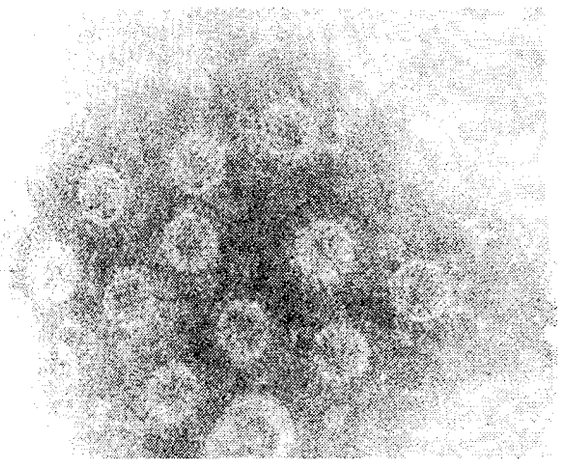
VIRUS-LIKE PARTICLES IN THE LIVER AND THEIR RELATIONSHIP TO AUSTRALIA ANTIGEN

SIR,—We found the article by Dr. Almeida and her colleagues (Dec. 4, p. 1225) very interesting, because we too have detected virus-like particles in the livers of 4 Australia-antigen (Au)-positive patients and have been struck by their resemblance to the inner shell of the Dane-particle form of circulating Au.[1]

The method used was simply to grind small pieces of liver tissue in a few drops of distilled water, apply a droplet of the suspension directly to a formvar-carbon-coated grid, and negatively stain with 2% sodium phosphotungstate at pH 7·0. Grids were examined in a Philips-300 electron microscope calibrated with a waffle grating replica.

The virus-like particles were detected in liver biopsy specimens of 2 renal-transplant patients on immunosuppression, and in post-mortem liver specimens from a



Typical aggregate of virus-like particles obtained from liver of Au-positive patient (×227,000).

patient with post-hepatic cirrhosis and from a patient with myelofibrosis who died of acute hepatitis. The particles, identical in all 4 cases, were delicate spherical virus-like bodies with an average diameter of 29·5 nm. (see accompanying figure). They were present singly and in aggregates surrounded by and held together by what appeared to be antibody, very similar in appearance to the liver particles described earlier by Almeida et al.[2] Free Au was detectable in some of the specimens (possibly derived from blood that was present in the liver tissue), but it was never seen in aggregated form, upholding the view of Almeida et al. that there are two separate antigen-antibody systems in this type of hepatitis. These particles could not be found in 4 control post-mortem liver specimens from Au-negative patients.

Thin-sectioning of the two liver biopsy specimens as part of a larger study [3] had revealed spherical particles, averaging 23 nm. in diameter, in the nuclei and occasionally in the cytoplasm of hepatocytes. The frequency of particles detected by negative staining correlated with the numbers seen in thin section. The size discrepancy could be due to differences in the techniques involved.

Morphological studies of Au in 36 sera from 17 patients with acute hepatitis, chronic active hepatitis, cirrhosis, or the chronic carrier state were carried out, by means of the technique of Kelen et al.,[4] to detect and concentrate the antigen. Dane particles in varying proportions were found in the sera of all but 2 of the patients—they were both chronic carriers. In 1 of the 36 sera—that of a renal-transplant patient—circulating aggregates of particles identical to those found in the liver were seen in addition to the regular Au, indicating that at times naked particles may be released into the bloodstream.

Measurements of the liver particles and the inner shells of a number of Dane particles showed a close agreement in size:

	No. of particles measured	*Average diameter*	*Size range*
Liver particles	90 (from 4 patients)	29·5 nm.	24·4–34·5 nm.
Inner shell of Dane particles	85 (from 10 patients)	29·7 nm.	26·8–35·7 nm.

On the basis of these data, we support the view that the liver particle gains a coat and is released into circulation as the Dane particle. The Dane particle may be the agent responsible for the infectivity of blood and blood products, all other forms of Au being excess coat material only. Whether the coat is necessary for infectivity—i.e., is a true viral envelope—or whether it is merely an overabundance of abnormal protein produced by the liver in response to infection is yet to be determined.

