SECTION OF STATE MEDICINE.

THE PRECIPITIN TEST IN MEDICO-LEGAL WORK.

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1. *Nature of Problem.*

In medico-legal work it is often of great importance to ascertain the source of a blood-stain—the species of animal that furnished the blood of which the stain is composed. For example, in murder cases, the defence often alleges that blood-stains found on the clothes of a suspected person, or on instruments in his possession or traced to him, are due to the blood of some lower animal with which he may have come in contact, perhaps in sport, or in the exercise of a butcher's trade. Until the method of which I now desire to speak came into use the difficulties in the solution of the problem as to the origin of a given blood-stain may be described as practically insuperable. It must be borne in mind that, in medico-legal work, where the life of a fellow-man hangs in the balance, nothing short of absolute certainty ought to suffice, and the only method hitherto available—the microscopic appearance of the blood corpuscles—falls far short of yielding the absolute certainty required. The stain when submitted for examination is practically always dry, and been so for days or weeks, not infrequently for months or years. The erythrocytes have become fused together, their outlines indistinct and distorted, their substance closely adherent to, or entangled in, the stained material.

In this country the mammals, the blood of which has most usually to be distinguished from that of man—viz., the ox, horse, sheep, pig, goat, and rabbit—possess red corpuscles differing so slightly from those of man that only a skilled microscopist, well versed in microscopic work, could hope to distinguish them with certainty from those of man in the perfectly fresh condition. There is no difference in shape, and the size varies within the narrow limits of a few microns. The red corpuscles of man average 7.7. μ, those of the horse 5.6, of the ox 5.6, of the sheep 4.5 to 5.0, of the goat 4.25, of the pig 4.6 to 6.0, and of the rabbit 7.0 to 7.5. In the fresh preparation, such differences are, of course, quite appreciable, and may be relied upon, when a sufficient number of unaltered corpuscles of each kind are available, so that a well-grounded average value may be determined. But how different is the case in medico-legal work!

Speaking from personal experience of many cases which I have examined I can say that it is extremely difficult to obtain from stains dried on fabrics or solid surfaces isolated corpuscles in a condition satisfactory for micrometry. Much depends on the fluid used for isolating the corpuscular elements. A large number of formulas have been suggested, of which the best that I have tried is a 32 per cent. solution of caustic soda or potash. But even careful maceration in this fluid yields so many forms, deformed in shape and differing widely in size, that even with stains of known origin it is difficult to convince oneself that any reliable distinction can be drawn by the aid of the microscope—and this, too, in stains that have only been quite a short time dried—a few hours in the incubator.

It is a commonplace of the books that the circular non nucleated erythrocytes of the domestic mammals can be readily distinguished from the larger, elliptical, nucleated, erythrocytes of birds, reptiles, and fishes—and a mere glance

suffices to tell the difference *in the fresh preparation.* But, when we come to the conditions that prevail in actual practice, the distinction is far from being such an easy matter as would at first sight appear. Shape and size may be veiled by distortion, nuclei when present may disappear or become hard to recognise—when present, may be simulated by highly refractive granules or dark central areas. Attempts to wash out the stains with water may have produced hæmolysis. From many blood-stains I have failed to isolate any red discs that could be relied upon to supply an answer to the question—Was this blood mammalian ? much to the surprise of jurists who had been led by the statements in the books to suppose that so much at least could be certainly ascertained and deposed to on oath.

We have advanced a long way since 1900, when the diagnosis of mammalian blood was the highest limit of certainty obtainable by the medical jurist. Two cases that have lately occurred in my own experience may serve as examples of what can be accomplished, thanks to the method of sero-diagnosis which I am about to describe. The clothes of a man who was accused of committing a murder having been submitted to me for examination, I found on one article only, the cap, a stain of blood. It was about the size of a threepenny-piece, and appeared to be a single drop which had fallen on the cap from above, and dried on the cloth undisturbed. The stain had evidently been there a considerable time, for, on microscopic examination of scrapings macerated in 32 per cent. soda the red corpuscles appeared so shrivelled and distorted that I could come to no conclusion as to their origin, merely noting that they were of mammalian character and seemed a little small for those of human blood. It did not, however, occur to me at the time that they were of other than human origin. On applying the precipitin test I found that they gave the reaction characteristic not of human, but of horse blood. This

result I duly reported, giving it as my opinion that the blood was of equine origin. On subsequent inquiry it turned out that the accused man was in the employment of a large horse-dealer, and was frequently engaged in assisting at operations performed on horses, more especially in the region of the mouth ; so that a drop of horse blood might readily have fallen unobserved on to his cap and dried there.

