treatment of tumor cells. However, an indication of potential antitumor activity of a drug can thus be demonstrated, especially if the activity is weak. The systemic antitumor activity of the drugs would be revealed by inhibition of growth of solid tumors, especially when treatment is initiated early, 24 h after tumor implantation. The antigenicity of the tumors toward the hosts used are not known; to determine these for the exploratory work carried out here would not be feasible. Except for our inexplicable 30-day survivor (this might be due to accidental s.c. rather than i.p. inoculation since a solid tumor but no ascites was observed in this control mouse at autopsy on day 30) in the case of Ehrlich ascites, not seen before in our laboratories[14], the tumors in control mice grew progressively to kill the host. Such continuous growth suggest a low antigenicity for these tumors in the present situation. In addition, the reported suppressive activity of cell-mediated immunity by the cyclosporins should favor tumor growth rather than tumor regression. Therefore, the antitumor activity seen appears real. Discrepancies in the response of solid versus ascites tumors to the same agents as observed in these experiments are not new and have been reported earlier for other antitumor agents[15,16]. Resorption, distribution and rapid metabolism may be responsible for this phenomenon.

In those experimental test systems which responded to treatment, the comparison of the 2 drugs reveals similar efficacy; although in some, but not all instances, CyC was somewhat more effective. In S180J the use of 0.5% carboxymethyl cellulose resulted in substantial loss of therapeutic effect, while toxicity in the form of weight loss was comparable to the ethanol-Tween 80-saline group.

In L1210 the use of divided doses of CyA and CyC (table) (one-half each of the dosage administered in the morning and afternoon, respectively) did not improve the antitumor effect but was less toxic to the host. The results obtained in this initial screening program are encouraging and justify further work with this new group of compounds.

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3 J.F. Borel, A. Rüegger and H. Stähelin, Experientia *32*, 777 (1976).
4 A. Rüegger and A. von Wartburg, Helv. chim. Acta *59*, 1075 (1976).
5 R. Traber, M. Kuhn, A. Rüegger, H. Lichti, H-R. Loosli and A. von Wartburg, Helv. chim. Acta *60*, 1247 (1977).
6 R. Traber, M. Kuhn, H-R. Loosli, W. Pache and A. von Wartburg, Helv. chim. Acta *60*, 1568 (1977).
7 M. Dreyfuss, E. Härri, H. Hofmann, H. Kovel, W. Pache and H. Tscherter. Eur. J. appl. Microbiol. *3*, 125 (1976).
8 T.F. Borel, C. Feurer, H.U. Gubler and H. Stähelin, Agents Actions *6*, 468 (1976).
9 R.Y. Calne, S. Thiru, P. McMaster, G.N. Craddock, D.J.G. White, D.B. Evans, D.C. Dunn, B.D. Pentlow and K. Rolles, Lancet *2*, 1323 (1978).
10 R.L. Powles, H. Clink, J. Sloane, A.J. Barrett, H.E.M. Kay and T.J. McElwain, Lancet *2*, 1327 (1978).
11 J.F. Borel, C. Feurer, C. Maguée and H. Stähelin, Immunology *22*, 1017 (1977).
12 J.F. Borel and D. Wiesinger, Proc. 11th Leukocyte Culture Conf., p.716. Academic Press, New York, San Francisco, London 1977.
13 J. Chihara, J. Hamuro, Y.Y. Maeda, Y. Arai and F. Fukuoka, Cancer Res. *30*, 2776 (1970).
14 G.S. Tarnowski, I.M. Mountain and C.C. Stock, Cancer Res. *33*, 1885 (1973).
15 W. Kreis, N.Y. Acad. Sci. *255*, 244 (1975).
16 I. Wodinsky, P.C. Merker and J.M. Venditti, J. nat. Cancer Inst. *59*, 405 (1977).

The effects of decreased glucose concentrations on the in vitro development of the post-blastocyst mouse embryo in a fetal calf serum- or bovine serum albumin-supplemented medium

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Summary. Decreasing the glucose concentration from 1.0 mg/ml to 0.25 mg/ml has no detrimental effects on post-blastocyst embryo development when either dialyzed fetal calf serum (20%) or bovine serum albumin (4.0 mg/ml) is used to supplement Eagle's Basal Medium (BME). Development is reduced in both serum- and BSA-supplemented BME devoid of glucose in comparison to glucose controls. Serum-supplemented media support better overall development than BSA-supplemented media.

