# THE PRECIPITATION TEST IN THE DIFFERENTIATION OF MEATS OF DOMESTIC AND GAME ANIMALS.

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# The Precipitation Test in the Differentiation of Meats of Domestic and Game Animals.

## Introduction

This work was undertaken not only because of the academic importance, but because of the potential value of a specific test that could be applied to the differentiation of meats of domestic and big game animals. In fact, the research was inspired and fostered by the officers of the Manitoba Game Branch, who pointed out that such a test would provide a further check on the depredation and activity of the incorrigible poacher. Frequently, the game guardian has some difficulty in obtaining a conviction against the illegal hunter of big game animals, although no doubt may exist in the officer's mind as to the origin of confiscated meat. It is not unusual, for example, for the culprit to claim that pieces of suspected meat are from sheep and not from deer. Again, the meat may be in such a condition at the time of seizure that even the experienced officer has difficulty in making a satisfactory or unquestionable determination by casual inspection. The development of a dependable test, sensitive enough to differentiate the flesh of related species, would obviate such problems.

Before assigning this task to the writer, Dr. J. A. Allen, pathologist of the Manitoba Game and Fisheries

Branch, made a preliminary review of the literature and decided that the precipitation method promised the greatest measure of success.

It was known, of course, that the precipitation test has been applied in the practice of meat inspection in America and in Europe, where horse meat was often fraudulently substituted for other and more expensive meats in the preparation of meat products. However, no mention was made as to its application to this specific problem. Furthermore, two of the principal governmental laboratories, dealing with related problems in Canada and in the United States, were consulted and their records and references indicated that no experimentation had previously been conducted in the differentiation of the meat of big game animals and that of the domestic food animals.

## Precipitins in General (Nuttall)

The importance of the use of the precipitin test as a practical method in the differentiation of albumins of different animals, was first realized by Ehrlich and Wassermann in 1900. The specific character of precipitins had already been indicated for bacterio-precipitins by Kraus, Bordet, Fich and Morgenroth in their work on Lactosera.

Ehrlich and Wassermann however widened the field. The principle underlying their work was the discovery that when a foreign protein such as blood serum was injected several times with intervals of a few days between injections, the body of the rabbit would develop a resistance to this protein in the form of antibodies in the body-fluids, particularly in the blood-serum. This blood-serum when removed from the rabbit and mixed with the specific protein that had been inoculated would cause a precipitate or, as it is usually called, "precipitum" to form. The serum possessing this property was termed antiserum, in contrast to the serum or antigen with which it was tested.

Nuttall summarizes the knowledge of precipitins very competently, extracts of which are given in the next few paragraphs.

Precipitins or Antibodies are found in the globulins. A great deal of work has been done in this field by various workers and the precipitins have been found in various globulins: pseudoglobulins, paraglobulins, and euglobulins; but have been rarely, if at all, found in the serum albumin. The precipitins occur in most of the organs of the body, particularly large amounts being found in the peritoneal lymph glands, also in the aqueous humor, and are transmitted to the offspring in utero when the mother is treated during pregnancy.

The regeneration of precipitins in the body after large bleedings is improbable due to anaemia and to the lack of the foreign substance which stimulated its production. The precipitins begin to disappear in the animal one month after the last injection, even when not bled; and also disappear in rabbits after prolonged treatment, in other words, the animal becomes immune.

The Precipitable Substance i.e. the protein inoculated and the precipitin may co-exist in the serum of immunified animals, at times still after bleeding. This can be detected by mixing the antisera.

The Precipitum, (as ascertained by various workers), is soluble in dilute acids and alkalis; is insoluble in water and in solutions of neutral salts and alkaline carbonates. Nolf, 1900, considered it a globulin. Precipitum from ox-serum was found to contain abundant phosphorus in organic combination, and gave the biuret, xanthoproteic, and glycoxylic reactions and was found to contain loosely-bound sulphur.

Heat above 60° C destroys the efficacy of both the precipitable substance (antigen) and of the antiserum.

## The Nature of the Precipitin Reaction

Eisenberg (1902), found that both substances combine quantitatively.

Muller (1902), and Leblanc (1901), obtained similar results pointing to the fact that both substances are present in the precipitum.

Strube (1902), like the others found a quantitative relation to exist between the interacting substances, for on adding 5 cc. of a blood dilution to different proportions of its homologous antiserum in dilutions of 1 : 10 to 1 : 5000 he obtained, as Nuttall has done, decreasing quantities of precipitum. Nuttall, however, recommends for medico-legal work keeping the amount of the antiserum constant while progressively diluting the antigen.

Welsh and Chapman, however, take another viewpoint. "Recognizing that the antiserum is the main source of the precipitate in a precipitin reaction and having regard to the exact quantitative relations of antiserum, antigen, and precipitate we have been able to arrange methods for the differention of proteins of closely related species.--Ewing, following a suggestion made by Uhlenhuth and others, tried the effect of progressively diluting the antiserum while maintaining the blood dilutions constant. He found that when added to various bloods in solutions of equal strength an antihuman serum in its highest dilution acted only upon human blood dilutions..... a method which we had independently devised, which though super-

ficially similar is fundamentally different from that of Ewing.

To a series of fixed quantities of each protein to be tested there were added progressingly diminishing amounts of the antiserum..... Our method was based on the experimental finding that the precipitable substance is contained in the antiserum and that there is a quantitative relation between the amount of the precipitate and the amount of the antiserum, provided the homologous protein is sufficient.. .... the weight of the precipitate is proportional to the weight of the antiserum engaged in the interaction..... If the protein of the homologous species be replaced by the protein of any heterologous, however closely related (as tested by the biological method), the weight of the precipitate from that quantity of antiserum is diminished."

It is this method and viewpoint of Welsh and Chapman's that has been followed on the whole by the writer, in this work.

Specificity of the Precipitins (extracted from Nuttall)

Strube thought that the quantitative differences of reaction is due to different species of blood not having identical constitution (obvious), but having

a closely related constitution, so that the antiserum for one reacts to a limited extent on the other.

It is assumed that the serum is composed of different albumins, (and there appears to be evidence of this), which we shall style (a), (b), and (c). Substance (a) is present in the heterologous species but (b) and (c) are not. With the homologous antiserum, (a), (b), and (c) are all precipitated. (The writer would like to add to this by suggesting that in groups of closely related animals, where group precipitation occurs, that (a) and (b) are precipitated and that where (a) alone is precipitated it shows only the general mammalian reaction).

## Historical Introduction

(Extracted from Nuttall)

1. The medico-legal use of precipitins was discovered almost simultaneously by Uhlenhuth, Wassermann, and Schutze (1901). They differentiated between man and other animals and found close relationship between man and baboons, the reaction taking place more slowly and to a lesser degree in the latter case.

Uhlenhuth and Nuttall (independently) found that putrid blood was just as specific as fresh blood. Nuttall also found that putrid antisera were specific.

2. Antisera in the Examination of Meats

From the preceding it seemed natural to conclude that meat extracts would react to corresponding haematosera for the reason that they contain blood.

Uhlenhuth (1901), obtained a positive reaction with anti-pig serum, tested upon the organs of a pig which had been dried for 18 months. Continuing this line of investigation he obtained positive results with the antisera for pig, sheep, horse, donkey, and cat blood, when these were tested upon the corresponding meats. He found that anti-sheep serum gave almost as much reaction with goat as with sheep, and less with beef. He found that mixtures of meat could be detected by the precipitin method in minced meat, in sausage and smoked meat (all uncooked of course).

The method of examination consisted in scraping the meat and extracting it with water or saline. An extract was suitable for testing when it foamed on being shaken. The extract was very cloudy and had to be cleared by repeated filtration through filter paper or a Berkefeld filter. If extracted with water, an equal volume of double normal salt solution had to be added to the watery extract before testing. In testing he added 10-15 drops of antiserum to 3 cc. of the saline meat extract.

Von Rigler (1902), used this method in the study

of meat adulteration. He prepared 20% watery extracts of the meat of seven species of animals (roebuck, hare, rabbit, horse, ox, pig, and cat) and injected 5-10 cc. thereof every 3 days subcutaneously during one month. Whereas normal rabbit serum had no effects on the meat extracts, the antisera were specific acting on extracts of mixed meats, as also upon some boiled and roasted meats of an homologous kind. (It is strange that the heat from cooking had no effect on the reaction).

Notel (1902) treated rabbits with horse serum, muscle juice, and muscle extract (in .1% soda solution) injecting subcutaneously every 2-3 days amounts of 10 cc. until 10-12 doses had been administered, after which 6 days were allowed to elapse before the animals were bled. He obtained the least effective antisera from the serum treated rabbits. (The writer has obtained similar results).

