cles[16]. The fact that heavy NA vesicles are also present in other tissues suggests that these vesicles may play a similar role in adrenergic nerve endings in general.

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The lectins from *Agaricus edulis.* Isolation and characterization

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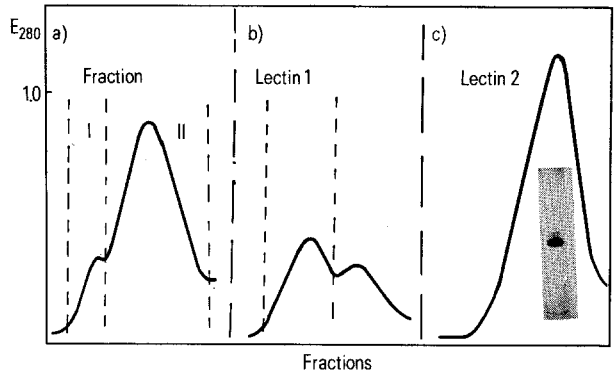
Summary. 2 lectins from the mushroom *Agaricus edulis* were isolated, after heating the crude extract at 75 °C, by ion exchange chromatography and gel chromatography using QAE-Sephadex A-50 and Sephadex G_{75}. Some hemagglutinating and physicochemical properties of the agglutinins are reported.

Lectins used to be commonly referred to as plant-seed, carbohydrate-binding proteins. However, they have also been found in bacteria, fish roes, snails, vertebrates and mushrooms[1,2]. Hitherto, lectins from the genus *Agaricus* have been isolated from *A. bisporus*[3] and *A. campestris*[4]. In the present paper we describe the lectins from *A. edulis.*

Materials and methods. Fresh mushrooms of the species *A. edulis,* collected in the streets of Berlin, were cleaned and dried at 50 °C. 50 g of the ground powder was suspended in 800 ml 0.9% NaCl-solution and heated for 10 min at 75 °C. The suspension was then cooled with water and centrifuged at 5000 × g for 10 min. The clear supernatant was treated with 59 g ammonium sulphate per 100 ml and stored overnight in the refrigerator. The precipitate was centrifuged at 6000 × g for 15 min and the sediment was suspended in 15 ml of distilled water. This solution was centrifuged at 15000 × g for 15 min to remove the last contamination. The clear dark brown solution was dialyzed against 0.3% NaCl solution. To remove the brown colored components, the solution was then applied to a column packed with 80 ml QAE-Sephadex A-50 equilibrated against 0.3% NaCl solution. All of the colored material remained on the column and the nearly colorless lectin fraction came through after the hold-up volume. The purified fraction was concentrated by ultrafiltration to a final concentration of 1% protein. Chromatography and rechromatography on a column of Sephadex G_{75} (90 × 1.5 cm) equilibrated with 0.9% NaCl solution revelaed 2 hemagglutinating lectin fractions. Both were desalted and freeze dried.

For polyacrylamide gel disc electrophoresis the method of Maurer[5] was used, which involved electrophoresis in columns of 7.5% gel in buffers of pH 4.3 and 8.9 respectively. Estimation of the subunits of the lectins was carried out in 10% polyacrylamide gel in the presence of sodium dodecylsulphate by comparison with marker proteins.

Hemagglutination assays were performed using a Takatsy microtitrator. Tests were performed with a 2% suspension of human erythrocytes type A and incubated for 1 h at room temperature. A hemagglutination inhibition test was carried out as described[6]. TCL for determination of the mol.wt of the lectins was performed with Sephadex superfine G_{200} and Sephadex G_{75} superfine at pH 7.8 and 4.8 as described by Andrew[7]. Carbohydrate content of the lectins was determined by the phenol-H_2SO_4 method with reference to glucose[8]. N-terminal amino acids were determined with the DNP-method of Fraenkel-Conrat[9]. Determination of sulfhydryl and disulfide groups was carried out with the reagent of Ellman[10]. Antisera were produced by immunizing rabbits with pure lectins. The animals were injected in



a Gel chromatography of the crude lectins from *A. edulis* on Sephadex G_{75} (1.5 × 90 cm). *b* Rechromatography of fraction 1. *c* Rechromatography of fraction 2 and the result of the disc electrophoresis of the purified lectin 2 (200 µg).

