A survey of domestic species of *Basidiomycetes* fungi for the presence of lectins in their carpophores

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Abstract

Preliminary investigations were conducted to determine the presence of active lectins in carpophores of fungi from the class *Basidiomycetes*, collected from natural localities in southern and south-eastern Poland. The degree of agglutination activity (expressed as the titre of agglutination) of aqueous extracts was determined at room temperature (18-20°C) and at +4°C in respect to human and animal erythrocytes suspended in physiological saline, part of which were additionally treated with proteolytic enzymes. From among the 104 tested species, extracts from 41 of them showed agglutination activity, among which 18 were high. In six cases, specific activity against human ABH group antigens was found. Extracts from 5 species agglutinated only animal erythrocytes, with pigeon erythrocytes being exceptionally sensitive to the lectins. Extracts from two species had distinctly higher agglutination activity at 4°C, which suggests that lectins of the "cold" agglutinin type are present in these species. Analysis of extracts from caps and stems showed that caps had a higher lectin content.

Key words: mushrooms, phytohemagglutinins, human and animal erythrocytes

INTRODUCTION

Although lectins (protein or glycoprotein compounds) have been known for about 100 years, they are still being discovered anew, and are the subject of the unfading interest of a growing number of specialists. In the light of current studies, the basis on which the properties of lectins are discussed is their ability to specifically bind to appropriate sugar receptors on the cell membrane. This binding may be blocked by the appropriate sugars which brings about the

deactivation of lectins (Franz et al. 1982). Presently lectins are being obtained from plants and microorganisms, as well as animals (from sponges to vertebrates) (Gold and Balding 1975).

The presence of lectins in higher fungi has already been known from 1893 when Kolbert (Lagarde 1971) determined, while studying an extract from *Amanita phalloides*, that in addition to a distinct hemolytic activity, agglutination also occurred. Initially, poisonous fungi were the object of study because their poisonous properties were deemed to be linked to the presence of hemolysins and agglutinins (lectins) (Abel and Ford 1907). Later studies showed that the fungi toxins do not belong to the same class of compounds as lectins. The presence of lectins with high agglutination activity was found in tasty, edible fungi such as: *Boletus edulis*, *Agaricus campestris*, *Cantharellus cibarius* (Tobiška 1964). During this period, studies of lectins from higher fungi were centered mainly on searching for lectins with specific activity toward blood group antigens and also on those showing mitogenic effects on human and animal lymphocytes cultured in vitro (Gold and Balding 1975).

The first attempts at isolating lectins from the carpophores of *Agaricus campestris* were carried out by Sage and Vazquez (1967). These authors determined the exact chemical structure of the lectin and some of its biological properties. This type of characteristic has also been given for lectins from several other higher fungi, among others: *Flammulina velutipes* (Tsuda 1979), *Aleuria aurantia* (Kochibe and Furukawa 1980), *Laccaria amethystina* (Guillot et al. 1983), *Xerocomus chrysenteron* and *Lactarius lignyotus* (Sychrova et al. 1985).

Information on the biological nature of lectins, their physiological role in the organisms in which they are produced, still remains scarce. The higher fungi are especially underexplored as a potential source of lectins. Complex studies on the content and nature of lectins occurring in higher fungi have been carried out in many European countries (Elo et al. 1951, Coulet et al. 1970a, b, Seeger and Wiedmann 1972), as well as in the United States (Bernheimer and Farkas 1953). In Poland, however, with the exception of the works of Raszeja (1964a, b), these types of studies have not been conducted. The information on the content of lectins in the species of the Polish microflora and their biological activity is incomplete. This study, based on the fungi from southern and south-eastern Poland, is an attempt to add to this body of information.

MATERIAL AND METHODS

The material used in this study was composed of species of higher fungi, collected from natural forest stands in southern and south-eastern Poland (Fig. 1). Their classification and nomenclature were based on Gumińska and Wojewoda (1985) with modifications according to Michael et al. (1983-1986).

