

in the PHA + LCM group occurred earlier and the mortality rate was also higher than in animals infected with LCM virus but not pretreated with PHA. It is known that, similarly to homograft rejection, it is the cellular immune response that gives rise to the neurological symptoms of i.cer. LCM virus infection and to the fatal outcome of lymphocytic choriomeningitis⁵. Findings of our experiment suggest that PHA treatment applied 1 day before i.cer. LCM virus infection enhances the cellular immune reaction of mice to the virus infection. Our results are in accord with the observation that i.v. PHA treatment accelerates the skin graft rejection in mice².

Zusammenfassung. Einmalige i.v.-Injektion von Phytohämagglutinin erhöht die zelluläre Immunreaktion der Mäuse gegen LCM-Virusinfektion.

Zs. BÁNOS, I. SZERI and P. ANDERLIK

Institute of Microbiology, Semmelweis University of Medicine, Hgyes Endre ut. 7-9, Budapest IX (Hungary), 19 March 1973.

⁵ J. HOTCHIN, *Monograph in Virology* (Ed. J. L. Melnick; S. Karger, Basel 1971), vol. 3, p. 57.

An Incomplete Anti-B Agglutinin in the Eggs of the Prosobranch Snail *Pila ovata*

The albumin glands of snails have recently become highly attractive objects of research, since they have been found to contain not only galactogen and enzymes of galactose metabolism¹, but also heterophile agglutinins to various animal cells², as well as a number of polyvalent iso-inhibitors of proteinases³. We have already studied the agglutinin and proteinase-inhibitor activities in the albumin gland of *Pomacea urceus*⁴. In more recent work on the eggs of the closely related prosobranch *Pomacea canaliculata*, we have shown that the agglutination pattern is virtually identical with that of *Pomacea urceus*. The results are shown in Table a. Inhibition is found in both cases with pig amnionic mucoid, peptone A sub-

stance, pneumococcus Type XIV polysaccharide and N-acetyl-D-glucosamine, although small differences were found for the agglutinins from the two sources (Table b). No immunological cross-reactions were seen, on the other hand, with the egg extract from *Pomacea canaliculata*

¹ W. FISCHER and H. WEINLAND, *Der Stoffwechsel der Galaktose und ihrer Derivate* (Thieme Verlag, Stuttgart 1965).

² O. PROKOP, G. UHLENBRUCK and W. KÖHLER, *Vox Sang.* 14, 321 (1968).

³ G. UHLENBRUCK, I. SPRENGER and I. ISHIYAMA, *Z. klin. Chem.* 9, 361 (1971).

⁴ G. UHLENBRUCK and G. STEINHAUSEN, *Blut* 25, 335 (1972).

Table a. Agglutination by red cell agglutinins from prosobranch snails of the genera *Pomacea* and *Pila*

Origin of red cells	Extract from Red cells	Titer against agglutinin extracts								
		PU Normal	PC	PO	PU Pronase-treated	PC	PO	PU RDE-treated	PC	PO
Human A		256	64	—	16,000	500	—	1000	256	—
O		256	64	—	8,000	256	—	4000	256	—
B		128	128	—	8,000	500	16	1000	128	16
Horse		2	4	—	2	8	—	2000	16	—
Bovine		8	4	4	512	256	128	4	8	8
Pigeon		—	4	—	2,000	500	—	500	64	—
Pig		256	64	4	500	1000	128	500	4000	16
Cat		—	8	32	500	32	128	1000	256	256
Sheep		128	—	—	500	4	—	1000	32	—
Rabbit		1000	500	32	500	2000	4000	500	1000	128
Bull frog		8	—	4	64	4	128	32	2	16

Table b.

