Serological Identification of Animal Proteins

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The discrimination of animal proteins is important in the field of forensic medicine. But recently the detection of proteins adulterated in livestock products has been occasionally sought.

As for the discrimination method of proteins, the physical and chemical processes or electrophoresis have been employed. But these methods cannot be easily made available for the discrimination of mixed proteins of miscellaneous origins.

On the contrary, the immunological discrimination of proteins can be carried out rather easily with a high sensitivity to obtain the more precise and higher specificity.

Therefore, the author has investigated the detection of animal species of meat proteins by the immunological discrimination method.

Preparation of antiserum

Two ways to procure antigen for the preparation of antiserum regarding the discrimination of meat proteins are known. One is to use the constituent parts of blood (serum and hemoglobin)[1], and the other is to make use of the muscular extract or the muscular constituents (actomyosin, myoglobin)[2,3].

Generally, the production of antibody against the muscular protein is rather difficult than that of against the serum protein. But it has succeeded in producing the species specific antibody of high titer by the improved immunization method, especially by utilizing the adjuvant meat antigen treatment[4].

The preparation of antigen was carried out as follows: (1) some raw meat minus their fatty portions was homogenized three times the amount of buffered saline (pH 8.2) in the blender during 5 minutes, (2) then the homogenates, after freezing and dissolution, was centrifugalized during 5 minutes (6000 rpm); (3) the supernatant fluid was used as the antigen and, (4) its protein concentration was about 1.5 per cent.

Generally, rabbit has been used for immunization but cattle, sheep, goat and chicken have been employed for the preparation of the antiserum which has been limited with the cross reaction.

The rabbit and chicken were immunized by single injection into their pads with the mixture of 1 ml meat extract described above (its protein amount was about 10 mg) and the same amount of Freund's complete adjuvant.

For sheep and goat, 5 ml of meat extract, and for cattle 10 ml of it, adjuvant treated respectively, were injected into their muscles two times at intervals of 3 weeks.

The antibody production was recognized 2 to 3 weeks after the immunization and generally, antiserum (titer 1:8–1:32) could be obtained at the end of 5 to 6 weeks from the immunization.

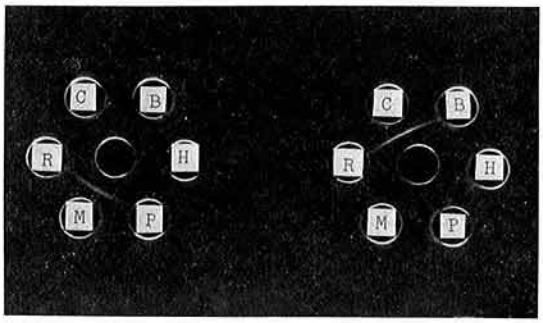
Method of serological test

The Ouchterlony's agar gel immunodiffusion

method was the best one of the routine test of serological reaction[5]. In our experiment, the agar gel had been prepared as follows: the thickness of gel was kept about 1.5 mm, the diameter of antigen and antibody-well were 6 mm, and the distance between them was 4 mm so as to get the clear precipitation bands.

As can be seen in Fig. 1, the antiserum was put in the central well, the antigens (the extract of subject meat) were placed in the surrounding wells and the agar gel plate was kept in the room temperature from 24 to 30 hours. Then the reaction was investigated.

Grabar's immunoelectrophoresis was used[5] to detect the positions of species specific antigens in a meat extract and Culliford's immunoelectrophoresis (reported in 1964)[6] was applied so as to shorten the reaction time. And the single radial immunodiffusion method may be applicable as a quantitative test[7].



Anti-Mutton Anti-Chicken

B : Beef extract
H : Horse meat extract
P : Pork extract
M : Mutton extract
R : Rabbit meat extract
C : Chicken meat extract

Fig. 1. Precipitation bands of species specific antisera on gel diffusion plate

Species specificity of antiserum

As noted in Table 1, the unabsorbed anti-meat sera have revealed a conspicuous species specific reaction against the meat extract antigen, but they have manifested the cross-reaction against the meat extract antigen of comparatively close relative animals.

