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Demonstration of Hepatitis B Surface Antigen by Orcein Staining in Paraffin Sections of Cirrhotic Liver

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Summary. Blood and liver from 44 and 30 patients, died in cirrhosis and other diseases, respectively, were tested for the presence of HB_sAg. Blood samples obtained at autopsy and in seven cirrhosis cases also before death were tested for HB_sAg by counter-electrophoresis. Detection of HB_sAg in hepatocytes was carried out in paraffin sections by the modified orcein staining technique of Shikata *et al.* Ten of 14 HB_sAg seropositive and 2 of 30 HB_sAg seronegative cirrhotic patients had orcein positive hepatocytes, which were not found in any liver specimen from the 30 non-cirrhotic seronegative patients. The orcein positive substance localized in the cytoplasm of hepatocytes, less often it was also seen in a few Kupffer cells. The hepatocellular carcinoma cells present in part of the livers studied did not contain any orcein positive substance. Histological changes in the cirrhotic livers showed no morphological indication of the presence of HB_sAg, except on staining with orcein. The modified orcein staining technique is a simple, handy procedure for use in any routine pathological laboratory and has the additional advantage of detecting HB_sAg also in stored paraffin blocks.

Key words: Hepatitis B surface antigen — Orcein staining method — Liver cirrhosis — Liver cell carcinoma.

Detection of the surface component of hepatitis B antigen (HB_sAg) in sera has been a routine procedure for several years, but demonstration of the antigenic material in the liver tissue was up to recently referred to research laboratories, because it required either immunofluorescence or electron microscopic techniques. Hadziyannis *et al.* (1973a, b) and later also others (Deodhar *et al.*, 1975; Gerber *et al.*, 1974b; Huang *et al.*, 1974; Korb, Huppertz, 1974; Vogel *et al.*, 1974) observed that liver cells showing a bright cytoplasmic fluorescence after staining with fluorescent specific antibody have a particular "ground-glass" appearance of the cytoplasm. However, "ground-glass" hepatocytes were chiefly found in symptomless carriers of HB_sAg (Deodhar *et al.*, 1975; Gerber *et al.*, 1974b; Hadziyannis *et al.*, 1973a, b). As the "ground-glass" appearance of liver cells is due to a quantitative increase of the smooth endoplasmic reticulum (SER), factors eliciting induction of detoxifying microsomal enzymes and hyperplasia of SER, e.g. chronic alcohol addiction, may also be responsible for "ground-glass" change (Klinge *et al.*, 1973). On the other hand, immunohistochemical studies have clearly shown that hepatocytes not exhibiting "ground-glass" cytoplasm may also contain components of hepatitis B antigen (HBAg) in both cytoplasm and nucleus (Akeyama *et al.*, 1972, 1974; Hadziyannis *et al.*, 1973a, b; Krawczynski *et al.*, 1972; Nowoslawski *et al.*, 1972).

On comparing liver sections showing HBAg specific fluorescence with conventional paraffin sections, Shikata *et al.* (1974) discovered that cytoplasmic

Table 1. Testing of cirrhotic and non-cirrhotic patients for HB_sAg by counter-electrophoresis and orcein staining

	Cirrhosis, HB_sAg seropositive				Cirrhosis, HB_sAg seronegative				Non-cirrhotic HB_sAg seronegative
	in life + at autopsy	in life only	at autopsy only	totals	in life + at autopsy	in life only	at autopsy only	totals	at autopsy only
Orcein positive	—	3	7	10	—	—	2	2	—
Orcein negative	—	1	3	4	3	—	25	28	30
Totals	—	4	10	14	3	—	27	30	30

structures in every respect similar to fluorescent cytoplasmic HB_sAg can be visualized by a modified orcein staining method and by Gömöri's aldehyde fuchsin stain as well. The specific affinity of these stains to HB_sAg was well documented. The results of Shikata *et al.* (1974) were subsequently confirmed by other authors (Deodhar *et al.*, 1975; Gerber *et al.*, 1974b; Vogel *et al.*, 1974).