Successful in vitro culture of post-blastocyst mouse embryos to the early somite stage has been accomplished[2,3]. Numerous factors influence the successful culture of these embryos and opinions differ as to which medium is optimal for studying post-blastocyst embryo development[4-6]. The specific growth factors contained within the milieu of serum that usually supplements a particular chemically defined medium are only slowly becoming apparent[7,8]. In our laboratory, fetal calf serum-supplemented Eagle's Basal Medium (BME) has proven optimal for blastocyst culture[9]. Supplementation of BME with crystalline bovine serum albumin (BSA) has also been useful in some studies[7]. Information regarding carbohydrate requirements and metabolism during the period of post-blastocyst development has been limited. In vitro hatching, attachment, and outgrowth of mouse embryos are influenced by media glucose concentrations[10,11]. The objective of the present study is to observe the effects of reduced glucose levels on the in vitro development of the post-blastocyst mouse embryo in dialyzed fetal calf serum- and bovine serum albumin-supplemented BME.

Mouse blastocysts were cultured in either dialyzed fetal calf serum- or BSA-supplemented BME containing various concentrations of glucose. BME devoid of a carbohydrate energy substrate was prepared from commercially available amino acid, vitamin, and phenol red concentrates, and a laboratory-prepared Earle's balanced salt solution[12] supplemented with antibiotics. Prior to the addition of glucose, 1 volume of BME was supplemented with 20% dialyzed fetal calf serum which had been previously heat-inactivated at 56 °C for 0.5 h. Dialyzed fetal calf serum (dFCS) was chosen as a medium supplement in order to minimize the presence of small carbon moieties[10]. Analysis of both the BME and dFCS indicated no detectable levels of glucose. A 2nd volume of BME was supplemented with 4.0 mg/ml BSA. BSA was chosen as a supplement to maintain an

Table 1. Effects of reduced glucose levels on embryo development in serum-supplemented media*

Glucose concentration (mg/ml)	Hatching from the zona pellucida (%)	Attachment to the collagen substratum (%)	Trophoblast cell outgrowth (%)	Differentiation to the egg cylinder stage (%)
1.00 (control)	89/118 (75)	88/ 89 (99)	88/88 (100)	16/88 (18)
0.75	95/118 (81)	94/ 95 (99)	92/94 (98)	17/92 (18)
0.50	84/117 (72)	81/ 84 (96)	81/81 (100)	14/81 (17)
0.25	93/120 (78)	92/ 93 (99)	92/92 (100)	13/92 (14)
0.00	112/123 (91)[b]	41/112 (37)[b]	18/41 (44)[b]	0/18 (0)[a]

* Embryos cultured in Eagle's Basal Medium (BME) supplemented with 20% dialyzed fetal calf serum. Each glucose concentration was replicated 5–6 times. Percentages reported at 120 h in vitro with superscripts a ($p < 0.05$) or b ($p < 0.01$) are significant in comparison to control glucose level using a statistical analysis which tests a hypothesis between population proportions.

Table 2. Effects of reduced glucose levels on embryo development in bovine serum albumin-supplemented media*

Glucose concentration (mg/ml)	Hatching from the zona pellucida (%)	Attachment to the collagen substratum (%)	Trophoblast cell outgrowth (%)	Differentiation to the egg cylinder stage (%)
1.00 (control)	84/110 (76)	6/84 (7)	4/6 (67)	0/4 (0)
0.75	71/ 96 (74)	6/71 (8)	4/6 (67)	0/4 (0)
0.50	74/100 (74)	3/74 (4)	0/3 (0)	-
0.25	90/109 (83)	6/90 (7)	4/6 (67)	0/4 (0)
0.00	20/103 (19)[b]	0/20 (0)	-	-

* Embryos cultured in Eagle's Basal Medium (BME) supplemented with 4.0 mg/ml bovine serum albumin (BSA). Each glucose concentration was replicated 6–8 times. Percentage reported at 120 h in vitro with a superscript b ($p < 0.01$) is significant in comparison to control glucose level using a statistical analysis which tests a hypothesis between population proportions.

essentially chemically defined medium[7]. Glucose was dissolved in separate aliquots of dFCS- or BSA-supplemented BME to yield final concentrations of 1.00 (control), 0.75, 0.50, 0.25, or 0.00 mg/ml.