Table 1. Serological specificity of unabsorbed anti-meat sera in gel diffusion test

Antiserum	Antigen										
	Beef	Mutton	Horse	Pork	Rabbit	Chicken	Turkey	Duck	Quail	Tuna	Soya*
Rabbit anti-beef	S**	C	·	C	·	·	·	·	·	·	·
Sheep anti-beef	S	·	C	C	·	·	·	·	·	·	·
Rabbit anti-mutton	C	C	·	·	·	·	·	·	·	·	·
Cattle anti-mutton	·	S	·	·	·	·	·	·	·	·	·
Rabbit anti-horse	·	C	S	C	C	·	·	·	·	·	·
Rabbit anti-pork	C	C	·	S	·	·	·	·	·	·	·
Goat anti-rabbit	C	·	C	·	S	·	·	·	·	·	·
Rabbit anti-chicken	·	·	·	·	·	S	C	C	C	·	·
Rabbit anti-turkey	·	·	·	·	·	C	S	C	C	·	·
Chicken anti-turkey	·	·	·	·	·	C	S	C	C	·	·
Rabbit anti-duck	·	·	·	·	·	C	C	S	C	·	·
Chicken anti-duck	·	·	·	·	·	·	·	S	·	·	·
Rabbit anti-quail	·	·	·	·	·	C	C	C	S	·	·
Chicken anti-quail	·	·	·	·	·	·	·	·	S	·	·
Rabbit anti-tuna	·	·	·	·	·	·	·	·	·	S	·
Rabbit anti-soya	·	·	·	·	·	·	·	·	·	·	S

* Saline extract of soybean protein
** S: Species specific reaction C : Cross reaction · : No reaction

Table 2. Antigenicity of heated meat proteins

	Pork				Mutton				Beef				Horse			
Temperature Time	15	30	45	60	15	30	45	60	15	30	45	60	15	30	45	60 min.
60°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
70	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+
80	+	−	−	−	+	+	−	−	+	+	−	−	+	+	+	+
90	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	±

This cross-reaction was generally not so strong as the species specific reaction, and it could be absorbed and eliminated by adding a small amount of meat extract which had revealed the cross-reaction to the unabsorbed antisera.

The absorbed antisera revealed only the species specific reaction so it can be used as a reagent for the practical identification of animal meats.

But the elimination of the cross-reaction from the rabbit unabsorbed antisera often resulted in the decline of titer of the species specific antibody.

Then we have tried crossing-immunization between the close relative animals to get the antiserum which reveals scarcely the cross-reaction and has the more active species specificity. The results are shown in Fig. 1 as examples in which the anti-mutton serum which has been procured from the cattle immunized with the mutton reacted with the mutton only, and did not react with the beef or other meat antigens. In the same way, the species specific antiserum for the discrimination of poultry meats has been procured from the immunized chicken[8].

Recently, soybean protein has been often mixed in the meat products as the so-called synthetic meat. Then we have tried to identify this protein with the antiserum obtained from the rabbit which had been immunized with the soybean protein extracted by hot water. (Table 1).

This antiserum did not react with the animal proteins and with the vegetable proteins of other plants (corn, peanut, rice, wheat, etc.) except soybean. But it did not reveal any reaction between the fibrous protein of soybean.

Antigenicity of heated meat proteins

The meats contained in the livestock products have been generally treated by heat in the manufacturing process. So the discrimination of heated proteins is actually an important problem. Therefore, we have investigated the changes of antigenicity of the meat proteins under some heating treatments with the antiserum obtained by raw meat immunization.

The results are noted in Table 2, and it has been shown that the stability of antigenicity against the heat is considerably different by the species of used meats. That is, the stability of the horse meat was the strongest, that of beef and mutton succeeded it, and the stability of pork antigenicity was little.

But antigenicity still remained in the meat antigens of every species even after the heat treatment of 70°C lasted for 30 minutes. This condition of heat treatment is generally used in the manufacturing process of ham and sausage. So this fact can prove that the immunological discrimination of manufactured meat proteins could be available to some extent.

The decline of the antigenicity of proteins by heat must be studied in the future but at present, it seems that one of the causes of this decline is attributed to the decreased soluble proteins which are concerned with the antigen—antibody reaction.

Warneck and Saffle said that the detection sensitivity of the immunological discrimination of meat proteins had been attained to the extent of 0.4–0.5 mg/ml. We have also succeeded in detecting the 36 times dilution of meat extract (meat 1: physiological salt solution 3) with the sensitivity of less than 0.5 mg/ml (calculated in terms of protein) so it is possible to detect the meats which had been mixed in the manufactured foods in the amount of more than 3 per cent of the total weight.

Consequently, our discrimination method could be sufficiently practicable and available.

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