NOTE

Histochemical and Immunoperoxidase Stains and Ultrastructure of Hepatitis B Surface Antigen Visualised in a Conventional Haematoxylin and Eosin Section: A Comparative Study*

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Hepatitis B surface antigen (HBsAg) can sometimes be visualised in conventional haematoxylin and eosin (H and E) stained liver tissue sections.[1-3] Cytoplasmic HBsAg in "ground-glass' hepatocytes of carriers has been described.[4] The present report describes a comparative study made on various histochemical and immunoperoxidase stains and the ultrastructure of certain morphologic forms of HBsAg which could be appreciated in the routine H and E stain.

MATERIALS AND METHODS

The materials consisted of paraffin blocks of formalin-fixed liver tissue collected at autopsy from 125 cases with primary hepatocellular carcinoma (HCC) which were found among 7,457 necropsies performed during the years 1969 to 1978 by the Department of Pathology. One-hundred tumours were associated with hepatic cirrhosis, three with cirrhosis and opisthorchiasis, and two with opisthorchiasis. The majority of cirrhosis cases were of the macronodular type. Patients ranged in age from 4 to 79 years, with a mean age of 46.3 years. There were 111 males and 14 females. One hundred and seventeen patients were Thai; seven, Chinese; and one, Burmese.

Haematoxylin and eosin-stained sections from all the cases were evaluated. Both liver and tumoral tissues were examined. The sections were studied microscopically for the presence of HBsAg in the cytoplasm of non-neoplastic liver cells as revealed upon routine H and E staining. Two cases of HCC associated with cirrhosis, in which HBsAg was visualised in the conventional H and E sections, were found.

Three-micron serial sections were cut from the paraffin blocks of the two cases. One of them stained with H and E. The remaining sections stained with orcein,[5] Gomori's aldehyde fuchsin,[6] chromotrope aniline blue,[7] Verhoeff-van Gieson's,[8] Masson's trichrome,[9] Macchiavello's,[10] and periodic acid-Schiff's (PAS) with and without diastase.[11] The presence of antigen was confirmed by direct immunoperoxidase stain.[12] Sections stained with H and E after examination and photography were decolorised and then stained using orcein technique. The stains were also performed for comparison in a case of chronic active hepatitis and a case of HCC with numerous HBsAg-positive ground-glass hepatocytes. Again, three-micron serial sections were cut from the paraffin blocks of formalin-fixed liver tissue obtained by surgical biopsy of the last two cases.

The antigen visualised in the routine H and E was studied by electron microscopy using material

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already blocked in paraffin.[13,14] HBsAg-positive ground-glass hepatocytes were also studied by electron microscopy.

RESULTS

Comparison of the sections stained with H and E and with orcein after decolorisation revealed that HBsAg could be visualised in the conventional H and E stain (Figs. 1 and 2). The presence of HBsAg was also confirmed by immunoperoxidase stain (Fig. 3). The antigen visualised in the H and E appeared as dense, purplish red inclusions in the cytoplasm of non-neoplastic hepatocytes. The inclusions were large and clearly outlined and they occupied a large portion of the cytoplasm. They were easily identified (Figs. 1A and 2A).

The results of various histochemical stains for HBsAg seen in H and E compared with those of HBsAg-positive ground-glass hepatocytes are shown in Table 1. The antigen visualised in H and E stained intensely with most of the stains, whereas HBsAg in the ground-glass cells revealed moderate or weak intensity of staining (Fig. 4).

Gomori's aldehyde fuchsin and Macchiavello's technique were found to be more sensitive than other stains. Using these methods, HBsAg visualised in H and E stained deeply. The antigen appeared as dense, clearly outlined, cytoplasmic inclusions surrounded by pale – staining cytoplasm when stained with Gomori's aldehyde fuchsin and it appeared to be surrounded by darker cytoplasm when stained using Macchiavello's technique (Fig. 5). The antigen appearing as ground-glass stained moderately with Gomori's aldehyde fuchsin (Fig. 4B) and lightly to moderately with Macchiavello's.