The carpophores were collected from May to November in the years 1977-1983. Specimens of each species were collected from at least two, often several to ten-odd, sites (collected at different times). Healthy, fully mature carpophores, as well as not fully mature ones (not yet releasing spores), were collected. Carpophores of all of the studied species were stored by freezing (-10°C), drying (at room temperature, under air-flow), part were also lyophilized.

Fig. 1. Collection sites of the studied fungi species

Extracts were prepared by separately homogenizing caps and stems in solutions of physiological saline at a weight ratio of 1:4 for frozen material, and 1:40 for dried and lyophilized material. The suspension was agitated for 3 hours at 35°C - 45°C , after which it was filtered and centrifuged to obtain a clear supernatant. The agglutination activity was tested against human erythrocytes (including group antigens ABH and Rh), as well as animal erythrocytes (bovine, pig, horse, mouse, rat and pigeon) and expressed as titre of agglutination. At the same time, comparative tests were done using erythrocytes which had been sensitized by proteolytic enzymes (trypsin, papain) according to Kobiela and Turowska (1969). In order to see if "cold" agglutinins were present, the agglutination reaction was carried out at $+4^{\circ}\text{C}$. All of the tests were conducted simultaneously with control reactions, in which the fungi extract was substituted with physiological saline in order to eliminate the possible effect of idiopathic agglutination of erythrocytes.

Table 1
Agglutination titres of the studied species of higher fungi determined by the dilution method

Active species of higher fungi (in systematic arrangement)**	Human erythrocytes gr.					Animal erythrocytes gr.						Specificity in respect to:	
	A ₁	A ₂	B	0	A ₁ B	C	P	H	M	R	Pg	human blood	animal blood
Family: <i>Ramariaceae</i>													
1. <i>Ramaria aurea</i> (Schaeff.: Fr.) Quél.	64	64		64	64		64		32	32	128		
2. <i>Ramaria flava</i> (Schaeff.: Fr.) Quél.	16		16	32	16	8	16	16	64		64		
3. <i>Ramaria pallida</i> (Schaeff.) Ricken	32	16		16	16	4	8	16	16		32		
Family: <i>Polyporaceae</i>													
4. <i>Albatrellus confluens</i> (Alb. et Schw.: Fr.) Kotl. et Pouz.	16			8		2		32		16			
5. <i>Daedaleopsis confragosa</i> (Bolt.: Fr.) Schroet.	0		0	0	0	0	0		0	2	8	0	R, Pg
6. <i>Laetiporus sulphureus</i> (Bull.: Fr.) Murrill	4096		4096	4096	4096	2048		8192			8192		
7. <i>Trametes hirsuta</i> (Wulf.: Fr.) Pilat	0	0	0	8		0	0			0	8	gr. 0	Pg
8. <i>Trametes versicolor</i> (L.: Fr.) Pilat	0		0	0	0	0	0	0	2	2	2	0	M, R, Pg
Family: <i>Boletaceae</i>													
9. <i>Boletus edulis</i> Bull.: Fr.	1024	1024	512	512	1024	128	512	1024			2048		
10. <i>Boletus erythropus</i> (Fr.) Krbh.	16	4		4	4	16	32	32	4	8	32		
11. <i>Boletus luridus</i> Schaeff.: Fr.	64	16	64	16	64	16	64	64	16	8	64		
12. <i>Leccinum scabrum</i> (Bull.: Fr.) S. F. Gray	8		8	16	8	2	8	16	0	8	32		
13. <i>Suillus bovinus</i> (L.: Fr.) O. Kuntze	0		0	4	0	0	0		0	0	8	gr. 0	Pg
14. <i>Tylopilus felleus</i> (Bull.: Fr.) P. Karst.	16		16	32	8	0	8	16	8		32		
15. <i>Xerocomus badius</i> (Fr.) Kuhn. et Gilb.	512		512	1024	512		512	1024			1024		
16. <i>Xerocomus chrysenteron</i> (Bull.) Quél	1024		512	512	1024	32	128	512			1024		
Family: <i>Paxillaceae</i>													
17. <i>Paxillus involutus</i> (Batsch: Fr.) Fr.	64		16	128	16	16	128	128	32	32	128		
Family: <i>Hygrophoraceae</i>													
18. <i>Hygrocybe conica</i> (Scop.: Fr.) Kummer	32	8	32	8	16	32	64	32	32	64	128		
Family: <i>Tricholomataceae</i>													
19. <i>Clitocybe ditopa</i> (Fr.: Fr.) Gill.	8		16	8	8	0	16			32	32		

