

No. 39

(NEW SERIES)

SCIENTIFIC MEMOIRS

BY

OFFICERS OF THE MEDICAL AND SANITARY DEPARTMENTS

OF THE

GOVERNMENT OF INDIA

THE APPLICABILITY TO MEDICO-LEGAL PRACTICE
IN INDIA OF THE BIOCHEMICAL TESTS FOR
THE ORIGIN OF BLOOD-STAINS

BY

LIEUT.-COLONEL W. D. SUTHERLAND, M.B., I.M.S.

(Edited by the Director-General, Indian Medical Service)



*Issued under the Authority of the Government of India by the Sanitary
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THE APPLICABILITY TO MEDICO-LEGAL PRACTICE IN INDIA OF THE BIOCHEMICAL TESTS FOR THE ORIGIN OF BLOOD-STAINS.

AT the instance of the Government of India I worked at the Medical College, Calcutta, for twelve months, with a view to ascertain whether the biochemical tests for the origin of blood-stains might be easily applied to medico-legal work in India. Most of what follows is deduced from the results obtained by me in Calcutta.

I. — Historical.

The question as to whether a blood-stain, produced as a *pièce à conviction* in a criminal case, is really due to human blood, is one that has much interested medico-legists ever since the importance of blood-stains came to be recognised. So early as in 1808 Jacopi is reported to have examined certain stains and declared that the microscope had revealed that these were due to bovine, and not to human blood. Mainly because the great Orfila did not believe in the accuracy of the microscope this statement did not attract the attention that it deserved. Orfila said¹ that he had often, when using the microscope, been unable to distinguish the blood of man from that of a pigeon, or indeed to be sure that it was blood that he had before him. Now, although in 1673 Leeuwenhoek had arrived at an approximately correct measurement of human blood cells,² the technique of the microscopists was still very defective in 1827, so that Orfila's statement should arouse less wonder than Jacopi's.

In 1829 Barruel³ asserted that he had accidentally discovered that the addition of concentrated sulphuric acid to a suspected stain caused the evolution of an odour, by which one could tell that the stain was really due to blood, and also could say what species the blood had come from. This story was received by Orfila in good faith,⁴ but in 1853 a relative of Barruel withdrew all claims that had been made for the "test". Yet in 1881 Dragendorff wrote of it, not

¹ ORFILA :—Nouveau mémoire sur le sang, considéré sous le rapport médico-légal. *Journ de chimie méd.* 1827, 365, 413 ; 1828, 105.

² MANDL :—Recherches médico-légales sur le sang. *Gaz. méd. de Paris.* 1842, x, 560.

³ BARRUEL :—Mémoire sur l'existence d'un principe propre à caractériser le sang de l'homme et celui de diverses espèces d'animaux. *Ann. d'hygiène,* 1829, 267.

⁴ ORFILA, BARRUEL and CHEVALIER :—Taches de sang : rapport médico-légal. *Ibid.* 1835, xiv, 349.



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merely as a matter of historic interest,¹ and now I see that Uhlenhuth and Weidanz² seem to think that there is really something to be said for it, but that its merits depend on the observer's keen sense of smell, and therefore do not warrant its use in medico-legal practice. So slowly does an erroneous observation die in medico-legal circles, the reason being, I think, that the *legal* type of mind, as distinguished from the *medical* type, is prone to accept, unquestioning, the statements of authority.

In 1862 Erpenbeck³ reported that the mere heating of a blood-stain to near charring point in a test-tube would enable one to fix the source of the blood that had caused it, by the odour evolved; but here, too, careful investigation showed the utter worthlessness of the "test".

As it appeared that Chemistry was powerless to aid the medico-legists in their endeavour to fix the source of blood-stains, they had recourse to the microscope, technique having become much improved; and at one time it seemed as if careful micrometric measurements were all that one needed to be able to distinguish with certainty between human and other mammalian red blood-corpuscles. Soon, however, doubts were expressed, by so great an authority as Virchow,⁴ as to the possibility of fixing the drying coefficient of the cells. Further observation seemed to set these doubts at rest: Virchow in 1875 published a report by Malinin⁵ of the accuracy of micrometric differentiation of blood-cells. Richardson in 1874⁶ was loud in his praise of this method of diagnosis; but he had to admit⁷ that he "was aware of the impossibility of distinguishing the blood of a man from that of a dog, but refrained from giving prominence to these facts, lest an improper use should be made of them in the defence of criminals". A strange and, I think, almost unique instance of a man of science having his soul vexed by thoughts of the possible effect of his discoveries.

¹ DRAGENDORFF:—Untersuchung von Blutflecken. in Maschkas Handbuch der ger. Medicin Tübingen, 1881-82.

² UHLENHUTH and WEIDANZ:—Praktische Anleitung zur Ausführung des biologischen Eiweissdifferenzierungsverfahrens mit besonderer Berücksichtigung der forensischen Blut- und Fleischuntersuchung, sowie der Gewinnung präzipitierender Sera. *Fenz.* 1909.

³ ERPENBECK:—Ueber chemische und microscopische Untersuchung auf Blutspuren in Criminalfällen. *Vierteljahrschr. f. ger. Med.* 1862, xxi, 15, 250.

⁴ VIRCHOW:—Ueber die forensische Untersuchung von trockenen Blutflecken. *Arch. f. path. Anat.* 1857, xii, 334.

⁵ MALININ:—Ueber die Erkennung des menschlichen und thierischen Blutes in trockenen Flecken in gerichtlich-medicinischer Beziehung. *Ibid.* 1875, lxv, 528.

⁶ RICHARDSON:—On the value of high powers in the diagnosis of blood-stains. *Amer. Journ. med. sci.* 1874, 68, 102.

Note on the diagnosis of blood-stains. *Monthly microscop. Journ.* 1875, xiii, 213.

⁷ WOODWARD:—On the similarity between the red blood-corpuscles of man and those of certain other mammals, especially the dog—considered in connexion with the diagnosis of blood-stains in criminal cases. *Ibid.* 1875, xiii, 65.

If the reader will take the trouble to refer to pages 53 and 55 of my monograph on blood-stains¹ he will see there elaborate tables of the measurements of the various animals' blood-cells, as estimated by the leading authorities, and he will note that, although these measurements are on the whole very near each other, yet they are only average measurements: the highest and the lowest for any given animal showing as great difference as exists between the average for that animal and the average for another species. He will observe, too, that the measurements are fixed for *fresh* cells. In a blood-stain the cells are dried and shrunken, and one cannot be sure that by treating them with this or that fluid they can be made to regain their original size. Of fresh blood examined by means of the microscope Masson with characteristic candour stated²: "Our experience has shown that, in the case of human blood, its differentiation from the blood of a pig, ox, or cat is easy; from the blood of a dog difficult; from the blood of a rabbit uncertain; and from the blood of a guinea-pig impossible." How much the difficulties are increased by the change of shape and size caused by drying, a little reflexion will show.

Micrometry having been tried in the balance and found wanting, endeavours were made to hit upon some other character of blood that might be of service for differentiation. Elaborate studies of the forms of the crystals of hæmoglobin derived from various animals' bloods were made; but the painstaking work of Michelsson³ showed that for forensic work these differences cannot be utilised. Cotton⁴ thought that the mere change of form caused by drying was a guide in the differentiation of the red blood cells of one species from those of another; but no one has verified his statement as to the differences produced.

Thus, up to the close of the nineteenth century there had not been discovered a sure and certain means of fixing the source of a blood-stain. It was then that the subject of "antisera" and their reactions began to be closely studied. In 1899 Tchistovitch had reported that by carefully treating animals with eel-serum, which is highly poisonous, he had managed not only not to kill them, but even to modify their blood-serum so that when brought in contact with eel-serum it caused a precipitate to form in it.⁵ In 1900 Myers reported⁶ that by treating animals with injections of egg- and serum-albumen he had obtained from them ant sera for these substances, so that the sera of the treated animals precipitated

¹ SUTHERLAND:—Blood-stains, their detection, and the determination of their source. *London*, 1907.

² MASSON:—De l'origine du sang en médecine légale. *Ann. d'hygiène*. 1885, 385, 530.

³ MICHELSSON:—Die Differentialdiagnose von Menschen- und Tierblut in der forensischen Praxis. *Blätter f. ger. Med.* 1906, 9, 116, 204, 285, 381.

⁴ COTTON:—*Bull. gén. de thérap.* 1901, cxli, 334.

⁵ TCHISTOVITCH:—Études sur l'immunisation contre le sérum d'anguilles. *Ann. Inst. Pasteur*. 1899, 406.

⁶ MYERS:—On immunity against proteids. *Lancet*, 1900, ii, 98.

solutions of these albumins. Uhlenhuth reported to the same effect¹ and then he, and—independently of him—Wassermann and Schütze reported that these facts could be turned to medico-legal use.²

The first experiments on precipitating antisera that were made in India were carried out in 1901 by Dr. E. H. Hankin, who reported them in the Annual Report of the Chemical Examiner, Agra, for that year.

In 1904 Marx and Ehrnrooth thought that they had shown that the agglutination of the blood-cells of one species by the blood-serum of another could be utilised as a preliminary test.³ Uhlenhuth is still of opinion that this is the case; I regret that my experiments, detailed in my monograph on blood-stains, have convinced me that this test is of no forensic value.

In 1900 Deutsch had proposed that the hæmolysins should be utilised as a guide, but for the method devised by him one needed intact blood-cells, and these are none too common in blood-stains, so his method met with no acceptance.⁴ In 1905 Neisser and Sachs proposed another method of utilising the hæmolysins, and this has been much used all over the world as a confirmation of the results obtained by means of the precipitin test.⁵

In 1909 Thomsen reported that the phenomena that are known as those of Anaphylaxis, and occur when certain animals (preferably guinea-pigs) receive, after some weeks' interval, a second dose of the blood of another species might be utilised in differentiating the blood of one animal from that of other species not nearly allied to it.⁶

Regarding these two tests I shall have a few words to say later; most of what follows concerns the precipitin test. The agglutination test I have not gone into since my previous work showed that it is not of value; but I am bound to say that the agglutinins have again come to the fore as a means of differentiating the blood of one individual from that of another of the same species.⁷

¹ UHLENHUTH:—Neuer Beitrag zum spezifischen Nachweis von Eiereiweiss auf biologischem Wege. *Deutsche med. Wochenschr.* 1900, 734.

Eine Methode zur Unterscheidung der verschiedenen Blutarten, im besonderen zum differential-diagnostischen Nachweise des Menschenblutes. *Ibid.* 1901, 82.

