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NOTES ON THE LARGE SCALE PREPARATION AND ON THE PROPERTIES OF ANTI-LYMPHOCYTE SERUM FOR USE IN MICE

TECHNIQUES

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SUMMARY

Mouse spleen and thymus cells have been used in the preparation of horse anti-mouse anti-lymphocyte serum (HAMLS). The cells were used either separately or in a mixture and three types of immunization schedules were used, viz. two-pulse, extended and chronic.

Antisera of marked immunosuppressive activity, as measured by the ability to prolong the life of skin homografts in mice, were obtained using all three schedules, the median survival time being, at best, 27·5 days for the chronic schedule, 26·7 days for the two-pulse schedule and 25·8 days for the extended schedule. The two-pulse and the extended schedules produced non-toxic antisera in a relatively short period of time but were uneconomic in terms of antigen and horses. The chronic schedule was preferred but after 10 weeks the development of unwanted antibodies precluded the further useful immunization of the horses.

INTRODUCTION

The work described in this paper was carried out in this laboratory over the past 3 years and is concerned with the large scale production and *in vitro* and *in vivo* testing of anti-lymphocyte serum for use in mice. This was part of a wider programme aimed at the production of immunosuppressive antisera for clinical use in man, as outlined by Long *et al.* (1969). In this particular study we set out to evaluate a variety of immunization schedules, dose levels, routes of antigen administration and the use of adjuvants. The use of non-human cells in raising antilymphocyte serum has the great advantage that the immunosuppressive potency of the sera produced can be measured directly in terms of their ability to prevent skin-graft rejection, as first demonstrated by Woodruff & Anderson (1963, 1964) in the rat, and since observed in the mouse system by Gray, Monaco & Russell (1964) and Levey & Medawar (1966a, b).

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Most previous work had been carried out in rabbits which we consider as unsuitable for really large scale production. By using the horse we hoped to produce large volumes of useful sera, and more importantly to provide data that would be applicable to the development of horse anti-human ALS.

MATERIALS AND METHODS

Cell preparation

Antigen—mouse spleen and thymus cells. A suspension of cells in Hanks's balanced salt solution (HBSS) was prepared from the thymus and/or spleen dissected from 6-week-old male and female Swiss Albino mice weighing between 17 and 21 g. For the series of experiments described here, thymus yielded $2{\cdot}4 \times 10^9$ lymphocytes per g wet weight of tissue (range $2{\cdot}2 \times 10^9$–$2{\cdot}9 \times 10^9$ cells) whilst the yield from spleen was $0{\cdot}7 \times 10^9$ lymphocytes per g wet weight of tissue (range $0{\cdot}4 \times 10^9$–$1{\cdot}0 \times 10^9$ cells). No attempt was made to remove erythrocytes from the cell suspensions or to fractionate the leucocytes in any way. The viability of the cells, as measured by the dye exclusion method (0·5% trypan blue prepared in isotonic saline) was rarely below 85%.

In vitro testing—mouse peripheral blood cells. Mouse platelets were obtained by differential centrifugation of 100 ml of whole mouse blood containing anti-coagulant (0·5 g EDTA–disodium salt and 4000 units of heparin). The blood was centrifuged at 1000 *g* for 3 min after which time the platelet-rich plasma was removed and the cells so obtained used for the platelet agglutination test at a concentration of 1×10^8 cells/ml.

The deposited cells were resuspended in their starting volume of phosphate buffered saline (PBS) pH 7·2, thoroughly mixed with 25 ml of 6% Dextran, molecular weight 110,000 (Glaxo Limited, Greenford, Middlesex) and allowed to sediment at room temperature for 30–60 min. The leucocyte-rich supernatant was removed and a portion used for the leucoagglutination tests at a concentration of 6×10^6 cells/ml.

The remainder of the supernatant was centrifuged at 1000 *g* for 5 min and the cell button washed in HBSS and finally suspended in HBSS to give a cell concentration of 6×10^6 cells/ml. The cells were used for leuco-cytotoxicity tests.

Finally, the erythrocytes were washed in isotonic saline and adjusted to a concentration of 0·1 ml of packed cells in 15 ml of isotonic saline. The cells were used for haemagglutination tests. Packed mouse erythrocytes prepared in this way were also used for absorption purposes.

