of 10 m*M* I. Such reduction was interpreted as the result of entrapment of propicillin molecules into micelles of I. A similar phenomenon has been reported in absorption experiments with other drugs (12, 13). While the absorption of antibiotics by the small intestine increased in the presence of surfactants, such promoted absorption depended on the nature of the surfactants rather than the physicochemical properties of the antibiotics. Interestingly, the existence of a significant interaction between antibiotics and surfactants appeared not to influence the absorption by the small intestine. Despite the entrapment of 90% of the anionic propicillin into micelles of II (5, 6), no reduced absorption was observed (Table I). In the presence of 10 m*M* I, absorption of the antibiotic was promoted with 30% of the anionic species incorporated into the micelles. These results suggest that alteration of the membrane permeability by nonionic and cationic surfactants induced the promoted absorption in the small intestine, concurring with the findings made by other laboratories (3, 4).

Walters and Dugard (14) demonstrated that the hydrophilic–lipophilic balance (HLB) of a surfactant represents an important property in determining the promoted absorption. However, our results from the intestinal experiments indicated that significant absorption enhancement was observed only with ether-type surfactants, suggesting that HLB cannot be the sole cause for the observed promotional effect in the intestinal absorption of β-lactam antibiotics. Alteration of the permeability sometimes can be a result of disruption of the membrane structure by surfactants (15–17). In the present study, however, no significant disruption of the membrane was detected by light microscopy. The change in membrane permeability with the surfactant, therefore, may reflect a reversible alteration of the diffusion barrier to lipid soluble, poorly ionized drugs as claimed by Davis *et al.* (3, 4).

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Acrylic Microspheres *In Vivo* VI: Antitumor Effect of Microparticles with Immobilized L-Asparaginase Against 6C3HED Lymphoma

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Abstract □ The antitumor effect of immobilized L-asparaginase was tested against lymphoid leukemia in mice with concomitant scanning of the L-asparagine level in serum. L-Asparaginase was immobilized in microspheres of polyacrylamide or polyacryldextran. These particles were used in C3H mice bearing the L-asparagine-dependent lymphoma (6C3HED). The tumor was maintained as an ascites tumor, 1×10^6 cells were injected intraperitoneally and on day 4 after inoculation, L-asparaginase was injected intramuscularly or intraperitoneally in microparticles. After injection of 5.0 IU ip of L-asparaginase in microparticles, partial remission was induced, generally, however, the cancer relapsed and killed the mice within 2–3 weeks. To obtain complete regression, it was necessary to inject 20 IU of L-asparaginase in microparticles intraperitoneally. The best therapeutic effect was obtained when the particles were administered intramuscularly. After injection of 5 IU the survival time was prolonged, but complete regression was not achieved. The best effect was obtained when the particles were given intramuscularly in two small doses (2.5 IU) at a 3-day interval. Such treatment induced complete regression; 10 out of 12 treated mice were completely cured and lived for several months. It is concluded that the L-asparagine level in serum has to be depressed to <20% of the normal level for at least 6–7 days to obtain complete regression of the tumor.

Keyphrases □ Acrylic microspheres—*in vivo*, antitumor effect of microparticles with immobilized L-asparaginase against lymphoma □ L-asparaginase—acrylic microspheres, *in vivo*, antitumor effect of microparticles with immobilized L-asparaginase against lymphoma □ Antitumor effect—acrylic microspheres, *in vivo*, microparticles with immobilized L-asparaginase against lymphoma

L-Asparaginase of bacterial origin has been used extensively during the last 10 years in the treatment of lymphatic leukemia (1–3) as either a complement to or in combination with chemotherapy. The remission of the tumors is dose dependent (3) and considered to be due primarily to the deprivation of the cells of L-asparagine

