THE BIOLOGICAL OR PRECIPITIN TEST FOR BLOOD CONSIDERED MAINLY FROM ITS MEDICO-LEGAL ASPECT[1].

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CONTENTS.

A POINT of great importance in medico-legal practice is the differentiation of human from other bloods. In dealing with fresh stains no difficulty has been found in distinguishing mammalian from avian and reptilian bloods. The more difficult operation of differen-

[1] The work on that portion of this paper which deals with the detection of blood dried on various fabrics and leathers, and the effects of age, soil, and certain chemical reagents (qualitatively) was carried out by Mr Sanger, partly in conjunction with myself, as the subject of his Thesis for the Degree of M.D. Subsequently I repeated our previous work and added all the quantitative determinations. We are indebted to Dr Nuttall for suggesting the main lines of this investigation and for the aid he has given us in our work.

G. S. G.-S.

tiating between mammalian bloods, for example between human and pig's blood, had up till the introduction of the biological, or preferably precipitin, test, except with the freshest material, baffled the ingenuity of the expert in legal medicine. In 1901 Nuttall (1. VI. '01) was amongst the first to draw attention to the possible value of this test for blood and serum in forensic practice.

Uhlenhuth, Ziemke, Biondi, and others on the Continent have worked extensively on the medico-legal side of the question, and the test has now been used in the courts of Germany, Italy, Spain, Norway, Roumania, Egypt, and the United States.

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.

Since their discovery the study of precipitins has attracted many investigators, and in consequence an immense number of observations have been made on the subject. The greater part of this work has been undertaken to elucidate points of scientific interest, and has been carried out with fresh material under the most favourable conditions. A study of the literature, however, reveals the fact that the various authorities are not all in agreement on some points of practical importance, as for example the effects of varying strengths of salt-solution, and of acidity and alkalinity. In a paper to be published shortly Nuttall has summarised the literature on the "precipitins" and other allied bodies, and recorded the results of very extensive observations on "the blood-relationship of animals."

Uhlenhuth, Wassermann, Ziemke, Biondi, and others have directed their attention to the application of the precipitin test in forensic medicine, and have studied the effects of many of the conditions subject to which the test would have to be applied in practice. Their researches have conclusively established the value of this method of determining the identity of blood-stains under a number of more or less adverse circumstances[1].

Up to the present no work has been done in measuring accurately the amount of "precipitum" formed, and thereby estimating the effects of various reagents and conditions on blood to be tested, and on antisera.

In this paper we have endeavoured to determine by the quantitative method introduced by Nuttall (5. IV. '02) the effects of age, heat,

[1] Working with 1 in 100 dilutions of pure serum Nuttall (VI. 1901) has shown that it is possible to distinguish the several bloods in a mixture.

putrefaction, filtration, and contact with soils, and chemical reagents, both on normal blood sera, and also on antisera. We have also applied the test to blood dried on various materials on which it might be met with in forensic practice, and have in most cases been able to devise methods to eliminate the various sources of error, due to the nature of the substances on which the blood has been deposited.

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.

A summary of the most striking of the cases in which this test has been applied in medico-legal inquiries will be given later.

Methods of producing Antisera.

It has been found that the result of the injection of the serum of almost any animal into a rabbit is to produce in the blood of that rabbit a body, which brings about the formation of a precipitate, when the latter's serum is mixed with the diluted serum of the former. This precipitate is technically spoken of as a "precipitum," and the serum of the treated animal as an antiserum to the species of animal from which the injected blood was derived.

The technique of the preparation of antiserum has been fully described by many workers, and is exhaustively considered by Nuttall in his paper to be published shortly.

In these experiments the anti-human serum, and some of the others, were prepared by intravenous injection, but much smaller quantities than those usually employed were found to suffice[1]. For example 18 c.c. of human serum injected in doses of 5, 5, 5 and 3 c.c. at intervals of 2, 3 and 4 days produced a powerful anti-human serum. The animal was bled 14 days after the last injection. Continental workers have used quantities ranging to hundreds of c.cs., and have frequently found that their animals stood the operation badly, whereas the above animal

[1] Powerful anti-ox and anti-sheep sera were made by injections of 9·2 and 12 c.c. in doses of 1, 2, 1, 3·2, and 1·5, 2, 2, 3·5, and 3 c.c. respectively. The intervals between the injections were 4, 4, 2 and 5 days in each case, and the animals were bled 7 and 10 days after the last injections.

and most of the others we have treated, continued to gain weight, and appeared to be healthy. Some of our control antisera were prepared by the intra-peritoneal method and quantities ranging from 30 to 60 c.c. were used. The animals stood the treatment very well.

Methods of preserving Sera and Antisera.

Many methods have been adopted by various observers for storing sera and antisera. The plan which we have always adopted in the case of antisera is as follows:

The blood is collected with all precautions against contamination in a large sterile Petri dish, which is tilted after the formation of a firm clot. The serum, which is expressed from the clot, is drawn up into small tubes about ·5 cms. in diameter and 10 cms. in length with the ends drawn out into fine capillaries. When full the ends are sealed and the tubes stored in an upright position. By this method a few drops of antiserum can, if necessary, be used at a time, and the tube again sealed without contamination. We have not noticed that antisera so sealed, and preserved at room temperature in the light, lose their properties any sooner than those kept in the ice-chest in the dark.

Chloroform, Trikresol, and Carbolic Acid have been used as preservative agents by some observers.

Fluid sera when obtained in a sterile condition were usually sealed in glass bulbs without the addition of preservatives, and when received in a putrid condition were similarly stored after filtration through porcelain.

Some fluid sera, especially those which were likely to have been contaminated, were stored in bottles with closely fitting stoppers, and putrefaction checked by the addition of a few drops of chloroform.

Blood dried on filter-paper and various fabrics has usually been kept in the dark at room temperature.

Methods of testing.

Qualitative.

When fluid serum could be obtained in sufficient quantities dilutions of 1 in 21 in ·6% saline solutions were generally used for testing. For testing samples of blood dried on various materials extracts were made by placing blood-stained pieces of these for some hours in small

quantities (1—2 c.c.) of distilled water, and afterwards adding equal volumes of 1·2 % salt solution. These extracts were generally found to be slightly tinted and clear, and to foam well on shaking.

In testing such solutions about ·5 c.c. were placed in small test-tubes of about 1 c.c. capacity contained in racks provided with a black background, and one to two drops of antiserum were run into each tube. Owing to its greater specific gravity the antiserum immediately flowed to the bottom of the tube. When the antiserum corresponded to the blood which was being tested a white cloud appeared at the junction of the fluids within a few minutes, and gradually developed into a dense cloud. After a few hours a well-marked precipitum was found at the bottom of the tube. In the following experiments controls were used in all cases. For example when tests were being made from suspected human blood at least two tubes of the extracted blood were prepared, to one of which was added anti-human serum, and to the other anti-ox or other antiserum. Frequently three or more controls were employed, normal rabbit, anti-horse, anti-ox, anti-sheep, anti-dog, anti-hedgehog, and anti-turtle sera being used for this purpose. If human blood was alone present the cloudings and precipitum appeared only in the tube to which anti-human serum had been added. Occasionally very faint cloudings and traces of deposit occurred in other tubes, used as controls, to which mammalian antisera had been added, indicating the "mammalian reaction." Nuttall (21. XI. '01; 20. I. '02) was the first to call attention to this mammalian reaction, and to show that the precipitating antisera are not strictly specific, but produce reactions with the bloods of nearly allied species. He pointed out that the more powerful the antiserum the more likely it is to include in its action distantly related animals.

Except in the case of closely allied species the cloudings resulting from this cause are slight, and from the point of view of forensic practice in this country may be neglected, though in lands where old-world monkeys are common it might be necessary to be provided with antisera for the prevalent species.

In fact it may be said that except under abnormal conditions antisera produce marked cloudings with their homologous sera only. The causes of the abnormal conditions just mentioned, when all antisera react more or less markedly in the same way, will be dealt with later.

This may be called the qualitative method.

Quantitative method.

In medico-legal practice fluid material would but rarely have to be tested, and consequently the qualitative method just described would have to be relied on.

For estimating the effects of age, putrefaction, and chemical reagents on fluid sera and antisera, the quantitative method devised by Nuttall (5. VI. '02) has been employed. The method has been fully described by him, but a short description is necessary here.

By means of an accurately graduated pipette ·5 c.c. of a 1 in 21 dilution of serum in normal salt solution is placed in a small, clean, dry test-tube, and later ·1 c.c. of antiserum is run in. The fluids are then thoroughly mixed by inverting the test-tube with the mouth closed by a carefully dried finger. This mixture is allowed to stand for 24 hours, by which time the precipitum has settled to the bottom of the tube. Should any have adhered to the sides it can be removed by gentle rotation or tapping. The supernatant fluid is then pipetted off, and the precipitum drawn up into a capillary tube (Plate XI. fig. 1^{7-9}). One end of this is sealed, and it is allowed to stand for 72 hours for the precipitum to again settle. By means of the instrument devised by Nuttall the volume of the capillary tube occupied by precipitum can now be estimated.

When using this method controls treated in the same way with other antisera were employed on several occasions. In other experiments only qualitative controls were made use of.

