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VARIABILITY OF HAEMATOXYLIN — BASIC FUCHSIN — PICRIC ACID (HBFP) STAINING OF INFARCTED MYOCARDIUM DUE TO TISSUE STORAGE

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Early myocardial infarction is usually detected grossly by utilising the nitro-blue tetrazolium (NBT) technique.[1] The normal areas stain dark blue and infarcted areas which lack the dehydrogenase enzyme remain unstained. In 1971, Lie *et al*[2] described a non-enzymatic histochemical method, the haematoxylin-basic fuchsin-picric acid (HBFP) technique, for demonstrating early stages of myocardial infarction. They found positive staining of infarcted myocardium from dogs as early as 30 minutes after coronary artery ligation and positive staining of infarcted human hearts as early as 6 hours following fatal coronary occlusion.

The HBFP technique is sensitive to early myocardial changes and can be carried out on formalin-fixed paraffin-embedded tissue. Although technically much care is required, it provides a vivid contrast between infarcted and normal fibres. There are several factors believed to affect HBFP positivity,[3] such as aging of unstained sections in room air,[4] and the precise timing of the differentiation step of this stain. This paper reports the results of the investigation of some of these factors.

MATERIALS AND METHODS

Myocardial infarction was experimentally produced in a dog by ligating the anterior descending branch of the left coronary artery. After 24 hours, the dog was killed by intravenous Nembutal and the heart removed immediately and fixed in 10% buffered formalin. Tissue taken from the area of infarction was routinely processed and embedded in paraffin. Normal heart muscle from another dog was used as a negative control.

Investigations were carried out to determine whether the following factors affected HBFP staining:

(1) storage of (a) unstained tissue sections and (b) blocked (paraffin embedded) tissue.

(2) drying of unstained sections at 60°C as compared to room temperature (approximately 28°C).

(3) The effect of storage and drying on the timing of the differentiation step was also studied.

1a. Effect of storage of tissue sections

Multiple sections were cut from paraffin blocks of infarcted heart muscle of the experimental dog and normal heart muscle of the control dog, and mounted on clean glass slides. They were stored at room temperature. After various time intervals of 1 day, 3 days, 1 week, 2 weeks, 3 weeks, 5 weeks and 6 weeks, sections were stained with HBFP and examined microscopically.

1b. Effect of storage of blocked tissue

Blocked material from infarcted and normal hearts were stored at room temparature. After the same time intervals as in Experiment 1a, sections were cut, stained with HBFP and compared with Experiment 1a.

2 The effect of drying at 60°C as compared to room temperature

The procedure of Experiment 1a was repeated with the difference that the tissue sections were stored at 60°C. Findings from the microscopical examination of the sections were compared with those from Experiment 1a.

3 Timing of the differentiation step

Sections were cut from blocks of infarcted and normal hearts and mounted on glass slides. They were divided into two batches. Both batches were stored for 10 days with one batch at room temperature and the other at 60°C. The HBFP method was performed as described by Lie *et al*.[2] The differentiation time with picric acid/acetone was varied from 5 to 30 seconds. The optimum differentiation time for freshly cut sections was found to be 15 seconds, and the differentia-

