THE BIOLOGICAL OR PRECIPITIN TEST FOR BLOOD, CONSIDERED MAINLY FROM ITS MEDICO-LEGAL ASPECT. II.

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(*Continued from p.* 291.)

(*From the Pathological Laboratory of the University of Cambridge.*)

The Influence of Heat on (*a*) *Anti-sera, and* (*b*) *Normal Sera.*

(*a*) *Anti-sera.* The effects of heat on anti-sera have been investigated by many observers and all agree that a temperature of 60° C. is not sufficient to destroy their efficacy. Detailed observations as to the effects of temperature do not seem, however, to have yet been carried out. The following table summarises some of the observations on this subject:

	Destroyed at	Resisted		Observer
Haemato-sera	70° C.	65° C.	Weakened	Bordet (1899)
,,	—	60° C.	No effect	Rostoski (1902)
,,	—	60° C. for ½ hr.	Still effective	Obermayer and Pick (1902)
,,	65° C. for 24 hrs.	60° C. for 48 hrs.	Weakened	Linossier and Lemoine (1902)
,,	68° C. for 2 hrs.	52° C.	No effect	Michaëlis (1902)
Anti-egg serum		60° C. for 1 hr.	Scarcely affected	Uhlenhuth (1900)

In order to determine quantitatively the effects of heating on the precipitum-forming property, specimens of anti-sera were heated in small sealed capillary tubes attached to the side of a thermometer in a water-bath.

Specimens of anti-ox serum were heated for 5 minutes each, and of anti-sheep serum for 1·5 minutes, at the temperatures given in the following table. Subsequently ·1 c.c. of each sample was added to ·5 c.c. of a 1—21 dilution of its homologous blood, and the resulting precipitum measured quantitatively.

After the process of heating, no visible change was noticed in the anti-ox serum till a temperature of 65° C. was reached, when the fluid became slightly opalescent. At 70° C. this opalescence was very marked, and at 75° C. the serum became gray, opaque, and solid. In the case of

the anti-sheep serum slight opalescence was noticed at 66° C., which became more pronounced at 68° C. The following table shows that a marked reduction in the precipitum-forming power coincided with the visible change.

When the slightly opalescent anti-serum was added to a serum dilution a slight cloudiness appeared throughout the fluid. The more markedly opalescent serum differentiated itself as it settled to the bottom of the tube as a very definite cloud. After shaking the tube the fluid appeared cloudy throughout, but remained in this condition, no precipitum settling to the bottom.

The precipitum settled most quickly in the unheated specimens, and the rate of formation of precipitum decreased as the temperature, to which the anti-serum had been exposed, increased.

Up to 60° C. no change in the precipitum-forming power was found in either the anti-ox or anti-sheep sera, and both gave no trace of precipitum when heated beyond 67° C. Between 60° C. and 67° C. the quantity produced in each case was diminished. The figures given are the mean of two estimations in each case.

	Anti-ox (heated for 5 minutes)			Anti-sheep (heated for 1·5 minutes)		
Temp.	Precipitum	Percentage	Remarks	Precipitum	Percentage	Remarks
37° C.	·0234	100		·0075	100	
40	·0234	,,				
45	·0234	,,				
50	·0234	,,				
55	·0234	,,		·0075	100	
56				·0075	,,	
57				·0075	,,	
58				·0075	,,	
59				·0075	,,	
60	·0234	100		·0075	,,	
61				·0056	74	
62				·0056	,,	
63				·0065	83	
64				·0037	49	
65	·0187	79	Slight opalescence	trace	?	
66				,,	?	Slight opalescence
67	·0103	42	,, ,,	·	0	,, ,,
68				·	0	Marked ,,
69				·	0	,, ,,
70	·	0	Marked opalescence	·	0	,, ,,
75	·	0	Opaque, solid			

(*b*) *Normal Sera.* But few experiments seem yet to have been made on the effects of heat on normal sera in regard to their power of producing precipitum with their homologous anti-sera. Some of these experiments have been carried out on undiluted specimens, and others on

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diluted, the results in the latter case not being strictly comparable with those in the former.

Serum	Destroyed at	Resisted		Observer
Eel	80° C.	58° C.	Less reaction	Tchistovitch (1899)
Fowl		70° C. for ½ hr.		Bordet (1899)
Diluted 1—100	100° C. 5 min.	55° C. ,, ,,	No effect	Nuttall (1901)
,, 1—10	65° C. 24 hrs.	60° C. for 4 days.	No effect	Linossier and Lemoine (1902)
Fowl's egg-albumin		56° C. for ½ hr.	No effect	Myers (1900)

The heating of specimens of undiluted ox serum (1 c.c. for 3 minutes), was carried out in the same manner as described for anti-sera. Subsequently 1—21 dilutions in salt solution were made, and tested with anti-ox serum.

