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Three Dimensional Reconstruction of Histological Sections from the Region of the Porta Hepats of a 11 Week Human Foetus

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INTRODUCTION

The biliary tree forms an intricate system of drainage ducts through which bile is transported from the liver to the small intestine. Essentially it consists of two parts: the intrahepatic (within the liver) and the extrahepatic (outside the liver). The biliary tree literally resembles the tributaries of a river, where smaller ducts join to form larger and larger ducts, the smallest of which is in contact with the liver cell, while the largest drains into the small intestine. Bile, which is produced by the liver cell flows through this intricate system of ducts to reach the small intestine.

The development of the biliary system in the foetus has fascinated embryologists for many years. It has been suggested that the intrahepatic and extrahepatic biliary system develop separately and connect at the porta hepatis region of the liver. However more recent evidence suggests that the intrahepatic and extrahepatic systems are in communication throughout gestation. Through an active remodelling process certain intrahepatic ducts are selected for development while the others are deleted. This process occurs at the porta hepatis region of the liver between 11 and 13 weeks of gestation and leads to the creation of an effective biliary drainage system. Errors in this remodelling process may be the cause of the condition called biliary atresia which results in improper biliary drainage, jaundice and later liver fibrosis in infants.

The porta hepatis is the region of the liver where the extrahepatic biliary tree ceases and continues as the intrahepatic. As this is a very important region in its development, we performed 3 dimensional reconstruction of the biliary tree in this region. As our work mainly involves histological techniques, in this project we have used images from such techniques to perform 3 dimensional reconstructions.

Three dimensional reconstruction of histological sections poses problems that do not arise with images of tomograms. One of the main problems is to align the sections so that the structures fall one behind the other in a natural order. Another difficulty is differences in staining intensity which affect the brightness of the images. The aim of this project is to study the feasibility of using simple serial, histological sections to construct a 3 dimensional image that will show the true situation within the organ.

MATERIALS

The paraffin embedded whole liver of an 11 week normal human foetus was serially sectioned at a thickness of 5 μ. The porta hepatis region was identified and every third section was routinely stained with hematoxylin and eosin. Thirty nine sections were included in the 3 dimensional reconstruction covering an area of 585 μ.

IMAGE PROCESSING

The sections were viewed under a Carl Zeiss (Germany) AXIOPHOT upright microscope. Images were obtained through a PROGRESS 3012 camera using the PROGRESS software option.

Alignment was done using a RGB monitor connected to the VIOB output of the IBAS 2.5 system (Kontron Elektronik, Germany). In order to align an image, the preceding image was converted to overlay and the current image aligned according to the overlay. Alignment was done at low magnifications of 2.5 using the outline and other clearly

visible structures in the image as references. Images were obtained at a magnification of 5. Eight bit images were obtained at a size of 1024 X 1024 and stored for later use.

After the alignment all further work with the images was done on the HDD monitor (FLEXSCAN F760i.W). Working with the IBAS, the images were reduced to a size of 512 X 512. Uneven intensities in the images were observed due to differences in staining which were corrected in by using the function normalize. The ducts in the biliary system were selected manually using the eraseoutside function in each image. The images thus obtained by selecting the ducts was stored as a separate sequence. As the stain used did not preferentially stain the ducts there was no difference in grey values between the ducts and other structures. In order to create the difference, the original images and the images of ducts alone were scaled differently and combined as in the following macro:

```
for i=1; i<40; i=i+1                    #i=image number
eraseoutside i, i+40, 300, 0            #selects the ducts
subtract 300, i, i+80, 2                #removes ducts from the
                                          original image
scalim i+80, i+120, 1,255,1,170,1,170,1
                                        #scales background to
                                          lower grey levels
scalim i+40, i+160, 1,225, 200, 255, 200, 255, 1
                                        #scales the ducts to
                                          higher grey levels
orim i+160, i+120, i                    #combines the two
                                          showing a difference
                                          in grey values between
                                          the ducts and other
                                          structures
```

THREE DIMENSIONAL RECONSTRUCTION

Mipron system version 2.0 (running on the Ibas computer) (Kontron Elektronik, Germany) was used for 3 dimentional work. Thirty nine images were used for the reconstructions shown in figures 1 and 2. Voxel size of the images were 1.08 μ in the X, 1.04μ in the Y and 15μ in the Z direction.