Mouse erythrocyte stroma preparation

Mouse erythrocytes from 120 ml of whole blood were added to 1000 ml of pyrogen-free distilled water containing 10 ml of a saturated solution of 20% Hibitane (I.C.I. Limited, Alderley Park, Macclesfield, Cheshire). Following the addition of 2 ml of a 1% acetic acid solution, the volume was adjusted to 1500 ml with pyrogen-free distilled water, and the mixture stirred and allowed to sediment for 24 hr. All manipulations were carried out at 4°C.

The stromata were washed repeatedly until they appeared a greyish white and the super-

natant was clear. When these conditions were achieved, the stromata were stored at 4°C until required.

Immunization of horses

In all the immunizing schedules described here, the thymocytes and/or spleen cells were administered in HBSS containing 100 units/ml of Streptomycin Sulphate and Polymyxin B Sulphate. All intravenous (i.v.) injections were administered in a volume of 400 ml, whilst subcutaneous (s.c.) and intramuscular (i.m.) injections were administered in a volume of 10 ml.

A total of 10 horses aged between 4 and 10 years and weighing approximately 650 kg, were immunized using three main schedules.

The first of these was the method (two-pulse) described by Levey & Medawar (1966a, b). This consisted of two intravenous injections spaced 14 days apart and the experimental animal was bled out on the 21st day. (See Table 1.) Four horses were immunized by this schedule (5942, 7273, 7131 and 7122). The latter two animals received much smaller doses than the previous two. A further two horses (7172 and 7209) were given a first injection of cells emulsified in adjuvant which was administered intramuscularly. This was followed 14 days later by a second dose of antigen divided so that one half was injected intramuscularly with adjuvant and the other intravenously without adjuvant. Aluminium phosphate (aluminium content 2·75 mg/ml) and Alhydrogel (aluminium content 6·5 mg/ml) were used as adjuvants. The cell dose was prepared in 5 ml of HBSS, an equal volume of adjuvant added and the cells emulsified and injected as indicated. (See Table 1.)

The second immunization schedule was an extension of the two-pulse method (hereinafter referred to as the extended schedule). The horses were given further intravenous injections of antigen, the timing of which was governed by the results of the *in vitro* studies and on the

TABLE 1. Horse anti-mouse anti-lymphocyte (serum two-pulse immunization schedule)

Horse No.	Day	Dose administered (No. of cells)	Source of antigen	Route	Adjuvant	Day of bleeding*
5942	0	82×10^9		i.v.	Nil	
	14	96×10^9		i.v.	Nil	21
7273	0	99×10^9		i.v.	Nil	
	14	83×10^9		i.v.	Nil	21
7131	0	1×10^9		i.v.	Nil	
	14	1×10^9		i.v.	Nil	21
7122	0	1×10^9	Mouse	i.v.	Nil	
	14	1×10^9	thymus	i.v.	Nil	21
7172	0	1×10^9		i.m.	Aluminium Phosphate	
	14	$0{\cdot}5 \times 10^9$		i.m.	Phosphate	
		$0{\cdot}5 \times 10^9$		i.v.	Nil	21
7209	0	1×10^9		i.m.	Alhydrogel	
	14	$0{\cdot}5 \times 10^9$		i.m.	Alhydrogel	
		$0{\cdot}5 \times 10^9$		i.v.	Nil	21

* Horses bled out.

TABLE 2. Horse anti-mouse anti-lymphocyte serum (extended immunization schedule)

Horse No.	Day	Dose administered (No. of cells)	Source of antigen	Route	Day of bleeding
6997	0	70×10^9		i.v.	
	14	51×10^9		i.v.	
			Mouse thymus		21
	56	63×10^9	and	i.v.	
			spleen		63
	112	80×10^9	cells	i.v.	
					119
5989	0	100×10^9		i.v.	
	14	86×10^9		i.v.	21, 23 & 25
	37	100×10^9		i.v.	46, 48 & 50
	56	100×10^9	Mouse thymus	i.v.	63, 65 & 67
	77	100×10^9		i.v.	
				i.v.	84, 86 & 88
	137	10×10^9		i.v.	144

general conditions of the horses. Large bleedings (8 litres) were usually taken 7–9 days after each of these injections. Two horses (6997 and 5989) were immunized in this way. (See Table 2.)