It is obvious that in these manipulations there are many possible sources of error unless the greatest care is exercised; with accurate dilutions and measurements of the quantities of fluid added together, most of these can, however, be excluded. If the diameter of the capillaries used is fairly constant, the only marked source of error which remains is the condition of the precipitum. Some precipita are flocculent and occupy much space, others pack into a fairly solid mass. It has been noticed, however, that either flocculent or compactly formed precipita are produced by the serum under examination, and that unless the conditions of the experiment are markedly changed the same serum always produces the same kind of precipitum.

In order to determine the range of experimental error in carefully made observations series of experiments were carried out, the details of two of which are given below. The results in all cases, both here and elsewhere, are expressed in cubic centimetres.

Results of measurements of precipitatum from four samples of a 1 *in* 21 *dilution of human blood and* 8 *samples of a similar dilution of ox serum, to which* ·1 *c.c. of their homologous antisera had been added.*

Human serum		Ox blood	
·0281 c.c.	Mean ·0293 c.c.	·0215 c.c.	·0233 c.c.
·0281 ,,		·0225 ,,	·0233 ,,
·0300 ,,		·0233 ,,	·0233 ,,
·0309 ,,		·0233 ,,	·0262 ,,

Mean ·0233 c.c.

The fluctuations above and below the means in the human series are ·0012 c.c. or 4 %, and in the ox series ·0029 c.c. and ·0018 c.c. respectively or 12 % and 7 %.

In order to arrive at the most trustworthy figures possible in most experiments two observations were made under identical conditions in the hope that by this means the experimental error might be reduced to a minimum.

In measurements of this kind, made with every care, probably a margin of 10 % must be allowed for experimental error.

Throughout these experiments the aim has been to indicate by measurements the effects of varying conditions on the formation of the precipitum as compared with controls. It must be stated, however, that with every precaution the measurements of the same set of materials on different days are not identical, although the proportions which the various members of the set bear to each other remain fairly constant. Hence though improvements in the technique of measurement may result in more accurate and constant figures, yet it is improbable that the general results will be materially altered.

Sources of error connected with properly preserved (1) *Antisera and* (2) *Sera to be tested.*

(1) *Antisera.*

Opalescent antisera. Of the continental workers, Uhlenhuth has published warnings against the use of opalescent antisera, which give cloudings with nearly all bloods, as has also been found by Nuttall. Uhlenhuth (11—18, IX. '02, p. 680) considered that they might be due to the animals being bled too soon after a meal. In this laboratory animals have been bled at all times, and very few opalescent antisera have been met with. We have noticed that in some cases the animals from which these opalescent antisera have been obtained have suffered from disease of the liver caused by *Cysticerci*. The very large injections practised by some foreign observers may account for some opalescent sera.

Filtration through porcelain, as Uhlenhuth (11—18. IX. '02, p. 680) and Rostoski (1902 b., p. 29) have shown, produces no effect.

Whatever the cause of this opalescence may be the sera are certainly untrustworthy.

Very powerful antisera may lead to false conclusions from producing comparatively large reactions in allied, or even distantly related bloods. A control tube containing homologous blood in a dilution approximately equal to that of the blood under examination would sufficiently guard against any mistake from this source.

Very weak antisera could only lead to the error of making a negative diagnosis when its homologous blood was in reality present.

Certain antisera which have been stored even in sealed bulbs kept in the dark in the ice-chest develop the property of causing cloudings in all serum dilutions to which they are added. Under these conditions they become utterly worthless.

(2) *Sera to be tested.*

Nuttall found that two specimens of monkey blood of *Cynocephalus* and *Macacus* caused with human antiserum precipita nearly equal to those of human blood. On enquiry as to the cause of death it was found that the former had died of intussusception, and the latter of dysentery, both diseases tending to produce concentration of the serum during life.

Measurements with anti-human serum by Nuttall and Strangeways[1].

Human	serum	...	100 %	
Macacus	,,	...	90 ,,	died of dysentery
Ourang	,,	...	80 ,,	
Cynocephalus	,,	...	70 ,,	died of intussusception
Mandrill	,,	...	50 ,,	(healthy)
Cercopithecus	,,	...	50 ,,	(healthy)

These results were repeatedly confirmed by qualitative tests. Certain other diseases also tend to produce differences in the precipitum-forming power of the serum (p. 285).

It would be but seldom that such conditions could lead to error in forensic practice.

Fluid sera employed for qualitative measurements unless kept in

[1] The varying amount of precipitum obtained with the different bloods is expressed in %; the reaction given with homologous blood (human, in this case) being taken as 100 %. We are indebted to Dr Nuttall and Mr Strangeways for these (unpublished) figures.

sealed bulbs or closely stoppered bottles would be likely to become concentrated from evaporation, and give too high a reading.

Nuttall has also noticed that certain blood dried on filter-paper obtained from the tropics failed to go into solution and therefore gave no reaction.

Methods of diluting blood for the purpose of testing.

It has been noticed by many observers that solutions of fluid, or dried, sera in distilled water become cloudy, and that after 24 hours a precipitate occurs. In ·5 c.c. of a 1 in 21 dilution of human serum in distilled water this precipitate amounts to about ·001 c.c. We have, however, found that including this precipitate ·1 c.c. of human antiserum produces a smaller quantity of precipitum with blood diluted with distilled water than with the same specimen diluted with normal salt solution. The mean of three experiments in each case gave ·0384 c.c. of precipitum in salt solution dilutions and ·0328 c.c. in watery dilutions.

All observers are agreed that physiological salt solution (·6 %) is the best diluent, giving a clear solution which does not tend to cloud or deposit any material on standing.

Two opposite opinions, however, have been arrived at as to the influence on the reaction of increasing quantities of salt. Linossier and Lemoine (21. III. '02), taking 1 in 20 dilutions added increasing quantities of salt and found that even 1 % of salt impeded precipitation, and 5 % completely prevented it.

On the other hand Eisenberg (v. '02, p. 307) considers that even 18 % of salt has no influence, and Rostoski (1902, b. p. 42) found no noticeable difference with 10 %.

Our experiments agree more closely with those of the last observers. We have quantitatively estimated the influence of salt in the following way. Tubes containing 1 in 21 dilutions of human serum with gradually increasing percentages of salt were arranged in a rack, and to each ·1 c.c. of anti-human serum were added. We found that the precipita in the tubes containing the most salt were more flocculent, and owing to the increased specific gravity of the medium took longer to settle (Plate XI., fig. 2).

Results of measurements showed a slight decrease to 7 % and later an increase, probably due to the fact that the more flocculent precipitum, though really less in amount, occupies a greater volume.

Results of increasing quantities of salt in human serum dilutions.

Percentage of salt	Precipitum c.c.	Percentage of precipitum as compared with ·6 % salt solution	Percentage of salt	Precipitum c.c.	Percentage of precipitum as compared with ·6 % salt solution
·6 %	·0643	100 %	8 %	·0693	107·7 %
1 ,,	·0571	88·8 ,,	9 ,,	·0673	104·6 ,,
2 ,,	·0638	99·2 ,,	10 ,,	·0770	119·7 ,,
3 ,,	·0554	86·1 ,,	12 ,,	·0686	106·6 ,,
4 ,,	·0639	99·2 ,,	14 ,,	·0737	114·6 ,,
5 ,,	·0618	96·1 ,,	16 ,,	·0730	113·5 ,,
6 ,,	·0635	98·7 ,,	18 ,,	·0821	127·6 ,,
7 ,,	·0630	97·9 ,,	Saturated solution	·0854	132·8 ,,

Experiments with sheep and anti-sheep sera, which form more compact precipita, show the diminution in volume plainly. In this experiment the tubes were all centrifugalised for the same length of time in order to diminish the error due to the increasing specific gravity of the solutions.

Similar experiments to above with sheep serum.

Salt	Precipitum c.c.	Percentage of precipitum
1 %	·0199	100 %
2 ,,	·0203	102 ,,
4 ,,	·0201	101 ,,
10 ,,	·0140	70·3 ,,
Saturated solution	·0122	61·3 ,,

Qualitative estimations showed that when the quantity of salt was increased above 5 % the antiserum did not sink to the bottom, and that clouding occurred at the top of the tubes, and also took longer in forming.

The strength of dilutions used in quantitative and qualitative tests.

Strangeways, working in this laboratory, has found that dilutions of serum less than 1 in 10 do not give proportionately as much precipitum as lower dilutions. He considers that in such dilutions the precipita are apt to be redissolved, and states that they are more flocculent, and do not settle well owing to the high specific gravity of the fluid. He has ascertained by experiment that dilutions of 1 in 15 to 1 in 30 give proportionately larger quantities of precipitum which settle sooner and more compactly. We have worked throughout in quantitative observations with dilutions of 1 in 21, which are convenient to make and give very satisfactory results.

In most cases it would be impossible to estimate the strength of extracts from dry materials, but generally this must be rather low. Uhlenhuth (25. VII. '01) employs as controls dilutions of blood dried on glass for known periods, and matches as far as possible the tint of the control to that of the extract under examination. By means of a series of bloods of various kinds dried at different dates he is able to procure a control of about the same age as the material he is investigating.

The influence of temperature on the formation of precipitum.