20. <i>Flammulina velutipes</i> (Curt.: Fr.) P. Karst	2048		2048	2048		0	1024		0	0	1024		P, Pg
21. <i>Laccaria amethystina</i> (Huds.) Cke. et Br.	8		4	2		0	16	16			16		
22. <i>Laccaria laccata</i> (Scop.: Fr.) Berk.	0		2	8		0	8		8	4	16		
23. <i>Lepista inversa</i> (Scop.) Pat.	32	32	64	16	16	2	64	32	0	0	64		
24. <i>Lepista nuda</i> (Bull.:Fr.) Cke.	32		512	128	32	32	128					gr. B	
25. <i>Mycena galericulata</i> (Scop.: Fr.) S. F. Gray	0	0	0	0	0	0	0	0	0		16	0	Pg
26. <i>Tricholoma portentosum</i> (Fr.) Quél	16			32	16	16	16	32					
27. <i>Tricholomopsis rutilans</i> (Schaeff.: Fr.) Sing.	512	256	512	512		64	256	64	32	32	256		
Family: <i>Entolomataceae</i>													
28. <i>Entoloma sinuatum</i> (Bull.: Fr.) Kummer	512	256	32	32		16	64		32	64	128	gr. A ₁ (A ₂)	Pg
Family: <i>Amanitaceae</i>													
29. <i>Amanita muscaria</i> (L.:Fr.) Pers.	64	128	32	512	32	16	32	128				gr. 0(A ₂)	
30. <i>Amanita vaginata</i> (Bull.: Fr.) Vitt.	0	0	0	0		0		4	4	8	64	0	Pg
Family: <i>Agaricaceae</i>													
31. <i>Agaricus arvensis</i> Schaeff.	128	128		128		8	128						
32. <i>Agaricus bisporus</i> (Lge.) Imbach	8		4	4									
33. <i>Agaricus campestris</i> L.: Fr.	128	128	128	128	128	64	256	256	256		512		
Family: <i>Cortinariaceae</i>													
34. <i>Cortinarius alboboviolaceus</i> (Pers.: Fr.) Fr.	32	32	16	16	8		32						
Family: <i>Strophariaceae</i>													
35. <i>Kuehneromyces mutabilis</i> (Schaeff.: Fr.) Sing. et Smith	64		32	32	64	4	0	128	128		64		
36. <i>Pholliota squarrosa</i> (Müll.: Fr.) Kummer	32		16	256		0			0	0	128	gr. 0	Pg
Family: <i>Russulaceae</i>													
37. <i>Lactarius deliciosus</i> (L.: Fr.) S. F. Gray	16		16	8		0	4	32					
38. <i>Lactarius vellereus</i> (Fr.) Fr.	512	512	512	512	512	0	16	32		0	0		
39. <i>Lactarius rufus</i> (Scop.: Fr.) Fr.	256		256	256		0	128	256			512		
40. <i>Russula cyanoxantha</i> (Schaeff.) Fr.	16	16	16	32	8		32	64	32	16	128		
41. <i>Russula queletii</i> Fr. in Quél.	0	0	0	0		0	16	128	32	16	128	0	Pg, H

** The tested species of higher fungi are systematized according to: Michael et al. (1983-1986) and Gumińska and Wojewoda (1985).