The second case was that of a man accused of a particularly brutal murder. On his "leggings" and trousers I detected a number of blood-stains, also on a knife which was found concealed in moist earth and was conclusively traced to the prisoner. A witness who remarked the appearance of the prisoner's knife, when he took it out to cut some twigs for a broom, was told by him that he had been killing a goat. From each of the articles mentioned I succeeded in obtaining a sufficiency of extract for the application of the test, and at once obtained the typical precipitation reaction with human antiserum, and a negative result with the other anti-sera against which I tested it. The human origin of the stain was thus proved. The murderer, whilst awaiting trial, confessed his crime, and has since suffered the extreme penalty of the law.

2. *General Nature of Precipitin Test.*

I will now briefly state how this valuable test originated. Its history will explain how it was that I came to take it up. Precipitins belong to the class of specific anti-substances or immune bodies. They were discovered by bacteriologists during the investigation of the blood serum upon which depends immunity against infective disease. It was whilst following in my own laboratory the results that had been achieved by Uhlenhuth (1), Wassermann and Schütze (2), and by Nuttall (3) in Cambridge that I was enabled to convince myself of the objective reality of the phenomena in question and of their ready applicability in practice. Having

tried the precipitin method in various ways and with every conceivable control, and having mastered the technique (which requires a good deal of time and perseverance), I brought the matter under the notice of the Irish Government in 1902, and have been entrusted by them with the conduct of such investigations in criminal cases.

During the seven years that have since elapsed I have been many times subjected to cross-examination with reference to the exact nature and reliability of the test. Save on one occasion I have always succeeded in explaining it to the satisfaction of the court. But, incidentally, I have found that even in our own profession there exists a great deal of misconception with regard to this matter. Passages from standard text-books on medical jurisprudence have been again and again quoted against me, and my attention has been called to the absence of any official recognition of the test on the part of the English Home Office. For these reasons I have thought it well to avail myself of the opportunity afforded by this Presidential Address to state in the clearest and least technical language I can command the nature of the test, the technique of its application, and the precautions that have to be taken in order to guard against error. I am writing not for experts or immunity but for those members of my own and the legal professions who, being without special knowledge of the subject, are desirous of knowing what has been and what can be accomplished by the aid of these new tests and how they are to be carried out.

As I have said above, precipitins belong to the class of substances called anti-bodies which arise in the blood and tissue liquids of animals (including man) as the result of the entrance of certain foreign substances into the animal economy. Not every foreign substance is capable of giving rise to an anti-body. In order to do so the introduced substance must possess a certain molecular complexity—must, in fact, be

similar in its general nature to the substances which the cell is in the habit of taking in as food, and for the admission of which it possesses what Ehrlich calls receptors. Extraneous substances of simple molecular constitution which are unlike food, and for which the cell does not possess any receptors, are incapable of giving rise to the formation of anti-bodies. Simple metallic substances, such as arsenic, alkaloidal substances like strychnine, and carbohydrates such as sugar and starch, are therefore incapable of giving rise to immunising anti-bodies, though a certain amount of immunity against poisons like arsenic or morphine can be produced by gradually acclimatising the system to their presence—a totally different matter.

In general terms it may be stated that the introduction into the system of foreign substances of albuminous or proteid nature leads to the formation of an anti-body which is found in the serum of the animal so treated. A substance capable of so acting is called an *antigen*. When antigen is brought into relation with serum containing anti-body a reaction takes place between the two, roughly comparable to that which takes place when an acid is brought in contact with an alkali. The two may be said to combine and neutralise each other. The effect may be at once perceptible to our unaided senses, or it may require to be demonstrated by special methods. The following are amongst the chief kinds of anti-bodies :—(1) antitoxins, (2) precipitins, (3) agglutinins, (4) opsonins, and (5) lysins.

Poisons of complex constitution and derived either from animals (snake and spider poison), from the higher vegetables (abrin, ricin), or from bacteria (diphtheria, tetanus) are the genetic bodies of antitoxins. When a toxin is brought into contact with its antitoxin no visible change is produced, but the combination is non-virulent, owing to the occupation of the haptophore group of the toxin, on Ehrlich's hypothesis.

