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# LOW ALLERGENIC GELATIN PREPARED FROM CHICKEN CARTILAGE BY ACID PROCESSING

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### **Backgrounds and Objectives:**

Gelatin is prepared from animal skins and bones by alkaline or acid processing, extraction, purification, and so forth. Gelatin has widely been used as an ingredient of medicines, cosmetics and foods, and regarded as safe, particularly nonallergenic.

However, studies of adverse reactions to live vaccines proved that gelatin caused allergic reactions. By the protein blotting method, Kelso et al. demonstrated the presence of anti-gelatin IgE in the serum of a child developing anaphylaxis to measles-mumps-rubella vaccine, in which gelatin had been used for stabilizing the virus<sup>1</sup>). Thereafter, many cases of allergic reactions to vaccines or to gelatin-containing foods have been reported <sup>2-5</sup>). Gelatin can, however, hardly be substituted by other materials because of its outstanding properties.

In the course of our studies to develop low allergenic gelatin, we found that denatured type-II collagen from chicken cartilage exerted an extremely low allergenic property.

The present study was to compare the allergenic properties of chicken-cartilage gelatin prepared by alkaline and acid processing.

# Materials and Methods:

Serum samples containing anti-gelatin IgE: Serum samples were collected from 15 gelatin-allergic and 15 healthy donors with informed consent as described previously.

*Gelatin and type-II collagen:* Two kinds of gelatin were prepared in our laboratory from chicken cartilage by two different processes; alkaline processing (designated alkaline gelatin) and acid processing (designated acid gelatin). Commercially available gelatin preparations were purchased at nearby retail shops. Type-II collagen was prepared from cartilage by limited pepsin digestion with or without alkaline pretreatment.

Assessment of allergenic properties of gelatin preparations: Allergenic properties of various gelatin preparations were determined with the serum samples from gelatin-allergic donors by chemiluminescence ELISA.

#### **Results and Discussions:**

The allergenic properties of various gelatin preparations are shown in Fig. 1. The allergenic property of commercially available gelatin preparations was significantly high (25.36 Ua/ml), whereas that of acid gelatin was significantly low (1.01 Ua/ml) and that of alkaline gelatin was intermediate (6.36 Ua/ml). No gelatin-specific IgE was detected in the serum samples from healthy donors (data not shown).





Fig.1. Allergenic properties of various gelatin preparations (Mean±SEM, \*:p<0.05, \*\*:p<0.01)

Fig.2. Allergenic properties of denatured type-II collagen by extension of the alkaline treatment period (Mean±SEM)

To study such difference, type-II collagen was prepared by alkaline treatment for different periods and subjected to determination of allergenic properties. As shown in Fig. 2, the allergenic properties increased with the period of alkaline treatment.

The previous studies showed that the alkaline processing hydrolyzed specific amino-acid residues of the gelatin molecule and altered the molecular conformation<sup>6)</sup>. As shown in Table 1, the isoelectric point and the number of ornithine residues of alkaline gelatin were different from those of acid gelatin and denatured type-II collagen without alkaline pretreatment. Alkaline processing may have altered the primary structure of gelatin, resulting in increased allergenic property.

## **Conclusions:**

We prepared two kinds of gelatin (alkaline gelatin and acid gelatin) from chicken cartilage and compared their allergenic properties using the serum samples from gelatin-allergic patients.

We found the following; (1) the chicken-cartilage gelatin, particularly that prepared by acid processing, was much safer than the ordinary gelatin preparations commercially available, and (2) such an allergenic property of gelatin might be induced by alkaline processing.

Table 1. Chemical properties of gelatin preparations and denatured collagen from chicken cartilage

and the second	No. of ornithine residues	
	pl	(per 1000 total amino acid residues)
Alkaline gelatin	5.3	3
Acid gelatin	8.4	0
Denatured type-II collagen <sup>a</sup>	8.5	0

\*prepared from chicken cartilage by limited pepsin digestion without alkaline pretreatment

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