A SURVEY ON THE DETECTION OF HORSEMEAT BY THE SEROLOGICAL PRECIPITIN TEST*

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A survey is presented on the use of the serological precipitin test for the detection of horse meat as an adulterant of beef. The results of a study on the application of the method of the analysis of cooked meats describe the limitations of the method, and emphasize the importance of knowing the history of processed meat products.

The development of a simple reliable control method for the detection of horsemeat as an adulterant of beef is of interest to both regulatory officials and meat processors. Of the various methods reported in the literature, notably the glycogen test[2], hexabromide value[1, 4], linolenic acid content[3, 8], and the serological precipitin test[6], only the latter method has found acceptance as a simple control procedure.

The serological precipitin test as used for the detection of horsemeat is based upon the formation of precipitins in the blood stream of rabbits that have been inoculated with horse serum or tissue extract (antigen). When the serum containing these precipitins is brought into contact with horse antigen under proper dilution conditions, a precipitin ring forms at the interface of the two liquids. Although there are varying degrees of cross reactivity between closely related species which produce non-specific flocculations, this factor is not critical in the present determination. Species specificity exists to form clear-cut flocculations at much lower dilutions of horse antigen than is required to form the non-specific reactions.

Although the precipitin test as such has been recognized for over fifty years, it is known that various modifications of the basic procedure are employed by different laboratories[7, 11]. The technique described by Kaplan[6] for the detection of horsemeat as an adulterant of beef has been used as a basic method throughout this study. It is the object of this paper to serve a two-fold purpose, first, to adapt the method more readily to control testing, and second, to discuss the limitations of the precipitin test in the analysis of cooked meats.

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PREPARATION OF ANTI-HORSE SERUM

A deterrent factor in the acceptance of the serological precipitin test as a control measure is the time and manipulative effort required to prepare a potent and specific anti-horse serum. Kaplan employs the multiple injection technique wherein rabbits are inoculated intravenously through the marginal ear vein every fifth or sixth day with sterile horse serum or tissue extract until the precipitin content is at a maximum. Proom[10] and Jones[5] propose the use of a single intramuscular injection of alum-precipitated horse serum. Anti-horse serum prepared in our laboratory by both methods was checked against known mixtures of horsemeat. It was found that although the serum obtained by multiple injections of horse antigen is in general satisfactory, the single injection alum antigen technique produces an anti-horse serum of greater potency and specificity.

Inasmuch as the preparation of antiserum by the multiple injection method takes approximately six weeks, and the alum injection method approximately twenty days, and since some laboratories do not have facilities for the injection and bleeding of rabbits, the present availability of commercial anti-horse serum is of considerable interest. Tests conducted on the commercial serum showed it to be comparable in potency and specificity to the anti-horse serum prepared by the alum antigen technique. Commercial anti-horse serum can also be stored in a deep freeze cabinet indefinitely and used for control testing as required.

EFFECT OF HEAT ON ANTIGEN

To elaborate further on the method under study, it was deemed desirable to investigate the limitations of the biological precipitin test in its application to cooked meats. It is generally understood that in the examination of cooked meat for adulteration, the basic need is for a test that will detect entirely denatured or partially denatured protein. Attempts have been made to produce an antiserum to heated horse flesh by immunizing rabbits with saline extracts of cooked meat[9, 10]. These attempts have not been successful. It, therefore, becomes apparent that in testing cooked meat for adulteration, by present test methods, misleading negative precipitin tests can be expected if the proteins of a commercial cooked meat product have been rendered insoluble to saline extraction.

In order to evaluate correctly the reliability of negative precipitin tests on cooked meats, it is necessary to know the conditions of processing. If the meat is cooked in an oven the problem is one of heat penetration. On the other hand if the meat is cooked in a water bath,

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denaturation occurs uniformly throughout the sample. Bearing this in mind, tests were carried out using normal anti-equine serum versus horse meat cooked under variable processing conditions.

HEAT PENETRABILITY

10-g samples of ground horsemeat were placed into a 100°C air oven for different time intervals. The samples were then extracted with saline and treated with normal anti-equine serum. Table 1 shows that for this particular weight and size of sample, the ground meat can be heated in excess of 150 minutes at 100°C and still produce a positive precipitin ring. This is undoubtedly due to the presence of native protein left in the uncooked portion of the meat patty.

In the course of the experiment it was also observed that cooking in the 100°C air oven destroyed the reddish color of the ground horse meat and enhanced the subsequent filtration of the antigen extract. Further investigation showed that heating horse meat samples for 10 to 20 minutes in a 100°C air oven results in an antigen extract that filters rapidly through Whatman No. 42 filter paper to give a clear filtrate without impairing the effectiveness of the antigen extract for precipitin formation. This simple technique eliminates the need for using centrifugation, vacuum, filter aids, and other antigen extract steps described in the literature. It is suggested that this modification can be used to advantage in control testing of meats for adulteration.

HEATING IN A WATER BATH

Precooked meat products such as sausages and frankfurters are generally processed by immersion in a 160°F to 165°F (71°C to 74°C) water bath until the center of the product attains a temperature of 153°F (67.2°C). The test method employed by Proom[10] was used to perform a series of tests in which ground horsemeat was shaped into sausage form, encased in cellophane bags, and immersed into constant temperature water baths for 30 minutes. The samples were then removed from their casings, extracted with saline, and filtered through Whatman No. 42 filter paper. As shown in table 2, sausages heated to 176°F (80°C) gave a positive precipitin ring.

