tions: 1. After platelet aggregation by Thrombofax has occurred, the sample is rapidly centrifuged to remove aggregates; then 0.2 ml of the supernatant is added to 0.8 ml PRP in the aggregometer: an immediate aggregation is invariably obtained, indicating the presence of an aggregating activity other than Thrombofax; this activity was found to correspond to about 1 μM ADP/10^8 platelets, measured as platelet-aggregating equivalent, according to WEISS and ROGERS[11]. 2. Adenosine, prostaglandin E_1, EDTA, apyrase, the enzymatic system phosphoenolpyruvate-pyruvate kinase, 2-deoxy-D-glucose and antimycin A (the last 2 substances used simultaneously) all inhibit platelet aggregation by Thrombofax at the same concentrations which inhibit aggregation by 2 μM ADP. 3. Both acetylsalicylic acid and indomethacin, at concentrations inhibiting platelet aggregation by 40 µg/ml collagen (Stago), strongly inhibit aggregation by Thrombofax. The aggregation by Thrombofax was also inhibited in plasma from normal people receiving the above anti-inflammatory drugs[12,13].

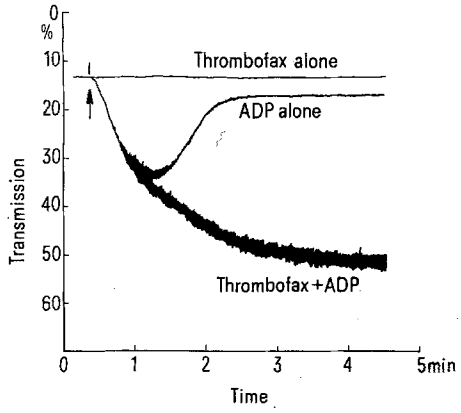


Fig. 3. Effect of a non-aggregating concentration of Thrombofax (diluted × 64 in isotonic saline) on human platelet aggregation by ADP ($2 \times 10^{-7} M$, final concentration).

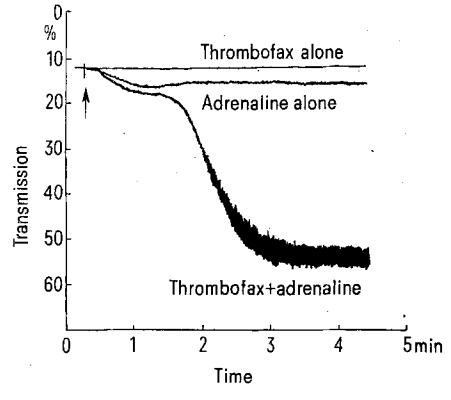


Fig. 4. Effect of a non-aggregating concentration of Thrombofax (diluted × 64 in isotonic saline) on human platelet aggregation by adrenaline ($10^{-6} M$, final concentration).

That Thrombofax induces the platelet 'release reaction' is also suggested by the following observations: 1. Thrombofax releases ^{14}C-serotonin from normal platelets: after 10 min aggregation, between 60% and 80% of the platelet-bound ^{14}C-serotonin is extruded. Acetylsalicylic acid and indomethacin ($2 \times 10^{-4} M$ final concentration) almost totally inhibit such a release. 2. Thrombofax also provokes the release of platelet factor-4 (PF-4) activity, measured by the method of HARADA and ZUCKER[14] as described by DONATI et al.[15].

In 12 normal subjects, the mean PF-4 activity released after 10 min aggregation by Thrombofax was 0.46 ± 0.08 PF-4 units/ml, which corresponds to about 80% of the mean total PF-4 activity (obtained by incubating PRP with Triton X-100[15]). Release of PF-4 activity, as well as aggregation by Thrombofax, are less pronounced at room temperature than at 37 °C. Acetylsalicylic acid and indomethacin ($2 \times 10^{-4} M$, final concentration) strongly inhibit the release of PF-4 induced by Thrombofax. The ingestion of 500 mg acetylsalicylic acid or 50 mg indomethacin provokes the same effect.

In conclusion, Thrombofax brings about platelet aggregation through the release of endogenous platelet ADP; ^{14}C-serotonin and PF-4 activity are released at the same time. FFA contained in the reagent seem to be responsible for the observed phenomena[16].

