T H E S I S.

The results of experimental work upon the Serological or Precipitin Test for the detection of Blood, considered from the Medico-Legal Aspect.

by

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THE IMPORTANCE OF THE PRECIPITIN TEST IN FORENSIC MEDICINE.

The laboratory examination of blood stains is one of the most important tasks, which a medical jurist is frequently called upon to undertake in the course of his work. The existing chemical tests, supplemented by those which may be carried out with the hand spectroscope, the micro-spectroscope and the microscope itself are such, that a very definite opinion may be given as to whether the substance composing a stain is that of blood. In medico-legal work, however, it is important that a definite opinion be expressed as to whether the blood composing a stain is the blood of a human being, as opposed to that of other animals belonging to the mammalian group. In order to arrive at such an opinion, the Precipitin or Serological Test must be employed. Hauser[1] has stated that "The responsibility "is very great when one makes a forensic blood examina- "tion by the sero-diagnostic method, and can only be "undertaken by those who are thoroughly conversant with "the method, and have at their command all the conditions "necessary for trustworthy work". Graham-Smith and F. Sanger[2] state as the result of their experiments with this test that "These experiments have led us to the "conclusion that with sufficient materials, and due "precaution to exclude the various sources of error, "there are but few conditions met with in forensic "practice under which human could not be readily "differentiated from other bloods. By this, however, "we do not mean to imply that a considerable "acquaintance with the action of precipitating "antisera on blood solutions is not necessary in the "successful application of this test". The test is now officially recognised by the law courts of several countries. There can be no doubt that when an/

an individual expresses an opinion that a blood stain is composed of human blood, he shoulders a very grave responsibility, as his finding may involve the life of an accused person on trial for murder. Experience, faultless technique and patience are therefore essentials before an examiner should express such a view. Having regard to the increasing importance of the test, it seems unfortunate that apart from the work of a few investigators, systematic research upon the medico-legal aspect of this line of examination, has been neglected in the British Isles. Graham-Smith and F. Sanger[2] have made comment on this matter, and have stated that "In this country, however, with the "exception of Nuttall and Grünbaum, none have yet worked "systematically on the subject in any of its aspects, "and in forensic practice it has been completely "neglected". A study of the bibliography from 1900-1924 reveals the fact that few British investigators have written at any length upon the test in relation to medico-legal work. This fact, in conjunction with the opinion of Nuttall[3] which he states at the conclusion of his far reaching work on Blood Immunity and Relationship, and is expressed as follows:- "The "exhaustive treatment which our present knowledge of the "precipitins has received, should prove of use to others, "and I hope that the work done will stimulate many to "further investigate the many problems which present "themselves", stimulated the writer to make investigations into some of the more obscure problems relating to the test in its medico-legal application.

II.

THE EVOLUTION OF THE TEST.

The history of the test is important and is of interest to the research worker. In 1897 Kraus[4] proved that by immunising an animal with injections of a culture of a microbe, a serum could be obtained from that animal, which when added to a filtered culture of this/

this microbe, produced a precipitate in it. Thereafter Bordet showed that if a rabbit be immunised by intraperitoneal injections of a milk subjected to a temperature of 67° centigrade, the rabbit will yield a serum which will precipitate this milk. Gengou[5] stated that the lactoserum produced by a goat, sheep or cow, gave the same reaction on the milk of a goat, sheep or cow, but that a difference was shown when human or mare's milk was tested. Tchistovitch[6], by giving certain animals doses of poisonous eel serum, was able to obtain a specific precipitating antiserum for eel serum. Nuttall[7] carried out a vast number of experiments to determine the degree of specificity of the precipitins. A summary of his experiments will reveal the extent of his work.

Antiserum for	Number of Tests.
Man.	825.
Chimpanzee.	47.
Ourang.	81.
Cercopithecus.	733.
Hedgehog.	383.
Cat.	785.
Reindeer.	69.
Hog-Deer.	699.
Antelope.	686.
Ox.	790.
Sheep.	701.
Horse.	790.
Donkey.	94.
Zebra.	94.
Whale/	

Continued.

Antiserum for	Number of Tests.
Whale.	94.
Wallaby.	691.
Fowl.	792.
Ostrich.	649.
Fowl egg.	789.
Emu egg.	630.
Turtle.	666.
Alligator.	468.
Frog.	551.
Lobster.	450.
Hyaena.	378.
Dog.	777.
Seal.	358.
Pig.	818.
Llama.	363.
Mexican deer.	749.
Total No. of Tests =	16,000.

It would appear from the result of his investigations that an antiserum of a given animal will produce a precipitate in the blood serum obtained from a similar animal or one closely related to it. He also demonstrated that "the degree and rate of blood reaction "appear to offer an index of the degree of blood "relationship, in other words, closely related bloods "react more powerfully (more precipitin) and more "rapidly than do distantly related bloods, provided the "latter/

"latter react at all.

"Like other lines of investigation, this one "appeared relatively simple at first; it is evident, "however, now that the phenomena of precipitation are of "an exceedingly complex nature".

For medico-legal purposes, advantage is taken of the fact, that by injecting an animal, usually a rabbit, with defibrinated blood of an unrelated animal, an antiserum is produced in the rabbit's blood. This antiserum will precipitate the serum of the animal whose blood was injected or of an animal closely related to it. This reaction constitutes the principle of the precipitin test. Uhlenhuth[8] must be credited with the distinction of being the first to publish results of tests of importance in relation to the value of the precipitin test and its possibilities in criminal work. He injected rabbits with human blood and tested the antiserum on nineteen different bloods, finding only a positive precipitin reaction with human blood.

Almost immediately Wassermann[9] demonstrated experiments which he had made with Schütze, similar to those of Uhlenhuth, at the Physiological Society Berlin (1901). He showed that with anti-human serum, a positive reaction was given only with human blood and the blood of the monkey.

From that time repeated experiments have been conducted by many investigators to determine the specificity of the precipitin test. In this connection the names of Schütze, Stern, Ziemke, Nuttall, Graham-Smith and Sanger among others should be mentioned. In 1901 Uhlenhuth[10] published results of experiments with the test, using blood stained articles. He obtained positive reactions with human blood stains upon a stick, sand, cotton, a coat, trousers, a hatchet and other articles. With anti-pig serum he obtained positive reactions with pig blood stains on linen.

Ehrlich[11] and others recognised the specific character/

character of precipitins. The majority of the authorities upon the precipitin test are agreed as to its specificity. Those holding this view are Nuttall, Wassermann, Schütze, Uhlenhuth, Florence, Kraus, Mirto, among many others. Sutherland[12] states that in a private communication, Wilcox expresses his favour of the test. Uhlenhuth in 1901[13] reported that his anti-human serum had identified human blood which had been dried for three months. It is of interest to note the results of his experiments with blood stains, the origins of which were unknown to him. By means of the test he was able to identify human blood upon a bludgeon, when mixed with sand, when imposed upon woollen cloth, the stain in this instance being four years old, and from stains on the fork and opening of trousers. He also tested for the source of putrefied blood of man, horse, ass, ox, sheep, dog, cat, goose, fowl, hare, rabbit, and stag, by employing the various specific precipitating antisera.

III.

THE TECHNIQUE OF THE TEST.

In order to master the technique of the precipitin test and to investigate certain problems in its application, the writer devoted a considerable period of time in the laboratory, studying the test in each of its stages. He fully realised that a careful study of the integral steps in the technique is essential, before an observer is in a position to carry out the test accurately and to appreciate and interpret the results obtained. It is obvious, that before the test can be applied to a stain, the substance composing it must be brought into solution. By steeping the stained material in normal saline, it is easy to obtain such if the stain be composed of blood, provided it has not been fixed by any agent. The writer confined himself exclusively to this solvent throughout/

throughout his tests and found it most efficient. Certain observers have recommended the use of certain chemical solutions to facilitate solution of a stain of old standing, or where the stain had been acted upon by tannic acid as in certain woods or leathers. The writer did not find this necessary.

Nuttall[14] states that he encountered bloods which would not pass into solution even after steeping in saline for 24 hours at 37°C. The blood stains used by the writer did not cause this difficulty even after great heat had been applied to them. Nuttall also found that bloods sent to him from hot countries where they had been dried on filter paper, refused to go into solution with saline.

It is essential that the solution of the stain be as free from haze as possible before applying the antiserum, lest a positive reaction be masked by an existing haze or turbidity. If the solution of the blood stain contains debris, it can readily be cleared by simple filtration or better still by centrifugalisation, the supernatant fluid being withdrawn by a pipette and the debris left at the bottom of the tube. A clear solution of the stain having been obtained, difficulty will be encountered in determining the strength of dilution of the blood serum which has been extracted. Since a drop of blood on an average occupies an area of a ten pfennig piece or a sixpence, a portion of the stain approximating one drop of blood should be excised. This estimation, of course, depends upon whether the blood has been imposed upon thick or thin material, whether or not it is absorbent, and if so, whether very absorbent. Another factor for consideration is whether the blood was in a partially clotted state when applied to the article under examination. The presence of clot may be seen in a stain, and experience will dictate what general allowance either way must be made to obtain an area of stain likely to have been covered by one drop of blood./

blood. When the stains under examination occupy a lesser area than that of a sixpence, an estimation should be made having regard to this rough standard. The determination of the degree of dilution of blood serum is necessary for medico-legal work, as the higher the dilution yielding a positive reaction the more specific is the test. There can be little doubt as to the delicacy of the test. Strangeways[15] has shown that "reactions may take place in dilutions of blood serum even over 1 in 1,000,000, for on adding a constant amount of antiserum to progressive dilutions, differences in the amount of deposit, measurable to the eye, were observable even in these highest dilutions, when the precipitin had been collected in fine capillary tubes".

We must then consider the method whereby the various strengths of the dilutions are effected, a portion of stain the size of a sixpence suspected of being composed of blood having been excised from clothing or other material. The excised stain is placed in six cubic centimetres of normal saline and allowed to steep for a period of twenty four hours. Since each cubic centimetre of saline contains approximately sixteen drops, and since the excised stain contains approximately one drop, the resultant dilution is approximately that of 1 in 100. Having obtained this dilution, it is simple to obtain dilutions of 1:1,000, 1:5,000, 1:10,000, and 1:20,000 which are necessary in the medico-legal application of the test. Such dilutions were used by the writer in most of his experiments. It is regrettable that there is at the present time, no method of obtaining an exact dilution of blood serum from a stain.

IV.

THE FOAM TEST AND COLOUR TEST IN RELATION TO THE ESTIMATION OF THE STRENGTH OF THE DILUTION OF BLOOD SERUM.

An extract of a stain having been effected, an indication as to whether blood serum is or is not present may be obtained by the application of the simple foam test./

test. Uhlenhuth was the first to mention this test, and Nuttall[16] favours its use. The test consists in blowing air into the solution by means of a fine pipette, when if serum is present a more or less persistent foam is produced. The greater the amount of serum present in the solution under test, the greater will be the amount of foam produced and the longer its persistence within limits. Nuttall states that as the result of experience he can estimate approximately the strength of the dilution. Below are the results of experiments conducted upon the foam test by the writer, who was investigating the importance of the test in relation to the accuracy of estimation of the strength of the dilution, by the amount of foam given and its duration. Blood stains composed of foetal blood dried upon filter paper for three months were used for the tests. The stain was extracted by saline in one and a half hours. Each test tube contained three cubic centimetres of each dilution. The foam test was applied by means of blowing through the solution with a pipette. The degree of blowing was uniform and lasted for a timed period of five seconds in each case.

Strength of Dilution.	Nature of Foam.	Blowing commenced.	Disappearance of Foam.	Duration of Foam.
1:50.	Copious large bubbled foam.	11.35 a.m.	12 p.m.	25 mins.
1:100.	"	"	Not recorded.	-
1:200.	"	"	2.30 p.m.	175 mins.
1:300.	"	"	12.10 p.m.	35 mins.
1:400.	"	"	11.55 a.m.	20 mins.
1:500.	Less copious large bubbled foam.	11.35½ a.m.	12.30 p.m.	54½ mins.
1:600.	Definite foam.	"	2 p.m.	144½ mins.
1:700.	"	"	11.40 a.m.	4½ mins.
1:800.	"	"	1.45 p.m.	129½ mins.
1:900.	"	"	12.30 p.m.	54½ mins.
1:1,000.	"	"	-	-

In/

In the dilutions 1:2,000, 1:3,000, 1:4,000 1:5,000, 1:6,000, 1:7,000, 1:8,000, 1:9,000, 1:10,000, 1:11,000, 1:12,000, 1:13,000, 1:14,000, 1:15,000, 1:16,000, 1:17,000, 1:18,000, 1:19,000, and 1:20,000 the foam produced was transitory and therefore its duration was useless from a practical point of view. The results of these experiments show that the duration of foam produced by applying this test is valueless as a means of estimating the strength of dilutions under test. The nature of the foam produced gave no reliable indication of the strength of the blood serum present. Dilutions of 1:50 to 1:400 all yielded a copious large bubbled foam. The duration of the foam also failed to indicate the strength of the dilutions. In a dilution of 1:50 the foam persisted for twenty five minutes, whereas a dilution of 1:200 yielded foam which persisted for nearly three hours. In the dilution of 1:600 the foam persisted for nearly two hours and a half. As the result of tests, it was found that the foam produced by shaking the solutions in test tubes gave a reliable indication of the presence of serum in the solution, by the formation of foam. The following tests were made to ascertain whether the extent and duration of the foam so produced would indicate the likely strength of the solution under test.

For this experiment foetal blood shed on filter paper twelve weeks previously was used. The blood was extracted with normal saline after steeping for one and a half hours. Each test tube was filled to a third of its capacity, and was shaken for a period of fifteen seconds, commencing at 10 a.m.

Date of Experiment - 26/2/25.

		Strength of Dilution.	Nature of Foam.	Shaking completed.		Duration of Foam.
Group 1.	1.	1:50.	Copious fine bubbled foam almost filling tube to extent of $\frac{2}{3}$ of remaining space.	10 a.m.	Foam last seen at 5 p.m. 27/2/25. Absent morning of 2/3/25.	31 hrs. +
	2.	1:100.	As above but filling tube to $\frac{1}{2}$ of remaining space.	"	"	31 hrs. +
	3.	1:200.	As above but filling tube to $\frac{1}{3}$ space.	10.1 a.m.	"	31 hrs. +
	4.	1:300.	"	10.2 a.m.	"	31 hrs. +
	5.	1:400.	As above - $1\frac{1}{2}$ cm. foam.	10.3 a.m.	"	31 hrs. +
	6.	1:500.	As above - $1\frac{1}{4}$ cm. foam.	10.4 a.m.	"	31 hrs. +
	7.	1:600.	As above - $1\frac{1}{2}$ cm. foam.	10.5 a.m.	"	31 hrs. +
	8.	1:700.	As above - $1\frac{1}{4}$ cm. foam.	10.7 a.m.	"	31 hrs. +
	9.	1:800.	As above - 1 cm. foam.	10.8 a.m.	Foam present 4.30 p.m. 2/3/25.	96 hrs. +
	10.	1:900.	As above - $1\frac{1}{4}$ cm. foam.	10.9 a.m.	Foam seen 5 p.m. 27/2/25. Absent morning 2/3/25.	31 hrs. +
Group 2.	11.	1:1,000.	As above - 1 cm. foam.	10.15 a.m.	Foam present 4.30 p.m. 2/3/25.	96 hrs. +
	12.	1:2,000.	"	"	"	96 hrs. +
	13.					

Continued.

	Strength of Dilution.	Nature of Foam.	Shaking completed.		Duration of Foam.
13.	1:3,000.	As above - ¾ cm. foam.	10.16 a.m.	Foam present 5 p.m. 27/2/25. Absent morning 2/3/25.	31 hrs. +
14.	1:4,000.	As above - ½ cm. foam.	10.17 a.m.	"	31 hrs. +
15.	1:5,000.	"	10.19 a.m.	4.30 2/3/25.	96 hrs. +
16.	1:6,000.	As above - ¼ cm. foam.	10.20 am	"	96 hrs. +
17.	1:7,000.	"	"	"	96 hrs. +
18.	1:8,000.	"	10.21 a.m.	"	96 hrs. +
19.	1:9,000.	"	"	"	96 hrs. +
20.	1:10,000.	"	10.22 a.m.	"	96 hrs. +
21.	1:11,000.	Layer of foam.	10.23a.m.	Foam disappeared 4 p.m. 26/2/25.	5 hrs. 37 mins.
22.	1:12,000.	Slight layer of foam.	10.24 a.m.	Foam disappeared 1.42 p.m. 26/2/25.	3 hrs. 18 mins.
23.	1:13,000.	"	"	"	3 hrs. 18 mins.
24.	1:14,000.	"	10.25 a.m.	"	3 hrs. 17 mins.
25.	1:15,000.	"	"	Foam absent 1.45 p.m. 26/2/25.	3 hrs. 20 mins.
26.	1:16,000.	"	10.26 a.m.	"	3 hrs. 19 mins.
27.					

Group 2. (Cont.)

Group 3.

Continued.

		Strength of Dilution.	Nature of Foam.	Shaking completed.		Duration of Foam.
Group 3. (Cont.)	27.	1:17,000.	Very slight layer of foam.	10.26 a.m.	Foam absent 11.10 a.m. 26/2/25.	44 mins.
	28.	1:18,000.	"	10.27 a.m.	Foam absent 11.5 a.m. 26/2/25.	38 mins.
	29.	1:19,000.	"	"	Foam absent 11 a.m. 26/2/25.	33 mins.
	30.	1:20,000.	"	10.28 a.m.	Foam absent 10.50 a.m. 26/2/25.	22 mins.

It is to be noted that the dilutions in Group 3 show a very much shorter persistence of foam than in groups 1 and 2. Group 2 exhibits a longer persistence of foam than Group 1. The results of this experiment indicate very definitely that the foam test produced by shaking is also useless to establish with any degree of accuracy the strength of the dilution under test, and that the duration of the foam produced, is such as to render the test valueless from a practical aspect, on account of the period required for observation.

The foam test is undoubtedly of value in indicating whether blood serum is present in the fluid, and by the density and copiousness of the foam so given, an experienced observer is able to ascertain in a very approximate manner the strength of the dilution of blood serum within limits. So far as the writer is aware these experiments have been conducted for the first time.

Uhlenhuth/

Uhlenhuth at first relied upon the colour test, and Ziemke also used this method to estimate the strength of the dilutions. Nuttall is of the opinion that the foam test is more reliable than the colour test and the writer also holds this view. In order to observe the colour range of dilutions of different bloods, and their response to the foam test, the following dilutions were made, examined, and reported upon. All dilutions were made from blood which had been dried on filter paper. The advantages of keeping blood in this manner are that the blood is easily stored, classified and preserved, that fairly exact dilutions may be made rapidly by normal saline, and that the solutions obtained are always clear. In all cases the blood was five weeks old.

(A) Sheep blood.

Strength of Dilution.	Colour.	Foam Test.
1:100.	Red.	Persistent foam.
1:1,000.	Yellow.	Persistent foam.
1:5,000.	V. Pt. Straw.	Fairly persistent foam.
1:10,000.	Almost colourless.	Transitory foam.
1:20,000.	Almost colourless.	Transitory foam.

(B) Foetal blood.

Strength of Dilution.	Colour.	Foam Test.
1:100.	Red.	Persistent foam.
1:1,000.	Yellow.	Persistent foam.
1:5,000.	Pale Yellow.	Fairly persistent foam.
1:10,000.	Almost colourless.	Transitory foam.
1:20,000.	Almost colourless.	Transitory foam.

(C) Maternal blood.

Strength of Dilution.	Colour.	Foam Test.
1:100.	Red.	Persistent foam.
1:1,000.	Pale Yellow.	Persistent foam.
1:5,000.	Almost colourless.	Fairly persistent foam.
1:10,000.	Almost colourless.	Transitory foam.
1:20,000.	Almost colourless.	Transitory foam.

The last experiment was repeated with maternal blood six weeks old and precisely the same results were obtained.

(D) Ox blood.

Strength of Dilution.	Colour.	Foam Test.
1:100.	Red.	Persistent foam.
1:1,000.	Pale Yellow.	Persistent foam.
1:5,000.	Faint Yellow.	Fairly persistent foam.
1:10,000.	Almost colourless.	Fairly persistent foam.
1:20,000.	Almost colourless.	Transitory foam.

(E) Sheep blood.

Strength of Dilution.	Colour.	Foam Test.
1:100.	Pale Pink.	Persistent foam.
1:1,000.	Pale Yellow.	Persistent foam.
1:5,000.	Almost colourless.	Fairly persistent foam.
1:10,000.	Almost colourless.	Transitory foam.
1:20,000.	Almost colourless.	Transitory foam.

(F) Pig blood.

