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AN INVESTIGATION INTO THE COMMON CAUSES OF BLOODSTAIN DETERIORATION

PART I

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Pioneer work by investigators like Landsteiner Jansky and Moss in the early years of this century led to the discovery of blood groups. Since then many workers have increased the number of blood agglutinogens, such as, M N S and P agglutinogens, Rh. factors, etc., which can be identified and differentiated by blood grouping techniques.

The blood group specialist is, in most cases, concerned with blood group determinations, for blood transfusion work, when he is solely interested in establishing the compatibility or otherwise of the donor and recipient bloods. The forensic scientist, on the other hand, deals with dried bloodstains, with a view to determine their source in cases of assault, rape or homicide and almost invariably confines himself to the determination of A B O groups.

One of the authors (S.S.), in the course of work, carried out in the Penang Laboratory has come across bloodstained exhibits which often do not give conclusive results as to their origin and blood groups. Such cases are commonly reported as being inconclusive due to putrefaction or deterioration of the bloodstains although there is no departmental information sheet on the causes and possibly the extent of decomposition caused by interfering substances. It was with a view to find out the common substances or conditions that cause the decomposition, and therefore, are responsible for the inconclusive results, that this investigation was undertaken. In all thirty-four conditions have been investigated and the findings are summarised.

Method of Securing Blood Samples

The supervisor of the Blood Bank, Penang was informed in advance of the type of blood required and when a donor of that group arrived at the bank he would notify one of us (O.Y.H.K.) who would arm himself with two vials each containing a few crystals of anticoagulant[1] (mixture of ammonium and potassium oxalates). After the supervisor had finished taking his sample; the delivery tube just before disconnection, was allowed to drip into our vials. The vials were then stoppered and the anticoagulant uniformly dispersed by a few gentle inversions of the vials. The agglutinin and agglutinogen titres of each sample of blood were next determined and the blood then used to stain filter papers as indicated below:—

1. **Stain Air Dried** — The filter paper was stained with blood and allowed to dry in an air-conditioned room.
2. **Stain Sun Dried** — The filter paper was stained as above and the wet stain placed in bright sunlight for about three hours (Temp. 96°F.).
3. **Bacteria and Blood** — Some Staphylococcus pyogenes, obtained from I.M.R. were suspended in deionised water. This suspension was employed to wet the filter paper which was allowed to stand for about five minutes. Then the bacteria impregnated paper (still wet) was stained with blood and kept moist for 24 hours by placing in a dessicator containing water.
4. **Polluted Blood** — Fresh blood was inoculated with the above bacteria, incubated at 37°C. for 24 hours and then used to stain filter paper.
5. **Stain Oven Dried** — Wet stain dried in an oven at 100°C. for ca. 3 hours.
6. **Moist Paper Stained** — Paper moistened with tap water and the wet area stained with blood. The blood gave two types of absorptions — one darker (more intense) than the other. Dried in air-conditioned room.

7. **Bloodstain Washed** — Stained filter paper, air dried and then held in running water (from tap) for approximately one minute.

8. **Perspiration and Bloodstain** — Paper stained with perspiration, dried and then blood stained. Dried in room.

9. **Blood and Soap Solution** — A 1% w/v solution was employed to wet a piece of filter paper. This was dried and then stained with blood.

10. **Bloodstain and FAB Solution** — Paper stained with 1% w/v FAB solution, dried and then stained with blood.

11. **Bloodstain Ironed** — Paper stained with blood, air dried and after ca. 2 hours ironed with an electric iron for approximately one minute.

12. **Bloodstain and Coconut Oil** — The paper was stained with coconut oil, allowed to dry and then counter stained with blood.

13. **Bloodstain and Vaseline Hair Cream** — The filter papers were smeared with vaseline, which was allowed to partially dry and then stained with blood.

14. **Bloodstain and Hydrogen Peroxide (10 vols.)** — Filter paper stained with blood and then hydrogen peroxide added drop by drop onto the wet stain. Gas was evolved and the wet stained area was foam covered. When the hydrogen peroxide was dropped onto a clean (free of blood) area of the filter paper no foam was formed.

15. **Bloodstain and Loam** — The filter paper was stained with blood and the wet area was covered with wet loam (taken from a flower bed) for 24 hours.

16. **Bloodstain and Clean Sand** — Same as above except that clean dry sand from the store was employed.

17. **Bloodstain and Starch** — The filter paper was soaked in starch solution (1% w/v), dried and then stained with blood.

