International Journal of Pharmaceutics, 17 (1983) 283–290
Elsevier

Tissue distribution and fate of free and liposome-encapsulated [^{125}Sb]sodium stibogluconate by gamma scintigraphy

L.S. Rao [1], J.G. Hardy [2] and C.G. Wilson [3]

[1] *Pharmaceutical Development, The Wellcome Foundation Ltd., Dartford, Kent,* [2] *Department of Medical Physics, and* [3] *Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham (U.K.)*

(Received May 12th, 1983)
(Accepted June 4th, 1983)

Summary

Gamma scintigraphy has been used to monitor the distribution and kinetics of elimination of free and liposome-encapsulated sodium stibogluconate following intravenous administration to rats. The results showed that the liposomal drug was localized and retained over a prolonged period in the liver and spleen region, whilst the free drug was subject to general distribution and rapid elimination via the kidneys.

Introduction

One of the most promising areas of application of the liposome drug carrier concept is in the treatment of disease conditions involving the reticuloendothelial cells. Very marked improvements in the efficacy of antimonial drugs, such as sodium stibogluconate (Pentostam), by encapsulation in liposomes have been reported in the treatment of experimental leishmaniasis in laboratory animals (Black et al., 1977; New et al., 1978; Alving et al., 1978; Alving et al., 1980). This effect is considered to be due to the fact that the phagocytic cells of liver and spleen, which are the foci of the parasite infection, are also implicated in the uptake of a major fraction of injected liposomes (Juliano and Stamp, 1975).

Correspondence: L.S. Rao, Pharmaceutical Development, The Wellcome Foundation Ltd., Dartford, Kent, U.K.

The evidence for localization of systemically administered liposomes has come from a variety of experimental studies, including external gamma scintigraphy of animals and patients after administration of liposomes labelled with gamma-emitting nuclides such as $^{99}Tc^{m}$, ^{131}I and ^{111}In (Jeyasingh, 1982; Hardy et al., 1980; Espinola et al., 1979). Although such labelling has given much useful information, the degree of correspondence between the patterns of distribution and the fate of the attached radiolabel and the liposomes themselves or the encapsulated drug must remain in doubt.

During work on the development of a liposomal formulation of sodium stibogluconate (SSG), the availability of a gamma-emitting isotope of antimony, ^{125}Sb (half-life, 2.8 years), led to an investigation of the possibility of using SSG incorporating the ^{125}Sb label. Although the gamma energy of the ^{125}Sb isotope is higher than the normal range of energies used in gamma scintigraphy, it was, nevertheless, found possible to image this isotope adequately. We report the results of our investigations on the distribution and kinetics of elimination of ^{125}Sb SSG in both the free and liposome-encapsulated forms in the rat.

Materials and Methods

Radiolabelled sodium stibogluconate

Sodium stibogluconate incorporating the ^{125}Sb isotope was synthesized using [^{125}Sb]antimony pentachloride from Amersham International. The final activity was about 40 MBq/g of the drug.

Preparation of the liposomes

One gram of a lipid mixture, comprising synthetic dipalmitoylphosphatidylcholine (Sigma, 98%)–cholesterol (Sigma, USP)–dicetylphosphate (Sigma) in the molar ratio 9 : 9 : 2, was dissolved in 50 ml chloroform. A mixture of 1 g of non-radioactive SSG and 0.5 g of SSG containing 20 MBq ^{125}Sb was dissolved in 15 ml distilled water by gentle warming. After adjusting to pH 6.5, this solution was added slowly to the chloroform solution of the lipid, which was maintained at 45–50°C and stirred vigorously.

The metastable emulsion was then transferred to a rotary evaporator and the chloroform expelled under reduced pressure at 45–50°C. The residual liposomal dispersion was made up to 25 ml using sufficient concentrated sodium chloride solution such that the final salt concentration was 1.8%. The liposomal dispersion was then isolated from the non-encapsulated drug as follows. The dispersion was centrifuged at 4000 rpm for 20 min. The clear supernatant was removed, and set aside for use as the free drug in the animal studies. The liposome sediment was redispersed using 1.8% sodium chloride solution and re-centrifuged. The very slightly turbid supernatant was removed and the liposome sediment once again redispersed and centrifuged as above. Finally, the supernatant was removed and the liposome sediment redispersed using 1.8% sodium chloride solution and, after adjusting to pH 6.5, stored at +5°C.