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IMMUNE COMPLEXES IN VIRAL HEPATITIS

SIR,—Several authors have suggested that Australia (Au) antigen/antibody complexes have a key role in the pathogenesis of viral hepatitis.[5,6] Such complexes have also been implicated in the pathogenesis of diffuse membranous glomerulonephritis.[7] We wish to report the finding of Au antigen and immune complexes in liver-biopsy specimens from patients with hepatitis. We examined 65 patients with various liver disorders. The results were as follows:

Au-positive hepatitis.—In 14 patients Au antigen was detected in the serum by counterimmunoelectrophoresis. In all 14 patients Au antigen could also be demonstrated in the liver by the indirect immunofluorescence technique. The antigen was located mainly on the nuclear membranes and in the cytoplasm of the liver-cells. A few foci were found along the sinusoids. In all 14 patients granular deposits of IgG and complement were found along the walls of vessels and sinusoids, suggesting the presence of immune complexes.

Au-negative hepatitis.—Au antigen could not be detected in any of the liver-biopsy specimens from 7 Au-negative patients with histologically confirmed hepatitis. However, all 7 patients, like the 14 Au-positive patients, showed granular deposits of IgG and complement along the vessel walls.

Other liver disorders.—Au antigen could not be found in the serum or the liver-biopsy specimen from any of the 44 patients with liver disorders other than acute or chronic hepatitis. 1 patient, however, showed another interesting phenomenon. He had pulmonary hæmorrhages and progressive loss of renal function, and after about 6 months he developed liver-function disturbances. Microscopic examination of a liver-biopsy specimen revealed no inflammatory changes. Immunofluorescence studies showed *linear* deposits of IgG and complement along the sinusoids and in the liver parenchyma. A kidney biopsy was performed. The kidney showed the histological picture of a focal necrotising glomerulonephritis. Immunofluorescence studies revealed the

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typical membranous deposit of IgG and complement. Immunofluorescence studies of the skin were negative.

Our findings in the hepatitis cases indicate that immune complexes are involved in both Au-positive and Au-negative viral hepatitis. The findings in the patient described above suggest that antibodies against basal membranes and/or reticular fibres may be cytotoxic for several, but not all, organs.

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FIBRINOLYTIC TREATMENT OF THE HÆMOLYTIC-URÆMIC SYNDROME

SIR,—The results of heparin therapy in severe cases of the hæmolytic-uræmic syndrome have been variable.[1] We have treated 3 patients with the plasminogen-activator streptokinase. All 3 recovered and are currently normotensive, with normal urinalyses and serum creatinines.

The initial dose of streptokinase (' Streptase ') was calculated as the number of units of streptokinase necessary to lyse the clot from 1 ml. of patient's plasma multiplied by the estimated plasma-volume. Following an intravenous injection of hydrocortisone hemisuccinate (50 mg.) to reduce the likelihood of febrile reactions, streptokinase was given in 2 ml. isotonic saline by constant infusion over 20 minutes. This dose was then given on an hourly basis in the same volume of fluid by pump over the course of therapy. Following therapy, the patient was heparinised at approximately 100 units per kg. given intravenously every four hours. Fibrin slides were made by a modification of the method of Kwaan and Astrup.[2] Plasminogen-rich and plasminogen-free bovine fibrinogen were produced by the method of Brakman.[3]

Case-reports

Patient 1.—A 3-month-old girl was admitted with a history of gastroenteritis followed by two days of anæmia, thrombocytopenia, and anuria. She was œdematous and in mild heart failure. Laboratory findings are shown in the table. No urine was present on bladder catheterisation. She received a five-day course of streptokinase therapy. During the second day she developed overt heart-failure and pulmonary œdema, but was managed successfully by 36 hours of peritoneal dialysis with no bleeding during the procedure. She began producing urine after three days of streptokinase therapy. At follow-up examination 10 months later she was normotensive, had a normal urinalysis, and serum-creatinine was 0·5 mg. per 100 ml.