The third group of horses (6762 and 6768) were immunized by a chronic schedule (suggested by Mrs I. Batty and Mr A. Thomson of these laboratories). This consisted of starting the antigen dose at a low level ($0{\cdot}5 \times 10^9$ cells) and increasing the dose at weekly intervals until $32{\cdot}0 \times 10^9$ cells had been reached. This course took 7–8 weeks to complete. The majority of the injections were given subcutaneously and the remainder intravenously. (See Discussion.) Horse No. 6762 received cells of splenic origin and Horse No. 6768 cells of thymic origin. (See Tables 3 and 4.)

Bleeding of horses and preparation of sera

Large bleedings were taken as indicated in Tables 1, 2, 3 and 4. In addition, small experimental bleedings (100 ml) were taken weekly for test purposes. Bleedings were taken from the jugular vein of the horse and the blood obtained was allowed to clot at room temperature. The blood clot was removed by centrifugation (750 *g* for 30 min) and the clear serum obtained. Sterilization of the serum was carried out by filtration through Carlson–Ford EKS sterilizing pads and inactivation was carried out by incubation at 56°C for 60 min. The serum was finally filled out aseptically into small volumes and stored at 4°C until required. Samples were checked routinely for pyrogenicity and sterility.

In vitro testing

Measurement of haemagglutinin, platelet agglutinin, leuco-agglutinin and leuco-cyto-

TABLE 3. Horse anti-mouse anti-lymphocyte serum (chronic immunization schedule)

Horse No.	Day	Dose administered (No. of cells)	Source of antigen	Route	Day of bleeding
6762	0	$0{\cdot}5\times10^9$		s.c.	0
	5	$1{\cdot}0\times10^9$		s.c.	5
	7	$2{\cdot}0\times10^9$		s.c.	7
	12	$4{\cdot}0\times10^9$		s.c.	12
	14	$6{\cdot}0\times10^9$		s.c.	
					18
	19	$8{\cdot}0\times10^9$		s.c.	
	21	$12{\cdot}0\times10^9$		s.c.	
					25
	28	$16{\cdot}0\times10^9$		s.c.	
	32	$24{\cdot}0\times10^9$		s.c.	32
	42	$24{\cdot}0\times10^9$	Mouse	s.c.	42
	46	$24{\cdot}0\times10^9$	spleen	s.c.	
					50
	53	$32{\cdot}0\times10^9$		s.c.	53
	56	$32{\cdot}0\times10^9$		s.c.	56
	60	$32{\cdot}0\times10^9$		i.v.	60
	63	$32{\cdot}0\times10^9$		i.v.	
					67
	74	$32{\cdot}0\times10^9$		i.v.	74
	77	$32{\cdot}0\times10^9$		i.v.	77
	81	$32{\cdot}0\times10^9$		s.c.	81
	84	$26{\cdot}0\times10^9$		i.v.	
					88
					91

toxicity was carried out. Modifications were incorporated into the established techniques enabling the tests to be carried out on the micro scale. All dilutions were carried out using microtitre diluting equipment (Flow Laboratories Limited, Ayrshire, Scotland) and plastic trays (Linbro Chemical Co., Inc., New Haven, Conn., U.S.A.). The diluent used for the haemagglutinin, platelet agglutinin and leucoagglutinin tests was PBS.

Each well in the plastic tray contained 0·025 ml of PBS. A volume of 0·025 ml of the HAMLS under investigation, was added to the first well and doubling dilutions from 1/2–1/2048 prepared using microtitre drum-head dilutors. Finally, 0·025 ml of the appropriate cell suspension was added to each well, the contents were mixed and incubated at 37°C for 90 min. Haemagglutinin was read with the aid of a hand lens ($\times 8$ magnification). Leucoagglutinin and platelet agglutination were read microscopically ($\times 80$ magnification). The end points were recorded as the highest dilution at which definite cell agglutination appeared.

