ANAPHYLACTOGENIC PROPERTIES OF PRODUCTS OF COMPLETE SOLUTION OF COLLAGEN

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It has been shown in guinea pigs and rabbits that the products of complete solution of collagen possess extremely weak anaphylactogenic properties, which do not prevent their clinical use.

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In recent years the possibility of completely dissolving collagen fibers in an acid medium after preliminary rupture of the intermolecular bonds in an alkaline saline solution has been demonstrated [3]. The various collagen preparations obtained by precipitation of the solutions (films, sponges, replicas of hollow organs) have been used by the authors experimentally for the repair of blood vessels and the bile ducts, for the treatment of burns, skin wounds involving extensive skin loss, wounds of the liver, and so on [5, 6].

The prospects of wide clinical application of these protein materials naturally bring up the question of their immunologic properties. Collagen is known to possess very weak antigenic properties [2, 7-12], evidently, because of the absence of aromatic amino acids in its molecule. The antigenic properties are depressed still further after tanning and sterilization of collagen by γ rays [7].

The object of the present investigation was to study the anaphylactogenic properties of collagen solutions obtained from the bovine dermis and of the fibers precipitated from solution in a pure form, and also as a complex with heparin, as used in plastic operations on blood vessels [5].

EXPERIMENTAL METHOD

Altogether two series of experiments were performed.

In series I, 10 guinea pigs weighing 280-300 g were immunized by a single subcutaneous implantation of 60 or 120 mg collagen precipitated from solution by acetone in the form of fibers (4 animals) or of a dried collagen-heparin complex (6 animals), so as to make the experimental conditions as close as possible to those of experimental and clinical collagen implantations. The reacting dose (0.2 ml of 0.3% collagen) was injected 30 days later intravenously. Bovine serum was injected as a control. The degree of the reaction was assessed by L. A. Zil'ber's method. The animals were sacrificed and control histological studies made.

To record the degree of the anaphylactic reaction objectively (graphic recording of the arterial pressure and respiration), the experiments of series II were performed on 11 rabbits, since analogous investigations on guinea pigs are impossible.

Rabbits weighing 3000-3200 g were immunized with 0.5% neutral collagen solution (8 animals), and a solution of collagen with heparin (0.1 ml heparin to 10 ml of 0.5% collagen solution; 6 animals). Immunization was carried out three times at intervals of 2 days in doses of 0.5 or 1 ml/kg body weight. As reacting dose, 0.5% collagen solution was injected in a dose of 5 ml/kg 20 days after the first immunization. Two control animals were injected with the same dose of collagen without preliminary sensitization. Yet another two immunized animals were injected with bovine serum in a dose of 1 ml/kg as reacting dose.

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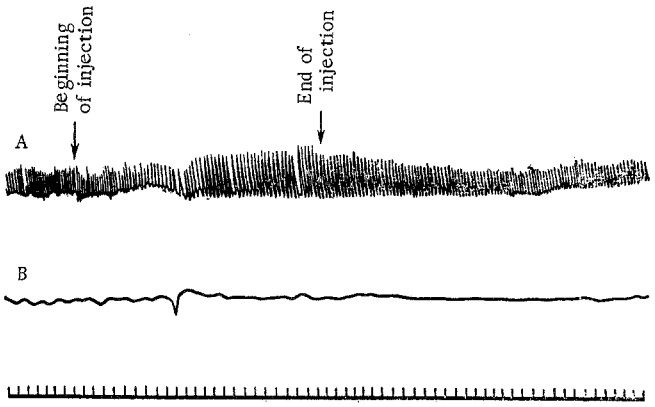

Fig. 1. Record of respiration (A) and arterial pressure (B) after intravenous injection of reacting dose of collagen.

EXPERIMENTAL RESULTS

In most animals of series I negative (–) or doubtful (±) results were obtained. No appreciable difference was found between the anaphylactogenicity of collagen or of collagen-heparin complex. In the control series the reaction was doubtful.

Histological investigation of the animals with weak anaphylaxis revealed moderate spasm of the bronchi. No marked changes characteristic of anaphylaxis (edema of the lungs, hemorrhage into the lungs or intestine, and so on) were observed. In most animals the histological picture was indistinguishable from normal.