Many authorities have published observations on this subject. Myers (14. VII. 1900) stated that the reaction took place rapidly at 37° C. Wassermann and Schütze (18. II. '01) and Michaëlis (9. X. '02, p. 734) confirm this observation, and Stockis (v. 1901) considers that 40°—42° C. is most favourable. Biondi (1902, p. 16) found the temperature to exert a considerable influence, but Linossier and Lemoine (1902) obtained reactions from 0°—58° C. Kister and Wolff (18. XI. 1902) contrary to all other observers state that there is no special difference in the reactions at room temperature and at 37° C.

Strangeways, working quantitatively, has shown that although the precipitum falls more rapidly at room temperature than at that of the ice-chest, and faster still at 37° C., yet finally the quantity of precipitum formed in each case is the same.

The relation of human serum to other body fluids.

Some of the materials used in these experiments had been preserved by the addition of a trace of chloroform for some months. This fact may render the figures given somewhat too low in comparison with fresh human serum. Specific reactions were produced, however, by all, though the reaction of amniotic fluid was very slight.

Anti-human serum No. I. was much more powerful than No. II., which moreover had undergone putrefaction.

Material	Anti-human No. I.	Anti-human No. II.	Percentages from means of these two	Anti-ox	Normal rabbit
1. Fresh human serum (2 days)	·0291 c.c.	·0197 c.c.	100 %	—	—
2. Old ,, ,, (8 months)	·0272 ,,	·0187 ,,	93·8 ,,	—	—
3. Placental serum (8 months)	·0150 ,,	·0112 ,,	54·5 ,,	—	—
4. Pleuritic exudate (2 weeks)	·0065 ,,	·0084 ,,	30·3 ,,	—	—
5. Hydrocele fluid (9 months)	·0046 ,,	·0037 ,,	16·7 ,,	—	—
6. Fluid from ovarian cyst (9 months)	·0018 ,,	trace	6·1 ,,	—	—
7. Amniotic fluid (9 months)	·0009? ,,	trace	3· ,, ?	—	—

Most observers have noticed that other body fluids, normal and pathological, as for example, pleuritic and ascitic exudates, hydrocele, ovarian, spermatocele and seminal fluids, synovia, albuminous and menstrual urine react with human antiserum; and in addition Nuttall (VI. 1901) observed that slight cloudings resulted with normal urine, as well as with nasal and lachrymal secretions.

The influence of age on blood to be tested.

Several observations have already been made on this subject. Uhlenhuth (25. VII. '01) found that dried human blood-stains 6 to 12 years old reacted with human antiserum in one minute. Biondi (1902) obtained positive reactions with dried human blood-stains 10—15 years old, but not with a specimen 20 years old. Ziemke (1901), however, was able to identify blood-stains 25 years old.

In order to make observations on this question we obtained blood-stained material from Scotland Yard, the museum of which contains the largest collection of forensic specimens in this country. The samples we obtained and tested have been tabulated in two classes according to the articles on which the blood was dried. The first class consists of metal, and the second of fabrics and leathers.

Blood dried on metal.

The results of experiments on 17 samples covering a period of 30 years, are arranged according to age in the following table. The number after each specimen refers to the catalogue of the Scotland Yard museum, and a short description of each is given in the appendix.

The reactions of all were neutral.

The following table refers entirely to weapons which had been preserved from rusting by the application of oil to the surface of the metal. This process had caked the blood into black masses, making it frequently difficult to say whether the mass consisted of blood and oil or rust and oil. In the majority of cases however it was possible to make certain of scraping off some blood. The material thus obtained was extracted with distilled water, and subsequently an equal volume of 1·2 % salt solution added to it. If necessary the solution was filtered through filter-paper, and tested in the way described.

Excellent results were obtained from these materials, and showed conclusively that the property of producing a precipitum with its appropriate antiserum is not lost by blood dried on metal even after 30 years have elapsed.

Instrument from which the blood was obtained	Age	Foam test*	Result with anti-human serum	Control serum	Remarks
1. Razor	5 months	good	marked cloud, 5 mins.	anti-turtle nil	This had not been preserved with oil.
2. Knife	few months	,,	,, ,,	,,	,, ,, ,,
3. Pocket-knife (554)	9 months	,,	marked cloud, 1 hour	anti-dog nil	,, ,, ,,
4. Razor (550)	10 ,,	,,	,, ,,	,,	,, ,, ,,
5. Hatchet (539)	1 year	,,	cloud, 20 mins.	,,	,, ,, ,,
6. ,, (544)	1 ,,	,,	marked cloud, 1 hour	,,	Much oil. Blood obtained from a crevice in the hatchet.
7. Pocket-knife (530)	1½ ,,	,,	marked cloud, 10 mins.	,,	Thin layer of oil.
8. Dagger in sheath (524)	1½ ,,	,,	,, ,,	,,	No oil.
9. Two knives (525)	1½ ,,	,,	,, ,,	,,	Very little oil.
10. Knife (494)	3 years	slight	,, ,,	,,	Much oil. On first occasion no reaction. Instrument again scraped; reaction good.
11. Chopper, knives (491), and oil-can	5 ,,	,,	nil	,,	Owing to the amount of rust and oil present it was impossible to say whether any blood had been scraped off.
12. Razor (423)	6 ,,	good	cloud, 20 mins.	anti-turtle nil	
13. ,, (357)	10 ,,	,,	cloud, 45 mins.	,,	See hat, Table p. 272, No. 2.
14. Pocket-knife (547)	11 ,,	fair	slight cloud, 1 hour	anti-dog nil	
15. Knife (17)	28 ,,	slight	marked cloud, 15 mins.	,,	Tried also with very strong anti-deer serum to see if any ruminant blood present; no reaction.
16. Razor (20)	28 ,,	,,	cloud, 15 mins.	,,	Much blood and oil.
17. ,, (19)	30 ,,	,,	marked cloud, 3 mins.	,,	Thickly smeared with blood; little oil.

* "Foam-test" refers to whether or no the blood dilution foamed. As Nuttall has found, it is a valuable aid to determining that blood has gone into solution.

In one case, No. 11, however, no reaction was obtained; the negative result was probably due to little or no blood being present on that part of the knife which was examined. The condition of the weapon was such that it was impossible to be certain that the material scraped from it was blood, but it was thought better to include it in the series, so as to point out the possibility of a mistake occurring under such circumstances.

It appears that the effect of oil on blood is to lessen the reaction. This is probably due to the blood being coated with a film of oil, and therefore not so easily passing into solution.

Blood dried on organic materials.

The experiments quoted below have been inserted here to show the effects of age on blood dried on organic fabrics, but further experiments (p. 287) indicate some of the fallacies which may arise from the character of the materials. It happened, however, that in the specimens chosen few were of such a character as to give rise to possibilities of error.

The blood-stained materials tabulated below were all obtained from Scotland Yard, and with them two series of tests were conducted, the antiserum employed in the second being more powerful than that in the first.

In the first series very small quantities were employed, but in the second the amount in each case was slightly greater. It was, however, not found possible on either occasion to obtain more than very small fragments, and, moreover, none of the specimens, with the exception of No. 11, were markedly encrusted with blood. The one exception, a specimen of hair, 28 years old, was in some parts thickly plastered, and gave well-marked reactions with each antiserum.

The following table shows that numbers 1, 2, 3, 4, 6, 8, and 11, or, 64% of the whole, gave well-marked reactions, their ages varying from 3 to 28 years. In No. 5 the paper was badly burnt and the capacity for reacting was probably destroyed by the heat. Nos. 7 and 10 produced alkaline solutions, and in each case the reaction with anti-human serum was very slight. This was probably due to the retarding influence of the alkali, which will be discussed later. At the time these experiments were carried out we were not aware of this action of alkalis. The negative result of No. 9 may have been due to its acidity. No. 12 failed to react, but we were unable to discover any reason for this. The controls in all cases were negative.

Material	Age	Foam test	Character of solution	Reaction to litmus	1st series		2nd series	
					Anti-human I.	Normal rabbit	Anti-human II.	Anti-ox
1. Lining of clothes	3 years	good	clear	neutral	marked cloud, 60 mins.	nil		
2. Felt hat	10 ,,	,,	cloudy, clear after filtering	,,			cloud, 15 mins.	nil
3. Printed paper	11 ,,	,,	clear	alkaline	cloud, 5 mins.	nil	cloud, 60 mins.	,,
4. Part of same paper	11 ,,	,,	,,	neutral			cloud, 15 mins.	,,
5. Same scorched	11 ,,	,,	,,	,,	nil	,,	nil	,,
6. Alpaca dress	11 ,,	,,	,,	,,	immediate cloud	,,	marked cloud, 10 mins.	,,
7. Braid	11 ,,	,,	,,	alkaline	slight cloud, 60 mins.	? cloud	slight cloud, 60 mins.	,,
8. Cardigan jacket	11 ,,	fair	cloudy, clear after filtering	,,			marked cloud, 30 mins.	,,
9. "Black Rep."	11 ,,	slight	clear	slightly acid	slight cloud, 60 mins.	nil	slight cloud, 15 mins.	,,
10. Cotton fabric, apparently washed	11 ,,	fair	,,	alkaline	slight cloud, 5 mins. no increase	,,		
11. Hair	28 ,,	good	,,	neutral	cloud, 30 mins.	,,	marked cloud, 5 mins.	,,
12. Wooden handle of chopper	28 ,,	none	,,	,,			nil	,,

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As so little material was available the results may be looked upon as most satisfactory, for it can scarcely be doubted that more distinct reactions would have been obtained had it been possible to make more extensive use of the specimens.

