Under the influence of cyclic GMP there was thus no change in the phasic nature of the course of wound healing, but granulation tissue and the surface epithelium were formed somewhat faster and cell proliferation and differentiation were activated. Compared with the control wounds there was no increase in the number of fibroblasts, but their number reached a maximum sooner after the operation. This led to an increase in the rate of collagen and fibril formation.

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QUATERNARY STRUCTURE OF MOUSE AMYLOID FIBRILS: DISSOCIATION OF FIBRILS INDUCED BY SODIUM DODECYLSULFATE AND ALKALI

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KEY WORDS: experimental amyloidosis; amyloid fibrils; quaternary structure; bonds between subunits; denaturing agent.

Among the main problems in the pathogenesis of amyloidosis that still remain unsolved are the mechanism of formation of amyloid fibrils and the directly connected problem of the character of the bonds responsible for maintaining the fibrillary structure. The chemical nature of the elementary subunits of amyloid fibrils has been studied intensively, and considerable progress has been achieved in this field [3, 8, 10-12, 14, 15]. However, the quaternary structure of fibrils, i.e., the character of the bonds between the subunits and the method of their packing in the fibril has not yet been studied. Some workers have suggested that the fibril contains S—S bonds between the subunits [1, 6, 7]. There is also evidence of the essential role of noncovalent bonds in fibrillary structure [14].

Some results of a study of the quaternary structure of the amyloid fibril obtained during an investigation of dissociation of fibrils under the influence of denaturing agents are described in this paper.

EXPERIMENTAL METHOD

Experimental amyloidosis was induced in CBA mice weighing 18-20 g by injection of 5% casein in 0.25% NaOH subcutaneously in a dose of 1 ml daily for 35-40 days. The mice were then killed and their spleens treated by methods described in the literature [9-13]. The spleens were homogenized in a knife homogenizer in physiological saline. The homogenate was centrifuged at 19,600g for 15 min and the supernatant poured off; the residue was again homogenized and centrifuged for 15 min as long as the supernatant contained protein, judging from its extinction at 280 μ. The final residue was homogenized in a glass homogenizer in distilled water and centrifuged at 48,000g on an ultracentrifuge for 1.5 h four times. The first supernatant was discarded and the rest were collected, pooled, and lyophilized. The resulting material was investigated under the electron microscope by the negative staining method.

Partially dissociated fibrils were obtained by treating the amyloid fibrils with 0.1 N NaOH with a protein concentration of between 1 and 10 mg/ml. Dissociation was carried out in a cold room on a magnetic mixer for 30 min and 2 and 6 h. The resulting solutions were adjusted to neutral pH with 1 N HCl.

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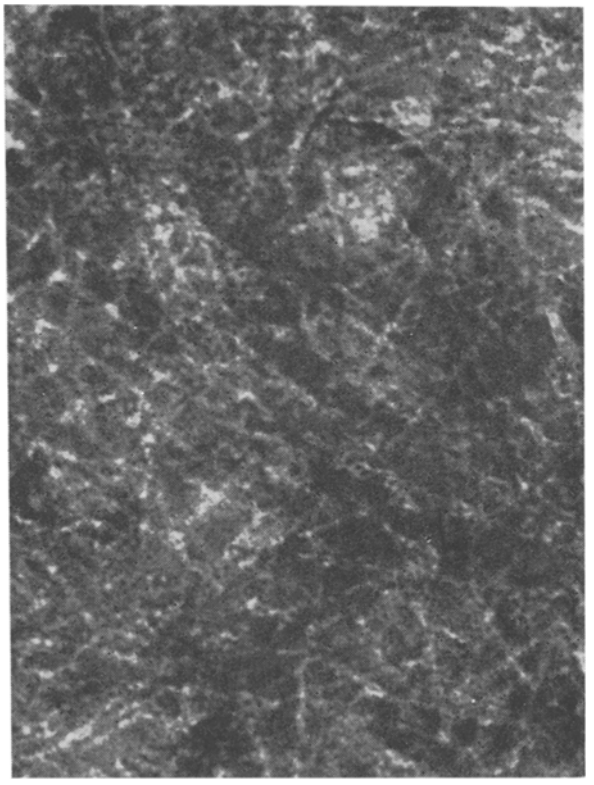

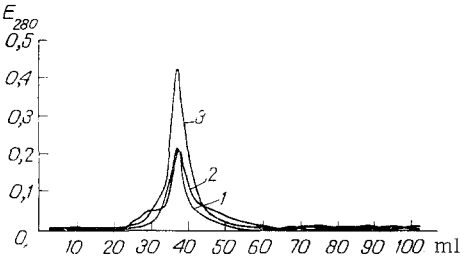

Fig. 1 Fig. 2

Fig. 1. Mouse amyloid fibrils. Electron micrograph. Negative staining. 20,000 ×.