Strength of Dilution.	Colour.	Foam Test.
1:100.	Pink.	Persistent foam.
1:1,000.	Pale Yellow.	Persistent foam.
1:5,000.	Almost colourless.	Fairly persistent foam.
1:10,000.	Almost colourless.	Transitory foam.
1:20,000.	Almost colourless.	Transitory foam.

In the next two experiments the stains were imposed on thick blanket material and the extract required to be centrifuged in order to render the solution clear.

(G) Partridge blood.

Strength of Dilution.	Colour.	Foam Test.
1:100.	Reddish Brown.	Persistent foam.
1:1,000.	Pale Yellow.	Persistent foam.
1:5,000.	Almost colourless.	Fairly persistent foam.
1:10,000.	Almost colourless.	Transitory foam.
1:20,000.	Almost colourless.	Transitory foam.

(H) Pheasant blood.

Strength of Dilution.	Colour.	Foam Test.
1:100.	Reddish Brown.	Persistent foam.
1:1,000.	Pale Yellow.	Persistent foam.
1:5,000.	Almost colourless.	Fairly persistent foam.
1:10,000.	Almost colourless.	Transitory foam.
1:20,000.	Almost colourless.	Transitory foam.

It will be seen from these experiments that the foam test was more or less constant in a general way, but that the colour test was unreliable even with bloods of the same age collected and stored in an uniform manner. The dilution of 1:100 of pig's blood was pink, that of sheep's blood pale pink, and that of partridge blood reddish brown. The writer made unsuccessful attempts to obtain colour matchings of several dilutions of various bloods at different ages, with dyes and colouring matters, in the hope that standard coloured solutions would be obtained, so that any given dilution of blood could be matched and the strength of the dilution disclosed. It was found, however, after repeated experiments that there were so many factors acting upon shed blood, that the colour of a solution gave no indication of its strength, except in a very rough and unreliable manner. The variations in colour of a known dilution of blood are due to the following factors among others:-

(1) The amount of haemoglobin present.

(2) The contamination of the blood stain with colouring matter or dye in the material or article on which the blood has been imposed.

(3) The age of the blood.

(4) The treatment of the blood stain e.g. washing, application of heat, etc.

(5) The amount of serum present in the blood.

(6) The presence of carbon monoxide.

On account of the variations in the colour of the same strength of dilution of various bloods, the colorimeter methods were not applied after the technique of their application was investigated.

The rationale of the precipitin test depends upon the presence of blood serum in the solution, not upon the presence of haemoglobin. The colour of a solution therefore is of no importance in estimating the strength of dilution of the blood serum present, except in/

in a very general manner. The application of the foam test or the shaking test would appear to be the best available methods for obtaining an approximate estimation of the likely strength of blood serum present in solution. The writer has found that the shaking test is quite as efficient as the foam test, as the air mixes with the liquid satisfactorily and it is simple to apply when one has a large amount of solution available. He is not aware that this has been noted previously.

V.

THE SELECTION OF CONTAINERS FOR THE FLUID UNDER EXAMINATION.

Having obtained estimated dilutions of blood, i.e., 1:100, 1:1,000, 1:5,000, 1:10,000, and 1:20,000, the examiner has next to consider the most suitable vessel into which they should be transferred before the antiserum is applied. The writer carried out many experiments in the application of the test to dilutions of blood serum contained in:-

(1) Wassermann tubes. Vide photograph No. 6 (Left).

(2) Dryer's tubes. Vide photograph No. 3 (Left).

(3) Durham's tubes. Vide photograph No. 3 (Right).

(4) Capillary tubes. Vide photograph No. 10.

(5) Long thin tubes. Vide photograph No. 4 (Right and Left).

(6) Troughs made with long cover glasses set in plasticene frames, (vide photograph No. 7) and in frames made from black wax. (Vide photograph No. 12).

(7) Troughs made with microscope slides set in plasticene frames. (Vide photograph No. 8).

He found that the precipitin reaction was shown equally well in Wassermann, Dryer and Durham tubes. The indication as to whether a Dryer or Durham tube should be used in preference to a Wassermann tube, lies in the amount of the solution available, as it is obviously preferable to half fill the former two tubes, than/

than to use the latter type of tube and have only sufficient solution at its base, when the reaction is more difficult to examine. The use of long thin tubes as in (5) was not considered satisfactory when photographs of the test were required, on account of the high lights given by the sides of the tube. When only a small amount of the solution is available the capillary tube method as suggested by Hauser[17] and W. R. Colles[18] and praised by Uhlenhuth should be utilised. (Vide photograph No. 21). This method yields satisfactory results. Many of the experiments carried out by the writer and embodied in this thesis, were made in this way. The advantage of using capillary tubes lies in the facts that only a very small quantity of the solution and antiserum/~~are~~ required, and that the tests can be made rapidly and accurately, the reaction being well exhibited. After the tests have been completed, the tubes should be destroyed, thereby ensuring that all the tubes used are fresh. Cleanliness of the tubes is essential, because the slightest haze on the tube wall may lead to error in the interpretation of the result of the test. Wash leather gloves should be worn, as the moisture and heat from the hands of the examiner may cause condensation on the glass. The technique adopted by the writer was as follows:-

The capillary tubes were boiled, dried, and viewed against a black surface to eliminate contamination. A series of watch glasses was arranged and filled with the various fluids to be tested. Another series of glasses was used, each containing the different antisera which were to be applied. One end of the tube was placed into the antiserum against which the fluid was being tested, until about a sixth to a quarter of its lumen was filled by capillary action. Thereafter the same end was placed into the fluid to be tested, and sufficient of it drawn up to fill three quarters of the lumen of the tube. Each end of the tube in turn was inserted

into/

into a block of plasticene. This procedure effectively sealed the ends. The tube was then inserted into a pellet of plasticene situated upon a ground glass plate, so that it stood vertically, the antiserum being uppermost. In this manner tests with dilutions of 1:100, 1:1,000, 1:5,000, 1:10,000 and 1:20,000 were made. As the antiserum mixed with the solution of blood serum, a haze developed and ultimately a flocculent deposit was observed. The writer did not observe the faint colourless ring at the point of contact of the solution under test and the related antiserum, which Lucas[18] states is easily detected, nor did he note the turbid ring when related antiserum was added. The writer always inverted the tube immediately the antiserum was added, so that it could mix intimately with the fluid under test. As soon as this was done an iridescent haze was noted when the two constituents of the fluid in the tube came into contact. Soon thereafter a haze developed and ultimately a precipitin appeared at the sides of the lowermost portion of the tube.

The reason for drawing up the antiserum into the tube, before adding the solution under test, was to prevent contamination of the antiserum in the watch glass by the solution, thereby permitting the antiserum to be used for further tests without risk.

Capillary tube method.

(1) Pig blood. Three months old.

1:100	1:1,000	1:5,000	1:10,000	1:20,000
Turbid.	Turbid.	Turbid.	Turbid.	Turbid.

All positive within 20 minutes. Anti-pig serum titre 1:20,000 used.

(2) Human blood. Four months old.

1:100, 1:1,000, 1:5,000, 1:10,000, and 1:20,000.

Tested with anti-pig serum titre 1:20,000.

All/

All negative after 24 hours.

(3) Ox blood. Three months old.

In above dilutions, tested with anti-ox serum titre 1:20,000.

All positive within 20 minutes.

(4) The above experiment was repeated, and the same results were obtained.

(5) Human blood (Maternal). Four months old.

In above dilutions: Tested with anti-ox serum titre 1:20,000. All negative after 24 hours.

Note:- Dilution 1:10,000 was thought to show a definite haze, but on investigation with a magnifying glass it was found that the apparent cloud was due to markings on the tube wall, thus indicating the necessity of scrutinising carefully the tubes before conducting the test.

It should be mentioned that with the use of the capillary tube, the haze is typical and develops rapidly when an antiserum is added to a related blood, but that the development of a flocculent deposit does not make its appearance within twenty minutes, being most frequently not observed until several hours had elapsed, in many cases not until after an interval of twenty four hours. Hauser[19] is of the opinion that when the test is carried out in this way it does not matter whether the proportion of antiserum be 1:30 or 1:3, for with the blood of many animals he never got a precipitate within twenty four hours.

The writer conducted experiments with improvised stands made with long microscope cover glasses set in both plasticene and black wax. Stands were also made in the same manner, with microscope slides. (Vide photographs No. 7, 8, and 11). By their use, one has a maximum surface over which to observe the formation of the reaction, with a very small quantity of the medium under test. The fluid is held out in a fine layer by the sides of the cover glasses or/

or microscope slides by capillary action, and when the layer of fluid is viewed against a black background, the haze is rendered very distinct and the flocculent deposit can be readily detected lying at the bottom. Care must be exercised in filling these narrow troughs to prevent soiling of their exterior, as being fragile they cannot readily be wiped or cleaned without the possibility of displacement of the fluid contents, and if such occurs the solution quickly dries upon the surface, giving rise to a misleading haze. This will be clearly shown by the photographs. In certain cases this soiling is unavoidable and when it occurs, the surface should be wiped lightly with gauze saturated in absolute alcohol, before viewing the results of the test. A very fine pipette should be employed for adding the fluids to these frames, as the space which should exist between the cover glasses or slides should only occupy the breadth of about three visiting cards. The use of black wax in the construction of the frames was found to be preferable to the standard green colour of plasticene, if photographs have to be taken, on account of the former eliminating high lights. (Vide photographs numbered 7 and 12).

VI.

METHOD FOR ARRANGING TUBES.

When a large number of tests are being conducted, it is necessary to have a reliable system of arrangement of the tubes, the contents of which are to be tested. Nuttall recommends the use of a wooden stand with racks and a vertically moveable black background for this purpose. The writer, however, prefers to use ground glass plates measuring eighteen inches square, on which to arrange the different series of tubes. The tubes are held in a vertical position on the plate by means of small pellets of plasticene, into which the ends of the tubes are embedded. This does

not/

not obscure the base of the tube other than the extreme tip, as the amount of plasticene used is small and is found to hold the tubes efficiently. The tubes are given numbers and are divided into different series, depending on the tests being made. Each series is provided with a distinguishing letter. The numbers are inscribed on the glass plate immediately opposite the tube to which it refers, whereas the letters are placed at the commencement of each row, thus:-

A	o 1	o 2	o 3	o 4	o 5	B	o 1	o 2	o 3	o 4	o 5
C	o 1	o 2	o 3	o 4	o 5	D	o 1	o 2	o 3	o 4	o 5
Controls:											
A	o 1	o 2	o 3	o 4	o 5	B	o 1	o 2	o 3	o 4	o 5
C	o 1	o 2	o 3	o 4	o 5	D	o 1	o 2	o 3	o 4	o 5

Large numbers of tubes can be placed on one plate of the size indicated. The background for examining the hazes and turbidities present in the earlier stages of a positive reaction is obtained by utilising a piece of stiff dull black cardboard, which can be placed in any desired position by means of the hand. The advantages of this method were found to be:-

(1) The ease with which the tubes could be handled and examined.

(2) That the plates with the tubes thereon could be removed from one place to another when desired, without danger of the tubes falling or their contents being displaced.

(3) That the plates after use could be scrubbed and the pencil marks removed.

(4) That the plates could be placed at any level to facilitate accurate examination of the tubes in an optimum light.

VII.

THE ANTISERUM.

Assume that suitable dilutions of blood have been made, and the type of tube or frame to be employed has been determined, attention must next be directed to the condition of the antisera to be used. Uhlenhuth emphasises the necessity of every antiserum used being proved effective and suggests that the preparation and testing of antisera for medico-legal purposes should be under state control. This is a most important suggestion. Its force is readily appreciated when an examiner in the British Isles makes an attempt to obtain antisera for medico-legal purposes. The writer encountered the greatest difficulty in obtaining supplies, having decided not to prepare the animals personally on account of certain circumstances. He found that supplies of antiserum were not available in Britain, but were only obtainable either from Germany or America. The antisera used in all experiments by the writer were supplied by Sächsisches Serumwerk, A. G., Dresden. The antisera were contained in hermetically sealed ampoules each containing one cubic centimetre, which were delivered in padded light tin tubes. The antisera were kept in an ice chest when not actually in use. Supplies were sent from Dresden at intervals to ensure their being in a fresh condition.

Before using any antiserum the contents of the ampoule were carefully scrutinised to determine their clarity. It was found that in certain instances the serum exhibited a haze after transit, but that on leaving the ampoules standing in a vertical position for twenty four hours, it became absolutely clear. In order to be quite certain that the serum to be used was perfectly clear, two drops were added to a tube containing a half cubic centimetre of normal saline. If no haze was produced, the serum was considered satisfactory for use. Examination of the antiserum before use is essential/

essential to eliminate the presence of clouding or opalescence. Reference to the latter condition will be made in some detail later. If the antiserum be not clear, it will impart a haze to every clear fluid to which it is added, thereby yielding misleading results. Uhlenhuth has suggested that some of the results of Kister and Wolff[20] and Strube[35] on non-homologous bloods with different antisera, may be explained by their having used opalescent antisera, their results being contrary to those of about forty other authors.

VIII.

THE MACROSCOPIC APPEARANCE OF THE PRECIPITIN REACTION.

The antisera having been found satisfactory, the dilutions of the stain available and the containers or tubes for the fluid having been prepared, the test may now be carried out, by the addition of the antiserum to the fluid under test. The writer in the bulk of his experiments added two drops of antiserum to about half a cubic centimetre of each dilution. When antiserum is added to a related diluted blood serum, depending on the point of contact between the fluids, it either runs down the side of the tube or drops directly on to the upper layer of the fluid column in the tube. In either case it sinks to the bottom and at the points of contact, a milky appearance is given almost immediately. This haze in time increases and becomes more dense at the base of the tube than in the lower half of the fluid in the tube. When the reaction is complete, and according to Uhlenhuth this should occur at the end of twenty minutes for medico-legal work, the fluid is clouded, except in the upper third of the fluid column, and a flocculent deposit is usually observed lying at the base of the tube. The deposit is formed by fine granules of precipitin which coalesce to form flocculi, and sink to the bottom of the tube as a precipitate. The small flocculent mass possesses a whitish colour as a rule. (Vide photograph

No. 5, tubes 2 and 7; also photograph No. 4, tubes 1 and 6). The above is descriptive of a positive reaction in the lower dilutions, but in the higher dilutions, the reaction is shown usually by the formation of a whitish cloud, but not necessarily by the formation of a precipitate, unless the dilutions have stood for about twenty four hours. Ziemke asserts that not infrequently a marked clouding may occur which does not necessarily lead to a deposit after twenty four hours. The writer agrees that this may be so in high dilutions but he has not observed it when dealing with dilutions of 1:100 to 1:1,000.

Uhlenhuth and Beumer[21] have found that in a dilution of 1:10,000 the commencement of cloudiness should be apparent in three minutes and with a dilution of 1:20,000 in about five minutes. From experiments carried out it would appear that no hard and fast rule can be enunciated in relation to the exact time when a cloudiness first exhibits itself, nor does this fact appear to the writer to be very important in medico-legal work, so long as the reaction is definitely positive at the end of twenty minutes. The writer has found that certain antisera although clear themselves, have imparted a very faint yellowish haze, when added to the lower dilutions of unrelated blood. Such a haze need never be confused with a positive reaction by an experienced observer, as its appearance is quite typical. Nevertheless the unskilled examiner should be warned against this condition lest it should lead to an erroneous interpretation of the result of the test. Uhlenhuth and Beumer[21] "state that the entire reaction should take place at "room temperature, and must be at an end within twenty "minutes, for forensic work. The reactions which occur "after half an hour are of interest to the biologist, "but of no use to the medico-legist, who is concerned "with the specificity of the test, and not with the "'mammalian reaction' as Nuttall calls it, which shows "the/

"the relationship which exists between various species".

Kraus[22] observes that 37°C. is the optimum temperature for the precipitin reaction. Other authorities as Nuttall, Wassermann and Schütze[23] and Myers[24] have confirmed this.

Strube, however, does not lay much stress on the time taken for the formation of a positive reaction and considers several hours may be necessary before it appears. The strength of the antiserum used should be noted. The writer used antisera of titre 1:20,000 for practically all his experiments. Both Uhlenhuth and Nuttall have drawn attention to the fact that over-powerful antisera may prove to be a source of error in medico-legal work, and Uhlenhuth, Kister and Wolff and Ewing have stated that when an antiserum is so powerful as to produce a reaction in unrelated bloods, its action should be weakened by diluting it with salt solution.

IX.

EXPERIMENTS WITH THE PRECIPITIN TEST.

The writer followed the suggestion of Uhlenhuth in regard to keeping a stock of blood stains of various ages, produced by the blood of different animals, so that a sample of blood of the kind alleged to be present in a stain under examination and of nearly the same age would be available. The bloods kept among others were those of pig, ox, sheep, and of the human, including foetal. These were dried on filter paper and the date inscribed. Human blood dried upon approximately thirty different types of fabric and upon many different kinds of wood and leathers was also at hand. In medico-legal work it is essential to have controls of the test, using different bloods. It is also necessary to test the blood solution under examination with the different antisera in succession until a positive reaction is obtained, if the blood is to be identified further than as being human blood or not./

not. The following experiments were carried out with human, foetal, and maternal, ox, sheep, pig and partridge bloods using anti-human serum, in order to study closely the reactions. The strength of the antisera used was titre 1:20,000.

A. Foetal blood. 8 weeks old. Anti-human serum used.

1.	1:100.	Markedly positive reaction at the end of 20 minutes.
2.	1:1,000.	
3.	1:5,000.	
4.	1:10,000.	1. Distinct cloud at the end of 10 minutes.
5.	1:20,000.	2. to 5. Slight cloud at the end of 10 minutes.

B. Foetal blood. 12 weeks old. Anti-human serum used.

1.	1:100.	Distinct cloud after 10 minutes.	Markedly positive reaction at the end of 20 minutes.
2.	1:1,000.	Slight cloud after 10 minutes.	
3.	1:5,000.	V. sl. cloud after 10 minutes.	
4.	1:10,000.	V. sl. cloud after 10 minutes.	

C. Maternal blood. 8 weeks old. Anti-human serum used.

1.	1:100.	Distinct cloud after 10 minutes.	Markedly positive reaction at end of 20 minutes.
2.	1:1,000.	Slight cloud after 10 minutes.	
3.	1:5,000.	Slight cloud after 10 minutes.	
4.	1:10,000.	Slight cloud after 10 minutes.	
5.	1:20,000.	Slight cloud after 10 minutes.	

D. Maternal blood. 12 weeks old. Anti-human serum used.

1.	1:100.	Distinct cloud after 10 minutes.	Markedly positive reaction at end of 20 minutes.
2.	1:1,000.	Slight cloud after 10 minutes.	
3.	1:5,000.	Slight cloud after 10 minutes.	
4.	1:10,000.	Slight cloud after 10 minutes.	
5.	1:20,000.	Slight cloud after 10 minutes.	

E. Maternal blood. 16 weeks old. Anti-human serum used.

1.	1:100.	Cloud after 10 minutes.	Markedly positive reaction at end of 20 minutes.
2.	1:1,000.	Sl. cloud " 10 "	
3.	1:5,000.	Sl. cloud " 10 "	
4.	1:10,000.	Sl. cloud " 10 "	
5.	1:20,000.	Sl. cloud " 10 "	

F./

F. Ox blood. 8 weeks old. Anti-ox serum used.

Dilution.	2 minutes.	5 minutes.	10 minutes.	20 minutes.
1:100.	V. Ft. Cloud.	Distinct Cloud.	V. Distinct Cloud.	Markedly positive reaction in all.
1:1,000.	"	"	"	
1:5,000.	"	"	"	
1:10,000.	No Cloud.	Very Sl. Cloud.	"	
1:20,000.	"	No Cloud.	Sl. Cloud.	

G. Sheep blood. 8 weeks old. Anti-ox serum used.

1:100.)
1:1,000.)
1:5,000.) Negative results after 24 hours.
1:10,000.)
1:20,000.)

H. Partridge blood. 12 weeks old. Anti-ox serum used. Extracted from thick blanket material, thereafter solutions were centrifuged.

1:100.)
1:1,000.)
1:5,000.) All negative after 24 hours.
1:10,000.)
1:20,000.)

The results speak for themselves, but comment is necessary in regard to certain differences in the reaction, when foetal blood was used as opposed to maternal blood. Halban and Landsteiner[25], have reported that foetal blood yields more precipitin with precipitating antiserum than does maternal, and that anti-human serum gives reactions with higher dilutions of adult serum than of foetal serum. We do not agree with the latter finding so far as our tests went, but we are in complete agreement with the former statement.

I./

I. Pig's blood. 8 weeks old. Anti-ox serum titre 1:20,000 used.

1:100.)
1:1,000.)
1:5,000.) Negative at the end of 24 hours.
1:10,000.)
1:20,000.)

