

pressure is below that of the atmosphere, whilst the patient's head and the anaesthetist are outside. It is easy to see that by either of these methods it will be possible to regulate the extent to which the lung recedes from the chest wall when the pleura is opened, and also, when required, to inflate it again so as to bring it in contact with the chest wall. But for our particular purpose it has this defect, that if one of the large bronchi is opened the physical conditions are obviously altered and the control over the lung could no longer be maintained. This objection does not, however, apply to cases where the foreign body is in the substance of the lung and where it may be possible after its extraction to hermetically close the incision in the lung by which it has been removed. But another objection at once suggests itself—namely, that the portion of the lung incised is sure to be septic, and therefore it must be a dangerous thing to complete the operation by sucking out the lung and then closing up the chest wall. Or, if not dangerous, it is at all events very likely to be followed by a septic empyema. The safest plan would be, if it were possible, to draw out the lung to the opening in the chest wall and secure it there by stitches, while at the same time accurately closing the pleura; in fact, treating it like a pulmonary abscess. It does not seem likely that these two methods would be largely employed in dealing with this class of cases. At present the apparatus is very costly, and of the cheaper forms which have more recently been devised, it does not appear that any as yet have been placed upon the market.⁵

A Presidential Address

ON

THE PRECIPITIN TEST IN MEDICO-LEGAL WORK.

Delivered before the Section of State Medicine, Royal Academy of Medicine in Ireland, on Jan. 28th, 1910,

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1. NATURE OF PROBLEM.

GENTLEMEN,—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.

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 has 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 micrometric 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 $7.7\ \mu$, 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 too many forms, deformed in shape and differing so 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 absent, 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 deposited to on oath.

We have advanced a long way since 1900, when the diagnosis of mammalian blood was the highest limit of certainty attainable by the medical jurist. Two cases that have lately occurred in my own experience may serve as examples of what can now 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 antisera 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 antisubstances or

⁵ A short description of these will be found in the last volume of Burghard's System of Operative Surgery, vol. iii., pp. 703-708.

immune bodies. They were discovered by bacteriologists during the investigation of the properties 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 in Germany by Uhlenhuth,¹ Wassermann and Schütze,² and by Nuttall³ 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 on 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 antibodies 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 antibody. 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 antibodies. 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 antibodies, though a certain amount of immunity against poisons like arsenic or morphia 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 a foreign substance of albuminous or proteid nature leads to the formation of an antibody 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 antibody, 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 antibodies: (1) antitoxins, (2) precipitins, (3) agglutinins, (4) opsonins (in part), 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 antibacterial 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 cytolytins, the genetic bodies 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 of this class and is extensively used for the detection of antigens and antibodies by the method first suggested by Bordet and Gengou⁴, more fully worked out by Wassermann⁵ 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 effect *only with its own antigen*, not with antigen derived from any other species of animal, save that which furnished the albumin 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 albumin from one species will react with antigen from a closely-allied species, as was pointed out by Nuttall⁶ in his admirable researches on blood relationship. For example, precipitins made by injecting human antigen will react with the albumin 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 controls, 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 albumin used in the injections. Errors from this source can readily be avoided by employing the antigen in highly dilute solutions (1 in 500–1000).

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 content in antibody. 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 evidence of the presence of antibody, 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. 10 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 Sanger⁶), that even specimens of antiserum that smelt badly on opening the tube, and the deposit from which contained anaerobic spores, gave quite useful precipitating reactions if care were taken not to stir up the sediment. Uhlenhuth⁷ 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 antiserum 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 0.05 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 1000 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 much 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 1000-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 a 100-fold dilution of antigen, can be made to yield conclusive results.

Highly potent antiserum when brought in contact with strong 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 1000, and by careful controls with as many other sorts of antigen as are available, in similar dilutions.

Nuttall¹⁰ has suggested and worked out a method of measuring the amount of precipitin 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 comes 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 test¹¹ 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 Abbé 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 large, considerable care must be taken not to use it too strong, lest pseudo-reactions 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 albumin. Should the amount of solution available be very small—less than 0.05 c.c., 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 an antigen different from the one under investigation. Thus, if human blood be suspected it is well to have in hand some rabbit serum anti to, say, ox-albumin. For obvious reasons the larger one's stock of these antisera the better, and I now endeavour to keep in stock those reacting with the albumin 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 15 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 (if there is one) containing the corresponding antibody in the second series, 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 antisera 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 highly 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 albumin 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 antiserum 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 satisfactorily 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 Uhlenhuth¹² 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 albumin differentiation, that introduced with another object by Bordet and Gengou⁴ 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 cultivable micro-organisms (typhoid, cholera, tubercle) and also when the infecting 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 succeeded¹⁴ by its means in demonstrating syphilis amboceptors in the humours of tabetics and paralytic dementes.