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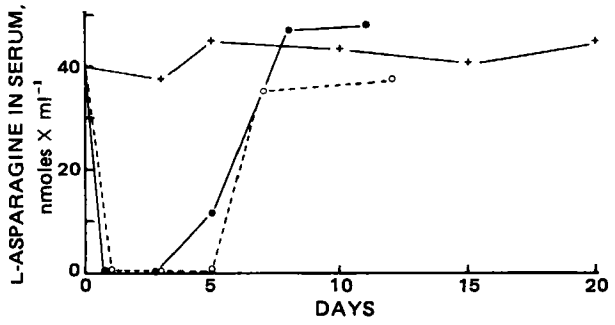

Figure 1—*L-Asparagine concentration in serum of healthy mice after a single intraperitoneal injection of L-asparaginase (5 IU/animal) in free solution (○) or in microparticles (●). Pooled serum from 4 to 6 mice was used for each time interval. The normal L-asparagine concentration (+) was obtained from a control group given physiological saline.*

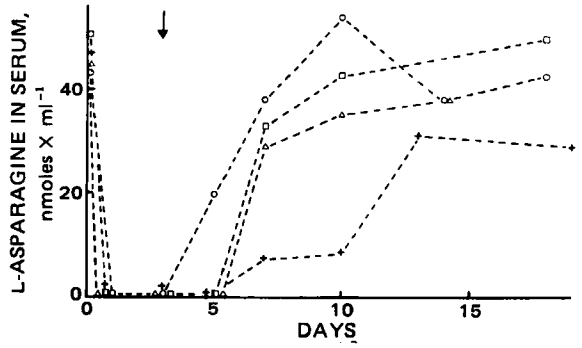

Figure 2—*L-Asparagine concentration in serum after intramuscular injection of L-asparaginase in free form, 5 IU/animal (○), 20 IU/animal (△), 2 × 2.5 IU/animal (□) and 2 × 2.5 IU/tumor-bearing animal (+). In the infected animals, 6C3HED cells (1 × 10⁶) were injected 4 days prior to the first injection on day 0. The arrow shows the time for the second injection of 2.5 IU of L-asparaginase in free form. Pooled serum from five mice was used for each time interval.*

and to some extent of L-glutamine (4). However, the short biological half-life of the enzyme necessitates frequent administration, which often leads to antibody production and hypersensitivity (5). This and other side reactions prevent the prolonged use of L-asparaginase with a subsequent relapse of the tumor and the development of L-asparaginase-resistant tumors.

The stability of enzymes against thermal denaturation and proteolytic degradation is generally improved when they are immobilized or polymerized. L-Asparaginase has been used successfully in immobilized form *in vivo* to prolong the duration of action in monkeys (6), rats (7), rabbits (8), and mice (9–11). When the enzyme is immobilized in porous microparticles of polyacrylamide with a mean diameter of 0.2–0.5 μm, the enzymic characteristics are essentially retained and the effects on the L-asparagine level in plasma is prolonged after intraperitoneal administration in rats (12). When the L-asparaginase microparticles are embedded in a polyacrylic gel the duration of action was prolonged up to ~25 days after implantation in the rat (13).

The present study was undertaken to correlate the L-asparagine-depressing activity of immobilized L-asparaginase in microparticles with the inhibition of tumor growth in mice, as well as to ascertain the optimal route of administration and the minimal dose of the immobilized L-asparaginase, to keep the serum L-asparagine concentration at a low enough level to prevent tumor growth in mice.

EXPERIMENTAL

Materials—L-Asparaginase[1] (E.C. 3.5.1.1), aspartate aminotransferase[2] (E.C. 2.6.1.1) (83 U/mg) isolated from porcine heart, and malic dehydrogenase[2] (E.C. 1.1.1.37) (2000 U/ml) from pigeon breast muscle were used without further purification. Acrylamide[3], *N,N'*-methylenebisacrylamide[3], *N,N,N',N'*-tetramethylethylenediamine[2], α-ketoglutaric acid[2], nicotinamide adenine dinucleotide[2] (NADH, reduced form) L-asparagine monohydrate[4], dextran[5], Nessler's reagent[2] (ammonia color reagent), and other chemicals were of analytical grade. Male mice[6] weighing 20–25 g were used throughout. The cell line used was the Gardner lymphosarcoma 6C3HED. It was grown in ascites form and was propagated every 7th day in C3H mice with 1 × 10⁶ cells suspended in a balanced salt solution ($CaCl_2$ 0.14 g, NaCl 8 g, KCl 0.4 g, $MgSO_4 \times 7 H_2O$ 0.2 g, KH_2PO_4 0.06 g, and water to 1000 g, pH 6.9).

