Forensic Science International, 39 (1988) 59–70
Elsevier Scientific Publishers Ireland Ltd.

POSTMORTEM STABILITY OF DNA*

WALTER BÄR[a], ADELGUNDE KRATZER[a], MARCO MÄCHLER[b] and WERNER SCHMID[b]

[a]*Institute of Forensic Medicine, Zürichbergstrasse 8, CH-8028 Zürich and* [b]*Institute for Medical Genetics, University of Zürich (Switzerland)*

(Received November 23rd, 1987)
(Accepted March 10th, 1988)

Summary

High-molecular-weight DNA was recovered postmortem in sufficient quantities from various human organ tissues as well as from blood, although not all organs were equally well suitable. Good DNA stability was found in brain cortex, lymph nodes and psoas muscle over a period of three weeks postmortem. Spleen and kidney showed good DNA stability up to five days postmortem but after longer periods, rapid degradation was observed. Yields of DNA from blood were not consistent because of the non homogeneity of samples. Blood clots were rich with DNA. Generally, the amount of degraded DNA correlated directly with the duration of the postmortem period. However in some cases, DNA degradation was already prominent after a short period. Case histories showed that high environmental temperature at the site of death and/or infectious diseases prior to death were the main factors for rapid autolysis. Gradual disappearance to complete loss of the long fragments (15–23 kb) was observed in DNA fingerprinting using the minisatellite probe 33.15. No extra-bands were noted, thus excluding erroneous conclusions. However, evidentiary value of older samples was lower.

Key words: DNA stability; Postmortem; Brain; Tissue (lymphatic, muscle, blood, kidney, thyroid); DNA-fingerprinting

Introduction

The detection of DNA polymorphisms considerably expands the means of discrimination. So-called 'DNA fingerprints', a banding pattern generated by hypervariable minisatellites are unique for every individual, except for monozygotic twins [1–3]. The bands segregate regularly. Therefore, familial relationship is reconstructible by comparing fingerprints of the parents or close relatives with that of a proband. Questions of identity in forensic cases often concern remains of humans who have been dead for various time periods. Data about stability of DNA and RNA is sparse [4,5]. Reports on DNA isolation from mummies of 2000 years of age [6] amplified the enthusiasm to "raise (genetically) the dead and buried" [7]. But according to

*Part of this work was presented at the 12th International Congress of the Society of Forensic Haemogenetics, 26–29.8.87, Vienna.

0379-0738/88/$03.50

investigations of Hughes et al [8], "hopes to clone the genes of the Iron Age Man" will probably not be fulfilled. Since high molecular weight DNA is considered to be prerequisite to obtain reliable RFLP-patterns, it is important to investigate the postmortem stability of DNA under various conditions. Postmortem decay of human bodies is an extremely complex and not yet fully understood process beginning with autolysis and putrefaction and is followed by aerobic and bacterial decomposition of organic material. Autolysis is a nonbacterial autodigestion by enzymes liberated from the lysosomes [9–12] whereas putrefaction is an anaerobic bacterial decomposition of proteins often accompanied by gas production. These two processes usually cannot be clearly separated and both show a maximum of activity at temperatures between 34–40 °C. Humidity of the air also influences the rate of autolysis [13]. Loss of enzyme regulation and lactic acidosis in autolysis enhances the activity of some enzymes, e.g. of the hydrolases [14]. Most of these enzymes are remarkably resistant to autolysis itself but are rapidly destroyed by bacteria. DNA in dead cells is degraded by 'nucleases', which belong to the large enzyme group of 'hydrolases'. They exist in two forms: as endonucleases and as exonucleases. The former decompose DNA by shearing it into smaller fragments whereas exonucleases detach one nucleotide after another from the terminal ends, thus gradually shortening the fragments. The fragmentation of high molecular weight DNA fragments should not lead to the non predictable appearance of extra bands in DNA fingerprints but this has yet to be demonstrated practically for a number of tissues. We investigated the yields of undegraded DNA by extraction of tissue specimens of brains, lymph nodes, liver, spleen, psoas muscle, kidneys, thyroid gland and blood of human bodies of various postmortem periods. DNA-fingerprinting was done on all samples to study possible postmortem changes of the banding patterns. Finally, preserving agents other than freezing for the storage of tissue specimen were tested in a few cases.