It is to Neisser and Sachs¹⁵ that we owe the introduction of complement-fixation into medico-legal practice. Their work was based upon the results of Moreschi¹⁶ 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 albumin, 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 inconceivably minute traces of albumin 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 albumin) 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 bacteria

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, Fish¹⁹ was enabled to obtain an antiserum (lacto-serum) 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 Laptès²⁰ claim to have traced the origin of samples of cheese. Similarly, in the case of egg-albumin, Myers and Uhlenhuth²¹ found that specifically different antisera 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 specific 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 albumin demonstrable was found to be derived from hens' 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 albumins of animal origin. Antisera have been made²³ 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. Hamburger²⁴ was able to obtain antisera differentiating the albumin from the casein of cow's milk. Uhlenhuth,²⁵ by injecting separately the white and the yolk of hens' eggs into different rabbits, was able to obtain antisera specific for each. Of more direct medical interest are the experiments of Weichardt²⁶ 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 albumin 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 albumin 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 connexion it is interesting to note that by injecting extracts of crystalline lenses from different animals, Uhlenhuth²⁹ found that the antisera 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 lenses of various animals (man, pig, fowl, frog) were found to react equally well with the extract of the lens of any animal. From this it would appear that the lens contains an albumin *sui generis*, specific for the

organ, but not for the species, and not sharing the biological properties of the blood-derived albumins 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 lens from the embryonic ectodermal epithelium. Römer³⁰ 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 reaction 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 albumin can still be obtained from them. As the result of their investigations carried out on Egyptian mummies from 3000 to 5000 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 states³¹ 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 6000 years old) an extract containing sufficient albumin 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 such as this, a somewhat general survey of the possibilities of the precipitin method would not be unwelcome to our Section of State Medicine.*

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* 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, dried for two years on the sole of a boot, was tested with several antisera and referred to its proper source.

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OBSERVATIONS ON CONJUNCTIVITIS.¹

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ON looking through the answers to the questions I set of the various forms of conjunctivitis I was struck with the enormous number of varieties that from the text-book standard might be enumerated. I wondered whether we were not adopting a sort of ready reckoner style, and whether the simple facts of conjunctivitis might not be lost sight of in the morass of nomenclature. There would be less to complain of if the names were consistently selected, either from the appearance or from the cause, or from the character of symptoms, but no such method is adopted. Some forms are described by names that are a survival of an older nomenclature, such as blennorrhœal, catarrhal; often names are chosen from the appearance of the discharge, mucopurulent, membranous, or from different characteristics, as nyctalopic, angular, follicular; others again from the exciting cause, pneumococcal, diplobacillary, gonococcal, so that without much difficulty one can place exactly the same condition in two or more categories. Instead, therefore, of accurate differentiation, confusion is rendered worse confounded.

I pray that we may be delivered from what used to be the deadly sin of dermatologists, among whom each man was a law unto himself as regards nomenclature, and few realised what his *confrères* were talking about. Let us not forget that conjunctivitis should mean no more and no less than inflammation of the conjunctiva. If, on the one hand, more should arise it is a sequela; on the other hand, for instance, a hyperplasia of the normal lymphoid tissue of the conjunctiva constituting follicles is not conjunctivitis until the follicles become inflamed.

The conjunctiva is that structure that extends from the margin of the skin of the lids to the limbus corneæ, beyond which it is represented by the corneal epithelium, thus constituting a sac. The epithelium at the lid margins is stratified and this form extends for a short distance. It soon becomes of cylindrical type comprising two layers, the deeper rather flattened, the superficial cylindrical. As it approaches the cornea it becomes again stratified, and this form covers the cornea. Goblet cells also form apparently from the deeper layer which discharge their contents, but do not regenerate and are themselves cast off. The sub-epithelial structure has been divided into two, both fibrous, the more superficial containing a large amount of adenoid tissue, while in the deeper, thicker, and much more densely fibrous connective-tissue corpuscles a large number of lymphocytes are normally present. True papillæ only form near the limbus corneæ. Arteries, nerves, and lymphatics run chiefly in the deeper fibrous layer.

