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AN INVESTIGATION INTO THE COMMON CAUSES OF BLOODSTAIN DETERIORATION

PART II

Effect of Perspiration and Saliva on Blood Grouping

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In Part II[1] of this work it was noticed that even sweat from a person whose blood belonged to group O had an intermediate effect on the blood agglutinins. Since some sweat is invariably present on bloodstained clothing, it was felt that the effect should be further investigated.

In this investigation, the co-operation of eight staff members, two from each blood group, was sought. Each person was supplied with 4 clean filter papers which had previously been marked with his name and respective blood group. The individuals were told to dab their bodies twice each day, once when they got to office in the morning and once again when they returned to work after lunch. They were to use the same filter paper for four days, i.e., each paper had received eight dabbing sessions before it was put away and a second one used instead. Despite the numerous dabbings, some of the filter papers when returned were noticed to be relatively clean. This was due to the fact that some of those selected to supply the sweat apparently did not perspire. To ensure that the filter papers contained group specific substances before they were counterstained with blood, four persons out of the above eight, each belonging to a different blood group were made to spit into different beakers. The saliva was then used to stain clean filter papers.

When the sweat-stained and saliva-stained filter papers were ready, four samples of oxalated blood, each belonging to a different group were obtained from the Blood Bank at Penang. These bloods were employed to stain filter papers as indicated below:—

	Filter Papers	Nos. of Bloodstained Filter Papers
Group A Blood	Clean	1
	Group A sweat-stained	2 and 3
	Group B „ „	4 and 5
	Group O „ „	6 and 7
	Group AB „ „	8 and 9
	Group A Saliva-stained	10
	Group B „ „	11
	Group O „ „	12
	Group AB „ „	13

Therefore each sample of blood stained thirteen filter papers in all. These were dried in an air-conditioned room and then kept aside for a two-week period, after which their agglutinin and agglutinogen titres were determined as described and Part II[1] and the Grouping of Saliva[2]. The results obtained are given in Tables I to VII.

DISCUSSION

In this piece of work precautions similar to those described in Part II[1] of the work were taken so as to minimise irregularities, and thereby maintain consistency of results.

The suppliers of sweat and saliva have previously been shown to be secretors[2].

The results obtained are best discussed under the two sub-headings:—

(a) Saline Extract.

(b) Anti-sera Extract.

(a) **Saline Extract** (Tables I - III) — These results provide an indication of the agglutinin

concentrations in each stain. Since the sweat and saliva of secretors are known to contain group specific substances[3], we would expect these to remove all or at least in part the corresponding agglutinins from the bloodstains by agglutination. In cases where this takes place, the saline extracts of the bloodstains would show a titre less than that for the corresponding control (bloodstain No. 1).

The above hypothesis is well substantiated by the results obtained (Tables I – III). The more complete removal of agglutinins by saliva may be alluded to the saliva containing a higher concentration of agglutinogens than sweat.

Although sweats from group O individuals are free from group specific substances, yet they do reduce the titres of the blood agglutinins (Tables I – III). The adverse effect is most probably due to the deteriorative effects of sweat on agglutinins. Therefore sweat from secretors would have a two fold effect on bloods, viz., deteriorative and agglutinin removal. The latter effect would only take place if the blood contains an agglutinin capable of reacting with the group specific substance of the sweat.

(b) **Anti-sera Extracts** (Tables IV – VI) — Extraction of bloodstains with the corresponding anti-sera and then a study of its agglutination abilities (with known cells) at different dilutions provides a basis for a comparative study of the agglutinogen concentrations that were originally present in the bloodstains. As a typical example let us consider:—

(1) filter papers stained with the sweat of a group A secretor, and

(2) filter papers stained with the saliva of a secretor whose blood belonged to group A.

(1) **Sweat-stained Filter Papers** — If the sweat-stained filter paper was counter-stained with blood of group A (taken from a different person), then according to theory, the blood-stained area should contain an agglutinogen concentration higher than that which would be present in the bloodstain on a clean piece of filter paper. This would be indicated by a decrease, relative to control (stain 1) in the agglutinations of the anti-A sera extracts.

A similar sweat-stained paper when counter-stained with blood belonging to group B should not show any decrease in its agglutinations with known B cells, although the anti-A sera extract would exhibit reduced/increased agglutination (relative to control) depending upon the relative concentrations of agglutinogen A and blood agglutinin a present.

