

ANAPHYLACTOGENIC PROPERTIES OF GELATIN AND ITS PRECURSORS

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ON OCCASION, questions have been raised relative to the allergenicity of edible gelatin. Some clinical reports have claimed its occurrence. Generally, however, gelatin has been considered as nonantigenic.

In addition, little attention has been given to the antigenicity of the parent products as distinct from the hydrolyzed gelatin. Traces of contaminants may elicit allergic symptoms, although gelatin itself may not be allergenic. It is for this reason that we included in this study the antigenic properties of bovine plasma, comminuted cooked ossein, and crude gelatin solution derived from the comminuted ossein, as well as food-grade gelatin and the most purified form—that used for intravenous injection.

MATERIALS

Bovine Plasma.†—In some pilot experiments, bovine serum and bovine plasma each reacted when used to challenge animals previously injected with comminuted ossein. Since the blood element reacting with the comminuted ossein was present in both the plasma and serum, bovine plasma was used exclusively in the experiments.

Comminuted Ossein.‡.—

Stage A: This preparation is made from bovine bone demineralized with 5 per cent hydrochloride, washed with water, and then treated with saturated limewater solutions at 60° F. for approximately four weeks for removing extraneous materials such as albumins, mucins, and chondrins and also to initiate hydrolysis of collagen to gelatin. The limed stock is then washed with cold water and dilute acid to approximately pH 6.0 to remove lime salts. At this stage the ossein is ready to extract with hot water at approximately 160° F. This is the material which, in its entirety, formed the basis for the preparation of crude gelatin raw material.

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This is the seventh in a series of studies on the Allergenicity of Modified and Processed Foodstuffs.

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†The bovine plasma and serum were generously supplied to us by Dr. M. S. Cooper, bacteriologist, Biological Production Section, Lederle Laboratories Division, American Cyanamid Company.

‡The comminuted ossein, crude, food-grade, and intravenous-grade gelatins were generously supplied to us by Dr. D. Tourtellotte, Director, Research Division, Charles B. Knox Gelatine Company, Inc. The gelatin solution contained 0.0108 gram protein nitrogen per cubic centimeter and the ossein 0.018 gram nitrogen per cubic centimeter.

Stage B: Two hundred thirty grams (40 per cent solids) of the stock was placed in 500 c.c. bottles with 290 grams distilled water added to fill, autoclaved at 15 pounds steam pressure for twenty minutes, then the cooked mix was poured into a Waring blender and ground to a uniformly comminuted mush. This comminuted material was rebottled, sealed, and reautoclaved at 15 pounds steam pressure for twenty minutes.

Crude Gelatin.—From the extraction of the boiling just described, an amount of crude gelatin liquors was placed in sealed bottles and subjected to autoclaving at 15 pounds steam pressure for twenty minutes. This provided intermediate material, hot water extracted, but not filtered free of the traces of insoluble and particulate organic matrix.

Food-Grade Gelatin.—This preparation represents the finished commercial dry gelatin dissolved in distilled water, 6 per cent w/v with 0.9 per cent sodium chloride added to physiologic concentration. The commercial dry gelatin used in these experiments was that supplied by the Knox Gelatine Company and is known as Knox Sparkling Gelatine, U.S.P.

*Intravenous-Grade Gelatin.**—Since this product is designed entirely for parenteral use, more exacting selection is made of hard bone starting materials to avoid spongy bone, which can carry traces of hemoglobin and plasma protein residues which might become factors in allergenic responses to the finished product. More exacting care is exercised throughout the intravenous gelatin process to insure complete sterility at every stage. This is accomplished by working in completely enclosed equipment and at temperatures and reactions which are inhibitory to even air-borne contamination. In the case of the intravenous solution, all filtrations are made through special asbestos sterilizing pads, followed by immediate autoclaving in the final sealed flasks used for parenteral administration.

METHODS

The precipitin or complement fixation tests cannot be used because the gelatin congeals on cooling and because the comminuted ossein has particulate matter in it. We believe the induction of anaphylaxis in the guinea pig and the use of the Schultz-Dale technique on isolated tissue to be the most suitable for studies on the antigenicity of these materials.

Normal guinea pigs,† weighing approximately 250 grams, which had never been fed beef or gelatin, were used for the anaphylaxis studies.

