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DNA fingerprinting of freeze-dried tissues*

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Summary. DNA profiling is usually unproblematic when carried out on material sampled shortly after death. Prolonged postmortem intervals and improper storage cause significant DNA degradation, however there are instances where DNA analysis becomes necessary months after the autopsy. In such cases the investigator may be able to revert to material initially stored for toxicological purposes. In some cases such specimens undergo freeze-drying before storage. We have therefore tested DNA fingerprinting of freeze-dried postmortem material. It was found that freeze-drying is a suitable method for preserving tissue samples for DNA profiling.

Key words: DNA fingerprinting – Freeze-drying – Postmortem tissue specimens – Identification

Zusammenfassung. DNA-Untersuchungen gestalten sich im allgemeinen unproblematisch, wenn das Untersuchungsmaterial kurzfristig nach dem Tode entnommen wurde. Längere postmortale Intervalle sowie inadäquate Lagerung können zu entscheidender DNA-Degradierung führen. Es finden sich aber immer wieder Fälle, wo sich die Notwendigkeit des DNA-Printing erst Monate nach der Sektion ergibt. Hier ist der Untersucher u. U. gezwungen, auf Material zurückzugreifen, welches ursprünglich für mögliche chemisch-toxikologische Untersuchungen asserviert worden war. Manchmal werden solche Proben vor der Einlagerung gefriergetrocknet. Aus diesem Grunde testeten wir die Möglichkeit des genetischen Fingerabdruckes an gefriergetrocknetem Leichenmaterial. Es ergab sich, daß die Technik der Gefriertrocknung auch in Hinblick auf DNA-Untersuchungen eine geeignete Probenvorbereitung ist.

Schlüsselwörter: Genetischer Fingerabdruck – Gefriertrocknung – Postmortale Gewebeproben – Identifikation

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Introduction

DNA profiling of cadaverous and stored material has been reported by several authors [1, 2, 5, 8, 11, 13, 16, 17, 23]. Such examinations are carried out for identification of blood samples [3, 22], identification of corpses, assignment of parts of corpses to one or more persons [14, 20], or, occasionally in cases of affiliation [9, 19]. The usual technique for storing tissues or isolated DNA is freezing [18], however investigations must sometimes be carried out several months after autopsy and it becomes necessary to revert to material initially stored for other purposes, most commonly for histological or toxicological examinations.

In principle it is possible to isolate DNA from formaldehyde fixed and paraffin embedded tissues [10]. Bär et al. referred to the use of buffered formalin [1], but formaldehyde fixation over a longer period may cause DNA degradation and foil any kind of DNA profiling. Storage of tissue samples in absolute alcohol or water-free acetone has also been found suitable. Bär et al. reported that the yields of DNA were comparable to those from frozen tissue [1]. Specimens stored for toxicological examinations are sometimes freeze-dried before storage. Our aim was to investigate whether this method of tissue preservation has a negative influence on DNA profiling.

Material and Methods

Post mortem tissues were sampled from routine autopsies (4 days post mortem at the latest): cardiac blood, myocardial tissue, brain cortex, muscle tissue, lung tissue and kidney. Freeze-drying was carried out on a Christ Delta I/IA (Christ, Germany) one day after autopsy at the latest using the technique described by several authors [6, 7, 21]. The freeze-dried tissues were stored for one year at −20°C. DNA fingerprinting was carried out as follows:

Extraction of DNA: approx. 1 g specimens of freeze-dried heart blood, brain cortex, muscle tissue, lung tissue and kidney were mechanically homogenized (Ultra-Turrax, IKA) in 10 ml nuclei lysis buffer containing 10 m*M* Tris-HCl pH 8, 400 m*M* NaCl, 2 m*M* EDTA pH 8. Blood samples were incubated in 50 m*M* KCl at 37°C followed by centrifugation (1000 × g). This procedure was repeated

until the pellet became colourless. DNA was prepared as follows: digestion with Proteinase K (500 µg/ml) and SDS (final concentration 0.5%), incubation overnight at 37°C; DNA was extracted twice in phenol/chloroform/isoamylalcohol (25:24:1), precipitated in 2 vol. of absolute ethanol, resuspended in 100 µl TE buffer and incubated at 56°C for 1 h. This was followed by precipitation in 20 µl 3*M* Na acetate and 500 µl abs. ethanol, one wash of the DNA pellet in 70% ethanol, resuspension of the dried pellet in TE buffer and dialysis for 3–4 hours.

