The analyses that have been made in detail while obtaining rabbit antisera could explain the rest of the experimental results. The sera against normal leukocytes were obtained through injection of mixed donor's leukocytes. The material used for immunization of another group of animals contained 97–100% paramyeloblasts. It was supposed that the established antigenic reduction did not touch, in the same degree, the elements of the white blood line of the patients investigated. Probably the antigen loss was most strongly expressed by the paramyeloblasts. It was admitted that under the influence of the leukemic agent from the antigenic profile of the paramyeloblasts a large part of the antigens was lost. Consequently the sera obtained against the paramyeloblasts did not contain all the antibodies with which the antigens of the remaining types normal and leukemic leukocytes would be connected.

Résumé. Les antigènes leucémiques de 40 malades atteints de leucémie aiguë et de 40 adults sains ont été étudiés à l'aide des sérums de lapin et par des méthodes d'immunodiffusion, d'immunoélectrophorèse et la réaction de fixation du complément. On a trouvé que les leucocytes de malades étudiés ont perdu une part des antigènes observés dans les leucocytes du groupe de contrôle des personnes normales.

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Studies on Phytohemagglutinins XXI. The Covalent Oligomers of Lysozyme — First Case of Semisynthetic Hemagglutinins

Lysozyme (3.2.1.17 mucopeptide N-acetylmuramoylhydrolase), is known to contain a combining site for linear oligosaccharides formed by N-acetyl-D-glucosamine[1,2]. In this respect it resembles the wheat germ agglutinin[3]. Similar sugar specificity is exerted also by hemagglutinins from other sources, e.g. the phytohemagglutinin II of the furze seeds (*Ulex europaeus* L.)[4] and the phytohemagglutinin from potato tubers (*Solanum tuberosum* L.)[5].

At present the assumption seems to be well justified[6] that the minimum of two sugar-combining sites in the lectin is necessary for the agglutinating activity. Recent findings[6] show that an increasing number of the combining sites in a lectin molecule, as effected by polymerization of the lectin, results in an increased hemagglutinating activity. All these facts imply that artificial preparation from monovalent molecules of molecules with more sugar-binding sites should give rise to semisynthetic agglutinins from nonagglutinating substances capable of binding carbohydrates.

In the present work lysozyme was polymerized by the action of glutardialdehyde[7] in an attempt to prepare a semisynthetic model agglutinin mimicking the action of phytohemagglutinins.

Materials and methods. A 1% water solution of glutardialdehyde (10 µl) was added to 100 mg of lysozyme (3 times crystallized chicken egg white lysozyme, Nutritional Biochemical Co., Cleveland, Ohio, USA) dissolved in 4 ml of 0.1 *M* phosphate buffer, pH 7.2. The mixture was intensively stirred for 1 min and then allowed to react for 24 h at room temperature. A small amount of a precipitate which formed was removed by centrifugation and the yellow supernatant was applied to a Sephadex G-100 column (2 cm × 200 cm) in saline (0.9% NaCl solution). Elution was effected by saline at a rate of 12 ml/h. Fractions of 4 ml were collected and the absorbance at 280 nm was measured.

Hemagglutinating activity was assayed by the serial dilution procedure, using a 2% saline suspension of the thrice washed erythrocytes. After 30 min at room temperature, the tubes were centrifuged for 1 min at 1000 rev/min (centrifuge Janetzki T 12) and observed macroscopically.

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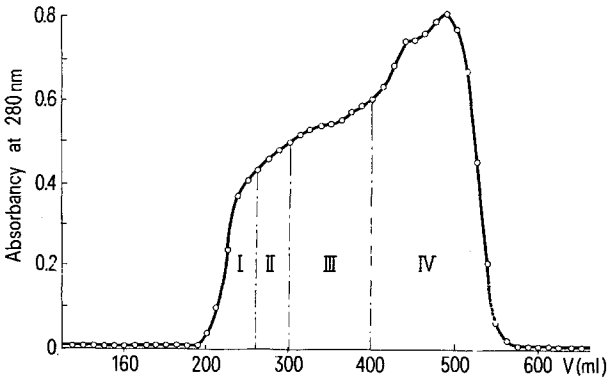

Fig. 1. Gel filtration of lysozyme oligomers on a Sephadex G-100 column (2 cm × 200 cm) in 0.9% NaCl. See text for details.

Erythroagglutinating activity[a] of lysozyme oligomers

	Yield (mg)	Erythrocytes				
		A_1	A_2	B	0	Rabbit
Original reaction mixture	90	16	16	16	16	0
Fraction I	5	128	128	128	128	0
Fraction II	11	32	32	32	32	0
Fraction III	32	2	2	2	2	0
Fraction IV	40	0	0	0	0	0
Lysozyme	—	0	0	0	0	0

[a] Expressed in serial dilution titers of 1% protein solutions.

Results and discussion. Results of the chromatography on Sephadex G-100 are shown in Figure 1. Protein-containing fractions were divided into 4 parts (I–IV) which were dialyzed against deionized water and lyophilized. The protein composition of the individual parts was analyzed by disc polyacrylamide gel electrophoresis in the dodecyl sulfate medium[8] and is shown in Figure 2. Determination of hemagglutinating activity of 1% solutions of the original reaction mixture and of the individual fractions I–IV after dialysis and lyophilization is summarized in the Table.

