UNIT VIII. DETERMINATION OF SEX OF ORIGIN, NONGENETIC MARKERS AND BLOOD COMPONENT PROFILING

SECTION 49. DETERMINATION OF SEX OF ORIGIN

49.1 Introduction

For some years, there has been interest in developing procedures which would enable the reliable determination of sex of origin of bloodstains and of certain other human tissues such as saliva and hairs. In some cases, such a determination could be informative and useful. Three general approaches have been used for this purpose, two of them cytological, and the third having to do with the measurement of sex hormone levels. The cytological techniques are concerned with the determination of either sex chromatin (Barr bodies), or of so-called F-bodies (Y-bodies) in cells.

49.2 Determination of Sex Chromatin

Sex chromatin bodies (Barr bodies) were discussed in section 1.2.4.4. These structures are characteristic of nuclei in certain cells from female mammals, including the nuclei of smooth muscle, adrenal cortex, and various epidermal cells. The structures may also be seen in polymorphonuclear leucocytes from peripheral blood smears of females, where they are sometimes called "drumsticks". These structures were first observed in cat neurons (Barr and Bertrand, 1949), and later in other cells and other species (Barr, 1957 and 1960). The nature of sex chromatin in cells is discussed by Moore (1966a), and he has also described its discovery and the earlier work in the field (Moore, 1966b). Davidson and Smith (1954) first noted sex chromatin structures ("drumsticks") in the neutrophil leucocytes of peripheral blood from females. These structures should be called "sex chromatin", or "X chromatin", though the latter term has not gained wide acceptance. Dr. Barr himself has discouraged the use of the term "Barr bodies".

Reports on the frequency of sex chromatin have varied, depending upon the tissue being studied. At least 100 cells must be examined according to most authorities, and some have recommended from 300 to 500. The variations may be due to tissue differences, and to differences in various technical factors. Other nuclear appendages may be mistaken for sex chromatin bodies by inexperienced observers as well. The somewhat subjective nature of sex chromatin determinations has been noted by Grob and Kupperman (1961), and they emphasized the importance of using reproducible techniques and experienced scorers.

In the earlier literature, a low frequency of occurrence of sex chromatin bodies was reported in cells from normal human males, but the general consensus of opinion some years ago seemed to be that normal male cells do not in fact exhibit the structures (Grob and Kupperman, 1961). As was noted earlier, there is evidence (Ohno, 1966) that the sex chromatin body structure represents an inactivated X chromosome (see in section 1.2.4.4). Thus, in people suffering from abnormalities of sex chromosome composition, the correlation between phenotypic sex and sex chromatin body frequency in nuclei may not hold up. These cases are comparatively rare in the population, but could lead to misinterpretation in medicolegal work. Discrepancies between phenotypic sex and chromatin body frequency may be observed in (1) chromatin negative females; (2) chromatin positive males; and (3) genetic mosaics. Phenotypic females who are chromatin negative are mainly of two types, those with Turner's syndrome, and those with testicular feminization syndrome. The former have various abnormalities, including ovarian agenesis, and karyotype analysis reveals that they have only 45 chromosomes, the missing one being an X. They are sometimes called "XO females". The latter group are phenotypically female, although they exhibit various abnormalities of sexual development. Karyotype analysis shows that they are chromosomally male, i.e., that they are XY and have 46 chromosomes. They are sometimes called "XY females". Males who are chromatin positive are often those who have so-called Klinefelter's syndrome. They have 47 chromosomes and an XXY sex chromosome composition, and they show a variety of abnormalities of sexual development. Genetic mosaics may be of either phenotypic sex, and different tissues of the same individual may show differences in sex chromatin pattern. Cytologic sexing by means of chromatin bodies has been reviewed by Grob (1970).

49.2.1 Sex chromatin determination in blood and bloodstains

Postmortem blood does not appear to be very suitable for sex chromatin determination, apparently because the leucocytes undergo lysis and degradative changes quite soon after death. Dixon and Torr (1956 and 1957) said that they had had no success with this technique in post mortem blood. Schilling (1960) reported that post mortem blood was good for up to about 6 hours after death for this determination.

Some reports have indicated limited success with bloodstains. De Bernardi (1959) studied bloodstains on hairs with some success. He said that at least 200 nuclei needed to be examined. Davidson (1963) described a procedure for the isolation of white cells from bloodstains, and examined a number of slides for chromatin bodies. Diagnosis of sex could not be made in every case, and he thought that the results of the determination should be taken only as an indication of possible sex. Ishizu *et al.* (1973) suggested that chromatin body determinations coupled with F-body determinations (see below) on the same material provided a more definitive conclusion than either cytological method by

itself. Caroff and Breton (1966) reviewed chromatin body sexing from a medicolegal point of view.

49.2.2 Sex chromatin determination in epithelial cells and hair

From a forensic point of view, the ability to determine sex in epithelial cells is probably most applicable to post mortem tissues and to exfoliated epithelial cells in saliva stains and traces. Dixon and Torr (1956 and 1957) noted that chromatin bodies could survive for a few weeks in post mortem epithelial tissues, and thought that this determination might be of value in some cases, such as for example with a body part which had been recovered and submitted for identification. They buried fetal tissue from a female in soil, and could still determine sex chromatin for about 4 weeks. Holzer and Marberger (1957) obtained somewhat similar results in their tests on post mortem tissues. Edwards and Cameron (1964) said that excellent preparations could be obtained from the mucosal epithelium of the urinary bladder, but that bodies even a few days old could give misleading results.

Sanderson and Stewart (1961) described a rapid, easy method for sex chromatin determination in buccal epithelial cells using aceto-orcein staining. Radam (1965) discussed the preparation of buccal epithelial cells for chromatin body determination. Renard (1971) described techniques for the preparation of epithelial cells from salivary and vaginal materials and for chromatin body determinations. Generally, 40% of the nuclei of cells of female origin recovered from various stains were chromatin positive. Ando (1973) examined buccal epithelial cells from salivary stains and traces for both Barr bodies and F-bodies (see below). The chromatin bodies were found to be less stable over the course of time than the F-bodies.

