pools contained IgM, IgG and IgA, whereas no immunoglobulins could be detected in the β-globulin pool.

From these data it is concluded that immunoglobulins are not responsible for the spermagglutinating activity of this serum. Of the proteins detected in the spermagglutinating fractions of the serum, only β-lipoprotein has been identified in all. Consequently, it is tentatively proposed that, in this serum (and, by comparison, in the other sera fractionated with 'Sephadex *G*-200' and DEAE cellulose), β-lipoprotein is the spermagglutinating agent.

To test this proposition, 0·5 ml. of serum and 0·5 ml. of horse anti-human β-lipoprotein (Hyland, batch 8413E-003A1) were incubated together overnight at 4° C. As a control, a similar mixture using anti-IgM was set up. The precipitates were removed by centrifugation and 0·3 ml. of each supernatant was incubated with 0·03 ml. of sperm suspension. After 4 h the β-lipoprotein test showed a score of 30/100 (equivalent to 1/8 dilution of the serum in non-agglutinating serum, Table 1), whereas the IgM test showed virtually complete agglutination of all sperm. It seems possible that the spermagglutinating activity remaining in the serum after reaction with anti-β-lipoprotein is due to activity in β-lipoprotein not precipitated by the specific antiserum used here. This explanation seems likely, for immunoelectrophoresis of the serum after reaction showed no β-lipoprotein detectable, when diffusion was performed with the anti-β-lipoprotein used for precipitation, but it showed β-lipoprotein detectable when diffusion was performed with horse anti-human serum (Hyland, batch 8040E004A1). It seems, then, that the spermagglutinins in this serum are β-lipoproteins.

Table 1. SPERMAGGLUTINATING SCORES OF SERUM DILUTED WITH:

Serum dilution	Saline		Non-agglutinating serum	
	1/2 h	4 h	1/2 h	4 h
Neat	94/100	Virtually complete	94/100	Virtually complete
1/2	22/100	52/100	20/100	Virtually complete
1/4	7/100	28/100	8/100	75/100
1/8	4/100	19/100	2/100	32/100
1/16	0/100	6/100	0/100	11/100
1/32	0/100	0/100	0/100	6/100
1/64	0/100	0/100	0/100	2/100
1/128			0/100	0/100

We conclude that the technique of micro-spermagglutination[1] can detect spermagglutinins which are not immunoglobulins. Further, because the spermagglutinins in the different sera examined by us appear to be similar, it is suggested that spermagglutinins detected by this technique are commonly not immunoglobulins.

This work was supported by a grant from the Population Council (New York). Some of it was performed in the laboratory of Dr A. G. Steinberg, Case Western Reserve University, Cleveland, Ohio, while one of us (B. B.) held a Harkness Fellowship. The serum was made available through the kind cooperation of Dr W. C. Weir, Cleveland, Ohio.

B. BOETTCHER
D. J. KAY

School of Biological Sciences,
The Flinders University of South Australia,
Bedford Park,
South Australia 5042.

Received April 18; revised June 30, 1969.

[1] Franklin, R. R., and Dukes, C. D., *Amer. J. Obstet. Gynec.*, **89**, 6 (1964).
[2] Rumke, Ph., and Hellinga, G., *Amer. J. Clin. Path.*, **32**, 357 (1959).
[3] Feltkamp, T. E. W., Kruyff, K., Ladiges, N. C. J. J., and Rumke, Ph., *Ann. NY Acad. Sci.*, **124**, 702 (1965).
[4] Boettcher, B., and Hay, J., *Proceedings of the Symposium on the Immunology of Spermatozoa and Fertilization* (Varna, Bulgaria, 1967, in the press).
[5] Flodin, P., and Killander, J., *Biochim. Biophys. Acta*, **63**, 403 (1962).
[6] Osterland, C. K., in *Methods in Immunology and Immunochemistry* (edit. by Williams, C. A., and Chase, M. W.), 2 (Academic Press, New York, 1968).

Suppression of the Haemorrhagic Component of the Schwartzmann Reaction by Anti-complement Serum

WE have previously described[1,2] the suppression of the haemorrhagic component of the Arthus reaction and the passive tuberculin reaction with antilymphocytic sera (in ref. 2 this was an antiserum against an extract of lymph node cells). The other inflammatory condition in the skin with a marked haemorrhagic component is the Schwartzmann reaction: the reaction developing at the site of an earlier intradermal injection of endotoxin when the animal is challenged with endotoxin, intravenously, 24 h later. This reaction has not been found consistently in guinea-pigs, although it can be readily produced in rabbits[3]; its mechanism is not well understood. We have investigated whether antilymphocytic sera suppress the Schwartzmann reaction and, if not, whether any of the heterologous antisera used in our previous experiments would suppress this reaction.

