The results described above indicate that the lymphoid cells obtained from mouse thymus may be divided into 3 groups according to the levels of free cholesterol and phospholipids: 1 group of total cells and 2 groups of cortisone-resistant cells, one group of which was obtained from control mice and another group of the cells from adjuvant-treated animals. It is widely accepted that within mouse thymus the cortisone-resistant lymphocytes (small population, about 5%) are mature cells and the cortisone-sensitive lymphocytes (large population, about 95%) are immature cells[17,18]. In this study, the group containing cortisone-resistant lymphoid cells from mouse thymus amounted to about 6% of total lymphoid cells. This proportionality suggests a similarity in the lipid composition between total and cortisone-sensitive thymic lymphoid cells. Cortisone-resistant lymphoid cells from the thymus of adjuvant-treated mice and from the lymph node of control animals belonged to the same group. The cortisone-resistant lymphocytes from mouse lymph node were reported to be almost exclusively thymus-derived cells[8,18]. In normal mice, therefore, there may be 3 groups of the lymphocytes derived from thymus: 1 group of immature cells and 2 groups of mature cells, such as the cortisone-resistant cells from thymus or mesenteric lymph node. The cortisone-resistant mesenteric lymphoid cells from control mice appear to be more mature than the corresponding thymic cells, since the cholesterol to phospholipid molar ratio was 0.95 for mesenteric cells and 0.71 for thymic cells, respectively[5-7]. Interestingly, the thymus-derived lymphocytes from mouse lymph node were shown to be separable into 2 subpopulations by cell electrophoresis, which indicated the presence of 3 subgroups of lymphocytes (1 group of immature cells and 2 groups of mature cells) in mouse thymus[19,20].

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Modification of lymphocyte response to phytomitogens by polycations and polyanions

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Summary. The stimulating effect on mitogen-induced lymphocyte response by polycationic compounds such as polylysine, DEAE dextran protamine and methylated albumin, is studied at different serum-protein concentrations and mitogen concentrations. It is suggested that the polar interaction between polycationic compounds and glycoproteins are of major importance for reactivity of lymphocytes to mitogens.

Normal serum is known to contain glycoproteins that interfere with the in vitro response of lymphocytes to phytohaemagglutinin (PHA) and concanavalin A (Con A)[1]. The α_2-glycoprotein fraction[2,3] prepared from normal human serum exhibits immunosuppressive effects, and likewise transcortin from normal serum inhibits the lymphocyte response to PHA[4]. From foetal serum, α-foetoprotein can be prepared in large amounts. This glycoprotein, which is also present in sera from patients with primary liver cancer[5], likewise has an immunosuppressive effect.

Serum-protein components with immunosuppressive activity are found in cancer patients and experimental animals bearing transplantable tumours. Transcortin is greatly increased in patients with breast cancer[4], and several inhibitory protein fractions and blocking factors are demonstrable in the serum from patients with various malignant diseases[6].

Characteristic of this group of α-glycoproteins is their relatively high sialic acid content which supplies the proteins with negatively charged groups. Focusing on the anionic nature of the immunosuppressive glycoproteins leads to the assumption that reduction of the negative charge by treatment with neuraminidase or polar interactions with polycations interferes with their effects. Treatment of rat lymph-node lymphocytes with neuraminidase, or addition of the polycations poly-L-ornithine or poly-D-lysine, enhances their response to PHA or Con A. This indicates that surface charge of lymphocytes is of importance for their mitogen-induced transformation[7]. Similarly, the consequence of polar interactions between anionic and cationic components in the cellular micro-environment needs consideration in order to try to estimate the extent of immunosuppressive activity in serum.

