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Abstract

Tissue localization of a subcomponent of the first component of complement (C1q) was examined in one postmortem case of HBs antigen (HBs Ag) positive hepatocellular carcinoma and in six cases of chronic hepatitis from liver biopsy specimens. The direct immunofluorescent method was used after fixation with 2% para-formaldehyde in concentrated ammonium sulfate. C1q localization was found in collagen fibers and the cytoplasm of fibroblasts in the connective tissues of specimens examined. The localization was particularly marked in the region of the fundal glands of the gastric wall. Apart from collagen fibers, other sites of localization included the surface membrane of lymphocytes, especially those cells of the mesenteric lymph nodes. In HBs Ag positive specimens, immune deposit-like substances appeared localized intra-hepatically and in the renal glomeruli. Since C3 and C4 were identified concomitantly, it indicates that these substances were indeed immune deposits. Despite the finding that C3 and C4 were identified together in the hepatic cell cytoplasm, C1q itself was not demonstrated in all hepatic cell cytoplasm.

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TISSUE LOCALIZATION OF C1q IN HBs ANTIGEN POSITIVE LIVER DISEASE PATIENTS BY DIRECT IMMUNOFLUORESCENT TECHNIQUE

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Abstract. Tissue localization of a subcomponent of the first component of complement (C1q) was examined in one postmortem case of HBs antigen (HBs Ag) positive hepatocellular carcinoma and in six cases of chronic hepatitis from liver biopsy specimens. The direct immunofluorescent method was used after fixation with 2% para-formaldehyde in concentrated ammonium sulfate. C1q localization was found in collagen fibers and the cytoplasm of fibroblasts in the connective tissues of specimens examined. The localization was particularly marked in the region of the fundal glands of the gastric wall. Apart from collagen fibers, other sites of localization included the surface membrane of lymphocytes, especially those cells of the mesenteric lymph nodes. In HBs Ag positive specimens, immune deposit-like substances appeared localized intra-hepatically and in the renal glomeruli. Since C3 and C4 were identified concomitantly, it indicates that these substances were indeed immune deposits. Despite the finding that C3 and C4 were identified together in the hepatic cell cytoplasm, C1q itself was not demonstrated in all hepatic cell cytoplasm.

A subcomponent of the first component of complement (C1q) (1) has recently been used as a reagent in the radioimmunologic method for detection of immune complexes in serum or other biologic fluids of various autoimmune diseases (2–5). It is postulated, however, that the progressive liver damage of chronic hepatitis type B may be due to an autoimmune reaction directed against the hepatocyte surface lipoprotein initiated by a hepatitis B virus (HBV) infection (6–9); but the localization of C1q in the liver tissue during chronic hepatitis type B and the precise role of the autoimmune mechanism in progressive liver damage are not yet clear (8, 9). With the object of clarifying the localization of C1q in the liver of chronic hepatitis type B and in other organs and tissues of man, the present authors used the direct immunofluorescent technique to examine this problem.

MATERIALS AND METHODS

Research specimens were prepared from frozen tissues of the liver, kidney, spleen, mesenteric lymph nodes, stomach, and small intestine of an autopsy case of hepatocellular carcinoma with hepatic cirrhosis. The serum was HBs antigen (HBs Ag) positive (immunoelectrophoretic method) (10) and alpha-fetoprotein positive (radioimmunoassay 1.3×10^4 ng/ml).

Liver biopsy specimens of three cases of HBs Ag positive chronic aggressive hepatitis (Type 2B) (11) and three cases of the same type of HBs Ag negative chronic aggressive hepatitis were also studied. HBs Ag in the sera was tested by the reversed hemagglutination method (RPHA) (12).

Examination of the tissue localization of Clq was performed by the authors' own technique (13, 14). Namely, saturated ammonium sulphate in 2% para-formaldehyde solution (para-formaldehyde dissolved in water to a final concentration of 2% then saturated with ammonium sulphate) was cooled to 4°C, and the resulting solution was applied for three minutes to fix tissue specimens. Specimens fixed by this technique were compared with acetone-fixed and unfixed specimens. All specimens were then stained with fluorescein isothiocyanate (FITC)-labelled anti-human Clq solution (Behringwerke Laboratory) by the direct immunofluorescent technique (15). The specificity of individual stainings was checked by standard blocking tests (15).

