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

exogenous oestrogen on tumour-associated immunity (TAI) in patients with prostatic cancer[8-10] has been evaluated and as such is the subject of this preliminary communication.

Ficoll-Paque (Pharmacia Fine Chemicals, Uppsala, Sweden) gradient separated peripheral blood leukocytes were obtained from 11 patients with a confirmed histological diagnosis of adenocarcinoma of the prostate, ranging in age from 57 to 82 years. Peripheral blood leukocytes at a concentration of 1×10^7 cells/ml in RPMI 1640 medium (Grand Island Biological Company, Grand Island, New York) containing 100 IU penicillin g/ml and 100 µg streptomycin/ml, untreated and treated with 20 µg/ml DES-P (Dome Laboratories, West Haven, Connecticut, Lot No. 813110), determined as the optimal inhibitory dosage from a dose-response curve[11], were incubated at 37 °C for 50 min in a mixture of 5% CO_2 in air. After incubation, cells were washed twice in RPMI 1640 medium and viability assessed by trypan-blue dye exclusion. Employing a modification[12] of the tube antigen-induced leukocyte adherence inhibition method of Grosser and Thomson[13], untreated and treated patients's leukocytes were reacted with 3M KCl-$(NH_4)_2SO_4$ extracts of allogeneic malignant prostatic tissue[12]; and the number of non-adherent cells counted in quadruplicate using a Standard Neubauer haemocytometer.

The effect of DES-P on TAI to allogeneic extracts of malignant prostate in 11 patients with prostatic cancer is shown in the table. Comparison of the significance of the difference in responsiveness of the patients' leukocytes untreated and treated with DES-P indicated a highly significant difference ($p < 0.02$). That this observed suppression of TAI in the presence of DES-P was not due to a cytotoxic effect of DES-P on the cells was shown by the observation that the viability (as determined by trypan-blue dye exclusion) of leukocytes incubated for 50 min in culture medium alone and that containing 20 µg/ml DES-P was essentially identical.

These preliminary observations demonstrate a significant suppressive effect of DES-P on TAI in prostatic cancer patients and are in consonance with earlier observations of suppression of PHA-induced lymphocytic blastogenesis. Suppression of in vitro cellular responsiveness by oestrogen, particularly TAI, raises further concern of the efficacy of oestrogenic therapy (as recently considered by Ablin[14]) for prostatic cancer.

Effect of diethylstilboesterol diphosphate (DES-P) on tumour-associated immunity in patients with prostatic cancer

Peripheral blood leukocytes	Nonadherent cells obtained with extract of allogeneic malignant prostate (mean ± SD %)	Significance
Untreated	30.9 ± 11.6	
Treated with 20 µg/ml DES-P	17.1 ± 8.8	$p < 0.02$

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Distribution of CSF (colony stimulating factor) in kidney of the mouse

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Summary. The activity of the colony stimulating factor (CSF) was measured in kidney subcellular fractions in mice. The highest activity was noted in microsomes. From other fractions, the cytosol had large amounts of CSF. On the basis of literature data, and the findings presented we suggest that the kidney is at least one of the organs of CSF biosynthesis.

The activity of colony stimulating factor (CSF) was found in many tissues, e.g. liver, lung, kidney[1-4]. On the other hand, kidney tissue was used as a feeder layer in agar culture of granulocytes and macrophages colonies[4]. In the literature, there is information that the kidney plays an important role as an endocrine organ secreting erythropoietin, thrombopoietin. Recently, some authors indicated the possibility of a common origin in both CSF and erythropoietin[5]. So we have decided to test this organ for CSF activity in the subcellular fractions, which may support the assumption of the biosynthesis of this factor in the kidney.

Material and methods. The experiments were carried out on kidneys obtained from 9 Swiss mice. The kidneys were fractionated according to Ali and Lack[6]. In accordance with their method, the determination of the activity of acid phosphatase[7] and cathepsin D activity[8] was checked.

CSF activity was tested according to Bradley and Metcalf[4], and expressed as the number of colonies of granulocytes/macrophages per mg of protein. Mice bone marrow cells were used at $1 \cdot 10^5$ cells/plate. Each sample was tested on 3 plates, so that each result is the mean value of 3 experiments.