In an earlier study the relation of 43 autopsy cases of liver cirrhosis to HB_sAg was analysed on the basis of serological finding (Bartók, Decastello, in press). In possession of the modified orcein staining technique proposed by Shikata *et al.* (1974), opportunity presented 1. to examine cirrhotic liver tissue for the presence of HB_sAg, 2. to compare the detectability of HB_sAg by immunoserological and histological methods and 3. to seek correlation between the presence of HB_sAg in the liver cells and changes in parenchymal tissue. The related findings are reported in this paper.

Material and Methods

Forty-four cirrhotic livers and 30 livers from patients died in other diseases were obtained at autopsy. Classification of cirrhosis was based on microscopic appearance of the liver and on clinical history. In every case, blood samples were taken at autopsy and were tested for HB_sAg and specific antibody (anti-HB_s) by counter-electrophoresis. Sera form seven cirrhotic patients had also been examined for HB_sAg before death.

HB_sAg was found in the serum samples of 14 cirrhotic patients. In 10 of the 14 cases blood samples were withdrawn exclusively at autopsy. In three cases HB_sAg-positivity had been detected one to two years before death, but all three samples were negative at autopsy. In a further case the sample obtained 19 days before death was negative, another sample withdrawn four days later was positive and the one obtained at autopsy was negative again (Table 1). Among the 14 cirrhosis cases 10 were identified as the cirrhotic phase of chronic aggressive hepatitis (active macronodular cirrhosis), three as inactive macronodular (cryptogenic) cirrhosis and one was alcoholic cirrhosis. HB_s-antigenemia in the latter patient was presumably due to coincidental infection related to blood transfusion. Hepatocellular carcinoma was supervening in four cases.

Thirty cirrhotic patients had no HB_sAg in the blood, three of them not even while alive (Table 1). Among the 30 patients eight had had active macronodular cirrhosis, nine inactive

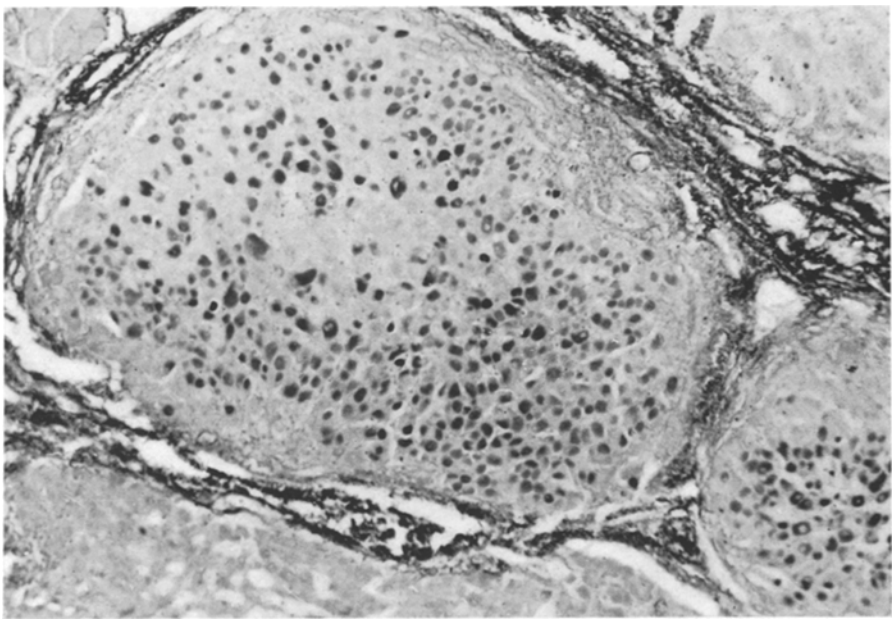

Fig. 1. Positive staining of large groups of hepatocytes. Modified orcein technique, ×120

macronodular cirrhosis, 12 alcoholic cirrhosis and one had secondary biliary cirrhosis. Hepatocellular carcinoma developed in five livers.

Among the 30 patients died in conditions other than cirrhosis no one showed acute or chronic hepatitis. All serum samples obtained in this group were HB_sAg negative (Table 1).

No anti-HB_s was demonstrable in any cirrhotic or non-cirrhotic patient serum.