Agglutinins and opsonins come under the head of anti-

bacterial substances, and so do bacteriolysins. The antigen is the micro-organism, and the effect of bringing antigen and antibody into contact is the agglutination, or phagocytosis in the presence of leucocytes), or solution, of the genetic organism, as the case may be.

Under the head of lysins come not only the bacteriolysins just referred to, but also cytolysins, the genetic body being cells foreign to the animal into the economy of which they are introduced. The most important of these are the hæmolysins, which are evoked by the injection of foreign red-blood cells. The effect of bringing the antibody (hæmolytic serum into contact with the antigen (blood corpuscles of the kind used for injection) is that the cell stroma is ruptured and the hæmoglobin diffuses through the liquid. This "laking" of the blood, as it is called, is the most easily observed of all phenomena, and is extensively used for the detection of antigens and antibodies by the method first suggested by Bordet and Gengou (4), more fully worked out by Wassermann (5) and his colleagues, and now generally known as complement-fixation.

For the present, however, we shall confine ourselves to the antibodies that more immediately concern us—viz., precipitins. The antigen of these is serum-albumin or globulin. Any albuminous tissue fluid, even urine (as was, I believe, first shown by Ruffer), can, when injected into an animal of another species, act as antigen. The effect of the addition of serum containing antibody to liquid containing antigen in solution is the production in the previously clear liquid of a haze or opacity which gradually thickens and condenses into little white flocculi. These gradually sink to the bottom and form a cloudy deposit technically called the *precipitum*.

Like all these substances, the precipitating antigen is *specific* in its action. This means that it will produce the above *only with its own antigen*, not with antigen derived from

any other species of animal, save that which furnished the albumen employed for the injection. This specificity is the keynote of all these reactions, and explains their utility in practice. It is not, however, absolute. An antibody produced by injecting albumen from one species will react with antigen from a closely-allied species, as was pointed out by Nuttall (3) in his admirable researches on blood relationship. For example, precipitins made by injecting human antigen will react with the albumen of the higher apes, sheep antibody will react with goat antigen, rabbit antibody will react with hare antigen, &c. But the reaction is, in comparative experiments with properly diluted material, found less distinct with the allied than with the identical antigen, and in this country, where identification of human blood is chiefly required, this source of error is not of much importance and can readily be eliminated by proper control, as will be explained later on.

Of greater importance is the so-called "mammalian reaction" yielded by certain highly potent antisera by virtue of which their reactions extend to antigens derived from animals rather widely removed biologically from those which furnished the albumen used in the injections. Errors from this source can readily be avoided by employing the antigen in highly dilute solutions (1 in 500–1,000).

3. *Technique.*

The first and most difficult part of the procedure is to obtain the antibody. For this purpose I have always used rabbits. As antigen I have sometimes used human blood obtained, with due precaution against contamination, from the placenta during and after parturition. This is troublesome to obtain, and must be used fresh, as it is seldom uncontaminated, and micro-organisms speedily make their appearance in it even when stored in the refrigerator. Where antigen blood or serum is difficult to obtain, or can only be had at long intervals,

it would be quite possible to dry it over sulphuric acid in a vacuum, or in the incubator soaked into filter-paper. Once dry it can be sterilised at 150° without loss of its antigenic property, and can then be stored indefinitely, and when needed brought into solution with normal saline solution, or if preferred with sterile distilled water to which an equal volume of 1.7 per cent. saline solution is added. What I now invariably employ is ascitic or pleural effusion, or hydrocele fluid obtained as aseptically as possible and stored over chloroform. The chloroform can be got rid of by pipetting some of the clear supernatant serum into a sterile petri-dish and leaving it overnight in the incubator, covered only with sterile filter paper. Blood of domestic animals can, of course, be readily obtained from the slaughter-house.

Some of the serum (3, 4, 5, or 10 c.c.) is injected into a rabbit, either intraperitoneally, or into the marginal vein of the ear, with all due precautions, and the injection repeated at intervals of four or five days until 25 c.c. have been introduced, when the animal may be bled from the ear and the serum tested as to its contents in anti-body. The process of bleeding is much facilitated by wrapping the ear in a piece of lint wrung out of hot water. The veins become dilated and the yield of blood is greatly increased. The blood from the divided vein is taken up into a Wright's pipette or allowed to rise into a small test-tube with an obliquely drawn out capillary end. Should there be obvious evidence of the presence of anti-body, such as the production of a distinct opalescent zone at the line of contact with the antigen serum diluted 1 in 100, then the injections are proceeded with till the rabbit has received 70 to 80 c.c. of antigen. Much smaller quantities are often effective. Ten to 14 days after the last injection the animal is chloroformed and bled either by dividing the vessels of the neck or by quickly opening the thorax, cutting the great vessels and rapidly pipetting off the