18. **Bloodstain and Tannic Acid** — The filter paper was soaked in an aqueous solution of tannic acid (1% w/v) allowed to dry and then stained with blood.

19. **Bloodstain and Dilute Acetic Acid** — The filter paper was soaked in dilute acetic acid (1% w/v), allowed to partially dry and then stained with blood.

20. **Bloodstain and Perfume** — Paper stained with "PATRA" perfume dried and then counter stained with blood.

21. **Dettol** — A 1% v/v solution was prepared. (a) Filter paper was wetted with dettol dilution, allowed to dry and then stained with blood. (b) A filter paper was stained with blood, allowed to stand for about 10 minutes and then the stained area wetted with dettol dilution.

22. **Carbolic Soap** — A 1% w/v solution was made. (a) A filter paper was wetted with the solution, allowed to dry and then stained with blood. (b) Stained a filter paper with blood, allowed it to stand for about 10 minutes and then wetted the stained area with the carbolic soap solution.

23. **Alcohol** — The filter paper was stained with blood, allowed to stand for 10 minutes and then wetted with 95% ethanol.

24. **Heat** — Two filter papers were stained with blood, allowed to stand at room temperature for about 5 minutes and then: (a) one paper was maintained at 150°C. for one hour, and (b) the other paper was placed for 5 minutes in an air oven previously adjusted to 200°C.

25. **Formalin** — A filter paper was stained with blood, allowed to stand for 10 minutes, and then wetted with formalin solution (20%).

26. **Betel Nut** — Gopal, the gardner was made to chew some betel nut and then discharge the coloured sputum onto a filter paper. After the spittle had dried it was counter stained with blood.

27. **Saliva** — A few filter papers were wetted with Gopal's saliva, allowed to dry and then blood stained. (Gopal's blood group is O).

28. **Rust** — Some iron filings were sprinkled onto filter papers, moistened with water and then allowed to rust. The paper with

rust covered filings was next stained with blood.

29. **Dessication** — Filter papers were stained with blood and kept in dessicators (containing concentrated H_2SO_4) for two days.

30. **Dilute Acetic Acid** — Filter papers were stained with blood, allowed to semi-dry (5 minutes) and then wetted all over with dilute acetic acid (1% v/v).

31. **Bacteria Coli** — (a) Bacteria impregnated papers were moistened with blood and kept moist for 48 hours. (b) Blood samples were innoculated with B. coli, incubated at 37°C. for 48 hours and then used to stain filter papers.

32. **Staphylococcus pyogenes** — Staining as in the case of B. coli conditions (a) and (b).

33. **Incubated Blood** — Samples of blood were incubated for 48 hours at 37°C. and then used to stain filter papers.

34. **Hæmolysed Blood** — Samples of blood were allowed to stand at room temperature (ca. 30°C.) until completely hæmolysed (no cells visible under the miscroscope) and then used to stain filter papers.

PLAN ADOPTED

Grouping tests and whenever possible simultaneous agglutinin titre determinations on the bloodstains from each group of blood were carried out at the following times:

(a) bloodstains one or two days' old.

(b) bloodstains two weeks' old.

(c) bloodstains two months' old.

Precipitin tests at dilutions of 1:1000; 1:2000 and 1:4000 were also performed on the two month old stains.

A further lot of four blood samples, one per blood group, were secured. These were used to study conditions not previously studied and also modifications of a few of the conditions already investigated with the first four samples of blood. The latter modified conditions were investigated to determine whether or not such modifications would accentuate their adverse effects.

METHOD USED

Each sample of fresh blood, unless otherwise stated, was separated by centrifugation into: (a) cells and (b) plasma. The cells were washed until clear (three times) with physiological saline and then a suspension (2%) in normal saline made. A typical agglutinogen and agglutinin titre determination is described below:

Agglutinogen titre of Group A Blood — O-sera and anti A-sera of known titres were each titrated with physiological saline as in the case of saliva grouping[2]. To the diluted sera an A-cell suspension (2% cells of blood to be grouped) was added and the Dreyer tubes incubated at 37°C. for one hour after which agglutination readings were taken with the aid of a microscope.

Agglutinin titre of Group A Blood — Two volumes (0.008 cc) of the plasma from this blood was diluted with physiological saline and then to one row of dilutions a known A-cell suspension was added and to the adjacent row a similar B-cell suspension added. The tubes were again incubated and read after one hour.