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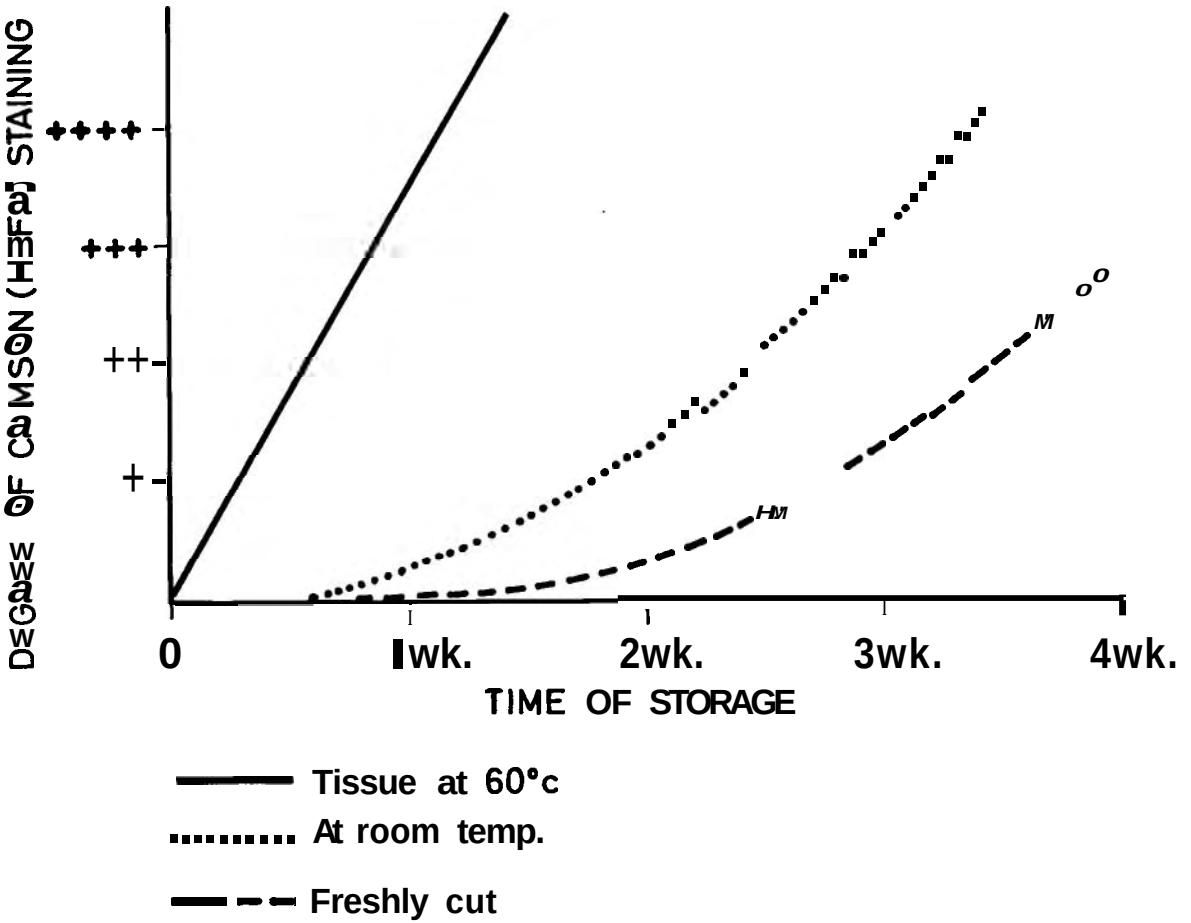

Fig 1. Variability of HBFP staining in relation to temperature and duration of storage

tion time of stored and dried sections were compared with this.

RESULTS

The findings of this study are summarised in Fig. 1. Freshly cut sections stained positively crimson only in the infarcted areas whereas sections which have been stored, irrespective of temperature, showed crimson staining of non-infarcted tissue as well. The extent of the positively staining areas also increased with increasing time duration of storage while freshly cut sections stained consistently. After two weeks there was a marked difference in the extent of staining between freshly cut sections and sections kept at room temperature indicating that stored sections had an unacceptable amount of false positive staining. Nevertheless, even freshly cut sections had an unusual increase in the extent of crimson staining after 4 weeks of storage of the tissue block, showing that storage of paraffin blocks also resulted in false positive staining, although to a lesser degree compared to stored tissue sections. It also became obvious very early in the experiment that drying at 60°C caused severe false positive staining and must be avoided.

In the timing study, it was noticed that after 10 days storage of sections, a longer differentiation time was necessary to obtain proper staining, compared to the time required when sections were freshly cut, showing that stored sections exhibited an increase amount of artifactitious background staining. The differentiation time was exceptionally longer when stored sections were, in addition, dried at 60°C.

DISCUSSION

This study showed that increasingly larger areas of myocardial tissue stained red (positively) with HBFP with longer periods of storage. It also took a much longer time for differentiation to remove the basic fuchsin dye from the normal areas. The differentiation step was critical, and could lead to either false positive or false negative staining. The implications of these observations are obvious when one realises that storage of tissue blocks and sections are common occurrences in the histopathological laboratory. In order to overcome the marked variability in HBFP staining that occurs with storage, it is important that both positive and negative controls accompany HBFP staining of tissue sections in histopathological practice. Drying at 60°C should be avoided.

The biochemical basis of the HBFP positive staining of infarcted tissue and the increasing positivity of stored sections is unknown. Lie *et al*[2] believe that the substance responsible for HBFP affinity is an unstable protein or protein-phospholipid complex. The HBFP stain must be considered empirical at present. Though the technical requirements may be rigorous, the method gives very precise, lucid staining if the procedure is strictly followed. The acetone/picric acid differentiation is the major factor affecting positivity or negativity. Other factors believed to affect HBFP staining include reagent freshness, timing of decolorisation, aging of unstained sections, thickness of muscle sections, tissue crushing or cutting artefacts and longitudinal versus cross-section fibres.

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