No visible change in the serum was noticed till a temperature of 56° C. was reached, when the serum became slightly opalescent. This opalescence increased between 63—67° C., and was still further marked at 68° C. All these specimens gave slightly cloudy solutions. At 70° C. the serum became very opaque, and at 75° C. white and solid.

The quantity of precipitum formed remained constant up to 50° C., but from 55° C. to 62° C. a marked diminution was noticed. At 63° C. a further reduction occurred, and at higher temperatures the formation of precipitum ceased. All solutions gave a good foam-test.

The figures given below are the mean of two estimations in each case.

Normal undiluted ox-serum heated for 3 minutes.

Temp.	Precipitum	Percentage	Remarks
Unheated	·0262	100	
40° C.	·0262	,,	
45	·0262	,,	
50	·0262	,,	
55	·0225	85	
56	·0225	,,	Slight opalescence
57	·0215	,,	,, ,,
58	·0215	82	,, ,,
59	·0206	74	,, ,,
60	·0187	71	,, ,,
61	·0187	,,	,, ,,
62	·0187	,,	,, ,,
63	·0122	46	Increased opalescence
64	.	0	,, ,,
65	.	0	,, ,,
66	.	0	,, ,,
67	.	0	,, ,,
68	.	0	Marked ,,
69	.	0	,, ,,
70	.	0	Opaque
75	.	0	,, and solid

These experiments, as far as they go, appear to indicate that an anti-serum can be exposed to a greater degree of heat than its corresponding serum without injury, and that the precipitum-producing property is completely destroyed in the latter at a lower temperature.

The effects of filtration of Normal Sera through "stone" filters.

It has been already shown (p. 279) that the substance of "stone" filters when allowed to act on serum exerts some influence on the serum exposed to it. In order to further test this point ox-serum was filtered through a new Berkefeld filter, and through a new clean Chamberland filter. After a certain quantity of serum had filtered through it was removed, and specimens from it diluted and tested. It was found that in the former case the precipitum-forming power was at first diminished, but returned to the normal after 110 c.c. had been filtered. No change was noticed during the passage of a further 300 c.c. through the filter.

In the latter case the precipitum-forming property diminished rapidly and fairly uniformly as the filter became choked.

Ox-serum.

Quantity filtered in c.c.	New Berkefeld filter		New clean Chamberland filter	
	Precipitum	Percentage	Precipitum	Percentage
Unfiltered	·0281	100	·0281	100
10	·0187	66		
20	·0210	74		
30	·0229	82	·0272	97
40	·0225	80		
50	·0229	82	·0272	97
60	·0229	82		
70	·0215	76		
80	·0225	80		
90	·0244	86		
100			·0245	87
110	·0225	80		
125	·0281	100		
140	·0272	96		
150			·0158	56
165	·0281	100		
200	·0281	100	·0158	56
250	·0281	100	·0114	40
350			·0114	40
400	·0281	100		

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The effects of the prolonged action of Antiseptics on Fluid Sera.

Unless fluid sera can be stored in a sterile condition it has generally been found desirable to add small quantities of antiseptics for the purpose of checking bacterial growth. In order to determine the effects of such antiseptics the following experiments have been carried out. Antiseptics in the proportions given below were added to fluid ox, and sheep sera, and allowed to act in sealed bulbs for 4 months. None completely checked bacterial growth. After this period dilutions of 1—21 in salt solution were made, and all were allowed to stand in open dishes for 2 hours in order that the volatile antiseptics should evaporate off. The results were compared with serum kept under the same conditions but without the addition of any antiseptic. The following table shows that in nearly all cases the precipitum-forming power was slightly reduced, but in a few completely destroyed.

The effects of the presence of these substances in fluids to be tested have been given previously (pp. 285—287).