Liposome characteristics

The degree of encapsulation of the drug in the liposomes was determined by gamma counting of the initial SSG solution, the various supernatants from the centrifugation steps and the final liposome dispersion.

The stability of the liposomal SSG preparation was assessed by subjecting the stored samples of the liposomes to centrifugation at 50,000 *g*, followed by two steps of redispersion and centrifugation in fresh 1.8% sodium chloride solution. The combined supernatants from the centrifugation stages as well as the final redispersed liposome pellet were assayed for activity using a gamma counter. The activity in the supernatants, expressed as a percentage of the total activity in the liposome sample, was taken as the free drug content of the stored liposomal dispersion.

Liposomes, prepared in a similar manner using non-radioactive SSG, were analyzed for size distribution using a Coulter Counter Model TA II fitted with a 30 μm orifice tube. The average volume diameter of the liposomes was calculated to represent the mean size of the liposomes.

Distribution studies in the rat

Healthy male Wistar rats were used for these studies. Each rat was anaesthetized with 20 mg pentobarbitone sodium, a jugular vein cannulated and 1.5 ml of the experimental preparation infused over a 2-min period. The solution of the free drug was administered to 6 rats each receiving 0.75 MBq ^{125}Sb. Four rats were each given 0.54 MBq ^{125}Sb in the form of liposomal SSG. Thus each rat received either 16.0 mg Sb as the free drug or 11.7 mg Sb as the liposomal formulation.

Imaging was undertaken using a gamma camera fitted with a pinhole collimator and the data recorded by computer. A 35% energy window was selected to detect the 428 keV gamma radiation of ^{125}Sb. Dorsal images ranging from 300 s to 1000 s duration were recorded with the un-anaesthetized animals restrained in Perspex tubes. The 6 animals receiving the free drug were investigated as 2 groups of 3. The first group was imaged at intervals over a period of up to 18 h, and the other group over a 4 h period but with more frequent imaging. The rats dosed with the liposomal preparation were imaged at intervals over a period of up to 150 h.

Quantitation of antimony distributions was achieved by defining regions of interest around the images using the computer. From each image, count rates were obtained for the whole body and the liver-spleen region. Corrections were applied for background counts.

To two additional rats was administered either [^{99}Tc^{m}]-labelled diethylenetriaminepentaacetic acid or [^{99}Tc^{m}]-labelled sulphur colloid in order to facilitate the interpretation of the antimony images. The former tracer is excreted rapidly via the kidneys and the latter material is taken up by the liver and spleen. These animals were imaged using a 20% energy window centred on the 141 keV radiation of ^{99}Tc^{m}.

Results and Discussion

Gamma counting of the initial SSG solution, the supernatants from the various centrifugation steps and the final liposomal SSG showed that the degree of encapsu-

