GROUP-SPECIFIC SUBSTANCES IN SWEAT.

A consideration of the medico-legal value of absorption methods applied to sweat-stains on clothing, with an account of the results obtained in fifty experimental cases,

by

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C O N T E N T S.

PART ONE.

PART TWO.

PART ONE.

Introduction.

It is now nearly fifty years since Landsteiner first demonstrated the presence of group-specific substances in human blood, and so opened the gateway to a fresh field of enquiry which has claimed the attention of many investigators. The main developments which have taken place are well enough known, and can be studied in any of the comprehensive surveys of the subject which are now available. It is sufficient here simply to remark on the widespread nature of the contributions made by these developments to biological knowledge and understanding. The extending fringe of the field includes much that is uncertain, or speculative, or beset with technical complexity, but a great deal has already been established and confirmed with a certainty which permits of practical application. This is notably so in connection with blood transfusion, but is true also of the methods which are available for the solution of a variety of medico-legal problems.

The forensic possibilities of blood grouping depend primarily on the proved constancy of the blood group in any one individual, on the known manner in which the blood groups are inherited, and on the fact that the group-specific factors are relatively stable and persistent substances, whatever their precise chemistry/

chemistry may be. The heredity of the blood groups has an obvious and natural application in cases of disputed paternity, but occasionally the same knowledge can be applied in more unusual circumstances, as is exemplified in the case which is described later in this thesis. The persistent nature of the group-specific substances and the evolution of a suitable technique for their detection made it possible to determine the group, not only of fresh blood, but also of blood stains; and it is from this point and in this direction that the study of blood-grouping has derived its forensic significance in criminal cases.

The earliest method applied to blood stains was the direct testing, with an extract from the stain, of fresh corpuscles from an individual believed to be the source of the stain. Agglutination of the corpuscles in such circumstances - (termed Landsteiner and Richter's reaction) - excluded the suspected individual. To this extent the test is valid though its scope is obviously very limited, and there appears to be no record of its actual use in a legal case. But the basic assumption of this early test, - namely that the agglutinins persist in a blood stain - has been confirmed by the findings of many investigators, although it has been realised that the persistence is by no means indefinitely prolonged, and that there is, in/

in fact, a fairly rapid falling off in agglutinin activity so that the passage of time may render their detection difficult or even impossible. In suitable cases, however, positive information can be obtained, and the first evidence of the kind to be accepted in a Court of Law was derived from the detection of agglutinins by Lattes, in 1916. Thereafter, the method was employed and recognised with increasing frequency, and the determination of the agglutinin content of a stain, if possible, still remains an essential part of any blood stain investigation.

By about 1920, the corresponding assumption that the agglutinogens also persisted in blood stains had been confirmed by means of absorption tests which resulted in a selective weakening of the appropriate anti-sera. Indeed, although there was some early divergence of opinion, it has become generally recognised that the agglutinogens are more persistent and more constantly detectable in stains than are the agglutinins. Moreover, absorption tests can be more easily standardised than the methods applicable to agglutinins. The detection of agglutinogens has tended therefore to become the primary object in the examination of a blood stain, while the earlier agglutinin techniques are regarded as confirmatory procedures which must always be included in the investigation/

investigation and may prove of decisive importance, but which cannot be relied upon to the same extent.

By 1930, it was recognised that group-specific substances A and B are present also in the cells of most of the organs of the body, and that, in the majority of individuals - (secretors) - water soluble group-specific substances can be detected in most of the body fluids and secretions. The technique employed for their detection in fluids or secretions is similar to the absorption methods used for the detection of agglutinogens in blood stains. If an agglutinogen is present, it will "inhibit" the activity of the corresponding anti-serum. These methods have been applied, therefore, in medico-legal cases involving stains other than those due to blood, - usually seminal stains, but also, in a limited number of cases, saliva stains on cigarette ends and even on the gummed edges of envelopes. Sweat is included among the bodily secretions which have been shown to contain the group specific substances A and B in secreting individuals, and it will be necessary to review the findings in respect of sweat more fully at a later stage.

Limitations to the Medico-legal value of Blood Grouping Tests.

To the forensic application of blood grouping methods to stains, of whatever nature, there are many limitations both as regards applicability and the value/

value of the results as evidence. The methods available, although reliable enough in expert hands, are by no means simple and straightforward, and the possibilities of technical error or mishap require to be guarded against most carefully. The interpretation of results must be accurately standardised, and controls are almost absolutely essential if misinterpretation is to be avoided. The difference between the group investigation of stains and the simple grouping tests which are applicable to fresh blood is perhaps not sufficiently appreciated by the legal and police authorities in their expectations on the subject or in their acceptance of results.

Apart altogether from such technical considerations, successful grouping may be rendered impossible by the smallness of the stain or its age, or by reason of contamination, or the conditions to which the stain has been subjected prior to examination. Such factors may preclude grouping tests altogether, and even when attempts are possible, it is most unwise to modify accepted standards of interpretation in order to arrive at a tentative result.

If the agglutinins in a stain have lost their activity and cannot therefore be detected, failure also to detect the presence of agglutinogens may indicate that the stain is of Group O, but could equally well be due to deterioration in any agglutinogen/

agglutinogen content originally present. In the same way, absence of agglutinogen from secretion stains may indicate group O, A or that the individual is a non-secretor, or that the agglutinogens have deteriorated. In all such circumstances, the findings will be essentially negative, and the expression of a positive opinion as to the group of the stain is rarely justifiable.

Limitations of a different nature are imposed by the known incidence of the blood groups. In the vast majority of stain investigations, a comparison is involved, and, as a rule, only the demonstration of a difference in the group of bloods from two or more situations is of positive value as evidence. The occurrence of blood or other stains of identical groups in such situations is usually no more than corroborative or suggestive. This limitation is well exemplified in a great variety of cases of homicide or assault where the clothing of the suspect is stained with blood of the same group as that of the victim. This may well be suggestive, even strongly so when the position and extent of the stain are considered, or when the group is a relatively uncommon one, but frequently the suspect has a plausible explanation for the presence of the stains on the basis that the blood is derived from an injury of his own person. In such circumstances, it is obviously desirable to ascertain the blood group of the/

the suspect, but under existing legislation in this country, it is rarely possible to obtain a sample of blood for this purpose. Consequently, blood grouping evidence is almost invariably presented without the blood group of the accused being known and as a result, the positive value of such evidence is diminished, in many cases to zero. This is a very major limitation, and there is no likelihood of the Law being amended to permit of blood samples being taken from a suspected or accused person for blood grouping tests which may furnish evidence against him. Any practical methods, therefore, which will obviate this outstanding difficulty to an appreciable extent, are worthy of serious consideration.

The Record of an Original Case.

I have regarded it as appropriate to commence with the foregoing brief survey of the development of medico-legal blood grouping methods and their limitations, because it is against that background that I wish now to consider an actual case which presented certain interesting and unusual features, and in which I carried out all the blood grouping investigations.

In September, 1946, an unmarried woman, aged 40 years, was murdered by manual strangulation. She had received a number of blows on the head which had bled/

bled freely. The post-mortem examination revealed also that she was pregnant, and the foetus was estimated to be of between two and three months' development. The fact of her pregnancy was important as an indication of motive, as the suspect was a married man, living in the vicinity, with whom the deceased woman was known to have been associating on intimate terms for some time previously. There was blood staining on the clothing of the deceased, and also on both sleeves and the right breast of the accused's jacket, on the brim of his hat, and to a slight extent elsewhere on his clothing. The accused explained the presence of these stains by asserting that he found the body lying already dead, and lifted it into a nearby shed.

In addition to the stained clothing, I had available for examination two samples of the blood of the deceased, and saline suspensions of material aspirated from the heart and liver of the foetus, all of which were obtained at the post mortem examination.

The blood group of the deceased, as shown by direct testing of her erythocytes with known antisera, and by confirmatory testing of known cells with her serum, was Group B.

By means of washing and separation and resuspension, it was possible to prepare a satisfactory red/

red cell suspension from the foetal material, and to test repeatedly with varying dilutions of known antisera. By these means, the blood group of the foetus was satisfactorily established as being Group AB.