The positive results of these tests practically agree with Uhlenhuth and Beumer[21] who state that when a 1:10,000 dilution is used, within 3 minutes, and when a 1:20,000 dilution is used, within 5 minutes, the beginning of a cloudiness should be apparent, and that the entire reaction should take place at room temperature, and must be at an end within 20 minutes for forensic work. The following further tests were made.

A. Sheep's blood. 10 weeks old. Anti-pig serum used. Titre 1:20,000.
1:100; 1:1,000; 1:5,000; 1:10,000; 1:20,000.
All were negative even after 48 hours. (Vide photograph No. 2.)

B_1. Maternal blood. 12 weeks old. Anti-human serum used. Titre 1:20,000.
1 c.c. of dilutions was added to each Dryer tube.
1:100; 1:1,000; 1:5,000; 1:10,000; 1:20,000.
All were positive within 20 minutes. (Vide photograph No. 3 Left.)

B_2. Maternal blood. 12 weeks old. Anti-human serum used. Titre 1:20,000. 1 c.c. of each dilution was added to each long thin tube. 3 drops of anti-human serum were added to each.
1:100; 1:1,000; 1:5,000; 1:10,000; 1:20,000.
All were positive within 20 minutes. (Vide photograph Left No. 4.)

B_3. Maternal blood. 12 weeks old. Anti-human serum used. Titre 1:20,000. Microscope slides mounted in plasticene frames, were used and 2 drops of antiserum were added to each. 1½ c.c. of each dilution were added/

added to each frame.

1:100; 1:1,000; 1:5,000; 1:10,000; 1:20,000.

All were positive within 20 minutes. (Vide photograph No. 7.)

B_4. Maternal blood. 12 weeks old. Anti-human serum used. Titre 1:20,000. Durham tubes were used. 1 c.c. of each dilution was transferred to each tube. 3 drops of antiserum were added to each.

1:100; 1:1,000; 1:5,000; 1:10,000; 1:20,000.

All were positive within 20 minutes. (Vide photograph No. 3 Right).

B_5. Maternal blood. Wassermann tubes used.

1:100;	1:1,000;	1:5,000;	1:10,000;	1:20,000.
7 drops serum.	6 drops serum.	3 drops serum.	Not done.	3 drops serum.

All were positive at the end of 20 minutes. The reason of the lack of uniformity in the amount of human antiserum added was due to there being different quantities of the dilutions in the tubes. (Vide photograph No. 6 Left).

B_6. Maternal Blood. Cover glasses mounted in plasticene frames were used. 1 c.c. of each dilution was added to each frame. 3 drops of anti-human serum were added to each of the following dilutions:-

1:100; 1:1,000; 1:5,000; 1:10,000; 1:20,000.

All were positive at the end of 20 minutes.

C_1. Ox Blood. 10 weeks old. Durhams tubes were used. Dilutions 1:100; 1:1,000; 1:5,000; 1:10,000; 1:20,000. 3 drops anti-ox serum were added to each tube containing 1 c.c. of each dilution.

All were positive at the end of 20 minutes. (Vide photograph No. 5 Right).

C_2. Ox Blood. 10 weeks old. Durhams tubes used. 1 c.c. of each dilution was added to each tube, thereafter 3 drops of anti-ox serum.

Dilutions 1:100; 1:1,000; 1:5,000; 1:10,000; 1:20,000 were used. All were positive at the end of 20 minutes.

Vide/

(Vide photograph No. 5 Left).

C_3. Ox Blood. Microscope slides in plasticene frames were used. 1½ c.c.s of each dilution were added. 2 drops of anti-ox serum were added to each.

Dilutions 1:100; 1:1,000; 1:5,000; 1:10,000 were used.

All were positive at end of 20 minutes.

C_4. Ox Blood. Cover glasses in plasticene frame used. ½ c.c. of each dilution was added to each tube. 2 drops anti-ox serum were added to each.

Dilutions 1:100; 1:1,000; 1:5,000; 1:10,000; 1:20,000 were used. All were positive at end of 20 minutes.

D_1. Foetal Blood. 12 weeks old. Long thin tubes were employed. 1 c.c. of each dilution was added to each tube. 3 drops of anti-human serum were added to each.

Dilutions 1:100; 1:1,000; 1:5,000; 1:10,000; 1:20,000 were used. All were positive at the end of 20 minutes.

(Vide photograph No. 4 Right).

D_2. Foetal Blood. Cover glass frames were used. 1 c.c. of each dilution was added to each frame. 2 drops of anti-human serum were added.

Dilutions 1:100; 1:1,000; 1:5,000; 1:10,000; 1:20,000 were used.

All results were positive at the end of 20 minutes.

D_3. Foetal Blood. Microscope slide frames were used. 2 c.c.s of each dilution were added to each frame. 3 drops of anti-human serum were added to each.

Dilutions 1:100; 1:1,000; 1:5,000; 1:10,000; 1:20,000 were used. All were positive after 20 minutes.

(Vide photograph No. 8.)

E_1. Pig Blood. 10 weeks old. 3 c.c.s of each dilution were added to each Wassermann tube. 6 drops of anti-pig serum were added to each.

Dilutions 1:100; 1:1,000; 1:5,000; 1:10,000; 1:20,000 were used. All were positive after 20 minutes.

(Vide photograph No. 6, Right).

E_2/

E_2. Pig Blood. Cover glass frame method was used. 1 c.c. of each dilution was added to each tube. 2 drops of anti-pig serum were added to each.

Dilutions 1:100; 1:1,000; 1:5,000; 1:10,000; 1:20,000 were used. All were positive after 20 minutes.

E_3. Pig Blood. Durham tube method. 1 c.c. of each dilution was added to each. 3 drops of anti-pig serum were added to each. All were positive after 20 minutes.

Dilutions 1:100; 1:1,000; 1:5,000; 1:10,000; 1:20,000 were used.

F_1. Human Blood. Microscope slide frames were used. 2 c.c.s of each dilution were added. 3 drops of anti-human serum were added. All were positive after 20 minutes.

Dilutions 1:100; 1:1,000; 1:5,000; 1:10,000; 1:20,000 were used. (Vide photograph No. 9.)

F_2. A dilution of 1:100 foetal blood 12 weeks old was treated with 1 drop anti-human serum. Capillary tube method. Definitely positive reaction obtained after 10 minutes. (Vide photograph No. 10, Right).

F_3. A dilution of 1:100 human blood 16 weeks old was used. 3 drops of anti-human serum were added to 1½ c.c.s of the dilution.

A positive reaction was obtained at the end of 20 minutes. (Vide photograph No. 11).

G. A dilution of 1:100 pig's blood about 17 weeks old was used. 1½ c.c.s were added to a cover slip frame and 3 drops of anti-pig serum were added. A positive reaction was obtained at the end of 20 minutes. (Vide photograph No. 12).

X.

EXPERIMENTS WITH OPALESCENT ANTI-HORSE SERUM.

Opalescent antiserum by imparting a turbidity to every clear solution to which it is added vitiates the test. By chance a quantity of anti-horse serum supplied to the writer was found to be opalescent in character; advantage was taken of the fact to conduct the following experiments,/

experiments, in order to ascertain the extent to which such an antiserum when added to an unrelated blood might simulate a positive reaction.

Pheasant Blood. 12 weeks old. Opalescent anti-horse serum was used. The blood was extracted from thick blanket and the solution centrifuged. (Vide photograph No. 1).

Dilutions used.	1:100. Turbidity.	in all immediately following the addition of the antiserum.
	1:1,000. Turbidity.	
	1:5,000. Turbidity.	
	1:10,000. Turbidity.	
	1:20,000. Turbidity.	

Further advantage was taken of the supply of opalescent anti-horse serum to demonstrate that when it is added to normal saline a definite haze is immediately developed, whereas an antiserum which is in good condition will not produce any haze, the saline remaining perfectly clear. This has been noted by Uhlenhuth, who furthermore states that a slight opalescence is usually perceptible when any antiserum is added to blood dilutions, the tube being viewed by strong transmitted light, but the clouding here referred to is much more marked. In the results recorded above it will be noted that the turbidity is uniform in all dilutions.

To 1 c.c. of ·85% saline 4 drops of opalescent anti-horse serum were added. These imparted a very distinct turbidity to the saline solution and produced a deposit after standing for 24 hours. (Vide photograph No. 1_A).

The presence of a deposit in this regard has not been recorded previously so far as the writer is aware.

To the same amount of saline in another tube 4 drops of anti-pig serum in good condition were added. The saline in this case remained clear, there being only a very slight yellow tinge imparted to the fluid, due to the pale straw colour of the antiserum. (Vide photograph No. 1_B.)

Nuttall[26], Uhlenhuth[27], Miessner and Rostoski[28] have encountered opalescent antisera. The true cause of an opalescent serum is apparently not yet beyond surmise. Uhlenhuth states that he has encountered the condition much more frequently in intravenously treated animals. Dr. Graham-Smith has found several rabbits yielding opalescent antisera to be affected by Cysticerci. Although the true cause of opalescence has not yet been established, this does not affect the position of the medico-legal examiner, who must be on his guard against its use. On applying such an antiserum to normal saline, a cloud of uniform character is almost immediately produced. It affects the whole fluid and is not restricted to the lower two thirds of the tube as is the precipitin reaction. (Vide photograph No. 1).

Observations. When any doubt arises as to whether the antiserum to be used is likely to impart a haze on account of its condition and not as the result of a positive action, the saline test as above should be made, prior to proceeding with the precipitin test. The writer feels satisfied, as the result of the many tests he has carried out, that certain antisera, although fresh, may impart a very slight haze when added to an unrelated blood, but this need never be confused with a definite positive reaction, when the examiner has sufficient experience. When a haze due to opalescence occurs, it shows itself immediately the antiserum is added, and does not increase in amount as the result of time.

XI.

THE APPLICATION OF THE PRECIPITIN TEST IN CRIMINAL CASES.

The Application of the Precipitin Test in a case of Homicide.

The age of the stain was eight weeks. A suspicious stain was found upon the shirt of accused. The strength of the extract from the stain, which was obtained with normal saline after steeping for 24 hours, was estimated/

estimated as being about 1:50 on account of the positive character of the foam test and the colour of the solution. The material on which the stain was imposed was teased out in saline with needles to facilitate the effecting of a solution of serum. The estimated strengths of the dilutions used for the test were:- 1:500, 1:1,000, and 1:3,000. It was not thought expedient to use the 1:50 extract as the colour was too deep and would probably mask the reaction. To preclude the possibility of the blood being that of the cow, control tests were made using anti-ox serum. Wassermann tubes were used.

Tube 1. 1:500 extract from stain. 4 drops anti-human serum added.	Tube 2. 1:1,000 extract from stain. 4 drops anti-human serum added.
Tube 3. 1:3,000 of extract from stain. 4 drops anti-ox serum added.	Tube 4. Normal saline. 4 drops anti-human serum added.
Tube 5. 1:1,000 dilution of human blood extracted from filter paper. Stain was of same date as the one under test. 4 drops anti-human serum added.	Tube 6. 1:500 ox blood extracted from filter paper, same age as stain under test. 4 drops anti-human serum added.
Tube 7. 1:1,000 dilution of ox blood extracted from filter paper, same age as stain under test. 4 drops anti-human serum added.	Tube 8. 1:100 dilution ox blood extracted from filter paper, same age as stain under test. 4 drops anti-ox serum were added.

The/

The saline remained clear on the addition of the anti-human serum which was in good condition.

Results.

(Vide photograph No. 13).

Tube 1. Positive reaction within 20 minutes.	Tube 2. Positive reaction within 20 minutes.	Tube 3. Negative after 3 hours.	Tube 4. Clear.
Tube 5. Positive reaction within 20 minutes.	Tube 6. Negative after 3 hours.	Tube 7. Negative after 3 hours.	Tube 8. Positive within 20 minutes.

The tests proved conclusively that the stain tested was composed of human blood.

The application of the Precipitin Test in a case of Murder.

A portion of stain was removed from a jacket, a vest and trousers of the accused man. These exhibited a dull dirty appearance, the whole of the areas occupied by the stains being mud covered. The size of the portions excised varied from one inch to about two inches square. These were placed in separate watch glasses, and steeped in a requisite amount of ·85% saline for a period of 24 hours. Thereafter the clothing was teased out by means of mounted needles, until the fabric in each instance formed a pulpy mass. The extract was then removed by means of a pipette into separate Wassermann tubes. The extract was found to be a mud coloured solution, containing a large quantity of earth-like material in suspension and responding to the foam test. The tubes were then placed in an electrical centrifuge, for fifteen minutes. On removal, the solid matter was found to be resting on the foot of the tubes, while the fluid above was clear, and exhibited a very slight yellow tinge. The clear supernatant fluid was transferred by means/

means of a pipette to fresh tubes and four drops of anti-human serum, titre 1:20,000 were added to each. A positive reaction was given in all within twenty minutes. Portions of the extract treated with anti-ox serum, titre 1:20,000, yielded a negative result. The latter test was applied because of a likely defence, that the stains had been produced by cow's blood which had splashed on to accused's clothing, while he was present during calving.

XII.

FURTHER APPLICATIONS OF THE TEST.

The application of the Precipitin Test to a stain fourteen and a half years old.

A portion of woollen undervest of a suicide, measuring one inch square, was excised and steeped in normal saline for twenty four hours. A positive reaction was obtained on the addition of four drops of anti-human serum, within twenty minutes. The extract was positive to the foam test. The estimated strength of the dilution used was 1:1,000.

This experiment was undertaken on account of the results of Hansemann[29] who obtained the precipitin reaction with mummy material 4,000 years old. Meyer J.[30] was successful with mummy material 5,000 years old. Friedenthal, however, failed with a mummy 500 years old. Biondi[31] obtained reactions with human blood stains which had dried 10-15 years, but not with those dried 20 years.

It would appear that dried blood may remain for very long periods without affecting the action of the precipitin test. Blood serum is apparently a very stable body so far as the effects of age are concerned.

Beumer[32] has applied the precipitin test for the medico-legal examination of bones, stating that provided the bones had not been roasted or incinerated, the test was of value in determining the source of the bone.

The application of the Precipitin Test to detect the origin of bone.

A square centimetre of human skull bone about a year old, having upon it a few specks of material resembling blood, was treated with 4 c.c.s of ·85% saline for twenty four hours. The estimated dilution was about 1:20,000. The foam test was positive. A positive reaction to anti-human serum was obtained within 20 minutes. A negative result was yielded when anti-pig serum was used. (Vide photographs No. 14 and 15).

XIII.

THE VALUE OF THE TEST WITH BLOOD MIXTURES.

Nuttall[33] in his most interesting work raises a point of great medico-legal interest. He carried out tests with a view to determining whether or not a mixture of several different kinds of bloods in solution would prevent a reaction taking place upon the addition of an antiserum which was effective when added to one of these bloods alone in solution. He found that when two to six different bloods were brought together into solution, so that each blood in the mixture was diluted to about 1:500 or 1:600, the presence of other bloods did not impede a reaction taking place between an antiserum and its homologous blood in the mixture. The antisera only acted, however, when a suitable blood was present in such a mixture.

Ziemke[34] repeated Nuttall's experiment and confirmed his findings.

Uhlenhuth tested an unknown dried blood mixture supplied to him by Beumer and by the use of the precipitin test diagnosed that it was composed of pig and sheep blood. Beumer thereafter stated that his diagnosis was correct, he knowing the composition of the stain which he had supplied to Uhlenhuth for examination. Having regard to the medico-legal importance of this finding, the writer conducted experiments with the undernoted results:-

Tube/

Tube 1.

Contained equal mixtures of ½ c.c. of 1:100 dilution of human blood and pig's blood. 2 drops of anti-pig serum, titre 1:2,000 were added. A positive reaction was obtained within 20 minutes.

Tube 2.

As above but 2 drops anti-human serum, titre 1:2,000 added. Positive result within 20 minutes.

Tube 3.

As above but 2 drops anti-ox serum, titre 1:2,000 added. Negative result after 24 hours.

Tube 4.

½ c.c. pig's blood dilution 1:100 alone. 2 drops anti-pig serum, titre 1:20,000 added. A positive and more intense reaction obtained than in tube 1 within 20 minutes.

Tube 5.

As above only 2 drops anti-human serum added, titre 1:20,000. Result negative after 24 hours.

Tube 6.

½ c.c. human blood dilution 1:100. 2 drops anti-pig serum, titre 1:20,000 added. Negative result after 24 hours.

Tube 7.

As above only 2 drops of anti-human serum titre 1:20,000 added. A positive and more intense reaction obtained than in tube 2.

(Vide photograph No. 16).

It can be asserted definitely that when dealing with mixtures of bloods, and on applying the antiserum to any related blood serum present in the mixture, a positive reaction will be obtained. In such a case the extent of the reaction will not be as great as when the antiserum is applied to a related blood serum alone.

XIV./

XIV.

EXPERIMENTS TO INVESTIGATE THE AMOUNT OF BLOOD STAIN NECESSARY BEFORE THE TEST CAN BE APPLIED SATISFACTORILY.

Experiments were conducted to demonstrate the fact that only a very small quantity of blood serum is required to carry out the precipitin test successfully. Frequently jurists fail to apply the test because they are of the opinion that the amount of stain available is insufficient for the purpose. Human blood dried upon filter paper for fifteen weeks was used. An area the size of a sixpence was excised to represent one drop of blood. The stain was halved, quartered, cut into eighths, then into sixteenths, and finally thirty-secondths.

The dilutions were obtained as follows:-

1. $\frac{1}{2}$ area in 6 c.c.s saline = 1:200 dilution.
2. $\frac{1}{4}$ " " 3 c.c.s " = 1:200 "
3. $\frac{1}{8}$ " " $1\frac{1}{2}$ c.c.s " = 1:200 "
4. $\frac{1}{16}$ " " $\frac{3}{4}$ c.c. " = 1:200 "
5. $\frac{1}{32}$ " " $\frac{3}{8}$ c.c. " = 1:200 "

The stains were steeped in ·85% saline for 24 hours.

Characters of Solution.

1. Persistent foam - very pale brown colour.
2. " " - " " " "
3. " " - " " " "
4. " " - " " " "
5. " " - " " " "

Next dilutions of 1:20,000 were made from the dilutions of 1:200.

The capillary tube method was used in testing the dilutions of 1:20,000.

		Durham tubes used.	Capillary tubes used.
Amount of Dilution used.		1:200 Dilution.	1:20,000 Dilution.
1 c.c.	1	3 drops anti-human serum titre 1:20,000.	"
½ c.c.	2	3 drops anti-human serum titre 1:20,000.	"
¼ c.c.	3	1½ drops anti-human serum, titre 1:20,000.	"
⅛ c.c.	4	1 drop anti-human serum, titre 1:20,000.	"
¼ c.c.	5	1 drop anti-human serum, titre 1:20,000.	"

All gave positive results within 20 minutes. It must be admitted that the carrying out of these tests took place under very favourable conditions. In certain medico-legal cases, when the solution of the stain is very dirty and as the result of centrifuging a copious deposit is obtained, difficulty might be encountered when dealing with very small amounts. In such a case the capillary tube method, which requires only one drop of fluid, would be suitable.

It appears therefore, so long as blood serum is present in a stain, irrespective of its amount, that with proper technique the precipitin test may be applied successfully.

XV.

THE SPECIFICITY OF THE TEST.

So far as all the previous experiments are concerned no doubt has been raised regarding the specificity of the test. The names of many authorities have already been cited who have expressed their favour of/

of the test and have lauded its specific action. The literature on the subject published in the year 1902, however, indicates that other observers did not hold the same view. These were Strube[35], Kister and Wolff[36], Linossier[37] and Okamoto[38]. The first mentioned found that anti-human serum produced a slight reaction with ox, sheep and pig bloods; Kister and Wolff that human blood is more strongly acted upon by anti-horse and anti-ox sera than by anti-sheep or anti-pig sera. Several authorities have attributed the latter findings to experimental error. Linossier found that anti-human serum precipitated the sera of ox, horse, dog, sheep, guinea pig and fowl, with the qualification that the reaction was incomparably greater with human blood. Okamoto found that all animal bloods gave negative results when anti-human serum was added, but that one seventh of the human blood stains that were tested by him, gave negative results on the addition of anti-human serum. The writer's findings based upon the foregoing tests proved to him that the precipitin test, as applied to various bloods, was rigidly specific in its action.