At the same time, FITC-labelled anti-human IgG, IgA, and IgM reagents (Behringwerke Laboratory) were also used by the same technique as that for FITC-labelled anti-human C3 and C4 reagents (Dakopats Laboratory).

RESULTS

Fixation by 2% para-formaldehyde proved extremely effective. The localization of Clq was thus studied using this fixation in various tissues obtained from the hepatocellular carcinoma autopsy case and for liver tissues of the six cases with chronic hepatitis.

Clq localizations in various tissues of the autopsy case

Liver. Clq was found with weak fluorescence in the intra-hepatic connective tissue and the cytoplasm of fibroblasts in portal triad. Moreover, Clq was localized in either a diffuse or granular pattern on the surface of the membrane of infiltrating lymphocytes in necrotic regions (Fig. 1). Furthermore, in some portal areas with exudation and hepatocellular necrosis, Clq was strongly demonstrated as an aggregation and/or an immune-like deposit. In these immune-like deposit areas, C3, C4, IgG and IgM were also found.

Kidney. Clq was less obvious than IgG in the region of the glomerulus, but was found in an identical degree to IgG as an immune-like local deposit in the region of the glomerular epithelial cells (Fig. 2-a). C3 and C4 were also identified in the same sites. Clq also was found in interstitial areas, being especially marked in the connective tissues of the distal tubule region. The distribution