Viewed through the electron-microscope, the antigen seen in H and E showed more electron density than that in the ground-glass cells as shown in the electron-micro-

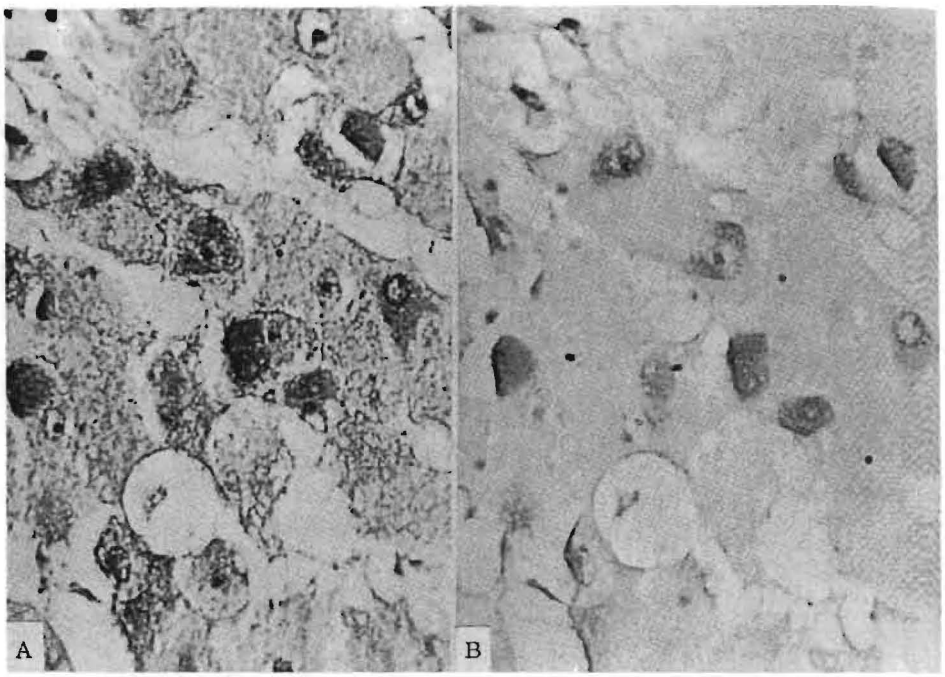

Fig. 1 HBsAg visualised in a conventional haematoxylin and eosin preparation: (A) HBsAg appearing as dense inclusions in the cytoplasm of non-neoplastic liver cells from case 1 (H and E stain, x450); (B) orcein staining of the same section illustrated in (A), performed after decolorisation of the H and E stain. The antigen inclusions are clearly seen presenting identical location and morphology as in (A) (x450).