Lectin	Human blood group specificity	mol.wt	Subunits	Carbohydrates	Cys/2	Stability
A. edulis I	no	60,000	4	18%	0	75 °C
A. edulis II	no	32,000	2	2%	0	85 °C
A. bisporus	no	64,000	4	4%	–	–
A. campestris	no	64,000	4	4%	4	85 °C

the footpads with a mixture of 10 mg lectin in 2 ml Freund's complete adjuvant once a week for 4 weeks. The animals were bled 14 days after the final injection. The fraction of immunoglobulins was isolated by precipitation with ammonium sulphate (degree of saturation 0.33). Double diffusion in agar was done using 1% agarose in 0.05 M sodium barbital buffer pH 7.4.

Results and discussion. Heating of the crude extract of *A. edulis* at 75 °C results in the precipitation of most of the proteins but not of the lectins. Gel chromatography of the heated lectin extract on Sephadex G_{75} results in a fraction of high molecular polysaccharide and 2 lectin-containing fractions. Rechromatography of the lectin fractions reveals 2 lectins (figure). The lectins are homogenous in disc electrophoresis and each of them shows a single band. The amount of lectins obtained from 100 g dried plant material was about 12 mg of lectin 1 and 280 mg lectin 2, respectively. Mol.wt estimated by thin-layer gel filtration was about 60,000 for lectin 1 and 32,000 for lectin 2. Mol.wt of the subunits of both lectins estimated by disc electrophoresis in the presence of sodium dodecylsulphate was about 14,000. The values for carbohydrate content obtained by the phenol-H_2SO_4 method were 18% for lectin 1 and 2% for lectin 2 with D-glucose as standard. The carbohydrates were not identified further. Neither cystein nor cystine was found in either of the lectins. We suggest that lectin 1 consists of 4 subunits and lectin 2 of 2 identical subunits which are noncovalently bounded. Only L-valine was found as N-terminal amino acid of lectin 2 (that of lectin 1 has not yet been determined). The hemagglutination of lectin 2 was studied in the presence of the following carbohydrates: D-glucose, D-mannose, D-galactose, L-fucose, L-rhamnose, D-glucosamine, D-galactosamine, N-acetyl-D-glucosamine, N-acetyl-D-galactosamine and N-acetyl-neuraminic acid. None of the sugars tested inhibited the hemagglutination of human erythrocytes in a final concentration of 0.1 M. 5% solutions of dextran, mannan (from yeast) and galactan (from *Lupinus albus*) showed no inhibition activity. The purified lectins showed no specificity for one of the human erythrocyte types. Erythrocytes of the types A, B and 0 were agglutinated. Blood group substances A and B were precipitated by the lectins. The activity of lectin 1 and 2 was unaffected by heating for 10 min at 75 °C and 85 °C, respectively. Also, incubation of the lectins in a solution of 6 M urea at room temperature for 60 min and in buffers from pH 2–11 had no influence of the hemagglutinating activity. When tested in immunodiffusion against the immunoglobulins IgA, IgG and IgM the lectins from *A. edulis* showed single bands against the immunoglobulins. That means the lectins are able to react with carbohydrate moities of the immunoglobulins. Also the hemagglutination of human erythrocytes by 1 mg lectin II per ml phosphate-buffered saline (titer 1:1024) was inhibited by equal parts of 0.2% IgA (titer 1:2) and 0.2% acid α_1-glycoprotein (titer 1:4). When tested in immundiffusion against the antiserum to lectin 1 and 2 a crude extract of *A. bisporus* (titer 1:128) revealed cross reaction of the lectins, showing relationships between the lectins. Some properties of the lectins from *Agaricus* are summarized in the table. Further studies of the structure and nature of the lectins from *A. edulis* are under way in our institute.

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Abnormal limbs *(abl)*, a recessive mutation affecting the tadpoles of *Xenopus l. laevis*

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Summary. 'Abnormal limbs' *(abl)* is a recessive and semi-lethal mutation introduced by a ♂ from the laboratory stock. Brachymely, syndactyly, polydactyly and brachydactyly are the main abnormalities encountered. They occur more frequently in the forelimbs than in the hindlimbs.

This mutation, provisionally called M_5, was found during the genetic analysis of somatic nuclei undertaken in our laboratory after nuclear transplantation experiments; it was introduced by a male from the stock. The heredity of the mutation has already been described[2].

10 matings were effected between heterozygous individuals of different generations; they have yielded 230 homozygous tadpoles out of 956, i.e. a percentage of 24.1, characteristic of a Mendelian recessive gene.

Description of the phenotype. The phenotype of the mutant tadpole is recognizable at about 22 days of development, i.e. at stage 52 of the normal table of *Xenopus l. laevis*[3]. The proximal parts of the most abnormal limb buds are usually wider and thicker in the *abl* tadpoles than in the wild-type