blood with which the thorax is filled into a sterile Petri dish which is propped up obliquely in the refrigerator till the serum has separated. The serum is then drawn up into sterile pipettes and sealed off and stored in the refrigerator. In practice it is difficult to avoid contamination by hairs, &c., and the serum will seldom keep at room temperature unless filtered through a small Berkefeld candle or treated with 1/10 of its volume of 5 per cent. phenol. I have found, however (in agreement with Graham Smith and Sanger) (6), that even specimens of antiserum that smelt badly on opening the tube, and the deposit from which contained anærobic spores, gave quite useful precipitating reactions if care were taken not to stir up the sediment. Uhlenhuth (7) found that mould-growths did not interfere with the specific power of the serum. The turbidity due to such growths must be allowed to subside or be got rid of by centrifuging. A plan that seems well worth trying is that recommended by von Eisler (8), of drying the antiserum on strips of black paper—the so-called *Naturpapier*. If only small quantities of antiserum are needed at a time, the animal yielding it can be kept alive for many months and its serum be maintained at a very fair level of potency by an occasional injection of antigen. As cachexia is, however, apt to supervene, and the *titre* has to be re-determined each time, this course is not to be recommended.

The process is tedious and liable to cause disappointment. Some batches of antigen prove toxic. I have had a specimen of pig-serum, freshly collected and to all appearance normal, which killed rabbits in doses as low as 2 c.c. intravenously injected. In other cases cachexia supervenes, more especially if the injections are massive (10 c.c. and more). It is important to make a practice of weighing rabbits at frequent intervals, as, if they waste, their serum is useless. Another frequent source of loss is anaphylaxis (9), which usually occurs at the second injection, especially if it has been a large one. Highly

immunised rabbits, the preparation of which has cost much time and trouble, sometimes die from unknown causes in the night, and their serum is thus lost. In order to avoid disappointment, if it is absolutely necessary to have some highly potent precipitating anti-serum in readiness by a certain date it is well to start preparing three rabbits at least eight or ten weeks beforehand.

Before applying it for medico-legal purposes, it is absolutely essential to ascertain the potency of the antiserum. For this purpose small test-tubes are used, about 5 cm. long by 5 mm. in bore. They are conveniently made fresh each time from glass tubing, and are placed in little racks with holes for half a dozen. These racks I have had specially made by a tinplate worker. With an ordinary drawn out capillary pipette I place in the bottom of each about 0.OS C.C. (3 drops) of the serum to be tested, and superpose on its surface with another pipette about 0.1 C.C. of antigen serum diluted with normal saline solution 10, 50, 100, 500, and 1,000 times. By way of control a second set of tubes is charged with the antiserum and treated in the same way with the serum of some other animal similarly diluted. Both antiserum and antigen should be absolutely clear, and the line of demarcation between them sharply marked. Personally, I have not been troubled by opalescent antisera, but if encountered they should be rejected.

Observation is made from time to time up to an hour, the tubes being left meanwhile at the ordinary temperature of the laboratory. The presence of a distinct line or zone of opalescence at the junction of the fluids indicates a positive result. The appearance is not unlike that produced by the nitric acid test as usually applied to albuminous urine. With a powerful antiserum and a strong solution (1: 100) of homologous antigen the opalescence gradually spreads through most of the supernatant fluid and resolves itself into flocculi which by the following day will be found to have sunk to the bottom of

the tube. When the antiserum is weak or the antigen very dilute (1 : 10,000–20,000) the zone may require careful scrutiny by a trained eye and suitable oblique illumination against a dark background for its detection.

I have found that antisera giving a distinct zone within an hour with antigen diluted 100-fold are quite strong enough for all practical purposes, though I am aware that stronger ones are considered necessary by the German workers. So completely can the test be controlled in actual practice that even comparatively weak sera, reacting only with 100-fold dilutions of antigen, can be made to yield conclusive results.

Highly potent antiserum when brought in contact with solutions of antigen may (and very often does) give rise to pseudo-reactions—*i.e.*, precipitates with non-homologous serum or stain extract. Errors so arising are to be avoided by using the antigen diluted to somewhere about the limit of the titre of the antiserum—not stronger at any rate than 1 per 1,000, and by careful controls with as many other sorts of antigen as are available in similar dilutions.