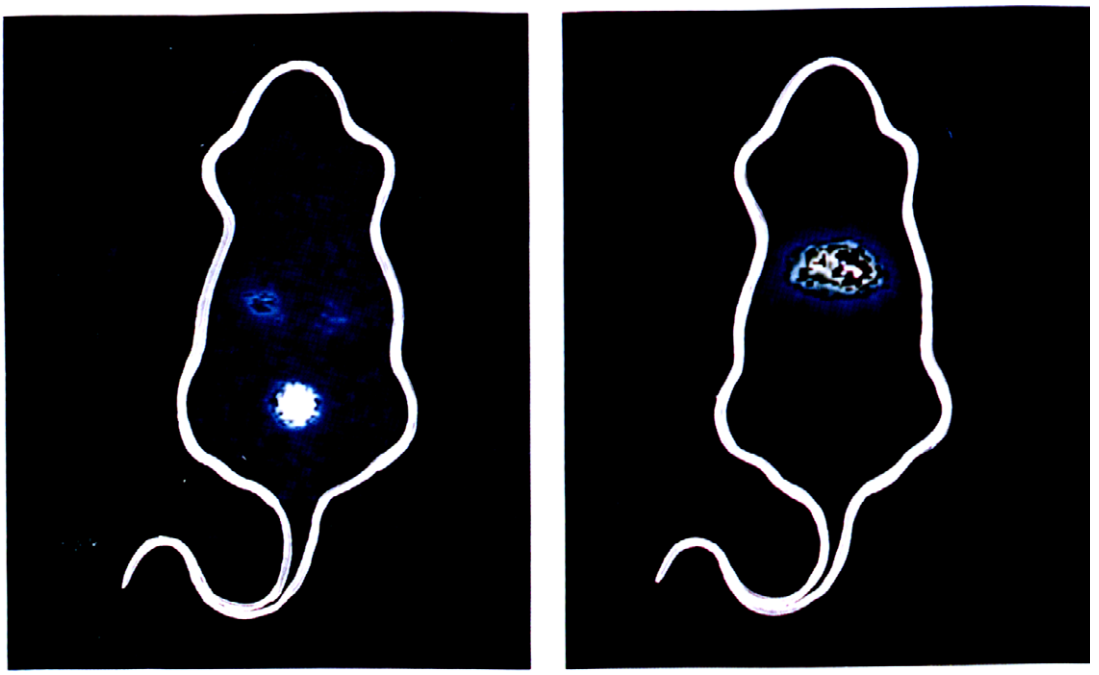

Fig. 1.

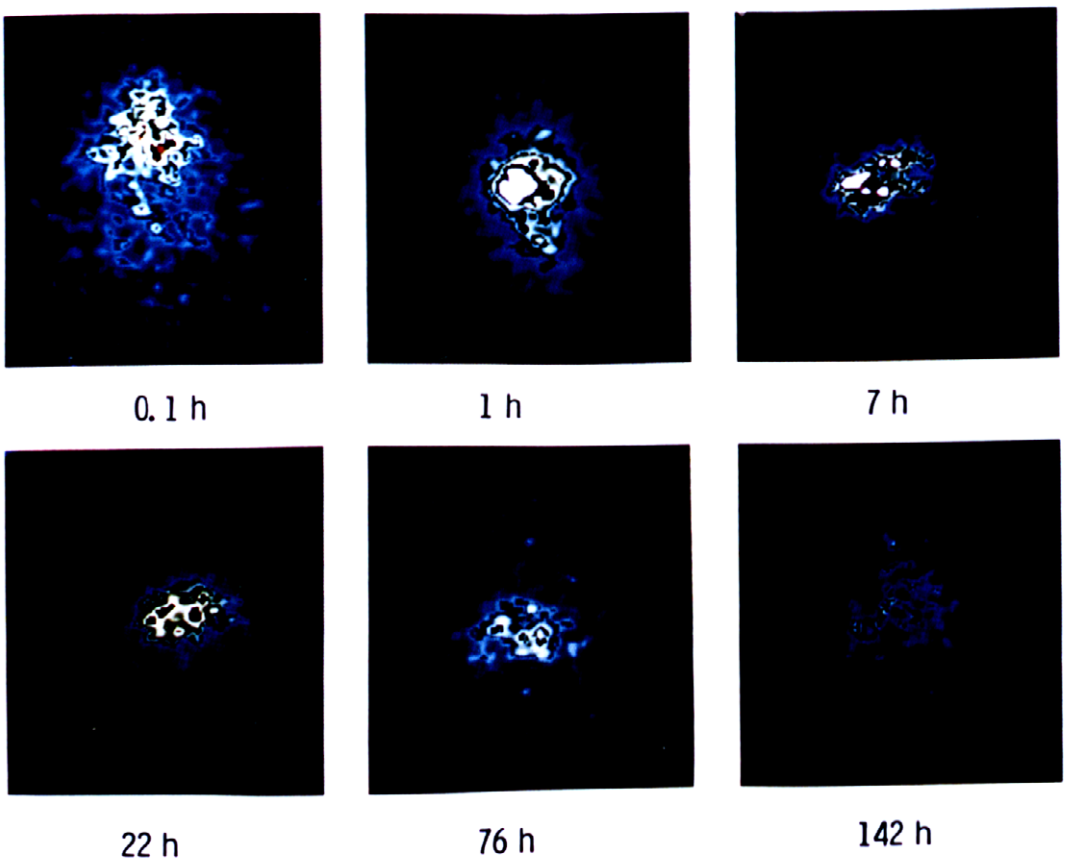

Fig. 3.