Experiments were conducted with anti-horse serum to determine whether it was rigidly specific, as it had been noted by the writer that previous research workers had recorded some disquieting results in this regard and he had incidentally noticed a suspicion of these, while pursuing other experiments. In May 1901 Nuttall[39] published results, having tested 24 bloods with anti-horse serum, when he found that only horse serum reacted to its antiserum. Uhlenhuth[40] published the results of having tested 24 bloods with anti-horse serum in July of the same year, when he found that no other bloods outside those of the horse and donkey reacted. In an article by Kister and Wolff[41] published in 1902, it is stated that tests on five bloods with anti-horse serum surprised them. They tested the blood of the horse, pig, ox, sheep and man. All bloods clouded on the addition of anti-

horse/

horse serum. Strong dilutions were used.

It should be clearly understood that the anti-horse serum used in the following experiments was in excellent condition, being clear and fresh, having been delivered after careful preparation.

All stains used were at least three months old, and were composed of human blood in the first two groups of tests. An area of stain the size of a sixpence was used. It was steeped in 6 c.c. normal saline. All stains were steeped for 24 hours.

Group A.

		Reaction with Litmus.	Treated with 2 drops anti-horse serum.	Control of saline and 2 drops anti-horse serum.
1.	Dull red woollen material.	Sl. acid.	Distinct haze.	All were perfectly clear.
2.	Light blue woollen material.	Sl. acid.	Dense haze	
3.	Bright red woollen material.	Sl. acid.	Faint haze	
4.	Pink woollen material.	Sl. acid.	Dense haze	
5.	Green woollen material.	Sl. acid.	Very faint haze.	

The dilutions were approximately 1:100. $\frac{1}{8}$ c.c. of each was used.

All hazes developed within twenty minutes.

Group B. White cotton - human blood, several weeks old.

	Dry Heat.	Reaction with Litmus.	Treated with 2 drops anti-horse serum.	Approximate dilution.	Control of saline and 2 drops anti-horse serum.
1	100°C. Exposed 1 hour.	Neutral.	Clear.	1:100.	All perfectly clear.
2	Exposed 2 hours.	"	Absolutely clear.	1:100.	
3	Exposed 3 hours.	"	"	1:100.	

½ c.c. of each dilution was used.

Group C.

(1) 2 drops of anti-horse serum when added to ½ c.c. of a dilution of 1:100 foetal blood four months old, did not produce any haze within 20 minutes.

(2) 2 drops of anti-horse serum when added to ½ c.c. of a dilution of 1:100 maternal blood four months old, did not produce any haze within 20 minutes.

(3) 2 drops of anti-horse serum were added to ½ c.c. of a dilution of 1:100 ox blood five months old, and produced a very decided haze within 20 minutes.

(4) 2 drops anti-horse serum were added to ½ c.c. of a dilution of 1:100 pig's blood five months old and produced a very faint haze within 20 minutes.

(5) 2 drops of anti-horse serum were added to ½ c.c. dilution of 1:100 of sheep's blood five months old and produced a very distinct haze within 20 minutes.

(6) 2 drops of anti-horse serum were added to ½ c.c. dilution of 1:100 of human blood five months old which had been made acid in reaction with 3 drops $\frac{N}{10}$ H_2SO_4, and produced a distinct cloud within 20 minutes.

Nos. 2 and 3 in Group A. and No. 3 in Group C. when viewed after 24 hours showed the presence of a slight flocculent deposit.

Observations./

Observations. Dilutions of human blood serum when acid in reaction exhibited turbidity after the addition of anti-horse serum. Dilutions of human blood serum, neutral in reaction which had been extracted from stains which had been exposed to dry heat of 100°C. for from one to three hours did not yield a turbidity on the addition of anti-horse serum. Neutral dilutions of ox, pig and sheep blood became turbid on the addition of anti-horse serum.

The experiments in Groups A., B., and C. were repeated using clear anti-pig serum.

The tests embodied in Group A, yielded dense turbidities in the presence of an acid reaction. These can be accounted for by the fact that the stains were steeped longer in saline, and as a result more dye was extracted, producing a more intense acid reaction.

Group B. Negative results were yielded.

Group C. The results were negative with the exception of test 4, which yielded a markedly positive reaction within 20 minutes.

XVI.

THE EFFECT OF THE NATURE OF THE REACTION OF BLOOD DILUTIONS UPON THE TEST.

Experiments were next made to determine the effect of the reaction of the fluid under examination, on the results of the test. The literature on the subject reveals conflicting views. A solution of normal blood is alkaline, but it was found by the writer that on testing dilutions of 1:100 up to 20,000, litmus paper did not reveal this, probably because of the high degree of the dilution of the blood. The use of an indicator was not found necessary, as when litmus paper failed to reveal the presence of an acid or alkaline reaction, the test was not affected. Tchistovitch[42] found that precipitation only occurred when the reaction was alkaline, if neutral, a slight opalescence was observable, and that no reaction took place when the dilution was acid.

Linossier/

Linossier and Lemoine[43] found that sulphuric acid lessened the reaction, it being entirely prevented by acid 4·9:1,000, that sodium carbonate greater than ·66:1,000 checked the reaction proportionately with the strength of the alkalinity. Rostoski[44] states that precipitation is impeded by an alkaline, and aided by an acid reaction. Di Mattei[45] states that the presence of H_2SO_4 interferes with the reaction. Graham Smith and Sanger[46], state that the precipitin reaction is inhibited by the presence of acids and alkalis. Vincent[47] states that the reaction is affected by acetic, citric, lactic, oxalic and tartaric acids if present 5 parts in 1,000, and by the following alkalis:- ammonia, caustic potash, and caustic soda.

The Nature of the Experiments conducted.

The human blood stains used were six weeks old. Many different samples of cloth fabrics were selected and thereafter the blood was imposed upon them.

The following investigations were made in each instance:-

(1) The nature and colour of the fabric.

(2) The appearance of the stain, having regard to its age, the type of fabric on which it was situated, and the colour of the material.

(3) The colour of the dilution of the stain obtained with normal saline.

(4) Whether the dye of the material on which the stain was imposed passed into solution along with the blood serum.

(5) In the latter event, whether the reaction of the solution was neutral, acid or alkaline.

(6) If acid or alkaline, whether the test was affected, and if so, to what extent.

The results of the experiments readily permit of tabulation as follows:-

GROUP I.

	Nature and colour of fabric.	Appearance of stain.	Colour of solution.	Reaction.	Strength of dilution.	Foam test.	Precipitin reaction.
1	Light blue woollen fabric.	Blackish matted stain.	Yellowish brown.	Very slightly acid.	1:100 approximately.	++	++
2	Light green woollen fabric.	Blackish coloured matted stain.	Greenish brown.	"	"	++	++
3	Carmine woollen fabric.	Dull red matted stain.	Pink.	Slightly acid.	"	++	++
4	Pink woollen fabric.	Dark brown matted stain.	Pinkish brown.	Moderately acid.	"	++	+++
5	Red woollen fabric.	Blackish matted stain.	Bright pink.	Moderately acid.	"	++	+++

I. Strength of Precipitin Reaction.

		P.P.T. Reaction.
1.)	)	1. V. distinct ++
) Acid	)	
2.)	)	2. " " ++
) reaction	)	
3.)	)	3. " " ++
) due to the	)	
4.)	)	4. Dense +++
) dye.	)	
5.)	)	5. " +++

It should be noted that in solutions 4 and 5 where the acidity was greater, the reaction was intensified. The strength of dilution in each case was made as uniform as circumstances would permit. An area representing the circumference of a sixpence was excised from each stain to represent one drop of blood. Each was soaked in 6 c.c. saline for 24 hours. Anti-human serum was used. For the next experiment solutions similar to those in Group I were neutralised with a weak solution of ammonium hydrate, the process of neutralisation/

neutralisation being tested with litmus paper in a very careful manner.

Group II.

		Precipitin Reaction.
1.	Solutions as	1. Distinct +
2.	above but	2. Distinct +
3.	neutralised	3. Distinct +
4.	with weak	4. Distinct +
5.	ammonium hydrate.	5. Distinct +

It will be seen that when the solutions were neutralised, all gave a positive precipitin reaction, on the addition of anti-human serum, but less marked than when the solution was acid. In dealing with solutions of blood stains on clothing, it will be found expedient to centrifugalise them, lest hairs or portions of debris be present, thus affecting their clarity. It is necessary to shake the tube, after neutralising the contents, lest some of the alkali used has adhered to the tube wall and later as the result of passing down into the fluid under test, the solution is rendered alkaline instead of neutral in reaction.

Group III. Stains same age as in Group I.

Anti-human serum used.

	Nature and colour of fabric.	Appearance of stain.	Colour of solution.	Reaction.	Strength of dilution.	Foam test.	Precipitin reaction.
1	Purple linen.	Blackish indefinite stain best seen in artificial light.	Brownish red.	Neutr.	1:100	++	+
2							

Continuation of Table.

	Nature and colour of fabric.	Appearance of stain.	Colour of solution.	Reaction.	Strength of dilution.	Foam test.	Precipitin reaction.
2	Navy blue woollen material.	Dull faint brown matted stain.	Pale brown.	Sl. acid.	1:100	+ +	+ +
3	Purple felt.	Brown matted stain.	Reddish brown.	Sl. acid.	"	+ +	+ +
4	Green cotton.	Blackish grey matted.	Greenish yellow.	Neutral.	"	+ +	+
5	Nigger brown buckram.	Reddish brown matted glossy stain.	Brownish red.	"	"	+ +	+
6	Multicoloured patterned cotton.	Milk chocolate colour.	Pale brown.	"	"	+ +	+
7	Light blue sateen.	Greybrown sl. shiny.	Straw.	"	"	+ +	+
8	Multicoloured cotton.	Brown.	Pale brown.	"	"	+ +	+
9	Pink sateen.	Reddish brown.	Pale brown.	"	"	+ +	+
10	Turquoise blue velvet.	Light brown stain.	Pale brown.	"	"	+ +	+
11	Lavender net cloth.	Brown matted	Tea colour.	"	"	+ +	+
12							

Continuation of Table.

	Nature and colour of fabric.	Appearance of stain.	Colour of solution.	Reaction.	Strength of dilution.	Foam test.	Precipitin reaction.
12	Pale blue linen.	Dark brown with light area - suggestive of serum.	Light brown.	Neutral.	1:100	++	+
13	Deep blue velvet.	Hard rough, brown stain.	Port wine.	"	"	++	+
14	Light brown velveteen.	Matted hard - dull brown.	Cinnamon.	"	"	++	+
15	Multi-bright coloured print.	Brown.	Reddish brown.	"	"	++	+
16	Shot pinkish yellow silk.	Brownish pink.	V. Ft yellow.	"	"	++	+
17	Bright multi-coloured print, light blue ground.	Greenish blue.	Dark brown.	V. Sl. acid.	"	++	++

Solutions numbered 2, 3. and 17, were next neutralised with weak ammonium hydrate. As in Group II it was found that although the reaction was definitely positive, it was not so marked after neutralisation of the fluid.

Having regard to the increase in the intensity of the reaction when the appropriate antiserum was applied to an acid solution, further tests were carried out to determine more definitely the effect of acidity or alkalinity on the precipitin reaction. Human blood

approximately/

approximately eight weeks old, which had been dried upon filter paper was utilised for the next series of experiments. The results yielded were regarded as being interesting, in that they differ with many previous opinions.

3 drops of anti-human serum, titre 1:20,000, were added to each of the three undernoted dilutions.

Group III.

Strength of Dilution.		Reaction.	Precipitin Reaction.
1	1:100.	Acid by addition of 3 drops of $\frac{N}{10}$ H_2SO_4.	+ + +
2	1:1,000.		+ + +
3	1:5,000.		+ + +

Human blood treated with 3 drops of anti-ox serum.

Strength of Dilution.		Reaction.	Precipitin Reaction.
4	1:10,000.	Acid by addition of 3 drops of $\frac{N}{10}$ H_2SO_4.	+ + +
5	1:20,000.		+ + +

Group IV. Human blood treated with 3 drops of anti-human serum.

Strength of Dilution.		Reaction.	Precipitin Reaction.
1	1:100.	Alkaline by addition of 3 drops of $\frac{N}{10}$ ammonium hydrate.	+ +
2	1:1,000.		+ +
3	1:5,000.		+ +

Human blood treated with 3 drops of anti-ox serum.

Strength of Dilution.		Reaction.	Precipitin Reaction.
4	1:10,000.	Alkaline by addition of 3 drops of $\frac{N}{10}$ ammonium hydrate.	Clear.
5	1:20,000.		Clear.

Group V. Human blood treated with 3 drops of anti-human serum.

	Strength of Dilution.	Reaction.	Precipitin Reaction.
1	1:100.	Neutral.	+
2	1:1,000.	"	+
3	1:5,000.	"	+

Human blood treated with 3 drops of anti-ox serum.

	Strength of Dilution.	Reaction.	Precipitin Reaction.
4	1:10,000.	Neutral.	Clear.
5	1:20,000.	"	Clear.

Ox blood, 7 weeks old, treated with 3 drops of anti-ox serum, titre 1:20,000.

Group VI.

	Strength of Dilution.	Reaction.	Precipitin Reaction.
1	1:100.	Neutral.	+
2	1:1,000.	"	+
3	1:5,000.	"	+
4	1:10,000.	"	+
5	1:20,000.	"	+

Group VII. Ox blood, 7 weeks old, treated with 3 drops of anti-ox serum.

	Strength of Dilution.	Reaction.	Precipitin Reaction.
1	1:100.	Acid by addition of 6 drops $\frac{N}{10}$ H_2SO_4.	+ +
2	1:1,000.	"	Clear.
3	1:5,000.	"	Clear.
4	1:10,000.	"	Clear.
5	1:20,000.	"	Clear.

Group VIII. Ox blood, 7 weeks old, treated with 3 drops anti-ox serum.

	Strength of Dilution.	Reaction.	Precipitin Reaction.
1	1:100.	Made alkaline with 6 drops $\frac{N}{10}$ ammonium hydrate.	Clear.
2	1:1,000.	"	Clear.
3	1:5,000.	"	Clear.
4	1:10,000.	"	Clear.
5	1:20,000.	"	Clear.

Observations. The tests in Group III revealed the fact that an acid reaction intensified the precipitin reaction, when antiserum was added to a related blood. An appreciable turbidity was produced in tubes 4 and 5 when unrelated serum was added to their contents.

In Group IV the alkalinity of the dilutions decreased the intensity of the reactions in tubes 1-3 and a haze was not produced in tubes 4 and 5 when an unrelated serum was added to their contents.

In Group V the positive reaction in tubes 1-3 was well marked, but not so definite as in tubes 1-3, Group III.

In/

In tubes 4-5 a turbidity was not produced on the addition of an unrelated serum. In this group the reaction of all solutions was neutral.

In Group VI with neutral solutions positive reactions were given with a related antiserum.

In Group VII when the solutions were made strongly acid, a positive reaction with a related antiserum was only given in tube 1.

In Group VIII when the solutions were made strongly alkaline no reaction was given on the addition of a related antiserum.

The anti-ox serum which was used in these experiments was clear and was tested in the following manner to find out the part played by the acid or the alkali and that played by the blood serum in the alteration of the precipitin reaction in their presence.

(1) Normal saline to which 10 drops of $\frac{N}{10}$ H_2SO_4 and 10 drops of anti-ox serum were added, remained perfectly clear.

(2) Normal saline to which 10 drops of ammonium hydrate and 10 drops of anti-ox serum were added, remained perfectly clear.

(3) Neutral normal saline to which 10 drops of anti-ox serum were added, remained perfectly clear.

It would appear therefore that the alteration in the normal precipitin reaction is not produced by the acid or the alkali in the above combination, but by the interaction of blood serum with these in the fluid under test. A moderately acid reaction increases the turbidity and it may simulate a positive precipitin reaction when blood is tested with an unrelated serum. When, however, the reaction of the fluid under test is intensely acid, the precipitin reaction does not occur, even when antiserum is applied to a related blood serum. A slight but definite alkalinity diminishes the extent of the reaction. An intensely alkaline reaction prevents the formation of

conflicting results, it is necessary to ensure that the solution under test is neutral, before applying the anti-serum. The reaction of all dilutions was ascertained by the writer before proceeding with any of the tests recorded in this contribution.

The following tests were made to determine whether acids or alkalis when added to dilutions of blood serum will produce a positive precipitin reaction on the addition of unrelated antisera.

Human blood about 8 weeks old was used. Anti-human and anti-pig sera were employed, of titre 1:20,000. (Vide photograph No. 17).

	1:100.			1:1,000.			1:10,000.		
			P.P.T.			P.P.T.			P.P.T.
A	1.	Neutral.	-	2.	Neut-ral.	-	3.	Neut-ral.	-
B	4.	Acid.	+ +	5.	Acid.	+ +	6.	Acid.	+ +
C	7.	Alkaline.	-	8.	Alka-line.	-	9.	Alka-line.	-
D	10.	Neutral.	+	11.	Acid.	+ +	12.	Alka-line.	+

The tubes in the rows marked A, B and C were treated with 2 drops of anti-pig serum, whereas the tubes in the row marked D were treated with 2 drops of anti-human serum. $\frac{1}{2}$ c.c. of dilution was added to each tube. Observations. The acid reaction was effected by adding 3 drops of $\frac{N}{10}$ H_2SO_4, the alkaline reaction by adding 3 drops of $\frac{N}{10}$ ammonium hydrate. Again it would appear that when an unrelated antiserum is added to a solution of blood serum which has a marked acid reaction, a turbidity is formed which closely simulates and is readily confused with a positive precipitin reaction.

XVII./

XVII.

THE EFFECTS OF EXPOSING BLOOD STAINS TO DRY HEAT.

The following tests were made to ascertain the effect of dry heat upon blood stains, in relation to obtaining a solution of blood serum from them after such exposure. Ferrai[48] has observed that no reaction can be obtained from blood that has been exposed to a temperature of 130°C. for an hour, 140°C. for 20 minutes, 150°C. for ten minutes, or 160°C. for from five to ten minutes. Biondi[49] confirms these findings. Mirto[50] found that bloods that have been exposed to a temperature of from 100°C. to 120°C. are soluble in normal saline and that the extract gives the reaction, but that exposure to temperatures between 130°C. and 140°C. if such has lasted half an hour, greatly reduce their solubility, and if the exposure has lasted an hour, prevent solubility and no reaction can be obtained.

White cotton cloth was used by the writer for these experiments. The age of the stains which were composed of human blood was eight weeks. The size of the stains exposed to heat was 1½" square.

Group A. Three blood stained samples of cloth were exposed to dry heat of 100°C. in an oven for a period of an hour. Thereafter, portions the size of a sixpence were excised and placed in tubes containing 6 c.c. of normal saline. Although steeping lasted for 24 hours, it was found that after steeping for 15 minutes, a reddish brown solution was obtained, which yielded a copious persistent foam on applying the foam test.

A second series of three samples as above were exposed to 100°C. for a period of 2 hours. Thereafter the extracts yielded a positive foam test.

A third series of three samples as above were exposed to a temperature of 100°C. for 3 hours. The foam test was positive.

Dilutions/

Dilutions of approximately 1:100 of all extracts from the above stains were made, also control dilutions of extracts from the same stains not exposed to heat. It was found that exposure to heat did not delay the obtaining of a solution of the blood serum from the stains, solutions from the heated stains being as readily obtained as those from the unheated stains. Anti-human serum was applied to the tubes containing the dilutions of blood serum extracted from the heated and unheated stains. All gave a positive reaction for human blood within 20 minutes. It was observed that there was no appreciable difference in the intensity of the reaction obtained with dilutions of the blood serum extracted from the stains that had been heated and those that had not been subjected to heat.

Observations. A solution of blood serum is easily obtained from a stain with normal saline, although the blood stain has previously been exposed to a temperature of 100°C. for from one to three hours.

Group B. Human blood stains on white cotton about 16 weeks were employed. Areas about 1" square were used.

	Temperature.	Dilution.	Foam Test.		Precipitin Reaction.
1	100°-110°C.	1:100.	+ + +	24 hours steeping.	+ +
2	100°-120°C.	1:100.	+ + +		+ +
3	100°-130°C.	1:100.	+ + +		+ +
4	100°-140°C.	1:100.	+ + +		+ +
5	100°-150°C.	1:100.	+ + +		+ +
6	100°-160°C.	1:100.	+ +		+ +
7					

	Temperature.	Dilution.	Foam Test.	Precipitin Reaction.
7	100°-170°C.	1:100.	+	+
8	100°- 180°C.	1:100.	+ After 72	+
9	100°-190°C.	1:100.	+ hours.	+
10	100°-200°C.	1:100.	+	+
11	100°-210°C.	1:100.	-	-
12	100°-220°C.	1:100.	-	-
13	100°-230°C.	1:100.	-	-

(Vide photograph No. 24 showing positive reactions given in stains numbered 6, 7, 8 and 10.).