Preparation of Microparticles Containing L-Asparaginase—Microparticles[7] of polyacrylamide (TC = 8-25)[8] or polyacryldextran (DTC = 11-1-75)[8] containing L-asparaginase were prepared according to a reported method (12, 14). L-Asparaginase, corresponding to 10,000 IU, was dissolved with the acrylic monomers (acrylamide, *N,N'*-methylenebisacrylamide or acryldextran) in 5 ml of 0.005 *M* sodium phosphate buffer, pH 7.4. After addition of the catalyst, ammonium peroxydisulphate (100 μl of a solution of 0.5 g/ml), the solution was poured into 200 ml of a mixture consisting of toluene and chloroform (4:1) and 0.5 g of a detergent[9].

The mixture was homogenized to produce a water–oil emulsion. The homogenizer was removed and the emulsion slowly stirred with a magnetic stirrer. The polymerization was started by adding 1 ml of the accelerator *N,N,N',N'*-tetramethylethylenediamine to the emulsion. During the whole procedure, oxygen was excluded from the system by bubbling nitrogen gas through the emulsion.

The microparticles were isolated by centrifugation, washed several times with buffer, and suspended in physiological saline after the last washing. Under the conditions used, the polyacrylamide particles (TC = 8-25) had a mean diameter of 0.1–0.4 μm, and polyacryldextran particles (DTC = 11-1-75) were 0.2–1.2 μm in diameter.

Preparation of Polyacrylamide Gel for Implantation—Microparticles of polyacrylamide (TC = 8–25) containing L-asparaginase (10 or 20 IU) were embedded in 200–300 μl of a polyacrylamide gel (TC = 16-25) prepared in the usual way in a small perspex box (1 × 1 × 0.7 cm). The gel tablet formed was washed with buffer and physiological saline,

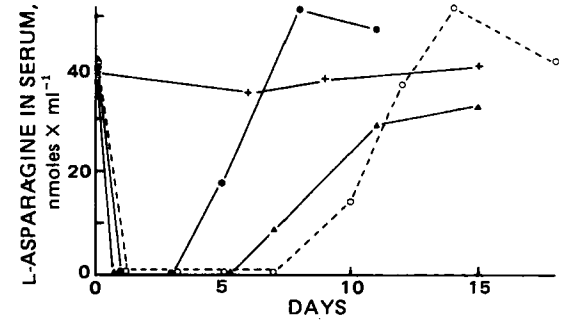

Figure 3—*L-Asparagine concentration in mice bearing the lymphosarcoma 6C3HED, administered intraperitoneally (1 × 10⁶ cells) 4 days prior to the start of the experiment. L-Asparaginase was injected intraperitoneally in free form (5 IU/mouse, ○) or in microparticles (5 IU/mouse, ●, 20 IU/mouse, ▲). A control group was given physiological saline (+). Each point is obtained from the pooled blood from 4 to 6 mice.*

[1] Asparaginase (Crasnitin) was obtained as a gift from Bayer (Sverige) AB, Stockholm.
[2] Sigma Chemical Co.
[3] Eastman Kodak Co.
[4] Merck Co.
[5] Dextran T40 (molecular weight: 40,000) was purchased from Pharmacia Fine Chemicals, Uppsala, Sweden.
[6] C3H mice, Bomholtsgård, Denmark.
[7] U.S. Pat. 4,061,466.
[8] The nomenclature is explained in a previous study (14).
[9] Pluronics F-68 (polyoxyethylene-derived polyoxypropylene) from Ugine Kuhlmann, Paris, France.