Dried and Fluid Sera preserved in the Laboratory.

Experiments undertaken with 10 specimens of human blood preserved on filter-paper in the laboratory of ages ranging between two years and two days showed by the qualitative method no obvious differences, either in the rate or degree of clouding, on the addition of anti-human serum. Controls with anti-ox and anti-sheep sera were negative.

A few quantitative experiments quoted below made on fluid sera, preserved by sealing in glass bulbs, indicate that such sera lose their strength to some extent, though differences exist in the rate at which this occurs.

	Anti-human No. I.	Anti-human No. II.	Per-centage	Anti-ox	Normal rabbit
Human serum (1 week)	·0291 c.c.	·0197 c.c.	100 %	—	—
,, ,, (9 months)	·0272 ,,	·0187 ,,	93 ,,	—	—
	Anti-ox			Anti-human	
Ox serum (mean of 8 exps., p. 264) sealed 1 year	·0233 c.c.		100 %	—	
,, ,, (mean of 3 exps.) sealed 2 years	·0239 ,,		102 ,,	—	
	Anti-fowl's egg, No. I.	Anti-fowl's egg, No. II.			
Fowl's egg albumen (2 days) ..	·0254 c.c.	·0162 c.c.	100 %	—	
,, ,, (9 months) ...	·0160 ,,	·0112 ,,	67 ,,	—	
,, ,, (14 ,,) ...	·0225 ,,	·0144 ,,	88 ,,	—	

Antidiphtherial horse serum four years and six months old preserved with trikresol was found to produce a good but somewhat flocculent specific precipitum amounting to ·0572 c.c.

In the above experiments anti-human serum No. I. was only a few days old, whereas No. II. was three and a half months old, and was moreover contaminated by bacterial growths. The first anti-fowl's egg serum was quite fresh and the second three months old.

All sera of the same kind do not give with the same antiserum identical precipita, nor even the sera of the same individual at different times in some cases, consequently an accurate determination of the influence of age is not possible. Our experiments however seem to point to a slight decrease in strength as the result of age, the human

serum and fowl's albumen experiments showing a decrease of precipitum of 7 % and 12 % after 9 and 14 months respectively. The fowl's albumen kept for 9 months shows a decrease of 33 %. It is, however, by no means easy to get accurate dilutions of egg albumen, and the relative weakness of the specimen may be due to this cause.

The two experiments just quoted also indicate that antisera lose some of their power, but not to the extent that some observers have stated. Some undoubtedly preserve their power of producing specific reactions after the lapse of 12 months. Others lose this property more rapidly, whilst some, as Nuttall has also found, become untrustworthy after a time, giving cloudings with all sera.

In considering the general results of these tables it appears that in the case of dried bloods time *per se* does not destroy their capacity for reacting with their own antisera. Judging from the control experiments with recently dried bloods we should think that the period between the addition of the antiserum and the formation of the cloud was increased, and the magnitude of the cloud diminished.

Fluid sera appear to deteriorate at any rate in some cases by keeping. It has been occasionally observed, however, in qualitative tests that old sera appear to react better than fresh ones.

The influence of putrefaction on sera and antisera.

Several observers have noted that blood even after putrefaction retains its power of forming a precipitum with its homologous antiserum. Uhlenhuth (1901), Nuttall (1901), and Biondi (1902), all obtained good reactions with putrid blood. Following a suggestion of Dr Nuttall's, in order to determine the influence of specific bacteria on serum, 1 in 21 dilutions in salt solution of ox and horse serum were inoculated with a series of organisms. Undiluted human pleuritic exudate was similarly treated. All were incubated for 5 days at 37° C. and then left at room temperature for 36, 50, and 40 days respectively; but the horse serum was allowed to undergo natural putrefaction also for the last 10 days. With the exception of the putrefactive bacteria none gave rise to very considerable growth, and in nearly all cases by the time of examination the organisms had sunk to the bottom, leaving the supernatant fluid clear. When necessary the fluids were filtered through filter-paper. All were slightly alkaline or neutral in reaction.

	Human pleuritic exudate (1—11)			Ox serum (1—21)			Horse serum (1—21) (contaminated)		
	Anti-human	%	Control anti-ox	Anti-ox	%	Control anti-human	Anti-horse	%	Control normal rabbit
	c.c.			c.c.			c.c.		
Control. No organisms	·0234	100	—	·0173	100	—	·0572	100	—
Putrefactive organism No. I.	·0280	119·6	—	·0140	80·9	—	·0713	124·4	—
,, No. II.	·0280	119·6	—	·0112	64·7	—	·0525	91·7	—
,, No. III.	·0280	119·6	—	—	—	—	—	—	—
Streptococcus	—	—	—	·0163	94·2	—	—	—	—
Putrefactive organism No. IV.	·0215	91·8	—	·0163	94·2	—	·0666	116·4	—
B. anthracis	·0206	87·8	—	·0150	86·7	—	·0591	103·3	—
Hofmann's bacillus	·0187	87·8	—	—	—	—	—	—	—
B. subtilis	·0187	80·0	—	—	—	—	—	—	—
B. typhi	·0187	80	—	·0140	80·9	—	·0657	114·8	—
B. diphtheriae	·0187	80	—	—	—	—	·0670	117·1	—
Putrefactive organism No. V.	·0187	80	—	·0084	48·7	—	·0582	101·1	—
Staphylococcus albus	·0187	80	—	·0169	97·6	—	·0754	111·5	—
B. coli	—	—	—	·0131	75·7	—	—	—	—
V. of cholera	—	—	—	·0112	64·7	—	·0670	117·1	—

In considering the above table in detail it is seen that the effects of various organisms on ox and human serum agree fairly closely with a few exceptions. The most striking are the putrefactive organisms I, II, and V. These differences may be due to the fact that growth in nearly all cases was less marked in the undiluted human, than in the diluted ox serum, the latter moreover was a year old and had been preserved in sealed tubes after filtration through porcelain. The effects on horse serum of the action of specific organisms combined with general putrefaction for 10 days agree with those of putrefactive organisms I, II, and III, on ox serum, in that the capacity for forming precipitum is increased.

It appears then from the few quantitative experiments we have made that the results of bacterial growth on sera differ, some reducing the quantity of precipitum produced and others raising it, neither action being however very marked. Such slight changes as do occur do not alter the specific character of the reaction.

Experiments were also made on human and other sera which had undergone natural putrefaction. Most of the materials had been kept for some time and consequently show the combined results of age and putrefaction.

	Material		Anti-human No. I.	Anti-human No. II.	Per-centage	Anti-ox	Normal rabbit
1.	Fresh human serum	(2 days)	·0291 c.c.	·0197 c.c.	100	—	—
2.	Old ,, ,,	(9 months)	·0272 ,,	·0187 ,,	93·8	—	—
3.	Putrid ,, ,,	(5 months)	·0262 ,,	·0169 ,,	88·1	—	—
4.	Putrid ,, ,,	(8 months)	·0150 ,,	·0140 ,,	59·4	—	—
5.	Putrid ,, ,,	(9 months)	·0131 ,,	·0150 ,,	57·4	—	—
6.	Putrid placental ,,	(9 months)	·0150 ,,	·0112 ,,	53·7	—	—
				Anti-ox		Anti-human	
7.	Ox serum	(1 year old) mean of 8 exps.		·0233 c.c.	100	—	
8.	Ox serum, putrid (,,	,,) mean of 3 ,,		·0233 ,,	100	—	

The above table shows that in some cases advanced natural putrefaction seems to exert little influence, for although the precipitum is decreased considerably in Nos. 4, 5, and 6, yet this is not the case in Nos. 3 and 8. All the specimens had been putrefying for the time given in each case. Though time may have influenced Nos. 4, 5, and 6, it is more probable that organisms whose growth deleteriously affected the serum were present.

Finally, from the few experiments we have done we are of the opinion that putrefaction to almost any extent does not affect the specific precipitum-forming body.

Since blood dried in small quantities does not undergo putrefaction to any appreciable extent this factor may be neglected in ordinary medico-legal work.

Experiments already quoted (p. 273) with contaminated anti-human and anti-fowl's albumen sera demonstrated that putrefaction in sealed tubes does not affect the antibody in them, as has also been found by Nuttall.

An experiment conducted on the same blood dilution with a normal and a contaminated sample of the same antiserum gave as a mean of four estimations in each case ·0433 c.c. and ·0436 c.c. of precipitum respectively.

Moreover putrid (filtered) sera when injected produce, as several of us have found, powerful and specific antisera, and Strangeways has shown that the power of antisera made with similar doses of fresh and putrid filtered sera is nearly identical.

The detection of blood in the presence of lime, mortar, and earth.

The wide distribution of these substances rendered it necessary to investigate their action on blood, since in medico-legal practice it might

often be necessary to test blood dried on, or mixed with, these materials.

