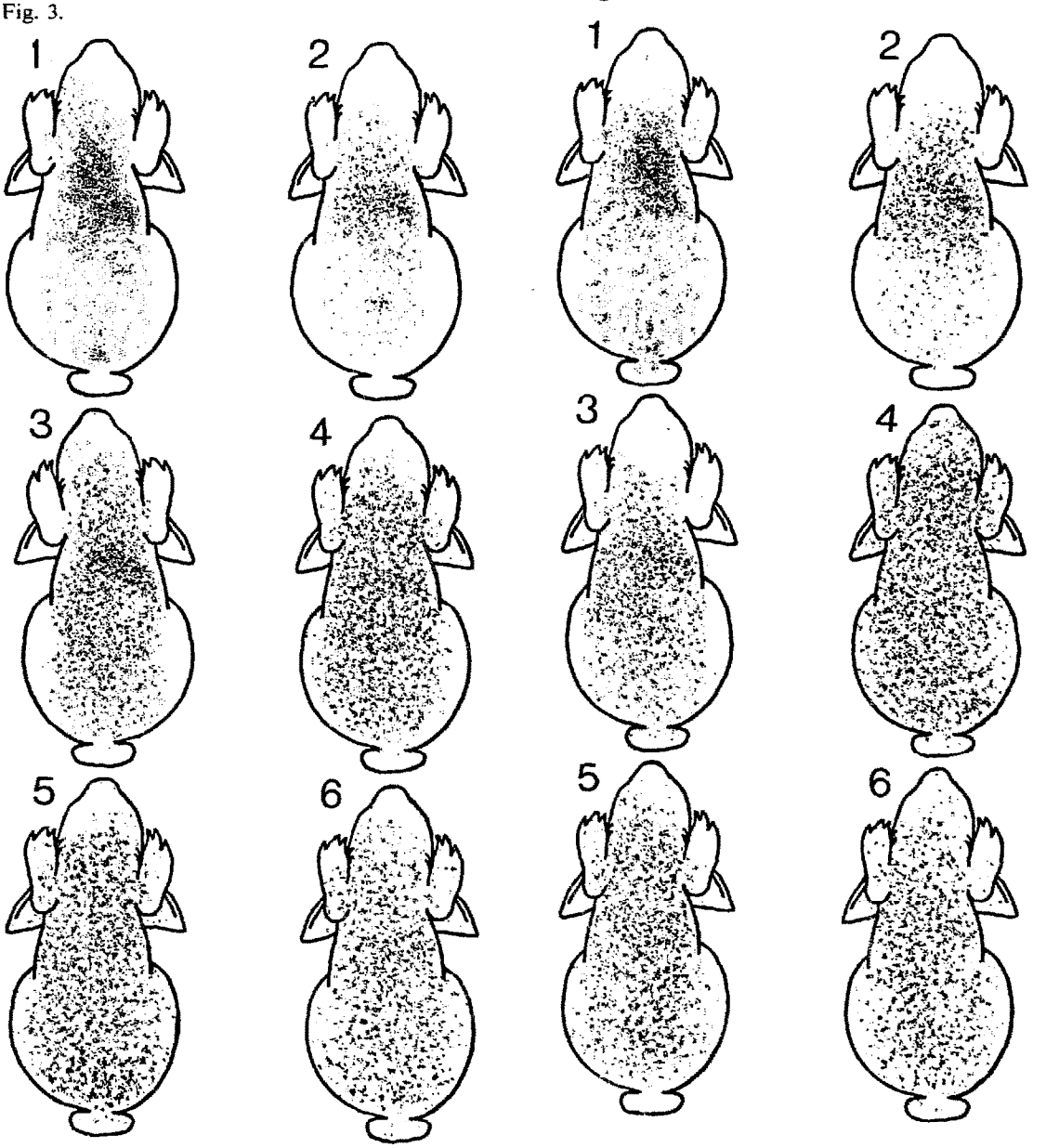


Fig. 2. Dialysis in horse serum of [^{75}Se]norcholestenol in a micelle system (○) or incorporated within polybutylcyanoacrylate nanoparticles (●).