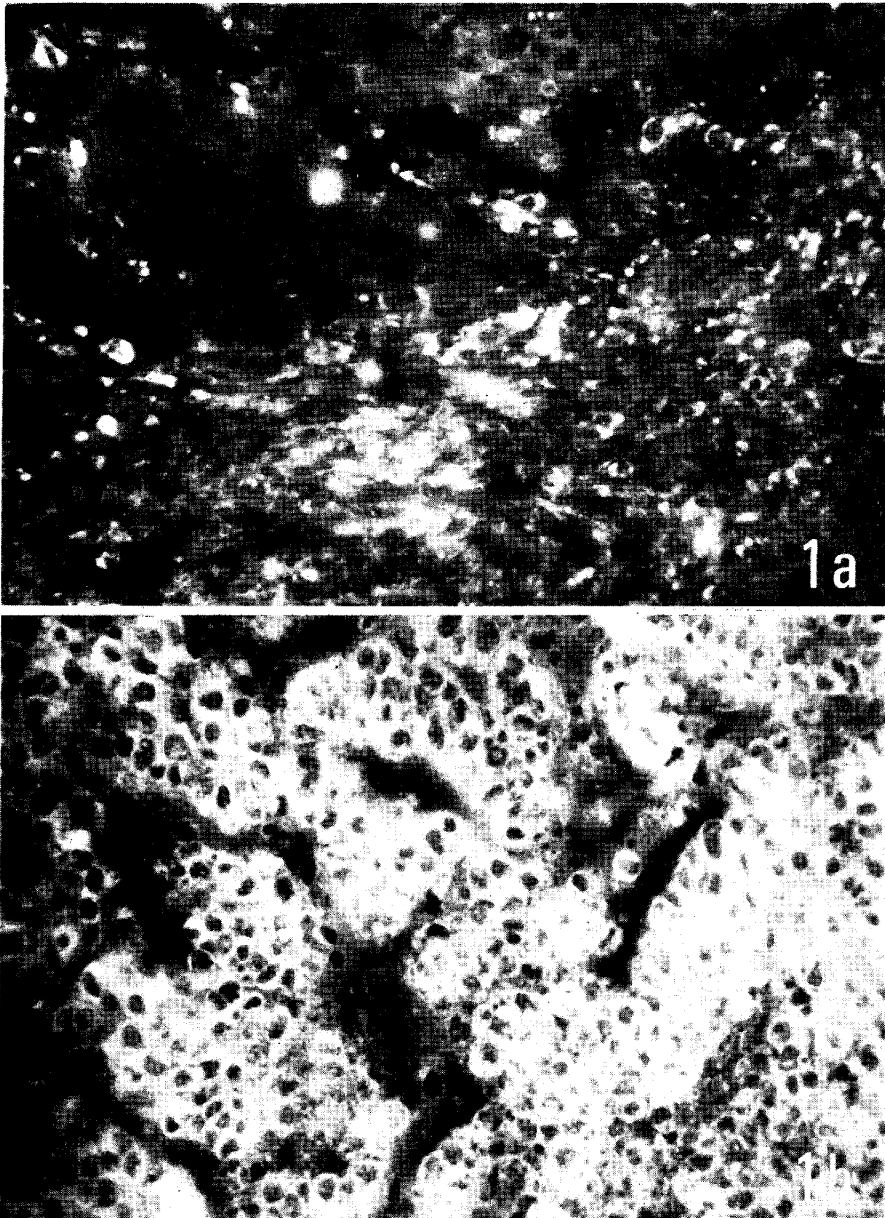


Fig. 1. Autopsy liver specimens from a patient with hepatocellular carcinoma. Fig. 1a. C1q was found in the collagen fibers and the cytoplasm of fibroblasts in connective tissue. An immune deposit-like substance was also localized in some places of the interstitial tissue. $\times 160$. Fig. 1b. Localization of C4 in the cytoplasm of carcinoma cells. $\times 160$.