Anaphylaxis was tested by the usual sensitization and shock procedures, with incubation periods of three to four weeks intervening. The sensitizing injections were made intraperitoneally and shock injections, unless otherwise specified, were given by the intravenous route. Whenever possible and when there was need for determining whether the absence of shock reaction was the result of absence of sensitization to a related material, the substance to which the animal was originally sensitized was employed as a second confirmatory

*Special gelatine solution, intravenous, Council of Pharmacy and Chemistry, J.A.M.A. 142: 32-33, Jan. 7, 1950.

†Supplied by Carworth Farms, New City, N. Y.

shock injection. A negative reaction obtained with a modified form does not justify the conclusion that allergenicity was not present unless proof is at hand that the animal had been sensitized in the first place. We believe this to be a sound method for determining the anaphylactogenicity of a modified protein.¹

An adequate number of animals must be used in these experiments. By using ten or more animals, chance of missing a low sensitizing potential is reduced.

Because of the character of the comminuted ossein, the particulate bone elements made it impossible for us to use this material for intravenous shock injection.

The Schultz-Dale experiment was used to supplement graphically the anaphylaxis experiments.

EXPERIMENTS

Eleven animals were sensitized with comminuted ossein and challenged with bovine plasma. The comminuted ossein evidently contained some blood element derived from the homologous bovine plasma, as a cross anaphylactic reaction was demonstrated in this series. However, when the order was reversed and the animals were given a sensitizing injection of bovine plasma and challenged with the comminuted ossein, there was no demonstrable evidence of a cross reaction in eighteen animals tested. When a second injection was given, using bovine plasma to which the animals had been sensitized, seventeen of the eighteen animals died in anaphylactic shock. This proved them to be highly sensitive to the plasma and not to the ossein (Table I). This indicates that the bovine blood elements present in ossein were there in extremely small amounts because, although there was enough present to sensitize, there was not enough present to shock plasma-sensitized animals.

TABLE I

NUMBER OF ANIMALS	SENSITIZING INJECTION	SHOCK INJECTION	RESULTS		SECOND SHOCK INJECTION	RESULTS	
11	Comminuted ossein 2 c.c. Ip.	Bovine plasma 0.2 c.c. Iv.	2 animals	++++			
			4 animals	+			
			5 animals	0			
18	Bovine plasma 1 c.c. Ip.	Comminuted ossein 2 c.c. Ip.	0		Bovine plasma 0.3 c.c. Iv.	17 animals	++++
						1 animal	0

Key to symbols:

+, Moderate dyspnea.

++, Dyspnea and convulsions.

+++, Dyspnea, convulsions, collapse, and recovery.

++++, Anaphylactic death with typically ballooned lungs.

Ip., Intraperitoneal.

Iv., Intravenous.

Table II shows data on attempts to sensitize animals to ossein and to determine whether or not highly purified intravenous-grade gelatin retained any of the blood-contaminating antigenic elements found in the ossein.

There was no evidence of cross reaction with the gelatin in fourteen animals sensitized with comminuted ossein and challenged with intravenous-grade gel-

TABLE II

NUMBER OF ANIMALS	SENSITIZATION COMMINUTED OSSEIN	SHOCK IV.-GRADE GELATIN	RESULT	CONTROL SHOCK BOVINE PLASMA	RESULT
14	2 c.c. Ip.	0.3 c.c. Iv.	0	0.3 c.c. Iv.	1 animal + + + + 1 animal + + + 6 animals + 6 animals 0

atin. When these same animals were subsequently challenged with bovine plasma, eight proved anaphylactic, showing that the comminuted ossein contained some blood element, whereas the intravenous-grade gelatin was completely devoid of it.

The next grade of purification after the ossein is crude gelatin. Nineteen animals were injected with crude gelatin and challenged with bovine plasma. There was no evidence that the blood elements were present in the crude gelatin (Table III). When these nineteen animals were subsequently challenged with crude gelatin they were all negative.

TABLE III

NUMBER OF ANIMALS	SENSITIZATION CRUDE GELATIN	SHOCK BOVINE PLASMA	RESULT	CONTROL SHOCK CRUDE GELATIN	RESULT
19	3 c.c. Ip.	0.3 c.c. Iv.	0	0.3 c.c. Iv.	0

In the next series (Table IV), nineteen animals were sensitized to bovine plasma and challenged with crude gelatin with negative results. A second challenging dose of bovine plasma showed them to be highly sensitive to bovine plasma.

