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 CHEESMAN[5] and tested extensively against proteinases by NORDEN[6].

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 eggs[7]. 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 system[8] 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 snails [9–11]. Similar anti-B reagents have been found in the eggs of fishes[12–15]. 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.

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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 action[1–8] although a couple of conflicting reports have been published[9–11]. 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 leucoagglutinating[12–15]. 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 isolated[16].

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.

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Table I. Characteristics of native kidney bean leucoagglutinin (La), leucoagglutinin oxidized with sodium periodate (OxLa) and heat denatured leucoagglutinin (DenLa)

	Lymphocyte stimulation[a] (μg/ml)	Leucoagglutination[b] (μg/ml)
La	3–5	5–10
OxLa	–	20–40
DenLa	–	–

[a] Amount of substance giving maximal stimulation[15]. [b] Minimum amount of substance giving detectable leucoagglutination[13].

Table II. Acute toxicity of kidney bean leucoagglutinin given as a single dose into the tail vein of adult CBA mice

Dose injected (mg/kg body wt.)	No. of animals treated	No. of animals surviving after indicated time period			
		5 h	1 day	2 days	2 weeks
8	28	13	7	4	4
4	21	21	21	19	19
2	10	10	10	10	10

commercially available preparations tested. On macroscopic autopsy, the animals killed showed greatly enlarged spleens and haemorrhages in the lungs, intestines and livers. Probably the acute toxic effects are caused by extensive vascular damage.

The results of the tests of immunosuppression are shown in Table III. As can be seen, the different preparations of leucoagglutinin all produced a slight prolongation of the graft survival time. However, this effect was only achieved by using doses near the lethal ones and, in addition, the effect was weak compared with that of RAMTG. The immunosuppressive effect was diminished, but not completely abolished, by inactivation of the lymphocyte-stimulating or leucoagglutinating activities by sodium periodate oxidation or heat denaturation. These results thus support those of previous investigations, indicating slight immunosuppression by kidney bean extracts[1–8]. Within 21 days the test animals did not produce antibodies against leucoagglutinin detectable by immunodiffusion in agar. It is possible that crude bean extracts are more potent as immunosuppressants than purified lymphocyte-stimulating fractions. It may thus be worth while to investigate fractions other than the leucoagglutinating and lymphocyte-stimulating ones from kidney beans for immunosuppressive effects. The prospective clinical use of the kidney bean leucoagglutinin for prolongation of graft survival seems to be highly questionable, due to its high toxicity and very weak immunosuppressive effect. However, other substances in the kidney bean extracts may be less toxic and more effective as immunosuppressants.

Table III. Number of recipients bearing visibly pulsatile cardiac grafts at different time periods after grafting

Treatment (mg/kg body wt.)		No. of mice with visibly pulsatile grafts after indicated number of days %					
		7	11	14	17	21	32
La	(2)	8 (100)	8 (100)	0 (0)	0 (0)	0 (0)	0 (0)
La	(4)	18 (86)	21 (100)	19 (90)	1 (8)	0 (0)	0 (0)
OxLa	(4)	18 (100)	12 (67)	7 (39)	0 (0)	0 (0)	0 (0)
DenLa	(4)	16 (94)	17 (100)	5 (29)	0 (0)	0 (0)	0 (0)
RAMTG	(150)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)
BSA	(4)	14 (100)	12 (86)	2 (14)	0 (0)	0 (0)	0 (0)

All test substances were injected i.v. into the tail veins, the treatment starting 1 or 2 days before the grafting and then continuing twice weekly until rejection. Control mice received 100 μg bovine serum albumin (BSA).

Results and discussion. The i.v. route of administration was chosen, because we wanted a uniform distribution of the substance in the test animals. The characteristics of the test substances are summarized in Table I. The native leucoagglutinin is strongly lymphocyte-stimulating Leucoagglutinin oxidated with sodium periodate is no longer lymphocyte-stimulating, although most of its agglutinating activity is preserved. The heat denatured leucoagglutinin was inactive in vitro. These 3 forms of the leucoagglutinin were used in order to elucidate the relationship between the in vitro activities and the in vivo effects.

The acute toxicity of the leucoagglutinin was rather high, which limited the range of doses usable in the test series. The results of the toxicity tests are summarized in Table II. There have been a few reports on the acute toxicity of commercially available PHA preparations. While a dose of 150–250 mg/kg phytohaemagglutinin-M (Difco Inc. or General Biochemicals Inc., USA) has been reported to cause significant mortality in mice[10,19], NORINS and MARSHALL[20] reported that 50–150 mg/kg of phytohaemagglutinin (Wellcome Research Laboratories, England) was sufficient to produce some mortality. Thus the leucoagglutinin is considerably more toxic than the

Zusammenfassung. Nach der Wirkung auf die Überlebenszeit von Transplantaten bei Mäusen zu schliessen, besitzt Leucoagglutinin aus *Phaseolus vulgaris* extrahiert, eine nur schwache immunosuppressive Aktivität. Sie ist nicht deutlich mit der lymphozytenstimulierenden oder agglutinierenden Aktivität verknüpft. Eine starke akute Toxizität von Leukoagglutinin wurde festgestellt.

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