J. Hyg., Camb. (1978) **81**, 405
Printed in Great Britain

e-Antigen: a link between immune response and infectivity in hepatitis B?

BY G. C. TURNER, HELEN T. GREEN AND VIVIAN D. BLUNDELL
Regional Public Health Laboratory, Fazakerley Hospital, Liverpool L9 7AL

(*Received* 21 *February* 1978)

SUMMARY

In a study of the distribution of e-antigen and anti-e in subjects whose blood was positive for hepatitis B surface antigen (HBsAg), patients with acute hepatitis B who were tested during the incubation period were all e-antigen-positive but after the onset of illness e-antigen was detected in only 11%. Persistence, and in some instances reappearance of e-antigen in those who became long-term carriers of HBsAg was associated with high titres of HBsAg. There was a high incidence of e-antigen in those conditions in which cell-mediated immunity may be depressed, including Down's syndrome and chronic renal failure.

The majority of HBsAg carriers identified as sources of infection were e-antigen-positive. A positive reaction for e-antigen is evidently associated with a defective immune response to hepatitis B virus infection which permits continued replication of virus in liver cells accompanied by high titres of HBsAg, numerous Dane particles and detectable DNA polymerase in the blood with consequently a greater likelihood of transmitting infection.

Although it cannot be assumed that anti-e positive carriers of HBsAg are not infective, it may be necessary, in the assessment of passive or active immunization for the control of hepatitis B, to take into account the e-antigen/antibody status of possible sources of infection.

INTRODUCTION

Since the first report in 1972 (Magnius & Espmark, 1972) of the demonstration of the e-antigen/antibody system in patients with hepatitis B virus infection, it has been confirmed (Eleftheriou *et al.* 1975; El Sheikh *et al.* 1975) that e-antigen can be detected only in blood samples that are positive for hepatitis B surface antigen (HBsAg). These two antigens are quite distinct with different physico-chemical characteristics but, in e-antigen-positive subjects, both can be demonstrated by immunofluorescence in the cytoplasm of infected hepatocytes (Trepo *et al.* 1976). The e-antigen is also distinct from hepatitis B core antigen (Takahashi *et al.* 1976) which can normally be demonstrated only in the nuclei of infected hepatocytes and not in the circulation. It has been suggested that e-antigen may be either a component of the complete Dane particle or host protein formed in the hepatocytes independently of Dane particles in response to hepatitis B virus infection. Blood samples that are e-antigen-positive frequently contain large numbers of Dane particles (Trepo, Bird & Zuckerman, 1977) and there is evidence

that a positive reaction for e-antigen identifies those HBsAg carriers who are more likely to transmit infection (Alter *et al.* 1976; Grady, 1976).

In this investigation, we have studied the distribution of e-antigen and its antibody, anti-e, in subjects with hepatitis B virus infection presenting either as acute hepatitis of limited duration or as long-term carriage of HBsAg including that associated with Down's syndrome and chronic renal failure, both conditions known to favour the development of long-term carriage of HBsAg. We have also demonstrated e-antigen-positive reactions in subjects who were probable sources of infection in outbreaks, or single cases, of hepatitis B.

METHODS

The subjects studied, from North West England and North Wales, were those whose blood had been found to be HBsAg-positive at the hepatitis reference centre, Regional Public Health Laboratory, Liverpool.

Serum specimens were tested for HBsAg either (until 1974) by immunoelectro-osmophoresis (IEOP) (White *et al.* 1971) or subsequently by reversed passive haemagglutination (RPHA) (Cayzer *et al.* 1974) using the Hepatest kit (Wellcome Reagents Ltd). RPHA titres were determined by testing doubling dilutions from 1/8 to 1/128000. In some instances, specimens were also tested by radio immuno-assay (RIA) using the Ausria II kit (Abbott Laboratories, Ltd), by Dr Elizabeth H. Boxall, Regional Virus Laboratory, East Birmingham Hospital.