² WASSERMANN and SCHÜTZE:—Ueber eine neue forensische Methode zur Unterscheidung von Menschen- und Säugetierblüt. *Berliner klin. Wochenschr.* 1901, 187.

³ MARX and EHRNROOTH:—Eine einfache Methode zur forensischen Unterscheidung von Menschen- und Säugetierblut. *Münchener med. Wochenschr.* 1914, 293, 696.

⁴ DEUTSCH:—Die forensische Serum-Diagnose des Blutes. *Centr. Bl. f. Bakt.* 1901 xxix, 661.

⁵ NEISSER and SACHS:—Ein Verfahren zum forensischen Nachweis der Herkunft des Blutes. *Berliner klin. Wochenschr.* 1905, 1388; 1906, 67.

⁶ THOMSEN:—Om Serumanafylaxiens Specificitet, og om Muligheden af at anvende denne i den mediko-forensiske Praxis til Adskillelse mellem Menneske-og Dyrblod. *Hospitalstidende.* 1909, 177.

⁷ v. DÜNGERN and HIRSCHFELD:—Ueber eine Methode, das Blut verschiedener Menschen serologisch zu unterscheiden. *Münchener med. Wochenschr.* 1910, 741.

Whether their supposed value for this purpose will stand the test of time remains to be seen; I have grave doubts on the subject.

II.—The Precipitin Test.

When we inject into an animal of species A. the blood, or blood-serum of another, not too closely allied, species B, then, in time, if the animal be immunisable—all animals of a species are unfortunately not so—we shall cause its blood to be so profoundly modified that, when we bring its blood-serum in contact with a dilution, and even a very high dilution, of the blood of species B, there is formed a precipitate. It is of no moment, from the medico-forensic point of view, whether the surface-tension of the colloid particles in suspension in the diluted blood is altered, or their electric charge is changed in kind, as the final result of the contact of the antiserum (the modified blood-serum of the immunised animal). The outstanding fact is that a precipitate is formed. Nuttall's very careful work¹ has shown that, although we have a "mammalian reaction" when *any* anti-mammalian serum is brought in contact with *any* mammalian blood-dilution, if time be given for the reaction to take place, and the dilution be not a high one, yet if the time- and dilution-limits fixed by Uhlenhuth² be observed, the expert has no trouble in distinguishing between specific and non-specific reactions: for the latter² are entirely eliminated. The mammalian reaction, which is of the highest importance to the biologist, is therefore no hindrance to the work of the medico-legist. The limits fixed by Uhlenhuth are these:—the dilution of the blood to be examined shall be at least a thousand-fold dilution, and the reaction of this dilute blood shall be evident within twenty minutes. Repeated observations by many competent workers have shown that these limits are all that are needed to give sure value to the test.

It was soon evident that dried blood-stains, on being treated with 0.85 per cent. salt solution gave a fluid which was to all intents and purposes a dilute blood, and reacted in a similar way. At first it was thought that the colour-index of the solution was a good measure of the height of its dilution, but Nuttall showed that it is not so to nearly the same extent as is the froth-index. When a solution of albumin is shaken, or has air blown through it, the amount of froth produced and its permanency vary directly as the strength of the solution. With a little practice one is able to treat an old dried blood-stain so as to obtain the required dilution of its extract.

¹ NUTTALL:—Blood relationship and blood immunity. *Cambridge*, 1904.

² UHLENHUTH and BEUMER:—Praktische Anleitung zur gerichtärztlichen Blutuntersuchung mittelst der biologischen Methode. *Zschr. f. Med.-Beamte*, 1903, pp. 185, 229.

Like all other tests used in forensic medical work the precipitin test has its limitations, but unlike most tests—*e.g.* van Deen's guaiacum test—its limitations are but few. The age of the stain, and the material stained have no influence on the results obtainable if the extract of the stain be rendered nearly neutral, as Graham-Smith and Sanger first pointed out,¹ and Vincent also observed.² Biondi showed that the material is of no moment,³ and Ponzio⁴ proved that even the blood of poisoned animals gives its normal reaction. Nuttall, Uhlenhuth and Biondi showed that it makes no difference whether the blood tested be decomposed or not—a matter of importance to workers in the tropics—and Ferrai,⁵ Mirto⁶ and Biondi showed that heat, even as high as 120°C. (248°F.) does not so alter a blood-stain that its extract ceases to give the reaction, when brought in contact with the appropriate antiserum. Biondi too first pointed out that the stains made when blood-sucking insects are crushed react according to the animal on which the insect has been feeding, an observation, confirmed by Uhlenhuth and by myself, which is of value for work in the tropics.

The few substances that cause an alteration of the constituents of a blood-stain so profound that its extract, if obtained, will not give the characteristic reaction are these:—mineral acids; corrosive sublimate; chloride of lime; sulphates of copper and iron; bisulphides of carbon and sodium; nitrate of silver; thymol; and permanganate of potassium. The extract in these cases will not react at all. It is not that it will give a positive reaction with some antiserum other than the appropriate one, consequently one source of error is even here eliminated.

I have been at some pains, here and in Europe, to verify the above-mentioned facts, and have been able to confirm them in all particulars.

So far as my results go, stains of 27 months' age in India give a good extract when treated with saline solution—those which do not do so readily respond to treatment with cyanide of potassium, the resulting extract having its excessive alkalinity corrected by the addition of tartaric acid,⁷ as Ziemke first recommended.⁷

¹ GRAHAM-SMITH and SANGER:—The biological or precipitin test for blood, considered mainly from its medico-legal aspect. *Journ. of Hygiene*, 1903, p. 258.

² VINCENT:—Le diagnostic médico-légal. Application de la méthode biologique. *Ann. d'hygiène*, 1904, p. 44.

³ BIONDI:—Beitrag zum Studium der biologischen Methode für die spezifische Diagnose des Blutes. *Vierteljahrschr. f. ger. Med.* 1902, Suppl. p. 1.

⁴ PONZIO:—La reazione biologica per la diagnosi specifica del sangue. *Gazz. med. siciliana*, 1904, No. 23.

⁵ FERRAI:—Sulla diagnosi specifica del sangue col metodo biologico in medicina legale. *Boll. R. accad. di med. di Genova*. 1901, p. 272.

⁶ MIRTO:—Sul valore del metodo biologico per la diagnosi specifica del sangue nelle varie contingenze della pratica medico-legale. *Riforma med.* 1901, pp. 855, 866.

⁷ ZIEMKE:—Zur Unterscheidung von Menschen- und Tierblut mit Hilfe eines spezifischen Serums *Deutsche med. Wochenschr.* 1901, 424, 731.

Here blood-stains are of necessity exposed to a higher temperature and brighter sunlight than in Europe, but even stains that were exposed for three weeks to the summer air and light, when treated with saline solution, soon yielded satisfactory extracts, and it is not likely that the stains in a criminal case here would be subjected to the influence of the weather for so long a period as three weeks. The reader does not need to be told that when blood-stains have been thoroughly washed out of the stained material, no satisfactory extract can then be obtained by treating the material.

Later I shall give fuller information as to the process and results of immunisation; here I would mention the animals that I worked with. *Rabbits*, which are the animals of choice in European and American laboratories, I worked with although they are neither cheap nor easy to get in the Calcutta market; *fowls* are cheap and plentiful, and, as my previous work had led me to believe that they are good sources of antisera, I used a number of fowls in my experiments; *geese* I tried, encouraged by the results of my work with fowls, but, probably because I did not push the doses of antigen—the substance injected, for which it is desired to obtain an antiserum—I did not obtain a satisfactory result from those that were treated; *goats* have been found to be unsatisfactory in Europe, but nevertheless I tried them here, as, if by any chance I managed to immunise a goat to a high degree, I would have a large stock of antiserum at my disposal; *monkeys* I tried, although I had previously failed to attain anything like the success reported by Uhlenhuth.¹ With fowls and rabbits I obtained satisfactory results; with goats and monkeys I had only failures.

The antihuman sera that I obtained reacted very well, but, as is the case in Europe, the antiruminant sera were only to a certain extent specific: with goat's, sheep's, buffalo's and ox' blood they gave reactions which differed only in degree. For more exact differentiation many expedients have been suggested as of service when an antiruminant serum has reacted with the extract of the suspected blood-stain. Hamburger² has recommended that the stain-extract be divided into several portions, each of which is then treated with a different antiruminant serum: that antiserum with which the most rapid and most marked reaction is obtained gives us the clue. Thus, if we have a stain, which on being extracted gives a reaction with an antiruminant serum, we treat separate portions of the extract with anti-ovine, -bovine, -hircine, etc., serum, and observe in which tube the reaction is most marked and rapid. That Hamburger's method of differentiation is of the highest value, I am convinced: the results of the examination of stains

¹ UHLENHUTH:—Ein Verfahren zur biologischen Unterscheidung von Blut verwandter Tiere. *Deutsche med. Wochenschr.* 1905, 1673.

² HAMBURGER:—Gerechtig onderzoek van bloed en andere lichaamsvochten. *Tijdschr voor strafrecht*, 1904, xvii, 82.

whose origin was unknown to me, but known to the sender, which is given on pages 18-20, shows this. Weichardt¹ had suggested that the "side-action" of an antiserum should be removed, by exhausting its power to cause this, as is done by the absorption-method with such success when allied bacteria are being differentiated. It appears to me that this method of dealing with an antiserum is not suitable for medico-legal work in India: the following experiments go far to show that the modification of the antiserum which is desired cannot always be obtained. An anti-ovine serum reacted well with a 1-1000 dilution of sheep's serum, and faintly with a similar dilution of goat's serum: to 1 c.c. of the antiserum was added 0.07 c.c. of goat's serum, and the mixture, after being thoroughly shaken, was left in the ice-chest overnight, and centrifugalised next morning, the supernatant fluid being decanted and tested. It was found that the reaction-power with goat's serum was no longer present, but the power of reacting with sheep's serum in 1-1000 dilution had also been lost—the modified antiserum was now useless for medico-legal work. Again, an anti-hircine serum that gave a good reaction with a 1-3000 dilution of its antigen, and a less rapid and less marked reaction with a similar dilution of sheep's serum, was treated with sheep's serum in the proportions noted above and shaken, kept on ice, and centrifugalised as described. The resulting fluid was found to be incapable of reacting with a 1-1000 dilution of sheep's serum, it is true, but, as it had also lost its power of reacting with a 1-1000 dilution of its antigen, it had become useless.