Materials and methods. Peripheral blood lymphocytes from normal volunteers were isolated by Ficoll-Isopaque flotation. The cells were cultured in medium Tc 199 supplemented with 2-18% heat-inactivated pooled normal human serum, 100 units ml^{-1} penicillin, 100 $\mu g\ ml^{-1}$ streptomycin, and 20 μmoles ml^{-1} HEPES buffer; 2×10^5 cells in 0.2 ml of the medium were added per well in Nunc microtitre

plates and cultured at 37 °C for 5 days, the last 24 h in the presence of 1 μCi ml^{-1} ^{14}C-thymidine. DEAE-dextran, mol.wt 2×10^6, Pharmacia, Uppsala. Poly-D-lysine, mol.wt 12×10^4, Miles-Yeda, Rehovot. Protamine sulphate was iridine sulphate supplied by Nordisk Insulin, Copenhagen. Methylated HSA was human serum albumin methylated by acid methanol for 24 h at 4 °C. The polycations to be tested were added to the wells in μl volumes and ceruloplasmin (Kabi), dextran sulphate mol.wt 5×10^6, Pharmacia, and chondroitin sulphate batch 93C-2360 Sigma, St. Louis, were similarly added to the wells. Mitogen-stimulated cultures (PHA (Difco) and Con A (Pharmacia)) as well as non-stimulated cultures were tested in triplicate or quadruplicate. The cpm of non-stimulated cultures was below 120 and not influenced by the addition of the polycations.

Results and discussion. In high concentrations, polycations suppress the PHA- and Con A-induced response of human lymphocytes as shown in table 1. However, at moderate concentrations, i.e. poly-D-lysine in concentrations of 4 μg ml^{-1}, DEAE-dextran 20 μg ml^{-1}, methylated human serum albumin 100 μg ml^{-1}, and protamine sulphate 500 μg ml^{-1}, comparable stimulations of the PHA response are seen. The lymphocyte response to Con A is stimulated to an even higher degree by the same polycations. Similar effects are seen on pokeweed-mitogen-stimulated cultures (data not shown). The polycation effect depends on the phytomitogen concentration and also on the serum-protein content of the test medium, as shown in figures 1a and 1b. It is seen that the enhancing effect induced by the polycation is highest at the lowest serum concentration. When a higher serum concentration is used, a higher concentration of phytomitogen is required to reach the same extent of polycation-dependent response augmentation. Supraoptimal lectin concentrations are known to be suppressive, and it was tested whether polycations would augment this effect. This was found not to be the case.

Protamine was found to be capable of a full stimulative effect on lectin responses when added 24 h after the initiation of the cultures. After 48 h only weak increase was observed, and later additions of protamine did not enhance the lymphocyte response, thus excluding a direct effect on the uptake of thymidine. The polycations tested in our experiments were not mitogenic in themselves, as judged by the thymidine uptake in cultures to which no mitogens were added. The effect of polycations observed was found not to be due to altered culture kinetics. Counting of viable cells recovered from cultures incubated with and without protamine sulphate showed that the compound had a minor toxic effect which, however, did not influence viable cell outputs from mitogen-stimulated cultures after 4 days. Pre-incubation of lymphocytes with protamine for up to 24 h, followed by washing of the cells, was nearly as effective as controls incubated with the compound for the total culture period.

In contrast to polycations, polyanionic compounds, i.e. the acid glycoprotein ceruloplasmin, and the acid mucopolysaccharide chondroitin sulphate, effectively inhibited the PHA- and Con A-induced response of human lymphocytes (table 2). Figure 2 shows titration experiments on the effect of ceruloplasmin of the same type as shown in figure 1 for protamine on the response of lymphocytes after the addition of various concentrations of PHA and Con A at different serum concentrations. In contrast to the polycation effect, the inhibitory effect is relatively independent of