Nuttall (10) has suggested and worked out a method of measuring the amount of precipitum formed in unit time, and comparing it in tubes containing the same antiserum with different antigens. This method does not seem to have been widely practised, and it appears to me that differences in the density of the precipitum might readily affect its apparent bulk, unless, indeed, it were concentrated by the centrifuge. Personally, I have not tried the method, and prefer to base my opinion upon the rapidity and distinctness with which the reaction come on in the zone of contact between highly potent antiserum and highly diluted antigen.

4. *Testing the Stain.*

Having obtained a satisfactory antiserum, the next step is to prepare an absolutely clear solution of the stain to be tested.

Before proceeding to do this, however, it is necessary to prove that the stain consists of *blood*. This must be done by the usual methods. I always use the benzidine test (11) first, and should this prove positive confirm it by the demonstration of hæmin crystals and the spectroscopic reactions of the pigment, using a small Browning spectroscope or the Abbe micro-spectroscope, if the amount is small. I always put up a preparation in 32 per cent. soda so as to observe the character of the corpuscles.

One must not lose sight of the fact that the precipitin reaction reveals the presence not merely of blood but of any albuminous substance, such as mucus, pus, semen, milk, or albuminous urine derived from the animal that has provided the antigen used for preparing the antiserum. The necessity for proving that the stain-producing substance is blood therefore remains.

This having been satisfactorily demonstrated, the next thing to do is to prepare a solution of the stain in normal saline solution, and to render it absolutely clear and bright by filtration and the centrifuge. If the amount of material is considerable care must be taken not to use it too strong lest pseudo-reactions should give rise to error. It should be colourless, or nearly so, foam on shaking, and give a slight but distinct reaction with the nitric acid test for albumen. Should the amount of solution available be very small—less than 0.05 cm.—the reaction must be carried out in small pear-shaped or lengthily oval pieces of capillary tubing, the liquids being introduced by means of finely drawn out capillary pipettes.

It is absolutely essential to carry out, side by side, with the actual test, a complete series of controls. For this purpose we need blood-stains of man and the chief domestic animals on various substrata. Sheets of filter-paper are the most convenient of these, but it is well to have the blood dried on

woollen, linen and cotton fabrics, also on leather, wood, and metal. This dried material should be of various dates so that one may select for control a specimen not more recent than that under investigation. In addition to the stains of known origin, one ought also to possess at least one other antiserum made with antigen different to the one under investigation. Thus, if human blood be suspected it is well to have in hand some rabbit serum anti to, say, ox-albumen. For obvious reasons the larger one's stock of these anti-sera the better, and I now endeavour to keep in stock those reacting with the albumen of the horse, ox, sheep, and pig. Lastly, one needs some serum from a normal non-immunised rabbit.

Two of the little stands are now taken, each containing six small test tubes. Each of the tubes in the first stand now receives 0.05 c.c. anti-human serum. With capillary pipettes about double the amount of one of the following dilute solutions is now added to each respectively :—

Test tube 1 receives extract of known human stain.
,, 2 ,, ,, ,, ox ,,
,, 3 ,, ,, ,, horse ,,
,, 4 ,, ,, ,, sheep ,,
,, 5 ,, ,, ,, pig ,,
,, 6 ,, ,, ,, stain under investigation.

The second stand of tubes is now charged as follows :—

Test tube 1 receives normal rabbit serum about 0.05 c.c.
,, 2 ,, anti-human ,, ,, ,, ,,
,, 3 ,, ,, ox ,, ,, ,, ,,
,, 4 ,, ,, horse ,, ,, ,, ,,
,, 5 ,, ,, sheep ,, ,, ,, ,,
,, 6 ,, ,, pig ,, ,, ,, ,,

About 0.1 c.c. of the extract of suspected stain is now

carefully superposed on each with a capillary pipette as above described.

The tubes are observed in fifteen minutes, and again at the end of an hour. Should the stain be of human origin only Nos. 1 and 6 of the first stand and No. 2 of the second stand should show a positive reaction. The negative results in the remaining tubes of the first stand prove that the antiserum used is specific—*i.e.*, will only react with its own antigen. The negative results in the second stand show that the stain is composed of specific human antigen reacting only with its own antibody.

Should the stain be of non-human origin it will, of course, react in the tube containing the corresponding anti-body in the second series (if there is any such), and the conclusion as to its nature can be confirmed by a proper series of controls.