Material and Methods

Tissue specimen of brain cortex, lymphatic node, liver, spleen, psoas muscle, kidney, thyroid gland and blood from 23 bodies of known postmortem age were collected from autopsy-cases of the University Institute of Forensic Medicine of Zurich. The bodies were not exposed to artificial ageing, but were 'naturally' aged cases recovered in the county of Zurich. Since the amount of nuclear mass varies considerably in different organ tissues from very high in lymphatic tissue to low quantities in skeletal muscle, four blocks of 50–400 mg of tissue were separately collected. They were kept at 4 °C if handled the same day or frozen at −20 °C until homogenization in a solution of 0.9% NaCl with a mechanical homogenizer of Popper. After centrifugation of the homogenized tissue at 3000 rpm for 5 min, the supernatant was discarded, the sediment resuspended and finally

incubated overnight at 37 °C in a solution of 1 ml of 10 mM Tris—HCl (pH 7.6), 10 mM EDTA, 100 mM NaCl (pH 8) and 2% SDS, 40 mM DTT, 40 μg/ml Proteinase K [15]. DNA-extraction was done by shaking the tissue vigorously three times for 10 min in equal volumes of a 1:1 mixture of phenol and methylene chloride. The sample extraction was terminated by a 4th extraction with 1 vol. of methylene chloride for 10 min. DNA was precipitated by adding 1/10 volume of a solution of 3 M Na-acetate and 2.5 vol. of ethanol (100%) followed by freezing at −80 °C for at least 2 h. Recovery of low quantities of DNA was achieved by centrifugation of the solution at 13 000 rev./min in a Eppendorf tube for 10—15 min. After vacuum drying for 30 min, the pellets were dissolved in appropriate volumes of TE-buffer of (pH 7.6) (usually in 20—400 μl). The DNA yield was estimated semi-quantitatively on testgels for each specimen by direct comparison with ethidium-bromide stained λ-DNA-markers of 50, 100, 200 and 500 ng DNA per μl. The mean value of the estimates of the 4 values per organ and case and its standard deviation were calculated and expressed in microgram of DNA per mg of crude tissue. These values were plotted against the postmortem period for each organ tissue (Figs. 1—5).

Approximately 0.2—1 μg of DNA was cut with 20 units of Hinf I at 37 °C, incubated overnight, electrophoresed in a 22 cm 1.2% agarose gel (SIGMA II A 6877) for 48—65 h and Southern blotted onto nitrocellulose filters (Schleicher and Schuell, 0.45 μm pore size). The ^{32}P-labelled probe was prepared from the human minisatellite M13 recombinant 33.15 after insert

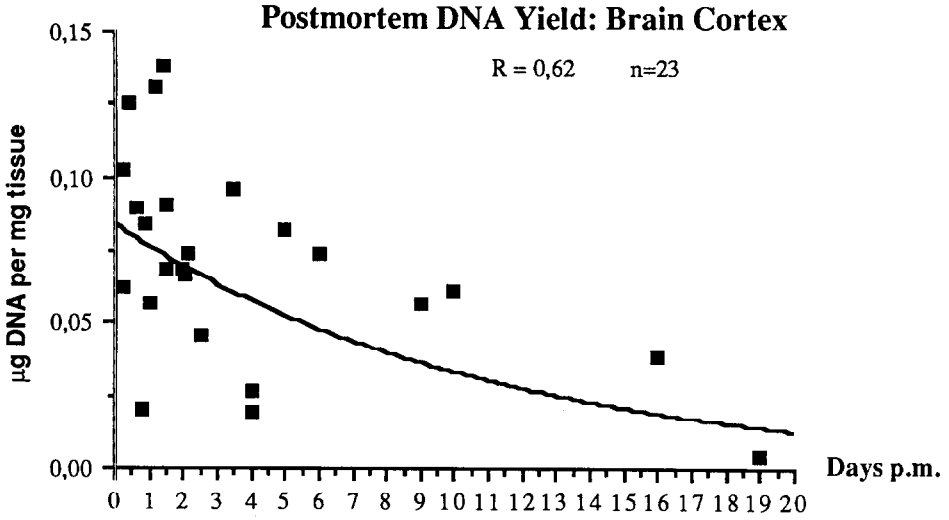


Fig. 1. Brain cortex: Yields of high molecular weight DNA of 23 cases expressed in μg DNA/mg crude tissue in relation to postmortem periods. A steady exponential decline with an acceptable correlation to the postmortem time was noted.

