Technical methods

Demonstration of myocardial infarction in putrefying bodies

G G P FECHNER, S SIVALOGANATHAN *University Department of Forensic Medicine, University of Leeds, St James's University Hospital, Leeds*

The demonstration of myocardial infarction becomes more difficult with increasing time after death. In morbid anatomy and forensic medicine in particular, it is not uncommon to encounter heavily putrefied bodies where the apparent cause of death is myocardial infarction. In these cases diagnosis is usually presumed because it cannot be proved.

Carle noted fluorescence of necrotic fibres in histological sections of myocardial infarcts stained with haematoxylin and eosin.¹ Siegal and Fishbein² and Al-Rufaie *et al*³ used this technique for the detection of early myocardial infarctions. We have used this technique to show established myocardial infarction in putrefying hearts.

Material and method

Samples from 10 hearts with macroscopical evidence of recent infarction were obtained from coroner's necropsies carried out in the Department of Forensic Medicine, University of Leeds. Samples from five normal hearts were used as controls.

Histological blocks were taken soon after necropsy, and the rest of each specimen was placed in an incubator at 20°C. Blocks were taken from each specimen daily for two weeks. The blocks were fixed in buffered 10% neutral formalin and embedded in paraffin. Sections (5 µm) were cut from each block and stained by the standard haematoxylin and eosin method. The stained sections were then examined using a Leitz epifluorescence microscope illuminated by a high pressure mercury lamp and H2 filter to provide blue light of 390-490 nm wavelength. This method was also used in putrefied bodies where myocardial infarction was thought to have been the cause of death.

Results

The areas of coagulative necrosis of infarction in the fresh specimens were easily recognisable. These areas appeared as yellow fluorescence under the

fluorescence microscope, with accentuation of the aggregations of necrotic contractile bands. The normal myocardial fibres had a background of light brown fluorescence (fig 1).

After two or three days in most cases the muscle fibres were disorganised, with fragmentation and segmentation of the myofibrils in all areas, both normal and infarcted. This made demonstration of the infarcted areas by means of light microscopy difficult. The areas of infarction were shown, however easily, under the fluorescence microscope by the yellow fluorescence. With increasing interval after death the background fluorescence of the normal fibres changed from the light brown colour to a yellowish colour, but the difference in intensity between the infarcted areas and the putrefying normal areas was still discernible (fig 2). Between seven and 12 days after death the difference between the normal and infarcted areas disappeared except for the aggre-

Fig 1 Bright yellow fluorescence of the myocardial infarction area one day after death.

Fig 2 Infarction area five days after death.

Fig 3 Infarction area (A) of the heart from a putrefied body.

gations of contractile bands, which retained their accentuated fluorescence. The control hearts did not show these features of differential fluorescence.

The technique was subsequently used in putrefied bodies. In the presence of moderate putrefaction it showed areas of infarcts. An illustrative case is described.

An 82 year old man was found dead in the middle of June, a few days after he was last seen alive. The body was moderately decomposed, as were the organs. Internal examination showed an enlarged heart, which weighed 500 g, with triple vessel disease with segmental calcification. The right coronary artery was completely occluded by what appeared to be a recent thrombus. The myocardium was hypertrophied and showed disseminated fibrosis. No evidence of recent infarction was discernible in the putrefied heart either macroscopically or microscopically. The fluorescence technique, however, showed a definite area of fluorescence in the posteroseptal region (fig 3), although the contrast was not as prominent.

Discussion

There does not seem to have been any systematic work on the demonstration of myocardial infarction in decomposed bodies. While working on the demonstration of early myocardial infarction Sahai and Knight commented that the acridine orange method appeared to be reliable for two or three days after death in warm weather and longer in refrigerated cadavers.⁴ Knight stated that the microenzyme staining of malate dehydrogenase could show early infarction in refrigerated cadavers four to five days after death.⁵ Zollinger used chromotropeaniline blue staining, but found that false negative results could not be excluded with a long interval after death.⁶ Rajs and Jakobsson found that autolysis made the haematoxylin basic fuchsin picric staining method unreliable.⁷

We believe that the fluorescence of haematoxylin and eosin stained sections is a useful way to demonstrate myocardial infarction in mild to moderately decomposed bodies.

References

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Requests for reprints to: Dr G Fechner, Department of Forensic Medicine, Clinical Sciences Building, St James's University Hospital, Beckett Street, Leeds LS9 7TF, England.

Linear track scanning of pathological tissue sections: an interdisciplinary approach to microscopy

J D DAVIES, C C JEAL, A R HOLTON *Department of Pathology, University of Bristol, Bristol BS8 1TD, and Department of Chemical Pathology, Bristol Royal Infirmary, Bristol*

Quantitative assessment of microscopical images may be achieved by manual point counting or more sophisticated stereology,^{1,2} dedicated microdensitometry, or automated image analysis.³ All these methods are either laborious or expensive.

Many changes in biological tissues are not homogeneous but exhibit a distinct polarity. The method described here aims to analyse such anisotropic variation in tissues. It is cheap and simple and gives both qualitative and quantitative evaluation of the optical density of constituents from linear tracks of sectioned tissue. The method entails the use of a photomicroscope and an integrating densitometer, which is designed primarily for the analysis of stained electrophoretic agarose gel tracks of proteins.

The equipment or its equivalent is readily available in most histopathological and chemical pathology laboratories, and thus the method requires no additional capital expenditure. In tightening economic circumstances these are considerable virtues. To illustrate the method we describe an examination of lymph nodes, but clearly there are much wider applications.

Material and methods

Twenty four lymph nodes from cats⁴ and humans⁵ were fixed in 10% aqueous formaldehyde, processed through alcohols and toluene, and then embedded in paraffin wax. Sections of 5 µm depth were cut on a rotary microtome, stained with Harris's haematoxylin and eosin, dehydrated, and mounted under glass coverslips in DPX.

The stained sections were photographed using a Zeiss Ultraphot III B (West Germany) with a 12V 100W tungsten halogen lamp running at 12V (3200/K) and Luminar macro lenses, taking care to use apertures giving even illumination of the whole field. Filtration was unnecessary with the film used, which was Kodak Super Speed 2751 duplicating film. This is a high contrast orthochromatic direct reversal film primarily intended for black and white line duplication. The film was cut to half plate, or 4 × 5 inch size (10.2 × 12.7 cm). Exposures were equivalent to minus 4.5 DIN. The film was thereafter developed (using Kodak Dektomatic diluted 1 in 4 for 2 minutes at 27°C) and fixed (using May and Baker Fixaplast diluted 1 in 3 for 1 minute at 30°C) in an Intercop 1614 VS processor and then washed at 20–25°C for 20 minutes.

Fig 1 Diagram showing a series of possible longitudinal tracks parallel to the long axis of a lymph node.