Solutions of earthy salts, mortar, and lime of various strengths were made in salt solution and tested qualitatively with various antisera to determine their action on serum. These actions vary to some extent with the quantity of serum added. In the following table the quantity added was one drop, since this was the unit chosen for qualitative experiments.

In this and other tables the following symbols have been used:

C = coagulation.
+ = marked cloud—full reaction.
\+ = less marked cloud.
× = medium cloud.
* = slight cloud.
*? = very slight cloud.

D = large deposit after 24 hours.
D = smaller ,, ,, ,,
d = smaller ,, ,, ,,
tr = trace of deposit.
• = no result.
— = no reaction.

Dilutions		Lime	Mortar	Calcium chloride	Calcium phosphate	Chalk	Sodium phosphate	Plaster of Paris	Alum	Caustic soda	Caustic potash
Saturated solution[1]	30 mins.	*	*	×	*	*	*	*			
	24 hrs.	d	d	d	*	d	d	*			
									1—25		
1—10		*	*	*?	*?	•	*	•	+	*	*
		*	*	*	•	•	•	•	**D**	*	*
1—100		•	•	•	•	•	•	•	×	×	×
		•	•	•	•	•	•	•	×	×	×
1—1000		•	•	•	•	•	•	•	×	*	*
		•	•	•	•	•	•	•	×	•	•
1—10,000		•	•	•	•	•	•	•	*	•	•
		•	•	•	•	•	•	•	•	•	•

The addition of serum to strong solutions of lime resulted in a general clouding, which later gave place to a dense cloud below, which would be hard to distinguish from a positive reaction. Mortar gave rise to a similar but smaller clouding. Calcium chloride and sodium and calcium phosphates caused cloudings in very strong solutions only. The actions of caustic soda and potash in certain solutions are very marked, and will be referred to again later. They are briefly mentioned here owing to their presence in earth.

At this point it should also be noted that strong lime and calcium solutions give rise on standing, even after filtration, to deposits of the salt at the bottom of the tube and a filmy layer on the surface.

[1] Where saturated solutions are mentioned the dilutions are 1—10 etc. of these.

It was found, however, that the difficulty in testing due to the presence of lime in mortar, plaster, and earth, could generally be eliminated in the following way. Solutions of all of the above substances were allowed to stand till the excess had settled to the bottom. The supernatant fluid was then pipetted off and filtered. Carbon dioxide gas generated by the action of dilute hydrochloric acid on chalk, and washed by passing through distilled water, was next passed through the fluid and the latter again filtered to free it from the presence of the precipitated calcium carbonate. By this procedure clear filtrates could be obtained in most cases, which remained so for an indefinite period, and produced no cloudings on the addition of sera.

The following formula explains the reaction :

$$Ca(OH)_2 + CO_2 = Ca\,CO_3 + H_2O.$$

Too much of the gas must not however be passed into the solution owing to the fact that excess of CO_2 renders the insoluble carbonate again soluble :

$$Ca\,CO_3 + H_2O + CO_2 = Ca\,H_2(CO_3)_2.$$

By quantitative experiments it was found that though this process caused a deposition of blood pigment from blood solutions, yet the property of producing precipitation on the addition of appropriate antisera was not in any way affected.

The action of dry and wet lime, etc.

Lime was intimately mixed with human blood and then spread on porcelain and exposed to the action of air for three months. The resulting compound turned a greenish colour. Solutions of this gave an immediate clouding on the addition of serum. After the passage of CO_2, however, and subsequent filtration, no reaction could be obtained with anti-human or other serum. Under these conditions it seems that unslaked lime completely destroys the reacting power of blood in contact with it.

Quantitative experiments over a shorter period bring out the destructive quality of lime and mortar very markedly. To ascertain the action on serum of dry and wet lime, mortar, brick, earth, etc. weighed quantities of one gramme of each were mixed with 1 c.c. of human serum and allowed to act for 4 days. Similar mixtures but with 10 c.c. of water added were also prepared and allowed to stand for 4 days, to determine whether any different action was excited by these

materials in the presence of water. At the end of this period all were made up to 1—21, by the addition in the former case of 20 c.c. of normal, and in the latter of 10 c.c. of double normal salt solution.

After applying the method of removing lime which has just been described quantitative estimations were made.

	Material		Anti-human	Anti-ox	%
1.	Control		·0403	—	100
2.	Chalk	dry	·0367	tr.	91
		wet	·0357	tr	89
3.	Red brick	dry	·0281	tr	70
		wet	·0347	tr	86
4.	Pasteur filter	dry	·0309	tr	77
		wet	·0319	tr	79
5.	Berkefeld filter	dry	·0291	tr	72
		wet	·0291	tr	72
6.	Earth	dry	·0244	tr	61
		wet	·0291	tr	72
7.	White brick	dry	·0262	tr	65
8.	Mortar	dry	tr	tr	0
		wet	·0028	tr	7
9.	Lime	dry	tr	tr	0
		wet	tr	tr	0

The above table shows that all the materials used in this series produced slight effects on the serum, but that mortar and lime completely destroyed its power of reacting. All had been ground up very finely before the addition of the serum.

The effects of the lime present in ordinary earths.

The next point of importance was to determine whether the amount of lime present in ordinary earths was sufficient to interfere to any serious extent with the reaction. For this purpose 9 samples of analysed earth were obtained, five from a field of gravelly land near Trowse, divided into five plots, and the others from different localities. The results of the analyses of these earths dried at 100° C. are given in the following table, arranged according to the percentage of lime present. We are indebted to Mr T. B. Wood for these analysed earths.

	I. Trowse, plot 1	II. Trowse, plot 2	III. Trowse, plot 3	IV. Trowse, plot 4	V. Wryde clay	VI. Needham salt	VII. Trowse, plot 5	VIII. Bentwick fen	IX. Littleport fen
Total lime	·91	1·01	1·43	1·46	1·48	1·57	1·97	2·95	4·39
Organic matter, loss by ignition	5·62	5·16	5·27	5·52	14·48	5·40	6·31	39·35	50·82
Calcium carbonate	1·16	1·39	1·94	1·98	—	—	3·06	—	—
Total phosphoric acid	·16	·18	·16	·18	·37	·19	·20	·30	·28
Total potash	·13	·14	·13	·14	1·32	·31	·13	·63	·56
Total nitrogen	·15	·11	·14	·53	·24	·24	·13	1·42	1·8

Strong solutions of the above after simple filtration were at first clear but showed a white filmy deposit after standing. The quantity of

this increased with the percentage of the lime present. On the addition of anti-human serum a thin cloud spread through the entire solution and gradually deepened, being considerably denser in No. IX than in No. I.

After the passage of CO_2 and filtration every solution was clear, with the exception of V and VIII, and produced no deposit on standing. The two mentioned were opalescent. No clouding occurred on the addition of anti-human serum.

Two sets of quantitative experiments were carried out with these soils. In the first 1 c.c. of finely divided soil was placed in a test-tube with 1 c.c. of human pleuritic exudate and 5 c.c. of water. After 4 days 5 c.c. of double normal salt solution were added to each, making a dilution of 1 in 11 of pleuritic exudate. In the second series 1 c.c. of dry earth was allowed to act for 4 days on 1 c.c. of pleuritic exudate. At the end of this period the specimens were diluted to 1 in 11 with salt solution.

These solutions were treated with CO_2 as described, and the precipita measured quantitatively.

Earth solution	Percentage of lime	Quantity of precipitum mean of the two observations	Percentage	Control anti-ox
No. I.	·91	·0404	100	—
No. II.	1·01	·0394	97·5	—
No. III.	1·43	·0319	78·9	—
No. IV.	1·46	·0389	96·2	—
No. V.	1·48	·0241	59·6	—
No. VI.	1·57	·0258	63·3	—
No. VII.	1·97	·0389	96·2	—
No. VIII.	2·95	·0314	77·7	—
No. IX.	4·39	·0383	94·8	—

The above table shows that the quantity of precipitum obtained did not decrease in proportion to the increase of the lime, which was apparently never present in sufficient quantity to materially affect the reaction. Excess of potash probably accounts for the low figures obtained in No. V, and possibly No. VIII (p. 284). In neither of these could a clear solution be obtained. Whatever may be the cause of the variations in the quantity of precipitum obtained, these experiments go to show that blood mixed with ordinary earth can be readily detected if present in sufficient quantity and that its specific character remains unaltered.

From our experiments on earth and lime salts we have drawn the following conclusions: (1) that the intimate mixture of lime with blood

completely destroys the latter; (2) that a clouding occurs in the solution of earth on the addition of serum; (3) that this is due principally to the presence of lime salts; (4) that the lime can be got rid of and the solution rendered clear, and not liable to clouding, by the passage of CO_2 and subsequent filtration; (5) that the passage of CO_2 in no way interferes with the reaction; (6) that the quantity of lime present in ordinary earth does not materially affect blood mixed with it.

The influence of chemical agents.

Observations on the reaction to litmus of extracts of coarse cloths and leather, as well as the possibility of the treatment of blood-stains in forensic practice with chemical reagents, made it desirable to investigate the action of such reagents on blood.

Acids.