Hair root sheath cells have been found to be suitable material for chromatin body sex determinations in many cases. Dixon and Torr (1956) found that hair root sheath cells provided excellent material in post mortem examinations. Schmid (1967) described a technique for sexing single head hairs from living persons. It was best if the hairs were examined at once, and not stored, in his experiments. Culbertson *et al.* (1969) also obtained reliable determinations from hair root sheath cells. In 30 people, 15 males and 15 females, Montanari *et al.* (1967) found that Barr bodies occurred in $29 \pm 5\%$ of the nuclei of these cells from females, while the values were very much lower in preparations from males ($6 \pm 2\%$). Bassett (1978) described procedures for sex chromatin determination in hair root sheath cells, and reported generally reliable and satisfactory results. He noted the importance of having experienced examiners score the nuclei, and the possibility of subjective error. He said further that preparations with sex chromatin frequency in the range of 10 to 40% of scorable nuclei should be considered inconclusive. Males and females showed sex chromatin in 5% and 57%, respectively, of their scorable nuclei.

Moore (1966c) discussed the determination of sex chromatin in connection with medicolegal problems.

49.3 Y-Body (F-Body) Determinations

In 1968, Caspersson *et al.* reported that metaphase chromosomes exhibited various fluorescence patterns after staining with fluorescent dyes, and it was suggested that the technique might provide a useful tool for chromosome mapping. These experiments were done on nonhuman material. Certain loci of the chromosomes were observed to bind quinacrine diHCl preferentially. In 1969, Zech found that the long arm of the human Y chromosome fluoresced brightly after staining human chromosome preparations with quinacrine mustards. The ability to observe a small fluorescent body in human cell preparations containing a Y chromosome, after staining with quinacrine diHCl, was quickly shown to be a property of a number of types of cells. In buccal epithelium, leucocytes and cultured skin fibroblasts, a large proportion of the cells from males (usually 25–50%) exhibited the fluorescent body (or F-body) after suitable preparation and quinacrine di-HCl staining (Pearson *et al.*, 1970; George, 1970).

49.3.1 F-Body determinations in dried blood and bloodstains

The current of opinion in the literature appears to be that F-body determinations can provide a highly reliable method of dried blood sexing if the procedures are carried out skillfully and if a sufficiently large number of leucocytes can be obtained for counting. A number of different techniques have been reported for these determinations. There is some variation in the estimate of the maximum age of a bloodstain that is still suitable for sexing by F-body counting. In addition, storage temperature and conditions as well as the nature of the substratum appear to affect the results obtained.

Phillips and Gaten (1971) reported successful results with bloodstains up to 10 days old on solid (nonabsorbent) substrata. About 86% of the leucocytes from male blood smears showed F-bodies, while the figure was only about 0.5% with female samples. In 1972, Phillips and Webster gave an improved technique in which 2 mM $MgCl_2$ was employed as an extraction medium instead of McIlvaine's buffer. In 1974, Phillips and Gitsham gave a procedure for bloodstains on absorbent substrata, and reported the results of a series of blind trial studies. Extraction techniques were not found very satisfactory for F-body determinations. Scraping the stain so as to cause a "dust" of bloodstain to fall upon the slide was the preferred technique. Reliable sexing was not possible in all the stains tested, but could be done in many cases. At least 100 nuclei had to be scored, they said. It was important to recognize, too, that readers had to become experienced in examining these preparations, and that there was an element of subjectivity in the determination. Müller *et al.* (1971) tested stains up to 32 days old and always found a higher F-body score in male bloods than in female bloods.

Schwinger (1972) could detect F-bodies in stains up to 30 days old. Blažek and Bráza (1972a) were able to determine the sex of bloodstains by F-body technique in stains 32 days

old. They said that the decay in the percentage of F-body positive nuclei from male stains was not a linear function of stain age. Aragones and Egozcue (1973) described a method which was applicable to stains up to 90 days old on absorbent or nonabsorbent surfaces. Brinkmann and Jobst (1973) found that 12.5% to 50% of cells from male bloodstains up to 4 weeks old contained F-bodies. It was possible to diagnose stains stored for 28 months at room temperature as being of male origin. Ishizu (1973) reported finding from 49% to 88% F-body-containing cells in blood smears from males and only 0% to 4% in those from females. Similar results were obtained with blood smears from newborns, and with cord bloods. Blood smears up to 5 months old from males showed more than 30% F-body positive cells. Schaidt and Krüger (1973) found that blood smears could be sexed by F-body technique for up to 19 days when kept at room temperature, but for up to 63 weeks if kept in a refrigerator. Bloodstains on textiles could be sexed for 8 weeks. Tröger and Liebhart (1974) found that male bloodstains stored for up to 15 months still showed detectable F-bodies. Thomsen (1975) gave an improved procedure for F-body determinations, the improvement consisting of a more effective filter combination for the fluorescence microscope.

Kringsholm *et al.* (1976) noted that they had found no overlapping between the sexes in the percentage of cells showing F-bodies. The highest female counts were always lower than the lowest male counts. They had not, therefore, observed any "false positive" males. Occasionally, a male blood could give a deceptively low F-body count, however, and this situation would result in a "false negative". A low F-body count had to be interpreted with caution, therefore, and could mean (1) a stain of female origin; (2) an older stain that was of male origin; or (3) a stain of male origin from a male with an abnormally low percentage of F-body positive cells. Kringholm *et al.* (1977) found that most bloodstains stored at room temperature for up to 21 weeks could be sexed by F-body technique. They did observe a few false negative results. Thomsen (1978) extended these aging studies to include stains kept at 5° and at 55°. False negative results appeared earlier in stains kept at either of these two temperatures than in those kept at room temperature, but there were no false positives. It appeared, therefore, that bloodstains retain their Y-body counts better at room temperature than at 5°, while the opposite appeared to be the case in the studies of blood smears (Schaidt and Krüger, 1973).

Schwinger and Tröger (1977) carried out blind trial studies using two different, independent scorers. In 21 stains up to 4 months old, the results of the two readers were very similar. Nanko (1980) has noted that F-body count in male blood smears fixed in methanol declines over the course of time, but the reason for the decline was not clear.

Wigmore *et al.* (1979a) reported on their technique for F-body determination in stains and on a series of blind trial sexing experiments. The subjective nature of the technique was emphasized, and the importance of carrying out case sample and control examinations on a "blind" basis was stressed. Cotton was found to be a problemetical substratum for bloodstains for this procedure, when compared with glass or nylon. It was difficult to obtain good results from bloodstains on wood or leather as well. In contrast to other reports, there was no significant reduction in the Y index of bloodstains as a function of age up to 138 days. In blind trial studies on casework materials, 65% of male bloodstains could be correctly identified, while no stains of female origin were misidentified as being male. Similar blind trial studies, with similar results, were reported by Wigmore *et al.* (1979b).