Heterologous antisera against the following guinea-pig tissue and serum components were prepared in rabbits and absorbed as described previously: (*a*) thymocytes[4], (*b*) lymph node cell extract[1], (*c*) guinea-pig whole serum[1], (*d*) guinea-pig γ-globulin[1], (*e*) guinea-pig granulocytes[2], (*f*) guinea-pig complement (C'_3) (ref. 5). The sera were the result of immunizing individual rabbits and were selected, in the case of anti-thymocyte and anti-lymph node extract serum, for strength of action in inhibiting contact sensitivity to dinitrochlorobenzene (a cell-mediated immune response) and the Arthus reaction. The anti-complement serum was prepared by Müller–Eberhard's method in the same way as in ref. 5.

Schwartzmann reactions were induced in 450–500 g albino (Hartley strain) guinea-pigs by intradermal injection of 1 mg lipopolysaccharide B from *Escherichia coli* 026 : *B*6 (Difco Laboratories, Detroit) dissolved in 0·1 ml. 0·15 M NaCl. Twenty-four hours later, 1 mg of the lipopolysaccharide solution was injected intravenously. Just before this intravenous injection, there was a marked red indurated inflammatory reaction (15–20 mm in diameter) at the site of injection. Between 3 and 4 h after the intravenous injection, a confluent or semi-confluent area of haemorrhage (10–15 mm in diameter) appeared in nine of twelve normal guinea-pigs; in two others, petechial haemorrhages were observed in the same area.

Table 1. PROPORTION OF GUINEA-PIGS SHOWING HAEMORRHAGIC LOCAL SCHWARTZMANN REACTIONS IF TREATED WITH 1 ml. HETEROLOGOUS ANTISERUM BEFORE INTRAVENOUS INJECTION OF ENDOTOXIN

Treatment	Proportion
Untreated controls	11/12
Anti-thymocyte serum	8/9
Anti-lymph node cell extract	9/9
Anti-granulocyte serum	10/10
Anti-whole guinea-pig serum	8/10
Anti-γ-globulin	6/10
Anti-complement (C'_3)	1/9

To determine the effect of the various heterologous antisera, 1 ml. was injected intravenously into each guinea-pig about 1 h before the intravenous injection of the lipopolysaccharide. Whereas the intravenous injection of such sera, at a similar time before the intradermal injection of antigen, completely suppressed the haemorrhagic component of the Arthus reaction[1], no corresponding suppression of this component of the Schwartzmann reaction by anti-thymocyte serum, anti-lymph node cell extract serum, anti-granulocyte serum and anti-whole guinea-pig serum was observed (Table 1). Haemorrhage was, however, suppressed in four of the ten animals treated with anti-γ-globulin serum, while anti-complement serum suppressed the appearance of haemorrhage in eight of the nine animals. Histological examination showed that there was no diminution in the granulocyte infiltration at the reaction site in animals so treated.

© 1969 Nature Publishing Group

The success in producing a local Schwartzmann reaction in guinea-pigs is attributed to the use of a higher dose (1 mg lipopolysaccharide intradermally followed by 1 mg lipopolysaccharide intravenously) than that used in the only other species, the rabbit, where this reaction has been previously obtained consistently using doses of the order of 10–100 μg. The failure to use higher doses in other species has given rise to the impression that the local Schwartzmann reaction is a peculiarity of the rabbit[3].

The failure to modify this reaction by heterologous antisera other than anti-complement serum, and in a few cases with anti-γ-globulin serum, separates this reaction from other inflammatory haemorrhagic reactions of the skin, such as the Arthus reaction and delayed-type hypersensitivity reactions of the tuberculin type, which have an allergic basis[1,2]. One anomalous finding is the failure to inhibit the haemorrhage of the Schwartzmann reaction with anti-thymocyte serum. The intravenous injection of this serum produces a marked fall in the level of circulating haemolytic complement sufficient to inhibit the development of the haemorrhagic component of the Arthus reaction[1], and the inflammatory components of chemical contact sensitivity and the non-specific reaction to the intradermal injection of turpentine[4]. Similar effects on contact sensitivity and turpentine inflammation by anti-complement serum have been reported previously[5]. This anomaly could be due to the fact that complement contains at least nine components. Anti-complement serum is directed mainly against the third component, whereas the fall in total haemolytic complement as a result of treatment with an antilymphocytic serum could be a result of a fall in other components of complement. Possibly, the Schwartzmann phenomenon needs the third component of complement (among others), while the Arthus reaction, cell-mediated immune reactions, and non-specific inflammation need other components not necessary for the Schwartzmann reaction—components which are "fixed" by the reaction between the antilymphocytic antibody and the lymphocyte cell membrane.

We therefore demonstrate for the first time the consistent production of a "true" Schwartzmann reaction in a species other than the rabbit; furthermore, we elucidate the difference between this reaction and the Arthus reaction in that it can occur despite a drop in the level of total haemolytic complement, produced by an antilymphocytic serum. We suggest, however, that certain complement components are involved, for it is suppressed by an antiserum directed chiefly against the third component of complement.