Table I—Antitumor Activity of L-Asparaginase Against 6C3HED Lymphoma [a]

Enzyme Preparation	Route of Administration	Survival Time, days	Mean Survival Time (± *SD*) or Median Survival Time (lower and upper quartile)	Treated 50-day Survivors
Control, particles	ip	10, 10, 10, 11	10.3 ± 0.5	0/4
Control, saline	ip	10, 10, 11, 11, 11, 11, 11, 12, 12, 13, 13, 13	11.5 ± 1.1	0/12
Microparticles, 5 IU	ip	14, 14, 15, 15, 15, 16, 16, 16, 16, 17, 18, 34	17.2 ± 5.4	0/12
Enzyme solution, 5 IU	ip	48, >50, >50, >50, >50, >50, >50, >50, >50, >50, >50,	>50	11/12
Microparticles, 20 IU	ip	16, 22, 44, 45, 50, 50, 50, >50, >50, >50, >50, >50	50 (44, >50)	5/12
Microparticles, (DTC = 11-1-75) 5 IU	ip	15, 15, 16, 17, 19, 35, 37, 47, 48, >50, >50, >50	37 (16, 48)	3/12
Microparticles[b], 5 IU + 6C3HED cells	ip	12, 13, 14, 16, 16, 16, 16, 16, 17, 17	15.3 ± 1.7	0/10
Enzyme solution[b], 5 IU + 6C3HED cells	ip	14, 15, 18, 18, 18, 32, 35, 42, 42	26.0 ± 11.7	0/9
Microparticles, 5 IU	im	11, 12, 14, 14, 14, >50, >50, >50	14 (12, >50)	3/8
Microparticles, 20 IU	im	31, 35, 38, 38, >50, >50, >50, >50, >50, >50, >50, >50	>50	8/12
Microparticles, 2 × 2.5 IU	im	31, 46, >50, >50, >50, >50, >50, >50, >50, >50, >50, >50	>50	10/12
Implant, 10 IU	sc	11, 11, 11, 12, 12, 12, 12, 13, 13, 13, 13, >50	12 (11, 13)	1/12
Implant, 20 IU	sc	10, 10, 10, 11, 11, 11, 12, 13, 14, 14, 14, 43	11.8 ± 1.7	0/12

[a] The mice were given 1 × 10^6 6C3HED cells ip, and on the 4th (or 4th and 7th) day after inoculation the mice were treated with different enzyme preparations by different routes. The survival time given is calculated from the day of inoculation. When not otherwise stated, the microparticles used in these experiments had the composition TC = 8–25. [b] The enzyme in free form or in microparticles was administered on the day of inoculation.

as earlier described (13), in order to free it from the remaining catalyst.

Assay of Native and Immobilized L-Asparaginase—L-Asparaginase activity was determined from the amount of ammonia produced by its reaction with the substrate L-asparagine at 37° (15). After the addition of Nessler's reagent, the absorbance was determined at 500 nm. Appropriate enzyme and substrate blanks were included in all assays. A standard curve was prepared with known amounts of ammonium sulphate.

Determination of blood L-asparagine was performed according to a fluorometric method (16). Blood was drawn each time from the tail vein or the orbital plexus from four to six mice. The blood samples were pooled and centrifuged. If not analyzed immediately the serum was frozen and stored at −18° until the analysis.

RESULTS

Serum L-Asparagine after L-Asparaginase Administration in Healthy and Tumor-Bearing Mice—After injection of 5 IU, ip of either immobilized or native L-asparaginase into the mice, serum L-asparagine dropped from a normal level of ~40–45 nmoles/ml to an undetectable level after 1 day and remained so for 4–5 days. Serum L-asparagine became normalized after days 6–7 as seen in Fig. 1.

Figure 2 illustrates the effects on the serum L-asparagine of native *L-asparaginase (2 × 2.5, 5, and 20 IU)* administered intramuscularly to healthy mice. A single injection of 5 IU im of the free enzyme lowered the serum L-asparagine level to zero and maintained it at zero for 3 days.