The blocks of liver tissue were fixed in 4% neutral formalin and were embedded in paraffin. In most cases two to four blocks were available from each liver. The sections were stained with H. and E. and with the modified orcein technique of Shikata *et al.* (1974). The blocks were embedded in the period between 1. 1. 1973 and 10. 6. 1975, and the orcein staining was performed in 1975.

Orcein staining was performed as follows: 1. oxidation in 0.3% $KMnO_4$ solution containing sulphuric acid (300 mg $KMnO_4$, 50 ml 0.6% H_2SO_4, 50 ml distilled water) for 5 minutes; 2. rinsing in distilled water; 3. bleaching in 1.5% oxalic acid solution for about 10 minutes; 4. staining in acid orcein solution (1 g orcein in 100 ml 70% ethanol, with pH adjusted to 1.0–2.0 by hydrochloric acid) for four hours; 5. short differentiation in absolute ethanol; 6. immersion in xylol and mounting.

Results

The liver tissue took on a homogenous light yellowish-brown orcein stain. The orcein positive substance stained dark brown or purplish brown and were sharply delineated thus being easily visible already in low power field (Figs. 1–4). Occasional paler staining did not affect perceptibility. The orcein positive substance localized in the cytoplasm of hepatocytes, but in three cases they were also seen in a few Kupffer cells. The nuclei of the hepatocytes did not take on the stain (Fig. 3) and no orcein positive substance was present in the hepatocellular carcinoma cells.

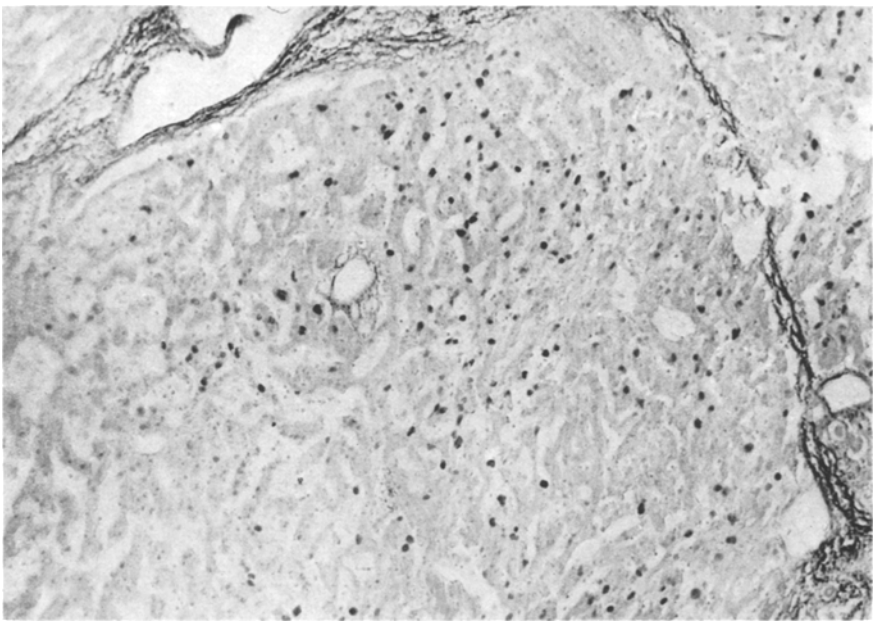

Fig. 2. Positive staining of irregularly scattered solitary hepatocytes. Modified orcein technique, ×100

Orcein-positive hepatocytes were found in 10 of the 14 HB_sAg-seropositive cases, of which seven were active macronodular cirrhosis, two inactive macronodular cirrhosis and one was alcoholic cirrhosis. Hepatocellular carcinoma was seen in three of the 10 cases. HB_sAg had been detected in the blood 1.5 years, 1 year and 15 days before death, respectively, in three cases, and at autopsy in seven cases (Table 1).

Among the 30 HB_sAg-seronegative cirrhosis patients two (one active and one inactive macronodular cirrhosis) had orcein positive liver cells. The inactive macronodular cirrhosis patient also had hepatocellular carcinoma. In neither case were serological examinations performed while the patient had been alive (Table 1).