Vallée and Nicolas (1903) have confirmed these observations. They speak of sero-precipitins in contra-distinction to musculo-precipitins.

Nuttall in his quantitative technique using serum for antigen rather than meat extracts and using antiserum prepared from injected serum obtained the following results in tests with anti-sheep serum, anti-ox serum, and anti-reindeer serum, i.e. the tests which correspond most closely with the writer's tests.

A. Anti-Sheep Serum

| | |
|---|---|
| 1. With sheep serum | 100 % |
| " ox serum | 75 |
| " reindeer serum | 35 |
| " hog-deer serum | 45 |
| 2. With sheep serum | 100 % |
| " goat serum | 39 |
| " ox serum | 37 |
| " antelope serum | 33 |
| " reindeer serum | 29 |
| " hog-deer serum | 22 |

B. Anti-Ox Serum

| | |
|---|---|
| With ox serum | 100 % |
| " antelope serum | 45 |
| " sheep serum | 36 |

C. Anti-Reindeer Serum (Rangifer tarandus)

| | |
|---|---|
| With reindeer serum | 100 % |
| " goat serum (sp.) | 38 |
| " hog-deer serum | 33 |
| " goat sp. serum | 18 |
| " antelope serum | 15 |
| " sheep serum | 15 |
| " whale serum | 28 |

Nuttall, in these tests, was merely trying to establish blood affinities and not to show the finer differences between various kinds of meat of closely related

species. His work was wholly with blood and blood-serums, not with meat extracts as is necessary in medico-legal work in testing meats. However, his work indicated that there might be sufficient difference in the species of closely-related game and domestic animals to bear further investigation.

## Methods

The following methods used by the writer fall into two large groups--A. The Preparation of the Antisera and Antigens and B. Tests of the Meat Extracts by the Different Antisera.

### A. The Preparation of the Antisera and Antigens

#### The Preparation of Protein (Serum) for Inoculation

1. Cow, Sheep

Flasks full of blood were obtained from the abattoir and kept in the refrigerator until the serum had separated from the clot--(24-48 hrs.). This serum, which was usually fairly clear, was then poured into test-tubes and centrifuged until the haemoglobin was thrown to the bottom of the tube. This clear serum was then poured into a bacterial filter. During the early stages of this work, a Berkefeld porcelain filter was used, but later it was found to be more economical of material to use a Seitz filter.

This sterile serum was stored in sterile flasks and

kept frozen until needed. Sterilizing the serum, as recommended by Nuttall and Uhlenhuth, was found to be advantageous, because in the few cases where this procedure was omitted there was a fairly high percentage of deaths in the rabbits inoculated. This was due in some cases to large abcesses in the abdomen which developed as a result of the unsterile serum. In a few cases the deaths were due to heavy infestations of parasites, hence care was taken thereafter, to procure as healthy rabbits as possible and to keep the serum sterile.

2. Deer, Moose

In this case, it being impracticable to obtain the blood from the animal, solutions of meat extract were made up as follows: On the average, 100 gms. of meat were cut up into small pieces and allowed to soak in 100 cc. of normal saline (.75%). If the meat contained a good deal of blood, more saline was used in proportion to the meat. It was found that alternate freezing and thawing helped to draw the blood out. When the meat was practically colorless (24-48 hrs.), the solution was filtered first through a coarse filter, then through the bacterial filter and kept frozen until used.

## Inoculation of the Serum into the Rabbits

Throughout this work the intraperitoneal method of injecting the serum was used exclusively and gave good results. This was the method preferred by Uhlenhuth and

Nuttall, although Strube claimed that the intravenous method was faster and yielded better antiserum. The rabbit was laid on its back on a specially designed board so that a wide rubber band was passed over its chest, under its foreleg and fastened under some nails on the other side of the board. A similar band held its thighs down with its legs spread apart, so that the abdomen bulged out between the two bands. In this position even the strongest rabbit was powerless, and the whole process could be easily carried out by one person.

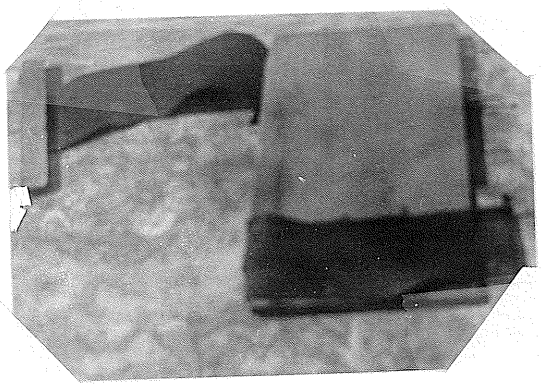
See Figure II

The area of the abdomen shaded was clipped free of hair and washed carefully with lysol. A 10 cc. syringe was found most practical and a mediumly fine needle was used with it, both of them being sterile. Nuttall recommended using a blunt needle and making an incision in the skin with a sharp scalpel, but that was found to be unnecessary. The skin was pinched up and the needle was inverted through it, then through the abdominal muscles, care being taken not to puncture the intestine. The serum was then injected, the needle withdrawn and the spot was daubed with lysol to prevent infection.

The amount inoculated was increased gradually from 3 or 4 cc. in the first inoculation to 10 cc. in the last one. Seven or eight inoculations were sufficient on the average. Nuttall made anywhere from 5-8 injections. Usually 7-12 days (sometimes 12 or 13 days when not convenient earlier) after the last inoculation, the rabbit

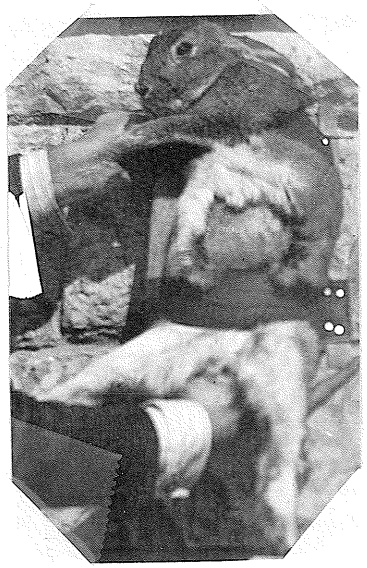
Figure

I



Rabbit Board

Figure II



Rabbit attached to the Rabbit Board,
showing abdomen shaved ready
for inoculation.

was tested by bleeding from the marginal ear-vein. This vein was made to stand out by rubbing the ear with xylol. The excess xylol was wiped off and the vein was pricked with a sharp needle, the drops being collected in a small 3 cc. test-tube. The blood was allowed to clot and the serum which rose to the top was poured into another test-tube and tested with antigen. If a good precipitate was formed the animal was ready to bleed, if not, it was inoculated once or twice again until the antiserum was strong enough.

An index of cards recording the dates of injections and date of bleeding was kept. There was one for each rabbit--as follows:

A. Rabbit I. (White) Injected with Cow Serum

| | | | | | |
|---|---|---|---|---|---|
| Sept. | 17 | 3 cc. | Centrifuged | Clear | Orange |
| " | 23 | 4 " | " | " | " |
| " | 26 | 5 " | " | " | " |
| " | 30 | 6 " | " | " | " |
| Oct. | 3 | 7 " | " | " | " |
| " | 7 | 8 " | " | " | " |
| " | 11 | 9 " | Filtered | Sterile | Red |
| " | 15 | 10 " | " | " | " |
| " | 29 | Bled--about 15 cc. extracted--rabbit | | | |

lived--strong antiserum--sterilized--frozen.

B. Same Rabbit

| | | | | | |
|---|---|---|---|---|---|
| Dec. | 3 | 3 cc. | Sterile serum | | |
| " | 9 | 4 " | " | " | |

| | | | |
|---|---|---|---|
| Dec. | 13 | 5 cc. | Sterile serum |
| " | 17 | 6 " | " " |
| " | 20 | 7 " | " " |
| " | 24 | 8 " | " " |

Break due to lack of serum

| | | |
|---|---|---|
| Jan. | 7 | 10 cc. |
| " | 17 | 8 " |
| " | 27 | 8 " |

Feb. 10 Bled--about 50 cc. extracted--rabbit lived--stronger antiserum--sterilized--frozen.

From the above record several conclusions were drawn.

1. The antiserum from B was stronger than from A in spite of the irregularity in the number of days between injections and the irregularity of the amounts injected. This may have been due to the rabbit having been inoculated previously i.e. there may still have been some antibodies present; or else the irregularity itself tends to make stronger antiserum.