Inhibition of haemagglutination by Red cells from	PU	PC	PO	PU	PC	PO
	Human A	Human A	Human B pronasetreated	Pig	Pig	Pig pronase treated
S XIV polysaccharide	4	128	—	—	2000	—
Peptone A substance	64	128	8	32	128	2
Pig amnionic mucoid	32	64	128	2	128	64
D-galactose	2	—	16	—	—	2
D-melibiose	8	—	32	8	2	—
D-glucose	—	—	2	16	—	2
L-rhamnose	—	—	8	—	—	ND
N-acetyl-D-galactosamine	2	—	—	16	—	—

PU, *Pomacea urceus*; PC, *Pomacea canaliculata*; PO, *Pila ovata*; RDE, neuraminidase (Behring Werke Marburg); ND, not determined.

and an antiserum prepared against the content of the albumin gland of *Pomacea urceus*. Furthermore, the eggs and albumin gland of *Pomacea canaliculata* contain an unusual polyvalent proteinase inhibitor ovorubin, a glycoprotein with a carotenoid prosthetic group, which was first isolated by CHEESMAN⁵ and tested extensively against proteinases by NORDEN⁶.

It was hence of interest to include a further related snail in the experiments, the prosobranch *Pila ovata*. The only material available from this animal was an egg mass kept for several years in the frozen state. Similar had, indeed, been used in the case of *Pomacea canaliculata*, but it seems that most of the relevant constituents of the albumin gland occur also in the eggs⁷. Agglutination and inhibition experiments made with saline extracts of the eggs revealed the presence of an incomplete anti-B (or anti-B-like) agglutinin, which reacted with human B cells only when these had been treated with proteinase (pronase) or neuraminidase. It also reacted with bovine red cells, which were capable of adsorbing it completely. Red cells of some other species also reacted and adsorbed the agglutinin, whereas others did not (Table a). A B-like antigen of plant origin containing thylacoids and fragments of the lamellar system⁸ also reacted with this anti-B-like substance. Other workers have already reported the occurrence of anti-B or anti-B-like agglutinins in certain snails⁹⁻¹¹. Similar anti-B reagents have been found in the eggs of fishes¹²⁻¹⁵. The relationships and the biological functions of these anti-B antibody-like substances have still to be established.

Zusammenfassung. In den Eiern der Schnecke *Pila ovata* wurde ein Agglutinin aufgefunden, welches in «inkompletter» Weise mit einem Blutgruppen-B-ähnlichen Antigen von verschiedenen Erythrozyten reagiert. Agglutinations- und Agglutinationshemmtiter werden mit denen von den verwandten Schnecken *Pomacea urceus* und *Pomacea canaliculata* verglichen.

G. UHLENBRUCK, G. STEINHAUSEN
and D. F. CHEESMAN

Department of Immunobiology, Medical University Clinic,
Kerpenerstrasse 15, D-5000 Köln 41 (Germany) and
Department of Biochemistry,
Bedford College, London N.W.1 (England), 26 March 1973.

- ⁵ D. F. CHEESMAN, Proc. R. Soc., B 149, 571 (1958).
- ⁶ D. A. NORDEN, Comp. Biochem. Physiol. 42b, 569 (1972).
- ⁷ G. UHLENBRUCK, I. SPRENGER and I. ISHIYAMA, Experientia 28, 242 (1972).
- ⁸ G. UHLENBRUCK and A. RADUNZ, Z. Naturforsch. 27b, 1113 (1972).
- ⁹ M. KRÜPE and H. PIEPER, Z. Immunforsch. 130, 296 (1966).
- ¹⁰ S. SCHNITZLER and R. KILIAS, Blut 20, 221 (1970).
- ¹¹ H. PIEPER and M. KRÜPE, Z. Immunforsch. 142, 141 (1971).
- ¹² G. UHLENBRUCK and O. PROKOP, Vox Sang. 12, 465 (1967).
- ¹³ K. JAROSCH, S. SCHNITZLER, O. PROKOP and G. UHLENBRUCK, Z. ärztl. Fortbild. 61, 758 (1967).
- ¹⁴ O. PROKOP, D. SCHLESINGER and G. GESERICK, Z. Immunoforsch. 132, 491 (1967).
- ¹⁵ S. SCHNITZLER, Acta biol. med. germ. 20, 256 (1968).