Digestion of DNA: 10 µg DNA was digested by 50 U of the enzymes HINF I, HAE III and Rsa I overnight at 37°C (at least 18 h).

Electrophoresis: Restriction fragments were separated in a 0.6% agarose gel (20 × 20 cm) in 1 × TBE at constant voltage (40 V) for 27 h.

Southern blot: Depurination was carried out in 0.3 *M* HCl for 20 min, denaturation in 0.5 *M* NaOH, 1.5 *M* NaCl twice for 30 min. DNA was transferred to a nylon membrane (Biodyne A, Pall and Oncor) overnight [12] which was soaked in denaturation solution for 15 min followed by neutralization of the membrane in 0.5 *M* Tris-HCl pH 8.0, 1.5 *M* NaCl for 15 min and washed in 2 × SSC for 10 min. Finally the membrane was baked at 80°C for 1 h.

Non-radioactive detection: Membranes were prehybridized and hybridized with the multi locus minisatellite probe B.E.S.T MZ 1.3 digoxigenin according to the method described in the Biotest-Manual [4], and with the single locus probe pYNH 24 according to the manufacturer's instructions [15]. Colour development: Incubation with antibody-alkaline phosphatase complex solution (Biotest or Boehringer), 1:5000, for 30–60 min and developed with BCIP/NBT (BCIP: 50 mg/ml DMF; NBT: 75 mg/ml 70% DMF).

Results and discussion

Freeze-drying is well known to toxicologists as a mild and effective technique for preparing human tissues for longer storage periods. There are two disadvantages of this procedure: evaporation and/or redistribution of volatile substances. However, these disadvantages should be unimportant for DNA profiling. We therefore examined DNA fingerprinting on specimens prepared in this way.

After 1 year of storage at −20°C, the patterns revealed by MZ 1.3 were virtually unchanged with the exception that high molecular weight bands exhibited reduced intensity, which was probably due to delayed post mortem sampling (Fig. 1). These findings correspond to those of Bär et al. [1] who reported a steady exponential decline of high molecular weight DNA with increasing postmortem interval. Due to the varying degrees of resistance against degradation the rate of decline varied from tissue to and our results demonstrated that the best results can be expected from lung tissue, muscle tissue and heart blood. In the special case of blood varying results were found which could not be explained by differences in the post mortem interval. The yield of DNA and the decreased staining intensity of high molecular weight DNA bands differed from case to case. This phenomenon may be due to artefacts, in particular to quantitative inhomogeneities caused by blood clots. Higher yields of DNA appeared to be associated with small or non-observable clots. Similar results were obtained with

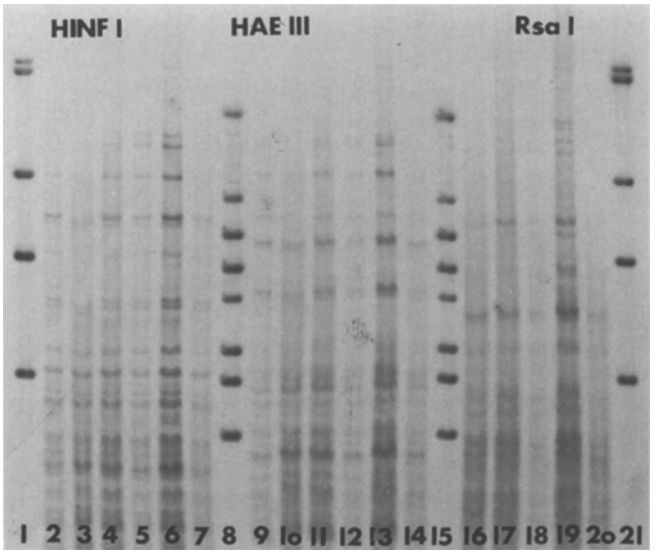

Fig. 1. DNA-fingerprinting on freeze-dried tissues: MZ 1.3 digoxigenin. *Lane 1, 21* – phage lambda DNA HindIII marker; *lane 2, 9* – heart blood; *lane 3, 10, 16* – myocard; *lane 4, 11, 17* – brain cortex; *lane 5, 12, 18* – muscle tissue; *lane 6, 13, 19* – lung tissue; *lane 7, 14, 20* – kidney; *lane 8, 15* – phage DNA BstE II marker. Tissues sampled from a male corpse 4 days after death, freeze-dried specimens stored for 1 year at −20°C

