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Studies on haemagglutination by different fungi

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Summary: 17 fungi representing dermatophytes, yeasts and moulds were examined for their capability to agglutinate red blood cells of fowl, g. pig, sheep, horse, cow and buffalo. The best agglutination was obtained by T. verrucosum, E. floccosum and A. niger. Yeasts showed negative results. The reaction took place after 60 to 90 minutes and elution occured after 2 to 3 hours at room temperature and at 37 °C, while it was stable at 4 °C. pH 7,2 was most suitable.

Zusammenfassung: 17 Pilzstämme (Dermatophyten, Hefen, Schimmelpilze) wurden auf ihre Agglutinationsfähigkeit gegenüber Erythrozyten vom Huhn, Meerschweinchen, Schaf, Pferd, Rind bzw. Büffel untersucht. Die stärkste Agglutination wurde durch T. verrucosum, E. floccosum und A. niger erzielt. Hefen zeigten negative Ergebnisse. Die Reaktion trat nach 60 bis 90 Minuten ein und die Elution verlief bei Raumtemperatur oder 37 °C über 2 bis 3 Stunden, während das System bei 4 °C stabil blieb. Das günstigste pH lag meist bei 7.2.

The haemagglutination was described by Hirst [2] and also by McClelland and Hare [3] in the same year. It was found that some viruses, notably those of the influenza group, cause the agglutination of the red blood cells of man, animals and birds. The virus particles attach themselves to receptors on the surface of the erythrocytes. This adsorption leads to the building of bridges between several erythrocytes as well as to the change of the electrical charge of their surface and consequently agglutination of the cells takes place.

In addition, the majority of fimriate bacteria e. g. Escherichia and Salmonella, bear fimbriae of a type that enables them to adhere to the red blood cells leading also to haemagglutination [1].

The present work is dealing with preliminary studies on various fungi as regard to their capability to agglutinate the red blood cells of different animals. Study of factors like pH, temperature and salt concentration was also considered.

Material and methods

Fungi representing dermatophytes, yeasts and moulds were tested for their capability to agglutinate red blood cells of fowl, g. pig, sheep, horse, cow and buffalo. These fungi were:

Dermatophytes: Trichophyton mentagrophytes, T. verrucosum, T. quinckeanum, T. equinum, T. rubrum, T. violaceum, Microsporum canis, M. gypseum and Epidermophyton floccosum. Moulds: Aspergillus niger, A. fumigatus, A. flavus and Penicillium sp. Yeasts: Candida albicans, Cryptococcus neoformans, Rhodotorula mucilaginosa and Trichosporon cutaneum.

The red blood cells were washed 3 times by saline solution and then resuspended in solutions of 3 different pH ranges, viz. citrate-phosphate buffer (pH 5,0 and 7,2) and tris buffer (pH 8,8). Similarly, suspensions of fresh cultures of the above mentioned fungi were made in the three buffer solutions. 0,25 ml of twofold dilutions of the fungal suspensions were pippetted into the haemagglutination plates to which an equal amount of 0,5% suspension of the red blood cells were added. The plates were incubated at 4 °C, 22 °C and 37 °C and were examined after 1—2 hours and on the second day. This was carried out so as to visualize the optimum pH, temperature and NaCl concentration for the haemagglutination reaction. The latter was investigated by testing the effect of 1%, 2% and 3% solution of NaCl on the reaction.

Results

It was found that the dermatophytes and Aspergilli could agglutinate the red blood cells of fowl and different animals, while the Penicillium sp. and the yeasts

showed negative reaction (Table I). The best agglutination was obtained by T. verrucosum, E. floccosum and A. niger. They agglutinated all the types of the red cells examined. T. rubrum and T. violaceum showed the best reaction with the red blood cells of g. pig. M. canis agglutinated the erythrocytes of all animals, whereas M. gypseum gave positive reaction only with red blood cells of sheep and horse. T. mentagrophytes showed weak agglutinability.

Table I. Haemagglutination of RBCS of different animals by fungi

Fungi	Red blood cells of					
	fowl	g. pig	sheep	horse	cow	buffalo
T. mentagrophytes	±		+		±	±
T. quinckeanum	+ + +	±	+	-	-	-
T. equinum	+	±	±	+	-	+
T. verrucosum	+ + +	+	+	+	+ + +	+ +
T. rubrum	+	+ + +	+	+	+ +	+
T. violaceum	+ +	+ + +	±	±	±	±
M. canis	+ + +	+	+	+	+	+
M. gypseum	-	-	+ ±	±	-	-
E. floccosum	+ + +	+ + +	+ +	±	+	+
A. niger	+ + +	+	+	+	+	+ +
A. fumigatus	+ + +		+	±	+	+
A. flavus	±		+	-	-	+
Penicillium sp.		-			-	-
C. albicans		-	-			-
Cr. neoformans						-
Rh. mucilagenosa		-				-
Tr. cutaneum	-	-			-	-

Haemagglutination was found to occur at 4°C., at room temperature as well as at 37°C. (Table II). The reaction was distinct after 60–90 minutes and elution was observed to begin after 2–3 hours at room temperature and at 37°C., whereas the reaction was stable at 4°C. up to the second day.

Table II. Effect of pH and temperature on the haemagglutination of fowl's red blood cells

Fungi	pH 5,0[1]			pH 7,2			pH 8,8		
	4°C	22°C	37°C	4°C	22°C	37°C	4°C	22°C	37°C
T. verrucosum	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +		-	
M. canis	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +		-	
E. floccosum	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+
A. niger	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ +		-
A. fumigatus	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +		-	-

The best results were obtained at pH 7,2. At pH 5,0 the control red blood cells showed also weak agglutination at the 3 different temperatures of incubation. At pH 8,8 complete negative result was obtained in case of A. niger and A. fumigatus after 30–40 minutes at room temperature, while positive reaction was seen in case of T. verrucosum, M. canis and E. floccosum. After 90 minutes only E. floccosum remained positive. At 4°C. A. niger and E. floccosum showed stable positive reaction; at 37°C. only E. floccosum was positive (Table III).

[1] the control red blood cells showed weak agglutination at pH 5,5 at the 3 different temperatures

Table III. Effect of NaCl concentration on the haemagglutination of fowl red blood cells at room temperature

Fungi	1%	2%	3%
T. verrucosum	+++	+++	++++
M. canis	+++	+++	+++
E. floccosum	+++	+++	++
A. niger	+++	++	++
A. fumigatus	+	+	+

The concentration of NaCl seeme to have little effect on the haemagglutination; however, higher concentration (3%) caused agglutination of the control red blood cells. Elution occurred also at room temperature while at 4°C. the reaction was stable.

Discussion

It is difficult to interpretate the aforementioned results because there is a lack of informations in the available literature concerning haemagglutination caused by fungi; however, these preliminary results may be useful to stimulate the interest in further studies in this direction. The present work showed that fowl red blood cells are suitable and dependable for testing the various fungi for haemagglutination and the reaction needs no special requirements as it can be done at pH 7,2 and at room temperature, thus, in the authors' opinion the pillars of the technique are easy to gain access formulating the simplicity of adopting this test.

Further investigations are also required for characterization of the chemical nature of the haemagglutinin and the cellular attachment sites for the fungi.

The fact that elution is delayed at 4°C. indicates that it is an enzymatic reaction. This needs also further investigation.

It is worthwhile to mention that haemagglutination caused by A. fumigatus was found to be inhibited by the addition of the homologous antiserum. This will be further investigated.

References

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