Supposing that either of these modified antisera had been stored and used, without preliminary testing of their powers, on the strength of the recommendation made by so competent an observer as Weichardt, then, in a case in which a faint "ruminant reaction" had been obtained one might have been led to conclude that neither goat's nor sheep's blood was present in the stain, when in point of fact either might be present, for all that one could say. *Furare in verba magistri* is a recognised good practice in matters legal; in matters medical it is a fruitful source of error.

The precipitate produced by the inter-reaction of a precipitin-antiserum and its antigen is soluble in excess of either antiserum or antigen, a fact which goes far to prove that the necessary condition of precipitation, besides a certain salt-content of the medium, is a change in the electric charge of the colloid particles held in suspension in the inter-reacting fluids. This fact Dehne has tried to utilise as a confirmation of the results obtained by the precipitin test. He recommended² that, when a precipitate had been obtained, there should be

¹ WEICHARDT.—Der Nachweis individueller Blutdifferenzen. *Hyg. Rundschau*, 1903, xiii, 756. Zur Frage des Nachweises individueller Blutdifferenzen. *Vierteljahrsschr. f. ger. Med.* 1905, xxix, 19.

² DEHNE.—Die spezifische Löslichkeit und ihre Anwendung bei der forensischen Blutuntersuchung. *Münchener med. Wochenschr.* 1907, 357.

added to the contents of the test tube some undilute antigen. If then the precipitate cleared up this would show that it was really a dilution of the antigen with which we had to deal in the first instance.

I have carried out many experiments on this point, and am of opinion that Dehne's suggestion is of no value for Indian medico-legal work. With a dilution of goat's serum a reaction was obtained with anti-ovine serum AO, and also with anti-hircine serum AH: Into one tube were put (1) dilute goat's serum, (2) AO and, after the reaction had occurred, (3) some sheep's serum; into another tube were put (1) dilute goat's serum, (2) AH, and, after the reaction had occurred (3) some goat's serum. Next morning the two tubes, that had been kept in the ice-chest overnight, presented identical appearances.

Again, an antibubaline serum AB reacted well with a high dilution of its antigen, and less markedly with a similar dilution of ox' serum. Into one tube were put (1) AB, (2) dilute buffalo's serum, and, after the reaction had occurred, (3) some buffalo's serum; into another tube were put (1) AB, (2) dilute ox' serum and after the reaction, (3) some ox' serum. The tubes were left in the ice-chest overnight and next morning were found to present identical appearances. No differentiation of kindred sera can be made on the results of these experiments.

Uhlenhuth has laid it down that ape's blood may be mistaken for human blood, so it was of great importance to investigate this point, although, so far as I am aware, it has not yet been alleged in an Indian murder trial that the blood-stains in question had been caused by blood that had come from an ape, and not from a man, as alleged by the prosecution. Two reasons for this occur to me. One is that the Hindus revere the *lungoor* (*semnopithecus entellus*). The other and more generally operative reason is that, even in India, the ape is not a domestic animal, and therefore is not at hand when most needed—by the defence: for Cicero's advice *causam mendaciunculis adspargere* is scarcely needed here in the East.

Through the kindness of the Superintendent of the Calcutta Zoological Garden, and the Principal Medical Officer of the Royal Colonial Army of the Netherlands-Indies, I have been able to examine blood-stains due to the blood of the following varieties of the apes of the Old World:—

- Orang utan*—*Simia satyrus*,
- Hooluck*—*Hylobates niger*,
- Siamang*—*Hylobates syndactylus*,
- Lungoor*—*Semnopithecus entellus*,
- Bonnet monkey*—*Semnopithecus mitratus*,
- Simpai*—*Semnopithecus melalophus*.
- Macaque*—*Macacus cynomolgus*.
- Pig-tailed monkey*—*Macacus nemestrinus*.
- Slender loris*—*Nycticebus tardigradus*.
- Rhesus monkey*—*Macacus rhesus*.

The stains were extracted in the usual way, and the extracts were diluted until they corresponded to 1-1000 dilutions of blood-sera. They were then all tested in a row, with many tubes of 1-1000 dilutions of human sera and stain-extract as controls. The rapidity and degree of reaction obtained in each tube were noted, when to its contents was added some antihuman serum of known specificity and potency. It was found that even in the case of the orang's blood-stain the reaction obtained was not "human", as it was not visible till after the expiry of twenty minutes; the human blood-stain extracts and dilute sera in each case reacted well before five minutes had elapsed. These experiments were repeated over and over again, the results being shown to those who did me the honour of visiting the laboratory, and in no case was a "human" reaction obtained with the extract of a simian blood-stain.

Uhlenhuth has had considerable success in the immunisation of monkeys with human blood, and in thus obtaining antihuman simian sera which, he says, react with human, but not with simian blood-dilutions, being thus of considerable value for differentiation¹. I have tried to immunise several monkeys, but with no success, which is somewhat strange, seeing that India is the home of the Rhesus, and that there was no fault in my technique, as I learned from correspondence with the distinguished originator of "cross-immunisation". The tabular statements given hereunder will show what was done with monkeys. I am in a measure consoled for this want of success by the results of the work, described above, on the differentiation of simian from human blood.

There remained the interesting question as to whether, by means of the precipitin test, one could differentiate the blood of one race of mankind from that of another. By the kindness of the undermentioned gentlemen I was able to experiment with the blood of the races mentioned:—

*Sent by Prof. Surveyor of the Grant Medical College, Bombay:—*Parsis, Gujaratis, Iranis, Goanese, Marathas of low caste.

*Sent by Dr. Leask, Singapore:—*Chinese, Japanese, Malays.

*Sent by Dr. Denier of the Pasteur Institute of Indo-China:—*Annamites.

*Received from the Eden Hospital, Calcutta, by the courtesy of Lieut.-Colonel C. R. M. Green, I.M.S.—*Europeans, Eurasians, Bengalis, Jews.

In all cases the serum of the race represented was diluted 1,000-fold and treated with antihuman sera obtained by injecting into fowls the sera of the various races mentioned as furnished by the Eden Hospital.

The results obtained were in all cases so similar that no difference of any medico-legal importance could be said to exist.

¹ UHLENHUTH:—Ein Verfahren zur biologischen Unterscheidung von Blut verwandter Tiere. *Deutsche med. Wochenschr.* 1905, 1673.

Where no racial difference was detected by the precipitin test, it would have been futile to seek for individual or sexual differences.

In order to test the medico-legal value of precipitating antisera in Indian practice it was necessary to have at my command antisera for the bloods of the following animals:—man, horse, dog, cat, sheep, goat, ox, buffalo and pig: the camel might be classed as a domestic animal of Asia, but its red blood-corpuscles differ from those of the other mammals by their shape, and therefore it was not necessary to have an antiserum for its blood.

From the Eden Hospital I got all the human placental blood that I needed, and from the Bengal Veterinary College, through the kind offices of Colonel F. Raymond, A.V.S., I got most of the mammalian bloods that I required. Although the air of Calcutta, being warm and very moist, is highly laden with bacteria, and has been condemned by Cunningham and Rogers as a great hindrance to bacteriological work, yet the nursing staff of the Eden Hospital, to whom in the nature of things the collection of placental blood had to be entrusted, were able by unremitting care to send me blood of which only a few specimens had undergone bacterial contamination.

From the blood-clot the serum was collected in the manner described by me elsewhere, and kept in the ice-chest. The ordinary type of American refrigerator will, I found, keep up a temperature of $3\cdot5\text{--}4\cdot5^{\circ}\text{C}$. ($38\cdot3\text{--}40\cdot1^{\circ}\text{F}$.) in the lower of its compartments, if the shelf between this and the upper compartment be perforated to permit the cooled air to descend. For the conservation of the antisera in full potency I tried various devices. The "dry method", by which the serum is dried over sulphuric acid or in the thermostat, and then sealed in glass tubes, which may contain an atmosphere of hydrogen, is a method which has been found to work well in the case of antitoxic sera. On being re-dissolved the dried serum yields an opalescent solution, and for this reason is of no use for forensic work with precipitin antisera. The "wet method," whereby an antiseptic is added to the antiserum which is then sealed in glass tubes and kept in the cold and dark, was at one time highly praised by Uhlenhuth; but I agree with Nuttall in thinking that the antiseptic can well be left out, if sufficient care is taken to keep the antiserum frozen in the dark until it is needed. This is the method that Ehrlich proposed years ago and no efficient substitute has been devised since then. Undoubtedly for Indian forensic work it is the method *κατ' ἐξοχήν*. Exposure to light and warmth very soon renders antisera of high potency quite inert: exposure to warmth alone, or light alone, acts in a similar way but with less rapidity. Of this I had proof in several cases in which I divided an antiserum into three portions, after titrating it with known dilutions of its antigen in order to fix its potency. One portion was kept frozen in the dark, another was kept in a drawer in the laboratory, and a third was kept hanging up in the laboratory exposed to light as well as

warmth. The frozen portion of the antiserum would be found to be as good as ever after many weeks—if time had permitted we might have kept it so for years ; the portion which was exposed to light and warmth would be useless in two or three days, and that protected from light useless in three or four days, whatever high potency the serum might have originally possessed. When I say useless I do not mean only useless from the forensic standpoint, but quite inert.

The thawing of the frozen antisera is apt, if it be carried out hurriedly, to give rise to occasion for grave error : a rapidly-thawed antiserum will look flaky and will cause a precipitate to appear when it is brought in contact with no matter what antigen-dilution. Anti-equine antiserum will under these conditions react with a dilute extract of a human blood-stain. The remedy for this is simple—never to do things in a hurry, and to standardise the antiserum each time it is used for a test, as to its potency and specificity. A slowly-thawed antiserum is clear and sure in its reactions. I am not aware that much stress has been laid on this point by other observers, but I am sure that workers in the tropics require this word of caution.

III.—The Precipitin Test—*continued.*

Uhlenhuth reported¹ that from two Rhesus apes he had obtained anti-human sera of 1-1600 titre, and from three Cercopithecus apes sera of 1-2000 titre. In his latest publication² he writes on this point, "Es muss allerdings hervorgehoben werden, dass diese Antisera wegen der Artverwandtschaft nicht sehr hochwertig waren"; but no one else has obtained antihuman simian sera of anything like this strength, so far as I am aware.

