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.

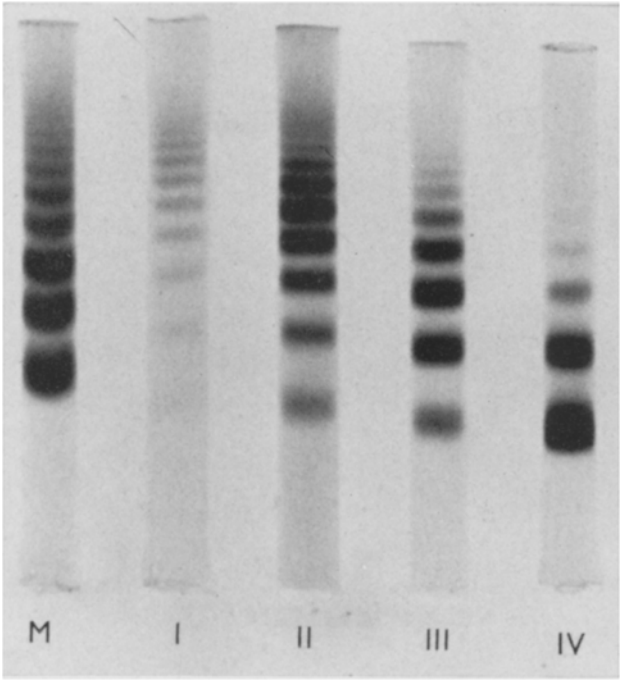

Fig. 2. Disc polyacrylamide electrophoresis, in dodecyl sulfate medium[8]. 7.5% Acrylamide with 0.1% sodium dodecyl sulfate in 0.1 *M* phosphate buffer, pH 7.2. Electrophoresis run for 3 h at a current 8 mA per tube (0.5 cm × 7 cm). M, original reaction mixture; I–IV, fractions I–IV.

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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Although an AN Ab are observed rarely in Swiss mice at 5 months, we selected only AN Ab negative mice as controls. Thymic tissue from these mice was fixed by glutaraldehyde, and osmium tetroxide, and was embedded in epoxy resin. Ultra thin sections were stained with uranyl acetate and lead citrate, and were observed using a Philips EM 300 microscope. In all SWAN C type viral particles were seen[4] in vacuoles of some of the epithelial cells.

We observed in 5-month-old SWAN mice an atrophy of the thymic epithelium, a reduction in the number of thymocytes, and invasion of macrophages and plasmocytes from the blood, fatty degeneration, and fibrosis. The epithelial cells had a reduced amount of ergastoplasm and electron dense granules: their nuclei became dense and pycnotic. Some of them were severely necrotic, and the majority were heavily vacuolated and contained lipid globules. Vacuoles containing electron dense flocular material were present in $3^1/_2$-month-old SWAN, but disappeared progressively with age. About 80% of epithelial cells in 5-month-old, and 5% in $3^1/_2$-month-old SWAN mice, contained electron-dense crystalline inclusions which appeared to have a finaly granular structure. The outline of the inclusions was always geometrical and represented a section through a geometrically regular structure; they were observed free in the cytoplasm, enclosed by a membrane, or in a vacuole. Small groups of 3 or 4 crystals were often observed, frequently close to the nucleus of the cell. No such crystals were ever observed in the thymic cells from control Swiss mice, with a normal level of thymosine-like activity in their serum.

DE VRIES and HIJMANS[5] drew attention to the possible role of the thymic epithelium in the prevention of autoimmune phenomena, when they studied anomalies in

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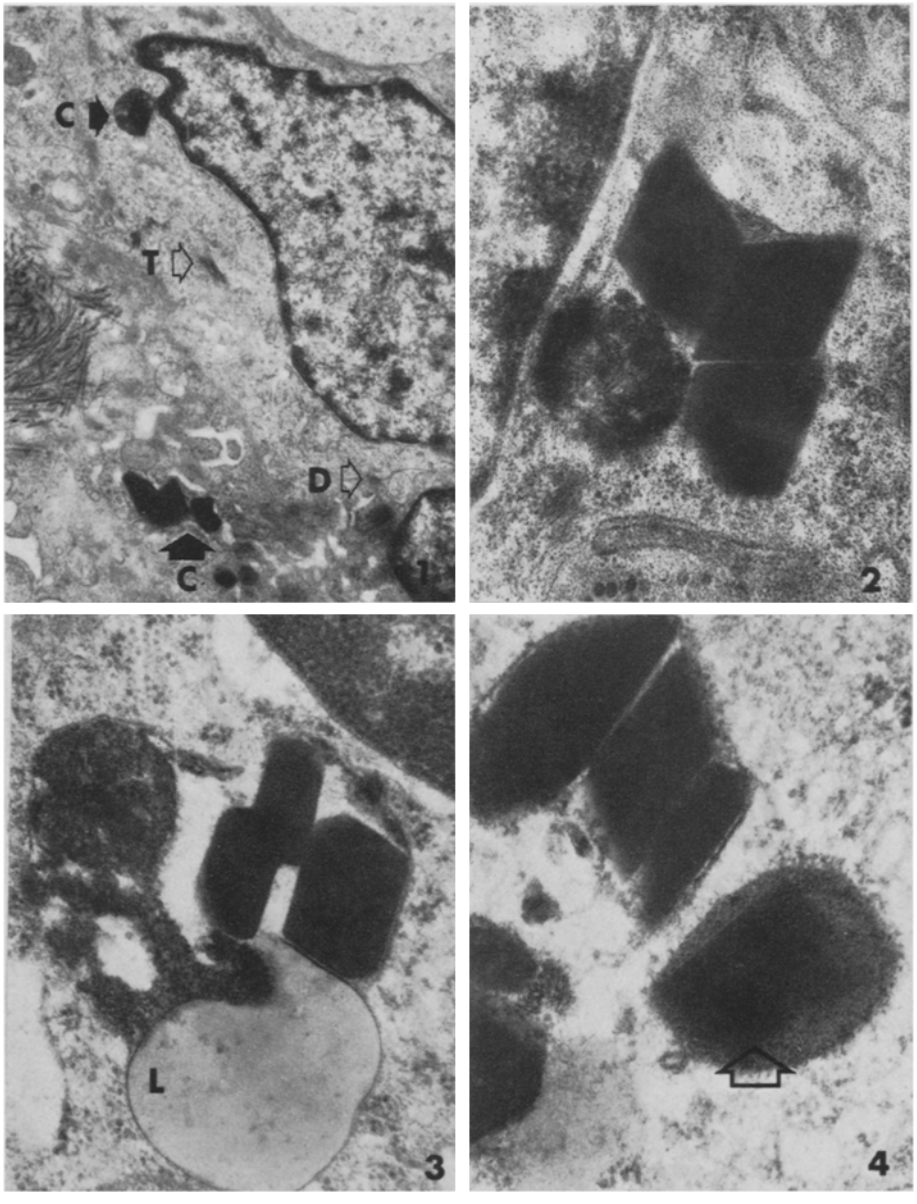