Let us return now to the two concrete cases mentioned earlier in this paper. In the case of the blood stain on the cap the amount of extract available for the test did not exceed 0.1 c.c. By the capillary tube method, however, I was able to superpose enough of it on the surface of five different anti-sera to assure myself that it reacted powerfully with the anti-horse serum, whilst it proved negative over anti-human, anti-ox, anti-sheep, and anti-pig serum, respectively. In another series of tubes I tested the specificity of the anti-horse serum used by running on to its surface a little slightly dilute stain extract from horse, man, ox, sheep, and pig, respectively, and obtained a reaction in the first tube only. The demonstration of the specific nature of the reaction was thus completed and the origin of the stain determined beyond all possible doubt.

In the other case above referred to, the extract from the several articles submitted at once reacted with anti-human serum, but not with sera anti to the albumen of the ox, sheep, pig, or horse. I had no anti-goat serum on which to try it,

but the anti-human serum I was using gave no reaction with extract of goat-stain, so that the positive result with anti-human serum was conclusive.

5. *Sensitiveness of the Test.*

Coming now to the delicacy of the method, my opinion, based on experience of many cases, is that if the operator has at his disposal a powerful anti-serum and good command of capillary-tube technique, the amount of albumin demonstrable by this test is amazingly small. The chief difficulty, in my experience, is not the smallness of the amount of antigen, but the difficulty of obtaining a satisfactory clear solution. Filtration of such minute quantities of stain extract is, of course, out of the question, and we must only have recourse to prolonged centrifugation at high velocities, which is usually successful.

I have never attempted to ascertain the smallest amount of antigen demonstrable by this means, but Uhlenhuth (12) says that it is easy to demonstrate 1/20,000th gramme, and that Hauser was actually able to demonstrate as little as 1/200,000th part of a gramme by the use of the capillary-tube method due to Carnwath (13).

My original intention was to refer in this address to the other sero-diagnostic method of albumen differentiation, that introduced with another object by Bordet and Gengou (4) and now well known under the name of complement-fixation. This reaction depends on the property possessed by mixtures containing antigen and the corresponding antibody, of fixing complement, and thus preventing the hæmolysis of sensitised corpuscles. It can be used for the detection of amboceptors (antibodies) in the presence of the corresponding antigen or for the detection of antigen in the presence of the corresponding amboceptor (antibody). It has been used successfully for the detection of infection by known and cultivated micro-organisms

(typhoid, cholera, tubercle) and also when the infecting micro-organism is unknown or uncultivable. Its principal application has hitherto been in the latter class of case, when it often affords the only available method of making the diagnosis. The infection to the recognition of which it has been mainly applied is that of syphilis. Wassermann first succeeded (14) by its means in demonstrating syphilis amboceptors in the humours of tabetics and paralytic dements.

It is to Neisser and Sachs (15) that we owe the introduction of complement-fixation into medico-legal practice. Their work was based upon the results of Moreschi (16), who first showed that complement is fixed by the union of precipitin with its homologous precipitinogen (antigen). Inasmuch as precipitins are generally looked upon as receptors of the second order provided with a precipitating and a haptophore group, and ordinarily act independently of complement, it is not exactly clear at first sight how the fixation takes place. It is certain that amboceptors are produced during immunisation with foreign albumen, and that, during the process of precipitation they unite with their corresponding antigen and throw down or fix complement. Those who desire precise information as to what goes on during the interaction of these specifically opposed substances are referred to Professor R. Muir's brilliant series of researches published in the *Journal of Hygiene*, and now collected under the title of "Studies in Immunity." Be the explanation what it may, the fact remains that complement is fixed by the union of almost inconceivable minute traces of albumen with its corresponding antibody. The quantity of the combining substances is far too small for any precipitate to be visible, yet the fact that the union has taken place may be proved by the absorption of the complement necessary for the re-activation of an inactive hæmolytic system.