That his luck was great appears from the fact that Professor Sachs of Frankfort on Maine, whose competence as a serologist is well known, has often tried to immunise monkeys with human antigen and as often failed to do so.³ Working in the native country of the *Macacus rhesus*, I have had no success, as the following tabular statement shows :—

No.	Total dose c.c.	Injected.	Treatment lasted days.	Potency of anti-serum obtained.
1	42.5	Subcutaneously . . .	28	1-100.
2	Died of pneumonia after 8th injection.
3	44	Intraperitoneally	24

¹ UHLENHUTH :—Ein Verfahren zur biologischen Unterscheidung von Blut verwandter Tiere. *Deutsche med. Wochenschr.* 1905. 1673.

² UHLENHUTH and WEIDANZ :—Praktische Anleitung zur Ausführung des biologischen Eiweiß-differenzierungsverfahrens u.s.w. *Fena.* 1909.

³ Private communication.

No.	Total dose c.c.	Injected.	Treatment lasted days.	Potency of anti-serum obtained.
4	52	Intraperitoneally	32	1-400.
5	104	"	32	1-50 about.
6	...	"	...	Died after third injection.
7	...	"	...	Died of serum disease after 7th injection.
8	113	"	45
9	52	"	33
10	...	"	...	Died after second injection.
11	164	"	45
12	66.5	Subcutaneously	46
13	79.5	Intraperitoneally	54
14	60	"	47
15	71	" (one dose intraven).	37
16	168	"	35
17	190	"	43
18	141	"	32

It might be argued that some of these apes received far too high doses of the antigen for one to hope for any result from the treatment: but here large doses and prolonged treatment are necessary, and the largest doses were given to big old apes. Frequent titration of their sera showed that the majority had not manufactured any antibodies; but the question of the immunisability of monkeys with the human sera obtainable here is scarcely definitely answered by these results, though the prospects of general success do not appear to be bright.

That the work of the medico-legist here renders it absolutely necessary to have at one's command a sufficient supply of antihuman simian serum, is not apparent in the light of the results obtained by me when testing simian and human blood-stains. Uhlenhuth is of opinion that one should have antihuman simian sera ready for use in cases in which the possibility of the presence of simian blood cannot be excluded. With diffidence I advance the opinion that such antisera

are not necessary, and that without them one can do all that is needed to further the ends of Justice.

Bruck's findings as to the differentiation of the blood of one race from that of another do not agree with mine ¹: he used the complement-deviation test as well. However, as my findings agree with those of such able observers as Linossier and Lemoine ² at Home, and Marshall and Teague ³ in the Far East, I think that the question of racial differences may be considered to be answered in the negative for good.

I have not found that the tubes which were introduced for precipitin work by Uhlenhuth have any advantages over tapering tubes which are used in Frankfort on Maine. These have the merit of being easy to make from a length of glass-tubing, though sometimes one comes across a bad glass here, which frosts as soon as it is heated and is therefore useless for the work. Tube-stands of various shapes have been devised by ingenious workers, but I have found that the ordinary and time-honoured pattern of test-tube stand is all that is required.

The capillary method, devised by Hauser, ⁴ of carrying out the test I have used several times, and found it easy enough to carry out. Carnwath's modification of it ⁵ I have not used, but am of opinion that it has advantages where a very small stain has to be tested. It may in the end supersede Hauser's method.

Here in India the stains that fall to be examined would usually be of fair size. In Europe they pride themselves on testing pin-point-sized stains, which in my opinion is a work of supererogation, when larger stains are present. In a recent note ⁶ I have shown how often cutting weapons are used by murderers in this country, and how the murderer is not content with striking one blow, but makes sure of his victim while he is about it. It will easily be understood that in such cases the victim bleeds freely.

In order to give the reader a good idea of what is meant by a positive reaction I asked Babu Behari Lal Das to draw the appearances in two tubes which I had used in an experiment. In one of these the reaction was positive, in the other negative, an antihuman fowl serum having been added to a dilution of a stain-extract, made by me from a human blood-stain in the one case, and from an

¹ BRUCK:—Die biologische Differenzierung von Affenarten und menschlichen Rassen durch spezifische Blutreaktion. *Berliner klin. Wochenschr.* 1908, 793.

² LISSIER and LEMOINE:—Essai de différenciation des albumines du sérum chez les animaux de même espèce, mais de races différentes. *C. R. Soc. de biol.* 1907, lxii, 4.

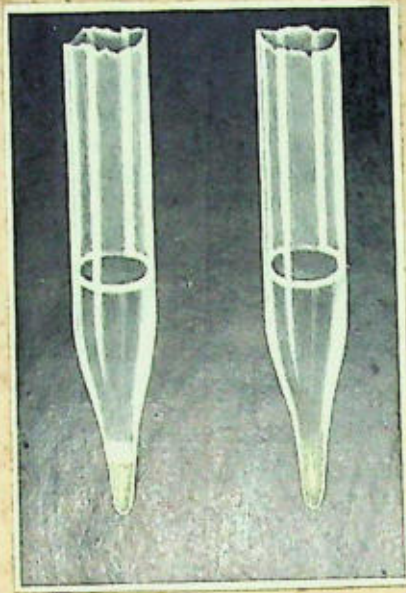
³ MARSHALL and TEAGUE:—A study of the precipitin and complement-fixation reactions. *Philippine Journ. of sci.* November 1908.

⁴ HAUSER:—Ueber einige Erfahrungen bei Anwendung der sero-diagnostischen Methode für gerichtliche Blutuntersuchungen. *Münchener med. Wochenschr.* 1904, 289.

⁵ CARNWATH:—Zur Technik der Untersuchung kleinster Blutspuren. *Arbeiten a. d. kais. Gesundheitsamte*, 1907, 27 Bd. Heft 2.

⁶ SUTHERLAND:—Medico-legal practice in the Mofussil. *Indian med. gaz.* 1910, 47.

ovine blood-stain in the other. The clear colourless appearance of the extracts in the tubes, to the taper bottoms of which the clear straw-coloured antiserum has



B. L. Das *red.*

THE PRECIPITIN-REACTION.

The contrast between the contents of the first tube, in which the antiserum has reacted with the stain-extract dilution, and those of the second, which remain clear at the point of contact of extract and antiserum, is well brought out in this plate.

sunk, is well brought out in the plate. It is not every case that gives such a well-marked reaction as that depicted here, which, however, is fairly typical of what one obtains when using a powerful antiserum and a highly diluted stain-extract.

When we know that our extract is at least diluted to the same strength as a thousand-fold dilution of the blood that caused the stain, or—to speak more accurately—of the serum of that blood, and a reaction does not occur within twenty minutes with the diluted extract, while with a thousand-fold dilution of the antigen of the antiserum that we are using we obtain a marked reaction well within this period, we are justified in concluding that in the stain before us there is not the antigen of our antiserum.

The task of the serologist here is somewhat harder than it is in a temperate climate: the animals require larger doses and more prolonged treatment, and at the end of this more of them will be found to be refractory. About 33 per cent. are either refractory or only slightly immunisable in Europe; here the percentage

of unsatisfactory animals is higher, and the disappointments of the observer more frequent. There a high-potency antiserum which will detect the presence of its antigen in a hundred-thousand-fold dilution—something like a wineglassful in a caskful—is not rarely obtained, and antisera of half this strength are easily enough obtained. Here the only antiserum that approached this strength was one of 1-40000 titre that I obtained from a fowl that had been treated with human (placental) serum. The following extracts from my notes show the difficulties which I met with in Calcutta :—

Animal.	Antigen.	Injected.	Total amount c.c.	Duration in days	Titre of Antiserum.
Fowl 23	Canine . . .	Intraperitoneal .	48.5	29	1-4000
" 24	Human . . .	" . . .	50	28	1-2000
" 25	Cat's serum . . .	" . . .	Died on	20th day . . .	Epizootic.
" 26	Horse's . . .	" . . .	"	23rd " . . .	"
" 27	Dog's . . .	" . . .	46.5 c. c.	29 days . . .	Useless.
" 28	Human . . .	" . . .	41	29 " . . .	1-3000
" 29	" . . .	" . . .	Died on	22nd day . . .	Epizootic.
" 30	Sheep's . . .	" . . .	40.5	23 days . . .	1-3000
" 31	Goat's . . .	" . . .	40	23 " . . .	1-3000
" 88	Human . . .	Intravenous . . .	Died on	8th day . . .	Epizootic.
" 89	" . . .	" . . .	"	9th " . . .	"
" 90	" . . .	" . . .	"	12th " . . .	"
" 91	" . . .	" . . .	11.5 c. c.	24 days . . .	1-12000
" 92	" . . .	" . . .	12	25 " . . .	1-40000
" 93	" . . .	" . . .	Died on	6th day . . .	
" 94	Camel's . . .	" . . .	" "	9th " . . .	
Rabbit 44	Cat's . . .	" . . .	7.75 c. c.	18 days . . .	1-3000
" 45	Dog's . . .	" . . .	8	18 " . . .	less than 1-1000.
" 46	Horse's . . .	" . . .	8.25	18 " . . .	1-2000
" 47	Sheep's . . .	" . . .	6.8	18 " . . .	less than 1-2000.
" 48	Human* . . .	" . . .	11.5	26 " . . .	less than 1-2000.

* Titre showed no improvement between 12th and 26th day.

The epizootic which carried off so many of the fowls—those that had received injections of foreign serum succumbed most easily—was caused by the pasteurella

known as the *bacillus avisepticus*. Dr. G. C. Chatterjee, Assistant Professor of Pathology at the Medical College, kindly treated many of my fowls with a vaccine of killed cultures of the microbe, and in some cases certainly this did good; but there is no doubt in my mind that the plan of campaign against this microbe should be based on rigid quarantine of all fowls bought in the open market in Calcutta, as recommended by Colonel Raymond, who saw and examined many of the fowls. Those fowls that have successfully passed the time-test, isolated from each other in cages, may then be taken to the laboratory and kept there while they undergo treatment with foreign serum.

It may be of interest to record my experience of treating goats with a view to obtain from them precipitin-antisera.

A black goat weighing 14 kilos. received the following doses of hydrocele fluid at three-day intervals—27·5; 30; and 35 c.c.; six days after the last dose of hydrocele fluid it was given 27·5 c.c. of human serum, then, after six days, 29·5 c.c. and after three days 15 c.c. All injections were made into the jugular vein easily and without untoward results. The serum, on being titrated on the tenth day after the last injection, was found to give a faint reaction with a dilution of the antigen, weaker than a thousand-fold. After a month's rest the goat was again treated, receiving at three-and-six-day intervals—30; 35; 33 and 30 c.c. of human serum intravenously. Shortly after the last dose it died of serum disease. A white goat weighing 14 kilos. received intravenously the following quantities of human serum at three-day intervals—30; 30; 34; 30 c.c. Just before its serum fell to be tested it contracted pneumonia and died.