Fig. 1. Epithelial cell with tonofilaments (T), desmosome (D) and crystalline inclusions (C). ×10,000.
Fig. 2. Cytoplasm of epithelial cells with crystalline inclusions. ×50,000.
Fig. 3. Cytoplasm of epithelial cell with vaccuole containing dense material, lipids, and crystalline inclusions. ×50,200.
Fig. 4. Crystlaline inclusion in a dense granule of epithelial cell cytoplasm. ×63,800.

	Titre of AN Ab [a]		Thymosin liver activity [b]		Crystals in epithelial cells [c]	
	Swiss	SWAN	Swiss	SWAN	Swiss	SWAN (%)
$3^1/_2$-month-old	0	8–64	128	16–32	0	10
5-month-old	0	64–2048	128	8–16	0	80

[a] Obtained by immunofluorescence. [b] Obtained by the technique of BACH et al.[3–9]. [c] % of epithelial cells with crystals.

the development of epithelial cells in NZB and (NZB × NZW) F1. A deficit in T cell functions has been observed moreover in both humans and animals with auto-immune disease[6,7], along with a definite reduction of thymosin-like activity in the serum[3,8,9].

The Table shows the presence of crystals, the level of serum thymosin-like activity and the titre of AN Ab for the Swiss and SWAN mice. In SWAN mice the level of serum thymosin-like activity falls between $3^1/_2$ and 5 months, whereas the number of cells with crystals increases over the same period, as does the number of mice positive in AN Ab. We suggest that the cytoplasmic crystals represent an intracellular build up of thymosin, or a precursor which cannot be secreted by the cell, perhaps due to lack or modification of an enzyme necessary for the activation of this hormone. Other examples of intracellular crystals representing storage of unsecreted protein have been documented[10–12].

This hypothesis will be tested by a more detailed investigation of the age at which the crystals first appear in the SWAN mice and by looking for similar formations in other autoimmune animals, such as NZB mice and perhaps in old mice of normal strains. In observations made on a few SWAN mice at an age of 1 year, crystals were present though less abundantly than in the 5-month mice.

Résumé. L'étude en microscopie électronique du thymus de souris autoimmunes SWAN de 5 mois dont le taux d'activité «thymosine like» est très bas, montre la présence d'inclusions cristallines dans le cytoplasme des cellules réticuloépithéliales. Ces cristaux évoquent la possibilité d'un défaut d'excrétion de l'hormone thymique par les cellules épithéliales expliquant la faible activité hormonale trouvée dans le torrent circulatoire de ces animaux.

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Antiandrogenic Suppression of Lymphocytic Blastogenesis: in vitro and in vivo Observations

The androgenic dependence of prostatic cancer and its treatment by antiandrogenic therapy by the administration of estrogen has been well documented since the classical studies of HUGGINS et al.[1]. However, the potential effects of such therapy on the immunologic responsiveness of the host to malignancy have not been delineated. Recently, ABLIN[2] alluded to the possibility that as estrogens result in a generalized stimulation of the reticuloendothelial system leading to what appears to be suppression of cell-mediated hypersensitivity reactions and enhancement of circulating antibody production that pallative hormonal therapy in patients with advanced

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Table I. Effect of diethylstilbestrol diphosphate (DEP-S) on the incorporation of ^{3}H-thymidine of peripheral blood lymphocytes stimulated with phytohaemagglutinin (PHA)

Mean ± S.D. × 10^{-4} Counts/min incorporation of ^{3}H-thymidine of 10^6 peripheral blood lymphocytes incubated with [a]			
PHA	PHA + DES-P	Without PHA	DES-P
7.5 ± 4.6	3.3 ± 2.3	1.3 ± 0.83	0.92 ± 0.38

[a] Data expressed as mean value ± 1 S.D. of triplicate determinations on 7 adult males.