In series II, regular changes in respiration were observed in most animals of both groups (immunized with collagen and collagen-heparin). In some animals 40-50 sec after the beginning of intravenous injection of the reacting dose of collagen the amplitude of respiration was increased, and in most animals the respiration rate was reduced by 33-50%. After 1 or 1.5 min, respiration returned to its original level (see Fig. 1). No differences were found in the respiration reaction between the animals of the experimental groups.

The results of measurement of the arterial pressure of the animals were varied in type. In four of the 8 animals of group 1 an extremely slight increase of arterial pressure was observed (by 2-6 mm) in step with the change in respiration. Later the arterial pressure gradually, and at different times – from 20 sec to 4 min – returned to its initial level and then remained unchanged until the end of the experiment. In 1 rabbit, after a very transient increase, a slight decrease of pressure (4 mm) compared with its original level took place, returning after 3 min to its initial value. In 2 animals 30-60 sec after the beginning of injection of the reacting dose a very brief fall of arterial pressure (by 3-4 mm) was observed. Finally, the tracing of the arterial pressure of one of the animals was unchanged. Among the animals of group 2, which were immunized with collagen-heparin, no changes of arterial pressure were found in 2 rabbits, while in the rest there was a slight increase similar to that described above. If bovine serum was injected as reacting dose instead of collagen, the arterial pressure remained unchanged and respiration was weakened only actually during the injection.

Hence, there was practically no decrease of arterial pressure such as is characteristic of the anaphylaxis reaction in rabbits [1].

Injection of the same dose of protein-free fluid (physiological saline) or of low molecular weight protein (serum albumin) caused no change in respiration or blood pressure. After injection of a macromolecular carbohydrate (dextran), with a molecular weight of 300,000, a slight increase of arterial pressure was observed in 2 animals and a small decrease in two others. A transient fall of arterial pressure was also found in one of the control animals receiving an injection of collagen without immunization. These results suggest that the variation in arterial pressure in this particular series cannot be regarded as a manifestation of anaphylaxis; they were evidently associated with the nonspecific action of the macromolecular solutions.

Most reports in the literature are based on the study of the antigenic activity of acid-soluble or neutral saline-soluble collagen fractions. The results of the experiments now described confirmed the hypothesis that alkaline-saline treatment preceding complete solution, although not breaking up the macromolecule, may cause certain changes in the molecular structure of the collagen and diminish its antigenic activity. Other evidence of this is the absence of allergic reactions in patients with collagen prostheses, sponges, and films. However, further clinical investigations of the allergenic properties of collagen are necessary.

Hence, the products of complete alkaline-acid solution of collagen, in the form of solutions, fibers precipitated from them, and collagen-heparin complex, possess extremely weak anaphylactic properties.

LITERATURE CITED

1. L. A. Zil'ber, Fundamentals of Immunity [in Russian], Moscow (1948).
2. V. I. Mazurov, Comparative Investigation of Soluble Collagen Proteins of Connective Tissue. Candidate dissertation, Moscow (1960).
3. E. V. Minkin and I. S. Shestakova, in the book: Scientific Transactions of Moscow Technological Institute of Light Industry [in Russian], No. 25, Moscow (1962), p. 52.
4. A. A. Tustanovskii, et al., Dokl. Akad. Nauk SSSR, 97, No. 1, 121 (1954).
5. A. M. Khil'kin, et al., Éksper. Khir., No. 1, 26 (1965).
6. A. M. Khil'kin, et al., Vestn. Khir., No. 3, 79 (1966).
7. F. Cervinka, et al., Folia Biol.,(Praha), 10, 94 (1964).
8. H. Grillo and J. Gross, J. Surg. Res., 2, 69 (1962).
9. P. Heller, et al., Proc. Soc. Exp. Biol., (New York), 101, 509 (1959).
10. M. Paz, et al., Proc. Soc. Exp. Biol., (New York), 113, 98 (1963).
11. S. Rothbard and R. F. Watson, J. Exp. Med., 113, 1041 (1961).
12. C. Steffen, Wien. Klin. Wschr., 75, 894 (1963).