Thomsen (1979) gave a brief review of bloodstain sexing by F-body technique, and said that it provided a useful technique in bloodstain analysis.

Curran (1976) reviewed a trial court case in New York in which opposing panels of experts disagreed over whether a bloodstain on a jacket could be reliably sexed by cytological techniques. The stain was about 22 months old when the tests were conducted. The trial court judge in this case held that the tests to determine the sexual origin of cells in the stain (sex chromatin and F-body) failed to meet the requirements for reliability and general scientific acceptance, and the testimony was not admitted.

49.3.2 F-Body determinations in other tissues

There are a number of reports on sex determination by F-body technique in hair root cells (Sakai, 1972; Ishizu, 1972; Brinkmann and Jobst, 1973; Tröger and Liebhart, 1974; Kringsholm *et al.*, 1977). Tröger and Liebhart (1974) found that hair root sheath cells were suitable only for up to about 5 days with stored hairs, but Ishizu (1972) reported successful results with hairs up to 150 days old, as did Kringsholm *et al.* (1977) with hairs up to 27 weeks old. Nagamori (1978) reported successful F-body sexing determinations in hairs without roots up to 4 weeks old, provided they were kept dry.

Cells from various tissues and organs, examined post mortem, have been found suitable for F-body sexing determination. Kovacs *et al.*(1972) obtained reliable results in looking at 100 cells from brain, heart, liver, spleen and bone marrow in 16 male and 10 female bodies. Blažek and Bráza (1972b) obtained similar results with various tissues, and they showed further (1972c) that another fluorescent dye called 1-[(6-chloro-2-methoxy-9-acridinyl)amino]-3-(dimethylamino)-2-propanol diHCl worked as well as quinacrine diHCl. F-body determination has also been described in the cells of cartilage, periosteum and marrow (Berghaus *et al.*, 1973) and in human tooth pulp (Sommermater, 1975).

Tröger *et al.* (1976) found that Y-bodies were detectable in significant percentages in buccal epithelial cells derived from cigarette butts smoked by males. 10% of the male cells still showed Y-bodies when the cigarette butts were 43 days old, while the female cells from the same source had never shown counts higher than 6%. Y-bodies can be detected in sperm cells (Sakai, 1972). Tröger and Eisenmenger (1977) did F-body analysis in vaginal epithelium to try and use their absence as a means of identifying the cells as being of female

origin. They said that stains had to be less than 4 weeks old, however, to justify such a conclusion.

Ishizu *et al.* (1973) described a technique for the consecutive determination of F-bodies and sex chromatin in the same sample material, which included saliva, hair roots and blood. The results of the two determinations, they said, were more definitive than those from either separate determination alone.

49.4 Sex Hormone Level Determinations

The use of pregnancy-associated hormones as bloodstain markers for blood derived from pregnant women was discussed in section 8.2.1. The idea of using male and female sex hormones (primarily testosterone and 17β-estradiol) as indicators of sex in dried bloodstains has been around for some years, but there appear to be very few published systematic studies on the subject. Among the most sensitive (and, hence, desirable for this purpose) methods of the assay of lower MW compounds such as steroids in complex mixtures is radioimmunoassay (RIA). This technique was discussed briefly in section 1.3.4.3.

Many RIA procedures have been described for steroid hormones, like testosterone, in serum (see, for example, Sheldon and Coppenger, 1977; Joshi *et al.*, 1979). In theory, these procedures should be adaptable to the analysis of bloodstains, and one could try to relate the sex of origin of bloodstains to the levels of one or a combination of the major sex hormones, especially testosterone, 17β-estradiol and progesterone.

The most extensive studies to date are those of Shaler (1975). He adapted serum RIA procedures for testosterone (T) and estradiol (E) to determinations in bloodstains, and then measured the "T/E" ratios in a selection of samples. The ratio was first determined on a "blind" basis in about 1,500 serum samples. The T/E ratio varied from 60 to 0.02. Analysis showed that values greater than 3.51 could be assigned as male with a high degree of probability. Predictability of sex based on the T/E was not 100% accurate, however; several male sera showed abnormally low ratios and several female sera showed abnormally high values. Unfortunately, nothing was known about the history or state of health of the donors. The ratio was next determined in about 250 bloodstains from 6 to 8 months old. Sex of origin was known, but the hormone level measurements were carried out on a blind trial basis. The predictability of sex was about the same as that found for the serum samples. It was noted that in the bloodstains, the T/E ratio separating males from females at the same probability level was 1.0, rather than the 3.51 seen with the serum samples. 98.9% of bloodstains from males had T/E ratios greater than 2.0, while 92.7% of stains of female origin had values below 1.0.

Recently, Szendrényi and Földes (1980) reported that they could successfully sex experimental bloodstains up to 6 weeks old by determining the testosterone concentration by RIA. Stains prepared from 32 males and 24 females were studied, and showed little change in testosterone concentration between 48 hours and 6 weeks of aging. The protein concentration of the samples was also determined, and it was recommended that the testosterone level be referred to the protein concentration when analyzing stains. Thus, testosterone levels expressed as pg/10 mg protein in stains correctly predicted the sex of origin in all cases in the limited number of stains analyzed.

Other hormones have been assayed in dried blood by RIA, but not for medicolegal purposes. Illig and Rodriguez de Vera Roda (1976) reported on an RIA technique for determining thyroid stimulating hormone (TSH) in dried blood spots. The method was apparently designed in order to enable samples from newborns to be forwarded to a distant testing laboratory in a convenient way.

SECTION 50. NON-GENETIC MARKERS AND BLOOD COMPONENT PROFILING

50.1 Introduction

Over the years there has been occasional interest in attempting to individualize blood using non-genetically determined components. Three general classes of components have been studied: (1) Components present in blood as the result of environmental exposures or of the ingestion of specific agents. This class includes primarily antibodies and drugs. The antibodies may be directed against blood group antigens, against specific pathogenic agents to which the individual has been exposed, or to allergens. This class also includes the hepatitis antigens. (2) Components present in blood as the result of normal metabolis processes. This class includes primarily metal ions and smaller MW metabolites. And (3) The serum proteins. Although serum proteins are genetically determined, and many are controlled by polymorphic loci, the kinds of studies being discussed here usually seek to find individual differences in the qualitative or quantitative patterns of a large series of the proteins.