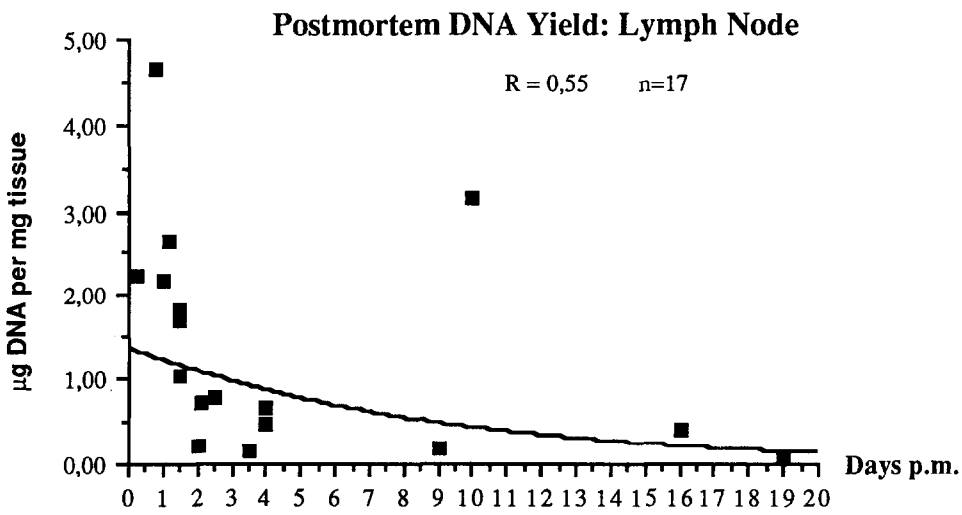


Fig. 2. Lymph nodes: Characteristics of yields of high molecular weight DNA of 17 unselected cases expressed in µg DNA/mg crude tissue in relation to postmortem periods were qualitatively similar to the ones of brain cortex, but yields were about 10–15 times higher.

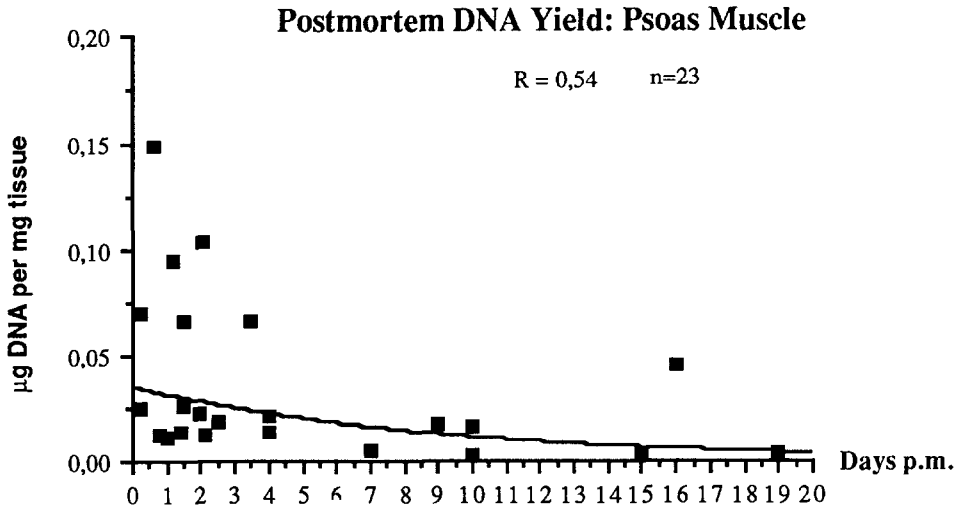


Fig. 3. Psoas muscle: Yields of high molecular weight DNA of 23 cases expressed in µg DNA/mg crude tissue in relation to postmortem periods. Yields were of the same order of magnitude as of brain.

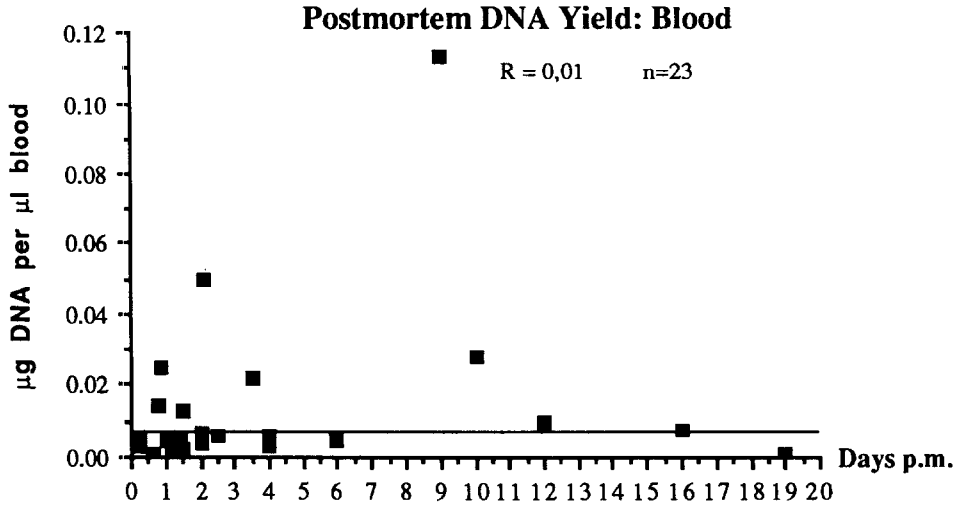


Fig. 4. Blood: Characteristics of yields of high molecular weight DNA of 23 cases expressed in µg DNA per µl blood. No correlation to the postmortem period was found because of inhomogeneous sampling due to the presence of small blood clots which were rich of high molecular weight DNA.

