LD_{50}. However, the suppression of tuberculin hypersensitivity with a well tolerated dose of rifampicin seems more significant. It demonstrates the efficacy of a single dose applied with the antigenic challenge and confirms the in vivo immunosuppressive action of this antibacterial agent.

In passing, another recently discovered effect of rifampicin might be mentioned, namely its marked antagonism to the toxic octapeptide phalloidin isolated from the poisonous mushroom *Amanita phalloides*[9].

Note added in proof. Depression of tuberculin sensitivity in guinea-pigs by chronic application of rifampicin (40 mg/kg for 30 days) has been reported by G. ALGEORGE and DORINA RUDESCU (Z. Immunforsch. exp. Ther *144*, 459, 1973).

Zusammenfassung. Rifampicin hemmte die Tuberkulinreaktion bei Meerschweinchen. Eine andere Manifestation zellulärer Immunität, die Abstossung von Hautallotransplantaten bei Mäusen, wurde erst bei Anwendung toxischer Dosen des Tuberkulostatikums vermindert.

G. L. FLOERSHEIM[10]

Department of Pharmacology, University of Basel, Basel (Switzerland), 12 July 1973.

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Studies on Phytohemagglutinins XV. Hemagglutinins of the Pea and the Lentil: Advantage of their Application in Comparison to Concanavalin A in some Agglutination Reactions

The exponentially increasing interest in the use of concanavalin A in studies of various biological systems, and some observations that the physico-chemically well characterized pea and lentil hemagglutinins can match or surpass concanavalin A in most of its applications[1–3], initiated the present comparative study. The aim was (1) to compare the agglutinating activity of the 3 phytohemagglutinins against various animal erythrocytes and (2) to supply basic characterization of the structural requirements of the carbohydrate-binding sites of these phytohemagglutinins under identical conditions.

Materials and methods. For our studies concanavalin A was prepared by the Sephadex adsorption procedure from the jack bean meal as described by AGRAWAL and GOLDSTEIN[4]. The pea and lentil hemagglutinins were prepared by the procedure described in our previous papers[5,6]. All the 3 phytohemagglutinins have similar molecular weights[5–7]. The hemagglutinating activity as well as the inhibitory activity of saccharides were determined by the methods referred to by TOBIŠKA[8].

For the comparison of the inhibitory activity of sugars, and the glycopeptide receptor from human erythrocytes (designated in the original paper as glycopeptide I.3[9], rabbit and dog red blood cells were used; all of them were agglutinated by all the 3 tested phytohemagglutinins. In the case of dog red blood cells, the hemagglutinin concentration was 4 times higher than the highest dilution at which hemagglutination was observed. With rabbit red blood cells, the highest dilution of the hemagglutinin solution which produced hemagglutination had to be used to find a perceptible inhibition by sugars.

Results and discussion. Results of the first part of the study are summarized in Table I. It can be seen that the pea and the lentil hemagglutinins show in all the agglutination reactions higher activity than concanavalin A, but differ in specificity towards erythrocytes of different

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Table I. Hemagglutinating activity of concanavalin A, pea and lentil hemagglutinins against erythrocytes of different origin

Species	Activity (titer)[a]		
	pea PHA[b]	lentil PHA[b]	concanavalin A
Dog (*Canis familiaris*)	1024	1024	512
Chicken (*Gallus domest.*)	256	256	64
Domestic pig (*Sus scroja domest.*)	2048	2048	8
Domestic goat (*Capra hircus*)	0	0	0
Cow (*Bos taurus domest.*)	0	0	0
Mouse (*Mus musculus*)	2048	4096	256
Rat (*Rattus norvegicus*)	2048	2048	64
Guinea pig (*Cavia porcellus*)	8192	4096	1024
Squirrel (*Sciurus vulgaris*)	32768	32768	1024
Rabbit I	65536	65536	1024
Rabbit II (*Oryctolagus cuniculus f. domest.*)	4096	4096	128
Vervet monkey (*Cercopithecus callitrichus*)	8192	16384	256
Man (*Homo sapiens*)	512	512	0
Frog (*Rana temporaria*)	2048	4096	64

[a] Titer of 1% phytohemagglutinin solution. [b] PHA, phytohemagglutinin.