There were two points of interest in these findings. The first was the confirmation by personal observation that the A and B agglutinogens are present and can be detected serologically in the erythocytes of the foetus at this early stage of development - certainly not more than three months. Only a few cases are recorded of the detection of agglutinogens at such an early period in foetal life. The second point was of greater practical importance in connection with the case, namely, the inference that the father of the foetus must have been a man of Blood Group A or Blood Group AB. Hence, although in this case the blood stains on the clothing were acknowledged to be derived from the deceased, the desirability of knowing the blood group of the accused was still of prime importance, for another, and less usual reason; and again, the impossibility of obtaining a sample of blood threatened to render the blood grouping evidence entirely inconclusive and even pointless.

Nevertheless, I proceeded to the grouping of the stained clothing of the accused using the technique which is described later in connection with my subsequent/

subsequent experiments. For my first test I selected a blood stained area of the lining of the left sleeve of the jacket. My reason for selecting this stain was that there appeared to have been some attempt at washing off the blood on the outside of the sleeve, but this had left the stain on the lining comparatively unaffected. Moreover, lining material is, in general, better to work with in these tests than a piece of woven worsted cloth. For a control, I used an adjacent, unstained portion of the sleeve lining. There appeared no reason to doubt that the blood had come from the deceased woman, and I anticipated results indicative of Group B. I was surprised, therefore, to find that there had been quite definite diminution in the agglutinin activity of both anti-A and anti-B sera, to a degree which might easily have given rise to an assumption that the stain was of Group AB, but for the control results. (See Table 1). In the control, the anti-A serum was almost equally reduced in potency, but the anti-B serum was virtually unaffected, a finding which also precluded any facile explanation on a basis of non-specific absorption.

Table 1./

TABLE 1.

Results of absorption tests on blood-stained area of lining from the left sleeve of accused's jacket, and on unstained area of lining as control.

Titre of Test Sera.	Anti-A Serum.	Anti-B Serum.
Unabsorbed	128	128
Absorbed with unstained area	8	64
Absorbed with blood-stained area	4	2

Absorption tests on a blood stained area from the outside of the jacket, on stains on the brim of the accused's hat, and on the stains on the clothing of the deceased all gave results indicating that the Blood was of Group B. (See Table 2). In the case of the stain on the hat, the result was confirmed by agglutinin tests which demonstrated the presence of anti-A agglutinin.

Table 2./

TABLE 2.

Results of absorption tests on blood-stained areas of

(a) the outer surface of accused's jacket;

(b) the brim of accused's hat; and

(c) the overall worn by the deceased.

Titre of Test Sera.	Anti-A Serum.	Anti-B Serum.
(a) The outer surface of accused's jacket.		
Unabsorbed	128	128
Absorbed with unstained area	64	64
Absorbed with blood-stained area	64	4
(b) The brim of accused's hat.		
Unabsorbed	128	64
Absorbed with blood stain	128	2
(c) The overall worn by the deceased.		
Unabsorbed	128	64
Absorbed with unstained area	64	64
Absorbed with blood-stained area	64	2

From a consideration of these results, I concluded that the unexpected findings from the stained area of lining were in fact due to the presence in the lining of agglutinogen A, and that this was probably derived from the sweat of the wearer of the jacket. This presumption was supported to some extent by the fact that the accused's employment involved/

involved considerable exertion, that the shirt available for examination had short sleeves which terminated above the elbow, and that the jacket was a well-worn working jacket. Considerable sweat staining of the lower sleeve lining was an obvious possibility, therefore, with a consequent agglutinogen content if the wearer was a secreting individual.

I next carried out absorption tests on an obviously sweat stained area of the lining, taken from the axilla. The results (see Table 3) were strongly suggestive of Group A, but the test suffered from the limitation that no proper control could be carried out, since there was apparently considerable sweat staining throughout the sleeve lining, and the lining of the jacket elsewhere was of a different material. It seemed improbable, however, that the lining material should so peculiarly affect the titre of the anti-A serum while leaving the anti-B serum unaffected, and on a basis of these tests, a tentative opinion was expressed that the habitual wearer of the jacket, presumably the accused, was of Blood Group A.

TABLE 3.

Results of absorption tests on sweat-stained area of lining from the armpit of accused's jacket.

Titre of Test Sera.	Anti-A Serum.	Anti-B Serum.
Unabsorbed	128	64
Absorbed with sweat-stain	8	64

Fortunately,/

Fortunately, a seminal stain was subsequently found on a pair of the accused's trousers. Group testing of this stain gave results which confirmed the opinion (see Table 4), and greatly strengthened my conviction that the accused was of Blood Group A, and could therefore be the father of the foetus discovered at the post mortem examination of the deceased.

TABLE 4.

Results of absorption tests on seminal stain on the trousers of the accused.

Titre of Test Sera.	Anti-A Serum.	Anti-B Serum.
Unabsorbed	256	64
Absorbed with unstained area	64	32
Absorbed with seminal stain	4	32

It is of interest to note that this case had many points in common with the case of Rex v. R. Smedley (September 1937). Smedley was charged with the murder of a young woman who was found dead in a field near her home, strangled by means of a necktie, and suffering also from multiple head injuries which had bled freely. Staining of the accused's clothing was due to his having carried the body for a short distance, and the blood of the deceased and the stains on the accused were both of Group O. That, however, was as/

as far as the blood grouping evidence could go. The deceased was found at the post-mortem examination to be three months pregnant, but no attempt appears to have been made to ascertain the blood group of the foetus. In any case, the value of such information would have remained problematical, for, as usual, the blood group of the accused could not be established.

It happens not infrequently that an illegitimate pregnancy provides a motive for the murder of a woman, and the corroborative value of "paternity" grouping tests, if possible, are obvious. If blood stains are also present, it becomes doubly desirable that information be available as to the blood group of the accused; hence the unusual interest and importance of the findings in the case which I have described.

Preliminary Experiments.

With such considerations in mind, I felt that the results obtained from the sweat stains in the case described were sufficiently clear-cut and of such positive value that further investigation was called for. If, in even a fair proportion of cases, the group of an accused person could be established from an examination of selected articles of his clothing, then to that same extent would one of the most serious practical limitations to the value of blood grouping evidence be overcome. The idea seems fairly obvious, but it was not one which I had heard or seen suggested in any discussion or reading on the subject.

For/

For a preliminary series of experiments, I selected six individuals more or less at random. Routine testing of the cells and serum of these individuals showed that two were of Group O, two were of Group A, one was of Group B and one was of Group AB, so that the selection proved fairly representative. At this stage, no attempt was made to determine whether the individuals were secretors or not. The respective blood groups having been established, I obtained from each a sweat-stained area, measuring approximately one square inch, from the lining of an outer garment. In five of the six cases, the area was cut from the armpit of the jacket sleeve; the exception, to which further reference will be made later, was an area taken from the lining of a waistcoat over the small of the back. The selected areas were tested by the absorption (or inhibition) technique subsequently described. No controls were included in this preliminary investigation, as the object was simply to ascertain whether, in a random selection of cases, the results were sufficiently suggestive to warrant a more extensive series of experiments.

In the two cases of Group O, no positive evidence was to be anticipated from a sweat-stain. Nevertheless I regarded these two cases as being of fundamental importance, inasmuch as the results obtained from/

from them would indicate whether or not a marked degree of non-specific absorption was to be expected from sweat stains. If so, the value of any further inquiry would be extremely problematical. In neither case, however, was there any great degree of diminution of the titre of the anti-sera, and the results (see Table 5) suggested that the factor of non-specific absorption was not one which would necessarily preclude the possibility of valid deductions in cases where group substances A or B were present in the stains. To this extent, therefore, they were encouraging and of positive value.

TABLE 5.

Results of absorption tests on areas of sweat-stained jacket lining from two individuals of Blood Group O.

Titre of Test Sera.	Anti-A Serum.	Anti-B Serum.
First case.		
Unabsorbed	128	128
Absorbed with sweat stain	64	128
Second case.		
Unabsorbed	128	128
Absorbed with sweat stain	64	64

In the two cases of Group A, (see Table 6) the effects of absorption of anti-B sera with the sweat stains were also relatively slight. In neither case was/

was the effective titre of the anti-B serum diminished to a degree which could be considered significant; such reduction as there was is quite commonly found after absorption with almost any sort of material, even when quite free of any stains whatever. This tended to confirm the impression that sweat stains, in the circumstances of these experiments, do not cause a misleading degree of non-specific absorption. On the other hand, the effective titre of the anti-A serum was markedly diminished in both cases. The figures represent a "shift to the left" of five places in one instance and four in the other. In subjects of known Group A, in whose cases the sweat stain had had little or no effect on Anti-B sera, it was difficult to believe that such definite weakening of the anti-A sera could be due to anything other than the presence of group substance A in the sweat stains of secreting individuals.