Several experiments were made with dilutions of both organic and inorganic acids in distilled water and salt solution. Dilutions from 1 in 10 to 1 in 100,000 were tested by dropping in serum and noting the effects up to 2 hours and after standing for 24 hours. In these observations one drop of antiserum was added to about ·5 c.c. of the dilution since this has been the quantity uniformly used in qualitative work.

The addition of larger quantities produced slightly different results, probably owing to the alkalinity of the serum itself, and moreover, perhaps for the same reason, the effects of different sera were noticed to vary slightly. This remark applies to all the following experiments of a similar nature.

With the inorganic acids a noteworthy phenomenon was observed. Strong solutions (1 in 10) in salt solution caused coagulation of the serum and destruction of the precipitating substance, whereas weak solutions (1 in 100) produced no result. Dilution between 1—500 and 1 in 10,000 caused more or less clouding, in the latter case taking place half-way up the tube. These cloudings probably resulted from the precipitation of the albumen by the dilute acid and were observable within a few minutes. With greater dilutions nothing occurred. It was also found that neutralisation previously with sodium carbonate prevented these cloudings and in some cases even dissolved them after they had been formed.

One example is given below in detail.

Dilutions of Nitric Acid in normal salt solution	Anti-human serum		Anti-human serum after neutralisation	
	1 hour	24 hours	1 hour	24 hours
1—10	coagulation	coagulated mass	—	—
1—100	very faint cloud	cloud	—	—
1—1000	marked cloud	cloud	—	—
1—10,000	slight cloud half-way up tube	—	—	—
1—100,000	—	—	—	—

The precise degrees of clouding caused by the various dilutions differ with different acids.

Some organic acids behaved differently, acetic, oxalic, and tartaric causing little clouding in dilutions of 1 in 10, but marked cloudings in 1 in 100 and in 1—1000.

Details of the action of tartaric acid :—

Dilutions of Tartaric Acid in salt solution	Anti-human serum		Anti-human serum after neutralisation	
	1 hour	24 hours	1 hour	24 hours
1—10	very slight cloud	slight cloud	—	—
1—100	,, ,, ,,	medium cloud	—	—
1—1000	medium cloud	marked cloud	—	—
1—10,000	—	slight cloud half-way up tube	—	—
1—100,000	—	—	—	—

The accompanying table shows the chief actions of acid dilutions in salt solution on serum. These effects are more marked when the dilutions are made in distilled water.

		Sulphuric	Nitric	Hydrochloric	Acetic	Oxalic	Tartaric	Picric	Carbolic	Citric	Salicylic sat. sol.	Chinosol
1—10	30 mins.	C	C	+	•	✱	✱	•	C	•	+	•
	24 hrs.	D	D	D	•	✱	✱	•	D	•	D	•
1—100		•	✱	•	✱	✱	✱	C	✱	✱	✱	✱
		•	✱	•	×	✱	✱	D	d	✱	•	✱
1—1000		✱	×	×	✱	×	×	+	•	×	•	✱
		✱	×	×	×	×	×	d	•	✱	•	✱
1—10,000		✱	✱	✱	•	✱	•	✱	•	✱	•	•
		•	•	•	•	•	•	•	•	•	•	•
1—100,000		•	•	•	•	•	•	•	•	•	•	•
		•	•	•	•	•	•	•	•	•	•	•

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Strong alkalis and salts.

Experiments with the more powerful alkalis showed that in strong solutions cloudings were also produced in them on the addition of serum. Ziemke (17. VIII. 1901) has recommended the use of ·1 % caustic soda in distilled water for extracting blood-stains under certain conditions. Our observations show that in such dilutions cloudings are apt to occur on the addition of any antiserum, and render it thus an unsuitable agent for the process. These cloudings are better marked in dilutions in distilled water that with those in salt solution.

The effects of dilutions of caustic soda are given in detail below :—

Solutions of caustic soda in salt solution	Anti-human serum	
	1 hour	24 hours
1—10	slight cloud	slight cloud
1—100	medium cloud	medium cloud
1—1000	slight cloud	—
1—10,000	—	—
1—100,000	—	—

The following table shows the actions of dilutions of alkalis and salts on serum :—

		Caustic soda	Caustic potash	Sodium carbonate	Ammonia	Ammonium sulphate	Ammonium tartrate	Sodium & potassium tartrate	Sodium acetate	Potassium cyanide 1—20	Sodium citrate	Magnesium sulphate	Potassium nitrite	Potassium chlorate 1—20	Borax
1—10	30 mins.	*	*	·	·	*	*	*	*	*	·	·	·	·	
	24 hrs.	*	*	·	·	*	*	·	*	*	·	·	·	·	
1—100		×	×	·	·	·	·	·	*	*	·	·	·	·	*
		×	×	·	·	·	·	·	·	*	·	·	·	·	·
1—1000		*	*	·	·	·	·	·	·	·	·	·	·	·	·
		·	·	·	·	·	·	·	·	·	·	·	·	·	·
1—10,000		·	·	·	·	·	·	·	·	·	·	·	·	·	·
		·	·	·	·	·	·	·	·	·	·	·	·	·	·
1—100,000		·	·	·	·	·	·	·	·	·	·	·	·	·	·
		·	·	·	·	·	·	·	·	·	·	·	·	·	·

Owing to the absence of any bad results from the addition of serum to sodium carbonate dilutions, we chose this reagent as being the most suitable for neutralising the effects of acids.

We next made some experiments to ascertain to what extent the acidity or alkalinity of the medium influenced the specific reaction. For this purpose both quantitative and qualitative experiments were conducted. The solutions in each case were made up in the following

way. Three series of 11 tubes were prepared, each containing ·5 c.c. of a 1 in 21 dilution of human serum in salt solution. To the first tube were added 5 drops of a solution of acid, to numbers 2, 3, 4 and 5, were added 4, 2, 3 and 1 drops of acid respectively. The sixth tube was not treated in any way. Numbers 7 to 11 received 1 to 5 drops of alkali respectively.

In the first series very small drops of a 1 in 100 dilution of hydrochloric acid was used, in the second series large drops of the same solution, and in the third series large drops of 1 in 10 solution of the same acid. Large drops of corresponding dilutions of sodium carbonate were used in series two and three.

In the quantitative experiments ·1 c.c. of anti-human serum was run into each tube, and in the qualitative one drop.

		Series I.			Series II.			Series III.	
No. of tube		Drops	Small drops	%	Large drops	%			
1.	1 in 100 Hydrochloric Acid	5	·0309	(61)	·0009	(2)	1 in 10 Hydrochloric Acid	nil	(0 %)
2.		4	·0319	(63)	·0018	(4)		,,	,,
3.		3	·0431	(85)	·0140	(27)		,,	,,
4.		2	·0431	(85)	·0187	(37)		,,	,,
5.		1	·0478	(93)	·0422	(83)		,,	,,
6.	Normal dilution of serum		·0507	(100)	·0510	(100)		·0516	(100 %)
7.	1 in 100 Sodium Carbonate				·0422	(83)	1 in 10 Sodium Carbonate	·0169	(31 ,,)
8.					·0441	(86)		·0150	(29 ,,)
9.					·0422	(83)		tr	?
10.					·0469	?		nil	(0 %)
11.					·0591	?		,,	,,

In series II the acidity and alkalinity varied from about 1—1000 to 1—5000 and in series III from about 1—100 to 1—500.

These experiments show that the presence of even small quantities of acid or alkali rapidly reduce the quantity of precipitum formed (see Plate XI., fig. 5). The apparent exceptions of numbers 10 and 11 of series II are due to the fact that the precipita produced were more flocculent and occupied more space than the more compact precipita elsewhere obtained. They also indicate that the presence of small quantities of acid or alkali do not alter the specificity of the reaction, for controls with anti-sheep serum were all negative.

Qualitative experiments undertaken on the same lines showed that with 1 in 100 solutions of acid and alkali, cloudings first occurred in the normal tube, next in the alkaline series, the times of their appearance increasing from No. 6 to 11. The last two showed faint traces of the specific reaction and also general opacity. On the acid side cloudings due to the acid rapidly appeared, but later specific cloudings were

superadded in Nos. 3 to 5. Control tubes tested with anti-ox serum showed general opacity in the last of the alkaline series and slight clouds in the acid series. Similar experiments with 1 in 10 solutions showed cloudings in the normal serum and first three specimens of the alkaline series only.

In the light of these observations it becomes necessary to test the reaction to litmus of all solutions which are to be examined and, if found decidedly acid or alkaline, to neutralise them.

It must also be remembered that the addition of strong acid or alkali to fluid or dried blood completely destroys it.

The effects of disease on the precipitum-forming power of serum.

Our experiments on this subject are only three in number but suggest that important differences may be found in diseased blood by means of this test. The following observations were made on sera from tuberculous cattle. The first required 2 c.c. of decinormal caustic soda per 100 c.c. of serum to give a pink tint with phenolphthalein, and the others 1·25 c.c. and ·8 c.c. respectively. Also the former required per 100 c.c. per 4·25 c.c. of decinormal caustic soda to produce a condition in which the serum was liquid when hot and solid when cold, and the latter 2 c.c. and 1·2 c.c. respectively. As a mean of three estimations in each case these sera produced ·0375 c.c., ·0328 c.c. and ·0244 c.c. of precipitum.