*The individual numbers denote agglutination titres of values 1:X (e.g. 1:16, 1:32, 1:128), 0 – lack of agglutination. Animal erythrocytes: C – bovine, P – pig, H – horse, M – mouse, R – rat, Pg – pigeon. Human erythrocytes: the following group antigens were taken into account: A₁, A₂, B, H.

Table 2

Agglutination activity of selected fungi extracts in respect to erythrocytes suspended in physiological saline (at 20°C and 4°C) and trypsin sensitized erythrocytes

Selected species of higher fungi (in alphabetical order)	Human erythrocytes						Animal erythrocytes					
	Group A ₁ +/-			Group O +/-			bovine			pig		
	E ₂₀	E ₄	E _t	E ₂₀	E ₄	E _t	E ₂₀	E ₄	E _t	E ₂₀	E ₄	E _t
1. <i>Albatrellus confluens</i> (Alb. et Schw.: Fr.) Kotl.	16	8	64	8	8	32	2	2	8	—	—	—
2. <i>Agaricus arvensis</i> Schaeff.: Fr.	128	128	—	128	128	—	8	8	—	128	64	—
3. <i>Agaricus bisporus</i> (Lge.) Imbach	8	8	32	4	8	32	—	—	—	—	—	—
4. <i>Agaricus campestris</i> L.: Fr.	512	512	2048	512	512	2048	512	256	1024	256	256	2048
5. <i>Amanita muscaria</i> (L.: Fr.) Pers.	64	128	1024	512	256	2048	16	32	256	32	128	512
6. <i>Boletus edulis</i> Bull.: Fr.	1024	1024	4096	512	1024	4096	128	64	1024	512	512	2048
7. <i>Boletus erythropus</i> (Fr.) Krbh.	16	16	64	4	16	64	16	8	32	32	16	128
8. <i>Boletus luridus</i> Schaeff.: Fr.	64	32	1024	16	32	1024	16	8	32	64	32	128
9. <i>Chalciporus piperatus</i> (Bull.: Fr.) Bat. = <i>Boletus piperatus</i>	0	2	4	4	4	16	0	0	0	0	2	4
10. <i>Clitocybe ditopa</i> (Fr.: Fr.) Gill.	8	2	8	8	2	8	0	0	8	16	4	32
11. <i>Entoloma sinuatum</i> (Bull.: Fr.) Kummer	512	512	2048	32	64	1024	16	16	128	64	64	256
12. <i>Flammulina velutipes</i> (Curt.: Fr.) P. Karst.	2048	2048	8192	2048	1024	4096	0	0	64	1024	1024	1024
13. <i>Fomes fomentarius</i> (L.: Fr.) Fr.	0	0	2	0	0	8	0	0	8	0	0	64
14. <i>Hydnum repandum</i> L.: Fr.	0	0	0	0	0	0	0	0	0	0	0	0
15. <i>Hygrocybe conica</i> (Scop.: Fr.) Kummer	32	16	128	8	8	64	32	—	—	64	—	—
16. <i>Hypholoma fasciculare</i> (Huds.: Fr.) Kummer	0	0	0	0	0	0	0	0	0	0	0	0
17. <i>Kuehneromyces mutabilis</i> (Schaeff.: Fr.) Sing. et Smith	64	32	128	32	32	128	4	2	16	0	0	16
18. <i>Lactarius rufus</i> (Scop.: Fr.) Fr.	256	256	1024	256	256	1024	8	2	128	128	128	256
19. <i>Lactarius vellereus</i> (Fr.) Fr.	512	512	2048	512	512	2048	0	0	16	16	8	32
20. <i>Laccaria laccata</i> (Scop.: Fr.) Berk. et Br.	0	0	32	8	0	64	—	—	—	—	—	—
21. <i>Laccaria amethystina</i> (Huds.) Cke.	8	0	16	2	0	16	0	0	0	16	16	32
22. <i>Laetiporus sulphureus</i> (Bull.: Fr.) Murr.	4096	4096	16384	4096	2048	8192	2048	2048	8192	—	—	—
23. <i>Leccinum scabrum</i> (Bull.: Fr.) S. F. Gray.	8	8	32	16	8	32	2	0	8	8	4	16
24. <i>Lepista inversa</i> (Scop.) Pat. = <i>Clitocybe inversa</i>	32	16	64	16	16	64	2	2	8	64	16	64
25. <i>Lepista nuda</i> (Bull.: Fr.) Cke.	32	64	128	128	128	512	32	16	128	128	16	256

