dose. No significant increase in the inhibitory effect was obtained at higher doses. No inhibition in PFC number and HA titre was observed in mice which were treated with LE beginning shortly before or after immunization.

Similar results were obtained with other B cell mitogens by Diamantstein et al.[9]. These authors suggest that stimulation of antigen-sensitive cells by B-cell mitogens a few days before immunization may lead to formation of a cell population which has lost its ability to react with antigens.

We propose 2 other possibilities for explaining the different effects of levan administered 24–48 h before antigenic stimulation and the effect of levan administration just before or after the antigen injection. a) LE injected before the antigen may diminish the antigen's availability to the immune system by decreasing the permeability of blood vessels[10]. b) LE injected before the antigen may block the RES (reticuloendothelial system), thereby preventing antigen processing. LE administered around the time of antigenic stimulation cannot block in time the already started antigen processing. Levan is known to have a variety of effects on macrophages[4,11,12], and these cells were shown to be responsible for some differences in immune response between high and low responder strains of mice[13].

The use of LE in the treatment of various pathological processes requires in some cases stimulatory, in others inhibitory effects on different immunological reactions. In the graft rejection process, the aim is to preserve the grafted tissue, while in tumor therapy the aim is to destroy the neoplastic cells. During the development of tumors, some immunologic reactions enhance, while others inhibit growth of the neoplasm. Studies of our group demonstrated that levan treatment inhibited tumor growth only when the daily injections were started within a few days after tumor-cell inoculation. Treatment with LE begun even 1 day before the tumor inoculation had an enhancing effect[14]. Similar effects were also noted with other immunomodulatory agents[15]. Whether the dependence of antitumoral activity on the time at which LE treatment is started, and the effect on antibody production of the period at which LE is given, are due to the same mechanism, is not clear.

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Immunoadjuvant activity of synthetic N-acetyl muramyl dipeptide

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Summary. The administration of phosphatidyl choline/cholesterol liposomes with entrapped N-acetyl muramyl dipeptide induced in guinea-pigs the development of delayed hypersensitivity to ovalbumin.

Several decades ago Freund and McDermott[1] discovered the immunoadjuvant property of mycobacteria with water-in-oil emulsion. The search for adjuvant active component of mycobacteria led first to the isolation of peptidoglycolipid fraction called Wax D[2,3]. Soon it became clear that the lipid is not essential for the observed activity, and water-soluble preparation of glycopeptide replaced mycobacteria in the Freund complete adjuvant for stimulation of delayed hypersensitivity[4–6]. Several groups independently then discovered that the minimal structure which is required for immunoadjuvant activity is N-acetyl muramyl L-alanyl-D-isoglutamine[7,8].

Chedid et al.[9] recently reported that some synthetic glycopeptides are even able to increase the humoral immune response when given in aqueous media instead of the usual water-in-oil emulsion. This, however, has not been observed so far for the development of cell mediated immunity (delayed hypersensitivity) where water-in-oil emulsion is needed to note the effect. The mineral oils used in Freund's adjuvants are not biodegradable, and they can be thus used only in the experiments on animals. Liposomes, phospholipid vesicles consisting of one or more lipid layers, appear to be promising as carriers of antigens to immunocompetent cells. Several recent studies have reported that liposomes increase the antibody response to a variety of protein antigens[10,11]. The main objective of the present study was to elucidate whether the liposomes can substitute mineral oils

Immunoadjuvant activity of N-acetyl-muramyl dipeptide

Compound tested	Dose	Delayed hypersensitivity
Ac-Mur-L-Ala-D-Glu-NH_2	200 µg	7.94
Phosphatidyl choline-cholesterol (8:2)		8.86
Pch-Ch + Ac-Mur-L-Ala-D-Glu-NH_2	200 µg	12.75*
FIA + Ac-Mur-L-Ala-D-Glu-NH_2	200 µg	13.82*
Freund's complete adjuvant (FCA)	1000 µg	13.58*
Freund's incomplete adjuvant (FIA)		7.34

Values represent the mean values for group of 8–10 guinea-pigs.
* Statistically significant results; $p < 0.05$.

in glycopeptide-induced delayed hypersensitivity of guinea-pigs to ovalbumin.