The stains were placed in a test tube into which a thermometer was inserted. The tube was passed downwards through the roof of the heat chamber. In this manner the temperature of the cloth was taken accurately, as the base of the thermometer and the stain were in the same position in the heat chamber. Each stain was placed in the oven when the temperature had reached 100°C. and remained there until the temperature recorded on the right of the first column of the table above was attained. Thereafter it was immediately removed, a portion the size of a sixpence excised, a dilution of 1:100 made up and tested with anti-human serum.

A positive reaction was obtained from a dilution of a blood stain brought from a temperature of 100°C. up to 200°C. It will be noted that a solution from the stains yielded a positive foam test in all cases of exposure up to 200°C., but that the obtaining of a solution which gave a positive foam test after steeping for 24 hours was only possible in tests 1-6. In test 6 the foam was not so copious as in 1-5. In tests 7-10 a positive foam test was only obtained after steeping in saline/

saline for 72 hours. In tests 11-13, the foam test was negative even after prolonged steeping. These tests show that a solution of the stain is more difficult to obtain, after the exposure of the stains to dry heat of 160°C. up to 200°C. After exposure above that temperature, a solution of the serum of the blood from the stain cannot be obtained even after prolonged steeping, as shown by the absence of a positive reaction to the foam test and precipitin test.

Group C. The effects of exposure of human blood stains about 16 weeks old on thick blue woollen material, to dry heat.

	Temperature.	Duration.	Foam Test.	Dilution.	Precipitin Reaction.
1	130°C.	15 mins.	++ after steeping 72 hours.	1:100.	++
2	150°C.	15 mins.	+ after steeping 72 hours.	1:100.	++
3	180°C.	15 mins.	"	1:100.	+
4	200°C.	2 mins.	"	1:100.	+
5	230 C.	5 mins.	- after long period of steeping. Specimen was scorched.	1:100.	-

All the solutions of the stains were slightly acid in reaction and before the human antiserum was added, they were neutralised with weak ammonium hydrate. All solutions were centrifuged.

Observations. The precipitin reaction can be obtained with blood serum extracted from a stain which has been exposed to dry heat of 130°C. for 15 minutes, 150°C. for 15 minutes, 180°C. for 15 minutes, 200°C. for 2 minutes. It was found that after exposure to a temperature of 230° for 5 minutes, the cloth was scorched. The extract

from/

from this stain did not yield a foam, and a positive precipitin reaction was not obtained.

Group D. Portions of navy blue serge cloth, on which human blood had been imposed sixteen weeks previously, were treated as in the experiments in Group C. It was found that after exposure to dry heat of 130°C. for 15 minutes, 150°C. for 15 minutes, 180°C. for 15 minutes and 200°C. for 2 minutes, a solution was obtained after steeping the stains in saline for 72 hours, which yielded a positive foam test and a positive precipitin reaction for human blood. Exposure of stains to dry heat up to 150°C. did not cause difficulty in obtaining a solution of blood serum. Above that temperature, namely from 160°-200° the stains required longer steeping in saline to effect a solution of blood serum. Above 200°C. a solution of blood serum was not obtained.

It should be noted that in Group D. all solutions were neutral in reaction.

In the foregoing tests 2 drops of anti-human serum, titre 1:20,000 were used.

Group E. Stains on white cotton about 24 hours old, which were subjected to the following temperatures of dry heat for the following periods, yielded the under-noted results:-

The stains were steeped in normal saline for 5 hours, in order to obtain an extract of blood serum.

	Temperature.	Duration.	Foam Test.	Precipitin Reaction.
1	130°C.	1½ hours.	+	+
2	140°C.	1¼ hours.	+	+
3	150°C.	¼ hour.	+	+
4	160°C.	¼ hour.	+	+

(Vide photograph No. 23).

It should be noted that the writer did not encounter difficulty in extracting blood serum with saline in/

in these experiments, contrary to the experience of Nuttall[14] who frequently found that blood dried on filter paper in hot countries would not pass into solution with saline after steeping 24 hours at 37°C.

XVIII.

THE EFFECTS OF STEEPING RELATIVELY FRESH BLOOD STAINS IN COLD WATER.

A.1. So far as the writer is aware, no systematic investigations have previously been made on this important medico-legal question. For the experiments human blood stains 24 hours old on white cotton were used. Areas of stain 1½" square were used.

	Period of steeping in water.	Appearance of cloth after steeping.	Duration of steeping in saline.	Foam Test.	Precipitin Reaction.
1	15 minutes.	Reddish brown.	Steeped in normal saline for 24 hours.	+ +	+ +
2	30 minutes.	"		+ +	+ +
3	45 minutes.	"		+	+
4	60 minutes.	"		+	+
5	1½ hours.	"		+	+
6	1½ hours.	"		+	+
7	2 hours.	"		+	+
8	2¼ hours.	"		+	+
9	2½ hours.	"		+	+
10	3 hours.	"		+	+
11	4 hours.	"		+	+
12	5 hours.	"		+	+

(Vide photograph No. 21 which shows results of tests 1, 3, 6 and 11. Photograph taken 24 hours after reaction was obtained).

The/

The stains were steeped in 20 c.c.s of distilled water.

Two drops of anti-human serum, titre 1:20,000 were added to each dilution of blood serum and in all a positive reaction was obtained within 20 minutes. After the stains had been steeped in the water for from 15 minutes to 1½ hours, a copious foam was yielded in the saline extract; whereas after steeping in water for from 1½ to 3 hours a fairly copious foam was produced in the extracts. The extracts made from the stains which had steeped in water for from 4 to 5 hours yielded foam, but not to such a marked extent as in the other instances. The dilutions of blood serum which had been extracted from the stains after steeping in the water for from 15 minutes to 1½ hours produced a more marked precipitin reaction than the dilutions of the extracts from the stains which had steeped for from 1½ to 5 hours, although these reactions were also definitely positive.

A.2. Tests were next made with the various waters in which the stains had been steeped. All the solutions were of a pale reddish brown colour, suggesting the presence of blood. A portion of each of the twelve waters was tested for the presence of albumen by the application of the heat test, with positive result. The precipitin test was applied to a second portion of each steeping and a positive reaction for human blood was obtained in all.

Esbach's Test for the quantitative estimation of albumen present in the steepings was made with the following results:-

1. Trace of albumen, too small in amount to estimate in the Esbach Tube.
2. and 3. Gave same result.
4. Contained ·25 gm. albumen per litre.
5. " ·45 " " " "
10. " ·5 " " " "
11. " ·4 " " " "
12. " ·5 " " " "

The amount of albumen increased proportionately with the duration of steeping. No. 11 is an exception but this may have been due to the fact that there was a smaller/

smaller amount of albumen in the stain originally.

Observations. The effect of steeping cloths stained with recent blood in cold water for a period of five hours did not prevent a positive precipitin reaction being obtained. Furthermore, although sufficient blood serum was left in the stains after steeping to obtain a positive reaction, an appreciable amount of blood serum was brought into solution with the water in which the stains were steeped, progressively increasing in amount after steeping for from 1½ hours up to 5 hours. Shaking or applying the foam test to the steepings themselves produced a copious lasting foam in all.

B.1. Effects of steeping old blood stains in cold water. The stains measuring 1½" square were eight or nine weeks old, and were steeped in 1½ oz. of distilled water. White cotton was the material used.

	Duration of Steeping.	Appearance of Stains after steeping.	Colour of Solution.	Foam Test.	Precipitin Reaction.
1	15 minutes.	Brownish red.	Very pale yellow.	+ +	+ +
2	30 "	"	"	+ +	+ +
3	45 "	"	"	+ +	+ +
4	60 "	"	"	+ +	+ +
5	1¼ hours.	"	"	+ +	+ +
6	1½ "	"	"	+ +	+ +
7	2 "	"	"	+ +	+ +
8	2¼ "	"	"	+ +	+ +
9	2½ "	"	"	+	+
10	3 "	"	"	+	+
11	4 "	"	"	+	+
12	5 "	"	"	+	+

The capillary tube method for the application of the test was used in this experiment.

B.2. Tests upon the steepings.

	Period of Steeping.	Colour of Steepings.	Heat Test for Albumen.	Foam Test.	Precipitin Reaction.
1	15 minutes.	Practically colourless.	+	+	+
2	30 "	"	+	+	+
3	45 "	"	+	+	+
4	60 "	Sl. yellow colour.	+ +	+ +	+ +
5	1¼ hours.	"	+ +	+ +	+ +
6	1½ "	"	+ + +	+ +	+ +
7	2 "	"	+ + +	+ +	+ +
8	2¼ "	"	+ + +	+ +	+ +
9	2½ "	"	+ + +	+ + +	+ + +
10	3 "	"	+ + +	+ + +	+ + +
11	4 "	"	+ + +	+ + +	+ + +
12	5 "	"	+ + + +	+ + +	+ + +

A positive precipitin reaction was given in all within 20 minutes. Capillary tube method was used.

The results of these experiments may be summarised as follows:-

Blood stains 24 hours old and those of 8 or 9 weeks, when steeped in cold water up to 5 hours retain sufficient blood serum to produce a positive precipitin action. The older stains apparently retained more serum than the fresher ones, presumably due to the greater fixation of the serum as the result of age. The extent of this fixation, although sufficient to retain serum in the stain after steeping up to 5 hours could be over-

come/

come when the stain so exposed was treated with saline for 24 hours, so that a positive precipitin reaction was obtained with the extract of blood serum. The extent of this fixation is more fully investigated in later experiments, when the stains were subjected to the action of warm and boiling water.

XIX.

EXPERIMENTS TO OBSERVE THE EFFECTS OF SUBJECTING RELATIVELY FRESH BLOOD STAINS TO WATER AT 50°C. IN RELATION TO THE PRECIPITIN TEST.

C.1. Blood stains which were 24 hours old on white cotton were used.

	Period of Steeping.	Foam Test.	Period to effect solution with saline.	Precipitin Reaction.
1	15 minutes.	+ +	1 hour.	+ +
2	30 "	+ +	"	+ +
3	45 "	+ +	"	+
4	60 "	+	"	+
5	1¼ hours.	+	"	+
6	1½ "	+	"	+
7	2 "	+	"	+
8	2¼ "	+	"	+
9	2½ "	+	"	+
10	3 "	+	"	+
11	4 "	+	"	+
12	5 "	+	"	+

The stains were steeped in 20 c.c.s of water, contained in glass beakers. These were placed in a water bath, the temperature of which was kept at 50°C.

C.2./

C.2. Tests made with the waters in which the stains had been steeped.

	Period of Steeping.	Foam Test.	Heat Test for Albumen.	Precipitin Reaction.
1	15 minutes.	+	+	+
2	30 "	+	+	+
3	45 "	+	+	+
4	60 "	+	+	+
5	1¼ hours.	+ +	+	+
6	1½ "	+ +	+	+
7	2 "	+ +	+ +	+ +
8	2¼ "	+ +	+ +	+ +
9	2½ "	+ + +	+ +	+ +
10	3 "	+ + +	+ +	+ +
11	4 "	+ + +	+ +	+ +
12	5 "	+ + +	+ +	+ +

Before applying the precipitin test, all the steepings were filtered, to clear the solutions of a small amount of debris, and on account of a slight mould which had formed on their surfaces, as these solutions had been kept in the laboratory for six days.

Observations. Steeping in water at a temperature of 50°C. extracted some of the blood serum from the stains, but less than when steeped in cold water for the same period, possibly on account of the heat fixing some of the albumen in the stains but not to such an extent as to prevent a solution of it being extracted readily by saline from the stains so treated.

D.1. Further tests to ascertain the effect of steeping cld/

old blood stains in water maintained at a temperature of 50°C. for varying periods. The stains used were about 16 weeks old. They were on white cotton. 20 c.c. of water was used.

	Duration of steeping.	Appearance of stain after steeping.	Colour of solution.	Foam Test.	Precipitin Reaction.
1	15 minutes.	Brown.	Pale yellow.	+ + +	+ +
2	30 "	"	"	+ + +	+ +
3	45 "	"	Straw yellow.	+ +	+ +
4	60 "	"	"	+ +	+ +
5	1¼ hours.	"	"	+ +	+ +
6	1½ "	"	"	+ +	+ +
7	2 "	"	"	+ +	+
8	2¼ "	"	"	+ +	+
9	2½ "	"	Almost colourless.	+ +	+
10	3 "	"	"	+	+
11	4 "	"	"	+	+
12	5 "	"	"	+	+

The tests in this experiment were made in capillary tubes. Positive reactions in all were given within 20 minutes.

D.2. The application of the precipitin test to the steepings in experiment D.1.

	Period of Steeping.	Foam Test.	Heat Test for Albumen.	Precipitin Reaction.
1	15 minutes.	+	+	+
2	30 "	+	+	+
3	45 "	+	+	+
4	60 "	+	+	+
5	1¼ hours.	+	+	+
6	1½ "	+	+	+
7	2 "	+ +	+	+
8	2¼ "	+ +	+	+
9	2½ "	+ +	+	+
10	3 "	+ +	+	+
11	4 "	+ +	+	+
12	5 "	+ +	+	+

The precipitin test was applied to a portion of each steeping by the capillary tube method and a positive reaction was obtained in all within 20 minutes. Observations. The precipitin reaction was obtained with extracts from blood stains sixteen weeks old, that had been steeped in water at a temperature of 50°C. for from 15 minutes to 5 hours, there being sufficient blood serum left in the stains in a state which will permit of it being brought into solution by normal saline, after such exposure. The steepings in which the stains had soaked for from 15 minutes to 5 hours all gave a positive foam test and a positive precipitin reaction, an appreciable amount of albumen derived from the serum being in solution, especially after steeping for 3, 4, and 5 hours.

It/

It must be remembered that in all these experiments, the stains used were produced by saturating the cloth with blood.

XX.

EXPERIMENTS WITH OLD BLOOD STAINS WHICH WERE SUBJECTED TO BOILING.

A I. Human blood stains were imposed on white cotton cloth. The blood was about 12 weeks old. Areas 2" square were used. The stains were placed in 200 c.c. distilled water, after it had attained boiling point.

	Duration of Boiling.	Appearance of Stain.	Colour of Solution.	Foam Test.	Precipitin Reaction.
1	5 minutes.	Red brown.	Yellow.	+ +	+
2	10 "	Light brown.	Straw.	+ +	+
3	15 "	"	"	+ +	+
4	20 "	"	"	+ +	+
5	25 "	"	"	+ +	+
6	30 "	"	"	+	+
7	35 "	Very light brown.	"	+	+
8	40 "	"	"	+	+
9	45 "	"	"	+	+
10	50 "	"	"	+	+
11	55 "	"	"	+	+
12	60 "	"	"	+	+

After steeping in saline for 24 hours, a ½ c.c. of extract of each stain was placed in Dryers tubes and 2 drops of anti-human serum were added to each.

Observations. It would appear that whatever effect boiling/

boiling has upon the stains, sufficient blood serum in a condition which will permit of extraction with normal saline is left in the stains after boiling for from 5 minutes to 1 hour to give a positive precipitin reaction.

A.2. The test was applied to portions of each of the boilings and a positive reaction obtained within 20 minutes. It should be noted that some of these solutions exhibited a haze. Those that exhibited a haze were those in which the boiling had been maintained for some considerable time. To determine the extent of the precipitin reaction, controls of the solutions without the addition of antiserum were contrasted with the tubes to which antiserum had been added in order to ascertain whether an appreciable increase in the turbidity had occurred, before regarding the reaction positive. Since this haze was regarded as being due to albumen, produced by the heat of boiling, it was not thought expedient to filter or centrifugalise the tubes.

B. A square of filter paper measuring 2" saturated with human blood dried thereon for 14 weeks old was boiled for ½ hour in 200 c.c. of distilled water, the stain being put in when the water had reached boiling point. An extract of the stain with saline gave a positive precipitin reaction within 20 minutes.

C. Further experiments to determine whether after boiling a stain, a positive precipitin reaction may be obtained.

A stain composed of human blood, 14 weeks old the size of two thirds of a sixpence was boiled in distilled water for ½ hour. On removal from the water it was placed in a test tube and 4 c.c. of normal saline were added. After 24 hours' steeping a slight foam was given on the application of the foam test. ½ c.c. of the extract was transferred to a Durham tube and treated with anti-human serum. A positive reaction was obtained within 20 minutes.

(Vide/

(Vide photograph No. 18).

A second experiment similar to the former one was made, the stain being boiled for one hour. A positive reaction was obtained from the extract within 20 minutes. (Vide photograph No. 19).

D. Tests with blood stains twelve weeks old which had been exposed to the periods of boiling indicated below, and which after boiling had been kept ten weeks. Each stain was steeped for 48 hours, although in many cases a foam was produced on shaking after treatment with saline, lasting half an hour.

	Duration of Boiling.	Colour of Solution.	Foam Test.	Precipitin Reaction.
1	5 minutes.	Yellow.	+ +	+
2	10 "	Straw.	+ +	+
3	15 "	"	+ +	+
4	20 "	"	+ +	+
5	25 "	"	+ +	+
6	30 "	"	+	+
7	35 "	"	+	+
8	40 "	"	+	+
9	45 "	"	+	+
10	50 "	"	+	+
11	55 "	"	+	+
12	60 "	"	+	+

The colour of the stains was faded brown, but after contact with the saline solution for ten minutes, they assumed a reddish colour, closely resembling that of fresh blood. All the stains were on white cotton cloth.

E./

E. Experiments conducted to ascertain whether boiling blood stains 24 hours old affects the application of the precipitin reaction. The stains used were of human blood which had been imposed on white cotton, twenty four hours previously. Portions 1" square were put into water after it had attained boiling point. The amount of water used in each case was 200 c.c.

	Duration of Boiling.	Appearance of Stain.	Colour of Solution.	Foam Test.	Precipitin Reaction.
1	5 mins.	V. Pale Brown.	Colourless.	-	-
2	10 "	"	"	-	-
3	15 "	"	"	-	-
4	20 "	"	"	-	-
5	25 "	"	"	-	-
6	30 "	"	"	-	-
7	35 "	"	"	-	-
8	40 "	"	"	-	-
9	45 "	"	"	-	-
10	50 "	"	"	-	-
11	55 "	"	"	-	-
12	60 "	"	"	-	-

The boilings were filtered after having stood for four days. The solutions were clear, and all gave positive reactions to human blood on the application of the precipitin test. (Vide photograph No. 22 showing the positive reactions obtained in the boilings of the stains numbered 6, 9 and 12).

Conclusions./

Conclusions. The effect of steeping stains in cold water appears to be that a greater amount of the blood serum on which the precipitin test depends is taken into solution by the water, than when heat is applied in the form of warm water at a temperature of 50°C. or in the case of boiling water, the amount of blood serum or albumen present being considerable in the case of cold water steepings. The treatment of stains whether fresh or old, by water at a temperature of 50°C. for from 15 minutes to 5 hours, did not interfere with obtaining a positive precipitin reaction, although less blood serum was retained in the former stains than in the latter. The heat apparently did not fix the blood serum to such an extent as to prevent its solution after prolonged steeping with saline, but it was sufficiently fixed to prevent the water in which the stain was steeped from extracting it all.

In the case of fresh stains, boiling quickly extracts the blood serum which has not yet become fixed in the stain. This extraction is so complete that neither a foam test nor a positive precipitin reaction was given on making a solution from the stain. In the case of old stains so treated, the albumen is probably more fixed in the stain and the process of boiling does not extract it to such an extent, permitting it to come into solution with normal saline after prolonged steeping.

This result is of importance in the application of the precipitin test in medico-legal practice, and so far as the writer is aware, it has not been commented upon in previous communications.

Further tests were made with blood stains 24 hours old. The stains were imposed upon washed white linen cloth, as the previous experiments had been made using fresh blood imposed upon new cloth obtained direct from the factory. It was thought expedient to wash the cloth prior to having the blood stains imposed upon/

upon it, before carrying out the following tests, in order to eliminate the possibility of the dressing of the cloth exerting any effect upon the blood serum. Each stain measured 1½" square and was boiled in 200 c.c. of distilled water, for respectively 15 minutes, 30 minutes and 60 minutes. Thereafter they were steeped in 10 c.c. of normal saline so that a solution of blood serum might be effected.

	Duration of Boiling.	Precipitin Reaction.
1	15 minutes.	Almost imperceptible haze after twenty minutes.
2	30 "	"
3	60 "	"

The hazes referred to were so indefinite that the writer would not have recorded them as positive reactions in a medico-legal case.

XXI.

EXPERIMENTS WITH BLOOD STAINS WHICH HAVE BEEN BOILED WITH SOAP.

A1. The stains were about 14 weeks old, were composed of human blood, and were upon white cotton material. Each stain was placed in 100 c.c. of distilled water to which 5 grains of castile soap powder had been added. The stains were placed in the water after it had attained boiling point. Stains measuring 1" square were used.

