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.

Results and discussion. The table shows that the highest activity of CSF is localized in microsomes, lower activity was found in cytosol, but mitochondria and lysosomes had less activity than homogenate of the whole kidney. The only traces of activity were noted in the nuclei and membranes. The main part of CSF found in microsomes may be logically explained by the fact that in these structures protein biosynthesis takes place. Higher activity of CSF in cytosol is probably connected with a transportation route from the microsomes to out cell compartment. The other subcellular fraction, and mitochondria and lysosomes, containing this activity may be due to contamination by fractions rich in CSF or by absorption of the protein. From the literature on the presence of large amounts of CSF in urine, and the findings presented may be further evidence that the kidney is the organ of biosynthesis of CSF.

Activity of CSF in kidney subcellular fractions of mice

Fractions	Colonies/mg of protein
Homogenate	60 (49-70)
Nuclei and membranes	5 (4-9)
Mitochondria	51 (39-80)
Lysosomes	43 (32-57)
Microsomes	139 (125-160)
Cytosol	67 (52-96)

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Evidence for in vivo protection against a rat sarcoma by allogeneic spleen lymphocytes[1,2]

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Summary. The protective effect of normal allogeneic spleen cells against the growth of a transplantable rat sarcoma was studied.

The transfer of allogeneic lymphoid cells exerts a stimulating action on the immunologic system of the recipient animal[3-10]. This action, termed 'allogeneic effect', involves the development of a specific graft-versus-host reaction in the lympoid organs of the host[3].

At the same time, a rejection response is produced by the recipient. It has been established that this effect is the result of a specific immunologic influence of the donor cells on either the host's B or T cells[3,4,11].

In a previous study, it was observed that pretreatment with allogeneic spleen cells contained in diffusion chambers inserted i.p. in the recipients significantly diminished the number of succesful ovarian allografts (7/24; 29.1%) in contrast to controls not receiving spleen cells (23/32; 71.8%; $\rho < 0.01$) (unpublished data). In the present experiment, the effect of enhancing the immune system by allogeneic cells has been used in a rat tumour system.

Materials and methods. Adult inbred rats of both sexes (strain b)[12] were used as hosts. Normal spleen and kidney cells were obtained from another inbred strain (strain l). A spontaneous transplantable sarcoma (sarcoma E 100)[13] maintained by successive passages in several strains since 1955 s.c., (in this case in strain l) was used as a source of tumor cells. This tumour is an encapsulated fibrosarcoma giving no metastasis and leading to the animal's death 30-40 days after grafting.

Several cell suspensions were prepared: a) alogeneic spleen cells were obtained by gently teasing the fresh, whole organ in a Potter-Elvehjem flask with Hanks-Simms solution. The suspension was centrifuged at $120 \times g$ for 15 min, the supernatant was discarded and cells were resuspended in Hanks-Simms medium. This operation was repeated twice, then the viable cell number was determined; b) tumor cells were obtained by trypsinizing fresh pieces of tissue (Trypsin Difco 1:250) in sterile conditions with Hanks-Simms solution; c) allogeneic kidney cells were obtained in the same manner as tumor cells from fresh, whole organ of rats from strain l. All cell suspensions were adjusted to a concentration of 7.5×10^6/ml. Cellular viability was determined in every case by the trypan blue dye exclusion test.

Suspensions of 1.5×10^6 cells in 0.2 ml of Hanks-Simms solution were put into a diffusion chamber. This consisted of a plastic (lucite) ring covered on each side with a Sartorius filter of 0.2 µM pore size. The diffusion chambers containing the cells were introduced through an abdominal incision into the peritoneal cavity of the rats under ether anesthesia.

Rats were divided into 4 experimental groups with diffusion chambers containing either: 1. allogeneic spleen cells; 2. sarcoma E 100 cells; 3. allogeneic kidney cells (as non-immunological control cells); 4. Hanks-Simms medium.

Size of sarcoma E 100 at 30 days after challenge in diffusion chamber bearing rats

Diffusion chamber containing	$\bar{x} \pm SE$	P
Allogeneic spleen cells	654 ± 102	
	$n = 8$	< 0.01
Sarcoma E 100 cells	906 ± 162	
	$n = 12$	NS
Kidney allogeneic cells	806 ± 215	
	$n = 12$	NS
Hanks-Simms solution (controls)	1301 ± 235	
	$n = 13$	

$\bar{x} \pm SE$: Mean tumor size expressed in $mm^2 \pm SE$. n, number of rats. Each group was compared wit the controls. P, value was obtained by Student's t-test. NS, not significant.