TECHNICAL NOTE

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Detection of Thyroglobulin in Bloodstains as an Aid in the Diagnosis of Mechanical Asphyxia

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ABSTRACT: Mechanical forces applied to the neck region are known to release certain amounts of thyroglobulin into circulation. In this experiment, an attempt was made to detect thyroglobulin in bloodstains as an aid in the diagnosis of mechanical asphyxia. Experimental bloodstains containing thyroglobulin at concentrations of 1, 2, 5, and 10 μg/mL were prepared on a sheet of filter paper. Small pieces of bloodstains, measuring approximately 2.4 cm^2 in area, were extracted with 0.1 mL of distilled water and the extracts were tested against an antihuman thyroglobulin serum by precipitation-electrophoresis. Bloodstains containing more than 1 μg/mL of thyroglobulin formed distinct precipitin lines for up to one month of storage, while bloodstains containing more than 5 μg/mL of thyroglobulin formed distinct precipitin lines for up to three months of storage. The present results suggest that the bloodstains can be utilized in the diagnosis of mechanical asphyxia.

KEYWORDS: pathology and biology, blood, thyroglobulin, bloodstains, mechanical asphyxia, throttling, strangulation

Leakage of certain amounts of thyroglobulin into the circulation has been demonstrated in some victims who sustained blunt force injuries in the neck region [*1-4*]. Radioimmunological studies showed that the plasma thyroglobulin levels were higher than 2 μg/mL in five out of twelve cases of throttling and strangulation and the highest one was 34 μg/mL [*4*]. Thus, the detection of thyroglobulin in plasma can serve as a helpful adjunct in the diagnosis of mechanical asphyxia.

In cases of mechanical asphyxia, the victims frequently show bleeding from the nostrils and mouth. It is quite possible that the bloodstains derived from the victims contain thyroglobulin since the protein released into the circulation can be distributed throughout the whole body in a matter of seconds. Therefore, the detection of thyroglobulin in the bloodstains found at

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crime scenes may afford a useful adjunct for the diagnosis of mechanical asphyxia in some cases, especially when the victim had been transferred to somewhere else.

In the present study, an attempt was made to detect the thyroglobulin in blood stains by precipitation-electrophoresis using an antihuman thyroglobulin serum.

Materials and Methods

Purification of human thyroglobulin and preparation of antiserum against human thyroglobulin were made as described earlier [*4*]. Heparinized blood samples were kindly supplied by the clinical laboratory of Nagoya University School of Medicine.

The purified thyroglobulin was added to four blood samples at concentrations of 1, 2, 5, and 10 μg/mL, respectively. These blood samples were dropped onto sheets of filter paper (Toyoroshi No. 2, Tokyo, Japan), allowed to dry at room temperature, and tested at appropriate intervals.

Prior to test, the stains were punched out by a puncher (diameter 5.5 mm) and ten discs were pooled followed by extraction with 0.1 mL of distilled water for a couple of hours at room temperature. Each extract thus prepared was submitted to the analysis. For reference, the

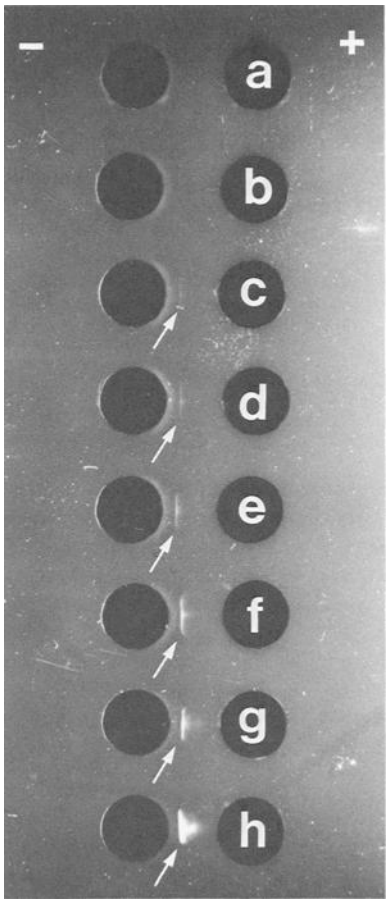

FIG. 1—*Sensitivity of the precipitation-electrophoresis method for detection of thyroglobulin. Arrows indicate the precipitation line formed. The concentrations of thyroglobulin were* (a) *0,* (b) *0.1,* (c) *0.2,* (d) *0.3,* (e) *0.5,* (f) *0.7,* (g) *1.0, and* (h) *2.0 μg/mL, respectively.*

hemoglobin concentration of each extract was determined according to the method of Van Kampen and Zijlstra [5].