I have some little experience of the test, and hope as time

and opportunity offer to accumulate some more and lay it before the Academy on a future occasion. Meanwhile, I will only say that complement-fixation is a very troublesome and time-consuming procedure when carried out with all the proper controls, and without them it has no scientific value. It is much more difficult than the precipitin reaction, and can hardly be carried out satisfactorily in a laboratory where any other work is going on. Moreover, it is excessively delicate, so much so that, as pointed out by Uhlenhuth, even the trace of protein contained in sweat may fix some of the complement. There are other sources of error. Thus, for example, there are present in various fabrics used for clothing, complement-fixing substances (17), the presence of which has to be specially tested for, and this complicates the already over-elaborate technique. This drawback may be got over in some cases by showing that the substances in question are heat-stable, which is not the case with blood, and in others by the use of artificial hæmolytic amboceptor instead of naturally hæmolytic serum as originally recommended. I am inclined to agree with Uhlenhuth when he says (*loc. cit.*) that the delicacy of the precipitin test (which can detect 1/20,000th gramme of foreign albumen) suffices for all practical purposes, and that it must seldom be necessary to invoke the aid of complement-fixation in medico-legal work.

Outside of medico-legal work there have been found several interesting and valuable applications for the precipitin method, which I will, in conclusion, briefly touch upon. There is, for example :—

(*a*) The diagnosis of bacterial infections by adding the serum of the infected animal (antibody) to the culture fluid of the suspected organism, filtered free from the bacterial bodies, but containing their soluble extract or metabolic products (antigen). If the two correspond, a specific precipitate is produced, the formation or absence of which can be

utilised for diagnostic purposes, as was first, I believe, shown by Wladimiroff in the case of glanders infection of the horse. The reaction has been found to work well in the case of typhoid, cholera and streptococci. This procedure has been very fully studied by R. Kraus, to whom we owe most of our knowledge on the subject (18).

(*b*) The recognition of the species of animal from which almost any given tissue or albuminous fluid has been obtained, not merely blood or serum. Thus, for example, by injecting cow milk serum into rabbits, Fish (19) was enabled to obtain an antiserum (lactoserum) which produced a specific precipitate with the serum of cow-milk, but not with that from other animals, and which could therefore be employed for diagnostic purposes. It would seem that even boiled milk can have its origin so determined. Working on these lines, Sion and Laptes (20) claim to have traced the origin of samples of cheese. Similarly, in the case of egg-albumen, Myers and Uhlenhuth (21) found that specifically different anti-sera are yielded by rabbits injected with the whites of the eggs of different species of birds. In this way it was found possible to determine whether a given sample of egg-white was produced by a common fowl, a duck, a turkey, or a plover.

A further application of this reaction, which has proved of the utmost practical value in meat-inspection, is that whereby the nature of a given sample of muscular tissue can be determined. Rabbits injected, not necessarily with muscle-juice, but with the blood or serum of, say, the horse, dog, or cat, yield antisera, which, when brought in contact with extracts of the muscle-tissue of these animals, give rise to highly specific precipitates. In this way it is possible to ascertain the species of animal from which a given piece of flesh was cut, and to refer the several components of a sausage to their respective origins. The German Public Health Administration has taken advantage of this discovery, and now actually prescribes the

use of the precipitin reaction in cases of suspected importation of forbidden kinds of meat, adulteration of sausages with horse or dog-flesh, &c.

Along these lines it would be possible to determine the origin of the various kinds of meat extract that are now on the market, provided that they had not been heated to such a point as to destroy their antigens. This has actually been accomplished in a few cases, and the claim that the extract was made from beef was substantiated in the case of one brand, whilst in another the only albumen demonstrable was found to be derived from hen's eggs! (22) An interesting and valuable field of work is thus thrown open to the food analyst who is possessed of the necessary biological training and the requisite facilities for animal experiment. Nor is the applicability of the method confined to albumens of animal origin. Antisera have been made (23) whereby the protein of the grains of cereals can be differentiated from that of the legumes.

(*c*) Different *kinds* of protein derived from the *same species* of animal can sometimes be thus differentiated. Hamburger (24) was able to obtain antisera differentiating the albumen from the casein of cow's milk. Uhlenhuth (25), by injecting separately the white and the yolk of hen's eggs into different rabbits was able to obtain antisera specific for each. Of more direct medical interest are the experiments of Weichardt (26) and Liepmann (27), who by injecting rabbits with placental syncytium were able (by the method of elective saturation) to obtain a serum capable of precipitating one kind of albumen only—that derived from syncytial cells. From this it would appear to be only a short step to obtain antisera exercising a specific action on cancer cells or the albumen derived from them, supposing (which is not certain) that such cells possess any specific properties not shared by the normal epithelium at their seat of origin. Experiments have been made in this

direction, but so far as I am aware without success (28). In this connection it is interesting to note that by injecting extracts of crystalline lenses from different animals, Uhlenhuth (29) found that the anti-sera so obtained did not react with the serum or extracts of other organs of the animal that provided the lenses used for the injections. Antisera made from the lense of various animals (man, pig, fowl, frog) were found to react equally well with the extract of the lense of any animal. From this it would appear that the lense contains an albumen *sui generis*, specific for the organ, but not for the species, and not sharing the biological properties of the blood-derived albumens of the rest of the body. This is a highly interesting observation when viewed in the light of what we know with regard to the origin of the lense from the embryonic ectodermal epithelium. Römer (30) has made use of it to support his theory that senile cataract is due to cytotoxic influences.

