strain of *B. megaterium* showed a similar inhibition against phage multiplication.

Experiments were carried out to gain an insight into the nature of the inhibitory factor. Results showed that the factor lost its biological activity when heated at 100° C for 10 min. There was no significant loss in activity on treatment with DNase or RNase, but proteolytic enzymes destroyed the inhibitory effect of the extract.

Purification of the extracts by column chromatography has been attempted to concentrate the biologically active compound. The factor can be adsorbed to CM-cellulose and eluted with a *p*H gradient. The active component exhibits the properties of a basic protein. We are trying to isolate the active principle in a pure state and study its structure as well as the mechanism of inhibition of phage multiplication. It would be interesting to investigate the role of such basic proteins in sporulation of micro-organisms.

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Effect of Anti-lymphocyte Serum on the Response of Human and Mouse Lymphocytes to PHA

THE immunosuppressive properties of anti-lymphocyte serum have attracted a great deal of attention. Most work has been carried out with antisera against lymphocytes of laboratory animals[1–3], but some clinical studies have also been made using anti-human lymphocyte serum (AHLS)[4]. Clinical work, however, is handicapped by the lack of method of assaying the immunosuppressive potency of AHLS *in vitro*. It has been shown that the stimulation of nucleic acid which is induced by phytohaemagglutinin (PHA) in human lymphocytes *in vitro* is broadly similar to that induced by antigens[5–8]. We have therefore studied the effect of anti-mouse lymphocyte serum (AMLS) on the response of mouse peripheral lymphocytes to PHA *in vitro* in the hope of establishing a correlation with the graft protecting potency of the serum. If a correlation could be established it seemed that analogous assays of AHLS might be used as a guide to its graft protecting potency.

All antisera were made in horses; the four anti-mouse lymphocyte sera (Nos 1–4) and three of the anti-human lymphocyte sera (Nos 5–7) were made at these laboratories; the fourth (No. 8) was a gift from Dr K. James. The immunization courses are shown in Table 1. The AMLS were assayed in *CBA* mice grafted with tail skin from *A* strain mice according to the technique of Billingham and Medawar[9]. Mice grafted on day 0 received subcutaneous injections of 0·5 ml. of AMLS on days 2 and 5; grafts were scored as rejected on the day the residual scab fell off and one half day was subtracted for the calculation of the mean survival time. Control groups were not injected.

Peripheral blood lymphocytes were collected from *CBA* mice, cultured and their response to PHA determined as described by Festenstein[10], except that ^{14}C-2-thymidine was added (0·075 μCi culture) on day 2 and the cells collected on day 3. Viable counts made when the cells were collected showed that 50–70 per cent of the lymphocytes survived. Human peripheral lymphocytes were separated from polymorphs by differential settling and overnight incubation[11]. Aliquots of washed cells were incubated with PHA for 48 h and the degree of stimulation was estimated by adding 0·2 μCi of ^{14}C-2-uridine/culture and incubating for a further 60 min; the cells were then collected by filtration and washed with trichloroacetic acid and methanol before scintillation counting in the usual way. All *in vitro* experiments were carried out with complement free media and the ALS was heated at 56° C for 30 min before use. ALS was added to the cells either with the PHA (AMLS) or 30 min before it (AHLS), preliminary experiments having shown that the activity was unaffected by prior addition.