None of the 30 HB_sAg-seronegative non-cirrhotic patients had orcein-positive hepatocytes (Table 1).

Quantity and Distribution of Orcein Positive Cells. Localization of the Orcein Positive Substance

In every case, orcein positive substance was detected only in part of the hepatocytes. In certain nodules large clusters of hepatocytes took on the dark stain (Fig. 1), while in others only single positive hepatocytes were found (Fig. 2), varying greatly in number between nodules, from 2–10 to many. The orcein positive clusters and solitary hepatocytes were randomly distributed in each nodule. Clusters of orcein positive liver cells were seen in a few nodules of

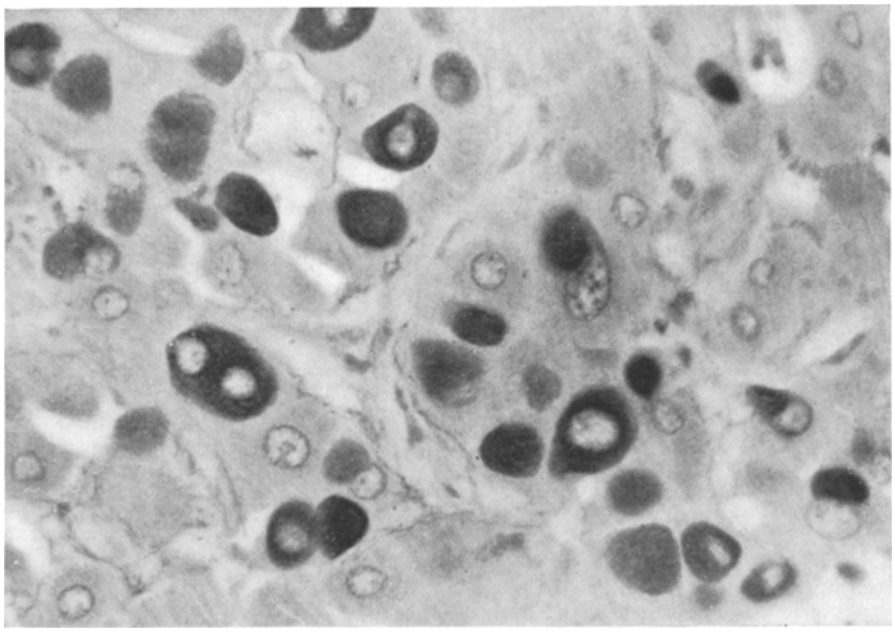

Fig. 3. Note annular arrangement of orcein positive substance around the nuclei of some hepatocytes and inclusion-like cytoplasmic aggregations of orcein positive substance in others. Note absence of nuclear staining. Modified orcein technique, ×700

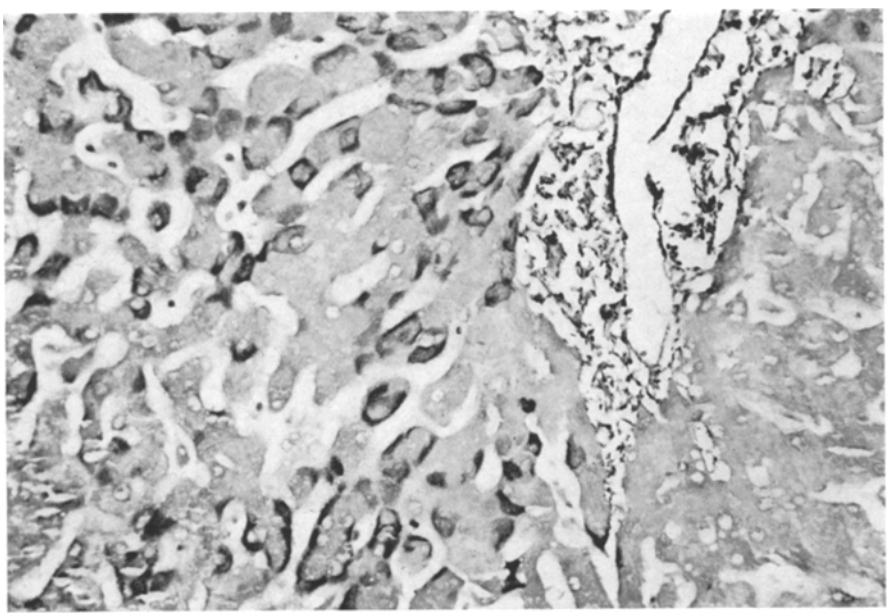

Fig. 4. Localization of orcein positive substance at the sinusoidal margin of hepatocytes. Modified orcein technique, ×300