Résumé. Le Thrombofax Ortho provoque l'agrégation des plaquettes humaines (ainsi que celles du chien, du cobaye et du rat) suite à la libération d'ADP endoplaquettaire. La ^{14}C-sérotonine et le facteur plaquettaire 4 sont aussi libérés au cours de la réaction. La fraction active du produit semble résider dans les acides gras libres.

G. DE GAETANO[17], J. VERMYLEN and M. VERSTRAETE

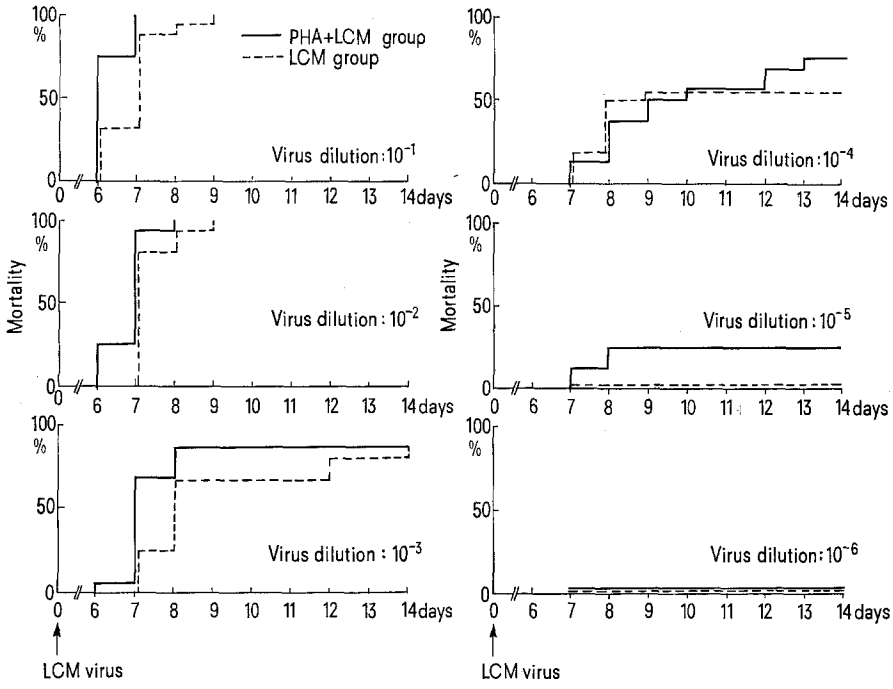
Laboratory of Blood Coagulation, Medical Research Department, University of Leuven, Leuven (Belgium), 27 March 1973.

[11] H. J. WEISS and J. ROGERS, Blood *39*, 187 (1972).
[12] G. DE GAETANO, M. B. DONATI and J. VERMYLEN, Int. J. clin. Pharmac. *5*, 196 (1971).
[13] G. DE GAETANO, M. CASTEELS-VAN DAELE, J. H. CLAES and R. EECKELS, Helv. paediat. Acta *26*, 423 (1971).
[14] K. HARADA and M. B. ZUCKER, Thromb. Diath. haemorrh. *25*, 41 (1971).
[15] M. B. DONATI, M. PALESTER-CHLEBOWCZYK, G. DE GAETANO and J. VERMYLEN, Adv. expt. Med. Biol. *34*, 295 (1972).
[16] The experienced technical assistance of Miss A. VANDENBUSSCHE is gratefully acknowledged.
[17] Fellow of the Katholieke Universiteit te Leuven, 1972/73. Present address: Laboratory for Haemostasis and Thrombosis Research, Mario Negri Institute, V. Eritrea, 62, I-20157 Milano (Italy).

Effect of Phytohaemagglutinin on the Course of Lymphocytic Choriomeningitis Virus Infection in Mice

The effect of phytohaemagglutinin (PHA) on different types of cellular immune response has been investigated by several authors. However, experimental data concerning the influence of PHA on the cellular immune response seem to be contradictory. This effect of PHA depends on the mitogenic activity of the preparation as well as on the dose and mode of its application[1,2]. The course of intracerebral (i.cer.) lymphocytic choriomeningitis (LCM) virus infection has been studied in our earlier experiments

[1] K. MARKLEY, S. W. THORTON and E. SMALLMAN, Proc. Soc. exp. Biol. Med. *139*, 37 (1972).
[2] GY. JÁNOSSY, GY. PETRÁNYI JR., and P. ALFÖLDY, Folia biol., Praha *15*, 453 (1969).



