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Determination of the Age of Bloodstains Using Immunoelectrophoresis

The study of the behavior of serum proteins in dried bloodstains is a subject of considerable forensic interest. Kind et al [*1*] estimated the age of dried bloodstains by a spectrophotometric method. Kind and Watson [*2*] estimated the age of bloodstains by ammoniacal bloodstain extracts. This paper reports on the determination of the age of a given bloodstain by using the technique of immunoelectrophoresis.

Serum proteins deteriorate gradually with respect to time. The experimental result of this study showed that the decomposition of various proteins of the serum has a direct relationship with the age of the bloodstains. The result of this study agrees with the fact that γ-globulin is the most stable of all the serum proteins, which was previously confirmed by Kabat and Pederson [*3*].

Materials and Methods

Anti-Human Serum

Emulsify fresh human serum with an equal volume of Freund's complete adjuvant (Difco) and distribute a total volume of 0.8 ml subcutaneously into the four footpads of an albino rabbit. Repeat at weekly intervals for a total of three injections. After one week from the date of the third subcutaneous injection, administer 0.4 ml of fresh human serum alone, intravenously. Repeat the intravenous injection for six consecutive days. After a lapse of one week test bleed the animal, collect the antiserum, and obtain the antiserum's titer.

Bloodstained Clothes

Specimens for this experiment were collected from bloodstained cotton clothes received in the laboratory over a period of one year. The age of the specimens collected was accurately known from case records. The method employed in this study was as follows.

Ten cuttings, one from each group of ten articles of the same age (for example, 15, 30, 60, 150, 300, and 365 days old) were collected. The project was commenced at 15-day-old bloodstains because the case articles are received in the laboratory 10 to 15 days after the occurrence. Elimination of the various natural antigenic substances would be possible only by the use of stains from the same textile material, namely cotton.

Preparation of Extract

Pull out a fiber of 1-cm length from the bloodstained portion of each of the ten clothes

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of the 15-day age group. Place the samples in ten different test tubes and add 0.05 ml of 0.85% normal saline. Prepare the extracts by teasing out the fiber. Repeat this process for other age groups. Always prepare the bloodstain extracts just before starting the experiment.

Immunoelectrophoresis

The methods described by Grabar et al [*4–7*] were followed in obtaining immunoelectrophoretic patterns. Select a buffer tank of suitable size which will accommodate seven microslides [*8*]. Perform electrophoresis on gel on 2.5 by 7.5-cm glass plates (microslides), using 1% agarose in Veronol® buffer (ionic strength 0.05, pH 8.6), which is composed of 5.65 g diethylbarbituric acid, 0.92 g sodium diethylbarbiturate, and 0.205 g sodium acetate. Make up to 1 liter with distilled water. Dissolve 1 g of agarose in 100 ml of Veronol buffer by boiling.

Spread 2 ml of agarose gel on each clean slide and keep the slide on a smooth surface to maintain uniform thickness of the gel layer. Cut two outer wells (diameter, 0.2 cm) and a trough (0.2 by 5 cm) in each plate, but the gel from the trough region should not be removed until after electrophoresis.

Add normal human serum into the wells of a plate and one extract from each of the six age groups into the wells of six gel plates, keep them in the buffer tank, and run the system. The duration of electrophoresis (7 V/cm) should be 90 min. After the completion of electrophoresis, remove the gel from the trough region, add anti-whole human serum to the trough, and place the plates for immunodiffusion inside a refrigerator. Do not disturb the plates for the following three days because the maturation of precipitation will continue until the third day. Dry the gel plates at room temperature and stain with amido-black solution which is composed of 45 ml methyl alcohol, 10 ml acetic acid, 45 ml distilled water, and 1 g amido-black. Destain in 1% glacial acetic acid and photograph the plates. Repeat this procedure for the remaining extracts.

Results

Not all the proteins seen in the immunoelectrophoretic pattern (IEP) of fresh normal human serum were found in those obtained from extracts prepared from stains of different ages. The serum proteins that are present in the IEPs of various age groups are shown in Table 1. The IEP of fresh normal human serum (Fig. 1) was compared with the IEPs of the bloodstain extracts of different age groups (Figs. 2-6). Extracts obtained from bloodstains of the same age group had the same variety of serum proteins. No precipitin band could be traced in the gel plates of the last group, the one-year-old bloodstain extract.

Gamma-globulin was present in the IEPs of the bloodstain extracts of all the age groups, including 300-day-old bloodstains. The other serum proteins were not present in the IEP of 300-day-old bloodstain extracts. The IEPs obtained in all these cases did not have other serum proteins, namely prealbumins, albumin, ceruloplasmin, α_1-lipoprotein, haptoglobin, and α_2-macroglobulin, which will be present in the normal human serum. In the case of β_1-globulins, only transferrin was present. When the bloodstains were more than 300 days old, γ-globulin was not present.