TABLE IV

NUMBER OF ANIMALS	SENSITIZATION BOVINE PLASMA 1 c.c. Ip.	SHOCK CRUDE GELATIN	RESULT	CONTROL SHOCK BOVINE PLASMA	RESULT
19		0.3 c.c. Iv.	0	0.3 c.c. Iv.	14 animals + + + + 2 animals + 3 animals 0

Food-grade gelatin was then tested for allergenicity (Table V). In the first twenty-six animals (Group I) food-grade gelatin was used as a sensitizing dose and bovine plasma as a challenging dose. There was evidence of the presence of bovine blood element in the food-grade gelatin in only two of these. In Group II, nineteen animals were injected and challenged with the same food-grade gelatin. There was no evidence of sensitization to the gelatin itself, although one showed the presence of some plasma element when the animals were given a second shock dose with bovine plasma.

In Group III, twenty-two animals were sensitized with bovine plasma and challenged with food-grade gelatin. In no instance was a blood element demonstrated in the food-grade gelatin when used as a challenging dose. However,

TABLE V

GROUP	NUMBER OF ANIMALS	SENSITIZATION	FIRST SHOCK	RESULT	SECOND SHOCK	RESULT
I	26	Food-grade gelatin 3 c.c. Ip.	Bovine plasma 0.3 c.c. Iv.	1 animal + + + + 1 animal + 24 animals 0		
II	19	Food-grade gelatin 3 c.c. Ip.	Food-grade gelatin 0.3 c.c. Iv.	0	Bovine plasma 0.3 c.c. Iv.	1 animal + + + + 18 animals 0
III	22	Bovine plasma 1 c.c. Ip.	Food-grade gelatin 0.3 c.c. Iv.	0	Bovine plasma 0.3 c.c. Iv.	20 animals + + + + 1 animal + + 1 animal 0

when these twenty-two animals again were challenged with the bovine plasma with which they were originally sensitized, twenty-one demonstrated profound anaphylaxis.

In a total of forty-five animals, therefore, there were only three instances, or 6.6 per cent, where sensitization to the blood elements was demonstrated by the food-grade gelatin. In twenty-two animals, however, when the food-grade gelatin was used as a shock agent in animals exquisitely sensitive to bovine plasma, there was no evidence that the contaminating blood elements were present in sufficient amounts to produce a shock reaction.

Therefore, in none of a relatively large series of animals was food-grade gelatin shown to be anaphylactogenic to itself. However, there was evidence that there is an extremely slight amount of the blood element found in the food-grade gelatin which occasionally was able to sensitize but never was able to produce shock reactions.

TABLE VI

GROUP	NUMBER OF ANIMALS	SENSITIZATION	FIRST SHOCK	RESULT	SECOND SHOCK	RESULT
I	10	Iv.-grade gelatin 2 c.c. Ip. 5 days later, 1 c.c. Ip.	Bovine plasma 0.3 c.c. Iv.	0	Iv.-grade gelatin 0.3 c.c. Iv.	0
II	30	Bovine plasma 1 c.c. Ip.	Iv.-grade gelatin 0.3 c.c. Iv.	0	Bovine plasma 0.3 c.c. Iv.	19 animals + + + + 2 animals + + + 3 animals + 6 animals 0

The most purified form of gelatin is the one used for intravenous injection. In Table VI, Group I, there were ten animals given two sensitizing injections of intravenous-grade gelatin, five days apart, and challenged with bovine plasma. They were consistently negative. When challenged with intravenous-grade gelatin again, they were also proved to be negative, indicating that this gelatin was devoid of allergenicity. In Group II thirty animals were sensitized with bovine plasma and in no instance was there any evidence of cross reaction when challenged with intravenous-grade gelatin. The animals were sensitive to a high degree, for twenty-four of the thirty showed anaphylaxis when challenged with the original bovine plasma.