The diluent used in the leuco-cytotoxicity tests was HBSS. A volume of 0·025 ml of this solution was added to each well, 0.025 ml of the HAMLS under investigation was added to the first well and doubling dilutions carried out from 1/2–1/2048, as previously described. Volumes of 0·025 ml of guinea-pig complement and lymphocyte suspension were added,

TABLE 4. Horse anti-mouse anti-lymphocyte serum (chronic immunization schedule)

Horse No.	Day	Dose administered (No. of cells)	Source of antigen	Route	Day of bleeding
6768	0	$0{\cdot}5 \times 10^9$		s.c.	0
					4
	5	$1{\cdot}0 \times 10^9$		s.c.	5
	7	$2{\cdot}0 \times 10^9$		s.c.	7
					11
	12	$4{\cdot}0 \times 10^9$		s.c.	
	14	$6{\cdot}0 \times 10^9$		s.c.	
					17
	18	$22{\cdot}0 \times 10^9$		s.c.	
	20	$22{\cdot}0 \times 10^9$		s.c.	
			Mouse		24
	27	$22{\cdot}0 \times 10^9$	thymus	s.c.	
	31	$24{\cdot}0 \times 10^9$		s.c.	31
					38
	41	$24{\cdot}0 \times 10^9$		s.c.	
	45	$24{\cdot}0 \times 10^9$		s.c.	45
					49
	52	$32{\cdot}0 \times 10^9$		i.v.	52
	55	$32{\cdot}0 \times 10^9$		s.c.	
	59	$32{\cdot}0 \times 10^9$		s.c.	59
	62	$32{\cdot}0 \times 10^9$		i.v.	
					66
	73	$32{\cdot}0 \times 10^9$		i.v.	73
					76
					80

the contents mixed and incubated at 37°C for 30 min. After this time, the supernatant from each well was removed and 0·025 ml of 0·2% trypan blue prepared in isotonic saline was added to each well. Counts were performed after a 5-min interval. The end point was taken as the highest dilution which showed a 50% viability count.

In vivo testing

Antisera having haemagglutinin titres of >1/1000 were absorbed with freshly prepared, packed mouse erythrocytes before use. Equal volumes of packed cells and antisera were incubated at 37°C for 60 min, after which time the cells were removed by centrifugation (1000 *g* for 15 min). This single absorption was usually sufficient to reduce the toxicity of the sera and allowed the mouse homograft test to be performed without deaths occurring in the grafted animals. Occasionally, toxicity persisted and exhaustive adsorptions were carried out using erythrocyte stroma. The stroma was removed by centrifugation at 100,000 *g* for 45 min.

The antisera were tested for immunosuppressive activity by the method of Billingham & Medawar (1951). Male CBA mice were grafted on day 0 with tail skin from male A strain mice (i.e. across an H_2 barrier). On day 2 and again on day 5, 0·5 ml of the HAMLS under

investigation was injected subcutaneously into groups of 6–8 mice. Bandages were removed either on day 9 or 10 and the grafts examined daily; each was scored as rejected when it was totally necrotic. The median survival time (MST) of grafts for each HAMLS was calculated. With every set of HAMLS assayed, one group of grafted mice was left uninjected (MST 11·6±1·3 days) and one group was injected with the Wellcome Research Laboratories standard of HAMLS of known graft protecting potency (MST 26·7±6·0 days). A good HAMLS will have an MST of over 20 days.

RESULTS

The *in vitro* and *in vivo* tests carried out on the sera of the horses immunized by the two-pulse schedule (Table 5) indicated that the high dose level of antigen produced antisera of good immunosuppressive potency (Horse Nos 5942 and 7273), whilst low dose levels of antigen produced, in general, antisera of very poor immunosuppressive potency (Horse Nos 7131 and 7122). The adjuvants used at the low dose level had little or no effect (Horse Nos 7122 and 7209). The *in vitro* results were as expected, high haemagglutinins and leuco-agglutinins and, in general, low platelet agglutinins; low cytotoxic figures for the poorly immunosuppressive antisera, and slightly raised titres for the immunosuppressive sera.

TABLE 5. Horse anti-mouse anti-lymphocyte serum (two-pulse immunization schedule). The results of *in vitro* tests and of the assay for immunosuppressive activity of sera

Horse No.	Day	HA*	PA†	LA‡	CYT§	MST (Days)‖
5942	21	1/1536	1/1	1/576	1/48	26·7±6·0
7273	21	1/1152	1/3	1/1152	1/48	22·7±3·0
7131	21	1/240	Nil	1/1048	1/3	16·5±2·0
7122	21	1/4608	Nil	1/1472	1/8	13·0±1·5
7172	21	1/36	<1/2	1/96	1/3	12·5±1·5
7209	21	1/144	1/2	1/640	1/12	16·0±2·0

* Haemagglutination titres. † Platelet agglutination titres. ‡ Leuco-agglutination titres. § Leuco-cytotoxicity titres. ‖ Median survival times in days in mouse homograft test, group of six to eight mice±SD.