Discussion

Gamma-globulin (antibody protein), the most stable of all the proteins [*3,9*], could survive 300 days after the coagulation of blood. This coincided with the basic feature of γ-globulin, which could revive to its original condition even after subjection to a temperature of 70°C or a varying pH range [*3,9*]. The immunoelectrophoretic analysis of the extracts

TABLE 1—*Serum proteins of the bloodstains of different ages.*

Serum Proteins	Age, in days						
	Fresh	15	30	60	150	300	365
γ-globulin	present	present	present	present	present	present	absent
β_2M-globulin	present	present	absent	absent	absent	absent	absent
β_2C-globulin	present	present	present	absent	absent	absent	absent
β_2B-globulin	present	present	present	present	absent	absent	absent
β_1-globulin	present	present	present	present	present	absent	absent
α_1-globulin	present	absent	absent	absent	absent	absent	absent
α_2-globulin	present	absent	absent	absent	absent	absent	absent
Albumin	present	absent	absent	absent	absent	absent	absent

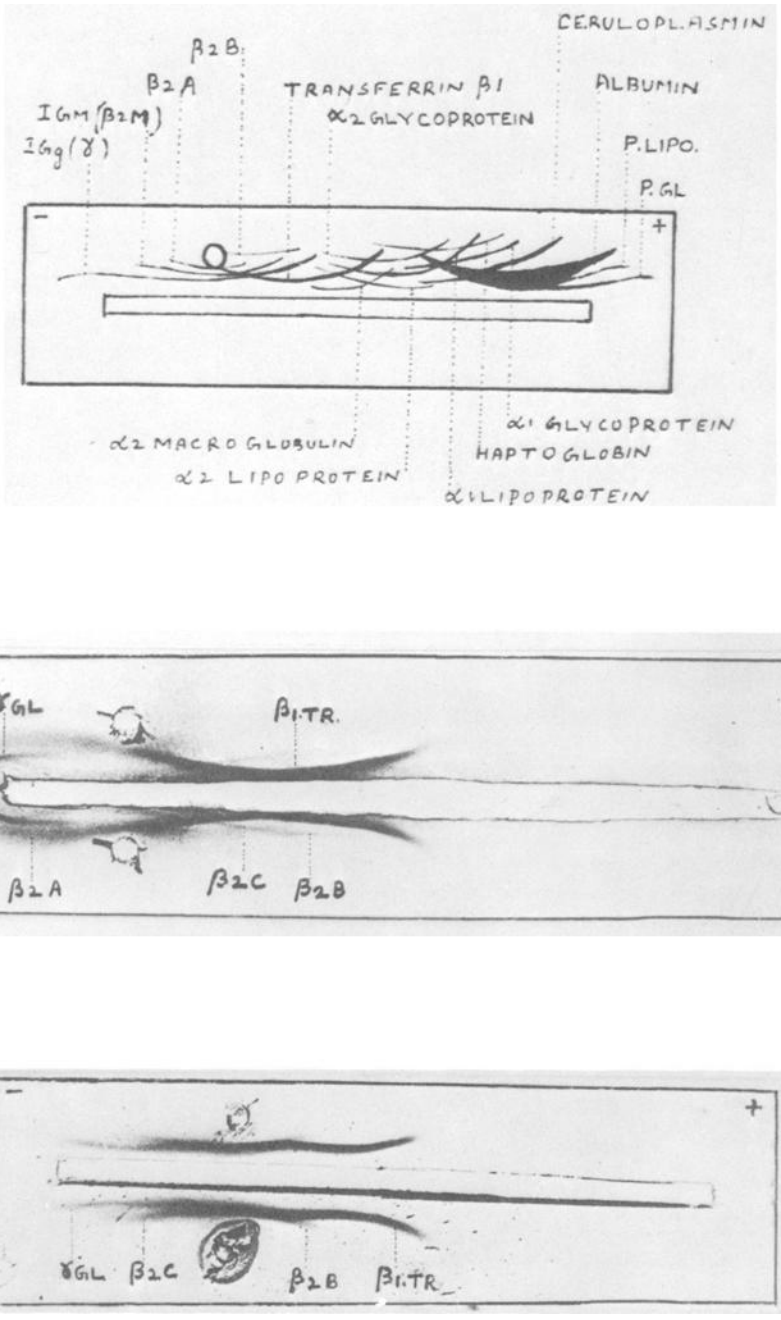

FIG. 3—*Photograph of IEP of a 30-day-old bloodstain.*

of one-year-old bloodstains did not reveal the presence of any of the serum proteins, including γ-globulin. Therefore, age estimation of bloodstains which are older than 300 days is not possible because of the deterioration of γ-globulin. Allowing the denaturing effect of seasonal fluctuations on the exhibits, the following conclusions could be drawn.