2. Obviously, that individual rabbit consistently gave good antiserum. There was, on the other hand, a rabbit inoculated with sheep antiserum that was treated in a very similar way to the rabbit above, but the antiserum, after both series of injections, was very weak. Evidently that rabbit was incapable of making good antiserum.

Nuttall had remarked on the fact that animals of

similar weight under identical treatment will yield antisera of different strengths. He warned against long-continued treatment of rabbits in the hope to strengthen the antiserum, as it would only lead to the disappearance of precipitins from the blood, in other words the animal would become immune.

## Extraction of Blood from the Rabbits

The fur covering the chest on the left side was all clipped off and the area daubed with lysol. A sterile 30 cc. syringe and a long sterile needle of large calibre were placed close to hand and the rabbit was put under ether. In this process a cone-shaped funnel of cardboard stuffed with cotton wadding and open at both ends was found very effective. A few drops of ether were poured onto the cotton and the rabbit's head was inserted in the wider opening of the funnel.

When it lost consciousness (testing by touching the eye with the forefinger until it stopped blinking) the funnel was retracted an inch or two, but kept near enough so that it could be replaced quickly, if necessary. The needle (detached from the syringe) was then inserted between the ribs at a point halfway down the length of the sternum and $\frac{1}{4}$-$\frac{1}{2}$ inch out from it on the left side of the rabbit. The left foreleg was extended straight out and as far back as possible so as to be out of the way. When the needle had penetrated into the thoracic cavity

it was moved gently around until the heart was directly beneath it (judged by the heat against the needle-point). Then the needle was thrust down and the blood came spurting out of it. The syringe was immediately fitted onto the needle and when it was practically full of blood i.e. approximately 50 cc., it was removed.

This blood was put into large sterile test-tubes, sealed with a cotton plug and allowed to clot. The serum was then poured into smaller sterile test-tubes to be centrifuged after which it was poured into a third set of sterile test-tubes and kept frozen. If it was to be kept for long it was sterilized as well, but for immediate use it was only found necessary in a few cases.

Nuttall's method of bleeding was quite different. He would bleed the animal to death by bending the rabbit's head back and slitting its throat across thus cutting through the jugular vein. The blood would spurt forth and was collected in a large sterile dish which was immediately covered. The serum when it separated out was pipetted into sterile glass bulbs drawn out at one end, which was then sealed over a Bunsen burner, care being taken not to heat the antiserum in the process. He claimed that serum which rose later should be preserved with chloroform or sterilized by a bacterial filter. The first serum would generally be sterile due to the clot retaining the microorganisms, some of the bacteria also being killed by the bactericidal property of fresh serum.

## The Preparation and Titration of Antigen for Tests

All the antigen was made in the same way to obtain as identical a concentration as possible in all cases. Approximately 90 gms. of meat were cut up fine and 30 cc. of saline were poured over it. This was let stand in the refrigerator until as much blood was drawn from the meat as seemed to be possible (24-48 hrs.). This was then poured into a filter to remove any sediment and later passed through a bacterial filter.

Macerating the meat with sand, as suggested by some authors, was found to be cumbersome and no more advantageous as the extracts prepared in the usual way were strong enough and in macerating not only the blood and muscle juices were extracted from the meat but also the fat, which would pass through the coarse filter and clog up the pores of the bacterial filter.

Leach, in summarizing papers by Uhlenhuth, Wassermann, Schutze, Gaujoux, etc., suggests using 50 gms. meat--100 gms. saline, first, however, extracting the fat from the ground-up meat by means of ether or chloroform. Nuttall, however, warns against the use of ether as a preservative for blood solutions as it interferes with the precitin reaction. Hence ether used for removing fat could also be objected to on these grounds.

The writer, however, discovered a simple way of removing the fat. Four samples of meat were prepared by

the Game and Fisheries Branch--for testing as unknowns. In this case, large quantities of fat were ground up with the meat. This constituted quite a problem until it was discovered that all the fat could be removed from the saline extracts by centrifuging, thus avoiding clogging up the bacterial filter.

In the case of beef and lamb i.e. animals which were bled to death, the meat naturally did not contain as much blood as the meat of animals killed by shooting, i.e. game animals. Therefore, the 3:1 solution was not always as concentrated in the former case as in the latter. But this was corrected as much as possible in the tests, by first titrating various dilutions of this 3:1 extract against its homologous antiserum--e.g. straight 3:1, 1:5 dilution in normal saline, 1:10, 1:20. The best dilution to use was found to be 1:10, in practically all cases. Then with that dilution further titrations were made with varying amounts of antiserum, antigen, and saline, to find the point at which the maximum amount of precipitate was formed and which was at the same time most economical of antiserum. In this way each antigen was examined and catalogued to indicate the best dilution to give maximum results with its homologous antiserum before being used in tests against other antisera.

The following titration test will perhaps serve to clarify the above statement. Sheep (antigen serum from the top of the clot) in 1:100 dilution was titrated

against sheep antiserum (mixed) and saline. The amount of the antiserum was constant--and varying quantities of saline and antigen were used.

| Antigen | Antiserum | Saline | AAmount of Precipitate |
|---|---|---|---|
| .5 cc. | .25 cc. | .00 cc. | .053 loose |
| .4 | .25 | .00 | .035 |
| .35 | .25 | .00 | .043 * |
| .3 | .25 | .05 | .035 |
| .25 | .25 | .1 | .027 |
| .2 | .25 | .15 | .024 |
| .15 | .25 | .20 | .02 |
| .1 | .25 | .25 | .017 |
| .05 | .25 | .3 | .012 |

This titration test, being the first, was more elaborate than necessary and later a more contracted form was used.

Then the sheep extract was tested. It was found that the 1:5 dilution gave the best results. This was then titrated against the antiserum, as above, but a narrower range was used.

| Antigen | Antiserum | Saline | Amount of Precipitation |
|---|---|---|---|
| .4 cc. | .2 cc. | .00 cc. | .015 |
| .35 | .25 | .00 | .018 * |
| .3 | .25 | .05 | .012 |

As the quantities starred yield the most precipitate they were used thereafter in any tests with either homologous or heterologous antisera. When much more antigen than .35 cc. was used there seemed to be an inhibitory force at work, and if much less antigen was used, it seemed to be insufficient to precipitate out all the precipitin in the antiserum--as interpreted according to Welsh and Chapman's views.

## The Method of Measurement

The antigen, antiserum, and saline, if any, were measured into and mixed by shaking in a small 3 cc. test-tube. This was allowed to stand for a minute or two until all the liquid settled to the bottom of the tube and the sides were fairly dry. Then the fluid was drawn up into a 1 cc. pipette graduated in tenths and hundredths. The relative quantities of antigen, antiserum were so regulated that there were always .6 cc. to be drawn up into the pipette. With careful pipetting only .02-.04 cc. (at the most) were left in the test-tube--the amount of precipitate from which would be negligible. Approximately the same amount was left in all the test-tubes in a test.

The chance for error there was eliminated in the end by averaging the results of a great many tests. Slender pipettes with a fine point would leave only .01 cc. in the test-tubes in most cases, and hence were the best for this purpose.

After the liquid was drawn up into the pipette, the tip of the latter was dried and dipped in melted wax a few times until the hole was thoroughly sealed, thus holding the column of fluid in place and in addition keeping it sealed from bacterial contamination. The finger was removed from the top of the pipette, which was inverted and inserted into a special rack where it was held rigid and upright. The height of the column of fluid had been previously adjusted so that the meniscus (the lower one, when inverted) was in the graduated area of the pipette. The precipitate when it formed would settle down on top of the meniscus, making the readings very simple, using the gradations on the pipette itself; thus avoiding any errors due to using ungraduated capillary tubes of varying calibre. The measurement could thus be made very accurately to hundreths, and with a fair amount of accuracy to thousandths, the error, if any, being .001 or less.

When some of the precipitate stuck to the walls of the pipette, it was dislodged by rotating the pipette between the hands gently but quickly a few times. This was usually necessary after 12 hours. If the pipette was badly jarred, the precipitate would slide down past the meniscus which would make the readings inaccurate and hence make a repetition of the test necessary. Hence great care was used thereafter in handling the pipettes.

This seemed to be a more accurate method than that of Welsh and Chapman who used fine test-tubes, although they were not described in the paper used as a reference here. Hence it was impossible to ascertain whether or not they were graduated.

Vaccine tubes were also used by the writer but were found impracticable due to the precipitate settling in the wide mouth of the capillary tube, rather than in the capillary tube itself, which was divided into hundredths. In some cases no amount of rotating would drive down all the precipitate into the capillary tube thus making accurate measurement impossible. Centrifuging was also tried in this connection but drove the precipitate along one side of the capillary and packed it more tightly than under normal circumstances. Hence that idea was abandoned.