only three livers, in other parts of which single hepatocytes were taking on the strain. In the remaining livers only single positive cells were found. Solitary orcein positive hepatocytes occurred in all or almost all nodules of six livers, in a minor part of the nodules in three livers and in only part of the blocks from further three livers. It is remarkable that three patients, who were HB_sAg-seronegative at autopsy (two had been HB_sAg-seropositive before death) had many orcein positive hepatocytes. Against this, several subjects found to be seropositive at autopsy had only very few orcein positive liver cells.

The orcein positive substance either formed round or oval cytoplasmic bodies, or filled the entire cytoplasm in part of the hepatocytes, while it surrounded the nucleus in an annular or semilunar manner in others (Fig. 3). Infrequently orcein positivity was localizing at the sinusoidal margins of the cells (Fig. 4).

Relation of Histological Finding to Orcein Positivity

"Ground-glass" hepatocytes were not found in any orcein positive cirrhotic liver. In serial sections alternately stained with H. and E. and orcein the orcein positive hepatocytes were difficult to identify, especially if they were scanty. H. and E.-stained sections showed no indication of any specific change in those areas in which orcein positive hepatocytes were numerous. Although the numbers of positive cells varied greatly between specimens, they appeared to be least numerous in the most severely damaged cirrhotic livers.

Among the four seropositive but orcein negative cirrhosis patients, the first had died in a very advanced stage of disease, the second had had a less advanced active macronodular cirrhosis, the third had had hepatocellular carcinoma extending over the greater part of the liver, and the fourth had shown extensive ischemic necroses. Apart from the latter non-specific damage, neither the above four livers, nor the livers of sero-negative and orcein negative macronodular cirrhosis patients showed notable qualitative or quantitative histological differences from orcein positive cirrhotic livers.

Discussion

It is known that HBAg consists of two components, a DNA-containing core antigen (HB_cAg), and a lipoprotein surface antigen (HB_sAg). In the serum, core antigen coated by surface antigen is localizing in the Dane particle, which probably corresponds with the infectious virion itself (Müller, 1975). According to immunohistochemical (Hadziyannis *et al.*, 1973a, b; Kater *et al.*, 1973; Müller *et al.*, 1973; Nowoslawski *et al.*, 1970, 1972) and electron microscopic examinations (Almeida *et al.*, 1973; Gerber *et al.*, 1974a; Huang *et al.*, 1974; Nowoslawski *et al.*, 1970; Sun *et al.*, 1974), HBAg may be present in both nucleus and cytoplasm of hepatocytes, but the nuclear antigen differs from the cytoplasmic antigen in respect of morphological features (Gerber *et al.*, 1974a; Huang *et al.*, 1974) and immunochemical specificity (Brzosko *et al.*, 1973). The cytoplasmic antigen found in the SER has been regarded as the HB_sAg, whereas the nuclear antigen as the HB_cAg (Gerber *et al.*, 1974a; Huang *et al.*, 1974; Müller, 1975).