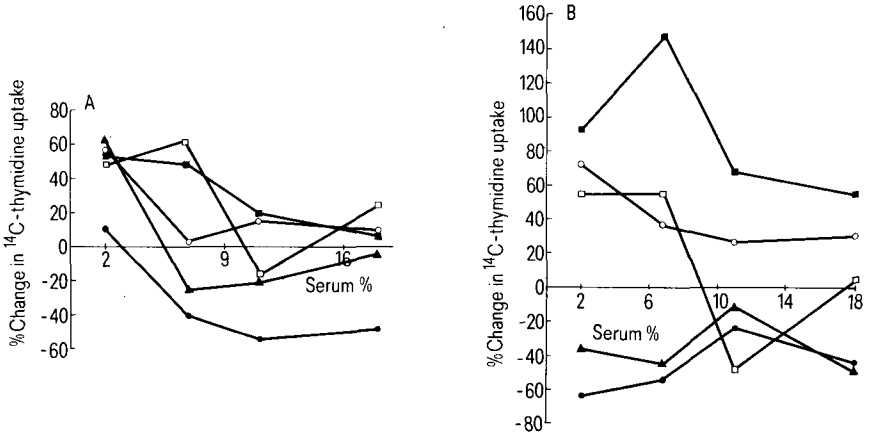

Fig. 1. The effect of 0.5 mg ml^{-1} protamine on PHA (A) and Con A (B) stimulated lymphocytes at different serum concentrations. Concentration of mitogens: 0.05 μg ml^{-1} (●); 0.25 μg ml^{-1} (▲); 0.50 μg ml^{-1} (□); 2.5 μg ml^{-1} (○); 5.0 μg ml^{-1} (■).The values are calculated as per cent change in ^{14}C-thymidine uptake relative to appropriate controls.

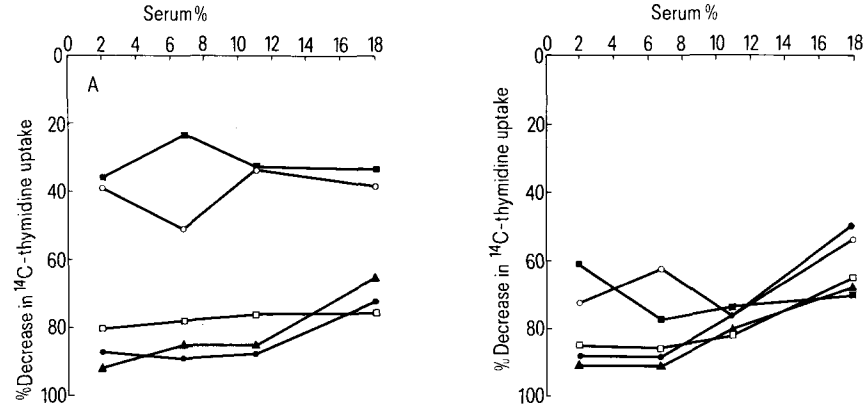

Fig. 2. The effect of 1.0 mg ml^{-1} ceruloplasmin on PHA (A) and Con A (B) stimulated lymphocytes at different serum concentrations. Symbols are explained in figure 1.

Table 1. Stimulative effect of polycations on PHA and Con A response of human lymphocytes

	Polycation added (ml^{-1})	Thymidine incorporation (cpm) PHA (0.5 μg ml^{-1})	Con A (0.5 μg ml^{-1})
DEAE-dextran	200 μg	16± 5 (−)	14± 4 (−)
	20 μg	2005±165 (96)	1920±123 (166)
Control		1022±140 0	720± 36 0
Methylated HSA	5 mg	180± 28 (−)	54± 12 (−)
	0.5 mg	13095±306 (226)	5590±211 (747)
	0.1 mg	6196±193 (54)	2600± 28 (294)
	0.01 mg	3484±149 (−13)	772± 22 (17)
Control		4017±182 0	660± 23 0
Poly-D-lysine	20 μg	3550±495 (−26)	108± 40 (−81)
	10 μg	11910±605 (148)	1932±218 (235)
	4 μg	9380±109 (95)	2897± 84 (403)
Control		4811±295 0	576± 39 0
Protamine sulphate	5 mg	47± 4 (−)	59± 13 (−)
	0.5 mg	8550±242 (113)	1278± 40 (94)
	0.1 mg	6933±376 (73)	1249± 20 (89)
Control		4017±182 0	660± 23 0

Table 2. Inhibitory effect of polyanions on PHA and Con A response of human lymphocytes