TABLE 6.

Results of absorption tests on areas of sweat stained jacket lining from two individuals of Blood Group A.

Titre of Test Sera.	Anti-A Serum.	Anti-B Serum.
First Case.		
Unabsorbed	64	128
Absorbed with sweat stain	4	64
Second Case.		
Unabsorbed	64	128
Absorbed with sweat stain	2	32

The figures for the case of Group AB were equally suggestive of a detectable content of both group substances A and B in the sweat stains (See Table 7). In this case, the "shift to the left" was six places in respect of the anti-B serum, but only four for the anti-A serum. This latter figure represents a shift which would ordinarily be regarded as significant, (and no doubt it was so in this case); but it was less than the figures for the other cases in which the presence of Group A substance in the sweat was to be anticipated. This was of interest because, although I deliberately excluded all consideration of the subgroups of A from these experiments I know that the particular individual is, in fact, of Group A_2B. In all absorption tests, absorption by Group A_2 substance is always less than by A_1. The results in this case suggested therefore, that even A_2 substance in sweat did produce a significant diminution of the anti-A titre, though its relatively weak absorptive power might conceivably give rise to anomalous results in actual practice. At all events, the result in this particular experiment gave an impression of consistency which was encouraging, and the apparently marked degree of absorption of the anti-B serum was a further feature of interest.

Table 7./

TABLE 7.

Results of absorption tests on an area of sweat stained jacket lining from an individual of Blood Group AB.

Titre of Test Sera.	Anti-A Serum.	Anti-B Serum.
Unabsorbed	256	128
Absorbed with sweat stain	16	2

The sixth case in this preliminary series was of Group B (see Table 8). The individual stated that he sweated most profusely on the back, and he therefore "donated" an area of lining material cut from the back of a waistcoat as being most suitable for my purpose. The results of repeated absorption tests, however, were inconclusive, as were those obtained from an area of armpit lining cut from the same suit, and I went on to test the saliva of the subject to determine whether he was a secretor or not. The results showed that he was a secretor. I next obtained an area of fresh sweat staining on clean blotting paper from the same individual, and carried out further tests. The results of these were highly suggestive of the presence of Group B substance only in the stain.

Table 8./

TABLE 8.

Results of absorption tests on areas of sweat stained lining, on the saliva, and on a fresh sweat stain from an individual of Blood Group B.

Titre of Test Sera.	Anti-A Serum.	Anti-B Serum.
(a) Waistcoat (lining).		
Unabsorbed	256	128
Absorbed with sweat stain	32	8
(b) Jacket sleeve (lining).		
Unabsorbed	256	128
Absorbed with sweat stain	128	32
(c) Saliva.		
Unabsorbed	256	128
Absorbed with saliva	256	0
(d) Fresh Sweat stain.		
Unabsorbed	256	128
Absorbed with sweat stain	256	16

I felt it necessary, therefore, to make closer inquiry into the history of the particular suit, which I had not actually seen. The suit was one which had been much worn and no doubt much sweat stained in India. But it had been dry-cleaned about a year before my experiments, and had been worn only on a few occasions in this country since the cleaning. It appears/

appears probable that these circumstances explain the failure of my absorption tests. The case, however, did make apparent the likelihood of certain obvious pitfalls and limitations to the method which I proposed to investigate.

Using the same methods, I carried out a further preliminary series of experiments on twelve more individuals of known blood groups - six of Group A, five of Group O, and one of Group B - with results which were not sufficiently different from the first series to warrant detailed tabulation. Two cases occurred in which the conclusions to be drawn were by no means clear cut, and one in which an absence of absorption was associated with a Group A individual who was a non-secretor, as proved from his saliva.

I also carried out a small number of similar experiments, using sweat-stained articles of underclothing, shirts and pyjamas, but with results which were comparatively disappointing. It appeared, not surprisingly, that garments which had been recently or repeatedly washed or cleaned are not suitable for the type of investigation which I was pursuing, and that the greater prospect of success lay in material from an outer garment which had not been cleaned or washed.

On the whole, the results of the preliminary experiments were such as to support the impression gained from the original case that in many instances it/

it might prove practicable to determine the blood group of an individual, in particular an accused person, from an examination of his outer clothing.

Review of the Previous Work on the presence of Group Specific Substances in Sweat.

The presence of group specific substances A and B in the sweat of secretors is, of course, well enough established, and while the preliminary experiments were in progress, I consulted the literature to ascertain what work had already been done in this connection and, in particular, whether any experiments or opinions were recorded which had the same medico-legal bearing as the one which was now concerning myself.

The presence of group specific substances in bodily secretions appears to have been first determined by Yamakami, in Tokio, in 1926, although the claim is probably open to dispute. His experiments were with saliva and seminal fluid, and all subsequent work has confirmed that these secretions (in secretors) do contain such substances in high concentration. Within the following five years, similar evidence of their presence was forthcoming in respect of almost every body fluid, although in varying concentration. During the same period and as a consequence of this work, the distinction between secretors and non-secretors was drawn.

Among the fluids investigated, sweat was included,/

included, but it does not appear to have been the subject of very intensive or widespread study. The most interesting reference, from my point of view, is to the work of Kan Itiosida who was amongst the earliest to demonstrate the presence of group specific substances in many body fluids, including sweat. In discussing his experiments, he does postulate the idea that the possibilities of identification were enormously increased by the fact that it would now be practicable to determine the group of sweat, urine, and faecal stains, as well as those due to saliva and seminal fluid. This is the only reference which I have found to the potential positive value of sweat stains for medico-legal purposes and I have found no record of sweat stains being in fact utilised in this manner.

Harley (1943) states simply that "the discovery "that the blood group factors occur in a water-"soluble form in the body fluids led to an extension "of the tests (for medico-legal purposes) to saliva "and semen stains", and that "the secretion stains so "far employed for medico-legal work are those of "saliva and semen." He emphasises the great limitation imposed upon the usefulness of blood grouping work in this country by the almost invariable difficulty in establishing the blood group of suspected and accused persons. He suggests, however, only the possible/

possible utilisation of cigarette stub saliva stains or the occasional seminal staining on the clothing of male prisoners as having a valuable application in this connection. He states that Wiener has suggested the use of urine, but there is no mention whatever of sweat.

In all the standard works on forensic medicine, there is either no reference at all to the subject of sweat staining, or it is mentioned only in a negative manner, as, for example, in Taylor's Medical Jurisprudence, (10th Edition 1947) where a warning is given that the presence of sweat in garments which have been worn close to the body may render the grouping of bloodstains on such garments quite worthless. "Recent Advances in Forensic Medicine" (1939) by Sydney Smith and Glaister, states that the following secretions "conform to group types: seminal fluid, urine, saliva, colostrum, milk, nasal secretion, vaginal secretion, lacrymal secretion, and others" - there is no specific reference to sweat stains. The grouping methods applicable to seminal fluid and saliva are discussed, but there is no indication that sweat stains can be or have been used for medico-legal purposes.

The recognised "Masters" have relatively little to say on the subject of sweat, and still less on the subject of sweat stains. Wiener (1943) refers to the studies of Lehrs (1930), Putkonen (1930), Yosida (1928), Schiff/

Schiff (1931), Thomsen (1931) and others, which demonstrated the presence of group-specific substances in a great variety of body-fluids, including sweat, and established the distinction between secreting and non-secreting individuals. He states in a general way that blood grouping tests can be of medico-legal value in cases where stains other than blood are found, but gives no specific indication that sweat stains have been so utilised in a positive manner. Nor does the contribution by Christensen, to which he refers, give any examples of this having been done. Wiener also gives a table (after Putkonen) showing the relative concentration of group specific substances in a number of body fluids, but sweat is not included in the table. Elsewhere, however, he states that sweat has a low content of group substances, and adds the warning that both sweat and urine are very apt to cause non-specific reactions because of their high salt content, although he does not make it clear why this should be so. Such substances as tobacco juice and gum do not, apparently, vitiate the results which can be obtained from the extremely small quantity of saliva present on cigarette ends and envelope flaps. Because of the possibility of non-specific reactions, he recommends for urine and sweat a special technique involving extraction with boiling distilled water, concentration of the extract, clarification by centrifugation,/

centrifugation, dialysis against running tap-water in a collodion or cellophane bag for several days, evaporation to dryness, redissolving in saline and, finally, removal of insoluble material by centrifugation, - after which, presumably, the tests may proceed. The technique hardly recommends itself as a regular, day to day procedure, and if such elaboration is strictly essential, then the time for the routine investigation of sweat stains for medico-legal purposes has not yet arrived. It was from this point of view that I considered certain of the negative results of my preliminary experiments so important and encouraging.