(*d*) Lastly, an interesting controversy has arisen as to whether a precipitin can be obtained from the tissues of Egyptian mummies that have been preserved for thousands of years. The inquiry was led up to by the ascertained fact that the origin of bones that have been buried for various periods can be revealed by the precipitin reaction, provided that an extract containing dissolved albumen can still be obtained from them. As the result of their investigations carried out on Egyptian mummies from 3,000 to 5,000 years old von Hansemann and Meyer maintain that their tissues react to anti-human serum with sufficient distinctness to enable their human origin to be so demonstrated. On the other hand, Uhlenhuth completely failed to obtain from 27 Egyptian and Peruvian mummies which he tested any extract capable of yielding a precipitin reaction. Indeed, he states (31) that he failed even with a mummy only 300 years old. In this negative result he is confirmed by Schmidt, of Cairo (32), who

was able to obtain from a mummy of the prehistoric period (at least 6,000 years old) an extract containing sufficient albumen to give a biuret reaction, but failing to yield a precipitate with human antiserum. The proteid matter would seem to have undergone some radical change during its long period of preservation.

In these latter observations I feel that I have wandered far from my proper subject. But I felt sure that in an opening address, as this, a somewhat general survey of the possibilities of the precipitin method would not be unwelcome to our Section of State Medicine.[a]

[a] A demonstration was then given of the mode of determining the *titre* of a specimen of anti-human rabbit-serum, and a stain of bovine origin was tested with several antisera and referred to its proper source. The stain in question had been dried for two years on boot leather.

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Dr. O'Farrell referred to the work done by Professor Muir in connection with the overlapping of the various serums. He desired to know if Professor McWeeney had tried heating the different serums, and driving off the complement, and seeing if the reaction came about by adding fresh complement. The possible presence in linen of some complement-fixation substance was easily understood, as the material in preparation being macerated for a long time much chemical changes must take place. Was it possible, he asked, that by bringing about a reaction in cancer cells an early diagnosis of carcinoma could be made ?

Professor Mettam said he believed he was correct in saying that Nuttall stated that simple frothing contained quite sufficient of protein material to give a reaction with the anti-serum. He did not quite agree with Professor McWeeney on the question of anaphylaxis. He thought it usually arose after the second dose when a major dose was given. In Professor McWeeney's cases the doses given were equivalent. He would like to draw attention to the explanation given by Vaughan. He took egg-white, and found it was composed of two bodies, one soluble in alcohol and one not. When injected, one was poisonous and one was not. The explanation of anaphylaxis was that in the minimum dose the poisonous was split off from the non-poisonous molecule, and the non-poisonous produced sets of anti-bodies, and the poisonous, being of so small a quantity, was harmless to the animal, or did insignificant damage. As a result of the injection of the non-poisonous portion a number of receptors were formed in the body. Then, when a larger quantity came in it was immediately split, and the non-poisonous parts of them were immediately attacked by the receptors, which liberated a large amount of the poisonous element, and this caused death.

Professor McWeeney, in reply, said the presence of acid or alkali, even in small quantities, completely upset the reaction. He had tried to reactivate inactivated precipitin serum with complement, but had failed. Precipitin serum stood heating

better than hæmolytic serum. It was evident that its precipitating group was much more stable than complement. He saw that his solution was quite colourless. It should foam briskly and give a slight, but distinct, nitric acid reaction. It should be worked down by degrees until it ceased to give a reaction. Of course, they were reduced to surmise as to what the actual dilution was. It had been found that serum taken from anaphylactic animals had no complement in it; and, inasmuch as complement was a most important constituent of human serum, it seemed to him more natural to account for the symptoms of the animals by the deprivation of an important element in the blood than by a purely hypothetical splitting of an albuminous substance, which seemed to him to belong to the realm of incompletely ascertained facts. His anaphylactic animals had had first a small dose, and then a second large one; the result was they died inside five minutes.