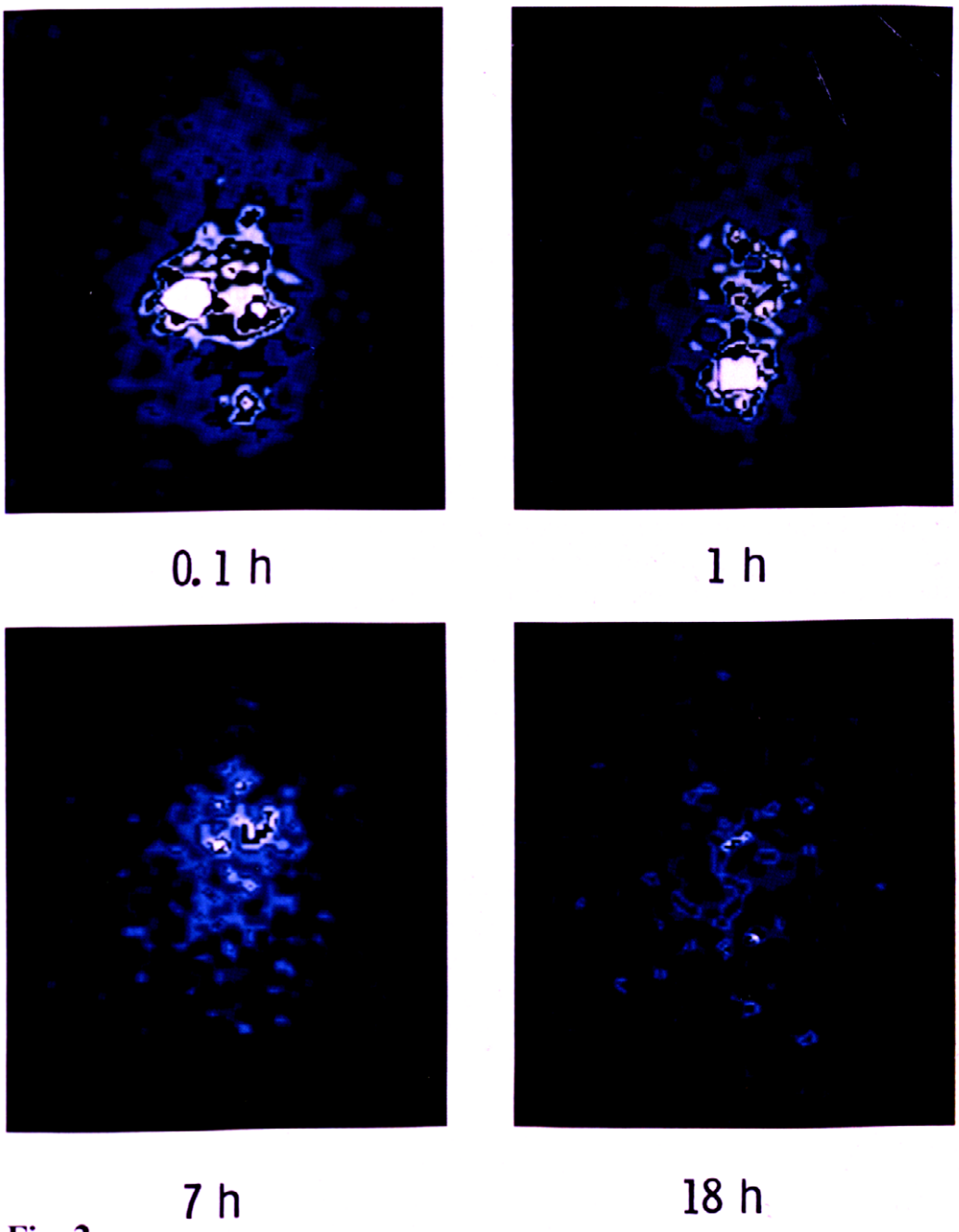

Fig. 2.

Fig. 1. Scintigraphic examination of rats after intravenous administration of [^{99m}Tc^{m}]diethylenetriamine-pentaacetic acid to identify the positions of the kidney and bladder and [^{99m}Tc^{m}]sulphur colloid to illustrate the position of the liver.

Fig. 2. Disposition of [^{125}Sb]sodium stibogluconate following intravenous administration as the free drug.

Fig. 3. Distribution of [^{125}Sb]sodium stibogluconate following intravenous administration of the liposomally-entrapped drug.

lation of SSG was about 27% and the antimony content of the final liposomal dispersion was 7.8 mg Sb (25.8 mg SSG) per ml. The first clear supernatant, diluted to 25 ml, contained 10.7 mg Sb (35.7 mg SSG) per ml and was used as the free drug in the animal studies. The free drug content of the stored liposome preparation (i.e. leaked drug) was 5.7% and 6.8% after 4 days and 54 days storage, respectively, at +5°C. The animal studies were carried out using liposome samples stored at +5°C for 10 days. The size analysis of a comparable liposome preparation (containing non-radioactive SSG) showed the average volume diameter to be 1.0 μm.