Agglutinin titre of Group A Bloodstain — (cf. Grouping of Saliva Stains[2]). About one sq. cm. of stained filter paper was cut into very small pieces, transferred into an extraction tube and extracted with the minimum quantity of physiological saline. The extract, free from suspended matter, was diluted in two rows of Dreyer tubes. To these known A-cell and B-cell suspensions (2%) in physiological saline were added and the tubes incubated at 37°C. for one hour after which the agglutination readings were taken.

Agglutinogen titre of Group A Bloodstains — (cf. Grouping of Saliva Stains[2]). Bloodstained paper was extracted with O-sera, the extract diluted with physiological saline and then mixed with A-cell and B-cell suspensions. Agglutination readings were then recorded. In each set of experiments a blank was performed by extracting clean filter paper and then treating the extract in a manner similar to extracts from bloodstained areas.

Precipitin Test — The bloodstain on each filter paper was extracted (overnight) with

physiological saline and then the clear extract diluted further with saline solution to yield dilutions of 1 in 1000, 1 in 2000 and 1 in 4000. These dilutions were then carefully layered, in three separate Dreyer tubes, onto some human anti-sera and the common surface observed three times for signs of opacity. The observations were spaced at ten minute intervals. A blank, extracting unstained filter paper was performed in each case.

DISCUSSION

In the section of "Method Used" only a very brief outline is given since a detailed account of essentially the same method has already been given elsewhere[2].

The possible contaminants chosen for study in the case of the first four blood samples were those that would commonly be expected to be present on articles stained with blood.

Before the precipitin reaction was performed, the human anti-sera was tested with known dilutions (v/v) of human sera and found to have an activity of 1 in 6000. The stain extracts were then diluted according to the foam test method[5], taking good care to use tubes of the same dimensions and the same pipette for preparing the different dilutions. It was found that with the exception of group A blood, even stain number 1, the reference standard did not give a precipitin positive reaction at the maximum dilution of 1 in 4000. The contaminants that produced adverse effects were heat (100°C.), tannic acid, acetic acid, heat (electric iron), perspiration, etc. These contaminants must have altered the nature of the serum proteins.

For all grouping work in this project fresh A and B cells were always taken from the same two persons so as to ensure that the "quality" of the cells was maintained at the same level throughout the project. Also the agglutination readings have been taken by the same person with the aid of the same microscope, thereby maintaining a uniform consistency in readings.

In experiments on agglutinin titre determinations, the blank was performed by diluting with physiological saline anti A and anti B sera of high agglutinin titres and then adding the A and B cells respectively. This determined the maximum agglutinating ability of the cells employed. Any agglutinations, with saline extracts from bloodstains, short of those shown by the blank may therefore be alluded to either the presence of agglutinins of low titres in the stains or the absorption of agglutinins by the filter paper carrying the stains. Since stain No. 1 in each type of blood has been taken as reference for comparison purposes, it may be logically inferred that any adverse deviations from stain No. 1 were due to decreases in agglutinin titres only.

In the extractions with sera, a standardised procedure was adopted with a view to minimise irregularities in results due to differences in stained areas and sera volumes used for extraction. Stains of one sq. cm. area were extracted with two drops of sera delivered from the same micro pipette. This procedure yielded approximately the same volume of sera in excess.

The results of stain number 1 in each case have been taken as a reference standard for all the other stains obtained from the same sample of blood. Therefore, a comparative study of the results obtained for other stains with those of stain number 1 would indicate any decrease in the agglutinogen/agglutinin potency.

Grouping tests on the first two samples of blood (groups A and B) were carried out with O–sera as the extraction medium and the results obtained in each case were found to conform to theory. With the third sample of blood (group AB), however, the stain extractions showed different degrees of cell agglutination although the fresh blood yielded equal titres for both the A and B agglutinogens and the O–sera used had equal agglutinin titre. Fresh stains of the same blood were again extracted, now employing anti–A and anti–B sera and this time the extracts agglutinated to the same extent with both A and B cells respectively, thus indicating the uneven removal[3] of agglutinins from the O–sera. A further sample (fourth) of blood (Group AB) was also found to "misbehave" with O–sera and yet comply with theoretical requirements when anti–A and anti–B sera were substituted

instead. As a result of the above it has been decided to replace the conventional O-sera (used in the Penang Laboratory since the commencement of blood group work) with anti-A and anti-B sera for all group determinations of bloodstained exhibits. Extractions with diluted anti-A and anti-B sera have been found to yield more conclusive results than those obtained by the use of undiluted sera.