	Polyanion added (ml^{-1})	Thymidine incorporation (cpm) PHA (0.5 μg ml^{-1})	Con A (0.5 μg ml^{-1})
Chondroitin sulphate	2 mg	1978±161 (−51)	469±58 (−29)
	1 mg	3236±145 (−20)	630±47 (−5)
	0.2 mg	3226±108 (−20)	836±14 (+26)
Control		4017±182 0	660±23 0
Ceruloplasmin	10 mg	89± 20 (−)	86± 8 (−87)
	5 mg	405± 33 (−)	280±35 (−58)
	2 mg	646±140 (−84)	305±35 (−54)
	1 mg	865±139 (−78)	292±25 (−56)
	0.2 mg	1812± 59 (−55)	383±29 (−42)
Control		4017±182 0	660±23 0
Dextran sulphate	200 μg	4805±296 (0)	354±54 (−41)
	100 μg	3593±403 (−25)	160±16 (−73)
	20 μg	6150±149 (+28)	58± 3 (−)
	5 μg	8001±283 (+66)	66±21 (−)
	1 μg	7267±203 (+51)	258±30 (−57)
Control		4811±295 0	598±24 0

All experiments are done in medium Tc 199 supplemented with 2% heat-inactivated pooled normal human serum containing 10^6 cells ml^{-1}. Values of cpm ± SD are shown. Figures in parentheses represent the percentage increase or decrease as compared with the control-stimulated cultures in the same experiment; (−) indicates more than 90% inhibition.

the serum concentrations within the range tested, i.e. from 2% to 18% serum added to the test medium.

The inhibitory effect of ceruloplasmin is strongest at low PHA concentration and the Con A response was reduced 60–90% by 1.0 mg/ml ceruloplasmin at all Con A concentrations within the range of serum addition to the medium.

The sulphated polysaccharide dextran sulphate, which is also a strongly negatively charged compound, showed inhibition of the Con A response in the concentration range from 1.0 to 200 μg ml^{-1} applied in the test. The PHA response was also inhibited at high dextran sulphate concentrations, but unexpectedly the response to optimal mitogen concentrations was enhanced at low concentrations of the polyanion. No effect of these polyanions on cell survival in culture was found.

The present studies on human lymphocytes agree with the observations of Novogrodsky[7] in rat lymph-node lymphocytes. Potentiation of the phytomitogen action has similarly been demonstrated for cytochalasin B, which in addition to its different cellular effects also reduces the negative cell-surface charge[8].

Several of these observations suggest that a large number of compounds which modify lymphocyte responses have the feature in common that they are either polyanionic or polycationic. Especially polycations are known to have a strong affinity for cell surfaces resulting in a series of effects, such as stimulation of uptake of macromolecules, uptake of RNA and DNA from virus, increased adhesion to surfaces and increased aggregation of cells. These properties of the polycations can be applied to the present study. The observed difference in stimulative activity comparing the nature of the polycations is in accordance with earlier experiments which showed that the binding affinity and the biological activity of polycations increase with increasing molecular weight and charge density[10].

In addition to this well-documented interaction between polycations and the cell surface, other interactions must be taken into consideration. Interaction between mitogen and serum factors may very well interfere with the mitogenic effect[11]. Con A is known to interact with serum glycoproteins such as ceruloplasmin. The data shown in figure 2 for ceruloplasmin inhibition of the Con A response suggest the possibility of such interactions, and similar mechanisms may interfere with the response to PHA. Alpha-foetoprotein, a glycoprotein with immunosuppressive activity[5], is assumed to exert an action similar to that of ceruloplasmin.

It is assumed that part of the stimulative effect of polycations is due to inactivation of serum glycoproteins interfering with the lymphocyte response. The inhibitory effect of polycations by low mitogen concentrations may indicate that at these low concentrations the phytomitogens are bound to serum proteins, and that the complexes are unable to interact with the cell surface.

The effect of charged compounds, such as the anionic glycoproteins and the polycations studied here, on the mitogen responses is complex and further investigations are planned to study this phenomenon. The present experiments suggest that 3 factors may be of importance: a) Interactions of polycations with the cell surface will enhance cell aggregation, thus activating blast formation. Glycoproteins inhibit this process. b) Interactions between mitogens and serum factors, exemplified by the interaction between ceruloplasmin and Con A, will modify mitogenicity. c) Cellular uptake of mitogens may be increased by positively charged compounds.

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Superiority of human complement for assaying bacterial lipopolysaccharides by their anticomplementary activity

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Summary. In assaying bacterial lipopolysaccharides (LPS) for anticomplementary activity, human complement (C) allowed detection of approximately 200 times smaller amounts of LPS than guinea-pig C. Pig C was slightly inferior to human.

