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.

7 LPS was different with the 3 species of C, the difference between the most and least active within each species of C was of the order of 10–20fold. The serum obtained from 4 of the laboratory staff showed there was no substantial difference between different samples of sera in their responses to the different LPS. Recent work[11,15] has amply demonstrated that LPS from different bacteria may vary widely in AC activity, while the AC activity of a given LPS may be altered by various chemical treatments[16]. The results here however show a striking difference between human and guinea-pig C in their sensitivity to the AC activity of LPS, the human being capable of detecting approximately 200 times lower concentrations of LPS than the guinea-pig. The underlying basis of this difference remains to be explained. LPS may exert its AC effect by both the classical and alternative pathways[17,18] and we have not yet investigated which way predominates under the conditions used. As compared with other methods for assaying LPS, the AC test has the advantages of cheapness, convenience and reproducibility. However, it would appear to be of the order of 1000fold less sensitive than the rabbit pyrogenicity test or the *Limulus* amoebocyte lysate assay[20]. Nevertheless, in certain defined circumstances, AC activity can provide a cheap and convenient assay for LPS and, in work to be reported elsewhere, we have used it to study a) the biodegradation of LPS by the slime mould *Physarum polycephalum* and b) the distribution and fate of LPS in marine sediments.

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Estimation of plasma thyroxine concentration in ducks in relation to different environmental and experimental conditions[1]

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Summary. Plasma thyroxine concentration was measured in ducks by the thyroxine-binding globulin technique. The assay allowed us to detect annual variations in thyroid activity as well as significant changes after starvation or cold exposure. No detectable thyroxine was formed in surgically thyroidectomized ducks.

In birds, as in other Vertebrates, protein bound iodine has been routinely used for the measurement of thyroid activity. Using these methods, Mellen and Hardy[2], and Mellen[3] have claimed that protein bound iodine ($PB^{127}I$, as well as $PB^{131}I$) was not significantly altered in chickens after cold stress, thiouracile-treatment, or thyroidectomy. Similarly, we were unable to detect any modification in $PB^{127}I$ of ducks reared for 1 or 30 days in a cold environment (+4 °C) or exposed to reduced (50%) or solid starvation[4].

More recently, using Sephadex column fractionations, we could show that in ducks, hens, pigeons and quail, the $PB^{125}I$ was not restricted to hormonal fraction (Astier[5–7]). A non-hormonal radio-iodinated and organic fraction (TCA precipitable but not extractible with N butanol) appeared in the plasma, preceding largely hormonal radio iodinated compounds and representing between 98 and 100% of the PB*I. The non-hormonal fraction decreased at 24 h after tracer injection, but between 1 and 14 days it was still equal to 30–50% of the PB*I. In addition, stable iodine determination confirmed the presence of 50% of non-hormonal iodinated proteins in the duck $PB^{127}I$.

The present studies were designed to estimate whether plasma thyroxine measurements, with the thyroxine-binding globulin (TBG) technique, could lead to a more accurate evaluation of thyroid function in ducks, and therefore allow the detection of variations in plasma thyroid hormone level in relation to various environmental conditions, even when PBI measurements failed to show any modification. The TBG method used here has already been used as a reliable assay for thyroid hormone concentration in chickens[8,9].

Material and method: The competitive protein-binding procedure used here was adapted from the original assay by Murphy et al.[10] and modified by Vigouroux[11].

Standard thyroxine solution: The standard curve was prepared by pipetting 20–60 µl of the working solution (10 ng/ml of L thyroxine dissolved in 95% ethanol) into polystyrene tubes and evaporating to dryness, under nitrogen at 37 °C.

Labelled thyroxine: L-^{125}I thyroxine (CEA Saclay, France; Sp. act. 40–50 mCi/µg), was dialyzed with rat plasma