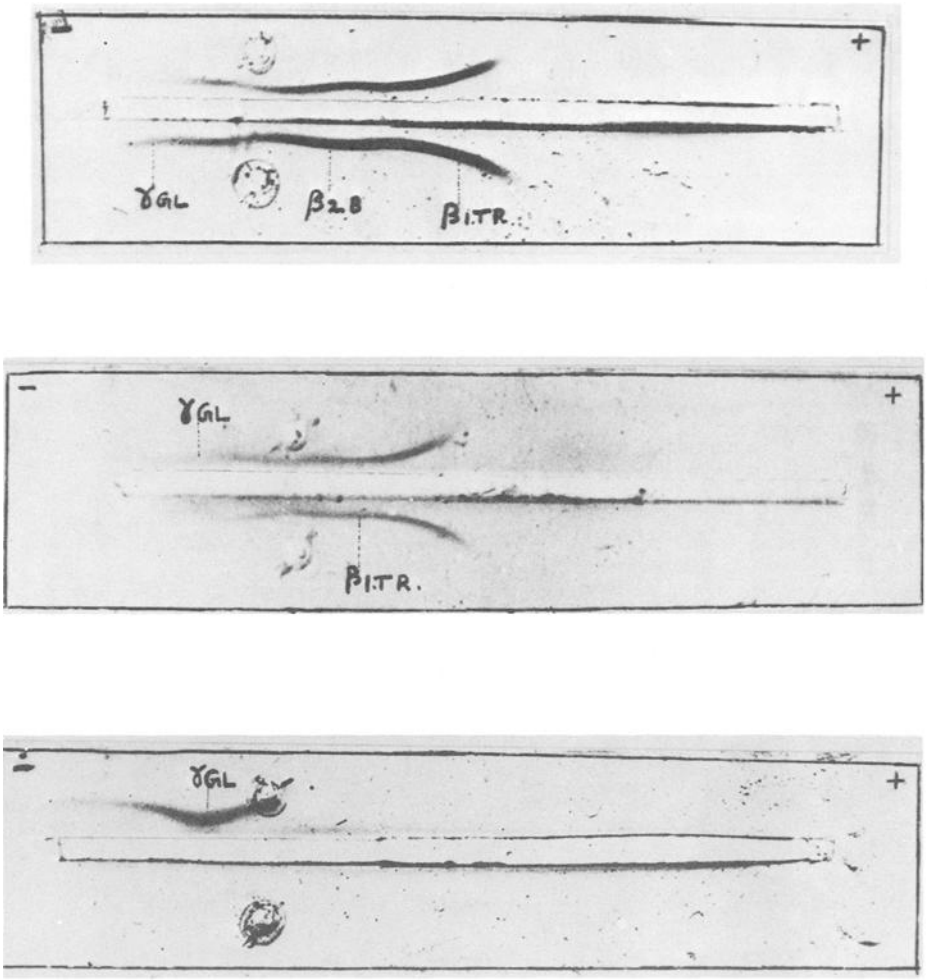

FIG. 6—*Photograph of IEP of a 300-day-old bloodstain.*

The presence of γ-globulin, β_2M, β_2B, β_2C, and β_1-globulin and the absence of other serum proteins in the IEP of any bloodstain would indicate its age as ranging between 10 and 15 days. In an IEP, if β_2M-globulin is absent, the age can be taken as 30 to 45 days. The IEP of a 60-day-old bloodstain would show the presence of only γ-globulin, β_2B, and β_1-globulin. If γ-globulin and β_1-globulin are present alone in the IEP of a bloodstain, its age would be about 150 days.

This study offers a simple and rapid technique for the approximate estimation of the age of bloodstains. While, in this report, I have focused my attention to the use of immunoelectrophoresis in the estimation of the age of dried bloodstains alone, it is possible that the principle involved may also be applied in the estimation of the age of semen.

Summary

The technique of immunoelectrophoresis was used to determine the age of bloodstains. The immunoelectrophoretic patterns (IEP) of bloodstains ranging from 15 days to one

year old were obtained by the use of high titer anti-whole human serum. The IEPs revealed gradual disappearance of β-globulins and γ-globulin with increase in the age of bloodstains. A comparative study of the IEP of normal human serum with those of the experimental bloodstains showed the absence of some of the corresponding proteins. The absence of a particular serum protein in the IEP of a given bloodstain will indicate the age of that bloodstain.

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