8–12-week-old random bred female Swiss albino mice were superovulated according to the method of Gates[13]. Immediately following human chorionic gonadotropin (HCG) injection, the animals were caged overnight with fertile males and examined the next morning for the presence of copulatory plugs which indicated mating. Approximately 90 h post-HCG injection, mated females were sacrificed by cervical dislocation and blastocysts were flushed from each excised uterus with approximately 0.3 ml of sterile glucose-free BME which had been previously gas-equilibrated with 5% CO_2 in air and warmed to 37 °C. Uniformly developed embryos were pooled and transferred through 3 successive drops of glucose-free medium. Approximately equal numbers of blastocysts were allotted to 30 × 10 mm sterile plastic Petri dishes containing 1.0 ml of experimental or control culture medium. Prior to the introduction of the embryos, the culture dishes were coated with a layer of reconstituted rat-tail collagen[3,14] and the media overlayed with 1.5 ml sterile paraffin oil and allowed to gas-equilibrate with a 5% CO_2 in air atmosphere at 37 °C. Culture dishes with embryos were placed into a 37 °C humidified incubator chamber in 5% CO_2 in air and left undisturbed for 72 h.

At intervals of 72, 96, and 120 h in vitro, mouse embryos were observed and scored with regard to the degree of development. Developmental stages included complete hatching from the zona pellucida, attachment of hatched blastocysts to the collagen substratum, trophoblastic cell outgrowth of attached blastocysts, and differentiation of the inner cell mass of attached embryos into an outer endoderm and inner ectoderm layer. Embryonic development for each observed event was expressed as the ratio of embryos that reached a particular stage of development to the total number of embryos that possessed the potential to reach that stage[11].

Mouse blastocysts were observed to hatch from the zona pellucida, attach to the collagen substratum, exhibit trophoblast cell outgrowth, and differentiate into 2 germ layers in serum-supplemented BME as has been previously reported[5,11]. Table 1 shows no significant effects on developmental ratios when glucose levels were decreased from 1.00 to 0.25 mg/ml in serum-supplemented BME. However, significantly higher hatching and lower attachment, outgrowth, and differentiation ratios were observed in the glucose-free medium. The fact that embryos did develop to the outgrowth stage in glucose-free BME may indicate that the embryos were utilizing endogenous glycogen stores for glycolysis for a limited period of time[15,16]. The reason for the significantly higher hatching ratio in glucose-free BME is unclear. Variable hatching ratios have been previously obtained in glucose-free BME in which we employed different lots of dialyzed fetal calf serum (unpublished data). Trends in attachment, outgrowth, and differentiation in these same media have paralleled the results presented here.

The results reported in this study are in disagreement with a previous report by one of the authors (R.J.W.)[10]. In that study, a deleterious effect of decreased glucose concentrations was observed. However the experiments were not identical. In the previous study, Minimum Essential Medium (MEM) was utilized and the concentration of dFCS was 10%. Either or both of these components may have influenced developmental events[4,5].

As seen in table 2, there were no detrimental effects of decreasing media glucose concentrations from 1.00 to 0.25 mg/ml in BSA-supplemented BME. Only hatching, attachment, and outgrowth occurred in glucose-containing media and the ratios in the latter 2 events were markedly reduced in comparison to comparable serum-supplemented media. The lack of attachment and subsequent outgrowth of hatched blastocysts in BSA-supplemented BME could be due to the absence of serum-derived cell attachment and/or outgrowth promoting factors[8,17]. In addition, a deficiency in certain serum steroid hormones (i.e. progesterone) in the chemically defined media may be important[7]. Ozias and Weitlauf[18] have suggested that progesterone may have a role in metabolic activation of glycogenolysis in delayed-implanting blastocysts. Thus embryos cultured in a medium

devoid of both glucose and progesterone may not be able to mobilize enough of their own glycogen stores to supply energy for hatching and subsequent development. This could explain why hatching was markedly decreased and was the only developmental event occurring in glucose-free BSA-supplemented BME. Further investigation into the energy related mechanisms involved in post-blastocyst development is currently being undertaken.