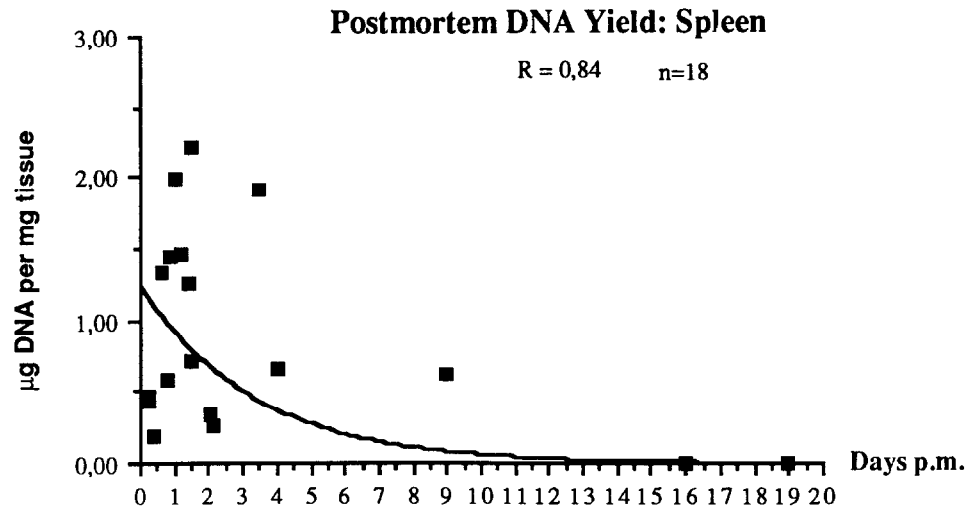
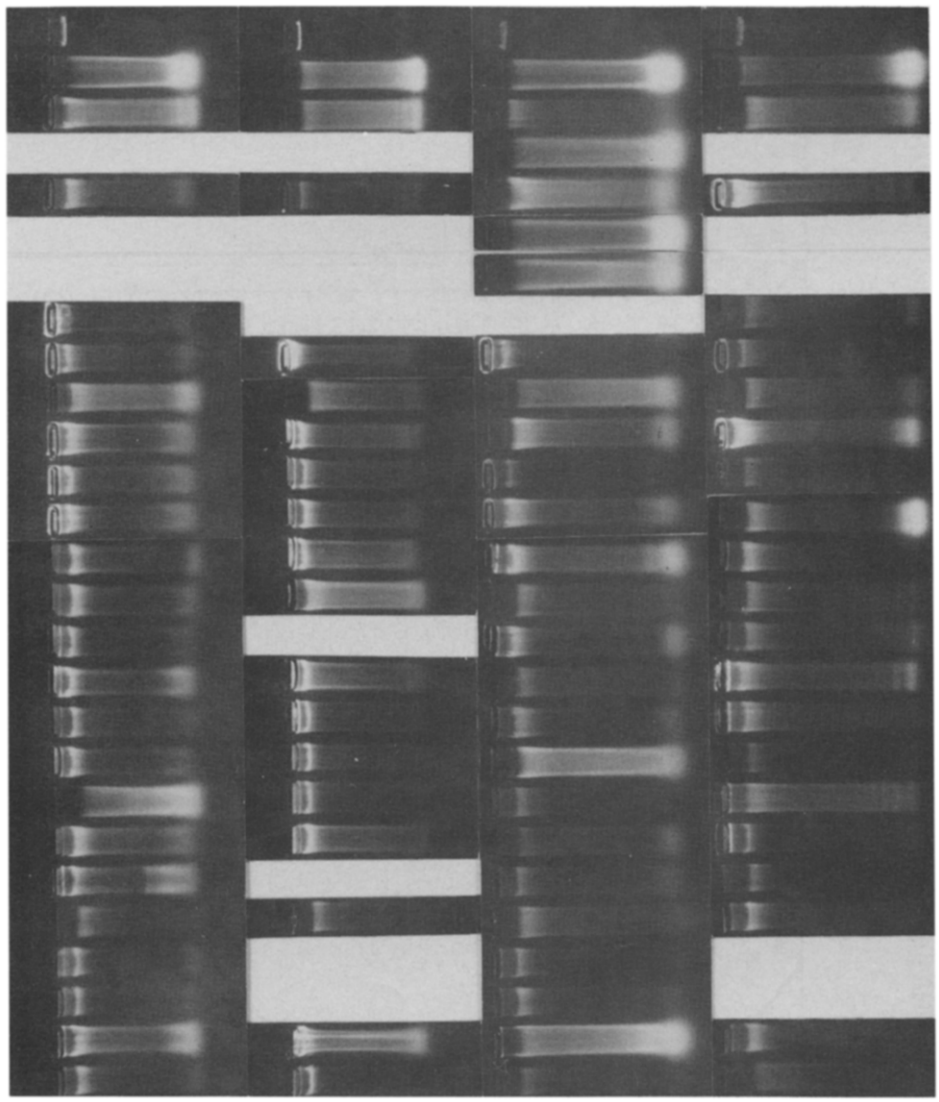


Fig. 5. Spleen: Yields of high molecular weight DNA of 18 cases expressed in µg DNA/mg crude tissue in relation to postmortem periods.