If, however, the sweat-stained paper was counter-stained with blood from a group AB individual, then the absorption of anti-A sera would increase resulting in a decrease of the extract agglutinations with known A cells. Agglutinations of the anti-B extract would correspond to those of the control.

Sweat from a group A secretor counter-stained with group O blood would only show changes in the saline extracts, unless the concentration of any group specific substances present is in excess of the corresponding blood agglutinin present.

(2) **Saliva** — The same sort of results would be expected from filter papers first stained with the saliva of a group A secretor and then counter-stained with blood.

From the agglutination results of sera extracts (Tables IV – VI) it will be noticed that those bloodstains containing saliva (Nos. 10 – 13) comply with the above theoretical requirements while the others (Nos. 2 – 9) containing sweat behave as though they were non-contaminated bloodstains (No. 1) or contaminated by substances that do not have agglutinin/agglutinogen reactions.

This apparent non compliance especially for sera extracts may be explained on the following basis:—

(a) That the bloodstain was on an area free of sweat.

(b) That the concentration of group specific substances in sweat is small and these in most cases have already been removed by agglutination at the time of counter-staining with blood.

Results — A summary of these cases where the results of grouping were inconclusive or the determined blood group differed from the group of the blood used to stain the filter papers is given in the following page:—

GROUP A BLOOD USED FOR STAINING

Stain No.	Contaminant	Sera Extract Result	Saline Extract Result
10	Saliva A	inconclusive	A
11	Saliva B	inconclusive	AB
13	Saliva AB	inconclusive	AB

GROUP B BLOOD USED FOR STAINING

Stain No.	Contaminant	Sera Extract Result	Saline Extract Result
2	Sweat A	B	AB
3	Sweat A	B	AB
8	Sweat AB	B	AB
10	Saliva A	inconclusive	AB
11	Saliva B	inconclusive	B
13	Saliva AB	inconclusive	AB

GROUP AB BLOOD USED FOR STAINING

Stain No.	Contaminant	Sera Extract Result
2	Sweat A	inconclusive
3	Sweat A	inconclusive
6	Sweat O	inconclusive
9	Sweat AB	inconclusive
10	Saliva A	inconclusive
11	Saliva B	inconclusive
12	Saliva O	inconclusive
13	Saliva AB	inconclusive

GROUP O BLOOD USED FOR STAINING

Stain No.	Contaminant	Saline Extract Result
3	Sweat A	A
5	Sweat B	B
8	Sweat AB	B
10	Saliva A	A
11	Saliva B	B
13	Saliva AB	AB

The results in the "blood group indicated" column (Tables IV - VI) were obtained by comparing the agglutinations of sera extracts from bloodstained areas with those of aera extracts from areas free of blood (Table VII). Where the difference in the agglutinations of the two corresponding sera extracts was greater than three places a definite group was reported. In all other cases the results were recorded as being inconclusive.

Grouping tests on saliva stains 10, 11 and 13 counter-stained with blood belonging to group A were in each case inconclusive. Stain number 10 and the corresponding blank showed a complete removal of agglutinin a and therefore, no conclusions were possible. The inconclusiveness in the case of stain number 11 was due to the saliva agglutinogen B being present in excess of the blood agglutinin b. This excess showed up in the anti-B sera extract while the deficient blood agglutinin b did not show up in the saline extract. From the blank for stain number 11, it may be inferred that the agglutinogen B in the saliva had a concentration either equal to or slightly greater than the concentration of the agglutinin b in the sera used for extractions. Again the case of stain Number 13, the B agglutinogen in the saliva of an AB secretor was

in excess of the blood agglutinin b and therefore, the latter did not show up in the saline extract. The B-cell agglutinations being in excess of A-cell agglutinations may be explained on the basis that at the time of stain extraction the B agglutinogen concentration was less than the A agglutinogen concentration, since the former agglutinogen had already been partly removed by the naturally occurring blood agglutinin b, while the saliva agglutinogen A, instead had a further addition from the blood.

With the blood of group B, the bloodstains containing sweat unlike those containing saliva, gave definite (clear cut) yet contradictory results for the sera and saline extracts. To account for these stain numbers 2 and 3 must have had a concentration of agglutinogen A (from sweat) just in excess of the concentration of the blood agglutinin a. This resulted in the complete removal (by agglutination) of the latter, hence its absence in the saline extract. The balance of the slight excess agglutinogen A did not exert any significant effect on the agglutinations of the anti-A sera extracts with known A cells. The results obtained for stain number 8 could be explained along the same lines.