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2 Y.C. Hsu, Devl Biol. *33*, 403 (1973).
3 Y.C. Hsu, J. Baskar, L.C. Stevens and J.E. Rash, J. Embryol. exp. Morph. *31*, 235 (1974).
4 A.I. Spindle and R.A. Pedersen, J. exp. Zool. *186*, 305 (1973).
5 A. McLaren and H.C. Hensleigh, in: The Early Development of Mammals. Cambridge University Press, London 1975.
6 B.H. Juurlink and S. Fedoroff, In Vitro *13*, 790 (1977).
7 J.A. DuBois, Thesis St. Bonaventure University, New York 1977.
8 S.B. Atienza-Samols and M.I. Sherman, Devl Biol. *66*, 220 (1978).
9 R.J. Wordinger and J.A. Kell, IRCS med. Sci. *6*, 12 (1978).
10 R.J. Wordinger and R.L. Brinster, Devl Biol. *53*, 294 (1976).
11 R.J. Wordinger and J.A. Kell, Experientia *34*, 881 (1978).
12 W.R. Earle, E.L. Schilling, T.H. Stark, N.P. Strauss, M.F. Brown and E. Shelton, J. natl Cancer Inst. *4*, 165 (1943).
13 A.H. Gates, in: Methods in Mammalian Embryology, p. 64. W.H. Freeman, San Francisco 1971.
14 R.L. Ehrmann and G.O. Gey, J. natl Cancer Inst. *16*, 1375 (1956).
15 S. Stern and J.D. Biggers, J. exp. Zool. *168*, 61 (1968).
16 C.B. Ozias and S. Stern, Biol. Reprod. *8*, 467 (1973).
17 R.J. Klebe, J. Cell Physiol. *86*, 231 (1975).
18 C.B. Ozias and H.M. Weitlauf, J. exp. Zool. *177*, 147 (1971).

Effect of human seminal plasma on tumour-associated immunity in prostatic cancer. A preliminary report

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Summary. Evidence of significant suppression of tumour-associated immunity in patients with prostatic cancer by human seminal plasma (HuSPl) has been observed. Collation of the immunosuppressive property of HuSPl in this and previous studies, together with recent studies demonstrating experimental induction of prostatic cancer by spermatozoa and the relationship of prostatic cancer to sexual activity are suggestive of an etiologic role for SPl in prostatic cancer.

In examining the natural history of adenocarcinoma of the prostate[1,2], we, as others, have been intrigued by the high incidence of occult carcinoma and wide variation in the age of onset of clinical disease. As possible explanations for these enigmas, the existence of the prostate as an immunologically privileged site due to its lymphatic anatomy, i.e., afferent lymphatics[3], or immunosuppressive properties of its hormonal and/or secretory milieu or tumour-elaborated factors (in the case of carcinoma)[4] has been hypothesized.

In an attempt to elucidate the role of these factors as contributory to the privileged status of the prostate, the effect of human seminal plasma (HuSPl) on cell-mediated tumour associated immunity (TAI) in patients with prostatic cancer[5–9] has been evaluated and as such is the subject of this preliminary communication.

Materials and methods. Peripheral blood leukocytes (PBL) were obtained from 25 patients with a confirmed histological diagnosis of adenocarcinoma of the prostate by Ficoll-Paque (Pharmacia Fine Chemicals, Uppsala, Sweden) centrifugation using a modification of the method of Boyum[10] as recently described[9]. PBL 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 100 µg streptomycin/ml, untreated and treated with 280 µg/ml HuSPl (obtained from 14 healthy adult males), determined as the optimal inhibitory dosage from a dose-response curve, 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[9] of the tube leukocyte adherence inhibition method[11], untreated and treated patients' leukocytes were reacted independently with 3M KCl-$(NH_4)_2SO_4$ extracts of allogeneic malignant prostate and bladder, as sources of specific and non-specific antigens[9]; and the number of non-adherent cells counted in quadruplicate using a Standard Neubauer haemocytometer.

Delineation of specific reactivity of prostatic cancer patients' leukocytes with malignant prostate (specific antigen) was made by concomitant reaction of the patients' leukocytes with malignant bladder as a source of non-specific antigen.

Results. The effect of HuSPl on TAI to allogeneic extracts of malignant prostate in 25 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 SPl when reacted with malignant prostate indicated a highly significant difference ($p < 0.01$).

As further shown in the table and, in agreement with previous studies of the tissue-specificity of TAI in prostatic cancer patients, the reactivity of the patients' leukocytes

Effect of human seminal plasma (HuSPl) on tumour-associated immunity in patients with prostatic cancer

Peripheral blood leukocytes	Non-adherent cells obtained with allogeneic extracts of malignant (mean ± SD %):		Significance (p)
	Prostate (specific antigen)	Bladder (non-specific antigen)	
Untreated	20.4 ± 13.1	5.4 ± 4.4	< 0.01
Treated with 280 µg/ml HuSPl	11.7 ± 8.1	5.8 ± 3.9	< 0.05
Significance (p)	< 0.01	> 0.05	