Schiff and Boyd (1942), in discussing the presence of group specific substances in the body fluids, include Putkonen's table which makes no mention of sweat, but elsewhere they list sweat as one of the body fluids which, in secretors, contains group specific substances plentifully. They go on to state, as a general proposition, that, in the case of dried stains resulting from any of the body fluids, the inhibition (absorption) tests as applied to dried organ preparations may be attempted. Successful results are reported from the stain on a cigarette butt and from the gummed edge of an envelope which has been licked by a secretor before sealing. Reference is made to the work of Hartmann (1941) in stating that/

that the small amount of substances present in urine may be sufficient to permit a diagnosis of the blood group, but there is no direct reference to the possible use of sweat stains for the same purpose.

Lattes (1932) points out that the methods applied to the grouping of blood stains may be extended to materials other than blood, notably saliva and seminal fluid. He also speaks of the possibilities which exist in stains due to mucus, sweat and other secretions, and quotes in this connection the contribution of Kan Itiosida which I have already singled out as being the only work which I have found which has a direct bearing on the potential positive value of sweat stains for medico-legal purposes.

From a perusal of the literature, therefore, I have come to the conclusion that, while the presence of group specific substances in the sweat of secreting individuals is well established, and while the idea of using sweat stains as a means of determining the blood group has been recognised, there has not, in fact, been a great deal of work done with sweat or sweat stains, and there has been little or no use made of sweat stains for medico-legal purposes. The probable reasons for this state of affairs are fairly obvious. Sweat is a fluid of relatively little interest to the pure serologist, and once the presence of group specific substances in sweat had been satisfactorily established/

established as an academic fact, there would be little inducement to devote much further attention to the subject. From a medico-legal point of view, the countries in which most of the blood grouping work has been done are countries in which there is no legal difficulty in obtaining samples of blood from accused persons. Hence the identification of the blood group by an examination of sweat stains would rarely be of practical importance. In this country, on the other hand, I feel that the value of blood grouping evidence would be very greatly enhanced if the results of sweat stain investigation were established as satisfactory and reliable, even although they could only apply in individuals of Groups A, B and AB who were also secretors.

PART TWO.

A Record of Experimental Work.

From all points of view I felt justified in proceeding with a more extensive and testing series of experiments. Obviously, the only satisfactory test was to see whether, and to what extent, I could in fact establish the blood groups of a number of unknown individuals by an investigation of sweat stains on their clothing. In all, I have recorded the results in fifty such cases investigated by the methods described in detail below. The cases were not selected in any way, but were, with two exceptions, a consecutive series of unknown individuals. One exception was a case in which the serum, after absorption with a dark blue lining material, was recovered in a condition resembling thick blue-black ink, and quite unsuitable for agglutination tests. The other was a case in which the absorption tests gave anomalous results. On inquiry, it appeared probable that these were ~~probably~~ due to the jacket having been worn by two individuals, the second of whom was unfortunately not available for testing purposes. These two cases were not included in the series.

Methods.

In each case, I obtained an area of sweat-stained lining from a jacket sleeve in the region of the/

the armpit. In some cases, the jacket was fairly new, in others it was old; in some, the lining material was fairly clean and the sweat staining not apparent, in others the material was very worn and dirty; in some, the jacket was still in fairly regular use at the date of the experiment, in others it had been laid aside for periods up to a year. A note was made of any such circumstance which could be regarded as a marked departure from the average. The actual area of material examined in each case was approximately one square inch.

As in all such tests, the prime essential is that one should be quite certain that the test sera and cells are suitable for the purpose. It is sometimes stated that the sera should be of high titre, but this doctrine need not be carried to extremes. In the present series no serum was considered satisfactory which did not produce definite agglutination at a dilution of 1 in 64; in fact, ~~almost~~ without exception the sera employed were effective at titres of 64, 128 or 256. The cell suspensions used were approximately 1 per cent in saline, and the sensitivity of the cells was verified before each test. Although the respective cells and sera were not obtained from the same source throughout - an ideal which has much to recommend it - it is possible to proceed with confidence if the above procedure is strictly observed as an initial step in each test.

Several/

Several methods have been recommended for ascertaining the effective titre of an anti-serum. I consider that the use of well-slides is simple and satisfactory; the various dilutions are easily prepared and the reading of results is facilitated. As a routine, ten well-slides were laid out in series on a white porcelain slab. To the first two, 5 c.mm. of the serum to be tested were added by means of a capillary pipette. To all the slides except the first, 5 c.mm. of saline were added. The serum and saline in the second slide were thoroughly mixed by repeatedly sucking up and expelling the fluid with the pipette, and 5 c.mm. of the mixture were transferred to the third slide. The same procedure was repeated throughout the series, 5 c.mm. of the mixture in the tenth slide being discarded. To each slide 5 c.mm. of a 1% suspension of appropriate cells was then added, so that each slide eventually contained 10 c.mm. of progressive dilutions of serum in saline ranging from 1 in 2 to 1 in 1024, each with an approximately 0.5% suspension of red cells which was gently stirred to ensure uniform distribution. Each dilution was covered with a moist watch glass to minimise evaporation, and the series was allowed to stand for one hour. At the end of that time, the watch glasses were removed, and each slide was rocked in a rotating manner. The red cells were examined

by/

by the low power of the microscope, and the greatest dilution at which definite agglutination occurred was noted. It is important that a satisfactory fixed routine be adopted for this determination of the effective titre, and that it should be strictly adhered to. My practice was to start my inspection at the highest dilution, and work my way downwards till definite and indisputable agglutination was observed. I then verified the presence of agglutination from the lowest dilution up to that point, and re-examination usually showed that the short lapse of time had sufficed to produce definite agglutination in the next highest dilution but no further. This final observation was taken as the titre of the particular serum under investigation.

For the test itself, the square inch of lining material was cut up with scissors to form a collection of small fragments. It is advisable to minimise as far as possible the production of mere fluff which tends to contaminate the serum recovered after absorption and may therefore necessitate centrifugation. The resultant collection of fragments was divided into two equal portions, each of which was placed loosely in a 30 x 7 mm. glass tube, appropriately labelled. A "modicum" of tested Anti-A serum was added to one tube, and a similar quantity of Anti-B serum to the other. It is not possible to define precisely a quantity/

quantity of serum which can be regarded as appropriate for all cases, even when, as in the present series, the area of material is approximately the same. Variations in the nature and texture of the stained material necessitate slight variation in the quantity of serum which must be added in order to permit the recovery of about 10 c.mm. after absorption. That is the objective, and it will, of course, be difficult or impossible if too little serum is added. On the other hand, the addition of an excess of serum results in incomplete absorption and therefore in misleading results. This is one of the critical points in all such experiments, and it is one which is perhaps insufficiently stressed in the literature, no doubt because descriptive precision is impossible.

The sera having been added, permeation through the test material was promoted by manipulation with a needle and by subjecting the contents of the tube to a reduced pressure, as has been recommended by Harley. This was simply done by connecting the tube to a water pump for several short periods of about 15 seconds each. By this means, bubbles of air were sucked from the interstices of the material, and more intimate permeation by the serum ensured. The moistened material was loosely packed into the bottom of the tube by tamping down with a fine glass rod. Thereafter, the tubes were corked, allowed to stand at room temperature/

temperature for an hour, and then placed in the refrigerator overnight, i.e. for about 18 hours. On the following morning, the tubes, having been removed from the refrigerator, were allowed to stand for a further hour at room temperature. The sera were then separately withdrawn by means of a capillary pipette, and if they have been judiciously added in the first place, about 10 c.mm. of each should be recovered. Each serum was laid out in progressive dilutions in a series of well-slides, as for the titre-determination previously described. When the unabsorbed (uninhibited) sera are the only controls being used, they should be set up in a parallel series at the same time. The addition of the appropriate cell suspensions and the subsequent procedure leading to the determination of the effective titre were then carried out in the manner previously described.