Brain Cortex
200 ng DNA
Lymph Node
200 ng DNA
Psoas Muscle
100 ng DNA
Blood
100 ng DNA

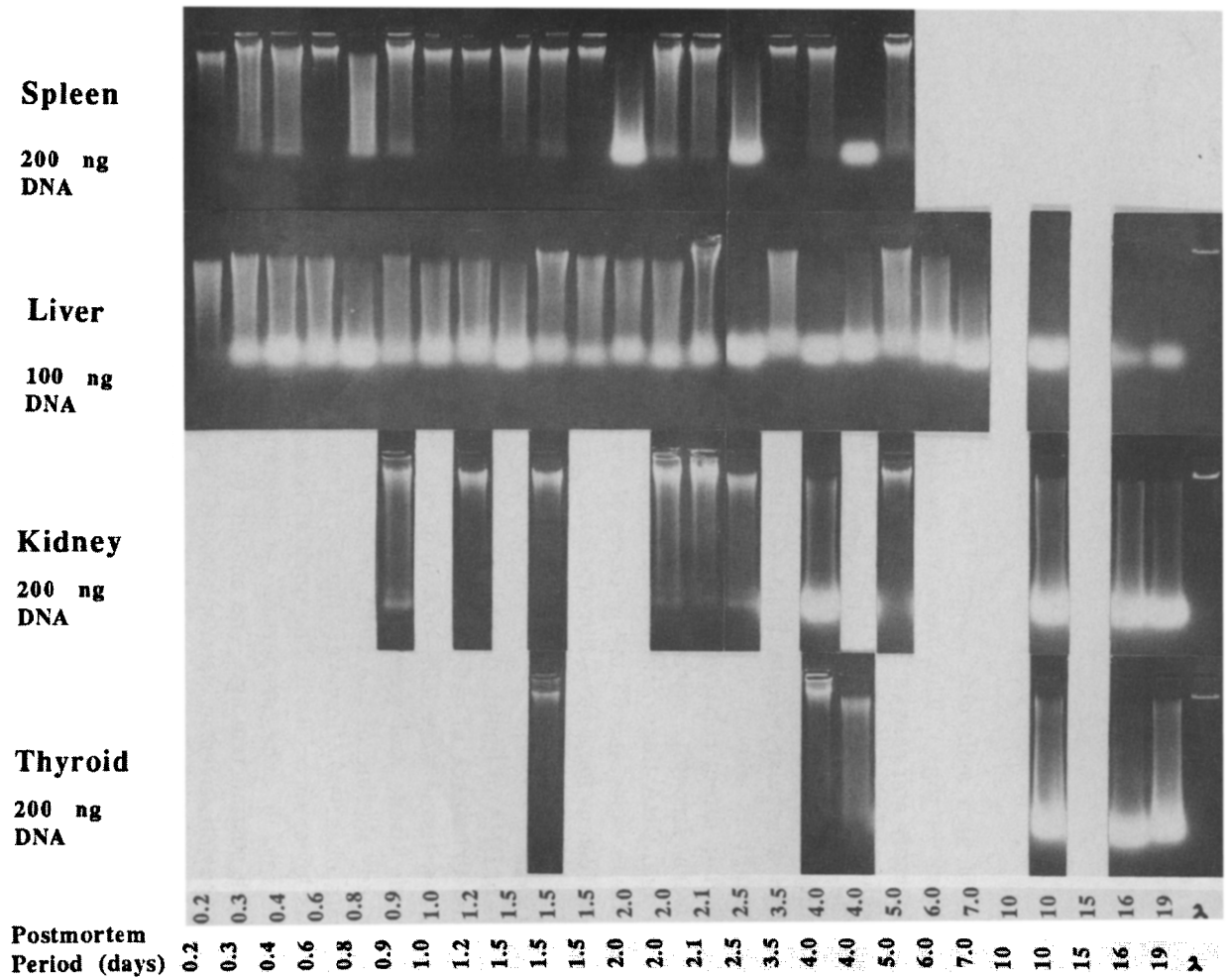


Fig. 6. The overall quantity and quality of the DNA extracted from postmortem tissue samples was conveniently evaluated on agarose gels comparing the samples with undegraded λ-DNA markers of known size. The quantitative estimates were however difficult since the transition of high- to low-molecular weight DNA was gradual. The amount of smearing DNA was correlated to the degree of DNA degradation. The portion of degraded DNA was notably higher in splenic and liver tissue, the latter already after a very short postmortem period. In general, increasing amounts of low molecular weight DNA were noticed with increasing postmortem periods of sampling. However, in some cases the spleens were devoid of high molecular weight DNA in an earlier postmortem period (2nd, 3rd, 5th and 4th day).