The *in vitro* and *in vivo* results of the horses immunized with the extended schedule are shown in Table 6. The *in vitro* tests performed on the antiserum produced by Horse No. 6997 indicated that it had responded very well to immunization; leucoagglutinins and cytotoxic figures were all high and the platelet agglutinins were low. However, *in vivo* tests for the immunosuppressive potency were disappointing, showing little or no activity. Horse No. 5989 on the other hand, while giving similar results as far as the *in vitro* tests were concerned, produced sera of good immunosuppressive activity from day 46 to 144, a period of almost 3 months.

The final two horses (Table 7) were immunized by the chronic immunization course. Horse No. 6768 (thymus cell antigen) showed high haemagglutinins, leucoagglutinins and cytotoxic titres, whilst the platelet agglutinins were low. The *in vivo* studies showed the immunosuppressive properties to be increasingly good from day 31 to 73, a period of 6

TABLE 6. Horse anti-mouse anti-lymphocyte serum (extended immunization schedule). The results of *in vitro* tests and of the assay of the immunosuppressive activity of horses immunized as shown in Table 2

Horse No.	Day	HA*	PA†	LA‡	CYT§	MST (Days)‖
6997	21	1/384	<1/2	1/258	1/64	11·3±1·0
	63	1/1536	1/4	1/768	1/160	13·8±2·1
	119	1/1152	1/8	1/3840	1/96	13·2±2·9
5989	21	1/92	<1/2	1/352	1/35	16·6±1·6
	46	1/576	<1/2	1/1536	1/96	18·2±2·4
	63	1/576	<1/2	1/2304	1/192	25·8±8·3
	84	1/2304	<1/2	1/2560	1/1216	20·9±5·1
	144	1/1152	1/2	1/8192	1/56	20·8±2·5
	373	1/288	<1/2	1/144	1/24	NT

NT, Not tested. * Haemagglutination titres. † Platelet agglutination titres. ‡ Leuco-agglutination titres. § Leuco-cytotoxicity titres. ‖ Median survival times in days in mouse homograft test, group of six to eight mice ± SD.

TABLE 7. Horse anti-mouse anti-lymphocyte serum (chronic immunization schedule). The results of *in vitro* tests and of the assay of the immunosuppressive activity of horses immunized as shown in Tables 3 and 4

Horse No.	Day	HA*	PA†	LA‡	CYT§	MST (days)‖
6762	12	1/1536	<1/2	1/52	1/6	15·3±3·8
	25	1/1536	1/10	1/576	1/10	20·2±3·6
	39	1/384	1/12	1/896	1/239	20·6±3·4
	53	1/1920	1/12	1/8064	1/840	24·5±4·5
	60	1/1152	1/16	1/8192	1/1024**	27·2±5·5
	74	NT	NT	1/8832	1/2048††	26·0±6·0
	91	1/4608	1/16	1/3840	1/2048	NT
6768	11	1/768	<1/2	1/36	1/32	16·0±1·9
	31	1/92	1/2	1/1536	1/96	19·2±3·6
	49	1/192	1/2	1/3072	1/80	19·5±2·1
	52	1/144	1/2	1/4624	1/256	23·5±2·9
	66	1/1920	NT	1/3200	1/1280**	26·8±4·5
	73	1/6144	NT	1/3584	1/320 **	27·5±5·0
	80	1/1152	1/6	1/3600	1/512**	24·0±6·0

NT Not tested. * Haemagglutination titres. † Platelet agglutination titres. ‡ Leuco-agglutination titres. § Leuco-cytotoxicity titres. ‖ Median survival times in days in mouse homograft test, group of 6–8 mice ± SD. ** Antisera toxic in mice, required erythrocyte absorption. †† Toxicity not completely removed after repeated absorptions with erythrocytes and erythrocyte stromata.

weeks. Horse No. 6762 (spleen cell antigen) showed slightly higher haemagglutinin, leucoagglutinin and cytotoxicity titres than its partner. Platelet agglutination was also slightly higher. The immunosuppressive properties of the antisera were very similar to Horse No. 6768, showing increased graft survival times from day 25 to 60, a period of about 5 weeks. From day 60 the antisera showed signs of toxicity.