Nuttall's method seemed much more cumbersome than the pipette method described above. Nuttall used .1 cc antiserum and .5 cc antigen dilution and mixed them in a small test-tube and left it standing for 24 hrs. the clear supernatant fluid was carefully pipetted off with a bent bulb capillary pipette. The precipitum and the small amount of fluid remaining were mixed and drawn up into thick glass capillaries with a lumen of approximately 1 mm. This was allowed to settle and then each end of the column of precipitate was marked on the glass with a glass pencil. Then the contents were blown out and the capillary was washed and dried. Then enough

clear water was run into the capillary from a thermometer graduated in tenths of a cc., (a small steel scale being used to measure the finer gradations), to replace the column of precipitate. The quantity was then read off the scale. This method however is rather complicated.

A similar technique was tried by the writer, using pipettes graded in thousandths to draw up the column of precipitum but usually the precipitum did not settle very well due to the narrowness of the lumen. Hence the coarse pipettes were used in preference to the finer, although the latter would be the best method, if perfected.

In the pipette method every precaution was taken to prevent the test from being spoiled by bacterial contamination. The antigen and the antiserum were sterilized by filtration and were kept frozen in sterile test-tubes which were carefully plugged. The saline, pipettes, and test-tubes were sterilized in an autoclave, the latter two being kept spotlessly clean so that any cloudiness or precipitate would show clearly.

The antiserum and saline remained perfectly clear always. The antigen, however, in the more concentrated solutions, would show, after a time,,bits of flocculent matter, which was probably coagulated albumin, perhaps due to the alternate freezing and thawing. This, however, was removed by centrifuging and the solution would stay clear until frozen again. The more dilute solutions of

meat extract such as were used in the tests did not act in this way. But due to lack of space in the refrigerator the meat extracts could not be kept frozen in the more dilute form. In this way all the reagents were kept perfectly free from flocculent matter that might be confused with precipitum.

## B. Tests of the Various Antigens with the Antisera

Welsh and Chapman, in their tests, used dried antisera exclusively. In a typical test they would use 0.01 gms. dried antiserum dissolved in .4 cc. of saline. To this they would add another 0.1 cc. of saline and 0.1 cc. of antigen, which was egg-white in 1:100 dilution with saline. In their tests they progressively decreased the quantity of antiserum, keeping the antigen constant, and increased the saline sufficiently at each step so as to always keep the amount of fluid in the tubes equal to 0.6 cc. When the antiserum used weighed only .0005 gms. a reaction occured only with the homologous antigen--that reaction of course, being very slight.

The same principle was used by the writer but the quantities were altered. By titration tests, such as were mentioned before in this work, it was discovered that with 0.35 cc. antigen, 0.25 cc. antiserum was the correct amount to use, to bring about the maximum precipitation. No saline was used in tests with those quantities.

In tests where the amount of hte antiserum was

decreased progressively, the saline was increased progressively so as to always keep 0.6 cc. liquid in the pipettes, as in Welsh and Chapman's method. Hence in a test with 0.1 cc. antiserum, the amount of saline was increased to 0.15, the amount of the antigen remaining fixed at 0.35. In this work the smallest amount of antiserum used was 0.05 cc.

The measurements of the precipitate in all the tests were made after 48 hours, approximately.

All the antigens were tested with normal rabbit serum and remained clear.

All the antisera were tested with saline and remained clear. The reason for this test was that Nuttall noticed antisera which had an opalescent appearance and reacted very strongly even with heterologous antigens, giving very inaccurate results. They clouded every blood dilution and even clouded normal saline. Dr. Graham-Smith, found several cases of it and some of the rabbits from which the blood was taken, were heavily infected with cysticerci, which was perhaps the cause of the opalescence.

The various antigens tested were as follows:

| | | |
|---|---|---|
| Beef | Bos bovis | (8) * |
| Moose | Alces americanus | (7) |
| Deer | Odocoileus virginianus | (7) |
| Caribou-Rangifer caribou | | (7) |

* Nos. refer to Bibliography.

| | | |
|---|---|---|
| Sheep | Ovies aries | (8) |
| Goat | Capra hircus | (8) |
| Fallow Deer (English) | Cervus dama | (9) |
| Swan | Olor sp. | (8) |

Tests with Anti-deer Serum (Virginia or White-Tail Deer--Odocoileus virginianus)

The first procedure was to test the various deer antigens (meat extracts), which had been prepared in the usual manner i.e. 90 gms. meat with 30 gms. saline. The antiserum used in these tests was from Rabbit No. 3 and was very strong. The first antigen tested was Deer Antigen No. 3. In trying to ascertain the best dilution in which to use it, 0.35 cc. of the antigen was mixed with 0.25 cc. of antiserum. These two quantities were maintained constantly while the dilution of the extract was varied.

| Antigen Dilution | Amount | Antiserum | Results in Precipitate |
|---|---|---|---|
| Straight extract | .35 cc. | .25 cc. | .035 cc. |
| 1:5 dilution of " | .35 " | .25 " | .052 " * |
| 1:10 " " " | .35 " | .25 " | .043 " |

The 1:5 dilution was the best and was used thereafter in the tests and the results with the relative amounts of antigen and antiserum i.e. .35 cc., .25 cc., were so good that no further titration was made. This No. 3 antigen was then tested against other deer antigens in what was

surmised to be their best dilutions, as a means of comparison. The same proportion of antigen and antiserum was used as before.

| | | | | | | |
|---|---|---|---|---|---|---|
| Deer antigen | No. 2 | in | 1:5 | dilution | brought down | 0.05 cc precipitate |
| " | " | No. 3 | " 1:5 | " | " | " 0.056 cc precipitate |
| " | " | No. 4 | " 1:10 | " | " | " 0.067 cc. precipitate |
| " | " | No. 1 | " 1:10 | " | " | " 0.07 cc. precipitate |

From this it seems that different antigens have different powers when acting on the antisera so as to produce precipitate. However, each antigen varies in itself from time to time, in the power to produce more or less precipitate. For instance, Deer Antigen No. 3 at times produced 0.52 cc. precipitate, at other times, 0.56, and at still others, 0.32 cc. precipitate only. This variation may have been due to differences in the room temperature. There was no other apparent reason. However, as the average of a number of tests was taken this discrepancy became more or less negligible.

In the following tests, the various antigens used had all been tested with their homologous antisera to ascertain the best dilution, and were used in that dilution in the tests.

Table I (over)

## Table I

## Anti-deer Serum (Rabbit 3)

.35 antigen )
.25 antiserum ) were used in these tests
.00 saline )

| Dilutions of Antigens | Antigens | Amount of Precip. 1 | Amount of Precip. 2 | Amount of Precip. 3 | Amount of Precip. 4 | Amount of Precip. 5 |
|---|---|---|---|---|---|---|
| Straight | Beef | .027 cc | .021 cc | .022 cc | .02 cc | .02 cc |
| 1:5 | Moose | .025 | .026 | .028 | .029 | .029 |
| 1:5 | Deer | .035 | .032 | .032 | .05 | .05 |
| 1:10 | Caribou | .03 | .025 | .028 | .035 | .045 |
| Straight | Sheep | .026 | .019 | .029 | .018 | .024 |
| 1:100 | Goat | .019 | .025 | .02 | .02 | .022 |

| Dilutions of Antigens | Antigens | Amount of Precip. 6 | Amount of Precip. 7 | Amount of Precip. 8 | Average Amt. of Precip. | % |
|---|---|---|---|---|---|---|
| Straight | Beef | .02 cc | .026 cc | .02 cc | .022 cc | 50% |
| 1:5 | Moose | .03 | .035 | .028 | .029 | 66 |
| 1:5 | Deer | .05 | .052 | .052 | .044 | 100 |
| 1:10 | Caribou | .04 | .041 | .035 | .036 | 82 |
| Straight | Sheep | .02 | .022 | .024 | .023 | 52 |
| 1:100 | Goat | .022 | .024 | .022 | .022 | 50 |

The difference between deer antigen and the extracts of any of the domestic meats is very clearly shown in these tests. Caribou seems to be most closely related to deer and moose comes next in order. The goat antigen which was used, was defibrinated blood which had been sterilized. By rights it should not be included in tests against meat extracts but was brought in to show the difference between the musculo-precipitins and the sero-precipitins. This

difference does not show in this test but is very clearly brought out in cases later on, such as with sheep-anti-serum.