In the present study the cytoplasm of the hepatocytes did take on positive orcein stain, whereas the nucleus did not. This accords well with the related findings of other authors (Deodhar *et al.*, 1975; Shikata *et al.*, 1974). The distribution pattern of the orcein positive hepatocytes and the cytoplasmic localization of the orcein positive substance were in every respect similar to those demonstrated by immunofluorescence (Akeyama *et al.*, 1972, 1974; Hadziyannis *et al.*, 1972; Kater *et al.*, 1973; Krawczynski *et al.*, 1972; Müller *et al.*, 1973; Nowoslawski *et al.*, 1972). Orcein positivity of the hepatocytes accorded well with cytoplasmic HB_sAg fluorescence in patients with various liver diseases (Deodhar *et al.*, 1975; Gerber *et al.*, 1974b; Shikata *et al.*, 1974). In identical sections stained with both methods, the cytoplasm of cells binding the fluorescent antibody were also taking on positive orcein stain (Shikata *et al.*, 1974). The above findings can be regarded as firm evidence that the orcein positive substance is identical with the HB_sAg, although the mechanism of the staining reaction is still unclear. Presumably, orcein (Deodhar *et al.*, 1975) and aldehyd-fuchsin (Shikata *et al.*, 1974) stain the disulphide bonds present in the cystine component of HB_sAg (Dreesman *et al.*, 1972; Sukeno *et al.*, 1972; Vyas *et al.*, 1972).

In the present study, orcein positive hepatocytes were found in 10 of 14 HB_sAg seropositive cirrhosis cases and in 2 of 30 HB_sAg seronegative cirrhosis cases, whereas all 30 non-cirrhotic, HB_sAg seronegative patients studied had orcein negative livers. The slight deviation between serological and tissular (orcein) positivity is not surprising, because the same was already observed with immunofluorescence method (Akeyama *et al.*, 1974; Cérat *et al.*, 1973; Krawczynski *et al.*, 1972; Müller *et al.*, 1973).

All HB_sAg seropositive patients except one with presumably coincidental infection had had either active or inactive macronodular cirrhosis and the two seronegative, orcein positive cirrhosis cases also came under this category. In the latter two cases only post-mortem blood samples were tested for HB_sAg. Among the seropositive and orcein positive patients three had HB_sAg exclusively in the blood samples taken before death, but not in those obtained at autopsy; Shikata *et al.* (1974) made consistent observations in two cases. One orcein positive patient had HB_sAg in only one of three blood samples taken within 19 days, viz. in the one obtained 15 days before death. Thirteen HB_sAg seronegative patients with cirrhosis of known etiology as well as 30 HB_sAg seronegative, non-cirrhotic patients had no orcein positive hepatocytes. In this light it seems convincing that in the two seronegative, orcein positive cases of macronodular cirrhosis, HB_sAg was present in the hepatocytes while its serum level may have been below the quantity demonstrable by counter-electrophoresis.

No orcein positive hepatocytes were found in four HB_sAg seropositive cirrhosis cases. Since the numbers of orcein positive hepatocytes varied widely within one and the same liver, their absence in the four cirrhotic livers may well have been due to sampling error. Another explanation also lies close at hand. In the cases studied by us, exclusively the cytoplasmic HB_sAg showed a positive orcein staining reaction, but others (Ahmed *et al.*, 1971; Almeida *et al.*, 1973; Gerber, Paronetto, 1974; Kater *et al.*, 1973; Müller *et al.*, 1973) reported predominance of the nuclear HB_cAg in hepatocytes of certain liver disease patients. This may well have been another cause of orcein negativity in the four HB_sAg

seropositive cirrhotic patients. Finally, difference of serum and hepatic levels of HB_sAg at the time of examination (Krawczynski *et al.*, 1972) can equally account for orcein negativity in seropositive patients and orcein positivity in seronegative ones. This accords well with the finding that the two cirrhotic patients who were seropositive before death, but seronegative at autopsy, had many orcein positive hepatocytes, whereas several cirrhosis patients with HB_sAg positive autopsy blood samples had only few.

It is known that in various parts of the world the frequency of HB_sAg is considerable greater in liver cell carcinoma patients than in the general population. This suggests that the HB virus may cause liver cell carcinoma in man, although no unequivocal explanation of the causal relationship has yet been found (Anthony, 1974). In the patient group studied in this laboratory, four HB_sAg seropositive cirrhotics developed hepatocellular carcinoma. Another liver cell carcinoma patient was HB_sAg seronegative, but orcein positive substance was found in his liver. In every case, however, the orcein positive substance was present exclusively in the noncancerous cirrhotic parenchyma, never in the tumor cells. Similar results were obtained in specific immunofluorescence tests by Hadziyannis *et al.* (1975). This supports the conclusion that in the hepatocellular carcinoma cases analyzed by the latter authors and ourselves, the replication of HB_sAg did not take place inside the tumor cells.