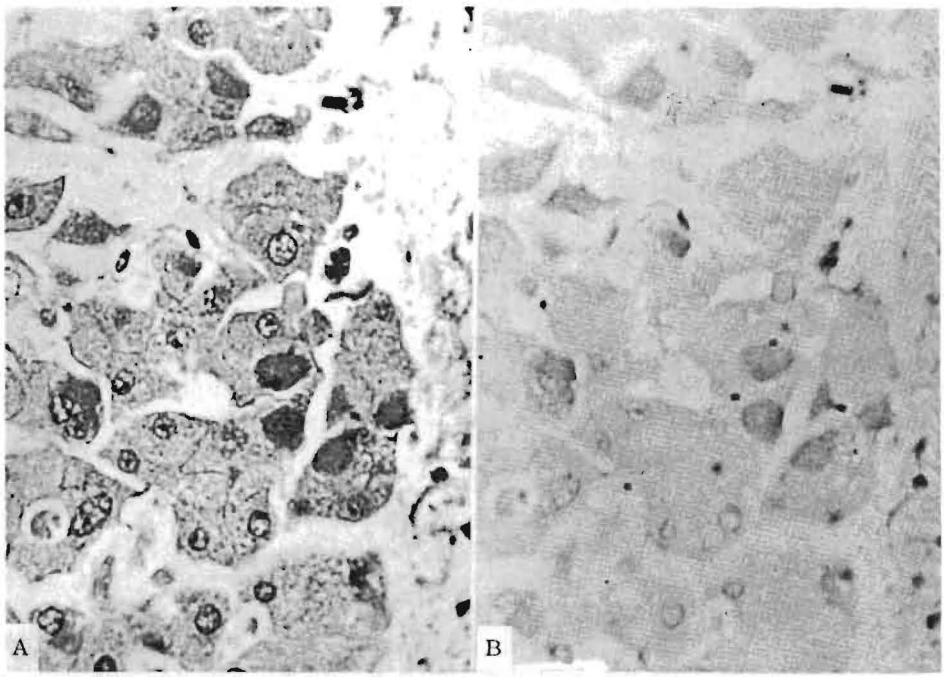

Fig. 2 (A) showing antigen inclusions in the cytoplasm of non-neoplastic hepatocytes from case 2 (H and E stain, x450); (B) orcein staining after decolorisation of the same section illustrated in (A). The inclusions observed are identical in site and morphology as those in (A) (x450).

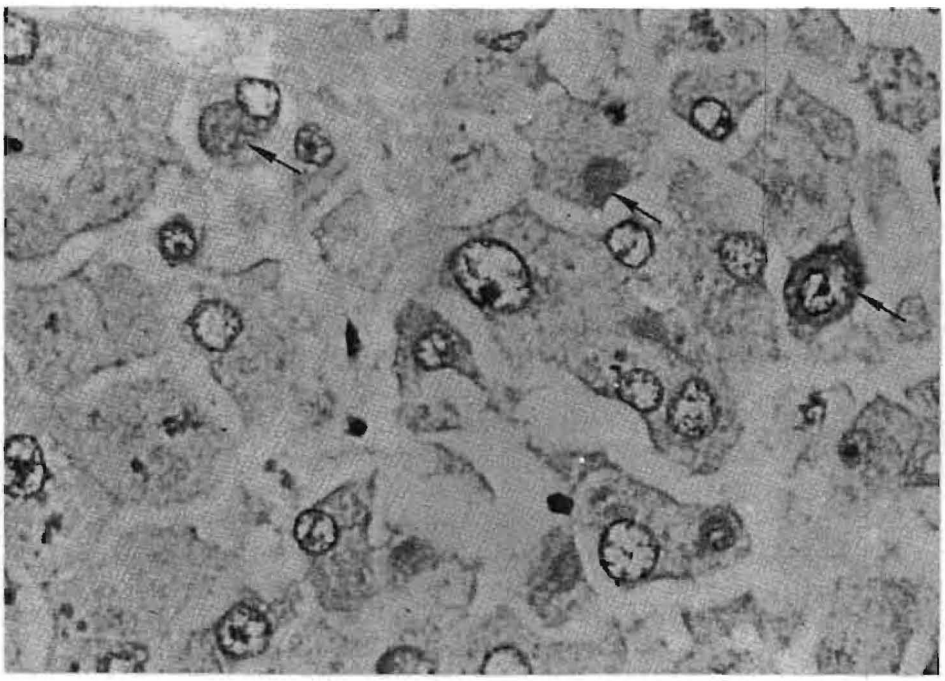

Fig. 3 Immunoperoxidase-positive HBsAg in the cytoplasm of liver cells (arrows). Paraffin section of a non-tumorous cirrhotic liver in case 2 of HCC (x450).

Table 1 Intensities of histochemical stains of HBsAg visualised in H and E compared with HBsAg-positive ground-glass hepatocytes.