DISCUSSION

Non-toxic, moderately good immunosuppressive antisera could be produced in the mouse–horse system, by a similar schedule to that described by other workers in the mouse–rabbit system (Levey & Medawar, 1966a, b), provided dose levels were adjusted to the body weight of the horses. If this dose level was decreased little activity as measured by the mouse homograft test was observed. The use of mineral adjuvants at these low dose levels did little to improve the situation. Although the unmodified two-pulse schedule produced good, non-toxic material it was for only a relatively short period of time. Often the horses reacted very badly to these large doses of antigen, and indeed one horse died whilst receiving its second injection of antigen. Following this experience, it was decided that horses immunized by this schedule should in future receive the antigen dose very slowly and that in addition the dose should be divided into two and administered to the horse with a short rest period between each half dose.

The *in vitro* results offered little guide as to the graft protecting activity. Jooste *et al.* (1968) showed that although all immunosuppressive antisera were cytotoxic, not all cytotoxic antisera were immunosuppressive and that the only definitive indication of activity at present was the power to prolong mouse skin homografts (Levey & Medawar, 1966a).

This was also demonstrated clearly in the present study by the two horses immunized by the extended schedule. Both horses produced antisera of varying activities. The leucoagglutinin and cytotoxicity titres of Horse No. 6997 were increased over a period of 120 days but the survival times of the skin homografts indicated antisera of poor immunosuppressive properties (MST 13·8 days). Horse No. 5989 also showed increased leucoagglutinin and cytotoxicity titres but considerable increases in MST's (20·8–25·8) of skin grafts were found for sera produced from the 63rd to 144th day of the schedule. The extended immunization schedule was capable of raising large quantities of active immunosuppressive antisera and although extravagant on antigen, was less expensive on horses than the two-pulse course. It is important to note that repeated intravenous injections of antigen over a period of 16 weeks produced non-toxic antisera, i.e. there was no evidence of the formation of unwanted antibodies other than red cell agglutinins which were absorbed out routinely if $>1/1000$.

The two horses, injected with spleen and thymus cell suspensions and immunized by the chronic immunization schedule both produced immunosuppressive antisera. The spleen cell suspensions although heavily contaminated with erythrocytes were as capable of producing active HAMLS as were the thymus cell suspensions. Removal of erythrocytes was not undertaken in this work as previous experiments in rabbits had shown that high haemagglutinin titres were produced irrespective of the concentration of erythrocyte contamination in the immunizing cell suspensions (Thomas & Edwards, unpublished results). From approximately day 60 onwards, both horses produced toxic antisera, killing several mice in the mouse homograft test. It was thought at the time that this effect might be due to anti-platelet activity but there was no evidence of petechiae at post mortem, as shown by Dr

A. W. Phillips of these laboratories, and blood smears showed platelets in abundance. In both animals the toxicity of the antiserum became progressively more serious. In the case of Horse No. 6768 receiving thymus cells, the toxicity could normally be removed with a single absorption with freshly prepared mouse erythrocytes. The sera from Horse No. 6762 were rather more difficult to deal with. Several absorptions with both mouse red cells and stromata being required to reduce toxicity to an acceptable level. As the course proceeded beyond 70–80 days both horses produced sera that could not be absorbed free from toxic antibodies even when preparations of mouse liver and lung were employed. The explanation for the toxicity of the sera is not clear but it appears spleen in particular contained minor antigens which eventually produced high titres of toxic antibodies. This type of course was undoubtedly the most economic in terms of antigen and horses. It produced antisera of good immunosuppressive activity over a considerable period and only the progressive increase in toxicity prevented the course continuing. There was no evidence of decay phenomenon in this work in contrast to the work of Jooste *et al.* (1968). This result also is in contrast to the results of Lance & Medawar (1970) testing sera produced by prolonged immunization of horses with spleen cells (Woiwod *et al.*, 1970). Their results indicated that a peak of activity occurred during which non-toxic sera of good immunosuppressive activity were produced. On one side of this peak the sera were non-toxic and inactive, whereas on the other side the sera were toxic and inactive. The explanation for the discrepancy in the results is not clear but may reside in the fact that while the antigen in both cases was spleen cells, in the mouse work fresh material yielding cells of high viability (85%) was obtained. In contrast to this, human spleens were removed many hours post mortem and of the cells obtained not more than 25% were viable. The use of such decayed material would conceivably make a considerable difference to the results obtained.

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