Summarizing, the orcein staining technique elaborated by Shikata *et al.* (1974) is very helpful for detecting HB_sAg in cirrhotic livers, but it does not inform about the presence of HB_cAg. The staining reaction is less specific than immunofluorescence and its mechanism is not yet clear. Nevertheless, the reliability of the orcein reaction has been verified by specific immunofluorescence tests (Deodhar *et al.*, 1975; Gerber *et al.*, 1974b; Shikata *et al.*, 1974). The great advantage of the orcein method is its simplicity, which renders it suitable for use in any routine pathological laboratory, especially as it can be employed for the examination of stored specimens, too. The greater part of the paraffin-embedded blocks used in this study have been stored since 1973, and Deodhar *et al.* (1975) even reported demonstration of HB_sAg with orcein in blocks stored since 1963. In possession of the orcein technique, the relationship between chronic liver disease and HB_sAg can be pursued directly in the liver tissue. The histological changes found by us in HB_sAg positive and HB_sAg negative cases of macronodular cirrhosis were in every respect similar to one another, thus orcein positivity was the sole indicator of the presence of HB_sAg.

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References

Ahmed, M. N., Huang, S. N., Spence, L.: Australia antigen and hepatitis. An electron microscopic study. Arch. Path. **92**, 66–72 (1971)

Akeyama, T., Kamada, T., Koizumi, T., Abe, H.: The localization of the Australia antigen in the liver by immunofluorescence. J. clin. Path. **25**, 1071–1074 (1972)

Akeyama, T., Kamada, T., Koyama, M., Abe, H.: Distribution pattern of hepatitis B antigen in the liver. Immunofluorescence studies. Arch. Path. **98**, 252–256 (1974)