	Duration of Boiling.	Appearance of Stain after Boiling.	Appearance of Solution of Stain.	Precipitin Reaction.
1	5 minutes.	Very faint brown.	Foam test could not be applied on account of the presence of soap. All solutions showed a distinct haze.	?
2	10 "	"		?
3	15 "	"		?
4	20 "	Almost Stainless.		?
5	25 "	Sl. V. Ft. Stain.		?
6	30 "	"		?
7	35 "	"		?
8	40 "	"		?
9	45 "	V. Ft. Brown stain at edges only.		?
10	50 "	"		?
11	55 "	One small Ft. Brown stain at edge only.		?
12	60 "	Very small brown stain in centre.		?

The capillary tube method was used in carrying out the test, as the extracts exhibited an appreciable haze due to the presence of soap in the stains. On account of the very narrow lumen of the capillary tube, the fluid when added showed only a very trifling haze. After the antiserum had been added and an interval of 20 minutes had elapsed, the result in each tube was noted and compared with a tube containing a solution of the stain under examination without the addition of the antiserum. In all cases a very decided turbidity was shown and was very much greater than in the untreated tubes. This may have been due either to a positive precipitin reaction given by the blood serum left/

left in the stain after boiling, or to the interaction between the antiserum and the soap present. In order to determine whether such an interaction takes place, a soap solution consisting of 2 grs. of soap powder boiled in 200 c.c. of distilled water was made, and to ·5 c.c. 2 drops of anti-human serum were added, the result being contrasted with a similar untreated solution of soap. It was found that in the first tube the haze was converted into a turbidity closely resembling a positive precipitin reaction. After 24 hours a slight sediment was seen lying at the foot of the tubes. The use of capillary tubes would appear to be the most suitable method to adopt when the fluids under test exhibit a haze which cannot suitably be removed or when it is inexpedient to remove it for the reason already mentioned.

After 24 hours all the tubes treated with antiserum showed a definite flocculent deposit, whereas it was absent in all the tubes containing the solution without the addition of antiserum.

B1. The steepings were next examined. All were very cloudy and contained curdled soap. Centrifugalising these was found to be useless as was filtration by ordinary filter paper. Shaking with methylated ether was valueless. Efforts were made to precipitate the soap with saline. The steepings were in each case diluted to twice their original bulk with saline and left for 24 hours. The saline caused the soap to form into a shreddy curd and thereafter by means of filtration with ordinary filter paper the solutions were examined and found to be in the following conditions:-

1. Not rendered sufficiently clear for the application of the precipitin test.
2. do.
3. do.
4. Very faint haze.
5. do.
6. do.
7./

7. Very faint haze.

8. do.

9. Not available.

10. Very faint haze.

11. do.

12. do.

Portions of 4-8 and 10-12 were transferred to capillary tubes, antiserum was added and the turbidity produced was contrasted with the fluid in the tubes containing similar untreated solutions. The results were such that although a definite haze or turbidity was produced, having regard to the presence of soap, a positive opinion could not be expressed by the writer.

As the result of these experiments it was found that when dealing with stains that have been subjected to a strong soap solution, great difficulty is encountered in applying the test and in interpreting accurately the results obtained, on account of the hazes in the solution due to the difficulty in obtaining complete precipitation of the soap. If all the soap be removed, there is a very real danger of removing a large amount of the blood serum from the stain which may be in the solution.

A2. Experiments to note the effects of boiling blood-stained white cotton cloth with 2 grs. castile soap powder. Portions of stained cloth measuring 1 sq. inch were used. Each stain was boiled in 200 c.c. distilled water. The age of the blood stains was 24 hours. All stains were put in the water after boiling point was reached.

	Duration of Boiling.	Appearance of Stain.	Appearance of solution of Stain.	Precipitin Reaction.
1	5 minutes.	Brown.	V. Ft. haze.	?
2	10 "	"	"	?
3	15 "	"	"	?
4	20 "	"	"	?
5	25 "	"	"	?
6	30 "	"	"	?
7	35 "	"	"	?
8	40 "	"	"	?
9	45 "	"	"	?
10	50 "	"	"	?
11	55 "	"	"	?
12	60 "	"	"	?

In these experiments similar results were obtained on the addition of anti-human serum, namely an increase in the existing haze. In view of the fact that a positive precipitin reaction was not obtained in extracts from similar stains boiled without soap, it was thought that the increase in the haze on the addition of antiserum was due entirely to the interaction between the soap and the antiserum, rather than the presence of blood serum. In the instance of the older stains, since a positive reaction was obtained from the extracts made from them after boiling, it was thought that the extent of the haze produced by the blood serum, when the related antiserum was added to the soapy extracts, was masked by the haze produced by the interaction of the soap and antiserum.

B_2. Experiments/

B_2. Experiments conducted with the steepings in experiment A_2.

In this case there being only two fifths of the amount of the soap used in experiment A_1 the solutions were clearer although after treatment with saline as in experiment B_1 practically all showed a faint haze. On the addition of 2 drops of anti-human serum, all when contrasted with controls of the respective steepings, were found to have yielded a turbidity within 20 minutes. (Vide photograph No. 20). The photograph referred to shows the reaction in the steepings of stains Nos. 8, 10, 11 and 12. Taken 24 hours after the reaction.

Observations. Having regard to the facts that the solutions tested were hazy originally, and that antiserum when added to a soap solution, definitely increases the haze without the presence of blood serum, these experiments indicate that the presence of soap would rather contraindicate the use of the precipitin test or if it is used, that the results obtained should permit of great latitude in their interpretation.

Uhlenhuth[51] found that wash water containing soap gave positive reactions with anti-human serum when it contained human blood. Graham-Smith[52], however, did not confirm this finding. So far as the writer is aware no systematic research on these lines has been previously conducted.

XXII.

EXPERIMENTS WITH BLOOD STAINS ON VARIOUS WOODS IN RELATION TO THE PRECIPITIN TEST.

The objects for investigating blood stains imposed upon wood, were to test the part played by the acids derived from certain woods, to ascertain whether the blood stains could be readily dissolved from the wood on which they were imposed, and finally to note whether the process adopted in the preparation of the wood, from the crude state to the finished polished state, might have an adverse effect upon the precipitin test.

The writer was able to obtain the following samples of wood for experiment:-

1. Oak.
2. Ash.
3. Beech.
4. Birch.
5. Maple.
6. Mahogany.
7. Pine.
8. Teak.
9. Walnut.

Samples of each of the woods, in the crude state, the part stained process, and the finished or polished state were used, numbering in all twenty seven specimens.

It was realised that these did not completely represent all woods, but it was thought that tests with this collection should provide sufficient data on the points desired for practical everyday purposes. Fresh human blood was applied to the surface of the twenty seven samples and this was allowed to dry for seventy two hours, so that the blood might have time to soak into the wood.

Blood-stained shavings from each of the twenty seven samples were taken. No special solvent was used, normal saline being employed. A solution of blood serum was readily obtained in a few hours, although all were allowed to steep for a period of twenty four hours, so that any chemical agent in the wood might also be fully brought into solution. All solutions were centrifuged after the shavings had been separated. The strength of dilution was approximately 1:100. Half a cubic centimeter of each prepared solution was transferred to a separate Dryer tube, so that the precipitin test might be applied and the results photographed. As will be seen from the table appended below, the only solutions which were found to be acid in reaction were:-

(1)/

(1) Oak, in its crude and finished states, but not in its part stained condition.

(2) Ash in its part finished condition.

(3) Walnut in each of its three stages, crude, part finished, and finished.

Two drops of anti-human serum were added to each tube, after the contents had been neutralised when necessary with a 10% ammonium hydrate solution.

All the reactions were positive after ten minutes.

(Table overleaf).

(Vide photograph No. 25).

1	2	3	4	5	6	7	8	9		
Oak.	Ash.	Birch.	Maple.	Walnut.	Pine.	Beech.	Mahogany.	Teak.	Type of Wood.	
Slightly acid.	Neutral.	Neut.	Neut.	Sl. acid.	Neut.	Neutral.	Neut.	Neut.	Crude State.	1
Neutral.	Very sl. acid.	Neut.	Neut.	V. sl. acid.	Neut.	Neutral.	Neut.	Neut.	Part finished state.	2
Slightly acid.	Neutral.	Neut.	Neut.	V. sl. acid.	Neut.	Neutral.	Neut.	Neut.	Finished state: polished.	3
1 +	+	+	+	+	+	+	+	+	Precipitin Reaction.	
2 +	+	+	+	+	+	+	+	+		
3 +	+	+	+	+	+	+	+	+		

Further tests were carried out to note the part played, if any, by the acid upon the intensity of the precipitin reaction. The intensity of the reaction given above with the originally acid solutions which had been neutralised, was contrasted with the reaction obtained when in their original acid state. It was found that when the tests were carried out without neutralisation, more definite turbidities were given, more especially in the case of Oak 1 and 3.

Results. The writer agrees generally with Biondi[53] who found that rough, polished, or varnished woods had no effect on the blood stains that had been deposited on them. He, however, noted that when an acid reaction is not neutralised before the test is performed, a greater turbidity is produced. He advises that before the test is carried out the extracts should be in a neutral state. The writer's experiments were conducted with a view to re-investigating fully this field of work, on account of the frequency with which blood stains upon wood are encountered medico-legally, and having regard to the lack of uniformity in the opinions expressed by previous writers touching the part played by an acid reaction in the application of the precipitin test.

XXIII.

EXPERIMENTS WITH BLOOD STAINED VARIOUS LEATHERS IN THE DIFFERENT STAGES OF THEIR MANUFACTURE IN RELATION TO THE PRECIPITIN TEST.

In order to determine the effect, if any, of leather upon the precipitin test, experiments were conducted using samples of leather in its various stages of manufacture. The various states of leather used were:-

A. Vegetable Tanned Leather.

Group 1. Leather after it has been tanned.

(a) Tanned hide - belly.

(b) Tanned Kip.

Group/

Group 2. Leather after tanning, but after being split for use in the uppers of boots.

(a) Kip after splitting.

Group 3.

(a) Flesh side of split.

Groups 2 and 3 are equal to Group 1.

Group 4. Leather after dyeing.

(a) Kip grain dyed.

Group 5. Finished black and brown leathers.

(a) Lining kip side.

(b) Coloured hide belly (chocolate colour).

(c) Coloured hide belly (brown colour).

(d) Willow kip side (chocolate colour).

(e) Willow kip side (finished) rich chocolate colour.

(f) Box kip (finished) black.

(g) Coloured hide belly (brown).

(h) Hide belly - grain dyed (light yellow).

(i) Hide belly after splitting (putty colour).

(j) Hide shoulder (chocolate colour).

(k) Golden brown - kip side (marled brown).

B. Chrome Tanned Leather.

Stage (1).

Leather after tanning.

Stage (2).

Leather after dyeing.

Stage (3).

Finished medium leather.

C.

(1) Patent colt leather.

(2) Patent enamel calf.

(3) Box calf.

(4) Glace kid.

(5) Willow calf.

Stains of human blood were imposed upon all these samples of leather. The undernoted results were obtained, with blood stains 24 hours old, when anti-human/

human serum was used. The dilutions used were estimated at 1:100.

A. Vegetable Tanned Leather.

		Nature of Leather.	Area of Leather used.	Reaction with Litmus.	Reaction with Indicator.	Colour of Solution.	After neutralisation and filtration.	Precipitin Reaction.
Group 1.	(a)	Tanned hide belly (light brown).	Surface shaving.	Acid.	Acid.	Yellow turbid.	Clear.	Haze and dense coagulum.
	(b)	Tanned kip. (Putty colour).	"	Sl. acid.	Sl. acid.	Clear.	Clear.	Faint haze.
Group 2.	(a)	Kip after splitting (putty colour).	"	Neut.	Neut.	Clear.	Clear.	Distinct haze.
Group 3.	(a)	Split of flesh side. (putty colour).	Excised.	Sl. acid.	Sl. acid.	Sl. turbid.	Clear.	-
Group 4.	(a)	Kip grain dyed.	"	Sl. acid.	Sl. acid.	Sl. turbid.	Clear.	-
		Lining/						

Continuation of Table.

		Nature of Leather.	Area of Leather used.	Reaction with Litmus.	Reaction with Indicator.	Colour of Solution.	After neutralisation and filtration.	Precipitin Reaction.
Group 5.	(a)	Lining kip side (pale putty colour).	Excised.	Neutral.	Neutral.	Colourless.	Clear.	Very faint haze.
	(b)	Coloured hide belly (chocolate colour).	Surface shaving.	Sl. acid.	Sl. acid.	Yellow turbid.	Clear.	Distinct haze.
	(c)	Coloured hide belly (brown).	"	V. sl. acid.	V. Sl. acid.	Faint yellow turbid.	Clear.	V. ft. haze.
	(d)	Willow kip side (chocolate colour).	"	Neutral.	Neutral.	Light yellow turbid.	Clear.	Dense haze.
	(e)	Willow kip side: finished: deep chocolate.	Excised.	Sl. acid.	Sl. acid.	Light brown turbid.	Clear.	V. Ft. haze.

Box/

Continuation of Table.

	Nature of Leather.	Area of Leather used.	Reaction with Litmus.	Reaction with Indicator.	Colour of Solution.	After neutralisation and filtration.	Precipitin Reaction.
(f)	Box kip (finished) (black).	Surface shaving.	Sl. acid.	Sl. acid.	V. ft. yellow clear.	Clear.	V. distinct haze.
(g)	Coloured hide belly (brown).	"	Sl. acid.	Sl. acid.	Grey turoid.	Clear.	Ft. haze.
(h)	Hide belly grain dyed (light yellow).	"	Acid +	Acid +	Light yellow clear.	Clear.	Dense haze.
(i)	Hide belly after splitting. Putty colour.	"	Sl. acid.	Sl. acid.	Grey turbid.	Clear.	Ft. haze.
(j)	Hide shoulder. Chocolate colour.	"	Acid +	Acid -	Grey: sl. turbid.	Clear.	Ft. haze.
(k)	Golden brown kip side (marled brown).	"	Neutral.	Neutral.	Light yellow clear.	Clear.	V. ft. haze.

B. Chrome Tanned Leather.

	Nature of Leather.	Area of Leather used.	Reaction with Litmus.	Reaction with Indicator.	Colour of solution.	After neutralisation and filtration.	Precipitin Reaction.
Stage 1.	After tanning: pale blue.	Surface shaving.	Faintly acid.	Faintly acid.	Yellow clear.	Clear.	Distinct haze.
Stage 2.	After dyeing: m. brown.	"	Neut.	Neut.	Clear light brown.	Clear.	V. Ft. haze.
Stage 3.	Finished medium leather: dark brown.	"	Sl. acid.	Sl. acid.	Ft. yellow clear.	Clear.	V. Ft. haze.

C.

	Nature of Leather.	Area of Leather used.	Reaction with Litmus.	Reaction with Indicator	Colour of solution.	After neutralisation and filtration.	Precipitin Reaction.
(1)	Patent colt (glace)	Excised.	Neut.	Neut.	Clear.	Clear.	Distinct haze.
(2)	Patent enamel calf.	Surface shaving	"	"	"	"	Ft. haze.
(3)	Box calf.	"	"	"	"	"	Ft. haze.
(4)	Glace kid	Excised.	"	"	"	"	Distinct haze.
(5)	Willow calf brown.	Surface shaving	"	"	"	"	Sl. haze.

The solutions which gave an acid reaction were neutralised with a weak solution of ammonium hydrate.

Each/

Each portion of leather was steeped in normal saline for three hours. The result of the precipitin reaction was recorded after twenty minutes.

The result obtained in A. Group 1. (a) was rather unique. Almost as soon as the anti-human serum was added to the dilution, a dense coagulum appeared at the base of the tube. About five to eight minutes thereafter a turbidity appeared in the middle third of the column of fluid, its appearance being suggestive of a positive precipitin reaction. The coagulum was regarded as being due to the presence of a large amount of tannin in the leather. The absence of a positive reaction cannot be accounted for in Groups 3 and 4. It appears, therefore, that with these three exceptions the precipitin reactions obtained were normal in character, and that the leathers used did not interfere with the test. A large amount of tannin is of course likely to give rise to error in the interpretation of results, as it undoubtedly gives rise to clouding, and care must be exercised and circumstances considered carefully, before an examiner should express a positive opinion regarding the source of the blood in a stain upon leather, as the result of the application of the precipitin test. Biondi[53] found that plain or coloured leather had no effect upon the blood stains deposited on them.

Graham-Smith and Sanger[54] report that tannin produces a marked clouding even in dilutions of 1:1,000 and thus interferes with the test. They consider that when large quantities of tannin are present the application of the precipitin test is rendered impossible, because of the clouding produced by the presence of tannin, masking the true reaction. The writer believes this is the first time that such extensive experiments have been made upon leathers in their various stages of manufacture.

SUMMARY/

SUMMARY OF OBSERVATIONS AND CONCLUSIONS.

As the result of applying the precipitin test on approximately a thousand occasions, about seven hundred tests of which have been described in this contribution, the following opinions have been arrived at.

The investigations made, have confirmed the work of previous investigators regarding the specificity of the precipitin test from the medico-legal aspect. The only antiserum used which yielded conflicting results was that of the horse.

The test is an extremely delicate one having yielded positive results for human blood when a dilution of 1:20,000 of a thirty secondth of a drop was used. The test may be used when only a very small amount of blood serum is available, provided that suitable technique is employed.

It may safely be applied when a stain is composed of a mixture of different bloods, each blood in the mixture responding to its related antiserum. A less definite reaction, however, is obtained when an antiserum related to one of the bloods in the mixture is added, than when a related antiserum is added to a dilution of a single blood.

For medico-legal purposes it is the most delicate and reliable test for proving the origin of blood serum present in a stain, but faultless technique and considerable experience are essentials in carrying out the test, and in interpreting the results yielded.

The precipitin reaction may be obtained with blood serum which has been dried for many years.

The foam test is only of value in estimating the likely strength of a dilution in a very approximate manner, the colour test being valueless in this respect.

The use of cover glass or microscope slide troughs is of service in performing the test when only a small amount of the fluid is available.

Capillary/

Capillary tubes may be usefully employed in cases where a very slight haze is present in the solution under test which cannot suitably be removed, or when it is inexpedient to do so, on account of removing also the blood serum present, as the lumen of the tube is so narrow that practically no haze is noticeable. In this way the results of a positive reaction are not appreciably vitiated. It is, however, wise to employ a control of fluid similar to the one under test but without the addition of antiserum, before determining the results of the test.

The use of an opalescent antiserum is a danger, on account of its simulating a positive reaction. Such serum will produce a turbidity in all solutions of blood serum to which it is added.

The behaviour of anti-horse serum was extraordinary and yielded conflicting results. When added to human blood neutral in reaction, the precipitin test was negative. When applied to human blood, neutral in reaction, which had been exposed to dry heat of 100°C. for one hour, a negative result was obtained. When applied to human blood which was acid in reaction a positive reaction was given. Positive reactions were obtained when pig, ox, and sheep bloods were treated with anti-horse serum, although the reaction of the bloods was neutral. When the above experiments were repeated using pig antiserum, a positive reaction was only produced in the case of pig blood. The writer cannot explain these results, as the serum used was in excellent condition and the technique was careful and thorough.

An acid reaction increases the intensity of the precipitin test, and simulates a positive reaction when antiserum is applied to an unrelated blood. When the solution of the stain is strongly acid, a positive reaction with ox blood and anti-ox serum was given only with a dilution of 1:100. A very strongly acid reaction appears to destroy the test, in dilutions above 1:100.

Alkalinity/

Alkalinity decreases the intensity of the precipitin test and when the reaction is strongly alkaline, the reaction is destroyed. A neutral reaction therefore is essential before applying the test, if reliable and satisfactory results are to be obtained. It would appear that the interference with the test, by acids and alkalis is due to their action upon the blood serum in the solution and not upon the antiserum applied.

Extracts of blood stains from woods and leathers, on account of the acid reaction which may be present, should always be neutralised before applying the antiserum.

There is a danger of encountering a simulated positive reaction in blood stains extracted from leather, on account of the presence of a large amount of tannin which will produce a cloud with any antiserum. Care and consideration are essentials before an examiner expresses an opinion as to the source of blood stains found upon leather, if an appreciable amount of tannin is present in the solution of blood serum obtained from the stain.

Blood stains exposed to dry heat of 100°C. for one, two, or three hours, yield a solution of blood serum with normal saline and the precipitin test is not interfered with. Exposure of a blood stain to a temperature of 100°C. and raised to 200°C. did not interfere with the reaction, although a solution of the serum was more difficult to obtain. Exposure of blood stains to 130°C. for 15 minutes, 150°C. for 15 minutes, 180°C. for 15 minutes, and 200°C. for 2 minutes yielded a solution of blood serum after steeping in saline for 72 hours. Exposure of blood stains to a temperature of 130°C. and 140°C. for 1¼ hours, 150°C. for ¼ hour and 160°C. for quarter of an hour did not vitiate the test.