26. <i>Paxillus involutus</i> (Batsch.: Fr.) Fr.	64	256	128	128	256	256	8	32	32	128	512	128
27. <i>Pholiota squarrosa</i> (Müll.: Fr.) Kummer	32	16	128	256	64	512	0	0	16	—	—	—
28. <i>Ramaria pallida</i> (Schaeff.) Ricken	32	16	32	16	16	32	4	4	32	8	4	64
29. <i>Ramaria aurea</i> (Schaeff.: Fr.) Quél.	64	32	128	64	32	128	—	—	—	64	64	128
30. <i>Russula cyanoxantha</i> (Schaeff. in Fr.) Fr.	16	128	64	32	128	64	—	—	—	32	128	128
31. <i>Russula emetica</i> (Schaeff.: Fr.) S. F. Gray	0	2	2	0	2	4	0	0	0	4	0	4
32. <i>Sarcodon imbricatus</i> (L.: Fr.) Fr.	0	0	0	0	0	0	—	—	—	—	—	—
33. <i>Tricholoma portentosum</i> (Fr.) Quél.	16	16	32	32	32	32	8	—	—	16	—	—
34. <i>Tricholomopsis rutilans</i> (Schaeff.: Fr.) Sing.	512	256	2048	512	256	2048	64	128	1024	256	128	2048
35. <i>Tylopilus felleus</i> (Bull.: Fr.) P. Karst.	16	16	64	32	32	64	0	0	2	8	0	2
36. <i>Xerocomus badius</i> (Fr.) Kühn. et Gilb.	512	128	2048	1024	1024	2048	—	—	—	512	256	2048
37. <i>Xerocomus chrysenteron</i> (Bull.) Quél.	1024	1024	4096	512	1024	4096	32	32	128	128	128	512

The numbers denote agglutination titres with values of 1:X (e.g. 1:16, 1:32, 1:128)

E₂₀ — erythrocytes suspended in physiological saline tested at 20°C

E₄ — erythrocytes suspended in physiological saline tested at 4°C

E_i — trypsinized erythrocytes, 0 — lack of agglutination, — not tested

RESULTS

Among the 104 tested species, extracts from 41 of them proved to have agglutination activity, with 18 showing particularly high activity, independent of the group antigen of the ABO system of the erythrocytes (Table 1). The presence of the most active lectins was found in the following species: *Laetiporus sulphureus*, *Boletus edulis*, *Xerocomus badius*, *Xerocomus chrysenteron*, *Agaricus campestris*, *Flammulina velutipes*, *Tricholomopsis rutilans*, *Lactarius rufus*, *Lactarius vellereus*, *Entoloma sinuatum*. It was also found that extracts from *Pholiota squarrosa*, *Suillus bovinus*, *Amanita muscaria* and *Trametes hirsuta* were especially active in agglutinating group 0 erythrocytes.

Table 3

Agglutination activity of selected species of higher fungi at different stages of development*

Species	Degree of carpophore development**	Stems	Caps
<i>Paxillus involutus</i> (Batsch: Fr.) Fr.	young fully mature	64 (8) 16 (8)	64 (8) 32 (16)
<i>Amanita muscaria</i> (L.: Fr.) Pers.	young fully mature	64 (16) 64 (32)	256 (32) 128 (64)
<i>Tricholomopsis rutilans</i> (Schaeff.: Fr.) Sing.	young fully mature	256 128 (2)	512 128 (2)
<i>Xerocomus badius</i> (Fr.) Kuhn. et Gilb.	young fully mature	512 256	512 256
<i>Lactarius rufus</i> (Scop.: Fr.) Fr.	young fully mature	128 (2) 128 (4)	256 (4) 128 (4)
<i>Russula cyanoxantha</i> (Schaeff.) Fr.	young fully mature	16 16	16 16
<i>Agaricus bisporus</i> (Lge.) Imbach	young fully mature	8 8	8 16
<i>Boletus edulis</i> Bull.: Fr.	young fully mature	1024 512	1024 1024
<i>Kuehneromyces mutabilis</i> (Schaeff.: Fr.) Sing. et Smith	young fully mature	32 32	64 32
<i>Lactarius deliciosus</i> (L.: Fr.) S. F. Gray	young fully mature	8 8	16 16