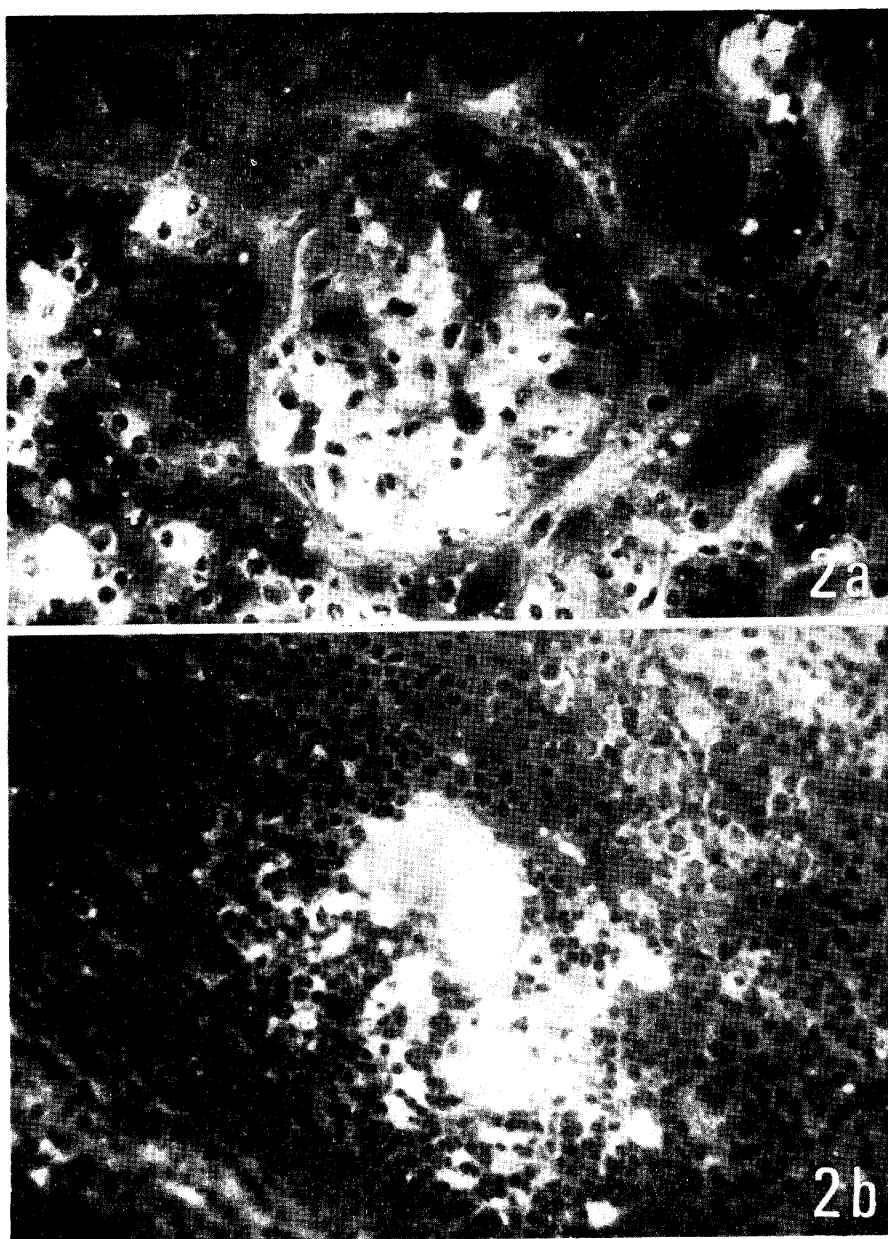


Fig. 2. Kidney and spleen specimens of the patient with hepatocellular carcinoma. Fig. 2a. Clq in the region of the glomerulus of the kidney. An immune deposit-like substance is localized to the region of the glomerular epithelial cells. $\times 160$. Fig. 2b. Clq is localized to the spaces between the central portion of the lymph follicles of the spleen. $\times 160$.

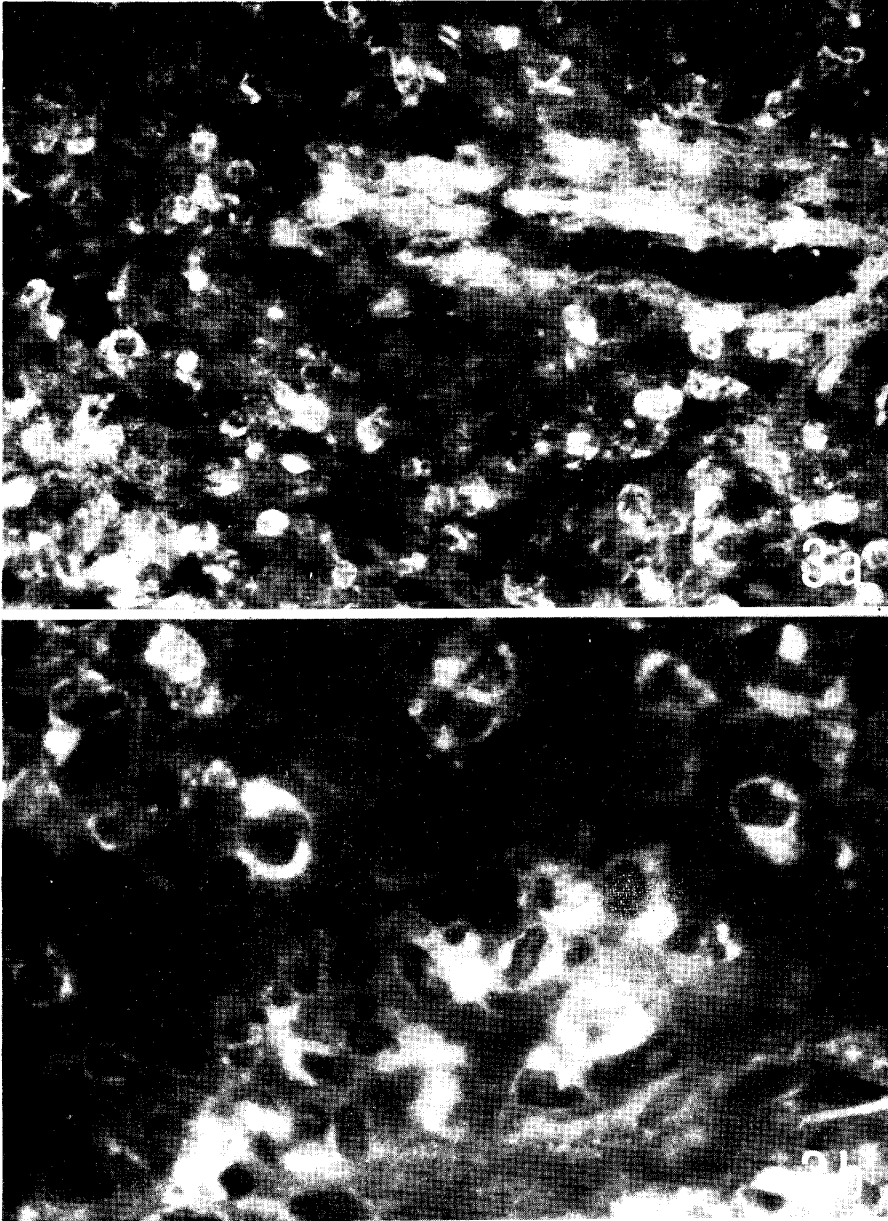


Fig. 3. Mesenteric lymph node of the same patient. Fig. 3a. Clq is distinctly more pronounced than in the spleen. Clq is demonstrated at the membrane surface of lymphocytes and in the connective tissue trabecule. $\times 160$. Fig. 3b. Lymphocytes with Clq. $\times 400$.