In a similar manner, all the remaining anomalous results could be explained.

From the above one could therefore reasonably conclude that in the case of bloodstains contaminated with the sweats of secretors, the results of grouping tests obtained would depend upon the relative titres of the agglutinogens of the contaminating fluids and the corresponding blood agglutinins.

Invariably with saliva which has a higher concentration of group specific substances, the grouping tests tend to show the presence of the excess agglutinogen.

Since with group AB and O bloodstains only sera and saline extracts respectively were made and their agglutinations observed, it may be argued that the erroneous/inconclusive results with these two bloods may not have arisen if the sera and saline extracts had been studied in each of these cases, just as is usually done when grouping bloodstains of unkonwn group.

In the opinion of one of us (S.S.) this extra labour would not have yielded any results better than those already obtained, for the following reasons:—

Group AB bloodstains — The stains of this blood when contaminated with stains of agglutinogen containing fluids would only alter the agglutinogen concentration of the bloodstain and therefore the saline extracts would not be expected to show any signs of agglutination with known A and B cells.

Group O bloodstains — Sera extracts from these stains would either yield no decisive agglutination results if agglutinogen concentration in the contaminant is equal to the corresponding agglutinin concentration in the blood (unless the contamination is by fluid from a group AB secretor), or if the agglutinogen concentration is in excess of that of the corresponding agglutinin, then the sera extract would only help to confirm the wrong results already indicated by the saline extract agglutinations.

From the summary given on page 264 it is obvious that the contaminant — saliva — has a marked effect! and in almost all cases yields inconclusive results. It can, however, be responsible for erroneous results, if during grouping, the saliva contaminated bloodstain were extracted with sera and saline and for the blank instead an area free of saliva had been unintentionally selected. Then stains such as number 11 (Tables I and IV) and numbers 10 and 13 (Tables II and V) would confidently have been reported as belonging to group AB although they actually belonged to groups A and B respectively. Thus even with the method of double check (sera extract with saline extract) erroneous results are possible.

The forensic scientist should, therefore, be extra careful when examining bloodstained articles in which the probability of contamination by fluids (saliva and seminal fluid) containing a relatively high concentration of group specific substances is present. Such articles would include handkerchiefs, materials used for gagging purposes, bloodstained clothing in cases of rape, etc.

RESULTS OF GROUPING TESTS CARRIED OUT ON FILTER PAPERS STAINED WITH BLOOD OF GROUPS A, B & O STAINS EXTRACTED WITH PHYSIOLOGICAL SALINE