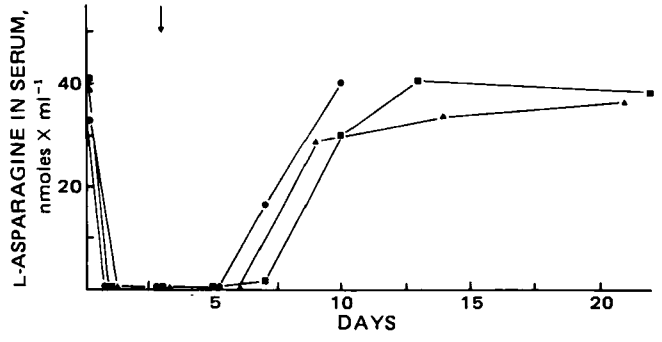

Figure 4—*Blood levels of L-asparagine in mice bearing 6C3HED cells (1 × 10^6 inoculated intraperitoneally 4 days in advance) after intramuscular injection of L-asparaginase in microparticles, 5 IU/animal (●), 20 IU/animal (▲), and 2 × 2.5 IU/animal (■). The arrow indicates the time for the second injection of 2.5 IU of L-asparaginase in microparticles.*

Injection of 2 × 2.5 IU and 20 IU of the free enzyme retained a zero L-asparagine level for up to 5 days. The effect on the serum L-asparagine of free L-asparaginase (2 × 2.5 IU) administered intramuscularly into mice bearing 6C3HED lymphoma is also shown in Fig. 2. The 6C3HED cells (1 × 10^6) were inoculated intraperitoneally 4 days prior to the first injection on day 0. The serum L-asparagine level was depressed to <10 nmoles/ml for 10 days due to the $t_{1/2}$-prolonging effect from the LDH-virus (lactate dehydrogenase-elevating virus) contamination of the cell line.

Figure 3 illustrates the effects on the serum L-asparagine of L-asparaginase (5 or 20 IU) administered intraperitoneally to mice bearing 6C3HED lymphoma. The 6C3HED cells (1 × 10^6) were inoculated intraperitoneally, and at day 4 after inoculation, native or immobilized L-asparaginase was injected intraperitoneally. After injection of the free enzyme, the L-asparagine level dropped from the normal value, was undetectable within 1 day, and remained so for 7 days. The serum L-asparagine level became normal after day 12, which means that the enzymic effect was significantly prolonged in the tumor-bearing mice, when compared with the effect seen in the healthy mice (Fig. 1). The same was seen after injection of 2 × 2.5 IU im as shown in Fig. 2. The prolonged effect is the result of the increased half-life of soluble L-asparaginase, which is caused by the LDH-virus contaminating the 6C3HED and other mouse lymphoma cell lines (4, 17). The increased $t_{1/2}$ of L-asparaginase is a secondary effect of the virus infection, and it is not seen when the 6C3HED cells are inoculated 1 hr after intraperitoneal injection of L-asparaginase (5 IU). In these animals the L-asparagine level followed the same time course as in noninfected mice, as shown in Fig. 1. Thus, the L-asparagine level was maintained at zero for only 5 days.

The therapeutic effect of L-asparaginase in tumor-infected mice is directly related to the duration of the enzymic effect—as will be discussed later—and thus to the $t_{1/2}$ value of the enzyme. In the mice infected with the 6C3HED cells on the same day, administration of L-asparaginase suppressed the growth rate of the lymphoma, but complete regression was not achieved. All the mice died after 14–50 days with grossly enlarged lymph nodes. However, when L-asparaginase was administered on day 4 after infection, 11 out of 12 mice were cured and survived 50 days as a result of the prolonged $t_{1/2}$ value of the enzyme. The results are summarized in Table I.

Immobilized L-asparaginase in microparticles administered intraperitoneally had the same effect on the L-asparagine level in mice bearing the 6C3HED lymphoma as in healthy mice (Figs. 1 and 3). Obviously, the LDH-virus contaminating the 6C3HED cells has no effect on the $t_{1/2}$ of immobilized L-asparaginase. Injection of physiological saline or microparticles without enzyme had no effect on the L-asparagine level.