Histochemical stains	HBsAg visualised in H & E	HBsAg-positive ground-glass hepatocytes
Gomori's aldehyde fuchsin	+ + + to + + + +	+ to + +
Macchiavello's	+ + to + + + +	+ to + +
Chromotrope aniline blue	+ to + +	+
Verhoeff-van Gieson's	+ + to + + +	+
Masson trichrome	+ + to + + +	+
Periodic acid-Schiff's	+ to + +	–
PAS-diastase	+ to + +	–

Intensities of stains are indicated as follows: + = weak, + + = moderate, + + + = strong, + + + + = very strong.

graphs of formalin-fixed paraffin-embedded tissues (Figs. 6 and 7). The antigen was poorly preserved. HBsAg seen in H and E was also visualised in one-micron epoxy-sections stained with toluidine blue (Fig. 8).

DISCUSSION

Shikata *et al*[5] stated that if a large amount of HBsAg exists in the cytoplasm it could cause some cytological changes or changes with regard to the stainability of the liver cells. However, they noted that the antigen could not be observed in the preparations routinely stained with H and E. But later on, in a study by Nayak and Sachdeva,[1] HBsAg was detected in the cytoplasm of hepatocytes by haematoxylin and eosin stain. In the present study, the antigen was easily identified using conventional H and E stain. It was proved to be antigen material by identifying the location and morphology of the structure with those subsequently seen to be positively stained with orcein in the same section. This had been performed by Nayak and Sachdeva.[1] However, the presence of antigen has to be confirmed by specific stains:[2] direct immunoperoxidase in the present study.

HBsAg visualised in H and E stained intensely using Gomori's aldehyde-fuchsin staining method. Shikata and his colleagues[5] stated that this method seemed to be more sensitive than orcein stain and probably stains the disulphide bonds of the antigen.

Borchard and Gussmann[7] detected liver cells containing HBsAg by using orcein, aldehydthionine and chromotrope aniline blue stains; they found that the orcein stain yielded the best results. The results were constant with the chromotrope aniline blue stain. The cytoplasm appeared greenish with occasional red-green metachromasia. In the present study, HBsAg seen in H and E stained weakly with chromotrope aniline blue.

The antigen visualised in H and E stained moderately with Verhoeff-van Gieson's technique.[8] This staining method was developed for staining elastic fibers. However, it also stained HBsAg in the liver tissue. The antigen visualised in H and E also stained moderately with Masson trichrome.

Inclusion bodies may be stained using Macchiavello's method.[10] It is of interest to note that HBsAg seen in H and E stained distinctively with this technique, appearing a dense purplish blue colour, with relatively ill-defined intracytoplasmic inclusions.

HBsAg visualised in H and E was weakly PAS-positive and diastase resistant. The PAS method before and after diastase treatment showed the presence of glycogen in the cytoplasm of some hepatocytes.