Table 1. PREPARATION OF ANTI-LYMPHOCYTE SERUM IN HORSES

Horse No.		Graft protection (MST days)*
	Anti-mouse lymphocyte serum	
1 (WRL No. 5942) EX 4896	Two intravenous injections of 80–90 × 10⁹ mouse thymocytes on day 0 and d y 14, bled day 21	23·7 ± 4·7
2 (WRL No. 7273) 27.10.67	,, ,, ,, ,, ,,	22 ± 3·3
3 (WRL No. 6997) 19.1.67	Four intravenous and intramuscular injections of 50–80 × 10⁹ mouse thymocytes on day 0, day 14 and then at intervals of 6 weeks; bled 7 days after last injection	15·5 ± 1·5†
4 (WRL No. 6768) 5.1.67	Fifteen intravenous and intramuscular injections given twice weekly, graded from 0·5 to 32 × 10⁹ mouse thymocytes/injection; bled 7 days after last injection	22·5 ± 3·3
	Anti-human lymphocyte serum	
5 and 6 (WRL Nos. 7207 and 7211) 14.4.67	Two intravenous injections of 10⁹ human peripheral lymphocytes given on day 0 and day 14; bled on day 21	
7 (WRL No. 6977) 14.4.67	Eight intravenous and intramuscular injections of human spleen cells during 6 months, graded from 2·5 × 10⁹ to 64 × 10⁹ nucleated cells/injection; bled 7 days after last injection	
8 (Edin. No. 3)	Eleven injections of human spleen or thymus cells during 12 months, graded from 5 × 10⁹ to 50 × 10⁹ nucleated cells/injection; bled 10 days after last injection	

* MST, arithmetic mean of survival times determined on at least twelve mice.
† Not significantly different from MST for uninjected mice of 12·5 days.

The graft protecting potency of the four AMLS is shown in Table 1 and the effect of the sera on the response to PHA is shown in Fig. 1. All four sera inhibited the response to PHA. The inhibition was not a result of cell death; microscopic examination of the cells showed that they were still intact, though untransformed. While the

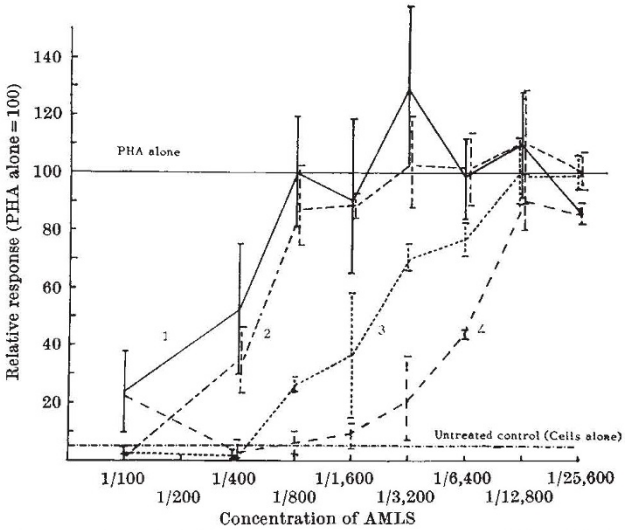

Fig. 1. The effect of AMLS on the response of mouse peripheral blood lymphocytes to PHA. The response to PHA alone is shown as a horizontal line; the values from four replicate experiments as vertical lines and those from single experiments as points. AMLS from: lightly immunized horses: horse 1 (———), horse 2 (— · — · —); moderately immunized horse: horse 3 (. . . . .); hyperimmunized horse: horse 4 (— — — —). Immunization courses are shown in Table 1.

courses of immunization could not be compared exactly, it appeared that the inhibitory activity of the serum increased as the course lengthened; the graft protecting power of the sera, however, did not, so that serum 3, which was inactive *in vivo*, had an *in vitro* activity intermediate between the two dose sera 1 and 2 and the hyperimmune serum 4.

The effect of AHLS on the response of human peripheral lymphocytes to PHA is shown in Fig. 2*a* and *b*. The effect is complex, but it seems to be composed of three elements. (1) An inhibition which extended to increasingly high dilutions as immunization proceeded. The cells were viable though untransformed and this reaction seems to be analogous to that seen in mouse cells. (2) An augmentation of the response by concentrations of AHLS lower than those required for inhibition (Fig. 2*a*). This effect was produced by sera 5 and 6, which did not stimulate uridine uptake in the absence of PHA. The degree of augmentation was variable and it is not yet certain that it was distinct from the augmentation produced by normal horse serum, though it did appear to be larger. (3) An augmentation of the response to PHA by concentrations of AHLS higher than those required for inhibition (Fig. 2*b*). This effect was seen only when sera were used which stimulated uridine uptake by lymphocytes in the absence of PHA[12] and it occurred for the same range of concentration irrespective of the presence or absence of PHA.