Interpretation of Results.

In interpreting the results, several factors had to be borne in mind. In the first place, although unstained material was investigated in certain cases (which are noted), the titres of the unabsorbed sera were the only controls employed routinely. This was done deliberately, because the case described early in this thesis indicated that in practice it may well happen that no reliable control material will be available./

available. Previous experience has convinced me that "shifts to the left" of two places are quite commonly due to non-specific absorption by the material itself, and that not infrequently the shift may be even greater. I decided, therefore, to ignore any shift of one or two places, and to regard shifts of even three or four places with suspicion, unless they occurred with one serum while the other serum remained relatively or completely unaffected. Such criteria are more exacting than those normally adopted for blood stains, but with blood stains it should be an invariable practice to use control material. They are not by any means more than can be expected from the average seminal stain, or from saliva. Moreover, they were in accordance with the results of my preliminary experiments, and by adopting them, I hoped to be able to "spot" groups A and B; a diagnosis of group AB would probably be much more tentative.

A further consideration to be borne in mind was that the fifty "unknowns" would certainly include a considerable percentage of Group O individuals, in whose cases no identification by positive absorption could be anticipated. From certain points of view, it would have been better to include only Groups A, B and AB in the series, but this, of course, would have necessitated prior selection by a referee if they were to remain even relatively unknown to me. On the other/

other hand, the inclusion of Group O cases in the series would have certain outstanding advantages. Not only would the blood groups of the test cases be absolutely unknown to me, but the results obtained from the Group O individuals would do much to determine the likelihood or otherwise of non-specific absorption proving a serious obstacle. In this way, the Group O cases would serve as a control for the series as a whole, and they would also contribute significantly towards the object of the experiments, namely the determination of the reliability of blood group diagnosis by absorption methods applied to sweat stains.

Furthermore, provision had to be made for the presence among the test cases of "non-secretors" who may be expected to form about fifteen to twenty per cent of any European group of considerable size. No specific absorption could be anticipated from the sweat stains of such individuals; the results should be the same as those given by Group O stains. But a check was necessary in order that any apparent error arising from this source should not be attributed either to faulty technique or to the unreliability of sweat stains for the purposes of the test; and, conversely, in order that any actual weakness in the method should not be glossed over by a convenient assumption that, in any particular case, an absence of absorption/

absorption might well be due to the individual being a non-secretor. For this reason, a sample of saliva was taken from each of the fifty cases and tested for its power to inhibit the activity of anti-A and anti-B sera by the following method.

After rinsing the mouth, the individual was asked to keep his mouth open and to allow the saliva to collect under the tongue. About 0.5 c.c. of saliva was then removed by capillary pipette and transferred to a 70 x 5 mm. test tube. The tube was immediately placed in a water bath and kept there at boiling point for ten minutes. This was done in order to destroy any salivary enzyme which might cause deterioration in the group specific substances on standing. The group specific substances themselves are, of course, thermostable. The saliva was then thoroughly mixed with an equal quantity of saline to reduce its viscosity, and the mixture was centrifuged. 10 c.mm. of the cleared, diluted saliva were then placed in each of two tubes, to which equal volumes of anti-A and anti-B serum were added respectively and thoroughly mixed. After standing for an hour at room temperature the two tubes of saliva-serum mixture were placed in the refrigerator overnight. After removal the following morning and standing for a further hour at room temperature, the fluids were regarded as being 1:2 dilutions of anti-A and anti-B sera, and titred/

titred as previously described. Using this method, I have found that the saliva of Group O individuals and non-secretors leaves the serum titre virtually unaffected while the saliva of secretors of Groups A, B and AB produces a marked lowering of the titre of the appropriate serum or sera, almost invariably to zero, and always to an unmistakable degree. By this method, therefore, all the test cases belonging to Groups A, B or AB were established as being secretors or non-secretors, so that due allowance could be made in considering the results.

The sweat stain tests were completed first, and the deduction made from them was recorded. Thereafter, the saliva tests were carried out routinely, irrespective of the sweat stain findings. Finally, a sample of blood was taken from each case under consideration and the blood group established by direct testing of a cell suspension with anti-A and anti-B serum. In this way, the validity of the sweat stain deductions was checked in each case, and errors due solely to non-secretion were detected.

Results.

The detailed results of absorption tests in the fifty cases are given in tabular form below. The numbers, e.g. 256, 64, 8 etc., indicate the dilutions at which the sera produced agglutination in cell suspensions of appropriate group. The effective titres of the unabsorbed sera are given first. The figures in respect of sera absorbed with sweat stains are followed by figures in brackets which show the extent of the "shift to the left" in each case, and are an index therefore of the degree to which absorption (or inhibition) of the serum has occurred. These latter figures, judged by the criteria previously discussed, formed the basis for the deduction drawn from the sweat stain investigation. The figures for the sera absorbed with saliva enabled a distinction to be made between the secretors and non-secretors of Groups A, B and AB and, together with the results obtained from the sample of blood, established the actual blood groups of the individuals, for comparison with the deduction already drawn. For secretors and non-secretors respectively, the symbols (S) and (N.S.) are used instead of the more usual (S) and (s).

Case No. 1 Name: J. Russell.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	128
Absorbed with sweat stain	128 (i.e. - 1)	64 (i.e. - 1)
Absorbed with saliva	256	128

Deduction from sweat stain investigation - Group O (or N.S.)

Blood Group, as established by testing red cell suspension and saliva - Group O.

Case No. 2 Name: D. Murray.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	256
Absorbed with sweat stain	128 (i.e. - 1)	128 (i.e.- 1)
Absorbed with saliva	256	256

Deduction from sweat stain investigation - Group O (or N.S.)

Blood Group, as established by testing red cell suspension and saliva - Group O.

Case No. 3 Name: D. Kerr.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	256
Absorbed with sweat stain	64 (i.e. - 2)	128 (i.e. - 1)
Absorbed with saliva	128	256

Deduction from sweat stain investigation - Group O (or N.S.)

Blood group, as established by testing red cell suspension and saliva - Group B (N.S.)

Case No. 4 Name: J. Lough.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	128
Absorbed with sweat stain	16 (i.e. - 4)	64 (i.e. - 1)
Absorbed with saliva	2	128

Deduction from sweat stain investigation - Group A.

Blood Group, as established by testing red cell suspension and saliva - Group A (S).

Case No. 5 Name: J. D. Steven.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	128
Absorbed with sweat stain	32 (i.e. - 3)	64 (i.e. - 1)
Absorbed with saliva	4	128

Deduction from sweat stain investigation - Group A ?

Blood Group, as established by testing red cell suspension and saliva - Group A (S)

Remarks: Jacket had not been worn for nearly a year.

Case No. 6 Name: N. Gorman.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	128	128
Absorbed with sweat stain	8 (i.e. - 4)	32 (i.e. - 2)
Absorbed with saliva	0	64

Deduction from sweat stain investigation - Group A ?

Blood group, as established by testing red cell suspension and saliva - Group A (S)

Remarks: Jacket had not been worn for about a year.

Case No. 7 Name: D. McLatchie.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	256
Absorbed with sweat stain	128 (i.e. - 1)	128 (i.e. - 1)
Absorbed with saliva	256	256

Deduction from sweat stain investigation - Group O (or N.S.)

Blood Group, as established by testing red cell suspension and saliva - Group B (N.S.)

Case No. 8 Name: R. R. Mitchell.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	256
Absorbed with sweat stain	128 (i.e. - 1)	256 (i.e. - 0)
Absorbed with saliva	256	256

Deduction from sweat stain investigation - Group O (or N.S.)

Blood Group, as established by testing red cell suspension and saliva - Group A (N.S.)

Case No. 9 Name: E. Petrie.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	256
Absorbed with sweat stain	16 (i.e. -4)	16 (i.e. - 4)
Absorbed with saliva	256	256

Deduction from sweat stain investigation - Group AB

Blood group, as established by testing red cell suspension and saliva - Group O.