Almeida, J. D., Gioannini, P., Scalise, G., Harrison, C. V.: Electron microscope study of a case of Australia antigen positive chronic hepatitis. J. clin. Path. **26**, 113–119 (1973)
Anthony, P. P.: Carcinoma of the liver in man. Int. Path. **15**, 29–44 (1974)
Bartók, I., Decastello, A.: Hepatitis B antigen in liver cirrhosis and hepatocellular carcinoma. Acta hepato-gastroenterol. (in press)
Brzosko, K., Madalinski, K., Krawczinski, K., Nowoslawski, A.: Duality of hepatitis B antigen and its antibody. I. Immunofluorescence studies. J. infect. Dis. **127**, 424–428 (1973)
Cérat, G., Richer, G., Viallet, A., Coté, J., Robert, J., Turgeon, F.: Detection of Australia antigen in liver biopsies by immunofluorescence. Canad. med. Ass. J. **108**, 981–984 (1973)
Deodhar, K. P., Tapp, E., Scheuer, J.: Orcein staining of hepatitis B antigen in paraffin sections of liver. J. clin. Path. **28**, 66–70 (1975)
Dreesman, G. R., Hollinger, F. B., Suriano, F. B., Fuijoka, R. S., Brunschwig, J. P., Melnick, J. L.: Biophysical and biochemical heterogenity of purified hepatitis B antigen. J. Virol. **10**, 469–476 (1972)
Gerber, M. A., Hadziyannis, S., Vissoulis, C., Schaffner, F., Paronetto, F., Popper, H.: Electron microscopy and immuno-electronmicroscopy of cytoplasmic hepatitis B antigen in hepatocytes. Amer. J. Path. **75**, 489–496 (1974a)
Gerber, M. A., Paronetto, F.: Hepatitis B antigen in human tissues. In: Schaffner, F., Sherlock, S., Leevy, C. M. (ed.): The liver and its diseases, p. 54–63. New York: Intercontinental Medical Book Corporation 1974
Gerber, M. A., Vernace, S., Popper, H.: Groundglass hepatocytes in hepatitis B antigen (HBAg) positive chronic hepatitis and cirrhosis. Gastroenterology **66**, 855 (1974b)
Hadziyannis, S., Gerber, M. A., Vissoulis, C., Popper, H.: Association of cytoplasmic HBAg with characteristic hepatocytes ("induction cells"). Gastroenterology **64**, 174 (1973a)
Hadziyannis, S., Gerber, M. A., Vissoulis, C., Popper, H.: Cytoplasmic hepatitis B antigen in "ground-glass" hepatocytes of carriers. Arch. Path. **96**, 327–330 (1973b)
Hadziyannis, S. J., Karvountzis, G. G., Menesis, E., Merikas, G. E.: Hepatitis B core antigen and antibody in primary liver cancer. Lancet **1975 II**, 418–419
Hadziyannis, S., Vissoulis, C., Moussouros, A., Afroudakis, A.: Cytoplasmic localization of Australia antigen in the liver. Lancet **1972 I**, 976–979
Huang, S. N., Groh, V., Beaudoin, J. G., Dauphinee, W. D., Guttmann, R. D., Morehouse, D. D., Aronoff, A., Gault, H.: A study of the relationship of virus-like particles and Australia antigen in liver. Hum. Path. **5**, 209–222 (1974)
Kater, L., van Gorp, L. H. M., Borst-Eilers, E.: Hepatitis: intrahepatic expression of Au antigen and immune complexes in liver tissue. Vox Sang. (Basel) **24**, 27–32 (1973)
Klinge, O., Kaboth, U., Winckler, K.: Feingewebliche Befunde an der Leber klinisch gesunder Australia-Antigen-(HB-Ag-) Träger. Virchows Arch. Abt. A **361**, 359–368 (1973)
Korb, G., Huppertz, A.: Lichtmikroskopische Leberzellveränderung als möglicher Hinweis auf einen positiven HB-Antigen-Befund. Klin. Wschr. **52**, 1132–1133 (1974)
Krawczynski, K., Nazarewitz, T., Brzosko, W. J., Nowoslawski, A.: Cellular localization of hepatitis-associated antigen in livers of patients with different forms of hepatitis. J. infect. Dis. **126**, 372–377 (1972)
Müller, R.: Natur, Eigenschaften, Vorkommen und Nachweis des Hepatitis-B-Antigens. Immunität und Infection **3**, 149–158 (1975)
Müller, R., Maess, J., Deicher, H.: Fluoreszenzserologischer Nachweis von Australia-Antigen in der Leber. Dtsch. med. Wschr. **98**, 1–3 (1973)
Nowoslawski, A., Brzosko, W. J., Madalinski, K., Krawczynski, K.: Cellular localisation of Australia antigen in the liver of patients with lymphoproliferative disorders. Lancet **1970 I**, 494–497
Nowoslawski, A., Krawczynski, K., Brzosko, W. J., Madalinski, K.: Tissue localization of Australia antigen immune complexes in acute and chronic hepatitis and liver cirrhosis. Amer. J. Path. **68**, 31–50 (1972)
Shikata, T., Uzawa, T., Yoshiwara, N., Akatsuka, T., Yamazaki, S.: Staining methods of Australia antigen in paraffin section. Jap. J. exp. Med. **44**, 25–36 (1974)
Sukeno, N., Shirachi, R., Yamaguchi, J., Ishida, N.: Reduction and reoxidation of Australia antigen: loss and reconstitution of particle structure. J. Virol. **9**, 182–188 (1972)

Sun, S. C., Anderson, K. E., Hsu, C. P., Kau, S. L.: Hepatocelluar ultrastructure in asymptomatic hepatitis B antigenemia. Arch. Path. **97**, 373–379 (1974)

Vogel, H. M., Henning, H., Lüders, C. J., Braun, H. H., Tripatzis, I.: Lichtoptischer Nachweis von HBAg im Leberbiotat? Vergleichende Untersuchungen von HBAg-Befunden im Serum mit Ergebnissen einer Aldehydthionin-Färbung. Z. Gastroent. **12**, 257–264 (1974)

Vyas, G. N., Williams, E. W., Klaus, G. G. B., Bond, H. E.: Hepatitis associated Australia antigen: protein, peptides, and amino acid composition of purified antigen with its use in determining sensitivity of the hemagglutination test. J. Immunol. **108**, 114–118 (1972)

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