Fig. 3.

Fig. 4.



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1 - 1 min
2 - 1 hour
3 - 1 day
4 - 1 week
5 - 2 weeks
6 - 3 weeks

Fig. 3. Scintiscans obtained following i.v. administration of [^{75}Se]norcholestenol incorporated within polybutylcyanoacrylate nanoparticles.

Fig. 4. Scintiscans obtained following i.v. administration of [^{75}Se]norcholestenol in a micellar system.

ticles than for the micellar system. The low accumulation of the nanoparticles in the liver region is somewhat surprising, since it is well known that colloidal particles are normally taken up preferentially by fixed macrophages (Kuppfer cells) after in-

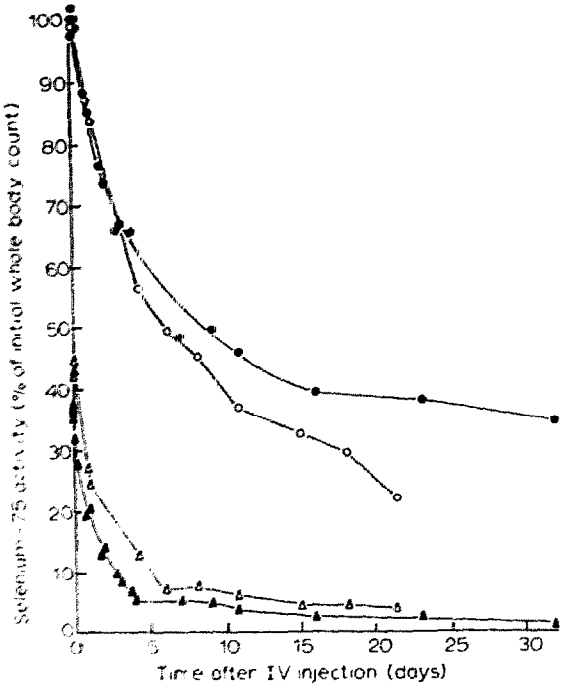


Fig. 5. Selenium-75 activity whole body and liver profiles, after intravenous injection into rabbits of [^{75}Se]norcholestenol (a) in a micellar system or (b) incorporated within polybutylcyanoacrylate particles. Micellar system: ○, whole body; △, liver. Nanoparticle system: ●, whole body; ▲, liver. (Mean n = 3, S.E.M. < 8%.)

travenous injection. Kreuter et al. (1979) have reported that between 60% and 90% of the total body activity was located in the liver following i.v. administration of polymethylmethacrylate nanoparticles. Clearly the polybutylcyanoacrylate nanoparticles can behave in a different manner, possibly because of a difference in particle size and surface characteristics (hydrophobicity) (Illum and Davis, 1982).

The clearance profiles for the intramuscular injection site show no significant differences for the two different systems (Fig. 9). However, the whole body profiles demonstrate a significant retention of the [^{75}Se]-activity in the body for the nanoparticle system which is especially pronounced 10 days post-injection. It may be noted too that this prolonged retention may not be caused necessarily by the persistance of nanoparticles since Couvreur (personal communication) has observed recently that the majority of the radioactivity provided by the intravenous injection of [^{14}C]-labelled polybutylcyanoacrylate nanoparticles was cleared from the bodies of mice after 24 h. Thus it is possible that the norcholestenol associated with the polybutylcyanoacrylate nanoparticles is distributed differently than that in the diluted micelle system on a cellular level, this difference in distribution being caused by the higher thermodynamic activity of the norcholestenol in the nanoparticle system. The resultant association of norcholestenol in different tissue or cell organelles possibly occurring at a very early stage following injection, may explain the prolonged retention of the [^{75}Se]norcholestenol.

Fig. 6.