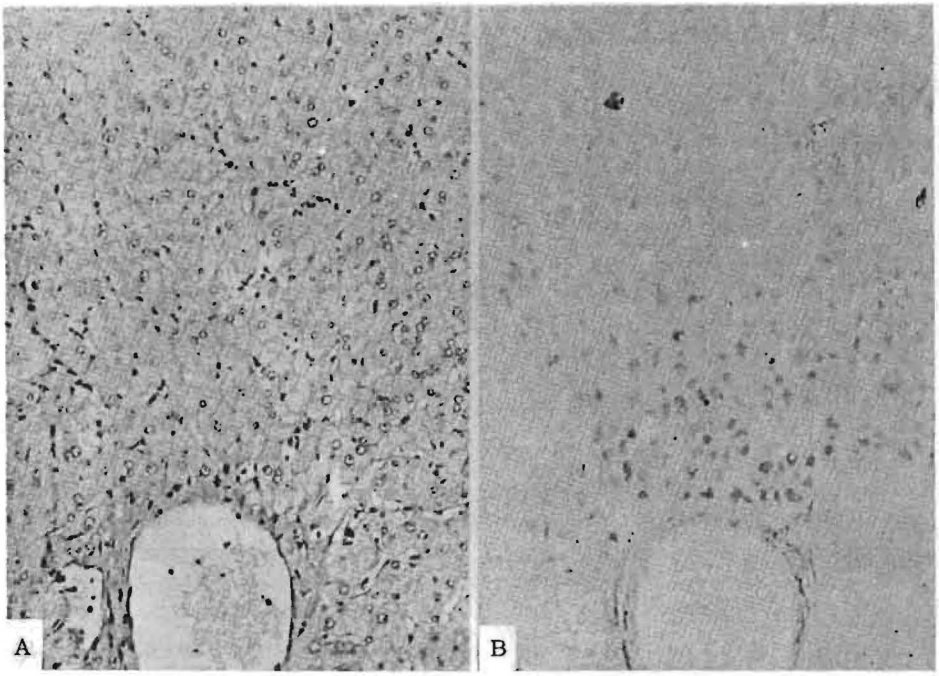

Fig. 4 Ground-glass hepatocytes: (A) showing liver cells with all or a discrete part of the cytoplasm replaced by a uniform granular change known as ground-glass cells. Note the non-ground-glass liver cells at the lower right-hand corner (H and E, x100); (B) the antigen in some ground-glass hepatocytes stained with moderate intensity using Gomori's aldehyde fuchsin (x 100).

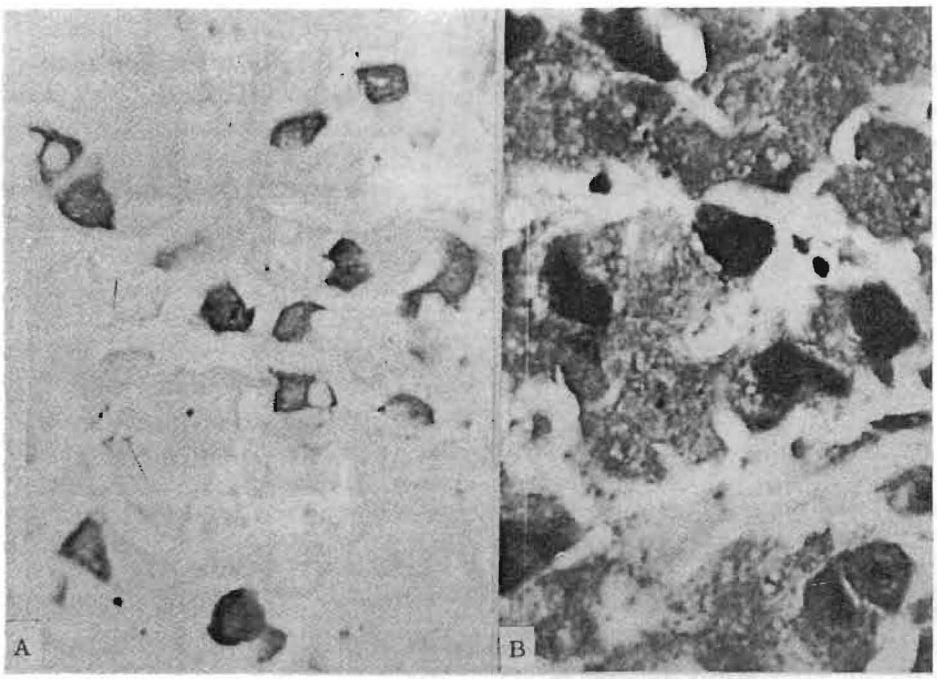

Fig. 5 HBsAg seen in H and E, case 2: (A) the antigen appears as dense inclusions surrounded by pale-staining cytoplasm (Gomori's aldehyde fuchsin, x450); (B) intracytoplasmic inclusions are surrounded by the cytoplasm which stained somewhat intensely with Macchiavello's (x450).

