positively charged liposomes do the same. By careful choice of route of administration and lipid composition, it is clear that adjuvanticity of liposomes may be decreased, but it is not clear whether liposome-entrapped materials can ever be protected from the immune response of the host.

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**Tumour Regression with Liposome-Entrapped Asparaginase: Some Immunological Advantages**

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The survival of certain malignant cells depends on the exogenous supply of L-asparagine, and in animal models administration of L-asparaginase results in low asparagine concentrations in blood, rapid tumour regression and complete cure (Broome, 1968). However, treatment of acute lymphocytic leukaemia in man with asparaginase has met with only moderate success (Capizzi *et al.*., 1970) and long-term treatment is hampered by toxic reactions and also by enzyme inactivation and the occurrence of anaphylactic shock promoted by anti-asparaginase antibodies. Previous work (Gregoriadis, 1975) has emphasized the enzyme- and drug-carrier potential of liposomes in therapy in terms of both avoidance of allergic (Gregoriadis & Allison, 1974) and other toxic reactions and homing of therapeutic agents to target cells (Gregoriadis & Neerunjun, 1975). In the present report possible advantages in the use of liposome-entrapped asparaginase have been investigated. *Erwinia* asparaginase (EC 3.5.1.1) (860 units per mg of protein) was generously supplied by Dr. H. E. Wade, Microbiological Research Establishment, Porton, Salisbury, Wilts., U.K. One unit of enzyme activity is defined as 1 µmol of substrate transformed/min at 25°C. The enzyme (12000 units) was dissolved by the addition of 1.0 ml of water, labelled with $^{125}$I (Gregoriadis *et al.*, 1974) and subsequently entrapped (Gregoriadis & Neerunjun, 1974) in liposomes composed of egg phosphatidylcholine, cholesterol and phosphatidic acid (negatively charged) or stearylamine (positively charged). Sonication (0.5–2.0 min) was carried out on negative liposomes, which were separated from the non-entrapped enzyme by molecular-sieve chromatography. Non-sonicated positive liposomes were centrifuged at 10000g for 45 min and the liposomal pellet was suspended in 10 mM-sodium phosphate buffer containing 1% NaCl. Of the enzyme used, 27.6–47.5% was entrapped in liposomes, which contained 33.0–57.5 units per mg of lipid. Lymphoma 6C3HED cells, kindly given by Dr. C. Wiblin of the Microbiological Research Establishment, were cultured intramuscularly (2×10⁶ cells per mouse) in the interscapular area of two to three C3H mice and transferred at 8-day intervals. Cells (4×10⁶) in 0.2 ml of Hank's balanced salt solution were injected intramuscularly as described above into C3H mice, which were divided 7–9 days later into groups of five and injected into the tail vein, the peritoneal cavity, the thigh of the hind leg or into the tumour itself with 0.2 ml of phosphate-buffered saline, non-entrapped or liposome-entrapped asparaginase (48–336 units in 0.2 ml). In other experiments, intact or
tumour-bearing mice were challenged intramuscularly with two to three injections of the enzyme (100 units per mouse) given at 10-day intervals. At 7 days after the last challenge these mice were injected intravenously with 500–1000 units of asparaginase, free or entrapped in negative liposomes, and observed for serum sickness.

All mice (total 20) treated with phosphate-buffered saline died in 17–34 days with grossly enlarged tumours. In contrast, in all mice (total 30) given non-entrapped asparaginase (48–168 units), and regardless of the route of administration, tumours regressed and were not palpable within 1–2 days after treatment. These animals survived for several months and were considered to be cured. On the other hand intravenous, intraperitoneal or intramuscular administration of asparaginase (48 units) entrapped in negative liposomes only moderately prolonged the survival of mice (total 30) to 27–40 days. Cure of mice (total 10) with entrapped asparaginase was achieved only when higher amounts (168 units) of the enzyme in negative liposomes were given intravenously. The same preparation injected into the tumour mass cured 60% of the animals (total 10), but there were no cures when treatment was given intramuscularly (total 5). Asparaginase entrapped in non-sonicated positive liposomes, however, and given intramuscularly or into the tumour mass did result in a 100% cure (total 15). These data suggest that liposomal asparaginase in blood can be reached by asparagine which presumably penetrates the lipid bilayers. Indeed, the concentration of asparagine (Cooney et al., 1970) in the blood serum and tumours of mice 3.5 and 24 h after intravenous treatment with non-entrapped as well as with entrapped asparaginase (300 units) was profoundly decreased. This was matched by a concomitant increase in the concentration of aspartic acid (Cooney et al., 1970). Non-sonicated positive liposomes given intramuscularly persist in the site of injection (Segal et al., 1975) and observed cures could be related to the slow release (Segal et al., 1975) of liposomal asparaginase into the circulation. Intravenous administration of non-entrapped asparaginase to mice (total 10) already pretreated with the enzyme led within minutes to anaphylactic shock and death. In contrast, similar mice (total 10) injected with liposome-entrapped asparaginase (500–1000 units) did not show any signs of illness for at least 48 h. Pre-immunized tumour-bearing mice injected with a therapeutic dose of non-entrapped asparaginase also died of serum sickness, but again administration of entrapped asparaginase into such animals did not produce anaphylactic shock. It is thus apparent that entrapped asparaginase not only starves asparagine-sensitive tumour cells to death but also, by virtue of its liposomal armour, prevents anti-asparaginase antibodies from interacting with the enzyme in the circulation. This avoidance of dangerous allergic reactions could render the use of asparaginase in cancer treatment immunologically safe.