*The tests were done using human A₁ erythrocytes.

**The carpophores were evaluated on the basis of their ability to release spores.

The numbers denote agglutination or hemolysis titres (numbers in parentheses) expressed as 1:X (e.g. 1:16, 1:32 etc.).

The extract from *Entoloma sinuatum* gave the strongest reaction with group A₁ erythrocytes, while the strongest anti-B reaction was demonstrated by the extract from *Lepista nuda* (Table 1). Extracts from 5 species agglutinated only animal erythrocytes. The most sensitive in these tests were pigeon erythrocytes, slightly less sensitive were mouse, rat and horse erythrocytes. The relatively least sensitive to the studied fungi lectins were bovine erythrocytes.

The Rh factor (only antigen D was taken into account) did not play an important role in the degree of agglutination activity of the studied material. Only in the species *Lepista inversa* did the presence of antigen D go along with enhancing the agglutination of erythrocytes of groups B, 0 and A₁B.

The studied fungi extracts clearly increased their ability to agglutinate both human and animal trypsinized erythrocytes (Table 2). The previously observed specificity of activity of extracts from *Pholiota squarrosa*, *Suillus bovinus*, *Amanita muscaria*, *Trametes hirsuta*, *Entoloma sinuatum* and *Lepista nuda* towards the group antigens H, A₁ and B was not found to increase after sensitizing the erythrocytes with trypsin. The trypsinized erythrocytes were, however, found to be sensitive to extracts from *Fomes fomentarius* and *Russula emetica*, which had not previously given a reaction in the medium of physiological saline and at room temperature. The lack of agglutination activity in the extracts from *Sarcodon imbricatus*, *Hydnum repandum* and *Hypholoma fasciculare* confirmed earlier suppositions about the lack of lectins in these species (Table 2).

Agglutination conducted at +4°C usually gave similar agglutination titres as those obtained at room temperature. Only in the cases of extracts from *Paxillus involutus* and *Russula cyanoxantha* did the titres significantly rise at the lower temperature, suggesting that "cold" lectins are present in these species (Table 2).

Comparison of the agglutination activity of extracts from carpophores at different stages of development encompassed only 10 species (Table 3). It was found that the agglutination titres of extracts of young carpophores of: *Paxillus involutus*, *Amanita muscaria*, *Tricholomopsis rutilans* and *Lactarius rufus* were higher than the titres obtained with extracts from fully mature carpophores of the same species. The mature extracts from the first two species, however, showed a greater hemolytic activity. No distinct relationship between the degree of maturity of the carpophores and their strength of agglutination activity was observed for the remaining species.

The analysis of the activity of extracts from caps and stems showed for most of the species a greater content, or higher activity, of lectins present in the caps (Table 3).

The comparison of extracts from dried and frozen carpophores which had been collected from the same site, did not show any significant differences in their agglutination activity. The frozen material, however, did show a significantly greater hemolytic activity.