Precipitation-electrophoresis was carried out by a modification of Culliford's method [6]. Two parallel lines of wells (diameter 5.5 mm) were punched out on an agarose gel plate across the direction of electrophoresis, approximately 1 cm apart. The anodal wells were filled with the antiserum, and the cathodal wells with each of the extracts or thyroglobulin standards. After electrophoresis for 30 min in the cold, the gel plate was washed overnight with normal saline and examined with a hand lens for the formation of precipitin lines under oblique illumination. Hemoglobin diffused in the gel was practically eliminated by this washing procedure. The washing procedure was omitted when standard thyroglobulin solutions were tested.

Results and Discussion

The highest dilution of the antiserum that formed an impeccable precipitin line against purified thyroglobulin (14 mg/mL) in the precipitation-electrophoresis was 1:32. The sensitivity of the method studied by using varying concentrations of thyroglobulin is shown in Fig. 1. Thyroglobulin in distilled water as little as 0.2 μg/mL could successfully be detected. As shown in Fig. 2, thyroglobulin in bloodstains could also be detected by this method, though the precipitin lines formed became less intense with the decrease in the concentration of thyroglobulin. Control bloodstains failed to form any visible precipitin lines even in the fresh state, indicating that the antiserum used was monospecific in strict language.

As shown in Table 1, bloodstains containing more than 1 μg/mL of thyroglobulin formed distinct precipitin lines for up to one month of storage, while bloodstains containing more than 5 μg/mL of thyroglobulin formed distinct precipitin lines for up to three months of storage. Hemoglobin concentrations determined for reference were 13.6 to 14.3 g/dL in the

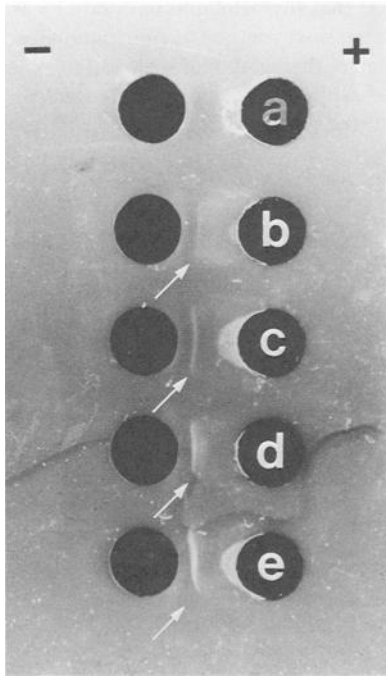

FIG. 2—*Demonstration of thyroglobulin in the extract of the bloodstains by means of precipitation-electrophoresis. Arrows indicate the precipitation line formed. The stains of control blood* (a), *the blood containing 1 μg/mL* (b), *2 μg/mL* (c), *5 μg/mL* (d), *and 10 μg/mL* (e) *of thyroglobulin were analysed.*

TABLE 1—*Analysis of thyroglobulin in the bloodstains of varying ages by precipitation-electrophoresis.*

Thyroglobulin in the Bloodstains, μg/mL	Results of Precipitation-Electrophoresis			
	Period of Storage			
	One Day	One Week	One Month	Three Months
Control	−	−	−	−
1	+	+	+	−
2	+	+	+	−
5	++	++	++	+
10	++	++	++	++

original blood samples, 5.1 to 7.3 g/dL in the extracts of the bloodstains in storage for one month, and 2.3 to 4.2 g/dL in the extracts of the bloodstains in storage for three months.

It is well-known that the sensitivity of the precipitation-electrophoresis depends in part on the potency of the antiserum employed and in part on the amount of the antigen available. The comparatively large size of wells punched out on an agarose gel plate that were able to accomodate large amounts of both the antigen and antiserum and the high potency of the antiserum seems to account for the remarkably high sensitivity of the present method.

In view of the recovery rates of hemoglobin, the extracts of the bloodstains in storage for one to three months approximately correspond to two- to three-fold dilutions of the original blood samples. Note that the extracts of the bloodstains still contain thyroglobulin not less than 0.2 μg/mL as judged by the visible precipitin lines. The failure of the extracts of older bloodstains to form visible precipitin lines may be ascribed to the diminished solubility of thyroglobulin or its antigenic degradation or both.

The present results suggest that thyroglobulin in dried bloodstains is fairly stable and can easily be detected by such a sensitive method as precipitation-electrophoresis. It is tempting to speculate that the detection of thyroglobulin in bloodstains may afford a useful adjunct for the diagnosis of mechanical asphyxia in some cases; especially when the victim had been transferred to somewhere, or the body was decomposed with a preserved bloodstain.

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