Two general approaches have been taken in these studies. In the first, a specific antigen, antibody, drug or component is detected and/or quantitated. In the second, the pattern of a series of components (usually serum proteins) in different individuals is examined. The individual components are not necessarily identified. Quantitative immunoelectrophoretic techniques have been employed in a number of these kinds of studies.

A number of different factors are involved in determining whether non-genetic markers and blood component profiles are truly individualizing markers. In the case of specific blood components, these include: (1) whether the presence and/or quantity of the component is constant in an individual over time, and over what length of time; (2) whether the presence and/or quantity of the component is detectable in dried bloodstains in a way that can be related to a whole blood sample; (3) the extent to which the presence or quantity of the component declines in dried bloodstains over time; and (4) what fraction of the population might be expected to exhibit the presence and/or quantity of the component detected. In the case of blood component profiles, there are similar considerations: (1) whether inter-individual differences exceed intra-individual differences at a given time, and over the course of time; (2) whether patterns in bloodstains accurately reflect the pattern in whole blood from which the stain was derived; and (3) to what extent the different patterns actually vary in a population. Work on non-genetic markers is, for the most part, at an early stage of development in this field. Additional studies will be needed to establish the individualizing value of many of the markers. There is not much doubt that the finding of certain specific non-genetic markers, such as HBsAg for example, in a bloodstain would be valuable in an investigation, and could prove helpful if a comparison were possible with the blood of the suspected depositer of the stain soon afterwards.

50.2 Detection of Specific Components of Blood

50.2.1 Antibodies

Among the first studies on the detection of specific antibodies in dried bloodstains are those of Kirk and his collaborators in the early 1960's. In 1963, Thornton and Kirk conducted studies on the detection of incomplete (Coombs reactive) anti-D in dried bloodstains up to 28 days old. They found that the antibody was detectable, and that it persisted for at least several weeks in dried bloodstains was judged by its titer against Rh+ type O cells. Leister and Kirk (1963) reported on the detection of rheumatoid arthritis factor in dried bloodstains using gamma-globulin treated latex particles as a detection system. Dried, powdered blood kept in vials was used for these experiments. They found that the factor was detectable in dried blood up to 150 days old, and that blood with an initially high titer tended to retain its activity in the dried state. The relationship of the factor being tested for in these studies to the Gm system (section 44) is not altogether clear. In 1964, Leister *et al.* reported a series of experiments on the detection of syphilis antibodies in dried blood. A microflocculation test (Kirk and Bennett, 1939) was used in these studies. 520 dried blood samples up to 140 days old were tested, and the loss of activity was greatest in the first five days. Not every sample retained activity equally well, but a higher percentage of initially strongly reacting samples gave positive results after 140 days than of samples which had reacted more weakly at the beginning.

More recently, King, Whitehead and Werrett have done studies on the detection of various antiparasitic and allergen-associated antibodies in bloodstains. King (1974a) and King *et al.* (1975) described the detection of antibodies to *Mycobacterium tuberculosis, Treponema pallidum* (non-specific "syphilis"), *Vibrio cholera* (cholera), *Toxoplasma gondii* (toxoplasmosis), *Trichomonas vaginalis* (trichomoniasis), *Candida albicans* (candidiasis) and *Toxocara canis* (toxocariasis). There were technical difficulties with the *Toxoplasma* and *Toxocara*. Approximately 75 bloodstains were tested with the other five antigens using an indirect fluorescent antibody technique. The majority of samples gave consistent positive and negative results in

separate readings, but some gave ambiguous results. The antibodies were stable in bloodstains for at least several months. In the sample of bloods studied, the discriminating power for the five antigens was calculated to be 0.71, roughly comparable to the ABO blood group system. Antibodies to *Mycobacterium tuberculosis* could be demonstrated in semen if it was not more dilute than 1:10. It was noted that the antibody titer in a positive group of bloods behaves as a continuous variable, and that scoring samples as "positive" or "negative" was an oversimplification. It was also noted, however, that the procedure lends itself to the profiling of a large number of different antigens to which antibodies might be expected. In interpreting the results, caution must be exercised because there are some cross reactions with related antibodies in a large series.

In 1976, Werrett *et al.* extended the antibody profiling studies to include allergen-associated antibodies. These antibodies are of the IgE class (section 1.3.3.2), and require very sensitive detection techniques because the circulating levels of IgE are very low compared to most other serum proteins (see in section 39). Commercially available RIA test kits for four allergens were used in the studies to detect the antibodies in experimentally prepared bloodstains. It was found that the antibodies could be detected in bloodstains on a variety of substrata, but that antibody activity declined more or less linearly to about 50% after 6 weeks. Using the four allergen-associated antibodies, it was possible to discriminate six different blood stains on a blind trial basis, and the results correlated with the reported allergic conditions of the donors. The results were considered to be preliminary but promising, since the range of different antibodies which one assumes could be detected in bloodstains (and for which reagents are available) is quite large.

King *et al.* (1976) reviewed the studies on antibody profiling of bloodstains. The promise and potential value of this approach were discussed. A large number of antimicrobial and allergen-associated antibodies could potentially be employed for this kind of profiling. An antimicrobial antibody profile reflects an individual's environmental exposure and vaccination history. The blood of full-grown adults can be differentiated from that of younger children in this way (King and Whitehead, 1975). In addition, menstrual blood was found to have a much higher antibody level relative to hemoglobin than the circulating blood of the same donor. Allergen antibody profiles could give information about the geographical origins or history of an individual. Information of potential value to an investigation might thus be provided from the examination of a bloodstain at a crime scene.

50.2.2 Drugs and plasma metabolites

The same kind of RIA techniques used to detect drugs in serum can be adapted for measuring drug levels in bloodstains. As noted in section 49.4, Shaler and his collaborators adapted RIA techniques for steroid hormone determination to bloodstain analysis. They have extended these studies to include the detection of a number of drugs as well. Shaler (1975) said that dilantin, a drug used to treat epilepsy, could be detected in a fragment of bloodstain equivalent to 10 $\mu\ell$ of blood, and that this represented an amount approximately 100 times larger than what was needed for detection. This work has since been extended to include detection of morphine (Mortimer *et al.*, 1978a; Smith *et al.*, 1980), digoxin (Mortimer *et al.*, 1978b), and barbiturates (Smith, 1980) in bloodstains.