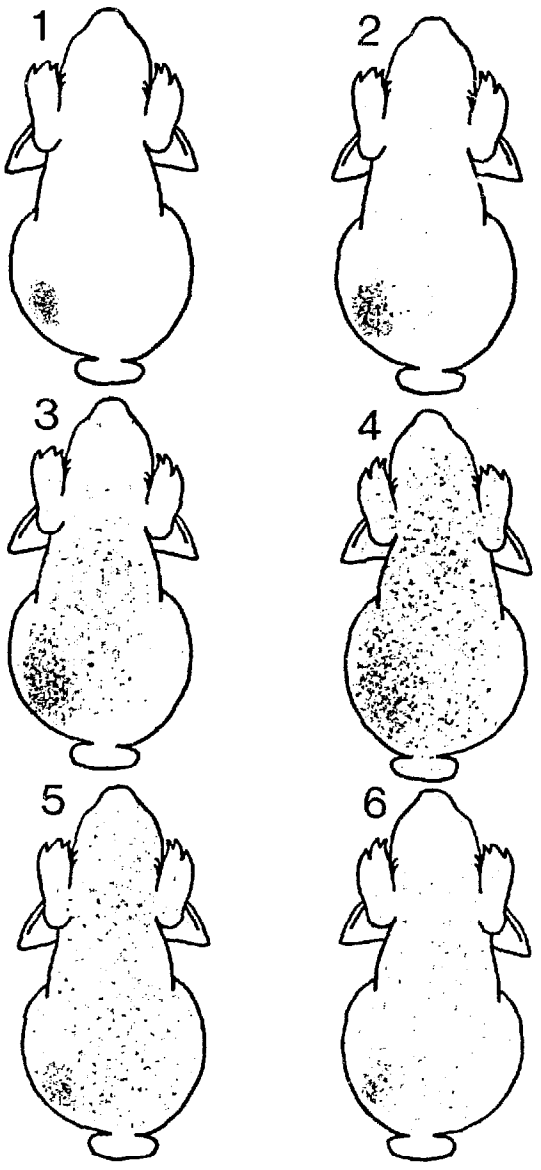
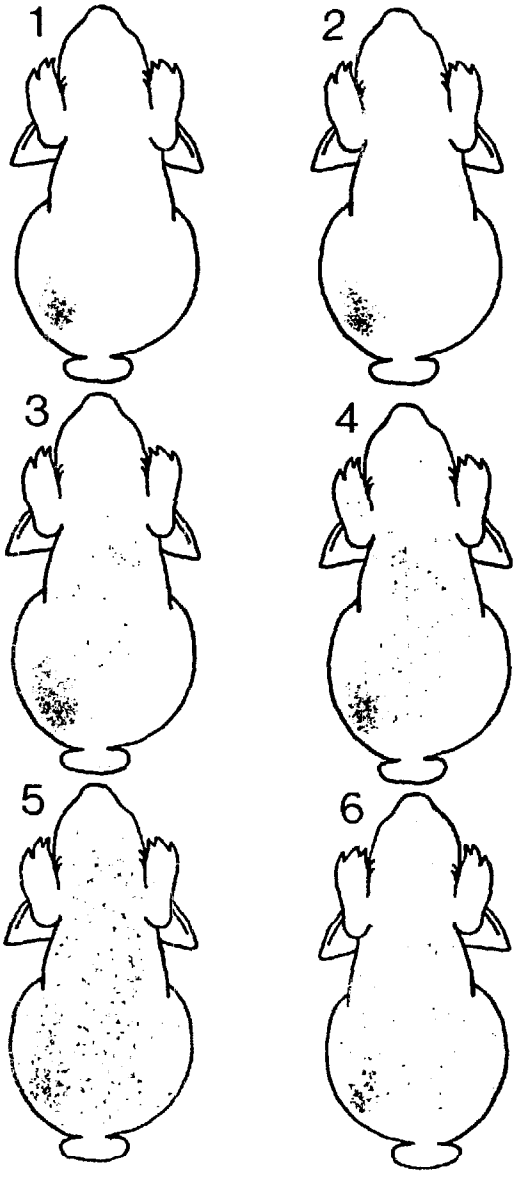


Fig. 7.