HBsAg in the ground-glass cells was detected by orcein stain.[5] The antigen stained weakly with Gomori's aldehyde fuchsin and Macchiavello's technique. The stains were very faint in chromotrope aniline blue, Verhoeff-van Gieson's and Masson trichrome staining methods. HBsAg in the ground-glass hepatocytes was PAS-negative.

An interesting finding in the present study was the identification of the antigen visualised in the H and E in one-micron epoxy sections stained with toluidine blue. This was not so for HBsAg in the ground-glass cells. With the electron microscope, the antigen seen in H and E revealed more electron density than that of the ground-glass cells. The reasons for these observation are not known, although the results of the present study suggest the accumulation of a greater amount of the visualised antigen in H and E stain than that appearing as ground-glass cells. Further investigation is recommended.

Summary

Various histochemical stains were performed on two autopsy cases of hepatocellular carcinoma (HCC) associated with cirrhosis, in which the hepatitis B surface antigen (HBsAg) was visualised in the cytoplasm of non-neoplastic liver cells using conventional haematoxylin and eosin (H and E) sections. For comparison, the stains were also done on specimens from one case with chronic active hepatitis and another of HCC with HBsAg-positive "ground-glass" hepatocytes. The antigen visualised in the H and E stained intensely in most of the stains, whereas HBsAg in the ground-glass cells showed staining of moderate or weak intensity. The antigen was also identified with immunoperoxidase stain.

Ultrastructurally, the antigen visualised in H and E revealed more electron density than that in the ground-glass. HBsAg seen in H and

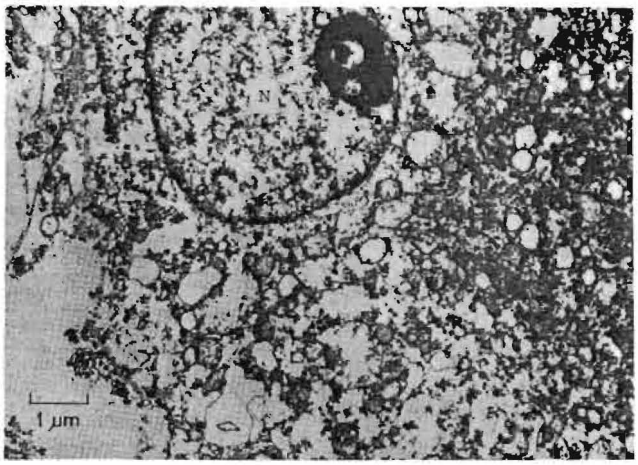

Fig. 6 Showing high electron density of the antigen inclusion visualised in H and E from case 1, occupying a portion of the cytoplasm close to the nucleus (N) of a liver cell (x10,560).

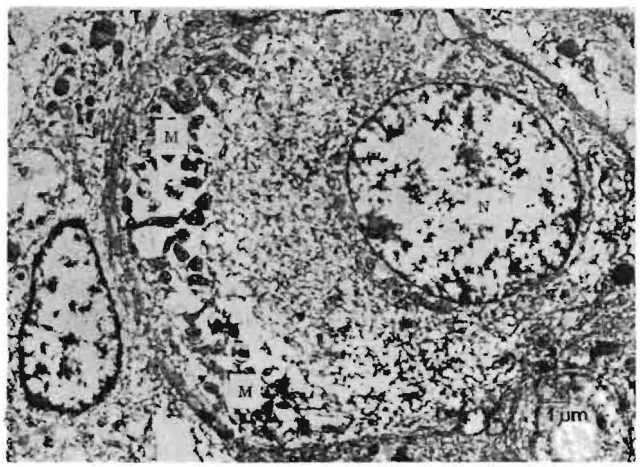

Fig. 7 HBsAg-positive ground-glass hepatocyte. Filamentous material surrounds the nucleus (N) with mitochondria (M) at the periphery of the cell (x7,040).