King (1974b) examined the possibility of profiling a series of plasma constituents of clinical importance in the individualization of bloodstains. Data on the levels of Na^+, K^+, Cl^-, Ca^{++}, Mg^{++}, PO_4^{3-}, protein, albumin, glucose, uric acid, urea nitrogen, cholesterol, SGOT (soluble glutamic oxaloacetic transaminase) and LDH (lactate dehydrogenase) were surveyed in this study. Generally, the intra-individual differences were too close to the population variances to make this "biochemical profile" of much individualizing value. He noted, however, that better analytical methods might improve the prospects for the use of this kind of profiling in forensic serology.

50.2.3 Hepatitis antigens

One of the best reviews of hepatitis serology may be found in the Nobel address by Blumberg (1977). The discovery of a detectable antigen associated with hepatitis B grew out of studies on the Ag lipoprotein polymorphism (section 45.1). A precipitating antiserum was discovered in the serum of a multiply transfused hemophiliac patient in New York which reacted differently from the known anti-Ag serums. Tested against a panel of sera, the antibody reacted with the serum of an Australian aborigine, and the antigen was given the name "Australia antigen", or "Au". The antigen was found to be very stable in stored sera, and was consistently positive or negative in the same person's serum collected in some cases over a number of successive years. It was noticed that all Au(+) sera came from transfused individuals, and by the end of 1966 it had been realized that Au was associated with acute viral hepatitis. Au could be transmitted by transfusion, and some of the people who received it in this way developed hepatitis. Some such people also developed anti-Au. This finding led to the mass screening of donor blood units for Au prior to any transfusions, and rejection of the Au(+) bloods. The virus responsible for hepatitis B is now known to contain the Australia antigen on its surface, and it is now termed hepatitis B surface antigen (HBsAg). The surface antigen can be removed to reveal a core antigen (HBcAg), and antibodies to both of these can be found in human sera. It turns out that HBsAg exhibits antigenic heterogeneity and a number of surface antigenic determinants (designated by various lower case letters) are known to be associated with the virus. Dmochowski (1976) has given a thorough and readable review of the entire subject of hepatitis, including information about the surface and core antigens and their serology. HBsAg may be detected by a variety of immunological techniques, immunodiffusion, crossed electrophoresis and complement fixation among them. Hemagglutination techniques, in which red cells or latex particles are

coated with anti-HBsAg and used as the test system, have also been described (see, for example, Archer, 1977).

HBsAg can be detected in dried blood. Hoste *et al.* (1977) described a series of studies on a number of experimentally prepared bloodstains using a "sandwich" RIA procedure for detection. HBsAg could be detected in stains up to 6 months old, though more material was needed for testing with the older stains. The presence of HBsAg is a fairly stable characteristic, and can persist for years in people who have it. The incidence of the antigen is fairly low in Western Europe and in this country, but much higher in some populations. HBsAg is sometimes found in so-called carriers, who appear to be healthy. Hoste *et al.* (1977) suggested that HBsAg could provide a useful nongenetic marker in bloodstains. Frazadegan *et al.* (1978) have described detection of HBsAg in blood dried on filter paper, although their interest was not in bloodstain testing *per se*. If HBsAg subtyping should become possible in bloodstains, an even greater discrimination among individuals will probably be possible. Richer *et al.* (1977) have shown, for example, that the frequency of the "e" antigen associated with HBsAg varies among different ethnic groups in the Montreal population.

50.3 Serum Protein Profiling

A number of workers have looked at serum protein profiles in different individuals using a variety of different techniques of separation and detection. The number of components resolved is dependent on the nature and resolving capability of the technique selected. In addition, some of the studies were carried out before the extent of serum protein polymorphisms was fully recognized (Unit VII). In any case, if the proteins being profiled are not identified, one can not be sure that one is not seeing an example of a known polymorphic serum protein when a difference is detected.

In 1953, Bernfeld *et al.* found differences in the serum protein profiles of a number of different individuals using moving boundary electrophoresis in a Tiselius cell (section 2.3.1). Brown and Kirk (1957) conducted similar experiments on dried blood using paper electrophoresis. They could distinguish some but not all the stains in a series prepared from ten different people. Laudel *et al.* (1963) could detect differences in the immunoelectrophoretic patterns in the sera of a small number of people after carrying out electrophoresis on cellulose acetate membranes and detecting the proteins with a horse immune anti-human serum.

Whitehead (1969) and Whitehead *et al.* (1970) explored the possibility of using antigen-antibody crossed electrophoresis (Laurell technique—see in section 2.4.3.2) as a means of finding individual differences in the serum protein patterns. Studies with bloodstain extracts indicated that individual differences could sometimes be seen. The technique was found to be especially valuable for distinguishing Hp phenotype (see in section 40), and the possibility of using the profiling technique to distinguish phenotypes in a number of different immunologically detectable polymorphic serum protein systems simultaneously was discussed. Phillips (1974) looked at a somewhat different approach to serum protein profiling. The quantity of various serum proteins in the sera of different individuals is known to vary. It was thought that these differences might be exploited as individualizing markers using specific antisera and immunochemical quantitation. Antigen-antibody crossed electrophoresis was considered too variable for this purpose, and a nephelometric quantitation procedure using an autoanalyzer was explored. The procedure was found to be unsatisfactory for bloodstain extracts and even to a great extent for older hemolyzed blood samples because of interference by hemoglobin. If suitable techniques could be devised for ridding the samples of hemoglobin, Phillips said, the technique might be very useful, and he recommended further study. Grunbaum and Hjalmarsson (1976) carried out preliminary experiments on aqueous extracts of bloodstains using isotachophoresis (section 2.5.2). There were differences in the patterns from four people, and these patterns did not change if the extracts were retested 24 hours later.

In 1976, Sweet and Elvins (1976a and 1976b) applied antigen-antibody crossed electrophoresis (which they call "crossed electroimmunodiffusion" or CEID—see in section 2.4.3.2) to the problem of distinguishing individual serum protein pattern differences. By selecting and scoring those peaks which showed completely different height ranges over several determinations among individuals being compared, it was possible to distinguish among ten persons in the study. There were five males and five females in the test group, and differences between males and females were noted in the patterns as well. There were a few serum proteins which showed significant differences in amount between the sexes, and it was suggested that analysis of these might provide a probable sex of origin estimate in bloodstains. Whitehead (1977), in a letter prompted by the work of Sweet and Elvins (1976a), pointed out some of the problems associated with serum protein profiling as a means of individualizing stains. He thought that considerably more work would be needed before profiling techniques would be suitable for use in actual casework, and that genetic markers would continue to provide the principal means of individualization available for some time. Sweet (1977) responded to Whitehead's comments in the same issue of *Science*, and did not, for the most part, disagree with them.