Sera	$\frac{NaOH}{10}$ per 100 c.c. to give pink with phenolphthalein	$\frac{NaOH}{10}$ required per 1000 c.c. to make serum liquid when hot, solid when cold	Precipitum
1.	2·0 c.c.	4·25 c.c.	·0375 c.c.
2.	1·25 ,,	2·0 ,,	·0328 ,,
3.	·8 ,,	1·2 ,,	·0244 ,,

Strangeways has made numerous observations (unpublished) on the differences in precipitum-forming power of the sera in disease.

The effects of antiseptics.

Lime, carbolic acid, and chinosol, might be taken under this heading but have already been discussed. We here propose to consider various volatile antiseptics, as well as such agents as formalin, mercuric perchloride, and copper sulphate, etc.

As an example of the important volatile antiseptics chloroform may be taken. In solutions containing much of this reagent on the addition of serum a white cloud, and later a deposit, occur. More dilute solutions give rise to slight cloudings. The results of experiments

with a series of such volatile antiseptics are given below. When only present in small quantities in preserved sera the possible error due to their presence can be eliminated by placing them in the incubator for half-an-hour to evaporate off the reagent. When present to a greater extent it was found that the supernatant serum above the deposit caused by them still retained its specific properties. This is in accord with what Nuttall has found.

		Corrosive sublimate 1—25	Copper sulphate	Formalin	Thymol	Chloroform	Alcohol	Benzol	Toluol	Xylol	Ether
1—10	30 mins.	C	C	+		×	C	×	✱	✱	✱
	24 hrs.	D	C	D		×	D	×	✱	✱	✱
1—100		×	C	✱	✱	✱	•	✱	✱	✱	•
		d	D	✱	✱	•	•	✱	✱	✱	✱
1—1000		✱	C	•	•	•	•	•	•	•	•
		✱	D	•	•	•	•	•	•	•	•
1—10,000		✱	+	•	•	•	•	•	•	•	•
		•	+	•	•	•	•	•	•	•	•
1—100,000		•	✱	•	•	•	•	•	•	•	•
		•	d	•	•	•	•	•	•	•	•

Corrosive sublimate and ferrous and copper sulphates were found to produce very marked effects. They apparently destroy the serum in contact with them and except when present in very small quantities it was found impossible to carry out the test. The effects of dilutions of corrosive sublimate and copper sulphate are given below.

	Corrosive sublimate and anti-ox serum		Copper sulphate and anti-ox serum	
Dilutions	1 hour	24 hours	1 hour	24 hours
1—25	immediate coagulation	large deposit	immediate coagulation	large deposit and cloud
1—100	dense cloud	,, ,,	,, ,,	,, ,,
1—500	cloud	cloud	coagulation and cloud	deposit & cloud
1—1000	,,	slight cloud	,, ,,	,, ,,
1—10,000	slight cloud	nil	marked cloud	,, ,,
1—100,000	nil	nil	slight cloud	small deposit

Silver nitrate causes an opaque white cloud on dilution with salt solution up to 1 in 10,000. Dilutions below this do not affect serum when added to them.

Formalin in 1 in 10 dilutions causes marked clouding, which increases till the whole contents of the tube are opaque white. Dilutions below 1 in 100 do not cause sufficient clouding to interfere

with the specific reaction. Solutions of thymol of 1 in 100 cause slight cloudings, but lower dilutions do not apparently affect sera.

Lysol and lysoform both cause great turbidity when added to salt solution even in low dilutions, and moreover even in very low clear dilutions the addition of serum causes clouding. No method has been devised for getting rid of these effects; consequently the presence of these substances except in very small quantities would render the test of doubtful value.

The effects of the reagents, which for the sake of convenience we have grouped under the heading of antiseptics, are very marked except in the case of the volatile class. Some of the latter when added in full strength to liquid sera produce heavy deposits, but the supernatant fluid retains its properties. Formalin and corrosive sublimate in strong solutions, as well as the sulphates of copper and iron and nitrate of silver in much weaker dilutions, completely destroy the precipitum-forming property. Lysol, lysoform, and similar antiseptics, owing to their property of forming cloudings with salt solution, render the application of the test of doubtful value in their presence.

The detection of blood dried on fabrics.

In order to determine to what extent the composition of different cloths influenced blood which had dried on them we procured a number of samples. Human blood was dropped upon these so as to leave some patches unaffected and others saturated. Subsequently the specimens were allowed to dry under natural conditions and were left undisturbed at room temperature and in the light for at least 30 days; some were not tested for nine months. First a series of control tests were carried out on unstained pieces of cloth in the following way. Small pieces 1 × 2 cms. were soaked overnight in 2 c.c. of distilled water. In the morning an equal quantity of double normal salt solution was added and the condition and reaction to litmus of the extract recorded. The majority of samples was found to be nearly neutral, some were distinctly alkaline, whilst most of the coarser materials were acid. About ·5 c.c. of each extract, if necessary after filtration, were placed in small test-tubes and 1 drop of serum added. No cloudings were noticed except in the markedly acid specimens. After neutralisation with sodium carbonate these also produced no effect on the serum. Certain solutions, especially the acid ones, were found to be opalescent, or slightly cloudy, before the addition of serum, but it was noticed that neutralisation tended to make these clearer. In all our experiments we have avoided shaking the extracts, as we frequently observed deposits and

	Material		Solution	Reaction to litmus	Anti-human serum		Anti-ox serum	
					Immediate	6 hours	Immediate	6 hou
1.	dress	(1 month)	clear	neutral	marked reaction	large deposit	—	—
2.	silk	,,	,,	,,	,,	,,	—	—
3.		,,	,,	,,	,,	,,	—	—
4.	fancy serge	,,	green	,,	,,	,,	—	—
5.		,,	clear	,,	,,	,,	—	—
6.		,,	,,	,,	,,	,,	—	—
7.	cloth	,,	,,	,,	,,	,,	—	—
8.	ure serge	,,	red	,,	,,	,,	—	—
9.	case	(9 months)	clear	,,	,,	,,	—	—
10.	andkerchief	(1 month)	opalescent	,,	,,	,,	—	—
11.	ticking	,,	,,	,,	slight reaction	,,	—	—
12.	velveteen	,,	green	alkaline	marked reaction	,,	—	—
13.	reen cloth	,,	,,	slightly acid	,,	,,	—	—
14.	flannel	,,	clear	acid	,,	,,	—	—
15.	at	,,	,,	,,	medium reaction	medium deposit	—	—
16.	duster	(9 months)	opalescent	,,	,,	,,	—	—
17.	canvas	(1 month)	,,	,,	,,	large deposit	—	—

When acid the solution was neutralised.

cloudy precipitates at the bottom of the tubes, which in some cases were very difficult to remove by filtration. After removing the supernatant fluid in solutions containing blood the tubes were, however, shaken to ascertain whether sufficient serum was in solution to produce marked foaming.

In testing for blood, stained patches were treated in the way described above and neutralised if necessary. Two small tubes of each solution were prepared. To one was added one drop of anti-human serum and to the other a drop of anti-ox serum. Two results of some of these experiments are given on the opposite page.

The detection of blood-stains on leather.

Several observations have been made with various samples of leather, which have been placed under a separate heading to more fully bring into prominence their peculiarities. It was found that nearly all gave acid reactions on solution. The degree of acidity, however, varied greatly, chamois leather being alkaline, suède kid glove only slightly acid, and the coarser leathers very decidedly acid. The addition of a drop of serum to the acid solutions produced clouding, and even coagulation with extracts of the coarser leathers. The latter also gave rise, especially if shaken, to bulky deposits in the original solutions.

Nearly all the solutions of leather could be neutralised and the blood-test satisfactorily employed. One class of leather was, however, a marked exception, namely, thick polished yellow leather. Solutions of this gave rise to extremely acid yellow fluids, whose colour deepened on the addition of alkali. It was found impossible to obtain the specific test for blood dried on it. At first it was thought possible that the blood was destroyed by the acid after solution, and extracts were made in alkaline salt solution to neutralise this effect. Even under these conditions no positive results could be obtained. Up to the present although many methods have been tried we have been unable to devise one which gives satisfactory results, and are forced to conclude that the mode of preparation of such leathers produces conditions which destroy the blood in contact with them. Under favourable conditions, when blood has been thickly deposited on the surface, it might, however, be possible to scrape it off and obtain a positive reaction. In the following table all solutions when necessary were neutralised, and filtered, before the addition of anti-human serum.

A series of experiments was also made to determine the effects of boot-blacking and polish. Blood-stains blackened over were hard to detect on the boot, but by neutralisation and filtration clear solutions

Material	Condition	Colour	Reaction	Anti-ox				Anti-human	
				Unneutralised		Neutralised		Neutralised	
				15 mins.	24 hours	15 mins.	24 hrs.	15 mins.	24 hours
Chamois leather	clear	clear	neutral	—	—	—	—	medium reaction	medium deposit
Suède kid glove	cloudy	,,	slightly acid	—	—	—	—	good reaction	large deposit
White ,, ,,	clear	yellowish	acid	slight cloud	slight cloud	—	—	,,	,,
Boot	,,	,,	,,	,,	slight deposit	—	—	,,	,,
Leather from inside shoe	cloudy	,,	v. acid	,,	,,	—	—	,,	,,
Patent leather	,,	,,	,,	,,	,,	—	—	,,	,,
Yellow leather	,,	,,	,,	coagulation	deposit	cloud	cloud	cloud	cloud

could be obtained, and yielded well-marked reactions. Polish also made no difference to the test.