Remarks: A repeat experiment gave the same result. No control material was available for investigation in this case.

Case No. 10 — Name: H. Russell.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	256
Absorbed with sweat stain	64 (i.e. - 2)	256 (i.e. - 0)
Absorbed with saliva	0	256

Deduction from sweat stain investigation - Group A ?

Blood Group, as established by testing red cell suspension and saliva - Group A (S)

Remarks: New jacket, very seldom worn.

Case No. 11 — Name: W. Watson.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	256
Absorbed with sweat stain	128 (i.e. - 1)	256 (i.e. - 0)
Absorbed with saliva	256	128

Deduction from sweat stain investigation - Group O (or N.S.)

Blood Group, as established by testing red cell suspension and saliva - Group O.

Remarks: A fairly new jacket, but worn regularly for past two weeks in hot weather. Lining clean but sweat stain obvious.

Case No. 12. — Name: J. Wylie.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	256
Absorbed with sweat stain	256 (i.e. - 0)	256 (i.e. - 0)
Absorbed with saliva	256	256

Deduction from sweat stain investigation - Group O (or N.S.)

Blood group, as established by testing red cell suspension and saliva - Group O.

Case No. 13 — Name: J. Fraser.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	128	128
Absorbed with sweat stain	0 (i.e. - 7)	4 (i.e. - 5)
Absorbed with saliva	0	128

Deduction from sweat stain investigation - AB

Blood Group, as established by testing red cell suspension and saliva - A (S)

Remarks: In this case, a control experiment, subsequently carried out on a piece of the same lining material which showed no staining, gave results indicating very considerable absorption - (- 3 in each case) - which was probably non-specific.

Case No. 14 — Name: W. A. Cargill.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	256
Absorbed with sweat stain	32 (i.e. - 3)	256 (i.e. - 0)
Absorbed with saliva	4	256

Deduction from sweat stain investigation - Group A

Blood Group, as established by testing red cell suspension and saliva - Group A (S)

Case No. 15 — Name: D. McCabe.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	256
Absorbed with sweat stain	128 (i.e. - 1)	256 (i.e. - 0)
Absorbed with saliva	256	256

Deduction from sweat stain investigation - Group O (or N.S.)

Blood group, as established by testing red cell suspension and saliva - Group A (N.S.)

Case No. 16 Name: N. C. Lawson.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	256
Absorbed with sweat stain	128 (i.e. - 1)	128 (i.e. - 1)
Absorbed with saliva	256	256

Deduction from sweat stain investigation - Group O (or N.S.)

Blood Group, as established by testing red cell suspension and saliva - Group O.

Case No. 17 Name: J. Grieve.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	128	256
Absorbed with sweat stain	32 (i.e. - 2)	64 (i.e. - 2)
Absorbed with saliva	128	256

Deduction from sweat stain investigation - Group O (or N.S.)

Blood Group, as established by testing red cell suspension and saliva - Group O.

Case No. 18 Name: F. Semark.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	256
Absorbed with sweat stain	32 (i.e. - 3)	4 (i.e. - 6)
Absorbed with saliva	128	0

Deduction from sweat stain investigation - Group B.

Blood group, as established by testing red cell suspension and saliva - Group B (S)

Remarks: The jacket was fairly new, and the lining clean. The sweat staining, though definite was not heavy. This was another case in which, though a definite deduction seemed justifiable, there appeared to be a considerable degree of non-specific absorption.

Case No. 19 Name: J. Morgan.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	128	128
Absorbed with sweat stain	8 (i.e. - 4)	128 (i.e. -0)
Absorbed with saliva	0	128

Deduction from sweat stain investigation - Group A

Blood Group, as established by testing red cell suspension and saliva - Group A (S)

Case No. 20 Name: M. Thomson.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	128	128
Absorbed with sweat stain	8 (i.e. - 4)	128 (i.e. - 0)
Absorbed with saliva	0	128

Deduction from sweat stain investigation - Group A

Blood Group, as established by testing red cell suspension and saliva - Group A (S)

Case No. 21 Name: A. McCall.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	128	256
Absorbed with sweat stain	32 (i.e. - 2)	16 (i.e. - 4)
Absorbed with saliva	128	0

Deduction from sweat stain investigation - Group B ?

Blood group, as established by testing red cell suspension and saliva - Group B (S)

Case No. 22 Name: H. Mantz.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	128
Absorbed with sweat stain	32 (i.e. - 3)	32 (i.e. - 2)
Absorbed with saliva	256	128

Deduction from sweat stain investigation - Group O (or N.S.)?

Blood Group, as established by testing red cell suspension and saliva - Group O

Case No. 23 Name: J. McRobb.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	128
Absorbed with sweat stain	64 (i.e. - 2)	32(i.e. - 2)
Absorbed with saliva	128	64

Deduction from sweat stain investigation - Group O (or N.S.)

Blood Group, as established by testing red cell suspension and saliva - Group O.

Case No. 24 Name: J. Gordon.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	128	128
Absorbed with sweat stain	4 (i.e. - 5)	32 (i.e. - 2)
Absorbed with saliva	0	128

Deduction from sweat stain investigation - Group A

Blood group, as established by testing red cell suspension and saliva - Group A (S)

Case No. 25 Name: C. M. Stewart.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	128
Absorbed with sweat stain	32 (i.e. - 3)	32 (i.e. - 2)
Absorbed with saliva	128	128

Deduction from sweat stain investigation - Group O (or N.S.)?

Blood Group, as established by testing red cell suspension and saliva - Group O.

Case No. 26 Name: S. D. Peters.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	128
Absorbed with sweat stain	32 (i.e. - 3)	32 (i.e. - 2)
Absorbed with saliva	128	64

Deduction from sweat stain investigation - Group O (or N.S.)?

Blood Group, as established by testing red cell suspension and saliva - Group O.

Case No. 27 Name: D. Delworth.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	64
Absorbed with sweat stain	128 (i.e. - 1)	32 (i.e. - 1)
Absorbed with saliva	256	64

Deduction from sweat stain investigation - Group O (or N.S.)

Blood group, as established by testing red cell suspension and saliva - Group O.

Case No. 25 Name: C. M. Stewart.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	128
Absorbed with sweat stain	32 (i.e. - 3)	32 (i.e. - 2)
Absorbed with saliva	128	128

Deduction from sweat stain investigation - Group O (or N.S.)?

Blood Group, as established by testing red cell suspension and saliva - Group O.

Case No. 26 Name: S. D. Peters.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	128
Absorbed with sweat stain	32 (i.e. - 3)	32 (i.e. - 2)
Absorbed with saliva	128	64

Deduction from sweat stain investigation - Group O (or N.S.)?

Blood Group, as established by testing red cell suspension and saliva - Group O.

Case No. 27 Name: D. Delworth.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	64
Absorbed with sweat stain	128 (i.e. - 1)	32 (i.e. - 1)
Absorbed with saliva	256	64

Deduction from sweat stain investigation - Group O (or N.S.)

Blood group, as established by testing red cell suspension and saliva - Group O.

Case No. 28 Name: W. Batty.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	64
Absorbed with sweat stain	32 (i.e. - 3)	64 (i.e. - 0)
Absorbed with saliva	8	64

Deduction from sweat stain investigation - Group A

Blood Group, as established by testing red cell suspension and saliva - Group A (S)

Case No. 29 Name: J. Peat.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	128
Absorbed with sweat stain	64 (i.e. - 2)	64 (i.e. - 1)
Absorbed with saliva	256	128

Deduction from sweat stain investigation - Group O (or N.S.)

Blood Group, as established by testing red cell suspension and saliva - Group O.

Case No. 30 Name: G. J. Glendinning.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	128	64
Absorbed with sweat stain	32 (i.e. - 2)	8 (i.e. - 3)
Absorbed with saliva	128	64

Deduction from sweat stain investigation - Group O (or N.S.)?

Blood group, as established by testing red cell suspension and saliva - Group O.

Case No. 31 Name: R. D. Moncur.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	128	64
Absorbed with sweat stain	128 (i.e. - 0)	64 (i.e. - 0)
Absorbed with saliva	128	64

Deduction from sweat stain investigation - Group O (or N.S.)

Blood Group, as established by testing red cell suspension and saliva - Group O.