REFERENCES FOR UNIT VIII

Ando, K. 1973. Studies on the sex identification with human saliva and saliva stains by the analysis of exfoliated oral epithelial cells. *Jpn. J. Leg. Med.* **27**: 356–367 §[1]

Aragones, J. and J. Egozcue. 1973. A method for sexing 90-day-old bloodstains in absorbent and nonabsorbent material *Acta Genet. Med. Gemellol.* **22**: 113–116

Archer, A.C. 1977. An improved haemagglutination technique for the detection of hepatitis B_s antigen. *Med. Lab. Sci.* **34**: 345–350

Barr, M.L. 1957. Cytologic tests of chromosomal sex. *Progr. Gynecol.* **3**: 131–141

Barr, M.L. 1960. Sexual dimorphism in interphase nuclei. *Am. J. Hum. Genet.* **12**: 118–127

Barr, M.L. and E.G. Bertram. 1949. A morphologic distinction between neurones of the male and female, and the behaviour of the nucleolar satellite during accelerated nucleoprotein synthesis. *Nature* **163**: 676–677

Bassett, W.A.G. 1978. Sex determination by sex chromatin identification in the hair root sheath. *J. Canad. Soc. Forensic Sci.* **11**: 221–228

Berghaus, G., U. Reifenberg and G. Dotzauer. 1973. Geschlechtsbestimmung an Skeletteilen. Nachweis der sogennanten Y-Fluorescenzkörperchen. *Z. Rechtsmed.* **72**: 255–268

Bernfeld, P., V.M. Donahue and F. Homburger. 1953. Characteristic individual electrophoretic patterns in humans. *Proc. Soc. Exp. Biol. Med.* **83**: 429–434

Blažek, J. and J. Bráza. 1972a. Determination of cellular sex in older blood spots and in hair follicle cells with the aid of acridine fluorochromes. *Soudni Lek.* **17**: 17–22 [incorporated in *Česk. Patol.* **8** (1972)]

Blažek, J. and J. Bráza. 1972b. Demonstration of Y-chromosome in necroptic material by fluorescent methods. *Česk. Patol.* **8**: 45–49

Blažek, J. and J. Bráza. 1972c. The method of sex determination in necropsies with the aid of 1-[(6-chloro-2-methoxy-9-acridinyl) amino]-3-(dimethylamino)-2-propanol dihydrochloride. *Česk. Patol.* **8**: 108–110

Blumberg, B.S. 1977. Australia antigen and the biology of hepatitis B. *Science* **197**: 17–25

Brinkmann, B. and U. Jobst. 1973. Bestimmung des Kerngeschlechts an biologischen Supren. Forensischer Beweiswert und Anwandungsbereich. *Z. Rechtsmed.* **73**: 1–6

Brown, C.L. and P.L. Kirk. 1957. Individuality of blood. Electrochromatophoretic patterns of dry blood. *J. Forensic Med.* **4**: 58–64

Caroff, J. and J. Breton. 1966. Le sexe chromatinien—intérêt médico-légale revue générale. *Ann. Med. Leg.* **46**: 98–105

Caspersson, T., S. Farber, G.E. Foley, J. Kudynowski, E.J. Modest, E. Simonsson, U. Wagh and L. Zech. 1968. Chemical differentiation along metaphase chromosomes. *Exp. Cell Res.* **49**: 219–222

Culbertson, J.C., N.A. Breslau, M.K. Moore and E. Eneel. 1969. Sex chromatin determination from hair. *J. Am. Med. Assoc.* **207**: 560–561

Curran, W.J. 1976. Forensic medical science: the continued problems of judges and juries. *New Engl. J. Med.* **294**: 1042–1043

Davidson, W.M. 1963. Leucocytes in blood stains, *in: Forensic Immunology, Medicine, Pathology and Toxicology,* Rept. of 3rd Intl. Meeting, Int. Congr. Ser. No. 80, Excerpta Medica Foundation, Amsterdam & New York

Davidson, W.M. and D.R. Smith. 1954. A morphological sex difference in the polymorphonuclear neutrophil leucocytes. *Br. Med. J.* **2**: 6–7

De Bernardi, A. 1959. Diagnosi istomorfologica di sesso su sangue imbrattante capelli. *Minerva Medicoleg.* **79**: 76–79

Dixon, A.D. and J.B.D. Torr. 1956. Sex chromatin as an acid to the identification of sex in forensic medicine. *Nature* **178**: 797

Dixon, A.D. and J.B.D. Torr. 1957. Sex determination of human tissues from cell morphology. *J. Forensic Med.* **4**: 11–17

Dmochowski, L. 1976. Viral type A and type B hepatitis. *Am. J. Clin. Pathol.* **65** (suppl. to No. 5): 741–786

Edwards, J.H. and A.H. Cameron. 1964. Postmortem nuclear sexing. *Lancet* **1**: 328

Farzadegan, H., K.H. Noori and F. Ala. 1978. Detection of hepatitis-B surface antigen in blood and blood products dried on filter paper. *Lancet* **1**: 362–363

George, K.P. 1970. Cytochemical differentiation along human chromosomes. *Nature* **226**: 80–81

Grob, H.S. 1970. Cytologic sexing, *in:* T.D. Steward (ed.), *Personal Identification in Mass Disasters,* Smithsonian Institution, Washington, D.C.