No attempt at differentiation of the subgroups of groups A and AB has been made in this piece of work.

One Day Old Stains — A comparison of the results derived from the twenty, one-day old stains, of each blood group was made and a summary of the conditions/contaminants lowering agglutinogen and agglutinin titres is given:—

Agglutinogens

BLOOD GROUP AB

Stain No.	Active Agent	Agglutinogen affected
2	Sun dried	A and B; A = B
5	Heat at 100°C.	A
7	Stain washed	B
11	Heat (electric iron)	A and B; A > B
15	Loam	A and B; A > B
16	Clean Sand	A and B; A > B
17	Starch	A and B; A = B
18	Tannic Acid	A and B; B > A
19	Acetic Acid	A and B; A = B
20	Perfume	A and B; A > B

BLOOD GROUP B

Stain No.	Active Agent	Agglutinogen affected
5	Heat 100°C.	B
6	Wet paper stained	B
11	Heat (electric iron)	B
18	Tannic Acid	B

BLOOD GROUP A

Stain No.	Active Agent	Agglutinogen affected
3	Bacteria	A
5	Heat 100°C.	A
6	Wet paper stained	A
11	Heat (electric iron)	A
15	Loam	A
17	Starch	A

From the above it could be reasonably deduced that bloods belonging to group AB are most prone to agglutinogen deterioration whereas group B and group A bloods suffer almost equal degrees of agglutinogen inactivation.

Agglutinins

BLOOD GROUP O

Stain No.	Active Agent	Agglutinin affected
2	Sun dried	a and b; a > b
3	Bacteria	a and b; a > b
4	Polluted blood	a and b; a > b
5	Heat (100°C.)	a and b; a > b
6	Wet paper stained	a and b; a > b
7	Stain washed	a and b; a = b
8	Perspiration	a and b; a > b
9	Soap	a and b; a > b
10	Synthetic Detergent	b
11	Heat (electric iron)	a and b; a = b
12	Coconut oil	b

Stain No.	Active Agent	Agglutinin affected
13	Vaseline	b
14	Hydrogen Peroxide	b
15	Loam	a and b; $a > b$
16	Clean Sand	a and b; $a > b$
18	Tannic acid	a
19	Acetic Acid	a and b; $a = b$
20	Perfume	a

BLOOD GROUP B

Stain No.	Active Agent	Agglutinin affected
3	Bacteria	a
5	Heat (100°C.)	a

BLOOD GROUP A

Stain No.	Active Agent	Agglutinin affected
2	Sun dried	b
3	Bacteria	b
4	Polluted blood	b
5	Heat (100°C.)	b
6	Wet paper stained	b
7	Stain washed	b
8	Perspiration	b
9	Soap	b
10	Synthetic Detergent	b
11	Heat (electric iron)	b
12	Coconut oil	b
13	Vaseline Hair Cream	b
14	Hydrogen Peroxide	b
15	Loam	b
16	Clean Sand	b
17	Starch	b
18	Tannic acid	b
19	Acetic Acid	b
20	Perfume	b

A survey of the above summary of agglutinin results indicates that bloods of group O and group A are most susceptible and blood of group B least susceptible to changes in their agglutinin potencies.

Effect of Age — Time does not have any significant effect on the agglutinogens although it does lower the titres of the agglutinins as indicated in the summary given below:—

Agglutinogens

BLOOD GROUP AB

no effect

BLOOD GROUP B

Stain No.	Condition	Agglutinogen affected
4	Polluted Blood	b
11	Heat (electric iron)	b
12	Coconut Oil	b
13	Vaseline Hair Cream	b
14	Hydrogen Peroxide	b
16	Clean sand	b

BLOOD GROUP A

no effect

Agglutinins

BLOOD GROUP O

Stain No.	Condition	Agglutinins affected
1	Air dried	a
2	Sun dried	a
3	Bacteria	a and b; a > b
5	Heat (100°C.)	b
6	Wet paper stained	a and b; a > b
7	Stain washed	a and b; a = b
8	Perspiration	a and b; b > a
9	Soap	a and b; a > b
10	Synthetic Detergent	a
11	Heat (electric iron)	a and b; a > b
12	Coconut Oil	a and b; b > a
13	Vaseline Hair Cream	a and b; b > a
14	Hydrogen Peroxide	a and b; a > b
15	Loam	a and b; a = b
16	Clean sand	a
17	Starch	a and b; a > b
18	Tannic Acid	a and b; a > b
19	Acetic Acid	a
20	Perfume	a and b; b > a