Surface distance shading was used for the images containing only the ducts without the background structures.(Fig. 1) The following parameters were used for the reconstruction:

Rotation X: 0.0 Increment 5.0
Rotation Y: 0.0 Increment 20.0
Rotation Z: 0.0 Increment 0.0
view Point: 1000
Grey Threshold: 100-255

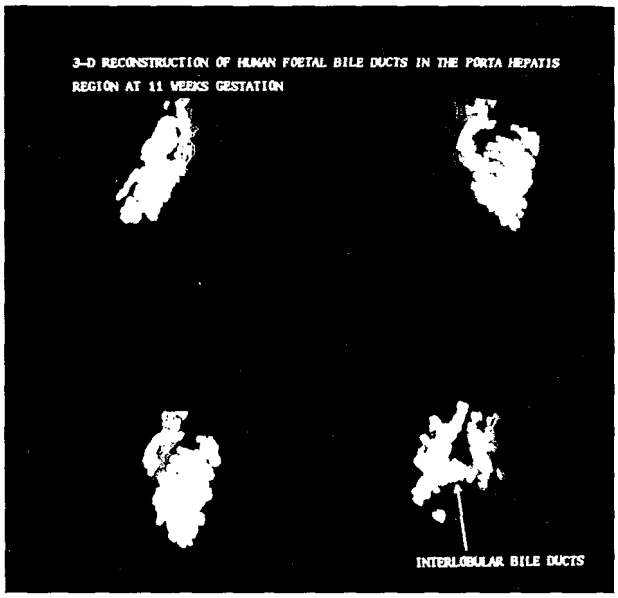

Fig. 1.

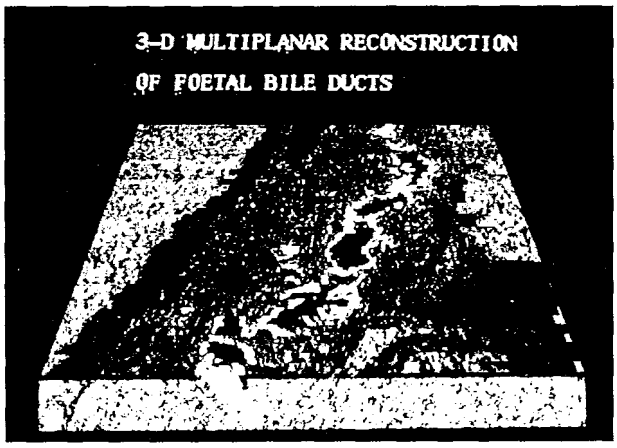

Fig. 2.

Output Resolution: 1.08

The 4 images in a clockwise direction from top left, show the following rotations:

1: X 0, Y 0,Z 0
2: X 10, Y 40, Z 0
3: X 15, Y 60, Z 0
4: X 50, Y 200, Z 0

Using the images which were created to show a grey level difference between the ducts and background structures multiplanar reconstruction was done using the same parameters as above. (Fig. 2) A cutplane has been used to show the continuity in

the lumen of the biliary system which is not seen in the other planes.

CONCLUSION

The 3 dimemsional reconstruction of this image sequence shows that by using very simple histological techniques in serial sections it is possible to study the behaviour of the object of interest in the real three dimensional situation. In this series we were able to show that many of the ducts led to dead ends, while one continued, maintaining an open tract from the first to the last section. Extension of the reconstruction in either direction, which we are currently pursuing, will add to the picture and provide useful information.

REFERENCES

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