A white goat weighing 13 kilos. received intravenously at three-day intervals 26, 26, and 30 c.c. of human serum. On the seventh day after the last dose its serum was tested and found to be absolutely inert. Eleven days after this it received 35 c.c. and six days thereafter 40 c.c. of human serum. Seven days after the last dose its serum was found to be still quite inert when brought in contact with a 1-100 dilution of human serum.

The following will give an idea of the work done with geese :—

151	Horse serum intraperitoneal and intravenous.	65 c.c. (two courses of treatment)	56 days	useless.
156	Sheep serum intraperitoneal	93	40	"
162	Human serum intravenous	(two courses)	52	"
		32		
163	" " "		died after 4th injection	
164	" " "	30·5 c.c.	49 days	"
165	" " "	31·5	47 "	"
166	" " "	36·5	47 "	"

(One dose intraperitoneal.)

It did not appear to me to be at the time expedient to go on with my attempts to immunise geese; but whether these are or are not refractory is a question to which the above data cannot be taken to give a definite answer. All that we can at present say is that here geese are not easily immunisable.

Hydrocele fluid was first used by Schütze as antigen; here it is very easily obtainable, and therefore it was of importance to test its capabilities as a source of antiserum. Unfortunately I have nothing good to say of it: its albumin-content is far too low for one to hope to be able to immunise animals with it unless one were to use relatively enormous doses, seeing that here the dose of serum as antigen must be somewhat increased.

The intraperitoneal injections were given as described by me elsewhere, as also the intravenous injections in the case of rabbits. For fowls I have found it of service to give four injections, the dose being adapted to the size of the fowl: first the right wing-vein, then the left, then the right cervical vein, and finally the left are the sites of injection. As all who have worked much with fowls are aware, it is not always easy to give an intravenous injection into the wing-vein, and there is much more chance of causing a hæmatoma when doing this than when giving, say, an intravenous injection in a rabbit even if the ear-vein be not chosen as the site. Geese of course are much easier to treat intravenously, but in them too the hæmatoma is almost the rule. I have not found that it entails a bad result, however, in the case of fowls treated with serum.

Ever since Uhlenhuth pointed out the importance of starving one's animals for a few hours before taking their blood for testing its titre, all observers have followed his lead. The beautifully clear serum that one obtains from the blood, allowed to clot in the cold—as described elsewhere—one can then pipette off into small sterile tubes, which are then sealed in the flame, and kept frozen till they are required.

The efficacy of the antisera obtained by me may be judged on perusal of the following tabular statement, which sets forth the results obtained by me when I examined blood-stains of whose origin at the time of examination I was ignorant. As in actual practice, the question that I desired to solve in each case was this: Is this stain due to human blood? If not, to what mammalian blood is it due, assuming that the appropriate antiserum is at hand?—

No.	Stains sent by	Opinion expressed by me after examination of the stain.	Information subsequently furnished by sender.
1.	Dr. C. L. Bose, Rai Bahadur, Assistant Chemical Examiner, Calcutta.	Not human*	Stain on blade of <i>dao</i> , on which human hair was found.
2.	Ditto	Human	Stain on clothes of a man accused of murder.

	Stains sent by	Opinion expressed by me after examination of the stain.	Information subsequently furnished by sender.
3.	Dr. C. L. Bose, Rai Bahadur, Assistant Chemical Examiner, Calcutta.	Human	On clothes of murdered woman.
4.	Ditto	"	On <i>sari</i> of murdered woman.
5.	Ditto	Not human*	Another stain in case of 4.
6.	Ditto	" *	Stain on quilt in case of 4 and 5.
7.	Ditto	Human	Stain on bedding on which corpse was found lying.
8.	Ditto	"	Stain on pillow-case, an exhibit in a murder trial.
9.	Ditto	"	Stain on a sheet in this case.
10.	Ditto	"	Stain on a man's <i>dhoti</i> in this case.
11.	Ditto	"	Stain on the woman's clothes in this case.
12.	Ditto	Not human	Stain made by cow's blood, 27 months old.
13.	Ditto	Human	Human blood-stain 14 months old.
14.	Ditto	Not human	Stain made by blood of pig from Orissa.
15.	Ditto	"	Stain caused by blood of pig from Bengal.
16.	Ditto	"	Stain of Calcutta pig's blood.
17.	Ditto	"	Buffalo's blood-stain.
18.	Ditto	"	Sheep's blood-stain.
19.	Ditto	"	Pillow-case stained with birds' blood.
20.	Ditto	Human	Stained cloth from a case of rape.
21.	Ditto	Not human	Cow's blood-stain.
22.	Ditto	Not human, ruminant—probably buffalo.	Buffalo's blood-stain.
23.	Ditto	Not human, may be sheep or goat or buffalo.	Sheep's blood-stain.
24.	Ditto	Not human	Pig's blood-stain.
25.	Ditto	Human	Human blood-stain.
26.	Ditto	"	Blood-stained garment from a rape case.
27.	Ditto	Human (examined first microscopically).	Blood mixed with feathers.
28.	Ditto	Human	Stain on drawers from a rape case.
29.	Ditto	"	Stain on trousers from a rape case.
30.	Ditto	"	Stain on skirt from a rape case.
31.	Ditto	"	Stain on chemise from a rape case.
32.	Ditto	Not human; cow or buffalo, probably cow.	Stain alleged to be of cow's blood.

* The real origin of this stain was not worked out, antisera for domestic animals' bloods not being at the time available.

No.	Stains sent by	Opinion expressed by me after examination of the stain.	Information subsequently furnished by sender.
33.	Dr. C. L. Bose, Rai Bahadur, Assistant Chemical Examiner, Calcutta.	Not human; cow or buffalo, probably cow.	Another stain in this case.
34.	Lieut.-Colonel Vaughan, I.M.S., Calcutta.	Human; not feline nor ovine.	Stain on paper that had been rubbed on cut muscle of dissected corpse.
35.	Ditto	Human	Cloth soaked in blood of putrefying corpse.
36.	Dr. E. H. Hankin, Chemical Examiner, Agra.	Not human; ruminant.	Stain from case in which a man was accused of having stolen and killed a goat.
37.	Dr. C. L. Bose	Human	Stain on coat of accused man.
38.	Ditto	"	Stain from bludgeon used by this man.
39.	Ditto	Not human	Stains stated by police to be probably of cow's blood.
40.	Lieut.-Colonel Nott, I.M.S., Howrah.	Not human nor ovine nor equine.	Stain of dog's blood.
41.	Dr. C. L. Bose	Human	Stain from a cot on which an injured man had lain.
42.	Captain F. P. Connor, I.M.S., Calcutta.	"	Stain from operation case.
43.	Ditto	Not human	Dog's blood-stain.
44.	Ditto	"	Stain of bird's blood.
45.	Ditto	Human	Human blood-stain.
46.	Captain H. B. Steen, I.M.S., Calcutta.	Not human	Stain made by raw beef-juice.
47.	Ditto	Human	Human blood-stain.
48.	Ditto	Not human	Bird's blood-stain.
49.	Ditto	"	"
50.	Ditto	"	Sheep's blood-stain.
51.	Ditto	Human	Human blood-stain.

The accuracy of the diagnosis made by me in each case shows that the antisera that I employed were of excellent quality, and that the technique of the examinations left nothing to be desired.

In most of the above-detailed cases, which were taken from actual medico-forensic practice, the Court before which the case was being tried would have been aided in coming to a decision by the information afforded by the precipitin test. In no other way could it be put beyond a doubt that the stains in question had, or had not, been caused by human blood.

Here, where advocacy knows no limits, it might be alleged that in a laboratory in which blood-stains of all kinds of animals, including man, are examined, the air might become so vitiated by minute particles of stain that the results obtained by means of the test could not be relied upon.

Nuttall long ago pointed out that when blood-stains that have not become completely dried are in close contact one with the other, their extracts may give fallacious reactions, by reason of the contamination induced by close contact. But stains that come up for examination are dry, and such, if left in close contact with other stains for months, do not become in any way modified by contact.

It is conceivable—and it is a fact—that human sweat, when it comes in contact with, say, a blood-stain due to horse's blood, might modify the extract of that stain so that it would give the human reaction: but, and it is a big but—when the stain extract is diluted up to the proper limits no such interference with the normal reaction of its constituents can occur: countless experiments by many observers have set this question at rest for good. If the extract were only a 1-20 dilution then errors might creep in; it must be fifty times as weak as this before it is tested in forensic practice.

IV.—The Complement-deviation Test.

Neisser and Sachs, who introduced this test, considered¹ that it may be profitably used as a confirmation of the results obtained by means of the precipitin test. Uhlenhuth, however, has all along been of opinion² that if the results yielded by the precipitin test are not confirmed by those obtained when the complement-deviation test is carried out, they should nevertheless be accepted. He found that he obtained deviation of the complement, and consequently a "positive reaction" when he examined by means of antihuman serum the following articles, taken from the collection in his laboratory:—jute-sacking; military foot-bandages, new and old; hemp-dust; earth; gravel; bark; hay; straw; bread; leather; hair of various kinds; woollen stockings; linen; various kinds of trouserings; felt hats; coats made of various materials; urine; pepton; pepton-bouillon; various undiluted sera. Neisser and Sachs replied that boiling of the extract would be found to simplify the test: if in one set of tubes unboiled, and in another boiled extract were tested, then if complement-deviation occurred in both it must be due not to the presence of the antigen, but to some vitiating influence being present.³ Further they insisted on the extreme delicacy of the reaction being utilised to get rid of disturbing factors: if the unboiled extract be very highly diluted only the specific antigen-reaction will be observed. So far as I can gather Uhlenhuth is not enthusiastic about the merits of the test, but admits that it is of some use.

¹ NEISSER and SACHS:—Ein Verfahren zum forensischen Nachweis der Herkunft des Blutes *Berl. klin. Wochenschr.* 1205, 1388, 1906, 67.

² UHLENHUTH:—Komplementablenkung und Blut-Eiweissdifferenzierung. *Deutsche med. Wochenschr.* 1906, 1244.

³ NEISSER and SACHS:—Bemerkungen zu der Arbeit von Professor Uhlenhuth über Komplementablenkung und Blut-Eiweissdifferenzierung. *Ibid.* 1906, 1580.