Schultz-Dale experiments, an example of which is shown in Fig. 1, graphically epitomize the studies of the antigenicity of gelatin. Virgin female guinea pigs sensitized to comminuted ossein were used in these experiments. The sensitized uterus was immersed in oxygenated Tyrode's solution. The uterine strip was challenged with food-grade gelatin (*A*). There was no evidence of reaction. After several minutes, the strip was challenged with bovine plasma (*B*). A very prompt maximal contraction ensued. The bath was changed with fresh Tyrode's solution (*C*). Bovine plasma was again placed in the bath (*D*). An immediate secondary contraction of lower amplitude ensued. The Tyrode's solution was again replaced (*E*). Bovine plasma was again placed in the bath (*F*). There appeared to be a refractoriness, followed several minutes later by a delayed reaction. The bath was changed again (*G*) and bovine plasma (*H*) was put in for the fourth time. The muscle showed complete refractoriness. Finally, without further change of bath, the muscle was challenged with histamine acid phosphate (*I*), which produced a maximal contraction.



Fig. 1.—Animals sensitized with comminuted ossein and tested with food-grade gelatin and bovine plasma. *A*, Food-grade gelatin; *B*, bovine plasma; *C*, washed; *D*, bovine plasma; *E*, washed; *F*, bovine plasma showing refractoriness and a delayed reaction; *G*, washed; *H*, bovine plasma, desensitization; *I*, histamine acid phosphate.

This experiment shows in a vivid manner that when the precursor (comminuted ossein) of the food-grade gelatin is used as a sensitizing agent, there is no evidence of antigenicity when challenged with food-grade gelatin, but there is definite evidence of sensitization to some element in the blood plasma. The secondary contraction at *D* and the delayed contraction at *F*, when the uterine strip was challenged twice with bovine plasma, suggest that the first challenge dose did not result in a complete refractoriness. This might be due to a multiplicity of antigens in the bovine plasma. The final refractoriness indicates complete desensitization of all the fractions in the bovine plasma. The reaction to histamine proves the validity of the desensitization by showing that the uterine strip retains its viability and optimal functioning.

SUMMARY OF EXPERIMENTS

I. (See Table VII.) Comminuted ossein was found to sensitize against bovine plasma in fourteen of twenty-five animals (56 per cent). Food-grade gelatin, refined from comminuted ossein, sensitized only three of forty-five animals (6.6 per cent) when challenged with bovine plasma. In none of thirty-two animals did intravenous-grade gelatin sensitize against bovine plasma.

TABLE VII. COMPARATIVE REACTIVITY OF CONTAMINATING BOVINE BLOOD ELEMENTS IN CRUDE AND REFINED GELATINE WHEN USED AS A SENSITIZING AGENT

NUMBER OF ANIMALS	SENSITIZATION	SHOCK	RESULTS		
			POSITIVE	NEGATIVE	PER CENT POSITIVE
25	Comminuted ossein	Bovine plasma	14	11	56.0
45	Food-grade gelatin	Bovine plasma	3	42	6.6
32	Iv.-grade gelatin	Bovine plasma	0	32	0.0

II. (See Table VIII.) In none of 141 animals sensitized with bovine plasma, comminuted ossein, crude gelatin, food-grade gelatin, and intravenous-grade gelatin was there any reaction when the animals were challenged with any of the forms of unrefined or refined gelatin.

TABLE VIII. NONREACTIVITY OF CRUDE AND REFINED GELATIN WHEN USED AS ANAPHYLACTIC SHOCK AGENTS

NUMBER OF ANIMALS	SENSITIZATION	SHOCK	RESULT
14	Comminuted ossein	Iv.-grade gelatin	0
9	Crude gelatin	Crude gelatin	0
19	Bovine plasma	Crude gelatin	0
19	Food-grade gelatin	Food-grade gelatin	0
22	Bovine plasma	Food-grade gelatin	0
30	Bovine plasma	Iv.-grade gelatin	0
10	Iv.-grade gelatin	Iv.-grade gelatin	0
18	Bovine plasma	Comminuted ossein	0

III. (See Table IX.) Whether the intravenous-grade gelatin was used as a sensitizing injection or as a shock injection, in no instance in fifty-eight animals was there any sensitization or shock manifested, even when they were cross-tested with bovine plasma.