Blood stains 24 hours old when steeped in cold water for from 15 minutes to 5 hours, still gave a positive reaction, as did the water in which they were steeped, the intensity of the reaction in the case of

the stains becoming less the longer the stain was steeped and greater in the case of the steepings. The above results were confirmed, using stains eight or nine weeks old, but these stains apparently retained more of the blood serum, it having probably become more fixed than in the case of fresh stains.

Subjecting blood stains 24 hours old to water at 50°C. for from 15 minutes to 5 hours, did not prevent a positive reaction being obtained. The steepings also yielded a similar reaction. Blood stains 16 weeks old yielded similar results but less blood passed into the water in which they were steeped.

Blood stains 12 weeks old yielded a positive reaction after boiling for from 5 minutes to 1 hour. Like results were yielded by the boilings. It would appear that whatever effect boiling has upon the stains, sufficient blood serum is left in them to permit of the precipitin test yielding a positive result. Similar tests carried out with stains 24 hours old, failed to yield positive reactions. It appears therefore that older stains become sufficiently fixed to retain enough serum, even after boiling, to yield a positive reaction, whereas fresh stains are not so fixed and quickly give up their blood.

The application of the precipitin test is not satisfactory when soap is present in the extracts of the stains, on account of the haze created by it, the undesirability of completely removing the haze, and the fact, that on the addition of any antiserum to a soap solution in which no blood serum is present, an increase in the density of the haze is produced.

For medico-legal purposes, the precipitin test may be applied with safety, under practically all conditions, the exception of course being when the blood serum has been destroyed by any agency. Such, among others, are efficient scrubbing or washing of the stain, charring or exposure to heat above 200°C. The blood/

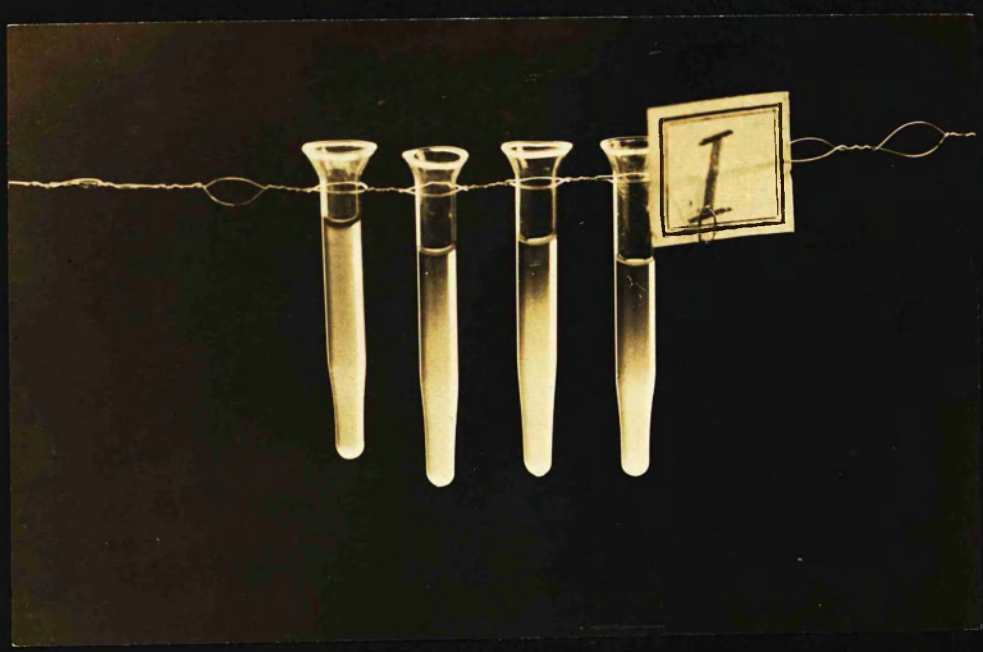
blood serum is a very stable body and is not readily affected under ordinary circumstances.

As much of the work recorded in this writing has been undertaken for the first time, it is hoped that the results obtained, will, by amplifying the subject from the medico-legal aspect, prove of service to those whose work demands the performance of the Precipitin Test in criminal cases.

APPENDIX OF PHOTOGRAPHS.

The following photographs depicting the absence and presence of the precipitin reaction have been appended, so that some pictorial conception of the nature of the reactions obtained in the experiments may be available. The very greatest difficulty was encountered by the photographer in taking these photographs, at the commencement, the high lights from the tubes and their surroundings masking the important points. Very prolonged and careful study was given before the first successful photographs were obtained. So far as the writer is aware only very few photographs of the precipitin reaction are available and these show the reaction with low dilutions of blood serum. Most of the photographs here submitted, show a positive precipitin reaction with dilutions of blood serum as high as 1:20,000.

PHOTOGRAPH NO. 1.



From left to right dilutions 1:1,000, 1:5,000, 1:10,000, 1:20,000 of pheasant blood added to Durham tubes, the amount varying from $\frac{1}{8}$-$\frac{5}{4}$ c.c. When 3 drops of opalescent anti-horse serum were added to each, an immediate turbidity formed. The photograph was taken half an hour after the addition of the antiserum. A flocculent deposit is not present. It illustrates the confusion which may exist between such a simulated reaction and a true positive precipitin reaction. The turbidity is uniform in all dilutions. The dense cloud formed almost immediately the opalescent serum was added.

PHOTOGRAPH NO. 1a.

Normal saline after opalescent antiserum has been added.

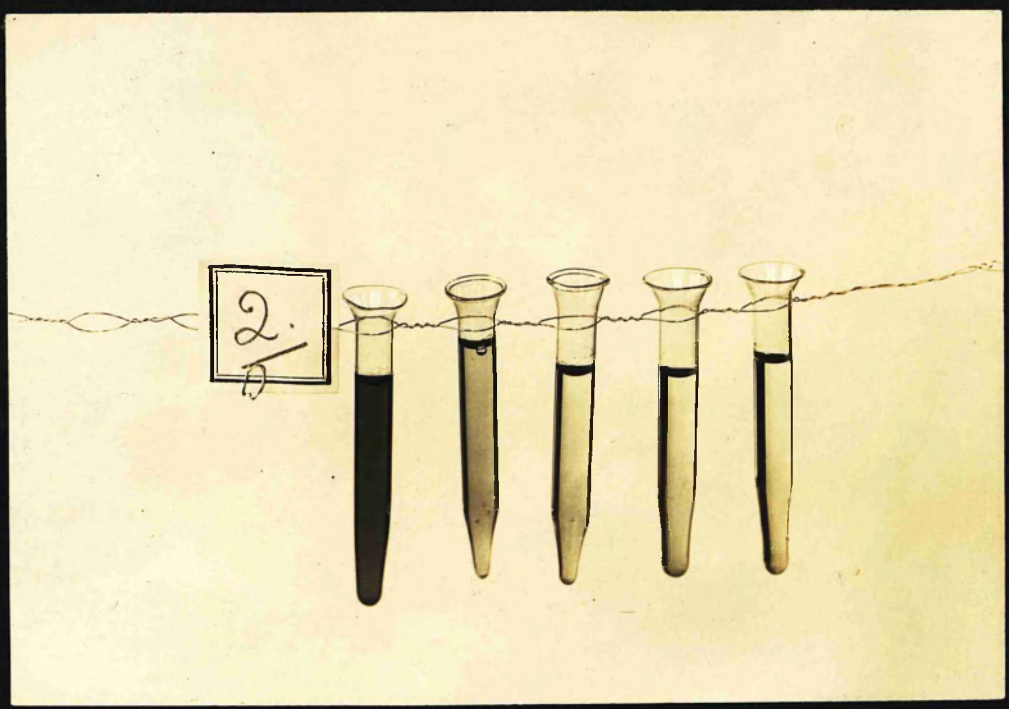
Note haze and deposit.

PHOTOGRAPH NO. 1b.

Normal saline after clear antiserum has been added.

The solution is clear, the shadow at the termination of the tube is not deposit, but is due to reflected light.

PHOTOGRAPH NO. 2.



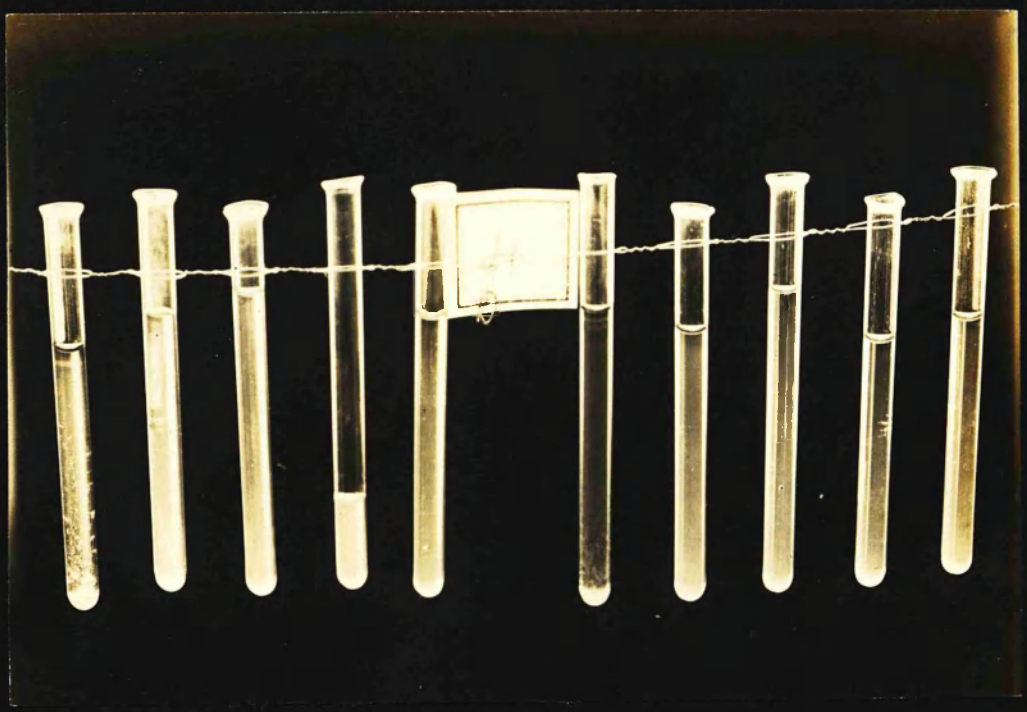
From left to right.

Dilutions of Sheep's Blood, 1:100, 1:1,000, 1:5,000, 1:10,000, 1:20,000. ¾ c.c. of each. 3 drops fresh anti-pig serum were added. No haze developed after 4½ hours. Note that No. 1 tube containing a dilution of 1:100 exhibits a dark shade due to the red colour of the solution. This degree of colour diminishes in tubes 2-4. Note contrast between previous photographs and this one, there being in this case no suggestion of clouding or haze.

PHOTOGRAPH NO. 3.

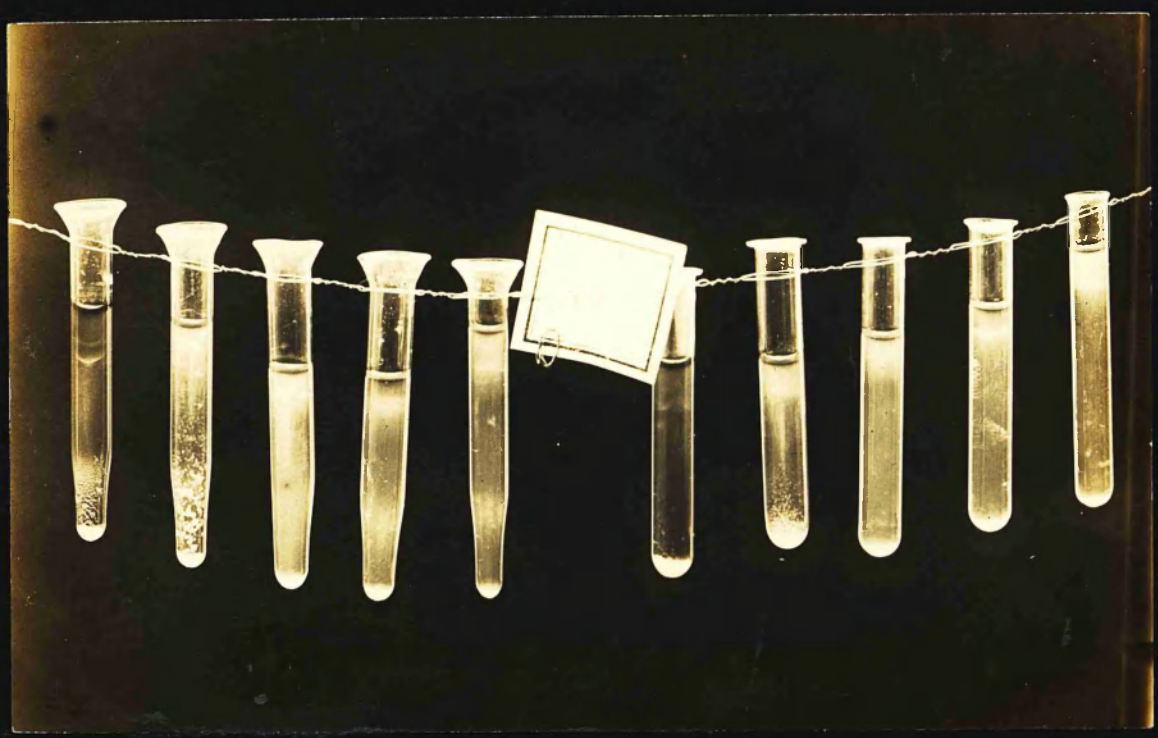
Left.	Right.
1, 2, 3, 4, 5. Dilutions of maternal blood, 1:100, 1:1,000, 1:5,000, 1:10,000, 1:20,000. Anti-human serum added. Note copious flocculent deposit in tube 1, and clouds and deposit in tubes 2-4. Photographs were taken ½ hour after the antiserum was added. Dryer's tubes were used.	1, 2, 3, 4, 5. Dilutions of maternal blood, 1:100, 1:1,000, 1:5,000, 1:10,000, 1:20,000. Note the typical reactions in all. Durham's tubes used.

PHOTOGRAPH NO. 4.



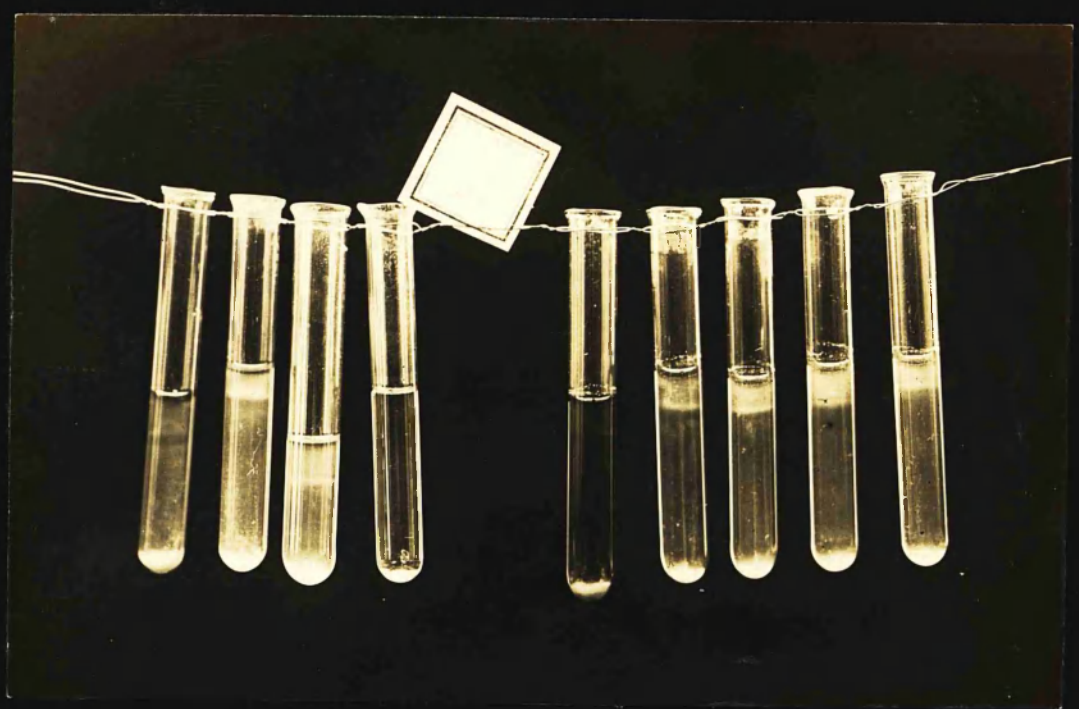
To the left of the photograph tubes 1-5 contain standard dilutions of human blood added to long thin tubes. To the right of the photograph, tubes 1-5 contain foetal blood in standard dilutions. 3 drops of anti-human serum have been added to all tubes. Photograph was taken ½ hour after the addition of the antiserum. It should be noted that the use of the long thin tube for photographic purposes is not so effective as the shorter and thicker tube, on account of the former increasing the high lights. Tubes 1Left and 1Right show the formation of the flocculent deposit in an efficient manner.

PHOTOGRAPH NO. 5.



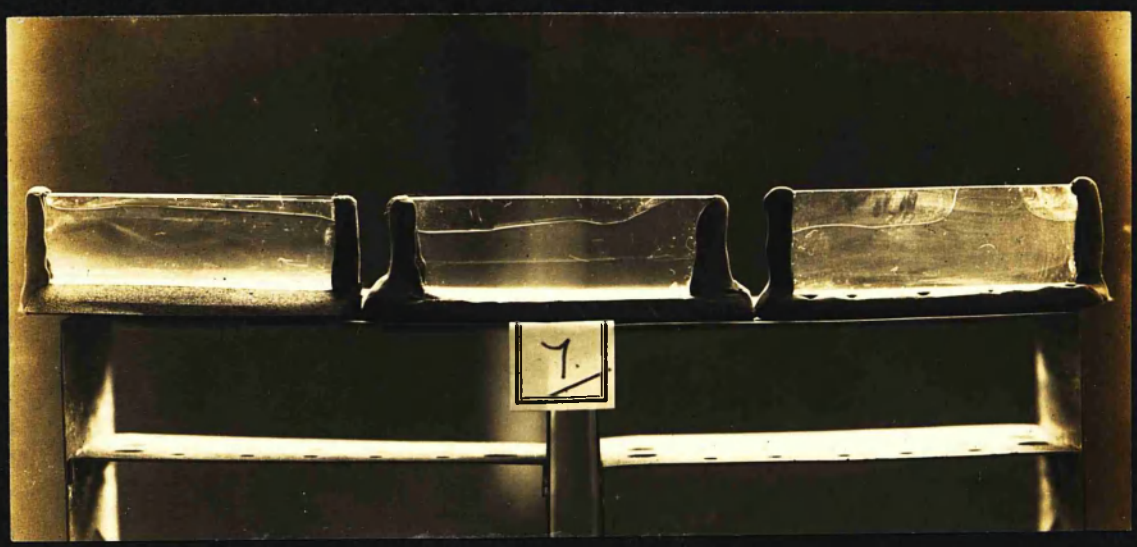
Left.	Right.
Standard solutions of ox blood and 3 drops of anti-ox serum, titre 1:20,000. All were positive after 20 mins. The photograph was taken 24 hours after. Note the rings of haze in the uppermost portions of the tube, due to commencing bacterial action. Tubes 1 and 2 show the flocculent deposit well.	Standard solutions of ox blood and 3 drops of anti-ox serum, titre 1:20,000. All were positive after 20 mins. The photograph was taken 24 hours after. There is a haze in the uppermost portions of tubes, due to commencing bacterial action.

PHOTOGRAPH NO. 6.