When this structure becomes inflamed it follows the usual course of inflammation from whatever cause arising, the difference being only of degree. The vessels dilate, the capillaries become crammed with leucocytes and red blood cells; in the veins the leucocytes collect along the vessel walls, and there is a general stasis. Then the leucocytes and the blood plasma transude; the lymphocytes normally present in the tissue enormously increase; the former wander through the tissues and show their characteristics of polymorphonuclear cells and reaching the surface are cast off as pus. Mast cells and plasma cells increase the effusion which produces the swelling known as chemosis. The goblet cells secreting mucus greatly increase as the inflammation proceeds, and these constituents go to form the "discharge." Sometimes the vessels become plugged or the fibrine-forming factors of the blood plasma that has transuded form a membrane on the surface of the conjunctiva involving the epithelial layer, or in more aggravated cases the coagulation

takes place in the structure of the lid, and the tissue becomes solid like wood.

Now this is briefly what occurs in varying degrees when the conjunctiva is inflamed. As a rule, the causes are so readily disseminated that the attack is general, but from some causes it may be more or less localised, and in a few it is strictly so.

Now, the incidence of some attacks of conjunctivitis may be fairly regarded in the light of an attack on the vitality of the patient by a foreign army of infection choosing the conjunctiva as a battle-ground, while other attacks represent a mutiny on the part of those whose special function is to preserve order. The first simile refers to the action of the various pathogenic micro-organisms that have been isolated, the second to neuropathic conditions, such as herpes, pemphigus, and perhaps phlyctenules, and if in your mind you carry further the similes you will recognise how closely the fact of victory or defeat or guerrilla warfare corresponds with actual facts of the inflammation. Now, as to these micro-organisms, if you will allow me to change my metaphor to the more mundane one of a police court, some have been caught red-handed and are convicted, and these include streptococci, pneumococci, gonococci, diplobacilli, Koch-Weeks and tubercle, while grave suspicion attaches to staphylococci, and circumstantial evidence is dead against Klebs-Löffler bacilli in membranous conditions, while several others are under supervision.

The chief agent in producing what used to be called mucopurulent conjunctivitis, catarrhal conjunctivitis, and "pink eye" is the Koch-Weeks bacillus. Quickly as it loses its malignancy, difficult as it is to grow, yet it is responsible for very big outbreaks, especially in schools where numbers are associated together, and though it may exercise its morbid influence at any time, yet it selects the springtime most frequently, possibly because then the conjunctiva is rendered more susceptible by pollen and dust. But it must not be forgotten that the pneumococcus is associated with a condition very similar to that of the Koch-Weeks bacillus, not so acute, I think, as an attack that sometimes is produced by Koch-Weeks, but more prolonged and less inclined to yield and with more constitutional disturbance. The anxiety, too, lest the corneal epithelium should become eroded should be much greater with the pneumococcus.

Streptococcal infection is not common; these micro-organisms are found in membranous conjunctivitis, of the responsibility for which the Klebs-Löffler bacillus is suspected, though it is not always found. Plugging of the vessels is said to occur in connexion with this infection, and the only time I have myself ever detected distinct plugging of a conjunctival vessel was in my own case. I had removed from a baby's eye a piece of grass over three inches long that was doubled on itself and lay concealed at the top of the upper conjunctival cul-de-sac. From the history of the case the foreign body had lain there (I am speaking from memory) for five or six weeks, and there was a very free discharge of what appeared to be thick pus from the conjunctival sac. As I was engaged examining the child with its head between my knees in the usual way, a fly settled on my forehead and I raised my left hand to flick it away, when I touched my eye with my finger. I did not pay much attention to it, not thinking from the circumstances of the case that the pus was likely to be particularly malignant, but a few hours afterwards irritation began in the eye, and the next day it was severely inflamed and chemosed, suggesting the possibility of gonorrhœal infection. The following day a black line like a hair could be seen in the swollen conjunctiva of the lower lid, and when I first saw it in the looking-glass I thought it was a hair, as also did another ophthalmic surgeon who tried to wipe it away with a pledget of wool. I had a good deal of constitutional disturbance, swelling of the pre-auricular gland, and continual chemosis and ptosis. Cultivations were taken from the secretion, but not at once, and the result was practically negative, but this was not surprising, as I had been using regularly germicide lotions. It was some three months before my eye could be considered quite well. Now, on reflection I believe my case to have been one of what is called Parinaud's conjunctivitis, cases of which I believe I was the first to describe in this country, though I did not recognise them as such when I wrote the account. No English text-book at that time, so far as I know, had described the condition. No observation I have ever made has given me such satisfaction as this, and I hope

¹ Founded on a lecture delivered at Oxford to the candidates for the diploma in Ophthalmology.