Grob, H.S. and H.S. Kupperman. 1961. Experiences with technics of chromatin sex determination. *Am. J. Clin. Pathol.* **36**: 132–138

Grunbaum, B.W. and S.G. Hjalmarsson. 1976. Isotachophoresis of bloodstains. *J. Forensic Sci. Soc.* **16**: 325–331

Holzer, F.J. and E. Marberger. 1957. Zur mikroskopischen Geschlechtsbestimmung an Leichenteilen. *Dtsch. Z. Gesamte Gerichtl. Med.* **46**: 242–249

Hoste, B., J. Brocteur, B. Donea and A. Andre. 1977. Identification of blood stains by non-genetic characteristics: the HBs antigen of viral hepatitis B. *Forensic Sci.* **10**: 229–234

Illig, R. and C. Radriguez de Vera Roda. 1976. Radioimmunologischer Nachweis von TSH in getrockneten Blutstropfen: mögliche Screening-Methode zur Entdeckung der Hyperthyreose bei Neugebornen. *Schweiz. Med. Wochenschr.* **106**: 1676–1681

Ishizu, H. 1972. Sex identification of hairs by Y-chromosome. *Jpn. J. Leg. Med.* **26**: 403–406 §[1]

Ishizu, H. 1973. Studies on sex identification of human blood and bloodstains. *Jpn. J. Leg. Med.* **27**: 168–181 §[1]

Ishizu, H., K. Ando, M. Nobuhara and Y. Mikami. 1973. Sex identification with a minimal sample by combining Y-chromatin identification and the X-chromatin detection method. *Jpn. J. Leg. Med.* **27**: 287–294 §[1]

Joshi, U.M., H.P. Shah and S.P. Sudhama. 1979. A sensitive and specific anzymeimmunoassay for serum testosterone. *Steroids* **34**: 35–46

King, L.A. 1974a. The identification of anti-parasitic antibodies in bloodstains using an indirect fluorescent antibody technique. *J. Forensic Sci. Soc.* **14**: 117–121

King, L.A. 1974b. The value of biochemical profiling for the discrimination of bloodstains. *J. Forensic Sci. Soc.* **14**: 323–327

King, L.A., D.J. Werrett and P.H. Whitehead. 1975. Antibody profiling of bloodstains. *Forensic Sci.* **5**: 144

King, L.A., D.J. Werrett and P.H. Whitehead. 1976. Antibody profiling of bloodstains. *Forensic Sci.* **8**: 151–154

King, L.A. and P.H. Whitehead. 1975. The differentiation of an adult's bloodstain from that of a child using an indirect fluorescent antibody technique. *Forensic Sci.* **6**: 197–203

Kirk, P.L. and C. Bennett. 1939. A rapid technique for syphilis testing with finger blood. *J. Lab. Clin. Med.* **25**: 86–88

Kovács, M., L. Harsányi and M. Sellyei. 1972. Y-body in cell nuclei of parenchymatous organs. *Z. Rechtsmed.* **71**: 104–107

Kringsholm, B., J.L. Thomsen and K. Henningsen. 1977. Fluorescent Y-chromosomes in hairs and blood stains. *Forensic Sci.* **9**: 117–126

Kringsholm, B., J.L. Thomsen, K.T. Henningsen and E. Niebuhr. 1976. Fluorescent Y-bodies in interphase nuclie: medicolegal aspects of false negative males. *Forensic Sci.* **7**: 171–172

Laudel, A.F., B.W. Grunbaum and P.L. Kirk. 1963. Individuality of human whole dry blood by immunoelectrophoresis on cellulose acetate. *J. Forensic Med.* **10**: 57–64

Leister, C.I. and P.L. Kirk. 1963. Rheumatoid arthritis factor. A sensitive

method for its detection for individualizing dry blood samples. *J. Forensic Med.* **10**: 157–161

Leister, C.I., J.I. Thornton and P.L. Kirk. 1964. Individualization of dry blood samples. Demonstration of syphilis antibody. *J. Forensic Med.* **11**: 31–35

Montanari, G.D., B. Viterbo and G.R. Montanari. 1967. Sex determination of human hair. *Med. Sci. Law* **7**: 208–210

Moore, K.L. 1966a. Introduction, *in:* K.L. Moore (ed.), *The Sex Chromatin,* 1–6, W.B. Saunders, Philadelphia & London

Moore, K.L. 1966b. The discovery of the sex chromatin, *in:* K.L. Moore (ed.) *The Sex Chromatin,* 7–15, W.B. Saunders, Philadelphia & London

Moore, K.L. 1966c. Sex chromatin and medicolegal problems, *in:* K.L. Moore (ed.) *The Sex Chromatin,* 453–464, W.B. Saunders, Philadelphia & London

Mortimer, C.E., F.P. Smith, L. Errichetto and R.C. Shaler. 1978a. Detection of drugs in a bloodstain. II. Morphine. Paper delivered at 30th Ann. Meeting, Am. Acad. Forensic Sci., St. Louis, MO

Mortimer, C.E., F.P. Smith and R.C. Shaler. 1978b. Detection of drugs in a bloodstain. III. Digoxin. Paper delivered at 30th Ann. Meeting, Am. Acad. Forensic Sci., St. Louis, MO

Müller, H., E.M. Bühler, M.G. Voegelin and G.R. Stalder. 1971. Eine neue Methode der Geschlechtsbestimmung in Leukozyten aus eingetrockneten Blutflecken. *Schweiz. Med. Wochenschr.* **101** (32): 1171–1174

Nagamori, H. 1978. Sex determination from plucked human hairs without epithelial root sheath. *Forensic Sci. Int.* **12**: 167–173

Nanko, S. 1980. Decrease of Y chromatin frequency with time after fixation of blood smear. *Forensic Sci. Int.* **15**: 1–2

Ohno, S. 1966. Single-X derivation of sex chromatin, *in:* K.L. Moore (ed.) *The Sex Chromatin,* 113–128, W.B. Saunders, Philadelphia & London

Pearson, P.L., M. Bobrow and C.G. Vosa. 1970. Technique for identifying Y chromosomes in human interphase nuclei. *Nature* **226**: 78–80

Phillips, A.P. 1974. The potential of nephelometric immunoprecipitin quantitation in forensic science. *J. Forensic Sci. Soc.* **14**: 135–136

Phillips, A.P. and E. Gaten. 1971. Y chromosome fluorescence in bloodstains. *Lancet* **2**: 371–372

Phillips, A.P. and C. Gitsham. 1974. The identification of male bloodstains by Y chromosome fluorescence *J. Forensic Sci. Soc.* **14**: 47–54

Phillips, A.P. and D.F. Webster. 1972. Improved Y-chromosome fluorescence in the presence of magnesium ions. *J. Forensic Sci. Soc.* **12**: 361–362

Radam, G. 1965. Zum Nachweis von Speichelspuren. *Beitr. Gerichtl. Med.* **23**: 226–235