Remarks: The lining in this case was marked by heavy sweat staining of recent origin. The lining was otherwise clean. The complete absence of absorption is of interest.

Case No. 32 Name: T. Y Murray.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	128	128
Absorbed with sweat stain	128 (i.e. - 0)	16 (i.e. - 3)
Absorbed with saliva	64	0

Deduction from sweat stain investigation - Group B

Blood Group, as established by testing red cell suspension and saliva - Group B (S).

Case No. 33 Name: J. Valentine.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	128	128
Absorbed with sweat stain	32 (i.e. - 2)	16 (i.e. - 3)
Absorbed with saliva	128	0

Deduction from sweat stain investigation - Group O (or N.S.)?

Blood group, as established by testing red cell suspension and saliva - Group B (S).

Remarks: The lining material in this case was blue in colour, but the recovered serum, although stained blue, appeared reasonably suitable for test purposes.

Case No. 34 — Name: T. Girdwood.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	256
Absorbed with sweat stain	32 (i.e. - 3)	64 (i.e. - 2)
Absorbed with saliva	256	256

Deduction from sweat stain investigation - Group O (or N.S.)?

Blood Group, as established by testing red cell suspension and saliva - Group O.

Case No. 35 — Name: A. Morrison.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	64
Absorbed with sweat stain	16 (i.e. - 4)	32 (i.e. - 1)
Absorbed with saliva	4	64

Deduction from sweat stain investigation - Group A

Blood Group, as established by testing red cell suspension and saliva - Group A (S)

Case No. 36 — Name: D. Marriott.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	256
Absorbed with sweat stain	128 (i.e. - 1)	256 (i.e. - 0)
Absorbed with saliva	256	256

Deduction from sweat stain investigation - Group O (or N.S.)

Blood group, as established by testing red cell suspension and saliva - Group O.

Case No. 37 Name: J. F. Smith.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	256
Absorbed with sweat stain	16 (i.e. - 5)	8 (i.e. - 6)
Absorbed with saliva	4	0

Deduction from sweat stain investigation - Group AB

Blood Group, as established by testing red cell suspension and saliva - Group AB (S)

Remarks: Lining material clean and free from dye or stains other than sweat.

Case No. 38 Name: T. Smith.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	256
Absorbed with sweat stain	8 (i.e. - 5)	64 (i.e. - 2)
Absorbed with saliva	0	256

Deduction from sweat stain investigation - Group A

Blood Group, as established by testing red cell suspension and saliva - Group A (S)

Case No. 39 Name: G. Traill.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	128
Absorbed with sweat stain	16 (i.e. - 4)	64 (i.e. - 1)
Absorbed with saliva	4	128

Deduction from sweat stain investigation - Group A

Blood group, as established by testing red cell suspension and saliva - Group A (S)

Case No. 40 Name: J. Eddie.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	256
Absorbed with sweat stain	32 (i.e. - 3)	128 (i.e. - 1)
Absorbed with saliva	16	128

Deduction from sweat stain investigation - Group A ?

Blood Group, as established by testing red cell suspension and saliva - Group A (S)

Case No. 41 Name: J. Williamson.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	256
Absorbed with sweat stain	64 (i.e. - 2)	64 (i.e. - 2)
Absorbed with saliva	256	256

Deduction from sweat stain investigation - Group O (or N.S.)

Blood Group, as established by testing red cell suspension and saliva - Group O.

Case No. 42. Name: A. Taylor.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	256
Absorbed with sweat stain	128 (i.e. - 1)	256 (i.e. - 0)
Absorbed with saliva	256	256

Deduction from sweat stain investigation - Group O (or N.S.)

Blood group, as established by testing red cell suspension and saliva - Group O.

Case No. 43 Name: G. Ross.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	256
Absorbed with sweat stain	64 (i.e. - 2)	128 (i.e. - 1)
Absorbed with saliva	256	256

Deduction from sweat stain investigation - Group O (or N.S.)

Blood Group, as established by testing red cell suspension and saliva - Group O.

Case No. 44 Name: W. McCartney.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	128
Absorbed with sweat stain	128 (i.e. - 1)	8 (i.e. - 4)
Absorbed with saliva	256	0

Deduction from sweat stain investigation - Group B

Blood Group, as established by testing red cell suspension and saliva - Group B (S)

Case No. 45 Name: J. Henderson.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	256	256
Absorbed with sweat stain	32 (i.e. - 3)	64 (i.e. - 2)
Absorbed with saliva	256	256

Deduction from sweat stain investigation - Group O (or N.S.)?

Blood group, as established by testing red cell suspension and saliva - Group O.

Remarks: Lining worn and very dirty as well as sweat stained.

Case No. 46. Name: J. W. Robson.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	128	64
Absorbed with sweat stain	8 (i.e. - 4)	32 (i.e. - 1)
Absorbed with saliva	0	64

Deduction from sweat stain investigation - Group A

Blood Group, as established by testing red cell suspension and saliva - Group A (S)

Case No. 47 Name: A. Smith.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	128	128
Absorbed with sweat stain	4 (i.e. - 5)	32 (i.e. - 2)
Absorbed with saliva	0	128

Deduction from sweat stain investigation - Group A

Blood Group, as established by testing red cell suspension and saliva - Group A (S)

Case No. 48 Name: J. Matthews.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	128	128
Absorbed with sweat stain	8 (i.e. - 4)	32 (i.e. - 2)
Absorbed with saliva	0	128

Deduction from sweat stain investigation - Group A ?

Blood group, as established by testing red cell suspension and saliva - Group A (S)

Case No. 49 Name: T. Flannigan.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	128	128
Absorbed with sweat stain	32 (i.e. - 2)	32 (i.e. - 2)
Absorbed with saliva	64	64

Deduction from sweat stain investigation - Group O (or N.S.)

Blood Group, as established by testing red cell suspension and saliva - Group B (N.S.)

Case No. 50 Name: J. Sutherland.

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed	128	64
Absorbed with sweat stain	16 (i.e. - 3)	32 (i.e. - 1)
Absorbed with saliva	0	64

Deduction from sweat stain investigation - Group A ?

Blood Group, as established by testing red cell suspension and saliva - Group A (S)

Case No. _____ Name: _____

Titre of Test Sera:	Anti-A serum	Anti-B serum
Unabsorbed		
Absorbed with sweat stain		
Absorbed with saliva		

Deduction from sweat stain investigation - _____

Blood group, as established by testing red cell suspension and saliva - _____

Summary of Results.

The individual test results are summarised in the following table which shows the actual group and the group as deduced from the sweat stain in each case. The success or otherwise of each experiment is indicated by one or other of the following symbols:-

⊕ = group correctly deduced with reasonable certainty. (In cases where a correct deduction was made that the stain contained neither A nor B substance, the group O cases are marked ⊕, while the non-secretors are marked + (N.S.))

(+/−) = group correctly deduced, but on very slender grounds and therefore with very considerable dubiety.

⊖ = group deduced wrongly.

TABLE/

Case No.	Actual Group	Deduced Group	Result
1	O	O or NS	⊕
2	O	O or NS	⊕
3	B (NS)	O or NS	+ (NS)
4	A	A	⊕
5	A	A ?	±
6	A	A ?	±
7	B (NS)	O or NS	+(NS)
8	A (NS)	O or NS	+(NS)
9	O	AB	−
10	A	A ?	±
11	O	O or NS	⊕
12	O	O or NS	⊕
13	A	AB	−
14	A	A	⊕
15	A (NS)	O or NS	+(NS)
16	O	O or NS	⊕
17	O	O or NS	⊕
18	B	B	⊕
19	A	A	⊕
20	A	A	⊕
21	B	B ?	±
22	O	O or NS	±
23	O	O or NS	⊕
24	A	A	⊕
25	O	O or NS	±
26	O	O or NS?	±
27	O	O or NS	⊕
28	A	A	⊕
29	O	O or NS	⊕
30	O	O or NS?	±
31	O	O or NS	⊕
32	B	B	⊕
33	B	O or NS	−
34	O	O or NS?	±
35	A	A	⊕
36	O	O or NS	⊕
37	AB	AB	⊕
38	A	A	⊕
39	A	A	⊕
40	A	A ?	±
41	O	O or NS	⊕
42	O	O or NS	⊕
43	O	O or NS	⊕
44	B	B	⊕
45	O	O or NS?	±
46	A	A	⊕
47	A	A	⊕
48	A	A ?	±
49	B (NS)	O or NS	+(NS)
50	A	A ?	±

The results of the series as a whole are summarised in the following table:-

Group	Correct (S)	Correct (N.S.)	Correct - Doubtful	Wrong	Total	%
O	14	-	6	1	21	42
A	11	2	6	1	20	40
B	3	3	1	1	8	16
AB	1	-	-	-	1	2
Total	29	5	13	3	50	
%	58	10	26	6		

Discussion.