Patient 2.—A 14-month-old girl had a history similar to patient 1. Initial laboratory values are shown in the table. Catheterisation yielded 10 ml. of urine in 5 hours. The patient received 56 hours of streptokinase therapy. After 24 hours' therapy she went into shock from hæmorrhage into the right thigh, stemming from a femoral puncture done at the referring hospital. This was treated successfully with packed red blood-cells. On the following day, hæmodialysis was carried out via a shunt inserted into the left groin. By the third day of therapy, this site also began to bleed with enlargement of the thigh. Therapy was then stopped, and an exchange transfusion performed to remove the remaining streptokinase. 3 days after stopping streptokinase, the child began to produce urine. At follow-up examination 4 months later the child was normotensive, with a normal urinalysis and serum creatinine of 0·6 mg. per 100 ml.

Patient 3.—A 22-month-old girl presented with a one-week history of gastroenteritis and bloody stools, followed by gross hæmaturia and oliguria. Her laboratory values are recorded in the table. A hippuran-^{131}I renogram was flat, there was no uptake of the isotope on renal scan, and there was no radioactivity in the bladder. As she passed a bloody stool on admission, streptokinase was deferred and she was dialysed via a shunt in the right arm. Over the next 2 days, her creatinine returned to its predialysis level (5·0 mg. per 100 ml.), the urine output fell from 250 ml. to 140 ml. a day, and the renogram remained flat. She had no stools over this period. Because of the likelihood of cortical necrosis she was given streptokinase for 102 hours. This was complicated only by mild oozing from a puncture site in the right groin, which was controlled by local pressure. After being started on heparin, she developed a staphylococcal abscess of the right parotid gland which responded to drainage and methicillin. Her urine output began to increase 24 hours after starting streptokinase. A renogram 2 days after stopping streptokinase revealed extraction and excretion of the isotope. An open biopsy of the left renal cortex was performed 19 days after discontinuing streptokinase. The biopsy revealed generalised mesangial thickening on light microscopy. By immunofluorescence, the mesangium contained large amounts of fibrin. Occasional capillary hyaline thrombi were found, but the majority of capillary loops were open. 2 months after therapy the child is normotensive, urinalysis is normal, and serum-creatinine is 0·6 mg. per 100 ml.

A kidney biopsy was obtained only from patient 3. Frozen sections examined by the histological fibrin slide technique revealed a striking increase in fibrinolytic activity in the renal cortex. Fibrinolysis was seen over the entire tissue section after only 2 minutes' incubation at 37°C. In tissue from 9 normal individuals and 80 patients with various renal diseases, including 2 patients with the hæmolytic-uræmic syndrome not treated with streptokinase, lysis was just detectable over single glomeruli at a mean of 31 minutes' incubation (range 10–54 minutes); complete lysis was not seen in any tissue until after 38 minutes' incubation.[4] Fibrinolytic activity was absent after incubation with plasminogen-free fibrin slides, indicating that the increase in lytic activity was due to increased levels of plasminogen activator and not free plasmin.

Discussion

Although the precise pathogenesis of the hæmolytic-uræmic syndrome is unknown,[5] experimental evidence suggests that the underlying pathological process leading to intraglomerular fibrin deposition involves capillary endothelial-cell injury, exposure of basement membrane collagen, platelet agglutination, and clotting.[6–8] We believe

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HÆMATOLOGICAL FINDINGS

Patient	Hb (g./100 ml.)	Platelet-count (per c.mm.)	Peripheral smear	P.T.T. (sec.)	Prothrombin-time (sec.)	Thrombin-time (sec.)	Fibrinogen (g./100 ml.)	F.D.P. titre	Serum-creatinine (mg./100 ml.
1	7·0	44,000	Microangiopathic	35	12·7	22·3	0·25	1/128	3·5
2	6·6	32,000	Microangiopathic	37·7	11·8	24·9	N.D.	N.D.	3·1
3	8·6	30,000	Microangiopathic	46·2	14·3	65·0	0·23	1/64	5·0
Normal	> 10·0	> 200,000	..	35–45	12–14	15–17	> 0·20	< 1/16	< 1·0

N.D. = not done. P.T.T. = partial thromboplastin time. F.D.P. = fibrin-degradation products.