The macroscopic picture of the agglutination was somewhat different from agglutination caused by most of the common lectins: a granular agglutinate formed showing a tendency to stick. The agglutination, whether effected by the original reaction mixture diluted 1:8 or by a solution of the fraction I diluted to the same activity, was not inhibited by 2% solutions of D-glucose, D-mannose, N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, L-fucose, D-galactose or cellobiose but was inhibited by glycopeptide I.3, a receptor for the pea lectin, obtained by KUBÁNEK et al.[9,10] from human erythrocytes. The inhibition by this glycopeptide was still effective at a concentration of 1.25 mg/ml. Hemagglutination titre of lysozyme oligomers was not influenced by EDTA solutions (phosphate buffer, pH 7.0) in a concentration range of 0.05–0.1 *M* and by 0.05–0.1 *M* solutions of $CaCl_2$, $CoCl_2$, $MgCl_2$, $MnCl_2$, $NiCl_2$ and $ZnCl_2$ in saline.

The separation on Sephadex G-100 of oligomers with different molecular weights enabled us to show that the hemagglutinating activity is associated with the higher oligomers of lysozyme (starting probably with the tetramer). Lower oligomers were not effective. This finding is in a good agreement with the results of LOTAN et al.[6] described for the polymerized soybean agglutinin.

It is to be expected that the possibility of preparation of the semisynthetic agglutinins will open new horizons for studies on the binding site interactions. The analogy between the hemagglutinating action of lysozyme oligomers and some phytohemagglutinins can be useful in the study of the agglutination phenomena and can contribute to elucidation of their mechanism. Thus the preparation of blood group specific agglutinins seems to be of interest, as well as the behavior of the synthetic substances towards lymphocytes and cancerous cells. Due to the high sensitivity of the agglutination reaction, the method of polymerization of monovalent ligand-binding proteins can become a useful tool for the detection of these proteins and, in general, for the investigation of cellular surface structures.

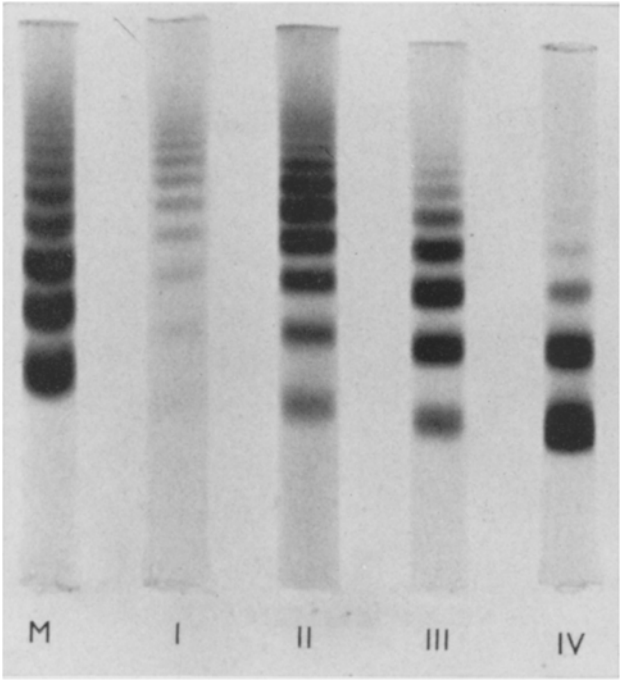

Fig. 2. Disc polyacrylamide electrophoresis, in dodecyl sulfate medium[8]. 7.5% Acrylamide with 0.1% sodium dodecyl sulfate in 0.1 *M* phosphate buffer, pH 7.2. Electrophoresis run for 3 h at a current 8 mA per tube (0.5 cm × 7 cm). M, original reaction mixture; I–IV, fractions I–IV.

Zusammenfassung. Die durch Einwirkung von Glutaraldehyd auf Lysozym entstehenden höheren Lysozym-Oligomere agglutinieren menschliche Erythrocyten aller A-B-O-Blutgruppen.

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Possible Relation Between low Thymosine-Like Activity in the Serum of Swan Mice (Swiss Antinuclear) and the Formation of Crystals in their Thymic Epithelial

Swiss antinuclear (SWAN) mice form a closed colony spontaneously develop high titres of antinuclear antibody. Immunologically and pathologically these mice represent an animal model for systemic Lupus erythematosus[1,2].

It has recently been shown that the thymosine-like activity in the serum of these mice falls between the 2nd and 6th month of life[3]. We have examined the thymuses of such mice by electron microscopy to see whether any ultrastructural modifications in the epithelial cells could be observed which might explain the early fall of the thymosine-like activity in these mice.

Ultrastructure studies were performed on SWAN mice aged $3^1/_2$ and 5 months with positive AN Ab (titres between 8 and 2048). Control studies were performed on Swiss mice of the same age and sex without AN Ab.

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