BLOOD GROUP B

Stain No.	Condition	Agglutinins affected
1	Air dried	a
2	Sun dried	a
4	Polluted Blood	a
5	Heat (100°C.)	a
6	Wet paper stained	a
7	Stain washed	a
8	Perspiration	a
9	Soap	a
10	Synthetic Detergent	a
11	Heat (electric iron)	a
12	Coconut Oil	a
13	Vaseline Hair Cream	a
14	Hydrogen Peroxide	a
15	Loam	a
16	Clean Sand	a
17	Starch	a
18	Tannic Acid	a
19	Acetic Acid	a
20	Perfume	a

BLOOD GROUP A

Stain No.	Condition	Agglutinins affected
5	Heat (100°C.)	b
8	Perspiration	b
10	Synthetic Detergent	b
11	Heat (electric iron)	b
12	Coconut Oil	b
13	Vaseline Hair Cream	b
15	Loam	b
18	Tannic Acid	b

From the above summary it is obvious that agglutinin a is more prone to deterioration with time than agglutinin b.

Two Weeks' Old Stains — With the second lot of blood samples a few additional conditions (Nos. 21 – 34) were investigated when the stains were two weeks old. This period was considered suitable since it was felt that is was the minimum time that lapsed before bloodstained exhibits are either made available, especially from distant and remote areas, or are normally examined, unless special circum-

stances warrant immediate attention. Further by the end of the two week period, both the contaminant and to some extent, the time factor would have already exercised their adverse effects, if any. Given below is a summary of the results:—

Agglutinogens

BLOOD GROUP AB

Stain No.	Active Agent	Agglutinogen affected
23	Alcohol	a and b: a = b
24a	Heat 150°C.	a and b: a > b
24b	Heat 200°C.	a and b: a ≤ b
25	Formalin	a and b: a ≥ b
28	Rust	b
32b	Bacteria	a and b: a > b

BLOOD GROUP B

Stain No.	Active Agent	Agglutinogen affected
24a	Heat 150°C.	b
24b	Heat 200°C.	b
25	Formalin	b
34	Hæmolysed Blood	b

BLOOD GROUP A

Stain No.	Active Agent	Agglutinogen affected
24a	Heat 150°C.	a
24b	Heat 200°C.	a
25	Formalin	a
28	Rust	a
29	Dessication	a
30	Acetic acid	a
32b	Bacteria	a

Agglutinins

BLOOD GROUP O

Stain No.	Active Agent	Agglutinin affected
21b	Dettol	a and b: a = b
22a	Carbolic soap	a and b: a > b
22b	Carbolic soap	a and b: a = b
23	Alcohol	a and b: a = b
24a	Heat 150°C.	a and b: a = b
24b	Heat 200°C.	a and b: a = b
25	Formalin	a and b: a = b
26	Betel Nut	a and b: a = b
28	Rust	a
30	Acetic acid	a and b: a = b
31a	Bacteria	a and b: a > b
31b	Bacteria	a and b: a = b
32a	Bacteria	a and b: a = b
32b	Bacteria	b
33	Incubated Blood	a
34	Hæmolysed Blood	a and b: a = b

BLOOD GROUP B

Stain No.	Active Agent	Agglutinin affected
21a	Dettol	a
21b	Dettol	a
22a	Carbolic soap	a
22b	Carbolic soap	a
23	Alcohol	a
24a	Heat 150°C.	a

24b	Heat 200°C.	a
25	Formalin	a
26	Betel Nut	a
27	Saliva	a
28	Rust	a
29	Dessication	a
30	Acetic Acid	a
31a	Bacteria	a
31b	Bacteria	a
32a	Bacteria	a
32b	Bacteria	a

Agglutinins

BLOOD GROUP A

Stain No.	Active Agent	Agglutinin affected
22a	Carbolic soap	b
22b	Carbolic soap	b
24a	Heat 150°C.	b
24b	Heat 200°C.	b
25	Formalin	b
26	Betel Nut	b
28	Rust	b
31a	Bacteria	b
32a	Bacteria	b
34	Hæmolysed Blood	b

The above results endorse the previous finding that agglutinin a is more unstable than agglutinin b.

In the assessment of potencies, (listed in the above summaries) only those conditions or contaminants which have produced a difference of one place or more in the agglutination titre, relative to that of the reference standard, have been considered to be deactivating.