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The very delicacy of the test, however, is held to be against it by such competent authorities as Muir and Martin,¹ and Friedberger.² Muir and Martin state that, in their opinion, it is only when one has at one's disposal antisera that are capable of inhibiting hæmolysis when such a small quantity as the one hundred-thousandth of a cubic centimetre of the antigen is present, that one should use the test in medico-forensic practice. Antisera of this very high potency are obtainable with difficulty in Europe. It remained to be seen whether they could be obtained here.

The test is based on the following facts:—If an animal be treated by means of injections of the blood, or—better—of the washed red blood-corpuscles of an animal of another species, it will, if it be immunisable, have its blood so modified that, when its serum is brought in contact with the red blood-cells of the species in question, these become dissolved. If this modified serum, however, be heated to 56° C. for half an hour it loses its power of dissolving these cells, to regain it if there be added to it some *fresh* serum—either that of the animal itself or that of certain other species. The heat had destroyed one of the constituents of the modified serum—the complement, but left unaffected another constituent, the hæmolytic antibodies that had been called into being by the injection of the foreign blood-cells. According as we follow the reasoning of the French or German schools of serology, whose protagonists are Ehrlich and Bordet respectively, we may regard the immune bodies as binders of the complement to the cells, or as rendering the cells liable to its action. In any case, for dissolution of the cells to take place we require complement and immune bodies to be present. We can by experiment fix the minimal dose of the treated animal's serum that is required to dissolve a given quantity of the red corpuscles in a given time: say that 0.001 c.c. of the heated serum of a rabbit, that has been treated with sheep's cells, will just dissolve 1 c.c. of a 5 per cent. suspension of these cells in saline solution within two hours, when to it has been added 0.1 c.c. of fresh guinea-pig's serum. Now, if we put into a test-tube the required quantity of guinea-pig's serum with some precipitin antiserum, and the sheep's cells-suspension, and the heated hæmolytic antiserum in the required quantities, we shall find that hæmolysis occurs—the cells are dissolved. If in another tube, in addition to the above-mentioned materials, we have put some serum of an animal of a species other than that for which the precipitin-antiserum is specific in its reaction, hæmolysis will also occur. But in a third tube, into which there has

¹ MUIR and MARTIN:—On the deviation of complement by a serum and its antiserum, and its relation to the precipitin test. *Journ. of Hygiene*, 1906, 265.

² FRIEDBERGER:—Zur forensischen Eiweissdifferenzierung auf Grund der hæmolytischen Methode mittelst Komplementablenkung nebst Bemerkungen der Bedeutung des Präzipitats für dieses Phänomen. *Deutsche med. Wochenschr.* 1906, 578.

been put the serum of the species for which the precipitin-antiserum is specific (the antigen of this antiserum, in other words) there will be no hæmolysis. The complement (fresh guinea-pig's serum) has in this tube been taken up by the mixture of the precipitin-antiserum and its antigen, and therefore has not been able to act with the immune bodies of the heated hæmolytic antiserum, which without it are inert.

The reader who desires further—and perhaps clearer—information on this point is referred to my monograph, in which I have endeavoured to set forth the various processes involved in as simple language as the highly technical nature of the subject allows one to use.

The great interest of the phenomena described lies in the fact that it is the presence of the antigen of the precipitin-antiserum that causes the complement to be deviated. If we are using an antihuman serum, then we know that its antigen must be present, if hæmolysis does not occur when we have put into the tube twice the minimal dose of the heated hæmolytic serum, and have very highly diluted the extract of the stain that we are testing. Using boiled and unboiled extract in parallel series of tubes we may safely conclude that it is no vitiating influence present in the material stained that has led us astray. If the precipitin test has already indicated the presence of human material in the stain extract we are now quite sure that our complement-deviation test has confirmed this observation.

But in the tropics the animals that are immunisable require large doses of antigen and prolonged treatment, and in the end do not yield antisera of very high potency. I have utterly failed to obtain the high-potency antisera that are, in Muir and Martin's opinion, needed to ensure accuracy. From the work that I had the privilege of doing in Frankfort on Maine I am sure that the high dilution of the stain-extract is a *conditio sine qua non* of accurate work with this test, and this entails the use of a very powerful antiserum. Here the most powerful antiserum that I obtained was able to cause deviation of complement when a ten-thousandth part of a cubic centimetre of its antigen was present, which is only a tenth of the strength demanded by Muir and Martin. And be it noted that this antiserum was the only one that I obtained of this strength—the others varied from 1-5000 upwards.

Here too we have another obstacle in the path that leads to success. For the accurate performance of the test the complement must be standardised: it must be known what is the smallest dose of the fresh guinea-pig's serum that is required to render active the ascertained minimal dose of the heated hæmolytic antiserum. This standardisation of the complement entails its exposure to light and heat, do what one will to avoid these causes of deterioration. Consequently it is hard to tell exactly how much the complement has lost power

between the time of its standardisation and the moment of its being used for the test. Of course, as it is so delicate it would be so used at once when its power had been fixed; but there is always a deterioration to be taken into account.

Naturally I endeavoured to find out whether the process of standardisation could be omitted, and the full power of the complement thus utilised for the test itself. I had no great hopes of success, since in Europe the sera of different guinea-pigs have been found to vary so much, but the point was well worthy of investigation here. The results obtained by me after doing numerous experiments show that, for the attainment of that accuracy which forensic work demands, one must not neglect to standardise the guinea-pig's serum just before using it for the test. The usual dose is 0.1 c. c. and in one case I found that this quantity was of itself able to cause dissolution of the standard quantity of sheep's red cells, while in several cases as much as 0.3 c. c. was required to reactivate the ascertained minimal dose of the hæmolytic serum: in other words, without experiment one cannot say what is the power of any given guinea-pig's serum.

With the comparatively weak antisera which I used the results obtained by me when doing the complement-deviation test were interesting, but by no means conclusive from the forensic point of view, and I much doubt the likelihood of this test ever becoming a matter of regular routine in India. For it to become so we should have to be able to obtain in large quantity very highly potent antisera, and this will, in my opinion, be always the *crux*, even if the problem of satisfactory conservation of the complement be solved.

For the determination of the fact that a patient is syphilitic the very similar test proposed by Wassermann, Bruck and Neisser is of the greatest value, and can, as I have found, be carried out here as well as in Europe. Clinical tests may lead one into error, but the error is easily rectified; in forensic work error must be absolutely excluded.

Even at its best the Neisser-Sachs test is, as its distinguished originators claimed, only confirmatory. I agree with Uhlenhuth in holding that it cannot be held to be corrective of the results yielded by the precipitin test. And that these are accurate here in India the reader will have already gathered.

V.—The Anaphylaxis Test.

The reports made by Rosenau and Anderson¹ led me to believe that the peculiar phenomena of anaphylaxis might well be turned to account in medico-legal practice, but before I was able to do any work on the subject I learned that Thomsen² had already carried out a number of experiments with

¹ ROSENAU and ANDERSON:—Studies upon hypersusceptibility and immunity. *Bull. No. 36 Hyg. Lab. U. S. Pub. H. and Mar. Hosp. Serv.* Further studies upon Anaphylaxis. *Ibid. Bull. No. 45.*

² THOMSEN:—*loc. cit.* page 4 *supra.*

this end in view. Since then the merits of the anaphylaxis test have attracted much attention, but out here it is hard to come by the literature, so I have had to trust to excerpts in the various journals, instead of to the original reports of the observers mentioned in the following lines.

It is now matter of common knowledge that guinea-pigs (and rabbits) are easily supersensitised by an injection of a foreign albumin. The condition does not make its appearance at once, but if after the 12-14th day the animal receives a second injection it will, in the majority of cases, react violently and rapidly.

The supersensitive condition, according to Rosenau and Anderson, who have done a large number of experiments, may last for three years, that is to say, during the whole life of the guinea-pig.¹ It begins after the 10-12th day, if the first injection have been made into the brain, in so small a dose as 0.001 c.c.; for intraperitoneal injection 0.01 c.c. is enough, even out here.

The why of the phenomena caused by the second dose of serum has been much discussed by competent observers. Richet, to whom we owe the term Anaphylaxis, believes² that a new substance is formed in the animal's blood. Rosenau and Anderson call this substance anaphylactin, and, since they found that the blood of a supersensitised animal causes a previously-untreated animal to become at once supersensitised, if the blood be drawn after the period of incubation has elapsed, it would appear that their idea has much in its favour. If the blood be drawn before the incubation period has elapsed, the animal into which it is injected does not become immediately supersensitive to an injection of the original substance.

On the other hand Friedberger believes that it is because there is produced an antiserum for the substance injected, and this combines with the substance at the second injection, forming a combination of antiserum and antigen, that the anaphylaxis occurs: the real state of affairs being an absorption of the complement of the animal's serum by the combination. This view is supported by the fact that Doerr and Russ found when working with dogs—as Friedberger and Hartoch had found in the case of guinea-pigs—that actively-supersensitised animals, when they show symptoms of anaphylaxis, have much less complement in their serum than normal, and passively-supersensitised animals at this time may have entirely lost their complement. Complement is not absolutely necessary for the maintenance of health, if it be true that during the first months of life babies have none in their blood; but it is easily conceivable that when once it is present its sudden diminution, not to speak of its total disappearance, will have a profound effect on the organism.

¹ ROSENAU and ANDERSON:—*Bull.* No. 50.

² RICHEL:—*Physiologie: travaux du laboratoire de M. Charles Richet. Tome vi. Anaphylaxie, alimentation, toxicologie. Paris. 1909, cited in Brit. med. Journ. 1909, i, 1254.*

Tsuru¹ is of opinion that it is because a *foreign* serum has been injected that the complement becomes less: he found that when guinea-pigs receive single doses of dog's or rabbit's serum, their complement becomes markedly decreased in amount, and further he found that the lessening of complement in actively-supersensitised animals is always very slight, as also in passively-supersensitised animals who have received a dose of blood from a sensitised animal of like species. On the other hand, if the sensitised animal be of another species, the loss of complement is marked.

Richet and Friedemann, and also Doerr and Russ, found that the injection of a serum mixed with its antiserum causes symptoms of anaphylaxis to appear. Friedberger² desired to remove the interference of the foreign serum, and to attain this end he allowed sheep's serum to react with anti-ovine serum, derived from a treated rabbit, *in vitro*. The resulting precipitate was most carefully washed, to get rid of the rabbit's and sheep's sera, and digested with fresh guinea-pig's serum, the mixture being then centrifugalised. The clear supernatant liquid was then injected into healthy, untreated, guinea-pigs, who all showed symptoms of anaphylaxis. If, on the other hand, the washed precipitate was digested with heated guinea-pig's serum, which as a consequence of being heated had lost its complement, and then the mixture, after being centrifugalised, was injected into guinea-pigs, these exhibited no signs of anaphylaxis. If these experiments are confirmed we may conclude that Friedberger's original contention is well-founded. In this case the phenomena of precipitation, complement-deviation, and anaphylaxis are all brought into line, as being primarily dependent on the formation in the serum of the treated animal of precipitins. Doerr and Moldovan,³ to judge from the short note on their work to which I have access, have found that where the precipitinogen is used up by the animal, no supersensitisation occurs.