Fig. 2. Elution curve of partially dissociated fibrils with different degrees of degradation from Sephadex G-200 column: duration of action of alkali 30 min (1), 2 h (2), and 6 h (3). Abscissa, volume of solution leaving column; ordinate, extinction of solution of denatured filbrils at 280 nm.

Electrophoresis of the purified, undissociated, and partially dissociated mouse amyloid fibrils in polyacrylamide gel with sodium dodecylsulfate (SDS) was carried out by Faibus' method [4] in 5.6% gel in Tris-acetate buffer. The test proteins were dissolved in dissociating buffer, pH 8.0, by boiling for 2-3 min in the ratio of 1 mg protein to 1 ml buffer. The dissociating buffer contained 2% SDS, 0.02% EDTA, 5% sucrose, 15 µg/ml pyronine, and 10 µmoles Tris in 1 ml. Amyloid fibrils (undissociated and partially dissociated) also were treated with dissociating buffer containing 1% mercaptoethanol besides the components listed above. Mercaptoethanol also was used in the dissociating buffer during electrophoresis of standard proteins. Electrophoresis was carried out for 1-1.5 h, during which time the pyronine zone moved through a distance of 75 mm. At the end of the experiment the gels were removed from the tubes and fixed alternately with three staining solutions containing acetic acid, isopropanol, and Coomassie in diminishing concentrations. The stained tubes of gels were placed in 7% acetic acid for keeping. Before the experiment a calibration curve was plotted for proteins with known molecular weights (mol. wt.): 200,000 — skeletal muscle myosin, 67,000 — serum albumin, 50,000 — heavy chains of immunoglobulins, 45,000 — ovalbumin, 25,000 — light chains of immunoglobulins, 12,400 — cytochrome, 1400 — chymotrypsinogen.

Chromatography on a column measuring 610 × 15 (D) mm with Sephadex G-200 was used for protein fractionation. Physiological saline was used as the eluant. The column was calibrated by proteins of known mol. wt.: 2 million — blue dextran, 480,000 — apoferritin, 45,000 — ovalbumin, 1450 — bacitracin. A sample of fibrils dissociated in 0.1 N NaOH for 30 min for 2 or 6 h, with a protein concentration of 2-3 mg, was applied to the column in a volume of 1 ml. The solution of dissociated fibrils was first centrifuged at 7000 rpm for 30 min and the small residue was discarded. The rate of elution was 5-7 ml/h. The elution profile was recorded directly by measuring extinction at 254 nm with a "Uvicord" instrument. Fractions of 3-4 ml were collected and their extinction measured at 280 nm on a spectrophotometer.

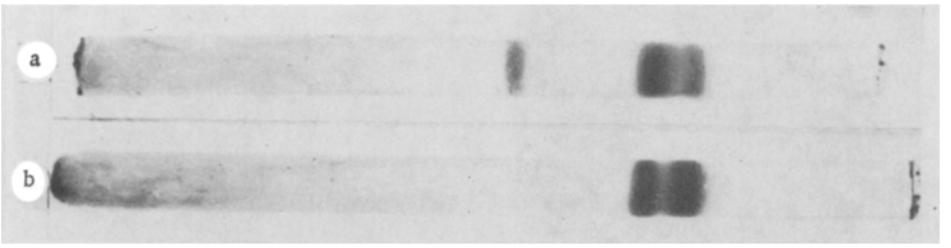

Fig. 3. Electrophoresis of native fibrils (a) and of fibrils partially dissociated with alkali (b).

EXPERIMENTAL RESULTS AND DISCUSSION

The purified preparations of amyloid fibrils consisted of cream-colored fluffy fibrous material, mildly adhesive, and reduced considerably in bulk as a result of gentle mechanical treatment. The mean yield was 2.6 mg of amyloid fibrils from 1 g mouse spleens.

Electron-microscopic analysis revealed typical amyloid fibrils in the preparations (Fig. 1).