Figure 4 illustrates the effects of microparticles with L-asparaginase administered intramuscularly in the scapular area of C3H mice bearing the lymphoma 6C3HED. L-Asparaginase (2 × 2.5, 5, and 20 IU) was in-

jected into 8–12 mice in three groups. After injection of 5 IU of immobilized L-asparaginase, normal serum values were obtained on days 8–9, whereas the normal level was reached on days 12–13 when 20 or 2 × 2.5 IU was administered. The results were the same in tumor-bearing and healthy mice. It is obvious that a prolonged effect on the plasma L-asparagine level is obtained by giving L-asparaginase in immobilized form intramuscularly, especially when the effects of 2 × 2.5 IU are compared in infected (Fig. 4) and healthy mice given free enzyme (Fig. 2).

The Growth-Inhibiting Effect of L-Asparaginase on Ascites Tumor—When studied in the ascites form, the tumor cells (1×10^6 cells) were generally given to groups of 12 C3H mice. After 4 days (or after 4 and 7 days), the mice were treated with different doses of L-asparaginase given by different routes or with saline or microparticles not containing any enzyme. Table I summarizes the survival times of differently treated groups. In untreated animals, the tumor grew rapidly and killed the hosts after 10–12 days. Immobilized L-asparaginase (5 IU ip given in microparticles) generally prolonged the survival time to 15–16 days. In a large dose (20 IU) or in polyacryldextran particles (from which enzyme can leak out) immobilized L-asparaginase had a longer effect and was able to cure five and three animals in respective groups.

As mentioned, the effect of native L-asparaginase is prolonged in infected animals and most of the mice (11 out of 12) were cured by 5 IU ip administered as shown in Table I for comparison. As expected and already discussed, the same dose did not cure the animals when the tumor was inoculated concomitantly with the administration of native L-asparaginase. These results are in complete accordance with the effect on the serum L-asparagine level shown in Figs. 1 and 3.

The tumor growth-inhibiting effect of immobilized L-asparaginase is improved when administered intramuscularly. The survival time of the different groups is shown in Table I. Three mice out of eight were cured when 5 IU of immobilized L-asparaginase was given intramuscularly. When 20 IU or 2 × 2.5 IU was administered, 8 and 10 animals, respectively, out of 12 survived for several weeks and were considered to be cured.

Intraperitoneal injection of acryldextran particles (DTC = 11-1-75) with L-asparaginase (5 IU) to C3H mice with 6C3HED cells, prolonged the survival time significantly compared with controls. Figure 5 and Table I summarize the results. After injection of particles with enzyme, normal serum values were obtained on days 9–10.

The Effect on Ascites Tumor by Implantation of L-Asparaginase in Polyacrylamide—L-Asparaginase in microparticles (TC = 8-25) was incorporated into a polyacrylamide gel (TC = 16-25). This gel, containing 10 or 20 IU of L-asparaginase, was then implanted subcutaneously on the backs of the mice.

The effect on the L-asparagine level is shown in Fig. 5. The serum concentration of L-asparagine fell from normal (40 nmoles/ml) to 18 nmoles/ml after 1 day and remained at this level during the rest of the experiment. On days 10–14 after inoculation of the cells (6–10 days after the implantation), all the animals died with one exception. This animal survived for several months and was considered to be cured. Table I summarizes the results. There were no significant differences between implants with 10 or 20 IU of L-asparaginase. These results indicate that it is necessary to depress the L-asparagine concentration at least to <10 nmoles/ml to obtain regression of the lymphoma.