Fig. 1 shows the relative positions of kidneys and bladder, and the liver-spleen region of the rat as visualized by the gamma camera using the [^{99}Tc^{m}]-labelled tracers. The distribution of the free SSG as a function of time after intravenous administration is illustrated by the sequence of images in Fig. 2. It can be seen that a rapid excretion of the free SSG via the kidneys occurred, with a high concentration appearing in the bladder by 1 h. Images recorded at later times show a generalized distribution of the material in the body with little drug remaining at 18 h. In contrast, the administration of liposome-encapsulated SSG led to an accumulation of the drug in the liver-spleen region, as shown in Fig. 3. This sequence of images shows the dominance of the liver-spleen area in accounting for the activity over a very prolonged period of time.

Plots of whole body activity retention against time for the free and liposomal drug formulations are shown in Fig. 4. It can be seen that about 50% of the administered free SSG was excreted within 2 h and only 13% of the drug remained

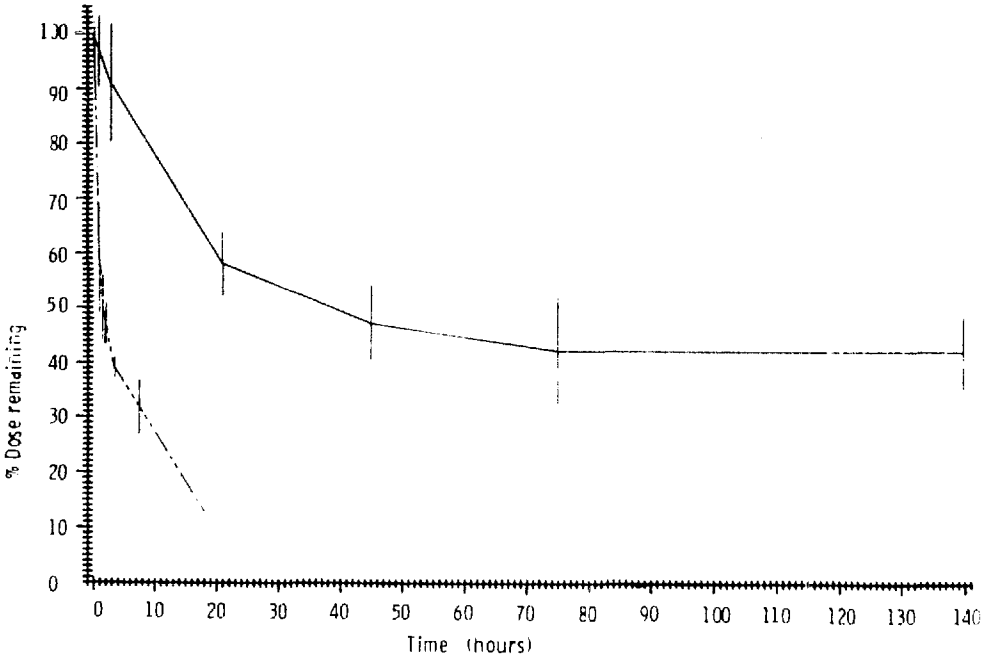

Fig. 4. Whole body clearance of [^{125}Sb]sodium stibogluconate following intravenous administration of liposomally-entrapped drug (solid line, n = 4 rats per group) or free drug (dotted line, n = 6 rats, 0–4 h; n = 3 rats 4.5–18 h post-dosing). Mean ± 1 S.E.M.