Renard, S. 1971. Determination of sex of exfoliated epithelial cells and its significance in forensic science. *J. Forensic Sci. Soc.* **11**: 15–20

Richer, G., D. Phaneuf, F. Boisvert, R. Guévin and A. Viallet. 1977. Difference in the distribution of e antigen among different ethnic groups in a population of blood donors. *J. Canad. Med. Assoc.* **116**: 757–759

Sakai, K. 1972. Investigation of fluorescent body in the human cells. *Jpn. J. Urol.* **63**: 658–671 §²

Sanderson, A.R. and J.S.S. Sterward. 1961. Nuclear sexing with acetoorcein. *Br. Med. J.* **2**: 1065–1067

Schaidt, G. and R. Krüger. 1973. Die Geschlechtsbestimmung an Blutproben und Blutspuren männlicher Personen. *Arch. Kriminol.* **151**: 41–48

Schilling, H. 1960. Zur Frage der Dauer der Nachweisbarkeit des Geschlechts an eingesandtem Blut, insbesondere an Blut von Leichen, Dissertation, Berlin (Humboldt), cited by O. Prokop, *Forensische Medizin,* Berlin, 1966

Schmid, W. 1967. Sex chromatin in hair roots. *Cytogenetics* **6**: 342–349

Schwinger, E. 1972. Geschlechtsbestimmung aus Blutspuren. *Z. Rechtsmed.* **70**: 157–162

Schwinger, E. and H.D. Tröger. 1977. Wie sicher ist die Geschlechtsbestimmung in Blutspuren? *Beitr. Gerichtl. Med.* **35**: 267–271

Shaler, R.C. 1975. The individualization of forensically important physiological fluids, Final Report, Grant #75-NI-99-0011, Nat. Inst. Law Enforcement Crim. Just., Law Enforcement Assistance Administration, U.S. Dept. of Justice, Washington, D.C.

Sheldon, P.E. and C.J. Coppenger. 1977. A rapid radioimmunoassay for serum testosterone. *Steroids* **30**: 149–157

Smith, F.P. 1980. Detection of drugs in dried bloodstains. IV. Barbiturates. Paper delivered at 32nd Ann. Meeting, Am. Acad. Forensic Sci., New Orleans, LA

Smith, F.P., R.C. Shaler, C.E. Mortimer and L.E. Errichetto. 1980. Detection of drugs in bloodstains. II. Morphine. *J. Forensic Sci.* **25**: 369–373

Sommermater, J. 1975. Contribution to nuclear sex determination by identification of the Y chromosome in human pulpal fibrocytes. *Forensic Sci.* **5**: 167

Sweet, G.H. 1977. Crossed electroimmunodiffusion and bloodstain investigations. *Science* **198**: 427

Sweet, G.H. and J.W. Elvins. 1976a. Human bloodstains: individualization by crossed electroimmunodiffusion. *Science* **192**: 1012–1014

Sweet, G.H. and J.W. Elvins. 1976b. Studies by crossed electroimmunodiffusion on the individuality and sexual origin of bloodstains. *J. Forensic Sci.* **21**: 498–509

Szendrényi, J. and V. Földes. 1980. Messung des Testosterongehaltes in Blutflecken zur kriminalistischen Geschlechtsbestimmung. *Z. Rechtsmed.* **85**: 263–267

Thomsen, J.L. 1975. Eine verbesserte Methode zum Nachweis des Y-Chromsoms in Blutspuren. *Z. Rechtsmed.* **76**: 81–86

Thomsen, J.L. 1978. Influence of the temperature on the detection of fluorescent Y-bodies in blood stains. *Forensic Sci.* **11**: 123–126

Thomsen, J.L. 1979. Y-chromosome detection in bloodstains. *Forensic Sci. Int.* **14**: 104

Thornton, J.I. and P.L. Kirk. 1963. Individualizing dry blood samples by demonstration of the Rhesus antibody. *J. Forensic Med.* **10**: 123–127

Tröger, H.D. and W. Eisenmenger. 1977. Eine Methode zum Nachweis von Scheidenepithelzellen. *Beitr. Gerichtl. Med.* **35**: 109–111

Tröger, H.D. and E. Liebhart. 1974. Zur zeitlichen Nachweisgrenze des Y-Chromosoms an Blutspuren and Haaren. *Beitr. Gerichtl. Med.* **32**: 159–162

Tröger, H.D., E. Liebhart and W. Eisenmenger. 1976. Wer hat die Zigarette geraucht? Bestimmung des männlichen Kerngeschlechts an Mundschleimhautzellen. *Beitr. Gerichtl. Med.* **34**: 207–209

Werrett, D.J., L.A. King and P.H. Whitehead. 1976. The detection of allergen-associated antibodies in bloodstains. *J. Forensic Sci. Soc.* **16**: 121–126

Whitehead, P.H. 1969. Antigen-antibody crossed electrophoresis. A new means of characterizing blood? Proc. 5th Int. Meeting Forensic Sci., Toronto, *in: Int. Microfilm J. Leg. Med.* **4**(4): card 10

Whitehead, P.H. 1977. Crossed electroimmunodiffusion and bloodstain investigations. *Science* **198**: 427

Whitehead, P.H., S.S. Kind, P.A. Morris, M. Davies and R. Cleevley. 1970. The examination of bloodstains by Laurell electrophoresis (antigen/antibody crossed electrophoresis). *J. Forensic Sci. Soc.* **10**: 83–90

Wigmore, R., P. Dolton and P.H. Whitehead. 1979b. The sexing of bloodstains using Y-chromosome fluorescence. Report of a "blind trial" involving 195 casework bloodstains. Tech. Note No. 113, Home Off. Central Res. Establ., Aldermaston, Reading, Berks., England

Wigmore, R., D.J. Werrett, L.A. King, P.H. Whitehead and A. Emes. 1979a. The detection of Y chromosomes in bloodstains—a reevaluation. *J. Forensic Sci.* **24**: 366–375

Zech, L. 1969. Investigation of metaphase chromosomes with DNA-binding fluorochromes. *Exp. Cell Res.* **58**: 463

Bibliographic Notes to References for Unit VIII

§¹ *Japanese Journal of Legal Medicine* (*Jpn. J. Leg. Med.*) has Japanese title *Nippon Hoigaku Zasshi*

§² *Japanese Journal of Urology* (*Jpn. J. Urol*) has Japanese title *Nippon Hinyokika Gakkai Zasshi*

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