Bacterial lipopolysaccharides (LPS) produce in animals or their tissues a wide variety of physiopathological changes known collectively as 'endotoxin' reactions. Some of these, such as the pyrogenic effect in rabbits[1,2], the killing of chick embryos[3,4] and the clotting of *Limulus* amoebocyte lysate[5,6] form the basis of bioassays for LPS. In seeking a cheap and convenient assay for the endotoxic activity of LPS we have reinvestigated the wellknown anticomplementary (AC) activity of these substances[7–10]. The main observation to be reported here is that AC tests with human complement (C) have proved to be about 200 times more sensitive than those with guinea-pig C.

Methods. All LPS samples were prepared by the Westphal procedure[11]. Those from *Escherichia coli* $O_{111}B_4$, O_{55} and O_{125} and from *Shigella flexneri* and *Salmonella typhi* were obtained from Difco Laboratories. Those from *E. coli* Lilly and from *Bordetella pertussis,* strain No. 18334 were prepared[12] in this laboratory. Freeze-dried guinea-pig C was obtained from Wellcome Research Laboratories, England. Fresh pig serum, from a single adult male animal, was provided by the Veterinary School, Glasgow University. Samples of human serum were obtained from laboratory personnel. Both the pig and human sera were stored in small aliquots at −70 °C. C was titrated by a conventional method with serial 2fold decreasing volumes of serum. In this system human, pig and guinea-pig C contained respectively about 50, 50 and 200 HU_{50} per ml of undiluted serum. To assay for AC activity 0.1 ml of each LPS sample, dissolved in Veronal buffer (VB)[13], was delivered in a series of 2fold decreasing volumes to 12 × 100 mm tubes and the volumes equalized to 0.9 ml with VB. To each tube was then added 0.1 ml C dilution containing 5 HU_{50}. The mixtures of LPS and C were incubated at 4 °C for 18 h followed by 1 h at 37 °C, when 0.5 ml of 1% sensitized erythrocytes were added and a further 30 min incubation at 37 °C given to detect active C. To facilitate the spectophotometric estimation of haemolysis, a modification of the procedure of Macmorine, Wardlaw and Weber[14] was used: the tubes were centrifuged for 5 min at 1500 × g and the supernates poured off the pelleted cells and discarded. The residual, unhaemolysed erythrocytes were then haemolysed in 3 ml distilled water and the optical density (OD) read in an EEL colourimeter with 430 nm filter. A graph of OD against log dose of LPS was plotted and the end point taken at 50% haemolysis. 1 anticomplementary unit (ACU) is defined as the weight of LPS which, when incubated with 5 HU_{50} of C under the above conditions, gives 50% haemolysis finally. The AC activity is expressed as the number of ACU per mg of LPS (ACU mg^{-1}).

Results and discussion. The table presents the results of AC assays of 7 different LPS against 5 HU_{50} of human, pig and guinea-pig C. Each recorded value is the geometric mean of at least 2, and usually 3, estimations. With guinea-pig C, the AC potencies were between 50 and 800 times lower (geometric mean 200), with *E. coli* $O_{111}B_4$ LPS the least active and *B. pertussis* LPS the most. Pig C responded similarly to human C, although the values were on average about ½, and *Sh. flexneri* was the most actively AC LPS. It will be noted that although the rank order of activity of the

Parallel titrations of anticomplementary activities of different species of LPS towards different species of complement

LPS	Anticomplementary activity (ACU mg^{-1}) towards complement from Human				Pig	Guinea-pig
	*A	B	C	D		
E. coli $O_{111}B_4$	33	14	26	33	20	0.2
E. coli O_{55}	21	14	26	33	40	0.66
Bordetella pertussis	158	89	63	100	80	4.0
E. coli Lilly	116	121	85	221	200	0.5
Shigella flexneri	250	237	224	168	143	0.66
E. coli O_{125}	50	n.t.	n.t.	n.t.	20	0.8
Salmonella typhi	200	n.t.	n.t.	n.t.	83	0.5

*A-D refers to different human sera from different individuals. n.t., not tested.