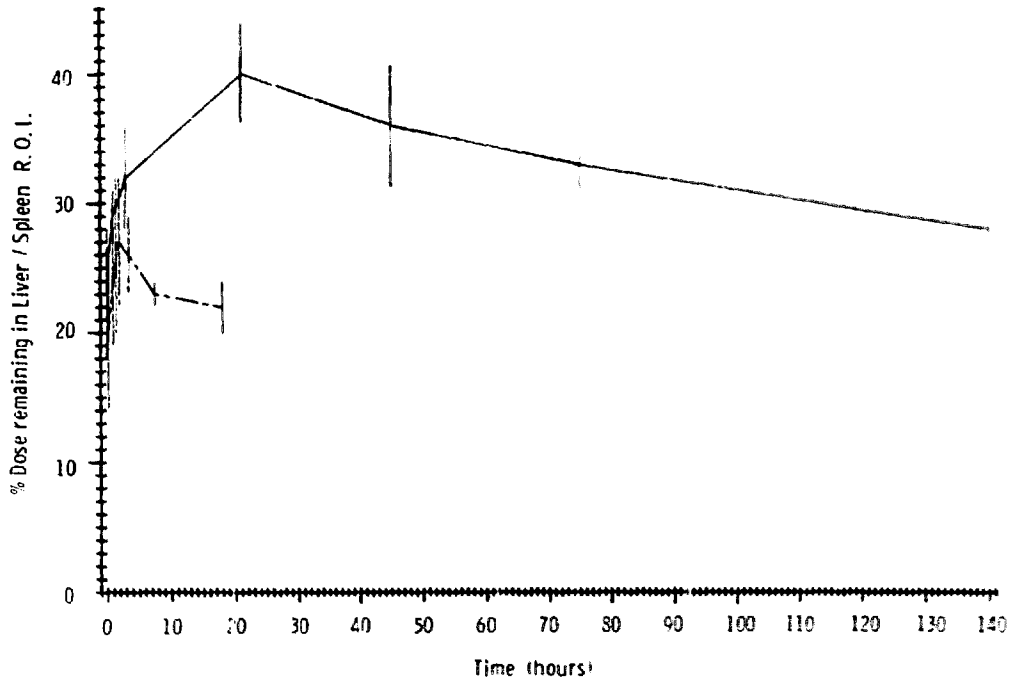

Fig. 5. Activity in the liver and spleen region of interest as a proportion of the whole body activity, following intravenous administration of [^{125}Sb]sodium stibogluconate as liposomally-entrapped drug (solid line, n = 4 rats per group) or free drug (dotted line, n = 6 rats, 0–4 h; n = 3 rats, 4.5–18 h post-dosing). Mean ± 1 S.E.M.

in the animals after 18 h. The liposomal drug, on the other hand, was eliminated much more slowly. There was a 50% retention of the drug in the body at 40 h post-injection and, even after 120 h, nearly 40% of the injected dose was still present.

The proportion of the whole body activity that was accounted for by the liver-spleen region as a function of time after injection is shown for both the free and liposomal drug in Fig. 5. With the free drug, approximately 25% of the activity in the body was in the liver-spleen area throughout the period of study. The activity in the liver-spleen region as a proportion of the whole body activity was about 40% at 20 h and 30% at 120 h in the case of the liposomal drug.

Rapid elimination of free SSG through the kidneys as shown in this study is in accord with previous clinical reports on Pentostam (Rees et al., 1980). This short half-time of SSG in the body with no specific uptake by the liver and spleen means that the drug needs to be administered frequently and in large doses. The changes in the distribution and half-time of SSG achieved by encapsulation in liposomes, as shown in Figs. 4 and 5, are indeed dramatic and fulfill the expectations of liposomal drug delivery in this context.

These results demonstrated indirectly the integrity of the liposomal formulation in circulation, since any loss of structural integrity would have released the drug which would then have been subject to rapid renal clearance as free drug. Since the elimination kinetics of the free and liposomal forms of the drug were so different, this study also served to demonstrate: the specific targeting of the liposomes to the

liver and spleen; and the gradual breakdown of the liposomes (or their integrity) within these organs such that antimony was subject to slow, but definite, elimination from the body.

These effects of specific targeting and prolonged residence of antimony in the liver and spleen can be expected to contribute to increased therapeutic efficacy of the liposomal form. This study, therefore, served to demonstrate the localization and kinetics of a liposome-encapsulated antimonial drug in an unequivocal manner. This has been achieved by gamma camera imaging of [^{125}Sb]sodium stibogluconate, which has not been reported previously.

Acknowledgements

We wish to thank Dr. W. Duncombe of Wellcome Research Laboratories, Beckenham for the synthesis of [^{125}Sb]sodium stibogluconate and help in the preparation and gamma-counting of the liposomes, and Dr. S.M. Gardiner and Mrs. A. Robinson of the Queen's Medical Centre for assistance in the animal studies.

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