		Ox serum		Sheep serum	
		Precipitum	Percentage	Precipitum	Percentage
Normal ox and sheep sera		·0356	100	·0140	100
Chloroform	1—1000	·0338	95	·0103	73
	1—500	·0328	89		
	1—100	·0187	55	·0093	65
Xylol	1—500	·0300	84		
	1—100	·0300	84	·0140	100
	1—25	·0281	79		
Benzol	1—1000	·0281	79		
	1—500	·0187	55		
	1—100	·0225	63	·0112	80
Toluol	1—100			·0140	100
Ether	1—500	·0347	97		
	1—100	·0244	68	·0084	60
Formalin	1—10,000	·0262	73		
	1—1000	•	0		
	1—500	•	0		
Alcohol	1—1000			·0075	53
	1—100			·0103	73
Lysol	1—500	·0187	55		
	1—100	·0169	47		
	1—25	•	0		
Lysoform	1—1000	•	0		
	1—100	•	0		
Chinosol	1—500	·0262	73	·0112	80
	1—200	·0262	73		

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The effects of the prolonged action of Acids and Alkalis on Fluid Sera.

Ox, and sheep, sera with acids and alkalis added in the proportions given below were kept under conditions similar to those just mentioned. After dilution, the solutions were neutralized, and then tested quantitatively. Control antisera were also used in all cases, but gave no reactions.

The effects of the presence of unneutralized acids and alkalis have been given previously (pp. 281—285).

The following table shows that, except in very small quantities, the prolonged action of inorganic acids completely destroys the precipitatable substance, but that organic acids do not exert so deleterious an influence. Strong alkalis act in the same way as inorganic acids.

		Ox serum		Sheep serum	
		Precipitum	Percentage	Precipitum	Percentage
Normal ox and sheep sera		·0356	100	·0140	100
Hydrochloric acid	1—1000			·0093	65
	1—500	trace	?		
	1—100	•	0		
Nitric acid	1—1000			·0103	73
	1—500	·0046	13		
	1—100	•	0	•	0
Sulphuric acid	1—1000			·0112	80
	1—100			•	0
Acetic acid	1—1000			·0112	80
	1—500	·0244	68		
	1—100	·0300	84	·0112	80
	1—10	trace	?		
Oxalic acid	1—1000	·0225	63	·0140	100
	1—500	·0244	68		
	1—10			·0028	20
Carbolic acid	1—1000			·0131	93
	1—100			·0112	80
Citric acid	1—1000			·0131	93
	1—100			•	0
Caustic potash	1—1000			·0122	87
	1—500	·0150	42		
	1—100	•	0	•	0
Caustic soda	1—1000			·0122	87
	1—100			•	0
Sodium carbonate	1—1000			·0103	73
	1—500	·0244	68		
	1—100			·0103	73
	1—10	·0244	68		
Ammonia	1—1000			·0140	100
	1—500	·0309	84		
	1—100	·0206	57	·0131	93
	1—10	·0065	18		

In April 1901 Uhlenhuth (25. IV. 01) published the results of experiments on the reaction of human blood to this test under conditions

likely to be met with in medico-legal practice. Three months later he carried out investigations on materials obtained from the public prosecutors (25. VII. 01). It was not until after his experiments had been made, and his results transmitted to the authorities, that he was informed as to the identity of the blood-stains which he had examined. Lists of the articles examined, the methods employed, and the results arrived at are very fully given by him (25. IV. 01, 25. VII. 01, and VII. 02). In every instance his diagnosis as to the presence, or absence, of human blood was correct, and in many cases he even went further, and was able to correctly name the animal from which the blood was derived in cases in which the stain was not that of human blood.

The following case may be cited as an example of the efficacy of this test. The clothes of a man suspected of having committed a murder were sent to Uhlenhuth for examination. The accused was also suspected of having slaughtered some sheep in a field a fortnight before the murder. The tests revealed the following facts.

	Human blood	Sheep's blood
Coat	6 places	6 places
Trousers	7 ,,	3 ,,
Waistcoat	4 ,,	0 ,,
Shirt	1 ,,	0 ,,
Hat	4 ,,	0 ,,

The evidence in court subsequently made it absolutely certain that the accused had killed the sheep, and he was also convicted of murder and sentenced to death.

His procedure in making these investigations was as follows. Extracts of the stains were made in salt solution, and tested by chemical and spectroscopic tests in order to determine whether the stains were due to blood or other substances. About 4 c.c. of the extract, foaming readily on shaking, were placed in a test-tube and five drops of human anti-serum run in. A positive reaction to human anti-serum was shown by marked clouding within a few minutes. When this did not occur the test was concluded to be negative, and another anti-serum was added. This proceeding was repeated till a marked clouding appeared with one of the anti-sera. When no positive reaction was shown with any of his anti-sera he considered that the stain was due to the blood of some animal to which he possessed no anti-serum.