DISCUSSION

It was shown earlier that immobilization of L-asparaginase significantly prolongs the depressive effect on the L-asparagine level in blood (6, 7, 9, 12). Potentially, an immobilized system would be of value to decrease the side effects seen when native L-asparaginase is used in high doses. The purpose of the present work was to correlate the effect on the L-asparagine level and the effect on an L-asparagine-glutamine-dependent lymphoma cell line. In addition, the purpose was to ascertain the optimal route of administration of the immobilized enzyme in microparticles and the minimal dose required to cure infected animals. The 6C3HED ascites tumor growing in C3H mice has been used, but it suffers from the disadvantage shared with other transplantable tumors growing in mice of contamination by the benign LDH-virus (14, 17). Unfortunately, this virus will, by some unknown mechanism, significantly increase the $t_{1/2}$ of native L-asparaginase. The duration of 5 IU L-asparaginase given intraperitoneally (measured by the period during which serum concentration of L-asparagine was undetectable) was increased from 5 to 7 days. However, the virus will have no effect on the duration of L-asparaginase in microparticles, which in essence is determined by the endocytic activity of the reticuloendothelial system (12). In an attempt to simulate the clinical situation, the effect of the enzyme was tested from day 4 after

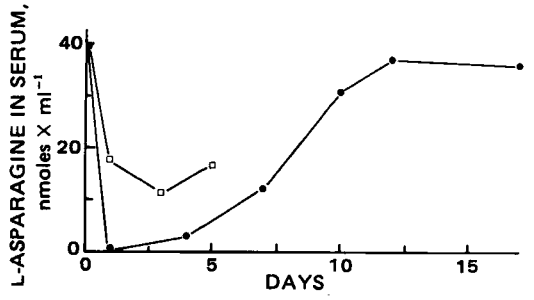

Figure 5—*L-Asparagine concentration in serum after a single intraperitoneal injection of L-asparaginase (5 IU/animal) in microparticles of polyacryldextran (●) or after implantation of 10 IU/animal sc on the backs of the mice (□). The mice were infected by the 6C3HED lymphoma, inoculated 4 days prior to the treatment. Pooled blood from 4 to 6 animals was used for each point.*

inoculation, when the virus already was sufficiently active and the number of tumor cells had significantly increased. This means that evaluation of the effect of the immobilized L-asparaginase unfortunately must be based on the comparison with the effect on the L-asparagine level obtained by giving the native enzyme on the inoculation day (before the cancer has proliferated in the host or in healthy mice).

When administered intraperitoneally, the efficiency of L-asparaginase immobilized in microparticles is equivalent to that of free enzyme, as depicted in Fig. 1. However, intramuscular administration clearly shows that the enzyme in immobilized form had longer L-asparagine-depressing activity than in free form (compare Figs. 2 and 4).

Within some limits the therapeutic effects of L-asparaginase in humans are dose related (3). The primary parameter of this effect, is the L-asparagine serum concentration, which has to be depressed to very low levels in order to prevent the growth of the tumor. We found that this level has to be <10 nmoles/ml in the C3H mice, while the normal value is ~40–45 nmoles/ml. Moreover, it is important that the L-asparagine concentration be kept at this very low level for at least 6–7 days to obtain complete regression of the tumor in the mice (measured as the survival time of 50 days). When the L-asparagine concentration was depressed to 0 values for only 5 days, the cancer growth generally recurred and the mice died within 2–3 weeks.

L-Asparaginase is very stably bound in the porous polyacrylamide microparticles and can therefore not interact directly with the tumor cells. It is obvious that the therapeutic effect of L-asparaginase is mediated *via* its L-asparagine-depressing activity.

In this study, the best L-asparagine-depressing effects were obtained with L-asparaginase in microparticles given intramuscularly. Table I, shows that the groups treated with immobilized enzyme contain more 50-day survivors than those treated with the same doses intraperitoneally. Particles administered intraperitoneally are rapidly cleared by the reticuloendothelial system and stored in the lysosomal vacuomes (18). The enzyme is thus secluded from the systemic circulation and cannot affect the L-asparagine level. The same will happen with microparticles injected intravenously (18), and to date no intravenous studies have been performed. When larger polyacryldextran particles were used intraperitoneally, the systemic effect lasted a little longer than with small polyacrylamide particles, which probably was due to enzyme leaking out from the polyacryldextran particles prior to their phagocytosis. It was shown earlier (14) that proteins immobilized in biodegradable polyacryldextran are not stably bound in such particles.