preparation of replicative forms (RF) by double digestion with EcoRI and HindIII and random oligolabling according to Feinberg and Vogelstein [16] using the commercially available Multiprime System of Amersham (RPN, 1601). Southern blots were prehybridized for 6 h, then hybridized overnight at 42°C in the presence of 50 μg/ml denatured salm sperm, 0.1% SDS, 6% (w/v) polyethylene glycol 6000 (Fluka), 45% formamid, 10× Denhardt's and 1 × SSC. After washing the filters with 1 × SSC for 10 min at room temperature and for 2 × 1/2 h at 65°C, they were autoradiographed for 1–5 days at −80°C using FUJI New RX or Amersham MP film and two KYOKKO-Folio PHOS-4 intensifying screens.

Results

Brain cortex

The yields of high molecular weight DNA per milligram of tissue in 23 cases are shown in Fig. 1. The mean values of DNA of the 4 extractions varied considerably and ranged from 0.14 to 0.004 μg per mg of crude tissue. A steady decline of the yields of DNA in relation to postmortem age was observed. The correlation coefficient of these two variables was R = 0.62. The overall quality of the extracted DNA, visualized as the amount of slow migrating high molecular weight DNA to faster 'smearing' DNA of lower molecular weight is shown in Fig. 6. The increasing amounts of low molecular DNA in direct relation to postmortem age was obvious although a few cases were strongly marked by DNA degradation independent of postmortem age. DNA fingerprints were obtained in all cases. About 10 mg of tissue in fresh cases and 60 mg in cases of a postmortem age of up to 3 weeks were sufficient for a DNA-fingerprint.

Lymphatic tissue

The yields of DNA of lymphatic node are shown in Fig. 2 for 17 cases.

Due to the abundance of cell nuclei in lymphatic tissue DNA yields per milligram of raw tissue were 0.09–4.6 μg of high molecular weight DNA per milligram crude tissue and were about 10–15 times higher than in brain cortex. A steady decline of the DNA yields in relation to the postmortem age was again observed. However, the correlation coefficient of these two variables was lower (R = 0.55). The qualitative aspect of the DNA extracts is shown in Fig. 6. The proportion of degraded to undegraded DNA correlated with postmortem age. The amount of 'smearing' was identical for each case to that observed in electrophoresed DNA from brain cortex. 1–10 mg of lymph node tissue contained enough DNA to obtain a DNA-fingerprint.

DNA yields per milligram of splenic tissue were about 2 times lower than those of lymph node tissue in 18 cases and varied between 0 and 2.2 μg/mg crude tissue (Fig. 5). Decrease of high molecular weight DNA was exponential with a good correlation to postmortem age (R = 0.84). In some

of the cases DNA degradation was more rapid than in lymph nodes. High molecular weight DNA was found in only small amounts after 4–5 days postmortem. However, DNA fingerprinting was still possible but the long fragments became very weak and eventually disappeared. In other cases, high molecular weight DNA was already completely missing after only 2 days. The soft consistency of the splenic tissue was pathognomonic in these cases and autolysis was usually also a prominent feature. Raw tissue (10–100 mg) were necessary to perform a DNA fingerprint with DNA from splenic tissue.

Psoas muscle

In the 23 cases, the meanvalues of the yields of 4 DNA extractions of psoas muscle are shown in Fig. 3. The yields of high molecular weight DNA per milligram of raw muscle tissue was of the same order of magnitude as the one of brain cortex and varied between 0.003 and 0.148 μg/mg. DNA stability was also similar with some rare exceptions probably caused by the differences in postmortem body cooling due to the different topographic anatomic localization of the two organs in the human body. The proportion of undegraded to degraded DNA was not generally worse than in brain. About 10 mg of tissue in fresh cases and 60 mg in cases of a postmortem age of up to 3 weeks were sufficient to produce a DNA-fingerprint.

Blood

Yields of high molecular weight DNA of the 23 postmortem blood samples varied considerably and ranged between 0.0005 and 0.12 μg/ml blood (Fig. 4). A direct correlation to postmortem age was not found (R = 0.01). DNA from postmortem blood showed a good overall-stability but samples drawn from vessels were not homogeneous. The presence of small clots considerably increased the DNA yields and was found to be one of the responsible factors for the great quantitative variations of DNA yields. Extractions from clots that were also present in decayed bodies gave very good results. DNA fingerprints – provided a sufficient DNA quantity was available – were of good quality.