From the relative percentages of the blood groups, and from the incidence (about 15 per cent) of non-secretors, it appears that the fifty cases formed a fairly representative series. The general nature of the results supports the belief, if such support is needed, that group specific substances are present in the sweat of secreting individuals, certainly to a lesser extent than in saliva and seminal fluid, but nevertheless detectable in sweat stains by absorption methods.

As was anticipated, the chief difficulty was caused by non-specific absorption. This, however, did not appear to be due solely, or even mainly, to the presence of crystalloids or other substances in the sweat itself. Indeed, the results do not suggest that such factors constitute an absolute barrier to the use of relatively simple methods in the investigation of sweat stains. In a high proportion of Group O cases there was very little absorption of either serum, sometimes none at all. In general, these were the cases in which the lining material was relatively clean apart from obvious sweat staining. As regards the remaining Group O cases, the absence of control experiments with material unstained by sweat makes it impossible to be dogmatic, but the impression remains that non-specific factors in sweat do not cause a degree/

degree of absorption which need be misleading if suitable criteria are adopted.

A factor of undoubted importance, however, is the manner in which different materials, in varying states of cleanliness or otherwise, can affect the titre of a serum, quite independently of the presence of group specific substances. Fortunately, such non-specific absorption tends to affect both anti-A and anti-B serum more or less equally, and is likely therefore to arouse suspicion. If anything, the tendency is for the anti-A serum to be weakened to a slightly greater extent than the anti-B, a finding which has been observed also in other applications of the absorption method. A suspicious attitude, however, will not suffice to prevent mistakes. The only way of overcoming the difficulties due to non-specific absorption is to carry out reliable control tests, and, as I indicated previously, this may be difficult or impossible when sweat-stained material is under consideration. In my series, I deliberately omitted to test control material for this reason, so that the results indicate the possibilities in the most unfavourable circumstances, which are, however, not likely to be exceptional in actual practice.

It would be too much to claim that the experiments were an unqualified success. In a considerable proportion of cases - about one-third of the whole - a correct "diagnosis" was arrived at only with extreme dubiety/

dubiety or not at all. Even among the cases in which a correct deduction was drawn with comparative confidence, there were some in which the degree of certainty would not have warranted the expression of a medico-legal opinion in the absence of control experiments. Nevertheless, the results contained much that was of positive practical value.

The following table shows the range of absorption found in the experimental series. The seventy-five instances in which no specific inhibition was to be expected include all the Group O cases and the non-secretors in respect of both sera, the Group A (S) cases in respect of anti-B serum, and the Group B (S) cases in respect of anti-A serum. The instances in which specific absorption was to be expected provide, of course, a smaller total - twenty-five - comprising only the Group A (S) cases in respect of anti-B serum, the Group B (S) cases in respect of anti-B serum, and the solitary Group AB (S) case in respect of both sera. As previously, the number of "shifts to the left" is regarded as an index of the degree of absorption.

TABLE./

TABLE.

Absorption Index	No specific absorption to be expected		Specific absorption to be expected	
- 0	15		0	
- 1	26	= 86%	0	= 4%
- 2	24		1	
- 3	7	= 10%	7	= 28%
- 4	2		10	
- 5	1	= 4%	4	= 68%
- 6	0		2	
- 7	0		1	

From the evidence afforded by this table, it appears reasonable to infer that, if an obvious sweat stain causes a degree of absorption less than is represented by a shift of three places, then it is almost certain that the stain contains no specific substance antigenic to the type of serum being used. If this is true of both anti-A and anti-B serum, then the stain is almost certainly from a Group O individual or a non-secretor.

Conversely, if the stain causes inhibition to more than three places, it is probable that that specific substance is present which is antigenic to the particular type of serum being used; and the degree/

degree of probability is very greatly increased - almost to certainty - if the strength of the opposite type of serum is left almost or completely unaffected by absorption with the sweat stain. If, however, both types of sera show considerable and more or less equal inhibition, then an expression of opinion is very inadvisable unless repeated tests and repeated controls are possible. Certain types of material, certain dyes, and very dirty material are all likely to cause a high degree of non-specific absorption. Two of the three wrong deductions made in the series were due to the occurrence of high degrees of non-specific absorption which were not checked against control experiments. It is obvious that for this reason a diagnosis of Group AB staining should only be made if satisfactory controls are possible.

Unfortunately, a considerable number of cases show what may be termed an intermediate degree of absorption, represented by a shift of three places. Even this degree of absorption is probably significant when the opposite type of serum is unaffected, but this intermediate range includes also cases in which there is no helpful discrepancy of this extent. Many of these prove to be of Group O, but it cannot be assumed that they all belong to this group; as is exemplified by the third complete failure in the experimental/

experimental series. It is probable also that A_2 substance might commonly be responsible for such moderate degrees of absorption and so cause errors in diagnosis. Unless there is a really significant difference between the two sera, or unless control tests are particularly convincing, I consider that no satisfactory opinion can be expressed on these cases which come within this intermediate range.

But although there are obvious pitfalls and limitations, the results of my experiments do support the view that the investigation of sweat stains may be of great practical value in medico-legal cases, and that considerable reliance can be placed on the results of absorption tests when these are of a clear-cut nature. Approximately two-thirds of the experimental cases were correctly allocated to Group A or Group B, or to a category which includes both Group O individuals and the non-secretors of other groups. In actual practice, the proportion of successes might well be less owing to the frequent occurrence of much-soiled material with which to work, but there is no reason to believe that the proportion would cease to be significant.

It appears, therefore, that much greater use should be made of sweat stains than is done at present. It is hardly necessary to detail at length the/

the circumstances in which they might prove of value - one can think of many. But, above all, the experiments confirm that it is possible, in a significant proportion of cases, to establish within certain limits the blood group of an accused person, and also whether he is a secretor, without having a sample of blood or a fortuitous seminal stain to work with, and without resorting to procedures which are over-elaborate for routine purposes. In expressing an opinion, however, it will usually be advisable to state simply that the stain appears to contain or to be lacking in group substance A or B, rather than to be dogmatic about the blood group of the individual. For it is always possible that a garment may have been worn also by another person, and the evidence on this point must be left to others.

In conclusion, I must emphasise that nothing in the results of these experiments can justify any neglect in carrying out control tests whenever this is possible. Furthermore, the results of a single test should not be relied upon when repeated tests are possible, as they usually will be in the case of sweat stains. And, finally, the results obtained from sweat stains should not be relied upon exclusively when it is possible to perform confirmatory tests on other stains, e.g. of seminal fluid, vaginal secretion or nasal secretion. **"Multa collecta probant quae singulatim non probant."**

SUMMARY.

1. The development of medico-legal blood grouping methods and their limitations are reviewed briefly. It is pointed out that the value of blood-grouping evidence is very frequently minimised or completely nullified by the absence of any evidence as to the blood group of an accused person.

2. The record of an actual case is presented in which sweat staining on the clothing of an accused person was of importance, in that it not only interfered with the grouping of the victim's blood stains, but also proved of positive value in establishing the blood group of the accused.

3. The results of a number of preliminary experiments are described and discussed. These experiments, together with the case referred to above, favour the impression that, in a significant proportion of cases, it might prove practicable to determine the blood group of an individual from an examination of his sweat stains.

4. Previous work and recorded opinions on the presence of group-specific substances in sweat are reviewed briefly. From this review, it appears that there has been comparatively little work done, and that little or no use has been made/

made of sweat stains for medico-legal purposes, certainly in this country.

5. The methods adopted and the results obtained in a series of fifty experimental cases are recorded in detail, and the results are presented also in a summarised form.

6. The results of these experiments confirm the belief that in a substantial proportion of cases it is possible to establish the blood group of an individual, within certain limits, by the investigation of the sweat stains on his outer clothing by relatively simple absorption methods.

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I/

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