Microparticles given intramuscularly are eventually taken up by invading macrophages and/or encapsulated by fibroblasts, which will prevent further effects on the systemic L-asparagine level of the immobilized enzyme. In the present case a small dose (2.5 IU im) was administered. Although such a small dose was effective, the duration was limited by the phagocytosis of particles; this was circumvented by giving a second small dose after an interval of 3 days. Such a regimen was shown to be significantly more effective than one single dose of the same amount of L-asparaginase. Ten out of 12 treated mice were completely cured and lived for several months.

In an evaluation of the optimal route of administration, the immunological properties of the immobilized enzyme should be considered. Preliminary results (19) indicate that L-asparaginase in microparticles given intramuscularly is less immunogenic than the native form of the enzyme as manifested by a lower antibody production in mice. In addition, no toxic effects have been detected after injection of moderate doses of microparticles in mice (20).

In summary, all the results indicate that the optimal effect of L-asparaginase is obtained when the enzyme is injected intramuscularly in immobilized form in small doses.

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Acrylic Microspheres *In Vivo* VII: Morphological Studies on Mice and Cultured Macrophages

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Abstract □ Intravenously injected microparticles of polyacrylamide were cleared from the circulatory system in mice predominantly in liver, spleen, and bone marrow in mice by macrophages belonging to the reticuloendothelial system. In these cells, particles were found in dilated secondary lysosomes. The lysosomotropic character of the particles was further demonstrated using cultured peritoneal mouse macrophages. Histological changes of the liver, spleen, and bone marrow detectable by light and electron microscopy could only be seen after administration of massive doses of microparticles corresponding to 160 mg/kg body weight. In such cases, a medium-coarse vacuolization of liver parenchymal cells could be seen 1–2 days after particle administration. After 3–5 days, degeneration and necrotic cellular alteration occurred in the liver, spleen, and bone marrow. One week after particle administration, regeneration started under formation of granulomas which replaced the necrotic areas. The tissues later became normalized (after 2–3 weeks), but small granulomas remained for several weeks. The damage was parallelled by changes in the liver and spleen weights. Electron microscopy of the liver revealed that the initiated vacuolization of the parenchymal cells was due to mitochondrial swelling with rupture of the mitochondrial cristae.

Keyphrases □ Acrylic microspheres—*in vivo*, morphological studies on mice and cultured macrophages, polyacrylamide, liver, spleen, marrow □ Macrophages, cultured—acrylic microspheres, *in vivo*, morphological studies on mice, polyacrylamide, liver, spleen, marrow □ Polyacrylamide—acrylic microspheres, *in vivo*, morphological studies on mice and cultured macrophages, liver, spleen, marrow

Microparticles of highly cross-linked polyacrylamide, or derivatives, have recently been introduced as a slowly degradable carrier system for immobilized enzymes *in vivo* (1). Such particles have a pronounced porous structure allowing substrates to freely penetrate the particles and interact with the immobilized enzymes. They are small enough (mean diameter 0.1–0.4 μm) to be injected intravenously without causing respiratory complications. After intravenous or intraperitoneal injection, the microparticles are taken up by cells of the reticuloendothelial system, essentially in the liver and spleen, where they are probably localized in the lysosomal vacuome, although no direct evidence has yet been presented. Microparticles with immobilized L-asparaginase have been used to depress the level of circulating L-asparagine with resulting growth inhibition of the ascites tumor 6C3HED in mice (2). In addition, particles containing dextranase have successfully been used to treat an artificial storage disease in mice, which is produced by polyacryldextran (3).

In all studies performed to date, no signs of acute toxicity have been detected. Thus, the growth rate and survival time have been unaffected, and no tissue incompatibility has been noticed, besides the normal encapsulation of implants or intramuscularly injected microparticles. Very large doses of microparticles (100–200 mg/kg of body weight), however, have produced a transient hepatosplenomegaly in mice, lasting 4–8 weeks, resulting from the localization of the spheres in the liver and spleen. The purpose of the present work was to study microscopically the possible morphological alterations of several organs following the administration of such provocative doses of

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