Table II. Inhibition effect of simple sugars and glycopeptide I.3 on hemagglutinating activity of concanavalin A, pea and lentil hemagglutinins towards rabbit and dog erythrocytes

Saccharide	Inhibition activity (titer)[a]		
	pea PHA[c]	lentil PHA[c]	concanavalin A
	Dog erythrocytes		
D-glucose	8	2	8
D-mannose	32	4	32
methyl α-D-glucopyranoside	32	4	32
N-acetyl-D-glucosamine	4	2	4
L-fucose	0	0	0
sialic acid[b]	0	0	7
D-galactose	0	0	0
glycopeptide I.3	32	64	32
	Rabbit erythrocytes		
D-glucose	4	2	16
D-mannose	32	4	32
methyl α-D-glucopyranoside	32	4	128
N-acetyl-D-glucosamine	8	2	8
L-fucose	0	0	0
sialic acid[b]	0	0	0
D-galactose	0	0	0
glycopeptide I.3	64	32	64

[a] Numbers indicate last degree of 2-fold serial dilution of 0.1 *M* sugar or 1% glycopeptide solution, which causes perceptible inhibition of hemagglutination by 4 minimum hemagglutinating doses in the case of dog erythrocytes and by 1 minimum hemagglutinating dose in the case of rabbit erythrocytes. [b] Sialic acid (N-acetylneuraminic acid) solution was neutralized with NaOH. [c] PHA, phytohemagglutinin.

origin. This difference is especially remarkable in human erythrocytes where concanavalin A is completely inactive.

For the comparison of the inhibitory activity or carbohydrates in hemagglutination, rabbit and dog erythrocytes were used. Rabbit erythrocytes were chosen for relatively high agglutination titers by all of the 3 agglutinins and the characteristic difference in agglutinability by concanavalin A and by the pea and lentil hemagglutinins. With dog erythrocytes, on the other hand, the difference in the agglutination activity by the individual hemagglutinins was the smallest one observed. For assays with simple sugars, the sugar components (D-mannose, N-acetyl-D-glucosamine, L-fucose, sialic acid and D-galactose) of highly active glycopeptide I.3[9] were chosen to show the possible share in the inhibitory power of the glycopeptide. In addition to them D-glucose and methyl α-D-glucopyranoside were examined. As shown in Table II, glycopeptide I.3, though isolated from human B-erythrocytes[9] exhibits a relatively high and nearly uniform inhibitory activity also in systems with rabbit and dog erythrocytes with no particular difference with regard to the hemagglutinins used. With low molecular carbohydrates, two important facts are apparent. Methyl α-D-glucopyranoside and D-mannose are the most active inhibitors of all the small carbohydrates tested. Most conspicuous is the activity of α-D-glucopyranoside in the case of the rabbit erythrocytes and concanavalin A. Other important fact is the low sensitivity of the lentil hemagglutinin towards simple carbohydrates that stands in contrast to its relatively high sensitivity towards the glycopeptide. This property is evidently due to somewhat different arrangement of the lentil agglutinin binding site with regard to the other two phytohemagglutinins. It is interesting to note that D-mannose is the only highly active monosaccharide from all the sugars tested that compose the glycopeptide. It seems likely that this sugar is also the main carrier of the glycopeptide inhibitory activity in erythroagglutination. The weak inhibition of the lentil hemagglutinin by low molecular saccharides on one hand, and a strong inhibition by the glycopeptide on the other, documents the fact that some of the phytohemagglutinins require in addition to the specific structure of the haptenic dominant also its incorporation into a more complex carbohydrate molecule, the components of which and their sterical arrangement are important for the completion of the binding interaction. Even more evident is this fact in the case of the hemagglutinin of *Phaseolus coccineus* L., that is inhibited by the glycopeptide, although no one of the monosaccharide components of the glycopeptide alone is active[9].

As shown in the present paper, the phytohemagglutinins of the pea and lentil that have been well characterized[5,6] are more active in agglutination of erythrocytes and display some important differences in activity towards erythrocytes from different animals. We hope that the application of these, as well as of other phytohemagglutinins, will help to elucidate the structure of the receptors responsible for agglutination and other important biological reactions. The possible application as anti-tumor agents in vivo of phytohemagglutinins exhibiting similar specificity but lower toxicity for the host than concanavalin A, represents another stimulus in this field of research[10].

Zusammenfassung. Phythämagglutinine der Erbse und Linse besitzen ähnliche biologische Eigenschaften wie Concanavalin A; ihre Aktivität ist jedoch in allen Agglutinationsreaktionen grösser als die von Concanavalin A.

L. Bureš[11], G. Entlicher, M. Tichá and J. Kocourek

Department of Biochemistry, Charles University, Albertov 2030, Praha 2, (Czechoslovakia), 12 June 1973.

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[11] Present address: 2nd Research Unit of Internal Medicine, Institute for Clinical and Experimental Medicine, Budějovická 800, Praha 4 - Krč, Czechoslovakia.