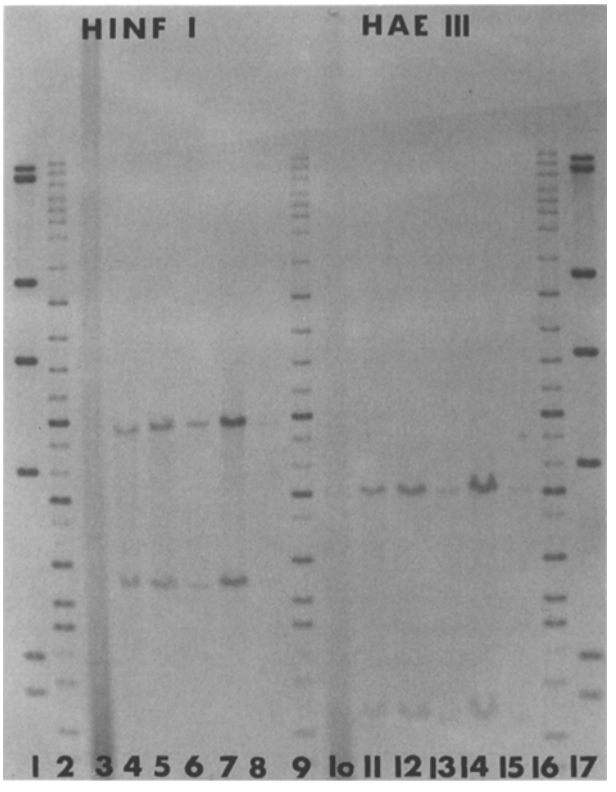

Fig. 2. DNA-fingerprinting on freeze-dried tissues: p YNH 24. *Lane 1, 17* – phage lamda DNA HindIII marker; *lane 2, 9, 16* – analytical marker wide range DG #1931 (Promega); *lane 3, 10* – heart blood; *lane 4, 11* – myocard; *lane 5, 12* – brain cortex; *lane 6, 13* – muscle tissue; *lane 7, 14* – lung tissue; *lane 8, 15* – kidey. Tissues sampled from a female corpse 3 days after death, freeze-dried specimens stored for 1 year at −20°C

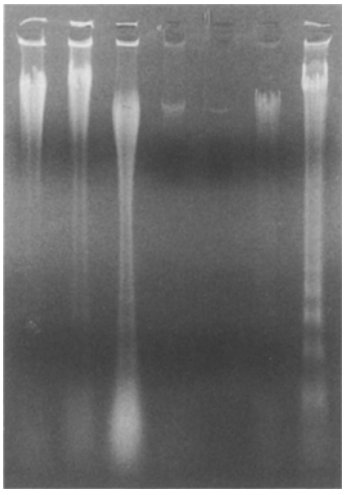

Fig. 3. Electrophoresis of undigested DNA on agarose gel. From left to right: *Lane 1* – brain cortex, freeze-dried; *Lane 2* – lung tissue, freeze-dried; *lane 3* – myocard, freeze-dried; *lane 4* – heart blood, freeze-dried; *lane 5* – lamda DNA; *lane 6* – heart blood, freeze-dried; *lane 7* – heart blood frozen. *Lane 1–4:* stored for one year, postmortem interval 4 days. *Lane 6–7:* stored for 3 days, postmortem interval 2 days

p YNH 24 (Fig. 2). Low intensity staining of the higher molecular weight region was extremely marked in the case of heart blood and kidney. The banding patterns of myocard, lung tissue, muscle tissue and brain cortex could be interpreted straightforwardly. Figure 3 shows the electrophoretical separation of undigested DNA on agarose gel. If one assumes that the smearing corresponds to the degree of DNA degradation, different stages of decomposition in one and the same corpse are apparent (lane 1–4). Comparison of freeze-dried blood to frozen blood is shown in lane 6 and 7. No significant difference in DNA degradation was detected. Different staining density does not result from different yields of DNA but from different amounts of the material extracted.

We conclude that freeze-drying is a suitable method for preparing tissue samples for storage so that not only toxicological analyses but also DNA fingerprinting can be carried out. We found no deleterious effect of this method on DNA profiling. Variations in the intensity of fingerprint bands resulting from different postmortem intervals and from the organs used as DNA source seem to be similar to those found in frozen tissues. In problem cases samples initially stored for other purposes can be examined.

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