			TABLE I — GROUP A BLOODSTAINS									TABLE II — GROUP B BLOODSTAINS									TABLE III — GROUP O BLOODSTAINS								
Filter Paper Number	Blood Group of Secretor	Blood Cells	1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	Blood Group Indicated	1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	Blood Group Indicated	1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	Blood Group Indicated
1	Nil	A	—	—	—	—	—	—	—	—	A	+	±	—	—	—	—	—	—	B	+	+	+	+	+	+	±	—	O
		B	+	±	+	+	+	±				—	—	—	—	—	—	—	—		+	+	+	+	—	—	—	—	
2	A	A	—	—	—	—	—	—	—	—	A	—	—	—	—	—	—	—	—	AB	+	—	±	—	—	—	—	—	O
		B	+	+	+	+	+	±				—	—	—	—	—	—	—	—		+	±							
3	A	A	—	—	—	—	—	—	—	—	A	—	—	—	—	—	—	—	—	AB	—	—	—	—	—	—	—	—	A
		B	+	+	+	+	+	+	—	—		—	—	—	—	—	—	—	—		+	—	—	—	—	—	—	—	
4	B	A	—	—	—	—	—	—	—	—	A	+	—	—	—	—	—	—	—	B	+	+	+	+	+	+	+	—	O
		B	—	+	—	—	—	—	—	—		—	—	—	—	—	—	—	—		+	—	—	—	—	—	—	—	
5	B	A	—	—	—	—	—	—	—	—	A	+	±	—	—	—	—	—	—	B	+	+	+	—	—	—	—	—	B
		B	+	±	—	—	—	—	—	—		—	—	—	—	—	—	—	—		—	—	—	—	—	—	—	—	
6	O	A	—	—	—	—	—	—	—	—	A	+	—	—	—	—	—	—	—	B	+	+	+	—	—	—	—	—	O
		B	+	+	+	+	—	—	—	—		—	—	—	—	—	—	—	—		+	+	—	—	—	—	—	—	
7	O	A	—	—	—	—	—	—	—	—	A	+	—	—	—	—	—	—	—	B	+	+	+	+	+	+	+	—	O
		B	+	+	+	+	+	—	—	—		—	—	—	—	—	—	—	—		+	+	+	+	—	—	—	—	
8	AB	A	—	—	—	—	—	—	—	—	A	—	—	—	—	—	—	—	—	AB	+	±	—	—	—	—	—	—	B
		B	±	+	—	—	—	—	—	—		—	—	—	—	—	—	—	—		—	—	—	—	—	—	—	—	
9	AB	A	—	—	—	—	—	—	—	—	A	+	—	—	—	—	—	—	—	B	+	+	+	+	+	—	—	—	O
		B	+	+	+	+	—	—	—	—		—	—	—	—	—	—	—	—		+	+	+	—	—	—	—	—	
10	A	A	—	—	—	—	—	—	—	—	A	—	—	—	—	—	—	—	—	AB	—	—	—	—	—	—	—	—	A
		B	+	+	+	+	+	+	—	—		—	—	—	—	—	—	—	—		+	+	+	±	—	—	—	—	
11	B	A	—	—	—	—	—	—	—	—	AB	+	—	—	—	—	—	—	—	B	+	+	+	+	+	—	—	—	B
		B	—	—	—	—	—	—	—	—		—	—	—	—	—	—	—	—		—	—	—	—	—	—	—	—	
12	0	A	—	—	—	—	—	—	—	—	A	+	—	—	—	—	—	—	—	B	+	+	+	+	+	+	+	—	O
		B	+	+	+	—	+	—	—	—		—	—	—	—	—	—	—	—		+	+	+	+	—	—	—	—	
13	AB	A	—	—	—	—	—	—	—	—	AB	—	—	—	—	—	—	—	—	AB	—	—	—	—	—	—	—	—	AB
		B	—	—	—	—	—	—	—	—		—	—	—	—	—	—	—	—		—	—	—	—	—	—	—	—	
Anti - A Anti - B	Nil	A	+	+	+	+	+	+	+	+		+	+	+	+	—	+	+	+		+	+	+	+	+	+	+	+	
		B	+	+	+	+	+	+	+	+		+	+	+	+	—	+	+	+		+	+	+	+	+	+	+	+	

\+ indicates agglutination.
± indicates beginnings of agglutination.
— indicates no agglutination.

RESULTS OF GROUPING TESTS CARRIED OUT ON FILTER PAPERS STAINED WITH BLOOD OF GROUPS A, B & AB AND ON THE BLANKS STAINS EXTRACTED WITH 1 IN 2 DILUTION OF ANTI-A AND ANTI-B SERA