Table II

Anti-deer Serum (Rabbit 3)

.35 cc antigen )
.1 cc antiserum ) used in these tests
.15 cc saline )

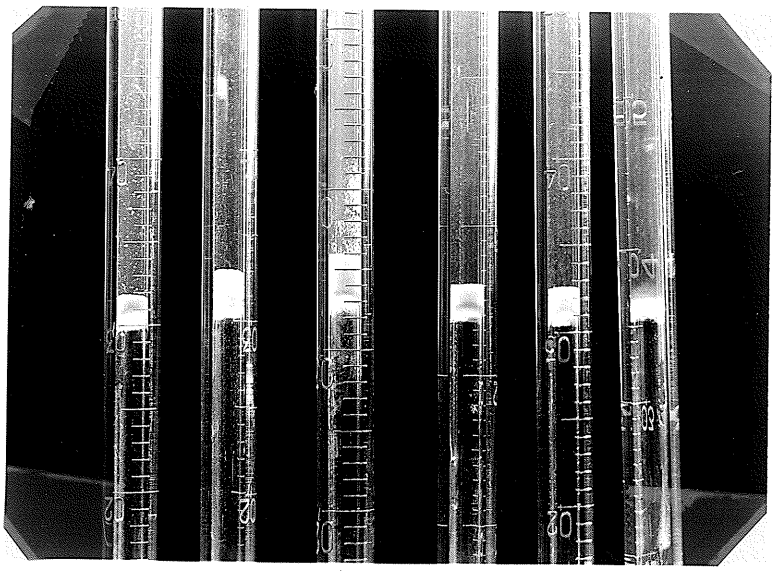
| Dilution of Antigens | Antigens | Amount of Precip. 1 | Amount of Precip. 2 | Amount of Precip. 3 | Amount of Precip. 4 | Amount of Precip. 5 |
|---|---|---|---|---|---|---|
| Straight | Beef | .005 cc | .005 cc | trace * | trace | .014 cc |
| 1:5 | Moose | .004 | .007 | .005 cc | trace | .017 |
| 1:5 | Deer | .015 | .015 | .014 | .015 cc | .025 |
| 1:10 | Caribou | .005 | .012 | .005 | .005 | .017 |
| Straight | Sheep | .004 | .005 | .005 | .005 | .015 |
| 1:100 | Goat | .004 | trace | trace | trace | not tested against goat |

| Dilution of Antigens | Antigens | | Average Amt. of Precip. | % |
|---|---|---|---|---|
| Straight | Beef | | .005 cc | 28% |
| 1:5 | Moose | | .007 | 41 |
| 1:5 | Deer | | .017 | 100 |
| 1:10 | Caribou | | .009 | 53 |
| Straight | Sheep | | .007 | 41 |
| 1:100 | Goat | | .002 | 11 |

*trace--less than .002

The test with this amount of antiserum seems to be even more specific than the first, the difference between the deer and the heterologous antigens being even greater.

Figure III



Test with Anti-Deer Serum

.35 cc antigen
.1 cc antiserum
.15 cc saline

| 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|
| Antigen--Cow | Moose | Deer | Caribou | Sheep | Swan |

In #6 there was no precipitate but the light glaring on the meniscus gave it that effect.

See page 30

Table II

Test #5

The moose antigen with the exception of the result checked in test #4, seemed to be giving more precipitate than the sheep--which was to be expected from the previous test.

Rabbit 3 is one which had been inoculated and bled six months previous to this series of injections.

The antisera in the following tests were very weak and the writer has merely included them for contrast.

Table III

Anti-deer Serum (Rabbit 1)

.35 antigen )
.25 antiserum ) were used in these tests
.00 saline )

| Dilutions of Antigens | Antigens | Amount of Precip. 1 | Amount of Precip. 2 | Amount of Precip. 3 | Amount of Precip. 4 | Average Precip. & % |
|---|---|---|---|---|---|---|
| 1:5 | Beef | .005*cc | .005*cc | .006 cc | .012 cc | not |
| 1:5 | Moose | .01 * | .01 * | .01 * | .014 | taken |
| 1:10 | Deer 4 | .02 * | .015 | .02 * | .02 | because |
| 1:10 | Caribou | .01 * | .009* | .015* | .005 | accuracy |
| 1:5 | Sheep | trace | trace | .015* | .008 | impossi- |
| 1:100 | Goat | faint trace | faint trace | faint trace | faint trace | ble |

* The precipitate here did not settle properly but was very light, and fine-textured, and formed a ring around the pipette at the meniscus i.e. there was either a hollow-cylinder of precipitate, or else a very light, loose precipitate. This made accurate measurement impossible.

One test was carried out with less antiserum, with the following results:

Table IV

Anti-deer Serum (Rabbit 1)

.35 antigen )
.1 antiserum ) were used in these tests
.15 saline )

| Antigens | Precipitate in cc. |
|---|---|
| Beef | trace |
| Moose | .004 |
| Deer | .009 |
| Caribou | trace |
| Sheep | trace |
| Goat | none |

Another weak deer antiserum was used in two tests. In this case, however, the precipitate settled properly.

Table V

Anti-deer Serum (Rabbit 2)

.35 cc antigen )
.25 cc antiserum ) were used in these tests
.00 cc saline )

| Antigens | Amount of Precip. 1 | Amount of precip. 2 | Average Amt. of precip. | Average % |
|---|---|---|---|---|
| Beef | .002 cc | .002 cc | .002 cc | 22% |
| Moose | .003 | .002 | .003 | 33 |
| Deer | .01 | .008 | .009 | 100 |
| Caribou | .004 | .002 | .003 | 33 |
| Sheep | trace | trace | trace | trace |
| Goat | faint trace | trace | trace | trace |

Conclusions: The results with the deer antisera on the whole are excellent even in the weaker antisera e.g.

Rabbit 2. It clearly shows a difference between deer extracts and those of domestic meats, and in addition, indicates that caribou and moose are more closely related to deer than cows or sheep.

## Tests with Anti-moose Serum--Alces americanus

Two antisera were used in these tests--one from a brown rabbit--the other from an angora rabbit. The meat extract was titrated against each antiserum and in both cases it was discovered that the 1:10 dilution of the extract gave the best results. Also the proportions .35 cc antigen, .25 cc antiserum, .00 cc saline gave the best results as in the case of the deer antigen.

In testing the strength of the extract in this case however, smaller quantities of antigen and antiserum were used--the reason being to conserve the antiserum.

| Antigen | | Antiserum Angora Rabbit | Saline | Results in Precipitate |
|---|---|---|---|---|
| Dilution | Amount | | | |
| 1:50 | .1 cc | .1 cc | .1 cc | trace |
| 1:10 | .1 cc | .1 cc | .1 cc | fair precip.* |
| 1:5 | .1 cc | .1 cc | .1 cc | trace |
| Straight Solution | .1 cc | .1 cc | .1 cc | trace |
| Straight solution | .5 cc | .1 cc | .1 cc | none |

* The 1:10 dilution being the best, it was then used in a fine titration.

| Antigen | Antiserum Angora rabbit | Saline | Amt. of Precip. |
|---|---|---|---|
| .35 cc | .25 cc | .00 cc | .028 cc |
| .3 | .25 | .05 | .023 * |
| .25 | .25 | .1 | .015 |
| .2 | .25 | .15 | .015 |

As usual the proportion .35 cc antigen to .25 cc antiserum gave the best results.

A similar fine titration test was carried out in the 1:10 dilution of the extract with the antiserum from the Brown rabbit. This antiserum was found to be almost as strong as the first one.

| Antigen 1:10 dil. | Antiserum Brown rabbit | Saline | Amt. of Precip. |
|---|---|---|---|
| .4 cc | .2 cc | .00 cc | .01 cc |
| .35 | .25 | .00 | .023 * |
| .3 | .25 | .05 | .012 |

* The precipitation was almost as great as from the antiserum from the angora rabbit.

The first tests were with sheep and moose antisera, as follows:

Table I

Anti-moose Serum (Brown Rabbit)

.35 cc antigen ) used in this test
.25 cc antiserum )

| Dilution of Antigens | Antigens | Precip. in cc. | % |
|---|---|---|---|
| 1:10 | Moose | .023 | 100 |
| 1:10 | Sheep | .009 | 39 |

Table II

Anti-sheep Serum (mixed)

.35 cc antigen ) used in this test
.25 cc antiserum )

| Dilution of Antigens | Antigens | Precip. in cc | % |
|---|---|---|---|
| 1:10 | Sheep | .018 | 100 |
| 1:10 | Moose | .005 | 27 |

Evidently the difference between sheep and moose antigens is quite marked.

This moose antigen had undergone putrefaction and was bacterially filtered.