DISCUSSION

The results obtained in this study have demonstrated the occurrence of lectins with distinct agglutination activity in respect to both human and animal erythrocytes in about 40% of the tested fungi species. In 6 species, this activity was found to be greater against human erythrocytes with a specific group antigen, or animal erythrocytes of a specific species. Fungal lectins which react specifically with a given human blood group antigen are widely known and described in literature. Among them the following lectins have been described: from *Clitocybe nebularis* (Tetry et al. 1953), *Fomes fomentarius*, *Marasmius oreades* (Elo et al. 1951, Coulet et al. 1970a), *Laccaria laccata* and *Laccaria amethystina* (Raszeja 1964a, Guillot et al. 1983). In the present study, such specific activity was noted in: *Entoloma sinuatum* (anti-A₁), *Lepista nuda* (anti-B) and *Suillus bovinus*, *Pholiota squarrosa* and *Trametes hirsuta* (anti-O), as well as *Amanita muscaria* (anti-O and A₂). The anti-A₁ activity of *Entoloma sinuatum* is confirmed by Lagarde (1971) both in respect to erythrocytes suspended in physiological saline and those treated with bromeline and suspended in bovine albumin. Coulet and Merland (1960) precisely characterize the specificity of activity of an extract from *Suillus bovinus* in respect to human erythrocytes of phenotypes O/D/NN and O/d/MN. The specific effect of *Amanita muscaria* extracts causing the agglutination of type O and A₂ erythrocytes has been described in many papers. Both in this study and that of other authors (Coulet et al. 1970a, Lagarde 1971), a high degree of variation in the degree of specificity of extracts from different collections was observed. No reports fully confirming the specific agglutination activity of the remaining 3 species were found in the available literature. Coulet and Merland (1960) report that *Lepista nuda* has a rather insignificantly increased activity against erythrocytes with the antigen phenotype O/d/MN. The lectin from *Pholiota squarrosa* is, on the other hand, considered by Elo et al. (1951) to be a lectin with a high, although inspecific (in respect to human blood group antigens) agglutination activity.

The agglutination of animal erythrocytes brought about by extracts from various fungi depended on not only the activity of the extracts, but also on the sensitivity of the erythrocytes of the different animal species. The significantly higher sensitivity of pigeon erythrocytes to most of the lectins, noticed in this study, is supported by the findings of other authors (Boyd 1963, Sage and Connett 1969, Sychrova et al. 1985). As is well known, avian erythrocytes differ from those of mammals mainly by the presence of a cell nucleus. These differences may also be reflected in the surface structure of the cell membrane and the, resulting from this, different nature or arrangement of surface receptors. There is, on the other hand, also the well known high sensitivity of the pigeon to pharmacologically active substances such as cardiac glycosides, which is made use of in pharmacological studies. At the same time it is

interesting to note the puzzling low sensitivity of bovine erythrocytes to most of the lectins, also noticed by Zaręba (1967).

The distinctly increased agglutination activity of the studied extracts against trypsinized erythrocytes fully confirms previous studies (Sage and Vazquez 1967, Lagarde 1971). By partially digesting the cell membrane of erythrocytes, proteolytic enzymes expose their additional surface receptors, which makes the reaction more complex, albeit less specific. This is probably the reason for the lowering of the degree of specificity of certain lectins in respect to erythrocytes which had been "sensitized" by proteolytic enzymes. Especially interesting is the fact that some of the fungi extracts, previously inactive in the physiological saline medium, became active against trypsinized erythrocytes. In this case, the nature of the lectin-erythrocyte bond may be the same type as the binding of the incomplete agglutinins. This fact should be checked by conducting the reaction between the fungal extracts and erythrocytes in high molecular weight mediums such as albumin, dextran etc. Lectins having the nature of incomplete agglutinins are known from literature (Boyd 1963, Raszeja 1964b). In her studies of lectins from higher fungi, Lagarde (1971) found that among 81 active species, 29 exhibited activity only in a bovine albumin medium or against erythrocytes treated with bromeline.