For example, in diagnosing a blood-stain on linen, given him by Prof. Beumer, he first added to the extract human anti-serum, then anti-sheep, and anti-horse, all with negative results. Finally he added

anti-pig serum, which gave a strongly positive reaction. The diagnosis of pig's-blood was confirmed by Prof. Beumer.

This method is open to the objection that a late clouding due to one of the anti-sera previously used may appear shortly after the addition of a subsequent anti-serum and give rise to a mistaken diagnosis. For this reason it would appear better to use smaller quantities in different tubes, and add a drop or two of an anti-serum to each tube.

Uhlenhuth always controlled his experiments by means of extracts of known dried bloods. In his papers he calls attention to the necessity in medico-legal investigations of proving the stains to be due to blood, and not albumen, or some other substance, by means of chemical and spectroscopic tests, and warns against the use of weak and opalescent anti-sera.

Ziemke (17. VIII. 01) made numerous experiments and demonstrated the possibility of differentiating human from other bloods, when dried on various materials. He frequently used soda solution (·1 %) as a solvent since he found that by this means he obtained a reaction when salt solution extracts gave none. The possibility of mistakes owing to the use of this solvent has already been mentioned (p. 283).

Whittier (18. I. 02) and Wood (24. IV. 02) have recorded instances in America in which the test has been made use of in legal cases. In both the presence of human blood was proved. The methods proposed by Nuttall were adopted. Austin (12. III. 03) in a paper on "the limitations of the Uhlenhuth test for the differentiation of human blood" showed that "other fluids of the human body, like effusions and exudates, were of little value" in the production of anti-sera. These facts, however, can have no bearing on the test in its medico-legal aspect, and but confirm the observations of others.

No attempt has been made to summarize the extensive literature on this subject, and these few citations are merely intended to emphasize the importance attached by foreign observers to this means of determining the origin of blood-stains in medico-legal investigations.

We must gratefully acknowledge the unfailing courtesy and kindness of Mr Henry, Chief of the Criminal Investigation Department of Scotland Yard, in placing at our disposal the unrivalled collection of medico-legal material in the Museum under his supervision.

Summary.

1. Powerful anti-sera may be produced by the intravenous injection of smaller quantities of serum than have hitherto generally been used (p. 260).

2. Nuttall's quantitative method affords a simple and fairly accurate means of determining the quantity of precipitum formed. By its means quantitative differences can be appreciated which are scarcely, or not at all, apparent in the tubes on inspection (p. 263).

3. Normal saline solution is the best diluent for normal sera, and 1—21 has been found to be a convenient dilution. Increase of salt has very little effect on the production of precipitum (p. 266).

4. The quantity of precipitum formed is not influenced by the temperature at which the experiment is conducted (*i.e.* between the temperature of the ice-chest and 37° C.) (p. 268).

5. In the case of dried bloods time *per se* does not destroy their capacity for reacting with their homologous anti-sera. Fluid sera appear to deteriorate slightly by keeping (pp. 269—274).

6. Putrefaction of the serum, or anti-serum, does not affect the production of a specific precipitum (p. 274).

7. Although the intimate mixture of lime and blood completely destroys the latter, the former is not present in sufficient quantity in ordinary earths to affect blood mixed with earth. The presence of small quantities of lime, however, gives rise to a clouding in solution, which can be got rid of by the passage of CO_2, and subsequent filtration (pp. 276—281).

8. The presence of even small quantities of acids, or alkalis, rapidly reduces the quantity of precipitum formed (pp. 281—284).

9. In diseased conditions a marked alteration may occur in the quantity of precipitum (pp. 265 and 285).

10. The volatile antiseptics produce little effect on sera, even after long contact, but formalin, corrosive sublimate, lysol, lysoform, the sulphates of copper and iron, and nitrate of silver, especially in strong solutions, exert a very deleterious action (pp. 287 and 358).

11. Blood dried on fabrics, and materials in common use (with the exception of certain leathers) may with adequate precautions be readily diagnosed (p. 290).

12. After an undiluted anti-serum has been raised to a temperature beyond 60° C. the capacity for producing precipitum is diminished, and

it is destroyed completely after exposure to 68° C. These effects seem to be produced at lower temperatures in normal undiluted sera (pp. 354–55).

13. The precipitum-producing power of normal sera is reduced by filtration through a Chamberland filter, but not by passage through a Berkefeld filter (as far as the experiment was conducted) (p. 357).

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