TABLE IV											TABLE V										TABLE VI										TABLE VII								
			GROUP A BLOODSTAINS								GROUP B BLOODSTAINS										GROUP AB BLOODSTAINS										BLANKS								
Filter Paper Number	Blood Group of Secretor	Blood Cells	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	Blood Group Indicated	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	Blood Group Indicated	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	Blood Group Indicated	Sweat/Saliva Stained Area	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256
1	Nil	A	+	−	−	−	−	−	−	−	A	+	+	+	+	+	+	+	+	B	+	−	−	+	−	−	−	−	AB	Nil	+	+	+	+	+	+	+	+
		B	+	−	−	−	−	+	+	+		+	−	−	−	−	−	−	−		+	+	−	+	−	−	−	−			+	+	+	+	+	+	+	+
2	A	A	+	+	−	−	−	−	−	−	A	+	+	+	−	−	−	−	−	B	+	−	−	−	−	−	−	−	Inconclusive	Sweat	+	+	+	+	+	+	−	−
		B	+	−	−	+	+	+	+	+		+	+	−	−	−	−	−	−		+	−	−	−	−	−	−	−			+	+	+	+	+	+	+	+
3	A	A	−	−	−	−	−	−	−	−	A	+	+	+	−	−	−	−	−	B	+	−	+	−	−	−	−	−	Inconclusive	Sweat	+	+	+	+	+	+	−	−
		B	+	−	−	+	+	+	+	+		+	+	−	−	−	−	−	−		+	−	−	−	−	−	−	−			+	+	+	+	+	+	+	+
4	B	A	+	+	−	−	−	−	−	−	A	+	−	−	−	−	−	−	−	B	+	+	+	−	−	−	−	−	AB	Sweat	+	+	+	+	+	+	+	+
		B	+	+	+	+	+	+	+	−		−	−	−	+	+	+	+	+		+	+	+	−	−	−	−	−			+	+	+	+	+	+	+	+
5	B	A	+	+	−	−	−	−	−	−	A	+	−	−	−	−	−	−	+	B	+	+	+	+	−	−	−	−	AB	Sweat	+	+	+	+	+	+	+	+
		B	+	+	+	+	+	+	+	+		−	−	−	−	−	−	−	−		+	+	+	+	−	−	−	−			+	+	+	+	+	+	+	+
6	O	A	+	+	−	−	−	−	−	−	A	−	−	−	−	−	−	−	−	B	+	+	+	+	+	−	−	−	Inconclusive	Sweat	+	+	+	+	+	+	+	+
		B	+	+	+	+	+	+	+	+		−	−	−	−	−	−	−	−		+	+	+	+	−	−	−	−			+	+	+	+	+	+	+	+
7	O	A	−	+	−	−	−	−	−	−	A	−	−	−	−	−	−	−	−	B	+	+	+	−	−	−	−	−	AB	Sweat	+	+	+	+	+	+	+	+
		B	+	+	+	+	+	+	+	+		−	−	+	−	−	−	−	−		+	+	+	−	−	−	−	−			+	+	+	+	+	+	+	+
8	AB	A	+	+	−	−	−	−	−	−	A	+	−	−	−	−	−	−	−	B	+	−	+	−	−	−	−	−	AB	Sweat	+	+	+	+	+	+	+	+
		B	+	+	+	+	+	+	+	+		+	+	−	−	−	−	−	−		+	+	+	−	−	−	−	−			+	+	+	+	+	+	+	+
9	AB	A	+	+	−	−	−	−	−	−	A	+	−	−	−	−	−	+	+	B	+	+	+	+	+	−	−	−	Inconclusive	Sweat	+	+	+	+	+	+	+	−
		B	+	+	+	+	+	+	+	+		−	−	−	−	−	−	−	−		+	+	+	+	+	−	−	−			+	+	+	+	+	+	+	+
10	A	A	−	−	−	−	−	−	−	−	Inconclusive	−	−	−	−	−	−	−	−	Inconclusive	−	−	−	−	−	−	−	−	Inconclusive	Saliva	−	−	−	−	−	−	−	−
		B	+	+	+	+	+	+	+	+		−	−	−	−	−	−	−	−		+	+	+	+	+	−	−	−			+	+	+	+	+	+	+	+
11	B	A	+	+	+	+	−	−	−	−	Inconclusive	+	+	+	+	+	+	−	−	Inconclusive	+	−	+	+	+	−	−	−	Inconclusive	Saliva	+	+	+	+	+	−	−	+
		B	+	−	−	−	−	−	−	−		−	−	−	−	−	−	−	−		−	−	−	−	−	−	−	−			−	−	−	−	−	−	−	−
12	O	A	+	+	+	−	−	−	−	−	A	+	+	+	+	+	−	−	−	B	+	−	+	+	+	−	−	−	Inconclusive	Saliva	+	+	+	+	+	+	+	−
		B	+	+	+	−	−	−	−	−		−	−	−	−	−	−	−	−		+	+	+	+	+	−	−	−			+	+	+	+	+	+	+	+
13	AB	A	−	−	−	−	−	−	−	−	Inconclusive	+	−	−	−	−	−	−	−	Inconclusive	−	−	−	−	−	−	−	−	Inconclusive	Saliva	−	−	−	−	−	−	−	−
		B	+	−	−	−	−	−	−	−		−	−	−	−	−	−	−	−		+	+	−	−	−	−	−	−			−	−	−	−	−	−	−	−

+ indicates agglutination.

± indicates beginnings of agglutination.

— indicates no agglutination.

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