FIG. 1. Red-cell creatine by the present method versus automated method. The line represents equal values by both methods.

The recovery of creatine added to red cells was determined by the manual method. In 15 experiments creatine was added to red cells: this could only be done by adding creatine to the water into which the cells were delivered before deproteinization. When 5 to 50 mg% of creatine was added to red cells in this way, 83% to 93% of the added creatine was recovered.

The observation of Ennor and Stocken (1948) that in the case of biological material containing thiol groups the colour reaction between creatine and diacetyl-l-naphthol is partially inhibited was confirmed in the case of red cells; this inhibitory effect may be as much as 20% and is abolished by the use of *p*-chloromercuribenzoate.

Red cell creatine in normal females was found by Griffiths and Fitzpatrick (1967) to have a mean value of 5.6 ($SD \pm 1.3$) mg% and in normal males 4.4 ($SD \pm 1.5$) mg%. These mean values are in agreement with those of Kurohara (1965), but in each group the spread is much greater than reported by this worker.

The higher values for normal females were found to be statistically significant. It is of interest that Hunter and Campbell (1918), in what is probably the earliest and, until recently, almost the only work on red cell creatine, also found this sex difference.

In subjects exhibiting increased erythropoiesis, such as those responding to treatment for anaemia, values for red cell creatine up to 50 mg% may be found.

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Method for obtaining both frozen and paraffin sections from the same liver biopsy

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Liver biopsy is now an established diagnostic technique (Sherlock, 1962). Tissue obtained by this method also provides valuable specimens for histochemical investigation. For diagnostic purposes formalin-fixed, paraffin-embedded material is commonly used, whereas many histochemical techniques, especially those demonstrating enzyme activities, require fixed or unfixed frozen sections. The division of a small needle biopsy specimen into two or more parts, however, accentuates the already critical problem of sampling and raises ethical problems because subtracting from the material to be embedded in paraffin may hinder the patient's diagnosis. It was therefore thought necessary to develop a technique for obtaining both frozen and paraffin sections from the total specimen.

MATERIAL

Eighty-five needle biopsies and 13 operative wedge biopsies of liver were studied. The patients suffered from a wide range of hepatic and non-hepatic diseases, and eight of the livers were normal.

METHOD

Depending on the histochemical requirements, the specimen, shortly after removal from the patient and preferably at the patient's bedside, is placed in an appropriate fixative or rapidly frozen as follows. The whole needle specimen, or a slice of a wedge biopsy 0.2 to 0.3 cm thick, is placed on a moistened 0.4 cm thick piece of 8% gelatin approximately 1 cm across. This in turn is placed on a piece of wet cork of similar thickness and slightly larger than the gelatin. The cork provides a convenient base for handling the specimen and for storage. A few drops of water are now put on a metal microtome chuck, and cork, gelatin, and specimen are placed on top of the water. The chuck is grasped with tongs and immersed, together with the specimen, in a hexane-dry ice mixture (Fig. 1). When the specimen is

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FIG. 1. The microtome chuck, cork, gelatin and specimen are quenched in a hexane-dry ice mixture.

frozen, in less than a minute, chuck and specimen are put into the cryostat chamber for 10 to 15 minutes to allow the temperature of the specimen to rise to that of the cryostat.

Alternatively, if a rapid freezing device is available, the cork, bearing gelatin and specimen, is speared with a scalpel and immersed in the hexane-dry ice mixture. A few drops of water are put on the chuck and the latter is cooled separately by means of the rapid freezing device. The cork is now placed on top of the chuck as the water begins to freeze.

The same rapid freezing procedures can be carried out if desired on previously fixed tissue.

When the temperature of the specimen has reached equilibrium with the cryostat temperature the required number of cryostat sections is cut. The frozen tissue with at least part of the gelatin is cut off the cork and allowed to thaw in formol saline at 4°C for at least four hours. Routine dehydration, clearing, and paraffin embedding are now carried out.

Unused frozen sections can be stored in the cold on slides or cover slips, sealed in plastic. If paraffin sections are not needed the cork can be cut off the chuck and used as a vehicle for storing the biopsy in a deep freeze. The remaining tissue can also be used for chemical studies.

COMMENT

By means of the above technique virtually the same tissue provides both frozen and paraffin sections. Routine diagnostic as well as special histochemical procedures can thus conveniently be performed on the biopsy. The rapid cooling and subsequent thawing in formol saline ensures that distortion of the specimen is kept to a minimum. Satisfactory results were obtained when the paraffin sections were stained with haematoxylin and eosin, by Perls' method for iron, and by the periodic acid-Schiff method, and when reticulin fibres were impregnated with silver by the method of Gordon and Sweet. Comparison of frozen and paraffin sections in cases with cholestasis showed that a considerable loss of bile pigments had occurred in the paraffin sections as compared with the frozen ones.

Although the above study was made on liver biopsies it is assumed that the technique is applicable to other tissues.

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