Material and methods. The synthetic N-acetyl muramyl L-alanyl-D-isoglutamine was prepared by the method to be published elsewhere[12]. Liposomes were prepared by conventional method. Mixture of phosphatidyl choline and cholesterol in molar ratio 8:2 were dissolved in chloroform and dried onto the surface of a flask, then saline solution of muramyl dipeptide was added. The mixture, after mechanical agitation which facilitate fragmentation of the miccelas, was sonicated for 30 min at a frequency of about 40 kHz. The immunoadjuvant activity was assayed on female albino guinea-pig injected into left hind foot-pad with 0.2 ml of ovalbumine-liposomes mixture, ovalbumine-glycopeptide mixture or ovalbumine-glycopeptide-liposomes mixture. For comparison, 2 groups of guinea-pigs were injected with a mixture of ovalbumine and either Freund's complete or incomplete adjuvant. 3 weeks later, the guinea-pigs were given an injection of 20 µg ovalbumine and the reactions 24 h later were read and graded from zero to a full intensity.

Results and discussion. From the data summarized in the table, it is evident that the administration of phosphatidyl choline-cholesterol liposomes with entrapped glycopeptide produced more pronounced delayed hypersensitivity to ovalbumin in guinea-pig than the administration of glycopeptide alone. The effect was comparable with the effect of Freund's complete adjuvants, or with the effect of mixture of glycopeptide with Freund's incomplete adjuvant (Bayol and Arlacel 4:1). Phosphatidyl choline-cholesterol mixture could thus substitute mineral oils of Freund's incomplete adjuvants in mixture with synthetic N-acetyl muramyl L-alanyl-D-isoglutamine for induction of cell-mediated immunity. This finding is not surprising since liposomes appear to be a good vehicle for transport of antigen preparation[13]. The exact role of liposomes in glycopeptide-induced delayed hypersensitivity is so far not clear. Recent morphological studies have established that liposomes are primarily phagocyted by macrophage system[14,15], where lysosomal system disrupts them to free entrapped material. The important role of activated macrophages in delayed hypersensitivity is very well known.

Our findings are in certain contrast with the results of Allison and Gregoriadis[10], who reported that in mice liposomes with diphteria toxoid did not cause the development of delayed hypersensitivity. The apparent reason for the discrepancy might be in the different experimental design and methods used for testing the delayed hypersensitivity. Our findings that Freund's incomplete adjuvant may be substituted by phosphatidyl choline-cholesterol liposomes might be of clinical importance, because of the great demand for compounds which would potentiate cell-mediated immunity.

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Effect of diethylstilboesterol diphosphate on tumour-associated immunity in prostatic cancer. A preliminary report[1]

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Summary. In continuing studies of the effects of oestrogen on immunologic responsiveness, preliminary evidence of significant suppression of tumour-associated immunity in patients with prostatic cancer has been observed.

The androgenic dependence of prostatic cancer and its treatment by androgen depleting therapy by the administration of oestrogen has been well documented since the classical studies of Huggins and Hodges[3]. However, attempts to delineate the potential effects of such therapy on the immunological responsiveness of the host to malignancy have only recently been made.

Following the suggestion by Ablin[4] that palliative hormonal therapy in patients with advanced breast or prostatic carcinoma may reduce the surveillance efficiency of their immunologic system, the effects of oestrogen on the in vitro reactivity of thymic dependent lymphocytes, as 1 parameter of the effects of oestrogen on immunological responsiveness were investigated. The results of these initial studies demonstrated significant suppression of the blastogenic response of normal human peripheral blood lymphocytes (PBL) to stimulation with phytohaemagglutinin (PHA) when the PBL were cultured in the presence of exogenous oestrogen (diethylstilboesterol diphosphate (DES-P))[5,6]. Suppression of PHA-induced blastogenesis was similarly observed after oestrogen therapy, compared with before, when PBL from patients with prostatic cancer were cultured in autologous serum[5,7].

In a logical extension of these initial studies, the effect of