Rate and time course of death in PHA-treated and non-treated animals in consequence of i.cer. LCM virus infection, during the parallel titration.

in mice treated with phytohaemagglutinin (Phytoclin, Wellcome Research Laboratories), and it was found that repeatedly given i.p. PHA failed to influence the cellular immune response of mice to i.cer. LCM virus infection[3]. In our present work the course of i.cer. LCM virus infection was studied in mice treated with a single i.v. injection of purified PHA.

Materials and methods. Experiments were performed on 5-week-old Swiss mice. Purified phytohaemagglutinin (Lot Br_7 Wellcome Research Laboratories) was administered and, in the case of a single i.v. injection, the LD_{50} was found to be 20 mg/kg for the mice. 150 mice were given i.v. injection of 10 mg/kg PHA. The following day parallel titrations were performed under identical conditions simultaneously on PHA-pretreated (PHA + LCM group) and untreated (LCM group) animals, using the WE strain of LCM virus. Tenfold dilution between 10^{-1} and 10^{-6} was used for determination of the virus titer. 16 animals of both groups were infected intracerebrally with each dilution. The remaining PHA-treated and an identical number of untreated animals served as PHA and control groups. The development of typical neurological symptoms of LCM virus infection, as well as the mortality rate, were observed in the virus-infected groups. 16 animals were sacrified from both the PHA and control groups 3 and 10 days after PHA treatment. Relative spleen weight of succumbed and sacrificed mice as well as the spleen index were determined in each group. Absolute lymphocyte counts were determined in the peripheral blood of uninfected animals from the PHA and control groups.

Results and discussion. Mortality of PHA treatment was 2% and occurred within 24 h after treatment. No further death was observed during the experiment in the PHA group. As compared with the untreated controls, PHA treated animals displayed higher absolute lymphocyte counts and significantly increased spleen weight on the 3rd day after treatment. Moderate increase of absolute lymphocyte count and spleen hypertrophy could be observed 10 days following the PHA treatment.

Rate and time course of death observed during the parallel virus titration in the PHA + LCM and LCM groups are summarized in the Figure. Comparing data of the PHA + LCM groups, it appeared that on applying the virus dilutions 10^{-1} and 10^{-3} death occurred earlier and the dilutions 10^{-4} and 10^{-5} caused higher mortality rate in the PHA + LCM group than in the LCM group. Typical neurological symptoms of i.cer. LCM virus infection were apparent before death in each animal succumbing during the parallel titration. Calculating the LD_{50} of virus with the REED-MUENCH formula, it was found to be $10^{-4.41}$ for the PHA + LCM group and 10^{-4} for the LCM group on the basis of our results. PHA-pretreated and untreated animals thus displayed different response to the same quantity of virus. Average spleen weight was higher in animals of the PHA + LCM group succumbing 6 to 9 days after LCM infection than in the LCM group. Spleen index was 1.17 in the PHA + LCM group and 1.0 in the LCM group.

Average spleen index and absolute lymphocyte count 3 and 10 days following PHA treatment in PHA and control mice groups

Mice groups	Average absolute lymphocyte count (days after treatment)		Spleen index	
	3	10	3	10
PHA	8000	6600	1.7	1.2
Control	5000	5000	1.0	1.0

A single i.v. injection of purified PHA caused spleen hypertrophy and rise in the absolute lymphocyte count in the peripheral blood 3 days after the treatment. This observation tallied with the experimental data of other authors[4]. Besides this known effect of i.v. PHA treatment, the changed response to i.cer. LCM virus infection of the animals could also be observed. Death of animals

[3] Zs. BÁNOS, I. SZERI, P. ANDERLIK and B. RADNAI, Experientia *25*, 1332 (1969).

[4] L. B. EPSTEIN and CH. W. SMITH, J. Immun. *100*, 421 (1968).

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 choriomeningitis[5]. 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 mice[2].

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, Hogyes 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 metabolism[1], but also heterophile agglutinins to various animal cells [2], as well as a number of polyvalent isoinhibitors of proteinases[3]. 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 substance, 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	Titer against agglutinin extracts								
Extract from Red cells	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.