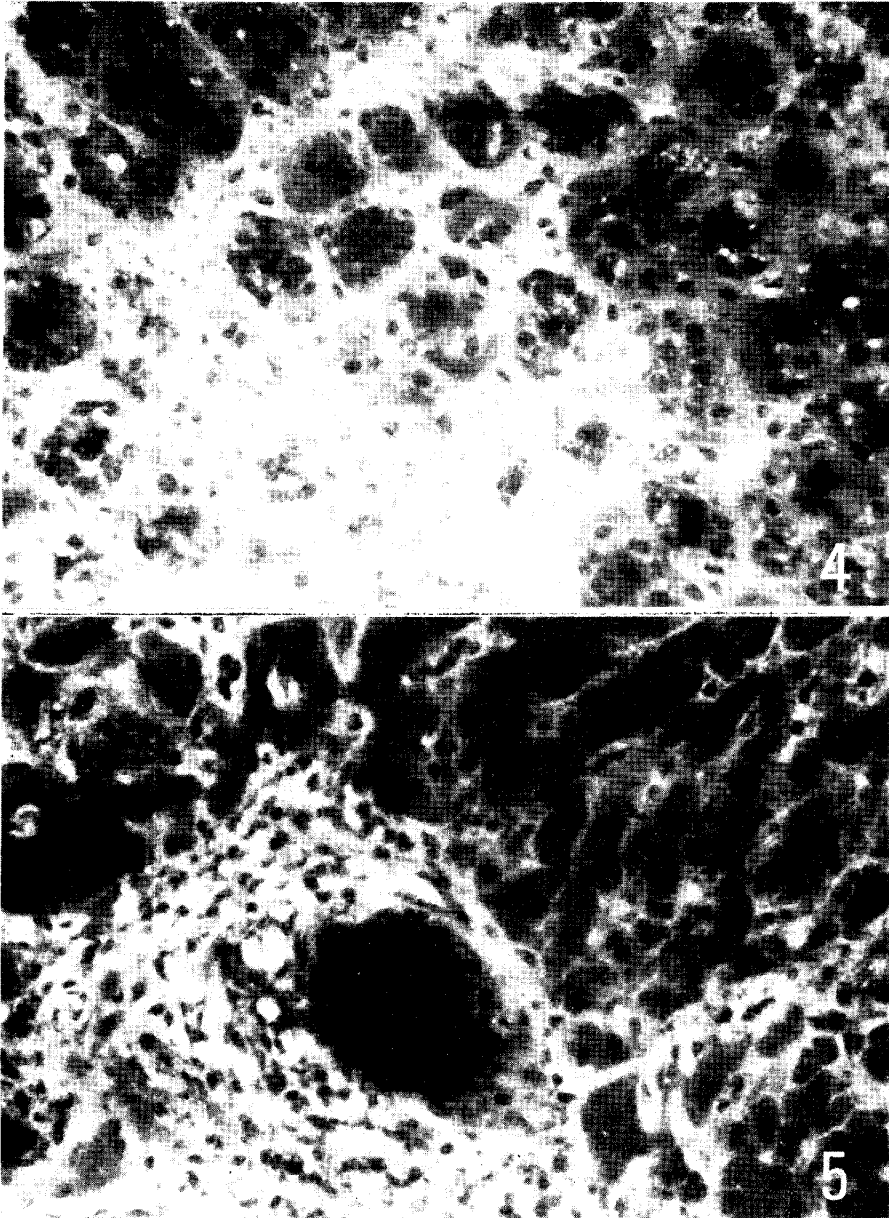


Fig. 4. Clq in the stomach wall of the patient with hepatocellular carcinoma. Clq is demonstrated in the interstitial tissue around the region of the fundal gland. $\times 160$.