Recently Pfeiffer⁴ has reported that when a guinea-pig has been rendered supersensitive to a given albumin, on receiving the second injection of this it shows, even when all other symptoms of anaphylaxis are absent, a marked lowering of the body-temperature—as much as 6.5°C. This lowering of temperature he and Mita have found to be a constant symptom, and in their opinion it may therefore be taken to be *the* sign when the test is performed in forensic medical practice.

One precaution must always be taken—the substance (serum) injected for the test must be heated to 56°C.—Uhlenhuth recommends 60°C.—for half an hour

¹ TSURU:—Die Komplementabnahme bei der aktiven und passiven Anaphylaxie. *Zeitschr. f. Immunitätsforschung u. exper. Therapie*. 1909. 4 Bd. Heft 5. cit. in *Münchener med. Wochenschr.* 1910, 806.

² FRIEDBERGER:—Eiweissanaphylaxie. *ibidem* cit. in eodem loco.

³ DOERR and MOLDOVAN:—Analyse des Präzipitationsphänomens mit Hilfe der anaphylaktischen Reaktion. *ibidem*. 1910. 5 Bd. Heft 1. cit. in eodem, 1025.

⁴ PFEIFFER:—*Ibidem*. 1909. 4 Bd. Heft 4. cit. in eodem, 705.

before it is used. Foreign unheated sera often cause untoward symptoms when injected into guinea-pigs, and thus the anaphylactic reaction might be simulated, especially in a case in which the test-serum was injected intracardially. Three of my guinea-pigs died soon after receiving comparatively small doses of sheep's serum (unheated). All observers insist on the necessity of preliminary heating of the test-serum, which when used should be warmed to near the body-heat of the guinea-pig. Time and again I have seen my rabbits and fowls suffer apparently severely, when they received a dose of unwarmed foreign serum intravenously, even if it had been previously heated to 56°C . for half an hour. In precipitin work this does not matter much; but it might absolutely mislead one when performing the anaphylaxis test.

So far as I was able to carry out work on the test I found it to be confirmatory of the results obtained by means of the precipitin test. Not all guinea-pigs showed signs of anaphylaxis—I had not then heard of Pfeiffer's work on the temperature-loss of otherwise apparently unaffected animals.

Thomsen, to whom belongs the credit of pointing out the medico-legal value of the phenomena of anaphylaxis, found that the reaction is specific for a group. Working with blood-stains of 2-3 months' age, that had been caused by the blood of man, pigeons, fowls, sheep, goats and monkeys, he found that while a supersensitised guinea-pig would react to a second dose of the substance to which it was supersensitive, or of a similar substance—a humanised guinea-pig would react to human or simian serum—it would not react to a substance far removed from the sensitising substance. Thus a humanised guinea-pig would not react to sheep's or goat's or fowl's serum. The following I take from my own notes:—

Four guinea-pigs supersensitised to human serum, by doses of 0.01 c.c., were given injections of 4 c.c. intraperitoneally after the period of incubation had elapsed: one died after 30 minutes; two were very ill, but recovered, and the fourth was apparently but little, if at all, affected—its temperature was not specially noted.

Two guinea-pigs supersensitised with 0.01 c.c. of human serum received intraperitoneally 4 c.c. of human serum on the 14th day: one died within 25 minutes, the other seemed to have recovered after 30 minutes, but was found dead next morning.

A guinea-pig that had received 3 c.c. of human serum, was on the 14th day treated with 1.5 c.c. of simian serum: it showed no signs of discomfort, but when it received, on the 118th day, 3 c.c. of human serum, it promptly reacted and died.

So far we may conclude that here, as in Europe and America, the condition of supersensitiveness persists for months; that it is a group-specific condition is likely, and that, apart from temperature-lowering, sometimes anaphylactic

symptoms are not elicited in previously sensitised animals is evident. More work requires to be done on this point.

The work done by Uhlenhuth and Haendel¹ shows that the age of the material makes no difference: they obtained the characteristic reaction in animals that had received doses of extracts made from a cow's placenta that was 30 years old; from a frost-bitten limb 53 years old, and from a collection of blood, 14 years old, that was completely decomposed, and, owing to its long exposure to light, had lost the power of reacting, by its extract, to a precipitin-antiserum.

Had one been able to supersensitise one's guinea-pigs with human or other sera, and then, as soon as a stain fell to be tested, to make an extract of the stain and use this as the test-substance, forensic work with the anaphylaxis test would have been rapidly done: a given stain could have been tested by this and the precipitin test on the day of its arrival at the laboratory. Unfortunately we cannot do this. A delay of at least fourteen days is required for the guinea-pigs, that have received injections of the stain-extract, to develop supersensitiveness. For these injections but a small amount of extract is needed, whereas if it be used for the test-dose we need many cubic centimetres of a strong extract, and for this a large stain is required. The cases in which we can use extract for the test-injections are thus few in number, for it appears to be of the utmost importance to sensitise a number of animals with the same stain-extract, or to test them therewith if they have been sensitised with normal serum: at least five animals would be required in a forensic case—three supersensitised with the extract, one untreated, and at least one supersensitised with some other material.

The results obtained, if confirmatory of the results yielded by the precipitin test, would be of value. It is probable that if exact measurements of the temperature of the animals were made, the observer would be able to detect specific reaction even when visible symptoms were slight.

Pfeiffer claims that he obtained excellent results when testing extracts that gave no reaction with a precipitin-antiserum, and, as we have seen, in one such case Uhlenhuth and Haendel obtained a good result. Much more work will have to be done on the test here before it can be said that its results, in the absence of confirmation by the precipitin test, are to be accepted.

VI.—Methods and results.

For carrying out these Biochemical tests in the tropics the first desiderata are apparatus for keeping the collected blood cool until sufficient serum has become separated from the clot, and for freezing the sera and antisera obtained: an ordinary American refrigerator-cabinet suffices for the first mentioned purpose, but a freezing-chamber is required for the second.

¹ UHLENHUTH and HAENDEL: in UHLENHUTH and WEIDANZ: p. 177.

For extensive work, or work in which sera sealed in capillary tubes are used, the "Frigo" apparatus, made by Lautenschläger of Berlin on the designs of Morgenroth, does not answer well, although by its means a very low temperature can be kept up at comparatively small cost, even in Calcutta. The freezing-chamber in this apparatus is small and must be removed from its bed of insulating material every time that the freezing-mixture of crushed ice and salt is renewed—twice daily, as I found. At the Medical College, however, there is a large freezing-chamber, with electric motor and piping for the circulation of the cooling medium,—ammonia. The temperature of the chamber, when once lowered, is kept from rising rapidly by the presence of a large brine tank in its interior. This chamber is all that is needed to make serological work in the tropics feasible, if not so easy as in more temperate climates.

The close proximity of the Eden Hospital to the Medical College is of advantage since the large supplies of placental blood, that are needed to keep up a sufficient stock of antihuman serum, may thus be obtained fresh daily. Undoubtedly clear placental serum is the material of choice for the treatment of animals from which it is desired to obtain antihuman sera. Uhlenhuth at one time, if not now, favoured the drying of blood and the storage of the dried blood, which might then be re-dissolved in saline solution when it was required for injection. This plan works well in Europe, but cannot be easily followed out here: the dried blood, unless air be totally excluded from it, would in a week be full of moulds in Calcutta, and—what is of just as much moment—the drying of the large quantities of blood that would be required would take up a great deal of the time of the observer's staff, and would be a work of supererogation where ice-chests and freezing-chamber are available.

All instruments and apparatus used in the laboratory must be carefully sterilised before use. This work was thoroughly carried out by my assistant, Dr. G. C. Mitra. The salt used for the saline solution must be pure chloride of sodium, and it must be dissolved in distilled water, the solution being boiled each morning to ensure its sterility.

For the preparation of the animals for injections and for the abstraction of blood an elaborate ritual is followed in some European laboratories. As this takes up a lot of time, I endeavoured to simplify the process, and succeeded in cutting it down to a thorough swabbing of the area that was to be the site of operation with pledgets of absorbent cotton-wool soaked in ether. None of the animals showed untoward results from this method of preparation, nor did the blood collected become more easily contaminated than when the directions of Nuttall were followed. In the vicinity of the College the tetanus-germ abounds, but none of my animals developed tetanus, although many were operated upon again and again. The site of the puncture, made when the injection was given, was immediately sealed with flexile collodion.

In my monograph, and in the foregoing pages, I have described all that one needs to know about the way in which the injections were given, and the blood abstracted. I shall now give some extracts from my notes to show how the animals were treated with antigen, and what results were obtained from the plan of treatment adopted.

Fowl 43 received injections of human serum, given intraperitoneally :—

On 12th March	10.5 c.c.
17th "	10 "
22nd "	10 "
27th "	10 "

On the 8th day after the last injection its serum was found to have a titre of 1-2,000 over, *i.e.*, it was more than powerful enough to cause a reaction in a 2,000-fold dilution of human serum within a few minutes, and did not react with any other antigen-dilution.

Rabbit 44: Feline serum intravenously :—

On 13th March	2 c. c.
16th "	2 "
19th "	2 "
22nd "	1.75 "

On the 8th day when its serum was titrated it was found to be 1-3000 over. Rabbit 71 was treated intravenously with ass' serum.

It received on 17th April a dose of 2 c.c.

20th "	2 "
23rd "	2 "
26th "	2 "
4th May	2 "

On the 7th day thereafter its serum was found to have a titre of 1-9000.

Rabbit 72 was treated in the same manner with human serum, receiving on 24th April 2 c.c., on 27th April 2 c.c., on 30th April 2 c.c., and on 6th May 1.5 c.c. Its serum on the 7th day thereafter was found to be inert.

Fowl 78 was treated with human serum as follows :—

On 8th May	1.5 c.c.	subcutaneously.
11th "	1.75 "	intravenously.
14th "	2 "	"
17th "	3 "	"
20th "	3 "	"
26th "	3 "	"

On 4th June its serum was found to have a titre of 1-3000.