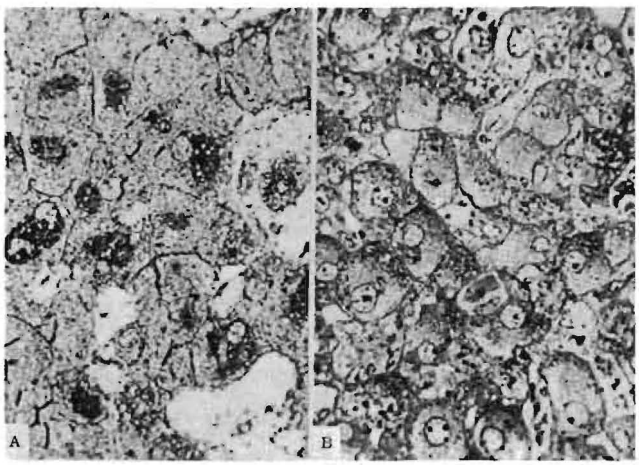

Fig. 8 One-micron epoxy-sections stained with toluidine blue: (A) HBsAg visualised in H and E is clearly seen as dense, large, intracytoplasmic inclusions (x450); (B) the antigen appears as ground-glass cells (x450).

E was also visualised in one-micron epoxy-sections stained with toluidine blue. The results suggest the accumulation of a greater amount of visualised antigen in the H and E stain than that appearing in ground-glass cells.

REFERENCES

1. Nayak NC, Sachdeva R. Localization of hepatitis B surface antigen in conventional paraffin sections of the liver: comparison of immunofluorescence, immunoperoxidase, and orcein staining methods with regard to their specificity and reliability as antigen marker. Am J Pathol 1975; 81: 479-92.
2. Sachdeva R, Nayak NC, Sama SK, Seth HN. Hepatitis B surface antigen in the liver-its appearance in conventional paraffin sections. Ind J Med Res 1976; 64: 1027-34.
3. Nayak NC, Dhar A, Sachdeva R, et al. Association of human hepatocellular carcinoma and cirrhosis with hepatitis B virus surface and core antigens in the liver. Int J Cancer 1977; 20:643-54.
4. Hadziyannis S, Gerber MA, Vissoulis C, Popper H. Cytoplasmic hepatitis B antigen in "ground-glass" hepatocytes of carriers. Arch Pathol 1973; 96:327-30.
5. Shikata T, Uzawa T, Yoshiwara N, et al. Staining methods of Australia antigen in paraffin sections. Jap J Exp Med 1974; 44:25-36.
6. Gomori G. Aldehyde fuchsin – a new stain for elastic tissue. Am J Clin Pathol 1950; 20:665-6.
7. Borchard F, Gussmann V. Detection of HBsAg-containing cells in liver biopsies by different stains and classification of positively reacting ground-glass hepatocytes. Virchows Arch A Pathol Anat Histol 1979; 384:245-61.
8. Sheehan DC, Hrapchak BB. Theory and practice of histotechnology. 2nd ed. St Louis: CV Mosby, 1980:196-7.
9. Masson P. Trichrome stainings and their preliminary technique. J Tech Meth 1929; 12:75-90.
10. Culling CFA. Handbook of histopathological and histochemical techniques. 3rd ed. London: Butterworth, 1974; 402-3.
11. Bancroft JD, Stevens A. Theory and practice of histological techniques. 2nd ed. Edinburgh: Churchill Livingstone, 1982: 189-90.

12. Huang SN. Immunohistochemical demonstration of hepatitis B core and surface antigens in paraffin sections. Lab Invest 1975; 33:88-95.

13. Zimmerman LE, Ramon LF. Application of electron microscopy to histopathologic diagnosis. Trans Am Acad Opth Otolaryngol 1972; 76:101.

14. Thakerngpol K, Damrongsak C, Stitnimankarn T. Ultrastructure of primary human liver cell carcinoma using material already blocked in paraffin. J Med Assoc Thailand 1973; 56:145-6.