Fig. 5. Clq in the biopsy liver specimen from a patient with chronic aggressive hepatitis type B. Clq is demonstrated in the connective tissue and on the membrane of infiltrated lymphocytes in portal triad. $\times 160$.

was more prominent in the walls of veins than in the arteries.

Spleen. Clq was detected in the spaces between the lymphocytes of the small splenic nodes, viz., the central portion of the lymph follicles (Fig. 2-b). Other sites included the membrane surface of peripheral lymphocytes, and a weak fluorescence was present throughout the connective tissue trabeculae.

Lymph nodes. The localization of Clq was demonstrated within lymph glands more distinctly than in the spleen (Fig. 3-a). Strong localization was observed at the membrane surface of lymphocytes, being particularly marked in cell groups that extended from the lymph follicle to the medulla (Fig. 3-b). Clq was also demonstrated in the connective tissue trabeculae.

Stomach. In comparison with other organs, the presence of Clq was vivid in the stomach wall, especially in the interstitial tissues around the fundal glands (Fig. 4). Clq appeared in "pooling" in the spaces around the gland cells but was not localized within the gland cells.

Small intestine. Similar localization was demonstrated in the intestinal connective tissue but was extremely poor in comparison to the stomach wall.

Clq was localized on the membrane surface of small and medium sized lymphocytes in the connective tissues of all the organs. Clq-positive small lymphocytes were found at the rate (per 100 small lymphocytes) of 10-15 in lymph nodes, 3-4 in the spleen, and 1-3 in the liver.

Clq-positive small lymphocytes predominated in the lymph nodes but the coexistence of C3 and C4 was hardly demonstrated in sites outside the liver.

Clq localization in biopsy liver specimens of chronic hepatitis

The localization of Clq in the liver was found in the same pattern as that described in the liver tissue of the autopsy case. Comparisons of Clq in the liver tissues of the three cases with HBs Ag positive chronic aggressive hepatitis and in the three cases with HBs Ag negative chronic aggressive hepatitis showed a predominance of Clq in the former due to pronounced aggregation and/or immune deposit-like localization (Fig. 5).

DISCUSSION

It is known that in the presence of Ca^{2+} , Clq (subcomponent of the first component of complement) as C1 in a trimolecular complex with C1r and C1s (16), initially combines with the immune complex of antigen and with IgG or IgM antibody in the classical pathway (17). Stroud *et al.* (18-20) have recently reported that their experiments on the composition of this subcomponent have shown it to be a protein resembling the collagen of connective tissues.

Thorbecke and others (21-24) reported the biosynthesis of Clq *in vitro* by human and monkey liver, spleen, bone marrow, and lung, and furthermore in macrophages isolated from peritoneal exudates and from lung washings. Their

conclusions were based on the detection of radiolabelled Clq precipitin lines developing with an anti-Clq antiserum in the presence of carrier serum or euglobulin. Colten *et al.* (25) also investigated Clq synthesis by a hemolytic plaque method. Although the cellular site of Clq synthesis was not identified, these workers suggested that since non-intestinal tissue, particularly those rich in lymphoid cells also produced Clq, a mesenchymal cell and not an epithelial cell was probably the site of synthesis. But renewed interest in the epithelial cell as a site of Clq synthesis has been generated by the experiments of Bing, Spurlocke and Bery (26). They demonstrated synthesis of macromolecular Cl and Cls in long-term primary suspension cultures of normal human colon, adenocarcinoma of the colon, and transitional epithelial cells of the bladder and urethra. It is obvious, therefore, that the definitive site of Clq synthesis is still a moot point in question.

The present results using a modified fixation technique clearly help to clarify the issue of the tissue localization of Clq. In regard to localization of Clq in liver tissue of chronic hepatitis type B, HBs antigen-antibody-immune complexes have been demonstrated in the serum of HBs Ag positive hepatitis (27), and it thus indicates that Clq is richly localized in liver tissue. The present results, however, showed no great difference between HBs Ag positive and negative hepatitis. An interesting finding was the marked immune-like deposit localization of Clq, especially in the HBs Ag positive cases. It is not possible to formulate a clear explanation of the localization of Clq, but the present work provides some important guidelines for further study, particularly in regard to the pathogenesis of chronic hepatitis.

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