Another moose antigen was used later on which had not putrefied. Both antigens gave identical results. Evidently, the putrifying had no detrimental effect on the strength of the specific reaction of the antigen, as Nuttall and other workers had discovered.

Table III

Tests with Anti-moose Serum (Brown rabbit)

.35 cc antigen ) were used in these tests
.25 cc antiserum)

| Dilutions of Antigens | Antigens | Amount of Precip. 1 | Amount of Precip. 2 | Average Amt. of Precip. | % |
|---|---|---|---|---|---|
| Straight | Beef | .003 cc | .012 cc | .007 cc | 38 |
| 1:5 | Moose | .016 | .019 | .018 | 100 |
| 1:5 | Deer | .014 | .015 | .014 | 77 |
| 1:10 | Caribou | .01 | .015 | .013 | 72 |
| Straight | Sheep | .005 | .01 | .007 | 38 |
| 1:100 | Goat | .001 | .02 * | .001 | 5 |

* Fallow Deer antigen in 1:10 dilution was used in test #2. The precipitate was very light and flocculent. It probably would have been equivalent to .015 cc packed precipitate.

Table IV

Tests with Anti-moose Serum (Brown Rabbit)

.35 cc antigen )
.1 cc antiserum ) were used in these tests
.15 cc saline )

| Dilutions of Antigens | Antigens | Amount of Precip. 1 | Amount of Precip. 2 | Average Amt. of Precip. | % |
|---|---|---|---|---|---|
| Straight | Beef | Trace | .008 cc | .004 cc | 57 |
| 1:5 | Moose | .003 | .012 | .007 | 100 |
| 1:5 | Deer | Trace | .01 | .005 | 71 |
| 1:10 | Caribou | .002 | .005 | .003 | 42 |
| 1:5 | Sheep | Trace | .003 | .002 | 28 |
| 1:100 | Goat | None | .006* | | |

* Fallow Deer antigen in 1:10 dilution was used again, but this time the precipitate packed down properly, and gave a more typical result.

Both of the tests #1 in the above Tables were carried out at the same time, and the reason for the amount of precipitate being so little as compared in each case with tests #2, which had been carried out together at a later date, was probably due to the fact that the room temperature was approximately 15° lower than usual--when the tests #1 were carried out. The writer can think of no other reason for it, as all the other factors were identical, in all the tests.

Table V

Anti-moose Serum (Brown Rabbit)

.35 cc antigen )
.05 cc antiserum) were used in this test
.2 cc saline )

| Dilution of Antigens | Antigens | Amount of Precip. | % |
|---|---|---|---|
| 1:5 | Beef | .002 cc | 25 % |
| 1:5 | Moose | .008 | 100 |
| 1:10 | Deer | .004 | 50 |
| 1:10 | Caribou | .002 | 25 |
| 1:5 | Sheep | Trace | |
| 1:10 | Fallow Deer | Trace | |

It can be seen from these tests that as the amount of antiserum is decreased, the range of antigens that it reacts with, becomes narrower. In other words, the test becomes more specific.

Table VI *

Anti-moose Serum (Angora Rabbit)

.35 cc antigen ) were used in this test
.25 cc antiserum)

| Dilution of Antigens | Antigens | Amount of Precip. 1 | Amount of Precip. 2 | Amount of Precip. 3 | Average Amt. of Prec. | Average % |
|---|---|---|---|---|---|---|
| 1:5 | Beef | .013 cc | .01 cc | .01 cc | .011 cc | 78 |
| 1:5 | Moose | .015 | .015 | .012 | .014 | 100 |
| 1:10 | Deer | .009 | .012 | .008 | .009 | 64 |
| 1:10 | Caribou | .011 | .015* | .01 | .012 | 85 |
| 1:5 | Sheep | .008 | .007 | .01 | .005 | 35 |
| 1:10 | Fallow Deer | .008 | .008 | .015* | .01 | 71 |

* Evidently this antiserum is not entirely dependable at

this dilution. In the next test, however, the antiserum seems to have acted normally.

Table VII

Anti-moose Serum (Angora Rabbit)

.35 cc antigen )
.1 cc antiserum) used in this test
.15 cc saline )

| Dilution of Antigens | Antigens | Amount of Precip. | % |
|---|---|---|---|
| 1:5 | Beef | Trace | |
| 1:5 | Moose | .008 cc | 100 |
| 1:10 | Deer | .004 | 50 |
| 1:10 | Caribou | .004 | 50 |
| 1:5 | Sheep | Trace | |
| 1:10 | Fallow Deer | Trace | |

Conclusions: From these tests it can be seen that deer and caribou are most closely related to moose and also that with good antiserum such as anti-moose (brown rabbit) there is a clearly marked difference between moose and beef antigens, and an even greater difference between moose and sheep.

Tests with Anti-sheep Serum

The first sheep antigen to be tested was sheep serum (from the top of the clot) and was diluted 1:100 with normal saline. First of all, there was a titration test to ascertain the right quantity of antigen and saline to use with .25 cc of antiserum. (This titration test was given before.)

| Reaction | Antigen | Anti-serum | Saline | Amount of Precip. |
|---|---|---|---|---|
| Precipitate forming in 12 minutes | .5 cc | .25 cc | .00 cc | .053 cc (loose) |
| | .4 | .25 | .00 | .035 |
| Precipitate * appearing in 4 minutes | .35 | .25 | .00 | .043 |
| | .3 | .25 | .05 | .035 |
| | .25 | .25 | .1 | .027 |
| | .2 | .25 | .15 | .024 |
| Precipitate appearing in 15 minutes | .15 | .25 | .2 | .02 |
| | .1 | .25 | .25 | .017 |
| Precipitate forming in 70 minutes | .05 | .25 | .3 | .012 |

The proportion of .35 cc antigen to .25 cc antiserum gives the maximum precipitation as always.

The next step was to test the strength of sheep meat extract. As in the case of the moose extract test in order to conserve the antiserum, in the rough test, only minute quantities of the reagents were used i.e. .1cc antigen, .1 cc antiserum, .1 cc saline. The various dilutions tested were 1:5, 1:10, 1:20--1:5 and 1:10 gave the best results, although 1:5 was slightly the better dilution.

The fine titration test was next in order. The dilution 1:10 of the extract was used.

| Antigen | Antiserum | Saline | Amt. of Prec. |
|---|---|---|---|
| .4 cc | .2 cc | .00 cc | .015 cc |
| .35 | .25 | .00 | .018 * |
| .3 | .25 | .05 | .018 |

The dilution 1:5 was tried with .35 cc antigen, .25 cc antiserum and gave .018 cc precipitate, same as the 1:10 dilution. The 1:5 dilution was the one used thereafter.

Table I

Anti-sheep Serum (Rabbit 3)

.35 cc antigen )
.25 cc antiserum ) were used in this test
.00 cc saline )

| Dilution of Antigens | Antigens | Amount of Precip. | % |
|---|---|---|---|
| 1:5 | Beef | .008 cc | 61% |
| 1:5 | Moose | Trace | Trace |
| 1:10 | Deer | .006 | 46 |
| 1:10 | Caribou | .006 | 46 |
| 1:5 | Sheep | .013 * | 100 |
| 1:100 | Goat | .02 * | 158 |

* The explanation of those results, lies in the fact that as the anti-sheep serum was prepared by injecting serum into the rabbit, sero-precipitins were formed. These sero-precipitins will act more strongly with serum dilutions than with muscle extracts, which is only natural, as the muscle extracts contain comparatively little serum. These sero-precipitins will even give more precipitate with heterologous antigens (provided they are dilutions of serum), than with a homologous antigen (if it is a muscle-extract dilution). The sheep antigen in this case being a muscle extract and the goat extract being a serum dilution (defibrinated blood), the reason for the above results is obvious.

As proof of the above statement the results of the

following test will speak for themselves:

Table II

Anti-cow Serum (mixed)

.35 cc antigen ) were used in this test
.25 cc antiserum )

| Antigen Dilutions | Antigens | Amount of Precip. | Reaction |
|---|---|---|---|
| 1:100 | Cow serum | .03 cc | Good precip. in 2 minutes |
| 1:5 | Cow extract | .016 | |
| 1:100 | Sheep serum | .02 | Good precip. in 5-10 min's. |
| 1:5 | Sheep extract | .002 | |

Table III

Anti-sheep Serum (Rabbit 2)

.35 cc antigen )
.25 cc antiserum ) used in these tests
.00 cc saline )

| Dilution of Antigens | Antigens | Amount of Precip. 1 | Amount of Precip. 2 | Amount of Precip. 3 | Average Amt. of Precip. | % |
|---|---|---|---|---|---|---|
| 1:5 | Beef | .007*cc | .008 cc | .012*cc | .009 cc | 82 |
| 1:5 | Moose | .005 | .005 | .006 | .005 | 45 |
| 1:10 | Deer | .005 | .006 | .003 | .005 | 45 |
| 1:10 | Caribou | .003 | .002 | .006 | .004 | 36 |
| 1:5 | Sheep | .005 | .016 | .011 | .011 | 100 |
| 1:100 | Goat | .022 | .022 | mink 1:10 none | .022 (goat) | 200 |

* In tests #1 and #3 the beef antigen gave more precipitate than the sheep antigen. Evidently this antiserum is not dependable at that dilution. In the next table, however, the results are good.