The problem of analysing the effect of temperature on the activity of lectins was undertaken by, among others, Coulet et al. (1969), Serafini-Cessi et al. (1980). The thermal resistance of fungal lectins is usually high and ranges from 50° to 80°C (Raszeja 1964b, Sage and Connett 1969). Lectins usually attain maximum activity at 20-37°C (Coulet et al. 1969). There are, however, reports of lectins behaving as natural "cold" agglutinins, that is, increasing their activity at +4°C. Among others, the lectins from *Glycine soja* var. *bansei* (Boyd 1963), *Phaseolus lunatus*, *Crotalaria mucronata* (Ottenssooser and Sato 1963) behave in this way. Among the higher fungi, this type of lectin has been noted in *Amanita regalis* (Lagarde 1971) and *Marasmius oreades* (Elo et al. 1951). The phenomenon of concomitant change or loss of specificity of lectins at lower temperatures should be underscored, as it has been recorded in lectins of seed plants (Ottenssooser and Sato 1963) and fungi (Elo et al. 1951). The authors attempt to explain this fact by changes in the structure of the bonds between the lectin molecule with the surface receptors of the agglutinated erythrocytes. The results of this study also suggest the presence of such lectins in *Paxillus involutus* and *Russula cyanoxantha*.

Many authors have found that the time and place of synthesis of lectins in higher plants are dependent on the stage of development of the plant (Kilpatrick et al. 1979). Lectins are usually stored in seeds, embryos or storage organs of mature plants. The analagous process of synthesis and storage of lectins in fungi has been studied only fragmentarily. Attempts have been made to analyze the lectin contents of the various parts of the carpophore and in relationship to the degree of their development. Coulet et al. (1970a)

and Mustier and Coulet (1965) noticed marked differences in activity between mature and immature carpophores of *Phallus impudicus* and *Mutinus caninus*, with the immature forms being more active. Dobovoy et al., cited by Coulet et al. (1970a) found agglutinins in *Lycoperdon pyriforme* only in its spores, and also noticed certain differences in the activity of stems and caps of *Amanita caesarea*, *Amanita muscaria* and *Laccaria laccata*. Also Orlow (cited by Raszeja 1964b) noticed that the hemagglutinating properties of caps and stems of the same carpophore differ quantitatively. Elo et al. (1951), however, did not find such differences in *Marasmius oreades*. Raszeja (1964b) in studies on the species *Laccaria laccata* var. *proxima* finds a greater accumulation of lectins in younger carpophores, while not noticing any differences in the activity of lectins in carpophores from different collection sites. Among the 50 species of fungi studied by that author (Raszeja 1964b), almost one half of them were characterized by greater agglutination activity of caps. As is evidenced by the cited studies, there is a wide range of opinions on this subject. The results of this study incline to the conclusion that caps of young carpophores are usually richer in lectins.

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Poszukiwanie lektyn w owocnikach krajowych gatunków grzybów z klasy *Basidiomycetes*

Streszczenie

Przeprowadzono wstępne badania zawartości aktywnych lektyn w owocnikach grzybów z klasy *Basidiomycetes*, pochodzących z naturalnych stanowisk Polski południowej i południowo-wschodniej. Określono stopień aktywności aglutynacyjnej (wyrażonej w postaci miana aglutynacji) wyciągów wodnych w stosunku do erytrocytów ludzkich i zwierzęcych zawieszonych w soli fizjologicznej oraz traktowanych enzymami proteolitycznymi w temperaturze pokojowej

(18-20°C) oraz w temp. +4°C. Z testowych 104 gatunków, wyciągi 41 gatunków wykazywały aktywność aglutynacyjną, w tym u 18 aktywność ta była stosunkowo duża.

W sześciu przypadkach stwierdzono specyficzność działania w odniesieniu do ludzkich antygenów grupowych ABH. Wyciągi 5 gatunków aglutynowały jedynie krwinki zwierzęce, przy czym erytrocyty gołębie okazały się szczególnie wrażliwe na działanie lektyn. Wyciągi 2 gatunków wyraźnie zwiększały swą aktywność aglutynacyjną w temperaturze +4°C, co sugeruje występowanie w tych gatunkach grzybów lektyn o charakterze „zimnych aglutynin”. Analiza aktywności wyciągów z kapeluszy i z trzonów wskazuje na większą zawartość lektyn w kapeluszach.