L. POLÁK
J. L. TURK

Department of Immunology,
Institute of Dermatology,
St John's Hospital for Diseases of the Skin,
Homerton Grove, London E9.

Received March 12; revised April 22, 1969.

[1] Turk, J. L., and Polák, L., *Lancet*, i, 130 (1969).
[2] Turk, J. L., and Polák, L., *Int. Arch. Allergy*, **34**, 105 (1968).
[3] Johnstone, D. E., Michaelson, S. M., Tuttle, L., and Howland, J. W., *Proc. Soc. Exp. Biol. and Med.*, **99**, 15 (1958).
[4] Turk, J. L., Willoughby, D. A., and Stevens, J. E., *Immunology*, **14**, 683 (1968).
[5] Willoughby, D. A., Polák, L., and Turk, J. L., *Nature*, **219**, 192 (1968).

Immune Complexes in Mice infected neonatally with Moloney Leukaemogenic and Murine Sarcoma Viruses

SEVERAL viruses have been reported to induce immunological tolerance in mice when administered in the immediate postnatal period. Among these are the lymphocytic choriomeningitis (LCM) virus[1,2] and the murine leukaemogenic viruses[3-5]. Moloney leukaemogenic virus (MLV) induces lymphocytic leukaemia in both newborn and adult mice[6]. Inoculation of adults with MLV results, however, in the development of both humoral and cell-mediated immune responses to the virus, whereas in neonatally infected mice these responses are greatly reduced or undetectable[4,7].

Oldstone and Dixon[8,9] have recently reported that mice neonatally infected with LCM virus are not completely tolerant to this agent, but make antibodies detectable only in renal glomeruli where they are presumably deposited in the form of antigen–antibody complexes. Our observations suggest that mice neonatally infected with MLV are also not completely tolerant, but have circulating antigen–antibody complexes which are deposited in renal glomeruli. Similarly, mice infected as newborns with the late lymphocytic component of murine sarcoma virus—Harvey (MSV-H), which is antigenically closely related to, or identical with, MLV—also develop immune complexes detectable in glomeruli.

BALB/c mice were inoculated with 0·1 ml. of MLV intraperitoneally within the first 18 h of life, or were in the third to ninth generation receiving vertically transmitted milk-borne MLV or the lymphocytic leukaemogenic virus separated from MSV-H ("late" MSV)[10]. Uninfected BALB/c mice housed in the same room as infected mice served as controls. Kidneys of 1–5 month old mice were studied by light microscopic and immunofluorescence techniques. Light microscopic sections were fixed in formaldehyde–acetic acid–alcohol, and stained with haematoxylin and eosin or with periodic acid–Schiff reagents. Tissues for immunofluorescence were rapidly frozen in liquid nitrogen and isopentane. Sections (4 microns thick) were washed in phosphate buffered saline (*p*H 7·1) for 1 h to remove any unbound globulin, stained for 30 min with fluorescein isothiocyanate conjugated goat anti-mouse γ-globulin, washed thoroughly and mounted in buffered glycerol. Similar sections were examined for the presence of MLV antigens by an indirect immunofluorescence technique, using hyperimmune rabbit anti-MLV serum and fluorescein isothiocyanate conjugated horse anti-rabbit globulin serum.

Deposition of mouse γ-globulin in glomeruli of MLV or "late" MSV infected animals was demonstrable when they were 70–90 days old. No differences were noted between groups receiving neonatal inoculation of MLV and those receiving vertically transmitted MLV or "late" MSV. Detectable γ-globulin first appeared along glomerular capillary walls in a finely granular pattern. By 100–120 days, deposits had become coarse and lumpy (Fig. 1). All glomeruli appeared to be involved, but other areas of

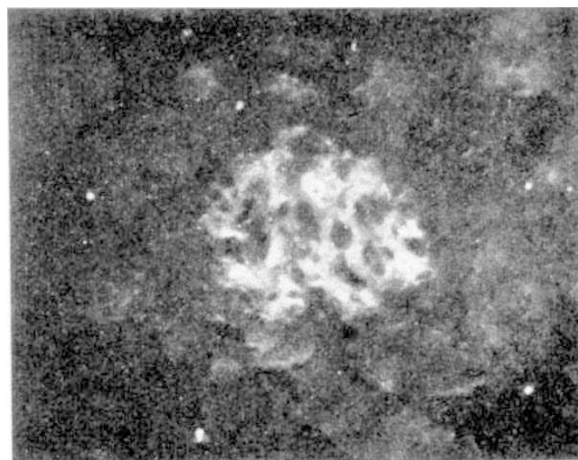

Fig. 1. Fluorescence micrograph of kidney from a 100 day old BALB/c mouse infected with MLV by vertical transmission, showing coarse, lumpy deposition of mouse γ-globulin in glomerular tufts. (Fluorescein isothiocyanate-conjugated goat anti-mouse globulin, × 450.)

© 1969 Nature Publishing Group