Table IV

Anti-sheep Serum (Rabbit 2)

.35 cc antigen )
.15 cc antiserum) were used in these tests
.10 cc saline )

| Dilution of Antigens | Antigens | Amount of Precip. 1 | Amount of Precip. 2 | Average Amt. of Precip. | % |
|---|---|---|---|---|---|
| 1:5 | Beef | .005 cc | .005 cc | .005 cc | 42 |
| 1:5 | Moose | .005 | .002 | .004 | 33 |
| 1:10 | Deer | .002 | .005 | .004 | 33 |
| 1:10 | Caribou | .005 | .003 | .004 | 33 |
| 1:5 | Sheep | .014 | .01 | .012 | 100 |
| 1:10 | Mink | Faint Clouding | Faint Clouding | | |

In the case of the mink no clouding showed until 24 hours --after which there was a faint clouding at the meniscus. This was probably the general mammalian reaction.

Table V

Anti-sheep (Rabbit 2)

.35 cc antigen )
.1 cc antiserum) were used in these tests
.15 cc saline )

| Dilution of Antigens | Antigens | Amount of Precip. 1 | Amount of Precip. 2 | Average Amt. of Precip. | % |
|---|---|---|---|---|---|
| 1:5 | Beef | .003 | .002 | .003 | 50 |
| 1:5 | Moose | Trace | Trace | Trace | |
| 1:10 | Deer | .002 | .002 | .002 | 33 |
| 1:10 | Caribou | Trace | .004 | .002 | 33 |
| 1:5 | Sheep | .005 | .006 | .006 | 100 |
| 1:10 | Mink | Faint Cloud-ing | Faint Cloud-ing | | |

Conclusions: None of the sheep antisera were as strong as they should have been, but indicate nevertheless

that after Goat antigen, Cow antigen reacts most strongly with sheep antiserum. The difference between sheep antigen and the antigens of deer, moose, caribou (game meats) is quite marked.

## Tests with Anti-cow Serum

In the titration of antigen to find the dilution which gives the maximum precipitate, the same method was used as for sheep and moose. The 1:10 dilution with saline was found to meet those requirements at first; but later on a weaker antigen was used, which could only be diluted 1:5 in order to bring about the same results.

### Table I

### Anti-cow Serum (mixed)

.35 cc antigen )
.25 cc antiserum ) were used in these tests
.00 cc saline )

| Dilution of Antigens | Antigens | Amount of Precip. 1 | Amount of Precip. 2 | Amount of Precip. 3 | Average Amt. of Precip. | % |
|---|---|---|---|---|---|---|
| 1:5 & 1:10 | Beef | .02 cc | .03 cc | .028 cc | .026 cc | 100 |
| 1:5 | Moose | .003 | .013 | .012 | .009 | 34 |
| 1:10 | Deer | .01 | .004 | .008 | .007 | 26 |
| 1:10 | Caribou | .011 | .013 | .005 | .01 | 37 |
| 1:5 | Sheep | | .026* | .01 | .018 | 68 |
| 1:100 | Goat | | .026** | .026 | .026 | 100 |

* It is impossible to explain why the amount of precipitate is so high in this case.

** This is another case of the sero-precipitin problem which was discussed before.

Table II

Anti-cow Serum (mixed)

.35 cc antigen )
.1 cc antiserum ) were used in this test
.15 cc saline )

| Dilution of Antigens | Antigens | Amount of Precip. | % |
|---|---|---|---|
| 1:5 | Beef | .01 cc | 100% |
| 1:5 | Moose | .002 | 20 |
| 1:10 | Deer | .004 | 40 |
| 1:10 | Caribou | Trace | Trace |

Table III

Anti-cow Serum (mixed)

.35 cc antigen )
.05 antiserum ) were used in this test
.2 cc saline )

| Dilution of Antigens | Antigens | Amount of Precip. | % |
|---|---|---|---|
| 1:5 | Beef | .002 cc | 100 |
| 1:5 | Moose | none | |
| 1:10 | Deer | Trace | Trace |
| 1:10 | Caribou | Trace | Trace |

In this case, as in all cases, if the antiserum is strong, progressively reducing the amount of the antiserum finally brings about the condition, in which the homologous antigen is the only antigen which has the power to produce any precipitate.

The following tables are compilations of results of tests with various antisera, some strong and some weak.

## Table IV

### Anti-cow Serum

.35 cc antigen )
.25 cc antiserum ) were used in these tests
.00 cc saline )

| Antigens | Anti-cow 1 Amt. of Precip. | Anti-cow 1 Amt. of Precip. | Anti-cow 2 Amt. of Precip. | Anti-cow 3 Amt. of Precip. | Anti-cow 4 Amt. of Precip. |
|---|---|---|---|---|---|
| Beef | .028 cc | .034 cc | .015 cc | .017 cc | .01 cc |
| Moose | .025 | .02 | Trace | .004 | Trace |
| Deer | .014 | .014 | .002 | .006 | Trace |
| Caribou | .018 | .016 | .003 | .004 | .001 |
| Sheep | .016 | .014 | .004 | .004 | Trace |
| Goat ) Mink ) Fallow) deer ) | G.032 | M Trace | M Trace | G.015 | F.D. Trace |

| Antigens | Anti-cow 4 Amt. of Precip. | | Average Amt. of Precip. | Average % |
|---|---|---|---|---|
| Beef | .01 cc | | .019 | 100 |
| Moose | Trace | | .008 | 42 |
| Deer | Trace | | .006 | 32 |
| Caribou | .002 | | .007 | 36 |
| Sheep | Trace | | .006 | 32 |
| Goat ) Mink ) Fallow) deer ) | F. D. Trace | | | |

## Table V

### Anti-cow Serum

.35 cc antigen )
.1 cc antiserum ) were used in these tests
.15 cc saline )

(over)

| Antigens | Anti-cow 1 Amt. of Precip. | Anti-cow 2 Amt. of Precip. | Anti-cow 4 Amt. of Precip. | Average Amt. of Precip. | % |
|---|---|---|---|---|---|
| Beef | .029 cc | .009 cc | .005 cc | .014 cc | 100 |
| Moose | .011 | Trace | Trace | .004 | 28 |
| Deer | .01 | Trace | Trace | .004 | 28 |
| Caribou | .008 | .002 | .002 | .004 | 28 |
| Sheep | .01 | Trace | Trace | .004 | 28 |
| Mink ) Fallow) deer ) | M Trace | M Trace | F.D. Trace | | |

Table VI

Anti-cow Serum

.35 cc antigen )
.05 cc antiserum ) were used in this test
.2 cc saline )

| Antigens | Anti-cow 1 Amt. of Precip. | Anti-cow 2 Amt. of Precip. | Anti-cow 4 Amt. of Precip. | Average Amt. of Precip. | % |
|---|---|---|---|---|---|
| Beef | .015 cc | .006 cc | Trace | .007 cc | 100 |
| Moose | .004 | Trace | None | .002 | 28 |
| Deer | .004 | Trace | None | .002 | 28 |
| Caribou | .005 | Trace | None | .003 | 43 |
| Sheep | .003 | Trace | None | .002 | 28 |
| Mink ) Fallow) deer ) | M Trace | M Trace | None | | |

Anti-cow serum 4, it will be noted, is very specific. It had been kept frozen for three or four months; but whether its specificity was due to that fact or not, was not known.

In these last three Tables--IV, V, and VI--two facts are shown very clearly in spite of the differences in the

strength of the antisera.

1. Beef antisera can be very definitely differentiated from the other antigens including those from game-meats.

2. Decreasing the amount of antiserum increases the specificity of the test as in Tables I, II, and III.

Conclusions on Section B

1. Progressively decreasing the antiserum in the tests increases their specificity.

2. To avoid trouble with differences in sero-precipitins and musculo-precipitins, in this type of work, it would be best to deal entirely with either one or the other. As it is practically impossible to obtain the blood from the big-game animals, and as the practical application consists entirely of identifying meats, the obvious course is to deal entirely with musculo-precipitins. In other words, it would be expedient to inject only meat extracts (not serum) into the rabbits in the preparation of antiserum. The antiserum prepared in this way is just as strong if not stronger than that prepared from serum. (e.g. anti-deer serum #3)

3. That any one of the antigens--beef, moose, deer and sheep--can be clearly differentiated from the other antigens by means of its homologous antiserum.