Kidney

Tissue of renal cortex was extracted in 13 cases. DNA yields ranged between 0 and 0.54 μg and dropped exponentially in relation to postmortem age (R = 0.84). Degradation of high molecular weight DNA was almost total after 5 days.

Thyroid gland

Thyroid gland tissue was available for DNA extraction in only 6 cases. Yields ranged between 0 and 0.07 μg/mg of raw tissue. Characteristics of degradation followed that of kidney tissue (Fig. 6) except for 1 case which showed high molecular weight DNA 19 days postmortem.

Discussion

The discovery of DNA polymorphisms represents a novel and powerful means of identification of bodies by reconstructing familial relations. The generation of individual banding-patterns, so-called DNA-fingerprints opens new potentials to the forensic serologists. Blood, semen as well as small tissue samples contain sufficient DNA to produce DNA fingerprints provided that the recovered DNA is of high molecular weight type. However, knowledge about postmortem DNA stability in cadaverous tissues of human origin is sparse. Fascinating reports about DNA recovery from Egyptian mummies gave rise to interesting speculations, but so far only DNA fragments of short length (< 3.5 kb) were found and cloned [6–8]. Gill [17] reported on successful sex determination of degraded DNA samples of blood stains up to 4-years old in which only low molecular weight DNA (< 10 kb) was present. We performed DNA extractions of various tissue specimens of human organs of postmortem ages varying between 6 h and 19 days. We found that in principal high molecular weight DNA can be recovered in high quantities from various organs as well as from blood, however not equally well from all organs. Good DNA stability was found in brain cortex, lymph

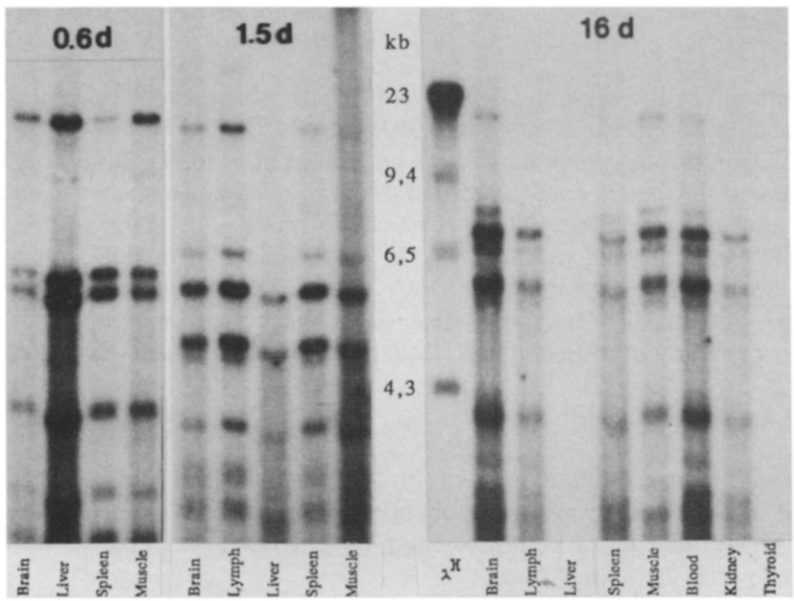


Fig. 7. DNA fingerprinting of postmortem tissue samples using the minisatellite probe 33.15 produced basically identical banding patterns for different organs. However, fading to complete loss of the longer bands was observed. This alteration could be directly related to the degree of autolysis, since organs with an early onset of autolytic alterations like liver and spleen showed fading of the long band already a few hours postmortem (0.6 and 1.5 days). The fingerprints of brain, muscle and blood were remarkably resistant whereas DNA fingerprints from liver and thyroid gave no results in a postmortem case of 16 days.

nodes, psoas muscle, and, partially in blood. Complete degradation of DNA was noted for liver tissue in a postmortem period of 24–36 h. Spleen, kidney and thyroid gland showed good DNA stability up to 5 days postmortem but after longer periods pronounced degradation was observed. Generally, the amount of degraded DNA increased proportionally to the length of the postmortem period (Fig. 6). In some cases however, DNA degradation of all organs was already prominent after a short period of time. Case histories showed that elevated environmental temperature at the site of death and/or infectious disease were the main factors for the occurrence of disproportionate autolysis. Since microbiological analysis was not performed on the tissue specimen from infectious cases, it was not possible to determine whether the advanced degradation was a consequence of bacterial growth or simply due to an elevated body temperature normally associated with infectious disease at the time of death.