Experiments with saline solutions of tannin show that it has a very deleterious action on serum, rendering the application of the test when it is present in large quantities impossible. Solutions of 1 in 20 to 1 in 500 produce instant coagulation of the serum, and 1 in 1000 produces marked clouding.

Detection of blood on materials not previously mentioned.

Ten examples of wall paper of various textures and colours, red, brown, yellow, blue, and green, were tested and gave typical reactions. All produced neutral solutions, some of which were tinted.

Extracts of blood dried on various kinds of paper, stones, flint, slate, coal, cork, string, straw, rubber, linoleum, as well as silver and copper coins, yielded satisfactory results.

Although one piece of oak on which blood had been thickly incrusted gave a marked reaction with anti-human serum, we failed to obtain any reaction with blood on two blocks of cedar and pine. The quantity present on each of these was exceedingly small, and the negative result was probably due to this cause.

Foreign observers working with similar materials to some of those we have just enumerated were able to obtain in most cases satisfactory results.

These experiments demonstrate that many substances in common use give acid solutions. In most instances the acidity is not so marked as to be of importance, but in some unless recognised and neutralised might be liable to lead to grave error. Extracts of certain substances are sufficiently alkaline to impede the reaction.

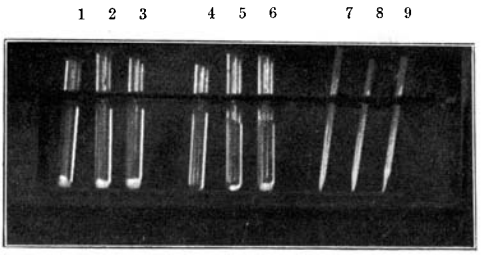

Fig. 1.

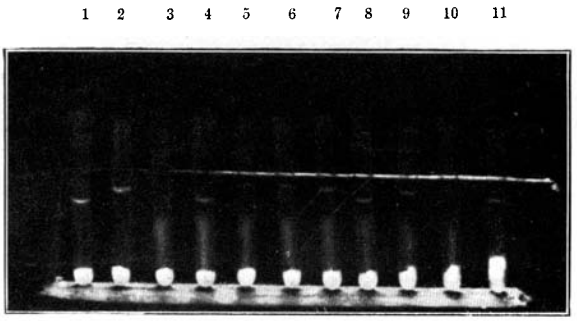

Fig. 2.

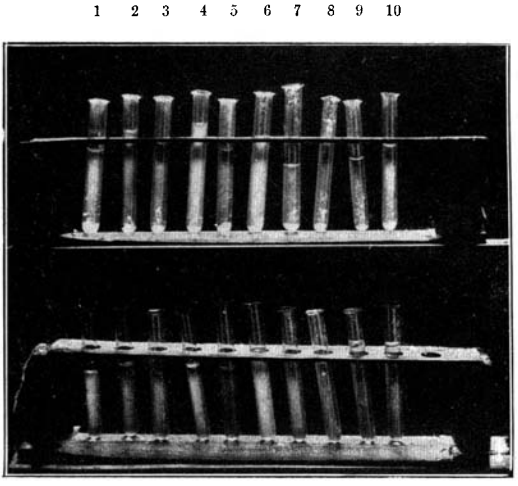

Fig. 3.

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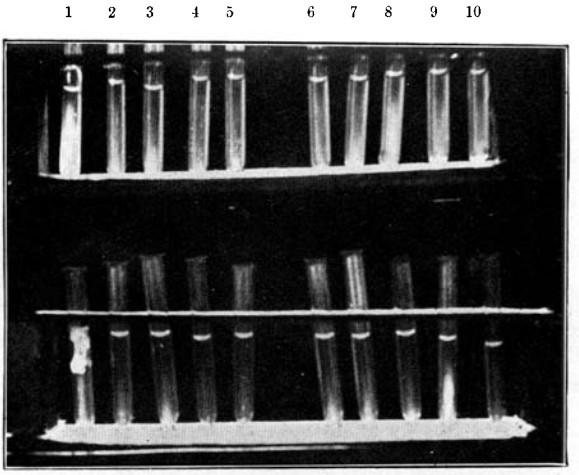

Fig. 4.

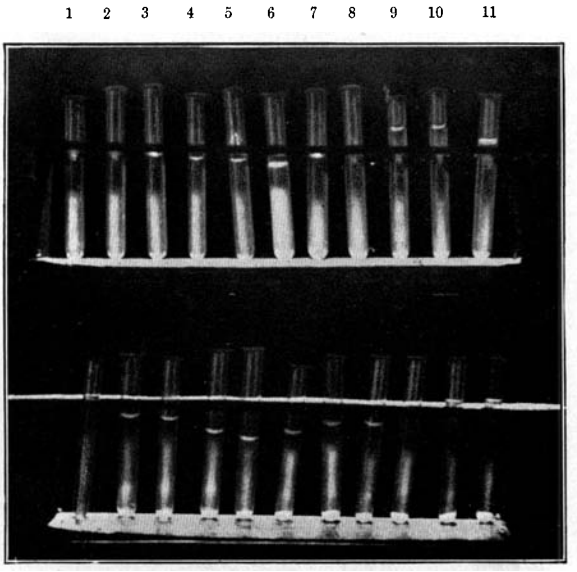

Fig. 5.

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EXPLANATION OF PLATE XI.

Fig. 1. No. 1 shows the precipitum with normal human serum of (1—21 in salt solution) and anti-human serum (·1 c.c.). No. 2 with putrid human serum (1—21) and antiserum, and No. 3 with normal human serum (1—21) and putrid anti-human serum. No. 4 shows a clear solution of human serum in salt solution (1—21). No. 5 shows the deposit resulting from the solution of human serum in distilled water (1—21). No. 6 the precipitum formed with human serum diluted with distilled water (1—21) and anti-human serum. Nos. 7, 8 and 9 show three capillary tubes such as are used in quantitative measurements, and containing precipitum.

Fig. 2 Shows effects of increasing quantities of NaCl on the formation of precipitum; each tube contains ·5 c.c. of a 1 in 21 dilution of human serum, and ·1 c.c. of anti-human serum. No. 1 contains ·6 % of salt, and those following 1 %, 2 %, 4 %, 6 %, 8 %, 10 %, 16 %, 18 %, and No. 11 is saturated with salt. Results of measurements are given on p. 267.

Fig. 3 Shows the specific precipitum in tests for human blood dried for a month on various materials. The lower series shows controls with anti-ox serum. The cloudings in the tubes are due to the opalescence of the solutions; and the various solid particles are portions of undescended precipitum.

No. 1 test for blood dried on silk handkerchief, No. 2 on tweed cloth, No. 3 on black dress fabric, No. 4 on dark green cloth, No. 5 on coarse green cloth, No. 6 on coarse red cloth, No. 7 on kid glove, No. 8 on blanket, No. 9 very coarse sack material, No. 10 on flannel. Nos. 11—20 show control tests with anti-ox serum: all negative.

The solutions whenever necessary were neutralised before testing.

Fig. 4. Nos. 1 to 5 show the effects on serum of dilutions of Hydrochloric acid in salt solutions of strengths of 1—10, 1—100, 1—1000, 1—10,000 and 1—100,000. No. 1 has a dense white cloud, No. 2 a slight cloud at the bottom, No. 3 a marked cloud, and the others are unaffected. Photographed after 6 hours.

Nos. 6—10 similarly illustrate the action of Tartaric acid, No. 1 (1—10) having a slight cloud, No. 2 (1—100) a medium cloud, No. 3 (1—1000) a marked cloud, Nos. 4 and 5 (1—10,000 and 1—100,000) are unaffected, the apparent deposit being due to the light.

Nos. 11—15 illustrate the action of Nitric acid. No. 1 (1—10) shows the coagulum, No. 2 (1—100) a very faint cloud, No. 3 (1—1000) a medium cloud, and Nos. 4 and 5 are unaffected.

Nos. 16—20 show the effects of Acetic acid. Nos. 1 and 2 (1—10 and 1—100) have slight clouds, No. 3 (1—1000) a medium cloud, and No. 4 (1—10,000) a marked cloud. No. 5 (1—100,000) is not affected.

Fig. 5 Illustrates the action of acids and alkalis on the formation of the specific precipitum. All the tubes contain ·5 c.c. of human serum dilution (1—21) in salt solution. Nos. 1—5 contain 5 to 1 drops of 1 in 10 Hydrochloric acid. No precipitates have been formed. No. 6 did not receive any acid or alkali. Nos. 7—11 contain 1—5 drops of 1 in 10 sodium carbonate solution; the quantity of precipitum shows a decrease along the series. Nos. 12—22 have been similarly treated but received drops of 1 in 100 acid and alkali respectively. The precipitum is seen to increase from 12 to 16 and decrease from 18 to 22. The slight clouding above the precipitum in each case is due to bacterial growth, the tubes having stood 48 hours.

We are indebted to Walter Mitchell, our laboratory attendant, for the time and attention he has bestowed on the photographing of these specimens.