The Effect of Kidney-Bean Leucoagglutinin on Homograft Rejection in Mice

There have been a number of studies of the effects of phytohaemagglutinin (PHA) extracted from kidney beans (*Phaseolus vulgaris*) on homograft rejection in various experimental animals. Most of the studies indicate that PHA has a weak immunosuppressive action¹⁻⁸ although a couple of conflicting reports have been published⁹⁻¹¹. Most of this work has been performed with rather crude, commercially available, PHA preparations, containing several different proteins. The well-known biological effects of PHA, i.e. lymphocyte stimulation and leuco- and erythroagglutination, have been shown to be produced by two kinds of glycoproteins, purely leucoagglutinating and both erythro- and leucoagglutinating¹²⁻¹⁵. The present study was undertaken to investigate the effects of a purified lymphocyte-stimulating leucoagglutinin on homograft rejection in mice. The effects of the leucoagglutinin were compared with those of rabbit-antimouse-thymocyte-globulin (RAMTG).

Materials and methods. Kidney bean leucoagglutinin (La) was prepared as described previously^{13,15}. Heat denatured leucoagglutinin (DenLa) was prepared by heating native La for 30 min at 100°C. This procedure caused precipitation of the leucoagglutinin. Periodate oxidation of La (OxLa) and lymphocyte-stimulation and agglutination tests were performed as described before^{13,15}.

RAMTG was prepared by immunizing rabbits with thymocytes from 2-week-old CBA mice. Each rabbit received 2 injections of $2 - 3 \times 10^8$ cells. The first injection was given intracutaneously and s.c., with the cells emulsified in Freund's complete adjuvant. The second injection was given i.v. without adjuvant. Serum was collected 7 days after the second injection and the IgG fraction was isolated¹⁶.

Two-month-old CBA mice weighing 24-28 g, were grafted with fetal hearts from C57 Black donors, as

described^{17,18}. The functional state of the grafts was assessed by examination under a stereomicroscope. Grafts showing no pulsatile activity 7 or 11 days after transplantation were considered to be surgical failures and were excluded from the test series.

- ¹ R. Y. CALNE, J. R. WHEELER and B. A. L. HURN, Br. med. J. 2, 154 (1965).
- ² K. MARKLEY, G. EVANS and E. SMALLMAN, Transplantation 5, 1535 (1967).
- ³ R. L. ST. PIERRE, J. B. YOUNGER and C. M. ZMIJEWSKI, Proc. Soc. exp. Biol. Med. 126, 687 (1967).
- ⁴ K. ONO, H. BLANCHARD, N. KASHIWAGI, R. N. M. MAC SWEEN, H. N. CLAMAN and T. E. STARZL, Surgery 67, 322 (1970).
- ⁵ S. S. STEFANI and C. D. MOORE, J. Immun. 104, 780 (1970).
- ⁶ C. D. MOORE and R. G. SLAVIN, Transplantation 11, 563 (1971).
- ⁷ K. MARKLEY, S. W. THORNTON and E. SMALLMAN, Proc. Soc. exp. Biol. Med. 139, 37 (1972).
- ⁸ W. ROSENAU, J. HABLER and M. GOLDBERG, Transplantation 13, 624 (1972).
- ⁹ M. W. ELVES, Transplantation 5, 1532 (1967).
- ¹⁰ B. KEHN and P. RIGBY, Nature 216, 182 (1967).
- ¹¹ M. LANDY and L. N. CHESSIN, Antibiotica Chemother. 15, 199 (1969).
- ¹² T. WEBER, C. T. NORDMAN and R. GRÄSBECK, Scand. J. Haemat. 4, 77 (1967).
- ¹³ T. WEBER, Scand. J. clin. Lab. Invest., Suppl. 111, 1 (1969).
- ¹⁴ L. W. ALLEN, R. H. SVENSON and S. YACHNIN, Proc. natn. Acad. Sci. 63, 334 (1969).
- ¹⁵ T. WEBER, C. T. NORDMAN and H. ARO, Biochim. biophys. Acta 263, 94 (1972).
- ¹⁶ M. JOUSTRA and H. LUNDGREN, *Proteins of the Biological Fluids* (Ed. H. PEETERS; Pergamon Press, London 1969), vol. 17, p. 511.
- ¹⁷ R. I. FULMER, A. T. CRAMER, R. A. LIEBELT and A. G. LIEBELT, Am. J. Anat. 113, 273 (1963).
- ¹⁸ K. P. JUDD and J. J. TRENTIN, Transplantation 11, 298 (1971).