The conditions/contaminants investigated may be classified under the following four headings:—

I. **Those exercising Marked Influence** — These are conditions/contaminants that deactivate either the agglutinogens or the agglutinins in everyone of the three possible bloods in each set of four studied.

II. **Those exercising Intermediate Influence** — These are conditions that lower the activity of either agglutinogens or agglutinins in at least two bloods out of the four of each set.

III. **Those exercising Slight Influence** — Under this category are included the conditions that effect either agglutinogen or agglutinin potency in only one blood out of a set.

IV. **Those exercising Doubtful Influence** — This class includes all those conditions in which apparent reduction of agglutinogen/agglutinin potency may be due to employing for extraction purposes areas of filter papers stained to different degrees.

MARKED INFLUENCE ON:

Agglutinogen

Heat 100°C.
Heat (electric iron)
Heat 150°C. }
Heat 200°C. } T F
Formalin }

Agglutinin

Bacteria
Heat 100°C.
Carbolic soap }
Heat 150°C. }
Heat 200°C. }
Formalin } T F
Betel Nut }
Rust }
Bacteria }

INTERMEDIATE INFLUENCE ON:

Agglutinogen

Tannic Acid
Starch
Rust } T F
Bacteria }

Agglutinin

Sun dried
Polluted Blood
Perspiration
Soap
Synthetic Detergent
Heat (electric iron)
Coconut Oil
Vaseline Hair Cream
Hydrogen Peroxide
Tannic Acid
Acetic Acid
Perfume
Alcohol }
Dettol } T F
Bacteria }
Hæmolysed blood

SLIGHT INFLUENCE ON:

Agglutinogen

Sun dried
Dilute Acetic Acid
Bacteria
Perfume
Dessication }
Alcohol } T F
Hæmolysed Blood }

Agglutinin

Starch
Dessication — T F
Incubated blood

DOUBTFUL INFLUENCE ON:

Agglutinogen

Moist Paper Stained
Stain washed
Clean sand
Loam

Agglutinin

Moist paper stained
Stain washed
Loam
Clean sand

Those conditions with "TF" against them have had a two week time factor incorporated in them, as distinct from the others without this factor.

In this project, an attempt has been made to study the effect of one condition/contaminant at a time and it has been shown that only a limited few out of the 34 conditions investigated have a marked effect and consequently render grouping tests inconclusive.

In practice however, two or more factors, such as sweat, starch, oil, heat, bacterial decay, rust, folding up of clothing with wet or semi-dry bloodstains, etc., are present on the great majority of bloodstained exhibits and their collective effect is at times so great that the more susceptible agglutinins (especially with the more abundant group O bloods) are completely destroyed and the less susceptible agglutinogens (if present) may be inactivated to such degrees that no sound conclusions are possible from a comparison of the clumpings (with A and B-cells) of their sera and saline extracts with clumpings of the blank (non-stained material) extracts.

It has been our experience on blood group determination work carried out on very dirty

exhibits, such as clothing from labourers, old felt hats and songkoks, turbans, etc., that the added A and B cells break up during the 37°C. incubation period, especially at the higher concentrations of sera extracts and also in the saline extracts where no dilution is carried out during normal routine grouping. The possible causes for this phenomenon are:—

(a) attack by bacteria that were initially present in a dormant state but have been nourished and consequently activated during the period of sera extraction (approximately 16 hours), and

(b) contamination of sera or physiological saline extracts with the "salts" which are normally present in sweat[4] (sodium chloride, urea, lactic acid, etc.) thus changing the "salt" concentration in the saline which now is no longer isotonic with the red cell contents. The resulting hypertonic solution would cause the cells to shrink, while attack by the bacteria would help to lyse the cell wall, the cell contents oozing out into the suspending fluid.

RECOMMENDATIONS

In all offences of a criminal nature, the bloodstained weapons or instruments, the clothing of both the injured person and the perpetrator, etc., should immediately be taken into custody by the investigating officer. He should then ensure that all the bloodstains are dry and in the case of articles of clothing, he would be well advised to air them inside a well ventilated room for a period of at least three hours, before folding them for packing purposes. The dried bloodstained articles should then be forwarded to the Department of Chemistry, without unnecessary delay. Soon after being received it would be advisable for the Chemist concerned to examine the bloodstains for their origin, blood group, etc., as per request by the investigating officer.

In the opinion of one of us (S.S.) if the above suggestions are closely adhered to at each stage, then inconclusive results of analysis would be reduced to a bare minimum and consequently the possibility of guilt or innocence of a person held in connection with a crime more readily established.

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