Practical Applications

From the conclusion arrived at under Section B, that any one of the antigens--beef, moose, deer and sheep--can be clearly differentiated from the other antigens by means of its homologous antiserum, it might seem at first glance that the problem of identifying unknown meats was

solved. This, however, is not entirely true, as may be seen from the following tests.

In testing an unknown meat this was the procedure:

In the first place, the antigen was prepared in the usual way 90 gms. meat to 30 gms. saline. This solution was then diluted 1:10 or 1:5 until it appeared to be of the same concentration (as judged by color and viscosity) as the dilutions of the known antigens, which were used in the tests.

A more accurate method was suggested by Welsh and Chapman but was not tried by the writer due to lack of time. To quote, " An unknown protein solution could be made comparable with the 1% protein solutions above employed (egg-white 1:100) by so adjusting the dilution that 0.1 cc should yield with trichloracetic acid a precipitate measuring between 1 mm. and 2 mm. in narrow tubes, as described by us. Then 0.1 cc of the unknown protein solution would contain approximately 0.0001 gm. of dried protein; and the test could be carried out by comparing this solution with similar dilutions of the homologous protein, and of a closely allied heterologous protein."

The method used by the writer, however, was fairly accurate due to experience in handling dilutions of meat extracts.

The unknown antigen was then tested with the various antisera--anti-cow, anti-moose, anti-sheep and anti-deer. The known antigens--beef, moose, deer and sheep--were each

run against the four antisera also, in order to provide a means of comparison in the results. If the four antisera used were all of the same strength, naturally the antigen would give the most precipitate with its homologous antiserum. As it is, the cow and the deer antisera were the only ones that were really strong enough. In these two cases, when mixed with the homologous antigen the precipitate from the homologous antiserum was much greater than that from heterologous antisera. The moose and sheep antisera, however, being weak, presented a different problem. Due to group precipitation in closely related species being so powerful, an antigen will give more precipitate when mixed with a very strong heterologous antiserum, than with its homologous antiserum, if the homologous antiserum is weak. The following test will bring out these points clearly:

Table I

.35 antigen )
.1 antiserum ) were used in this test
.15 saline )

| Antigens | Anti-cow serum 1 Amt. of Precip. | Anti-moose serum Amt. of Precip. | Anti-deer serum 3 Amt. of Precip. | Anti-sheep serum Amt. of Precip. |
|---|---|---|---|---|
| Beef Test 1 | .027 cc | .007 cc | .012 cc | .004 cc |
| Beef Test 2 | .027 | .002 | .012 | Trace |
| Moose Test 1 | .01 | 001 | .02 | .004 |
| Moose Test 2 | .004 | .01 | .01 | .003 |
| Deer Test 1 | .009 | .009 | .033 | .004 |
| Deer Test 2 | .007 | .002 | .025 | .002 |
| Sheep Test 1 | .016 | .005 | .014 | .01 |
| Sheep Test 2 | .015 | Trace | .012 | .007 |

Figure IV

Test with Beef Antigen

.35 cc antigen
.1 cc antiserum
.15 cc saline

1 2 3 4
Antisera--Cow Moose Deer Sheep

Figure V

Test with Moose Antigen

.35 cc antigen
.1 ccc antiserum
.15 cc saline

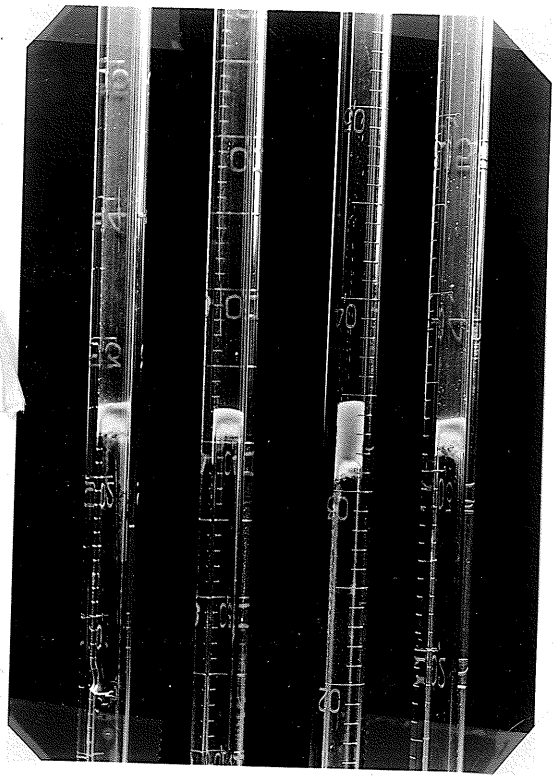
1 2 3 4
Antisera--Cow Moose Deer Sheep

See page 49

Table I

Tests Nos. 1

Figure VI

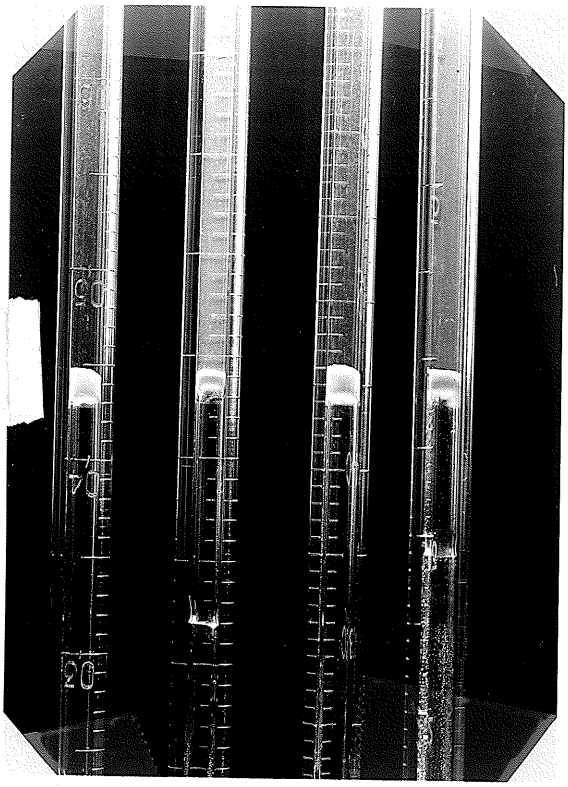


Test with Deer Antigen

.35 cc antigen
.1 cc antiserum
.15 cc saline

1 2 3 4
Antisera--Cow Moose Deer Sheep

Figure VII



Test with Sheep Antigen

.35 cc antigen
.1 cc antiserum
.15 cc saline

1 2 3 4
Antisera--Cow Moose Deer Sheep

See page 49

Table I

Tests Nos. 1

The moose antigen given almost as much precipitate with anti-cow serum as with anti-moose, and more precipitate with anti-deer serum.

The sheep antigen gives more precipitate with both anti-cow serum and anti-deer serum than with anti-sheep serum.

## Comment

As the question stands now it is only possible to distinguish beef and deer antigens. But if the moose and sheep anti-sera were made just as strong it is probable that it would be possible to distinguish moose and sheep antigens also. These antisera could be stored in the dry form (dried in vacuo) and used when necessary. However, the strength of the antisera is depleted by long-storing so that a time-limit would have to be set.

## Summary

1. Progressively decreasing the antiserum in the tests progressively increased their specificity.

2. To avoid trouble with differences between sero-precipitins and musculo-precipitins in this type of work, musculo-precipitins should be dealt with entirely. In other words, it would be expedient to use only meat-extracts for injection into rabbits in the preparation of antiserum.

3. In tests with the various antisera only beef and deer antigens can be differentiated from each other and from the other antigens due to the strength of the anti-cow and anti-deer sera.

4. It is impossible to differentiate clearly moose and sheep antigens from each other and from the other antigens when tested by the various antisera. This is due to the fact that group-precipitation in these closely related species is so powerful that an antigen will give more precipitate when mixed with a very strong heterologous antiserum than with its homologous antiserum--if the latter is weak, as it was in the case of anti-moose and anti-sheep sera.

5. It is probable that if the anti-moose serum and the anti-sheep serum were as strong as the anti-cow and anti-deer sera it would be possible to identify unknown extracts of sheep and moose, as it is now possible to do with deer and cow.

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