DNA-fingerprinting using the minisatellite probe 33.15 was performed on all of the samples. Approximately 0.2–1 μg of DNA was used to obtain a fingerprint. Basically identical banding patterns were found for different tissues of the same individual (Fig. 7). But gradual disappearance – fading to complete loss – of the long fragments in the 15–20-kb range was noticed. This alteration could not strictly be correlated to the postmortem age of the samples but appeared to be a direct consequence of autolysis. Organs commonly showing a higher degree of autolysis like the liver, spleen and kidney sometimes showed this alteration already after a few hours postmortem. Fragmentation of DNA by nonspecific hydrolysis affected primarily the longer fragments which statistically were better 'targets' than the shorter fragments. Since this hydrolysis produced polynucleotides of shorter but – as statistical events – never of identical length, no extra bands were to be expected in a fingerprint and therefore erroneous conclusions should be avoided. However, by losing bands, the evidentiary value of the result was lower and background radioactivity increased because the random fragments – dispersed along the electrophoretic track – contained at least a few tandem-repeats and were therefore able to generate a weak hybridisation signal. Most important, cases with complete loss of high-molecular-weight DNA never showed banding-patterns after hybridisation.

Finally, storage of tissue in absolute ethanol or waterfree acetone preserved high molecular weight DNA, the yields were of comparable size as from frozen tissues and DNA-fingerprinting was always possible. However, DNA was not extractable from tissue stored in a 4% formalin solution because non-buffered formalin was used. Goelz et al. [18] showed that DNA degradation was important in tissue kept in non-buffered formalin, but good preservation was noted for paraffin embedded tissue priorly stored in buffered formalin. If correctly preserved, important quantities of postmortem tissue specimen would therefore be available for different research purposes using DNA technology.

Acknowledgements

The probe 33.15 is the subject of patent applications and commercial enquiries regarding this probe should be directed to ICI Diagnostics, Gadbrook Park, Rudheath, Northwick, Cheshire CW9 7RA, England. A. Kratzer was supported by the EMDO Stiftung, Zürich.

References

1 A.J. Jeffreys, V. Wilson and S.L. Thein, Hypervariable 'minisatellite' regions in human DNA. *Nature*, 314 (1985a) 67–73.
2 A.J. Jeffreys, V. Wilson and S.L. Thein, Individual-specific 'fingerprints' of human DNA. *Nature*, 316 (1985b) 76–79.
3 A.J. Jeffreys, J.F.Y. Brookfield and R. Semeonoff, Positive identification of an immigation test-case using human DNA fingerprints. *Nature*, 317 (1985c) 818–819.
4 T.L. Wood, G.D. Frantz, J.H. Menkes and A.J. Tobin, Regional distribution of messenger RNAs in postmortem human brain. *J. Neurosci. Res.*, 16 (1986) 311–324.
5 L. Madisen, D.I. Hoar, C.D. Holroyd, M. Crisp and M.E. Hodes, DNA banking: The effects of storage of blood and isolated DNA on the integrity of DNA. *Am. J. Med. Genet.*, 27 (1987) 379–390.
6 S. Pääbo, Molecular cloning of ancient egyptian mummy DNA. *Naure*, 314 (1985) 644–645.
7 A.J. Jeffreys, Raising the dead and buried. *Nature*, 312 (1984) 198.
8 Margaret A. Hughes and D.S. Jones, Body in the bog but no DNA. *Nature*, 323 (1986) 208.
9 G. Strassmann, Über Leichenveränderungen, autolytische, Fäulnis- und Verwesungsvorgänge. *Dtsch. Z. gerichtl. Med.*, 3 (1924) 359–371.
10 O. Neubauer, Abbau von Gewebseiweissen (einschl. der Autolyse). *Hdb norm u path Physiol* Bd 5, Springer-Verlag, Berlin, 1928, p 721.
11 E. Müller, Der Zelltod. *Hdb Allg Path* II/1, H.W. Altmann, Springer-Verlag, Berlin, Göttingen, Heidelberg, 1955, p 613.
12 W. Gössner, Untersuchungen über das Verhalten der Phosphatasen und Esterasen während der Autolyse. *Virchows Arch.*, 327 (1955) 304.
13 F.P. Mackie, The microscopical changes occurring in organs after death. *Ind. J. Med. Res.*, 16 (1929) 827.
14 H.C. Bradley, Autolysis and atrophy. *Physiol. Rev.*, 18 (1938) 173.
15 P. Gill, A.J. Jeffreys and D.J. Werret, Forensic application of DNA 'fingerprints'. *Nature*, 318 (1985) 577–79.
16 A.P. Feinberg and B. Vogelstein, A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.*, 132 (1983) 6–13.
17 P. Gill, A new method for sex determination of the donor of forensic samples using a recombinant DNA probe. *Electrophoresis*, 8 (1987) 35–38.
18 S.E. Goelz, S.R. Hamilton and B. Vogelstein, Purification of DNA from formaldehyde fixed and paraffin embedded human tissue. *Biochem. Biophys. Res. Commun.*, 130 (1985) 118–126.