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NAME
CH/FI
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1 - 1 min
2 - 1 hour
3 - 1 day
4 - 1 week
5 - 3 weeks
6 - 10 weeks

Fig. 6. Scintiscan obtained following i.m. administration of [^{75}Se]norcholestenol incorporated within polybutylcyanoacrylate nanoparticles.

Fig. 7. Scintiscan obtained following i.m. administration of [^{75}Se]norcholestenol incorporated within a micellar system.

The study shows that nanoparticles can effectively modify the fate of a model lipophilic drug in the body. It also demonstrates the potential of gamma-scintigraphy as a technique for studying the delivery capabilities of various dosage forms.

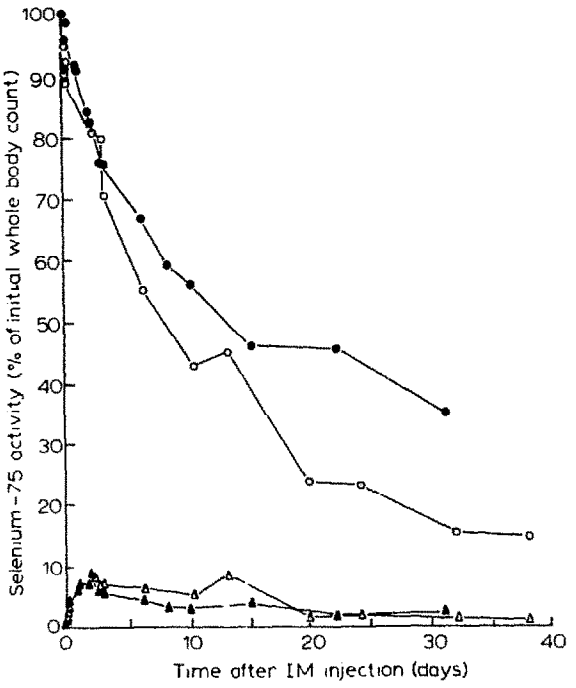

Fig. 8. ^{75}Se-activity whole body and liver profiles after intramuscular injection into rabbits of [^{75}Se]norcholestenol (a) in a micellar system or (b) incorporated within polybutylcyanoacrylate nanoparticles. Micellar system: ○, whole body; △, liver. Nanoparticle system: ●, whole body, ▲, liver. (Mean n = 3, S.E.M. ≯ ±7%.)

A recent study by Maincent (1982) on the oral administration of vincamine in solution and bound to polycyanoacrylate nanoparticles has shown that the nanoparticle system had a greater bioavailability than the solution. We believe that our proposal of a higher thermodynamic activity of the drug in the nanoparticle system is an explanation of these interesting findings.

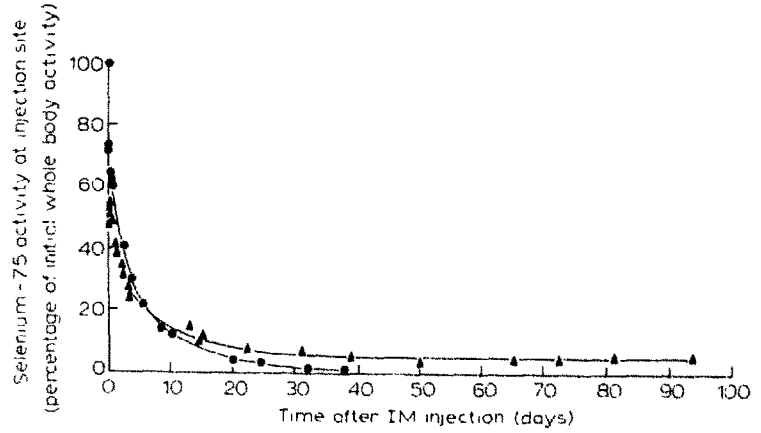

Fig. 9. ^{75}Se-activity clearance from the injection site, after intramuscular injection of [^{75}Se]norcholestenol in micellar system and when incorporated within polybutylcyanoacrylate nanoparticles, into rabbits. ●, micellar system; △, nanoparticles. (Mean n = 3, S.E.M. ≯ ±11%.)

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