TABLE IX. ABSENCE OF ANY ANAPHYLACTOGENIC REACTIVITY OF INTRAVENOUS-GRADE GELATIN

NUMBER OF ANIMALS	SENSITIZATION	SHOCK	ANAPHYLAXIS
14	Comminuted ossein	Iv.-grade gelatin	0
24	Bovine plasma	Iv.-grade gelatin	0
10	Iv.-grade gelatin	Bovine plasma	0
10	Iv.-grade gelatin	Iv.-grade gelatin	0

COMMENT

Gelatin does not exist as a natural protein but is derived from hydrolysis of the insoluble connective tissue protein collagen which exhibits no demonstrable antigenic effects.

The nonantigenicity of gelatin was thought to be due to a deficiency of aromatic groupings of its amino acid constituents, tyrosine and tryptophane. There are several other possible explanations for the immunologic inertness of this protein. It is deficient in carbohydrate groupings. It may be excreted rapidly in the urine after intravenous injection into the animal. It is usually prepared from collagen by prolonged treatment with boiling water or steam and this treatment may well be sufficient to destroy any antigenic power of the preparation.

In explanation of the foregoing are several investigations² which showed that the introduction of aromatic groupings, including tyrosine, into gelatin does not produce a compound which has antigenic powers comparable with those of the majority of proteins.

Similarly, the failure does not appear to be due solely to lack of tyrosine even when carbohydrates are added.³

With reference to its rapid excretion via the kidneys, Haurowitz and his co-workers⁴ believe that the major part of ingested gelatin or gelatin derivatives is so rapidly excreted in the urine that little remains in the body to induce antibody formation. Gelatin and several other proteins with molecular weights of less than 70,000 are rapidly excreted in the urine by anesthetized dogs.⁵ Proteins such as egg albumin and Bence-Jones protein, both of which are excreted by the kidneys, are known to be fully antigenic. Loss by excretion in the urine, according to Wormald,⁶ cannot be the only factor that determines the nonantigenicity of gelatin.

The last explanation (that is, the effect of prolonged heating in boiling water), we believe, has validity because it has been shown that albumin heated in the presence of water becomes coagulated and does have a definite reduction in antigenicity.⁷

The studies detailed in this article demonstrate that gelatin is a nonantigen and that any antigenicity found in its crude precursor (comminuted ossein) was due solely to contamination with bovine blood elements. In no instance did crude or refined gelatin serve as an anaphylactogen. The presence of contaminating blood elements was found in 56 per cent of the experiments with comminuted ossein and in only 6.6 per cent of the experiments with food-grade gelatin. The anaphylactogenicity of the blood elements present in the comminuted ossein and crude gelatin mixtures persisted despite the heat and hydrolytic treatments during the process of gelatin preparation.

In no instance was any contaminating blood element found in the highly purified gelatin used for intravenous blood replacement.

CONCLUSIONS

1. Within the limits of our experiments, we can state that gelatin is non-anaphylactogenic.

2. The antigenicity attributed to gelatin is related to a contamination by some blood elements of the species from which the gelatin is derived. This antigenic element is present in both the plasma and serum and apparently is highly resistant to heat.

3. The presence of blood contaminants in comminuted ossein was demonstrated in the majority (56 per cent) of tests on animals sensitized to this precursor of refined gelatin. It was found to a negligible degree (6.6 per cent) in sensitization tests with the food-grade gelatin. It was completely eliminated in the intravenous-grade gelatin (Knox).

4. The blood contaminant in the comminuted ossein and the food-grade gelatin was present in so minute an amount that it did not induce any anaphylactic reactions in animals sensitized to the whole plasma.

5. Because no authentic case of gelatin sensitization has been demonstrated in man, we were unable to obtain gelatin antibody-containing blood for human studies. We therefore must assume that because it is nonanaphylactogenic for the animal, it must be nonallergenic for man.

6. Any allergenicity in man that might be attributed to gelatin is due to contamination with blood elements of the species from which it is derived and not to antigenicity of the gelatin itself.

In recent studies, Maurer^{8, 9} reports that he found normally occurring antibodies to gelatin in man. He also found them in herbivorous as well as carnivorous animals. This naturally occurring antibody is therefore not related to the diet of the animal. It may arise as a result of some relation between the collagen system and gelatin metabolism but this is uncertain. The antibody is not concerned in the production of the wheal reaction and apparently is not an anaphylactic antibody.

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