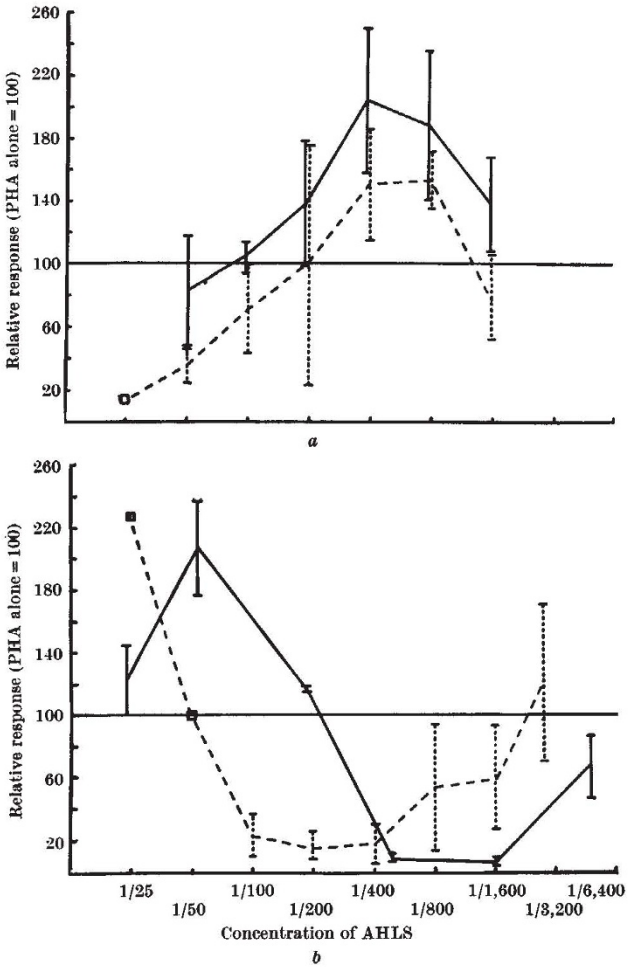

Fig. 2. The effect of the immunization course on the inhibitory activity of AHLS and variation of the PHA response with concentration of AHLS. The response to PHA alone is shown as a horizontal line, values from three replicate experiments shown as vertical lines, values from single experiments shown as points. *a*, Lightly immunized horses: horse 5 (———), horse 6 (— — —); *b*, hyperimmunized horses: horse 7 (— — —), horse 8 (———). Immunization courses are shown in Table 1.

The cytotoxicity of the AMLS prevented the use of these sera at concentrations high enough to establish whether or not mouse lymphocytes were stimulated in the same way.

The inhibition of the response to PHA by both AMLS and AHLS is in contrast to the stimulation reported by Greaves *et al.*[13]. They, however, used only concentrations of AHLS which induced a marked stimulation of DNA synthesis, and they removed the antiserum before adding the PHA. In the experiments described here, the antiserum remained in the cultures and, in these conditions, inhibition was observed at concentrations as low as one hundredth of those which would induce DNA synthesis. The results of Greaves *et al.* seem to be analogous to the augmented responses to PHA which we observed in the presence of sera 5 and 6 (Fig. 2*a*). This response to some samples of AHLS and to normal horse serum may be a form of immune response by the cells to normal horse serum which is only expressed in the presence of PHA; if so, it would be analogous to the stimulation of intercellular reactions reported *in vitro*[14,15].

The response of human peripheral lymphocytes to PHA can be measured equally well by determining the incorporation of either ^{14}C-2-uridine or ^{14}C-2-thymidine (unpublished work of B. M. and J. A. C. P.); it would therefore seem that the inhibitory effect of ALS on the response to PHA is similar in both human and mouse lymphocytes. Because the inhibitory effect of AMLS was not correlated with *in vivo* activity it is very unlikely that the graft protecting potency of AHLS can be determined in this way.

Discrepancy between *in vivo* and *in vitro* activities has also been noted by James[16] and it implies that the antibodies involved are heterogenous[17]; the variation in the discrepancy implies that the proportions of the antibodies in the serum also vary as immunization proceeds. Whether this is the result of the complexity of the antigen, or of the sequential synthesis of different classes of globulin with different biological properties[18], is a matter for conjecture.

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