Treatment of the preparations of fibrils with 0.1 N NaOH caused them to dissolve without residue. On neutralization of the solution, however, often a small residue was thrown down and was discarded after centrifugation. Chromatography of the product of fibril dissociation with alkali on Sephadex G-200 revealed the presence of only one component with mol. wt. of 500,000-600,000, according to the calibration data (Fig. 2). This result means that the fibrils obtained were highly purified and that the structure of the amyloid fibrils consisted of high-molecular-weight aggregates with mol. wt. of about 500,000. Data on the kinetics of dissociation of the fibrils under the influence of alkali confirm the view that under these conditions the fibrils break up, not into random fragments, but into definite, stable high-molecular-weight subunits. In fact, if the dissociation time was increased from 30 min to 6 h, only one peak remained on the elution curve, with a constant elution volume, corresponding to constancy of the molecular weight of the subunits. Dissociation of native fibrils and of fibrils partially dissociated with 0.1 N NaOH in 2% SDS, followed by electrophoresis of the dissociation products in SDS, revealed that the fibrils and their high-molecular-weight subunits are composed of two polypeptides with mol. wt. 11,000 and 15,000, in above equal proportions (Fig. 3). Disulfide bonds evidently do not participate in the association of these polypeptides in the composition of the fibrils, for the presence of 1% mercaptoethanol in the dissociating buffer had no effect on the molecular weight of the dissociation products. During electrophoresis of dissociation products of fibrils in SDS, both in the presence and in the absence of mercaptoethanol, besides the two principal bands, corresponding to mol. wt. of 11,000 and 15,000, a weaker band corresponding to a polypeptide with mol. wt. of 32,000 was found, differing from the bands with mol. wt. of 11,000 and 15,000 by its reddish-lilac color, such as is often given by glycoproteins when stained with Coomassie. If SDS was used to treat fibrils partially dissociated with 0.1 N NaOH, on subsequent electrophoresis only bands with mol. wt. of 11,000 and 15,000 were found. At least two possible explanations of this result can be given: 1) molecules, possibly glycoproteins, with mol. wt. 32,000 are not components of the fibrils but are an accompanying impurity and are deposited in the residue which is discarded after neutralization and centrifugation; 2) these molecules are also subunits of the fibrils, but they are not components of high-molecular-weight subunits with mol. wt. of 500,000-600,000 formed under the influence of alkali. Which of these explanations is correct only further experiments will show.

There is evidence in the literature that the elementary subunits of mouse amyloid fibrils are polypeptides with mol. wt. of 7200 [5], 8600 [2], 8700, and 9700 [3]. The results of the present investigation indicate that fibrils of the same type contain at least two types of polypeptide chains with mol. wt. of 11,000 and 15,000 simultaneously. These poly-

peptides in the composition of the fibrils have been shown to be associated into definite stable high-molecular-weight subunits with mol. wt. of 500,000-600,000. Disulfide bonds between the chains evidently play no part in maintenance of the quaternary structure of the amyloid fibrils.

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COMPENSATORY HYPERTROPHY OF THE OVARY IN ALLOXAN DIABETES AND ADRENORECEPTOR BLOCKADE

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KEY WORDS: alloxan diabetes; hypertrophy of the ovary; adrenoreceptors.

It was shown previously [3] that compensatory hypertrophy of the adrenal gland in alloxan diabetes is considerably reduced in rats and virtually absent in mice. This result indicated a disturbance of interaction between the pituitary and adrenals in diabetes. It was therefore interesting to study the course of compensatory hypertrophy of the ovary (CHO) in diabetes. Such an investigation could shed light on the state of interaction between the pituitary and gonad. Considering that adrenoreceptor blockade alleviates the course of experimental diabetes in rats [1, 2] and has a favorable effect on human diabetes [4], it was also decided to study the effect of obsidan (propranolol) and of phentolamine on CHO in rats with diabetes.

EXPERIMENTAL METHOD

Experiments were carried out on adult male albino rats initially weighing 207 g. A single subcutaneous injection of alloxan was given in a dose of 15 mg/100 g body weight. Two hours before injection of the alloxan solution, some of the animals were given an intraperitoneal injection of obsidan (3 mg/rat) or phentolamine (2 mg/100 g body weight). After the second day the adrenoblockers were injected intramuscularly: obsidan in the previous dose, phentolamine in a dose of 5 mg per rat. After the 10th day of the experiment the drugs were given on alternate days. Twelve days after injection of alloxan, the left ovary

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