TABLE 1—EFFECT OF HEATING IN A 100°C AIR OVEN

Time of heating minutes	Precipitin reaction	Heating clear extract to boiling
60	(+++)	turbid
90	(+++)	turbid
120	(+++)	colloidal suspension
150	(++)	colloidal suspension
180	(+)	faint colloidal suspension
240	(−)	clear (no protein)

Meat mixtures, as noted in table 3, were then cooked in a water bath at 158°F (70°C) for 30 minutes, extracted with saline, and the antigen extracts tested with commercial anti-horse serum. The results obtained point out that the serological precipitin test is applicable to the detection of horsemeat adulteration in mildly processed meat products.

TABLE 2—HEATING CELLOPHANE ENCASED SAUSAGES IN A WATER BATH

Bath temperature	Heating time (minutes)	Precipitin reaction	Phosphomolybdic protein test on extract
60	30	(+++)	thick flocculent precipitate
70	30	(+++)	thick flocculent precipitate
75	30	(++)	flocculent precipitate
80	30	(++)	fine flocculent precipitate
90	30	(−)	clear
100	30	(−)	clear
unheated control	—	(+++)	thick flocculent precipitate

SUMMARY

The serological precipitin ring test for the detection of horsemeat as an adulterant of beef is discussed relative to its use as a simple reliable control method. Commercial anti-horse serum is suggested for use by laboratories lacking facilities for the injection and bleeding of rabbits. Emphasis is placed on knowing the processing history of a cooked meat product before considering a negative precipitin test for horsemeat as being reliable.

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TABLE 3—DETECTION OF HORSEMEAT IN MILDLY COOKED MEAT PRODUCTS

Sample	Precipitin reaction
90% salami + 10% horsemeat	(++)
90% frankfurters + 10% horsemeat	(++)
90% bologna + 10% horsemeat	(++)
100% horsemeat	(+++)
100% salami	(−)
100% frankfurter	(−)
100% bologna	(−)

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REPORT OF COMMITTEE ON FROZEN FOOD SANITATION*

This past year your Committee on Frozen Food Sanitation continued its investigations dealing with "Regulations Governing Sanitation of Roadside Stands Dispensing Frozen Desserts" and with "Regulations Governing Sanitation of Frozen Foods Other Than Ice Cream."

The 1952 report contained a survey made by O. A. Ghiggoile on conditions existing at these stands in the United States along with a summary of recommendations for sanitary control. S. R. Howe submitted a report with respect to Legislation in Canada Governing Sanitation of Roadside Stands Dispensing Frozen Desserts. Mr. Howe continued his Canadian survey during the past year and reports as follows:

"In the report submitted last year with respect to the above mentioned subject, observation was made that in the case of the larger cities of six provinces, the inspection and enforcement of legislation dealing with the sanitary control of roadside stands were under the control of municipal authorities. Hence, as a means toward securing more complete information, the appropriate officials in seven representative Canadian cities were approached, including those in the three largest cities, namely Montreal, Toronto and Vancouver. Replies received from the Medical Health Officers in Charge indicate the following:

1. (a) Six cities exercise definite sanitary control over the manufacture and sale of ice cream and related frozen products, as well as ice cream mix.

(b) One city has no regulations or by-laws for such enforcement and consequently exercises very little control.

2. In five cities the bacterial content, including coliform, of ice cream, ice cream mix and semi-frozen products is regularly and systematically checked. (The Federal Food and Drug Standard of not more than 100,000 bacteria per gramme is the basis of enforcement.)

3. Two cities furnish each counter freezer operator with definite written instructions with respect to the cleaning and sterilizing of equipment and utensils.

4. One city requires that all ice cream manufactured within its limits must be made from milk or cream produced by herds on farms which are under their inspection. This means that ice cream mix made at outside points cannot be purchased by firms operating within the city limits.

It was found that the Medical Health Officers of the seven cities recognize the need of continual supervision of sanitary conditions maintained by counter freezer operators, particularly those making and selling soft ice cream direct from their machines, such as "Dairy Queen" and similar products. Officers in our two largest cities are in the process of revising their present regulations in order to meet the present changes and modifications in the manufacture and sale of frozen desserts. Some are concerned as to the handling of "soft" ice cream left in machines at the close of business each day and one officer asked a somewhat pertinent question: "Should permission be granted for it to be hardened, kept overnight and then remelted and sold the next day?"

In many instances, machines are sold by agents or manufacturers without any instructions as to the proper care and procedure for cleansing, dismantling and sterilizing. The co-operation of firms concerned in such matters would be of advantage and assistance to all concerned.

Finally, several officers expressed the opinion that some responsible legislative body, such as a Federal Government, should establish a code of sanitary requirements covering the manufacture and sale of all frozen desserts, which could be adopted by a municipality or province as a basis for regular inspection and control.

In 1952 J. A. King reported on a survey of State Regulations Affecting Frozen Foods other than Ice Cream. In this survey he was unable to find references to commercial frozen food which would insure the consumer against thawing and refreezing and no references were made in regard to temperature, age or transportation requirements for commercial frozen food.

During the past year J. A. King and S. E. Smith have been surveying frozen food packers as to their feeling in regard to the suitability of present regulations. At the present time these results are not ready for reporting.

Any comments on this report and suggestions whereby our future activities may be made more effective will be welcomed by your committee

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O. A. Ghiggoile
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