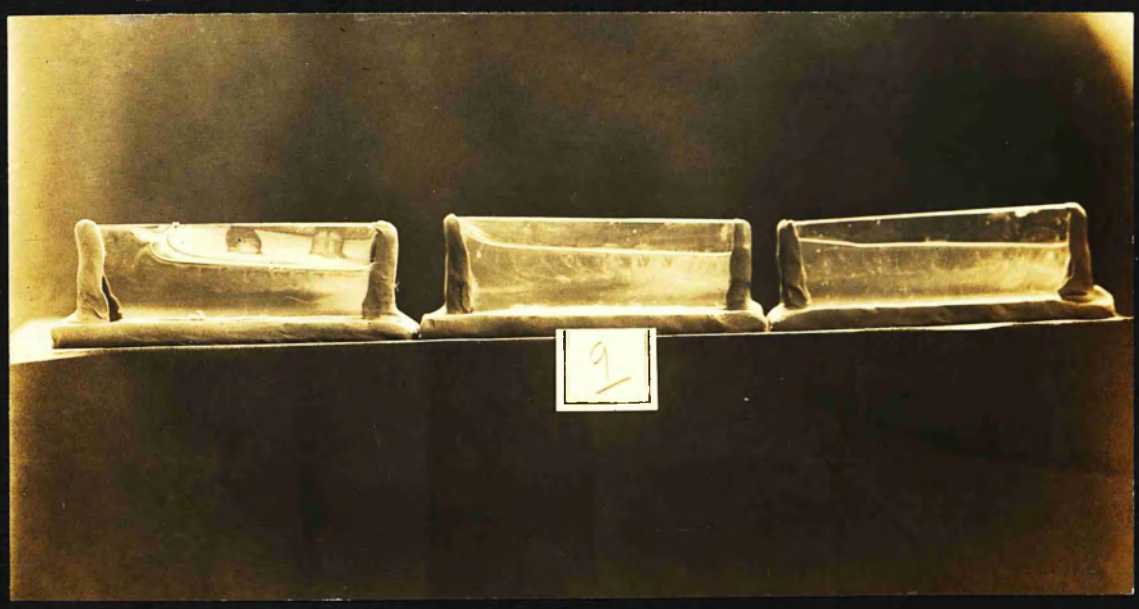
Left.	Right.
Human blood in dilutions of 1:100, 1:1,000, 1:5,000, 1:20,000 and 5 drops of anti-human serum. The deposits are well seen in these photographs. The photograph was taken 24 hours after. Note the hazes in tubes 1, 2, 3, due to bacterial action.	Pig's blood in dilutions 1:100, 1:1,000, 1:5,000, 1:10,000, 1:20,000 + 5 drops of anti-pig serum. No. 1 tube is dark, due to the deep colour of the blood solution. Deposits are shown in all the tubes. Hazes are present in the upper portions of tubes, 2, 3, 4, 5, due to bacterial action. Photograph taken 24 hours after.

PHOTOGRAPH NO. 7.



Microscope slide troughs. From left to right 1:100, 1:1,000, 1:5,000 dilutions of maternal blood, to which 3-4 drops of antiserum have been added. The photograph was taken 1 hour after the addition of antiserum. Note in frame 1 that the greenish colour of the plasticene throws a high light, the same is seen on the far side of tube 2, and in tube 3. Note the haze on the glasses above the column of the fluid, due to wetting and drying. The clouds and deposits can, however, be seen.

PHOTOGRAPH NO. 8.



Microscope slide troughs. Frames, 1, 2, 3, contain dilutions of 1:100, 1:1,000, 1:5,000 of foetal blood and 2 drops anti-human serum, titre 1:20,000. The photograph was taken ½ hour after. Note the haze above the level of the fluid in frame 1, due to the drying of the liquid material on the outer sides. Flocculent deposits and hazes can be seen in all.

PHOTOGRAPH NO. 9.

Microscope slide troughs. The thicker glass seems to show the clouds more definitely. Photograph was taken an hour after the addition of the anti-human serum, titre 1:20,000. Dilutions of 1:5,000, 1:10,000 and 1:20,000 of maternal blood were used.

PHOTOGRAPH NO. 10.

1:100 foetal blood, 12 weeks old, treated with 1 drop of anti-human serum, titre 1:20,000. The control on the left is normal saline and 1 drop anti-human serum. Note the cloud in the right capillary tube and flocculent deposit at the base of the tube. Also note the plasticene plugs at the lower terminations of the capillary tubes. The photograph was taken after 24 hours.

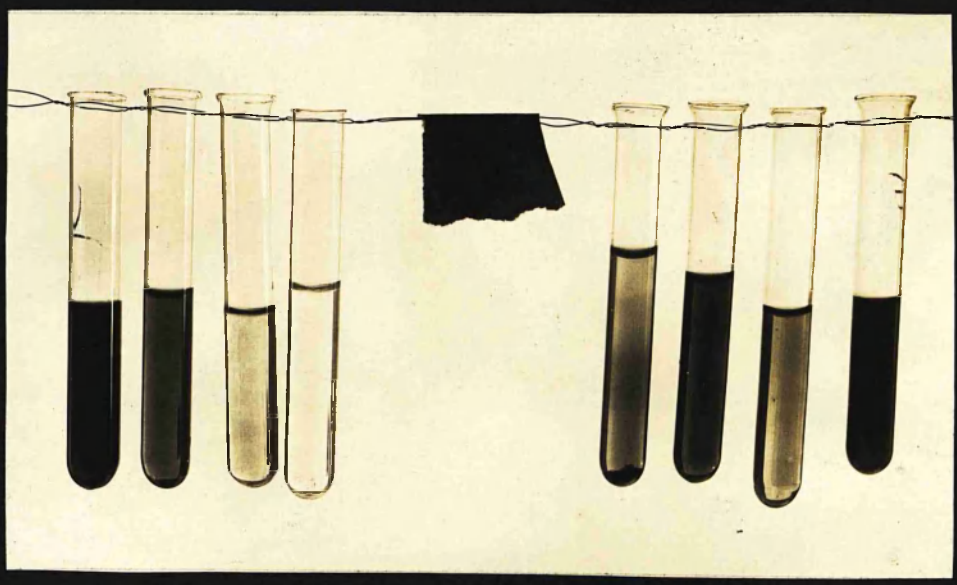
PHOTOGRAPH NO. 11.

1:100 human blood treated with 2 drops of anti-human serum. The photograph was taken after 24 hours. The cover slip trough was used. Note the haze in the fluid and the flocculent deposit. The haze above the fluid level is due to the drying of the fluid on the inner aspects of the cover slips.

PHOTOGRAPH NO. 12.

1:100 pig's blood + 2 drops of anti-pig serum. The photograph was taken after 24 hours. The cover slip method was used. Note the haze in the fluid and the flocculent deposit. The haze above the fluid level is due to the drying of the fluid on the inner aspects of the cover slips. Black wax frames were employed. Less reflected light is shown.

PHOTOGRAPH NO. 13.



Wassermann tubes were used. 1:500 extract from human blood stain with addition of 4 drops of anti-human serum.

Tube 1 is dark, due to the colour of the fluid. The cloud is obscured, but the deposit can be faintly seen.

1:1,000 extract from human blood stain with addition of 4 drops of anti-human serum.

Tube 2. The cloud is also obscured by the colour of the fluid, but the deposit is faintly seen.

1:3,000 extract from human blood stain with the addition of 4 drops of anti-ox serum.

Tube 3. Negative.

Normal saline + 4 drops of anti-human serum.

Tube 4. Negative, showing antiserum was in good condition.

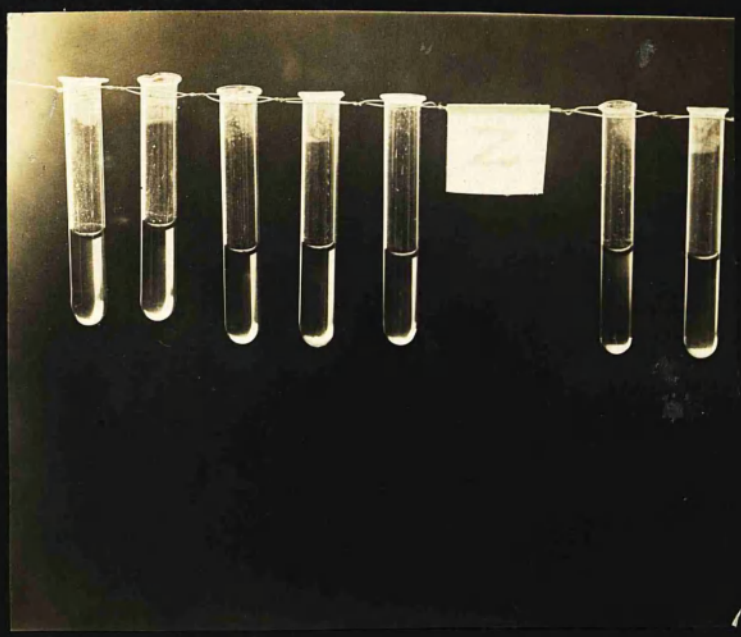
PHOTOGRAPH NO. 14.

Extract from human skull bone with the addition of anti-human serum. Note the flocculent deposit, also the dense haze in the upper portion of the tube, due to bacterial action, the specimen having to be held over for 72 hours prior to photographing.

PHOTOGRAPH NO. 15.

Extract from human skull bone with addition of anti-pig serum. Note the absence of deposit, but copious haze in the upper part of the tube, for the above reason. A negative result after 6 hours.

PHOTOGRAPH NO. 16.



Tube 1. Equal mixture of dilutions of human and pig bloods, with the addition of 2 drops of anti-pig serum.

Tube 2. As in tube 1 but 2 drops of anti-human serum were added.

Tube 3. As in tube 1 but 2 drops of anti-ox serum were added.

Tube 4. Dilution of 1:100 of pig blood, to which 2 drops of anti-pig serum were added.

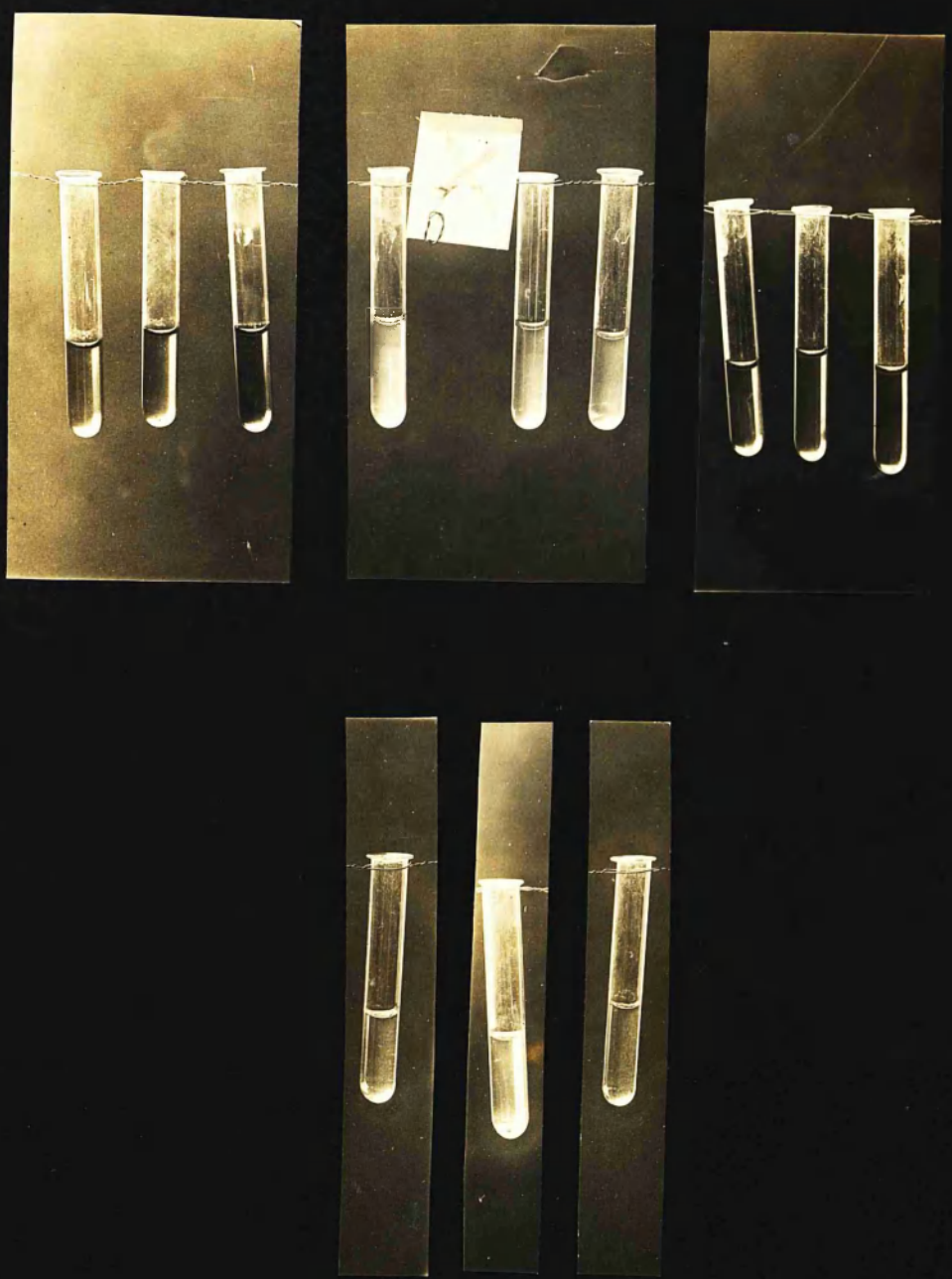
Tube 5. As in tube 4, but 2 drops of anti-human serum were added.

Tube 6. Dilution of 1:100 of human blood, to which 2 drops of anti-pig serum were added.

Tube 7. As in tube 6, but 2 drops of anti-human serum were added.

The results are not satisfactory owing to the dark colour of the liquids, and the incomplete elimination of high lights which are very difficult to avoid in photographing small glass tubes. The tubes read from left to right. A definite deposit can be seen in tubes 4 and 7.

PHOTOGRAPH NO. 17.



Tubes 1-3 are negative. Tubes 4-6 show a marked cloud. Tubes 7-9 were negative, but in the photograph owing to high lights a positive reaction is simulated. In reality this was not so. Tubes 10-12 are positive, but there was insufficient transmitted light to show them up well in the photograph. These photographs are not regarded as being satisfactory, but on taking a photograph of small suspended tubes, it is very difficult to obtain a suitable and evenly distributed light. The photograph was taken after 24 hours.

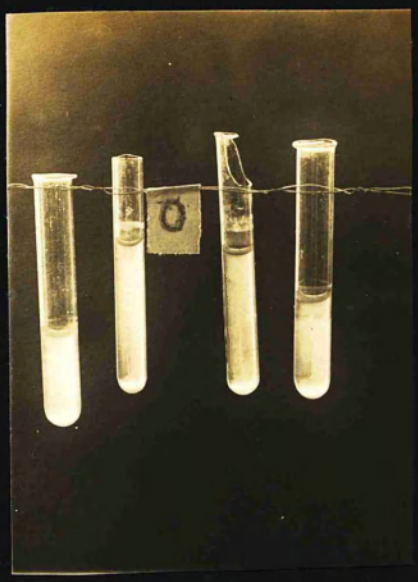
PHOTOGRAPH NO. 18.

Extract of human blood stain, 14 weeks old, from cloth boiled for ½ hour, treated with 3 drops anti-human serum, titre 1:20,000.

PHOTOGRAPH NO. 19.

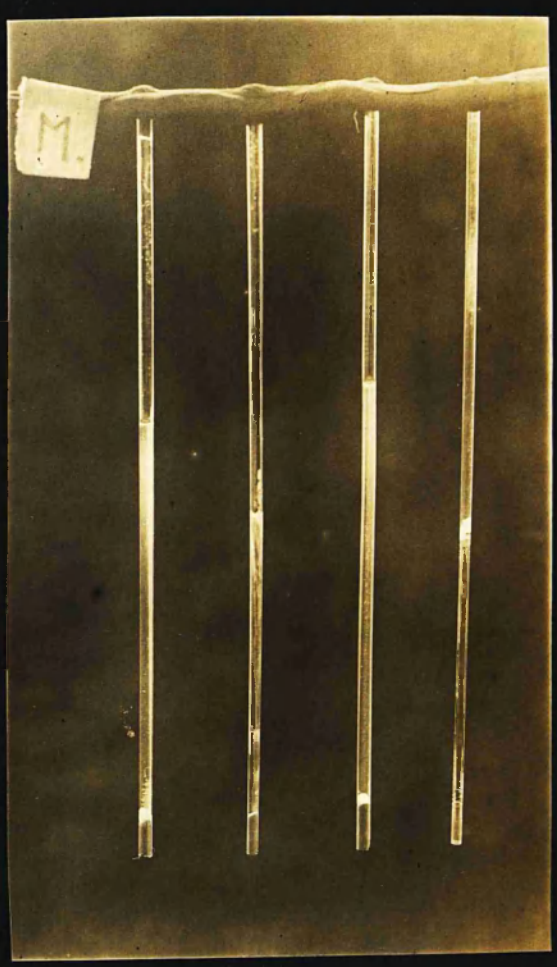
As above, but stain was boiled for 1 hour.

PHOTOGRAPH NO. 20.

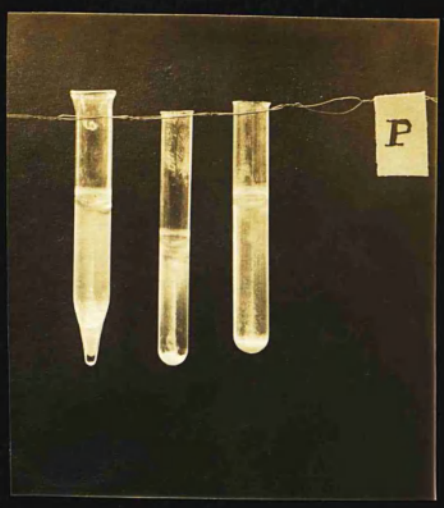


From left to right. The boilings of stains numbered 8, 10, 11 and 12. Photograph was taken 24 hours after the addition of 2 drops of human antiserum. Titre 1:20,000. The blood stains had been boiled in the above soapy waters for 40 minutes, 50 minutes, 55 minutes, and 1 hour respectively.

PHOTOGRAPH NO. 21.

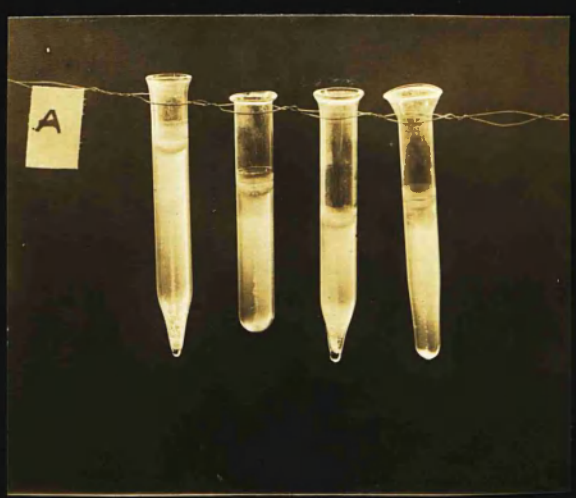


PHOTOGRAPH NO. 22.



From left to right. The results of the precipitin test applied to the boilings of stains numbered 6, 9 and 12. Note the positive reactions. Flocculent deposits are present. Photograph was taken 24 hours after. The blood stains had been boiled in the above waters for 30 minutes, 45 minutes and 60 minutes respectively.

PHOTOGRAPH NO. 23.



From left to right.

Tube 1. Extract of human blood serum from a stain exposed to a temperature of 130°C. for 1¼ hours.

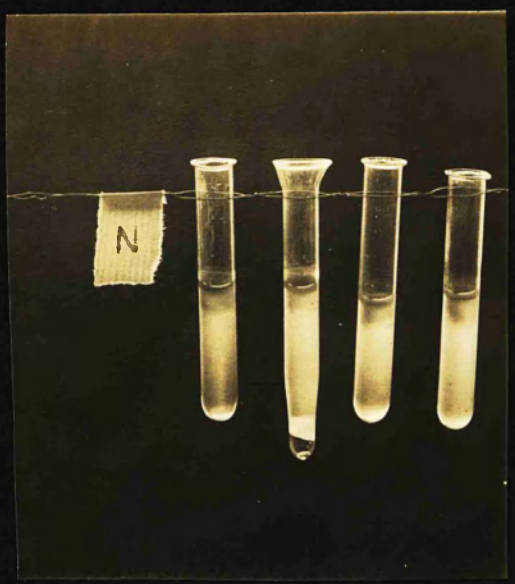
Tube 2. Extract of human blood serum from a stain exposed to a temperature of 140°C. for 1¼ hours.

Tube 3. Extract of human blood serum from a stain exposed to a temperature of 150°C. for ¼ hour.

Tube 4. Extract of human blood serum from a stain exposed to a temperature of 160°C. for ¼ hour.

All show positive reactions after the addition of anti-human serum.

PHOTOGRAPH NO. 24.



From left to right.

Tube 1. Extract of human blood serum from a stain which was exposed to a temperature of 100°C. and raised to a temperature of 160°C.

Tube 2. As in tube 1, but stain was exposed to a temperature of 100°C. and raised to a temperature of 170°C.

Tube 3. As in tube 1, but stain was exposed to a temperature of 100°C. and raised to a temperature of 180°C.

Tube 4. As in tube 1, but stain was exposed to a temperature of 100°C. and raised to a temperature of 200°C.

All show positive reactions after the addition of anti-human serum.

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