Fowl 83 received doses of hircine serum as follows :—

On 12th May	7 c.c.	intraperitoneally.
17th "	7 "	"
22nd "	3.5 "	subcutaneously.
27th "	2.5 "	intravenously.

On the 7th June it yielded a serum of 1-4000 over titre.

Fowl 91 received human serum in the following doses:—

On 1st June 2 c.c. intravenously.

4th	„	2.5	„
7th	„	2.5	„
10th	„	2.5	„
14th	„	2	„

Its serum-titre on the 25th June was 1-12000.

Fowl 135 received ovine serum thus:—On 19th July 2 c.c., on 23rd 1.5 c.c., on 26th 2.5 c.c., and on 30th 2.5 c.c., all intravenously. On 9th August its serum was found to be inert.

Rabbit 144 received intravenous injections of human serum as under:—

On 26th July	1.25	c.c.
29th „	1.25	„
2nd August	1.5	„
6th „	1.5	„

On the 16th August its serum was found to be inert.

Rabbit 34 received intravenous injections of human serum as follows:—

On 10th February	2	c.c.
13th „	2	„
16th „	1.5	„

On 22nd February its serum was found to have a titre of 1-2000, so it was given another injection—2 c.c., and on 1st March the titre had risen to 1-4000.

Rabbit 35 received doses of human serum intravenously as under:—

On 10th February	2	c.c.
13th „	1.5	„
16th „	2	„

Its serum-titre on 22nd February was almost *nil*. It was given an injection of 2 c.c. then and the serum was again tested on 1st March and found to be very weak, its titre being less than 1-500.

Two fowls—139 and 140—received hircine serum intravenously as under:—

139	22nd July	2.5	c.c.	same date	140	2	c.c.
	26th „	2.5	„			2.5	„
	30th „	2.5	„			2.5	„
	3rd August	2.5	„			2.5	„

Titre 13th August 1-4000.

Nil

Rabbit 99 received washed sheep's red blood-corpuscles intravenously as under :—

On 9th June	1.5	c.c.
15th „	2	„
21st „	2	„
26th „	2	„

These amounts represent, of course, the indicated quantities of a suspension of corpuscles in saline solution, the saline being added in the original amount of the serum that it replaced.

On 6th July the serum was tested and found to be capable of causing complete dissolution of the standard amount of sheep's corpuscles—1 c.c. of a 5 per cent. suspension in saline solution within 2 hours at 37° C.—when it had been heated for half an hour at 56° C. and reactivated by the addition of fresh guinea-pig serum. The working hæmolytic dose of this antiserum was 0.085 c.c. of a 1-50 dilution, the minimal hæmolytic dose being 0.0085 c.c.

Rabbit 82 received doses of a normal suspension of red corpuscles intravenously as follows :—On 11th May 3 c.c., 15th 3 c.c., 19th 2 c.c., 24th 2 c.c. It aborted on 30th May, but was not at all ill, so on 4th June it was given 2.5 c.c. and its serum, on being titrated on 18th June, was found to have a minimal hæmolytic dose of 0.002 c.c.

We see, then, that every attempt at immunisation of an animal is to be regarded as an interesting experiment here as in Europe : one cannot predicate of any animal that it will, or will not, yield an antiserum. Even if it be not refractory, one cannot guess what the titre of its serum will be. All that one may with confidence affirm is that if one uses care, is patient, does not strive to hurry matters, and treats a number of animals with human or other serum, one will have one's reward in the shape of a high-potency antiserum from some of these animals, and that the antisera thus obtained will be in every way satisfactory from the point of view of actual forensic medical practice.

There is no royal road in serological work, and the pitfalls in the path of the observer, especially in the tropics are—as I have indicated—many ; but with care, patience, and practice, which is said to make perfect, these may be avoided and the desired goal may be reached at last.

There remains to be said a word as to the most expeditious method of obtaining an answer to the questions that arise when a suspected stain comes to be examined : is this a blood-stain ? and, if so, to what blood is it due ?

Uhlenhuth appears to consider that the guaiacum-test (van Deen's¹) is of considerable value as a negative test. I do not think that it is

¹ van DEEN : Tinctura guajaci und ein Ozonträger, als Reagens auf sehr geringe Blutmengen. *Arch. f. d. holländ. Beitr. z. Nat. u. Heilk.* 1861-64. iii. 228.

worth one's while to use this test, which has so much against it as a positive test—as I have recounted in my monograph—when one has the peroxide of hydrogen test (Zahn's)¹ at one's command. If there be no froth developed by the contact of the reagent with a small fragment of the stain, then one is not inclined to believe that blood is likely to be present, and this belief will be much strengthened if the application, to another fragment of the stain, of the leucomalachite-green (Adlers')² test, or of the hydrazin (Riegler's)³ test, gives a negative result. If either of these tests gives a positive result then the aid of the spectroscope should be sought, the spectrum of hæmochromogen being so easily obtained and so characteristic. In the case in which this spectrum is obtained, the employment of the Teichmann test (hæmin crystals) is not necessary. It remains to fix the form of the blood-corpuscles present in the stain by examining a fragment under the microscope. Then an extract, made in the usual way, is tested with antihuman serum, if we have found in the stain cells which are obviously mammalian red blood corpuscles. In case there be any doubt about this,—it is not every stain made by bird's blood that can be at once diagnosed as such,—the use of the antiserum will help us.

In a series of test-tubes we have :—

Stain-extract diluted to at least 1-1000, a similar dilution of the antigen of our antiserum, a 1-1000 dilution of an extract of a stain known to have been caused by the antigen-blood, similar dilutions of extracts of stains known to have been caused by other bloods, similar dilutions of other antigens, saline solution alone, and finally a tube in which is the diluted extract of the suspected stain to which instead of the antiserum is added the serum of an untreated animal of the species from which the antiserum was taken. If now we have within the time-limit, a reaction in the tubes, treated with antiserum, in which are : stain-extract that is being tested, known human serum dilution, and known human blood-stain extract; but none in any of the other tubes, we have convincing proof that we are dealing with a human blood-stain. The following protocol will perhaps make this clearer :—

Tube	Stain-extract dilute.	Antihuman fowl serum	Reaction.
„ 2,	Human blood-stain extract dilute	„	„
„ 3,	Human serum 1-1000	„	„
„ 4,	Equine serum 1-1000	„	No reaction.
„ 5,	Canine serum 1-1000	„	„

¹ ZAHN: Die Anwendung des Wasserstoffdioxyds zum Nachweis von Blutflecken. *Corr.-Bl. f. schweizer Aerzte* 1871, i, 322.

² ADLER, O. and R.: Ueber das Verhalten gewisser organischen Verbindungen gegenüber Blut, mit besonderer Berücksichtigung des Nachweises von Blut. *Zeitschr. f. physiol. Chemie.* 1904, 59.

³ RIEGLER: Ref. in *Pharm. Cent. Halle* 1905, 355.

Tube 6, Fehne blood-stain extract dilute	Antihuman fowl serum	No reaction.
„ 7, Bovine blood-stain extract dilute	„	„
„ 8, Saline solution	„	„
„ 9, Stain-extract dilute	Normal fowl serum	„

Now if we take some of our stain-extract and inject it into five guinea-pigs and, on the 15th day thereafter, we give to three of these a dose of human serum, to one a dose of, say, equine serum, and to the fifth canine serum; and we take three other untreated guinea-pigs and give them the three kinds of sera mentioned, we shall have a good control of the results of our precipitin test. Supposing that two of the now humanised previously-treated animals show marked symptoms—probably all three will show a fall of temperature—and none of the others are apparently a whit the worse for the injections of serum that they have just received, we have strong confirmatory proof of the correctness of our diagnosis of the origin of the blood-stain in question.

The preliminary fixing of the facts that the stain is due to blood and that this blood is mammalian, would in actual practice be carried out by the Chemical Examiner; the serological tests would have to be carried out at one Central Laboratory in Calcutta.

VII.—Summary.

From what has been said we may draw conclusions that may be set forth briefly as follows:—

1. By means of the peroxide of hydrogen and leucomalachite-green tests we may ascertain whether blood is present in the stain before us, and confirm this observation by employing the spectroscope.
2. Then, by means of the microscope we may ascertain whether the red blood-corpuscles present are those of a mammal.
3. Having arrived at knowledge of their being those of a mammal we can, even in the tropics, tell whether that mammal is man or one of the domestic animals.
4. For this purpose we can apply the *precipitin test*. The antisera required for this test we can obtain by treating fowls and rabbits with the bloods of man and other animals. Our animals need a more prolonged course of treatment and higher doses than is the rule in temperate climates; and more of them will be found to yield worthless antisera. Still, with patience and care we shall have a good supply of the needed antisera, and may obtain these most economically by treating our animals by means of intravenous injections.
5. The antihuman sera will enable us to ascertain whether or not the stain to be examined has been caused by human blood. The bloods of

apes, even of those very closely related to man—*e.g.*, the Orang Utan—may thus safely be distinguished from human blood.

6. The bloods of the various ruminants may be distinguished one from the other by the modification of the test that was introduced by Hamburger.
7. The age of the stain, at least up to 27 months, makes no difference: all stains are fairly easy to extract, and the extract is not affected by the age of the stain.
8. The antisera must be kept frozen and protected from light in order that they may not lose their power.
9. The work of preparing antisera can only be carried out where (1) we have a large freezing chamber; (2) we have a lying-in hospital near the laboratory, as human placental blood is needed, in large quantities and as fresh as possible, for the treatment of our animals: the vital question being that concerning the presence or absence of human blood, and this requiring for its answer large quantities of anti-human serum.
10. It is not likely that antisera of the strength required for the *complement-deviation test* will ever be obtained in sufficient quantity to enable one to carry out this test in Calcutta, as a matter of routine in the case of the very numerous blood-stains that would fall to be examined by the expert. If antisera of proper strength be obtained they could be used to obtain confirmation—not correction—of the information obtained by the use of the precipitin test.
11. The *anaphylaxis test* promises well. More work on it is needed to establish clearly its value in all circumstances and under the most varying conditions, as demanded for forensic medical work. Its use would necessitate a delay of at least fourteen days between the receipt of the suspected blood-stains and the carrying out of the test.
12. The biochemical tests in forensic medical practice must be performed by an expert.

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THE APPLICABILITY TO MEDICO-LEGAL PRACTICE
IN INDIA OF THE BIOCHEMICAL TESTS FOR
THE ORIGIN OF BLOOD-STAINS

BY
LIEUT.-COLONEL W. D. SUTHERLAND, M.B., I.M.S.
(*Edited by the Director-General, Indian Medical Service*)



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