Serum specimens were tested for e-antigen and anti-e by immunodiffusion in dextrose-agarose gel (Magnius & Espmark, 1972). Wells for sera under test and for e-antigen-positive and anti-e-positive control sera were filled three times at 2-hourly intervals. Tests were read after incubation at 37 °C for 18h and at ambient temperature for a further 6 days. Positive reactions consisted of precipitin lines which varied in strength from one serum to another but which gave reactions of identity with positive control sera. Strong positive reactions for either e-antigen or anti-e were demonstrated in some sera that had been stored for up to 10 years.

The *ad/ay* subtypes of HBsAg-positive sera were determined by the method of Green & Turner (1975).

RESULTS

Acute hepatitis B

Sera from 72 consecutive cases of acute hepatitis positive for HBsAg investigated between January 1976 and June 1977 were tested for e-antigen and anti-e. e-antigen was detected in the incubation period or early in the acute phase of the disease in 8 (11%) patients; in 5 (10%) of 48 males and 3 (12%) of 24 females. Anti-e was not detected in any of these patients either in the acute phase of the disease or in convalescence. Detection of e-antigen clearly depended on the examination of serum collected early in the disease. Of the 8 e-antigen-positive cases, 2 were detected in the incubation period, 5 within the first week after the onset of jaundice and 1 in the only patient in the series who did not develop jaundice.

One patient in this series is worthy of note. A woman, aged 29, was e-antigen-

Table 1. *Distribution of e-antigen and anti-e in HBsAg-positive subjects who were probable sources of hepatitis B infection*

Nature of acquired infection	Subtype	Number of infected persons	Probable sources of infection	Subtype	Number of subjects	Number with positive reactions for: e-Antigen	Anti-e	Neither
Acute hepatitis B:								
Among patients in mental subnormality unit	*ad*	5	HBsAg carriers among patients in unit	*ad*	5	5	0	0
Among staff and relatives of patients on haemodialysis	*ay*	12	HBsAg carriers among patients on haemodialysis	*ay*	10	10	0	0
Among other hospital staff	*ay*	1	Infant, HBsAg carrier	*ay*	1	1	0	0
	ad	1	Patient, HBsAg carrier	*ad*	1	1	0	0
In recipients of HBsAg-positive blood transfusions	*ay*	4	Blood donors	*ay*	4	2	1*	1
	ad	2	Blood donors	*ad*	2	0	2*	0
HBsAg carrier state:								
In infants	*ay*	2	Mothers, acute hepatitis B at term	1 *ay* 1 n.t.	2	0	0	2
	ay	1	Mother, HBsAg carrier	*ay*	1	1	0	0
	ad	2	Mother, HBsAg carrier	*ad*	1	1	0	0

n.t., Not tested.

* Tests done on blood samples taken about 2 years after the donations that were the probable sources of infection.

Table 2. *e-Antigen and anti-e in 68 blood donors in relation to HBsAg titre*

HBsAg titre*	No.	e-Antigen-positive	Anti-e-positive
64000 or more	12	3 (25%)	6 (50%)
1024–32000	35	0	15 (43%)
512 or less	21	0	12 (57%)
Total	68	3 (4%)	33 (49%)

* By reversed passive haemagglutination.

negative in the acute phase and became within 3 months of onset HBsAg-negative by RPHA although weakly positive by RIA. She was tested again 8 months later when she was 7 months pregnant and was found to be strongly positive for both HBsAg and e-antigen. Liver function tests were normal and she was free of symptoms of liver disease. She was still positive for HBsAg and e-antigen at term and her baby was given 100 mg of anti-HBs immunoglobulin at birth and at 1 month. The child was HBs-negative at birth and at 4 months but when next tested at 6 months was strongly positive for HBsAg and e-antigen. The HBsAg-positive sera from the mother and from the child were of subtype *ay* (Table 1). Six months after the pregnancy the mother was still strongly positive for HBsAg and e-antigen.

In this series, detectability of e-antigen in the acute phase of the disease was not related to subsequent development of HBsAg carrier state. Of 36 patients who were followed up, 3 (8%), including the pregnant woman, were still HBsAg-positive but with normal liver function tests, 9 months or more after onset. In none of the 3 was e-antigen detected in the acute phase. On the other hand, of the 8 e-antigen-positive patients, 6 became HBsAg-negative within 6 months, 1 died and 1 was not followed up.

Similar findings in acute hepatitis B have been reported from London (Gibson & Ruparelia, 1977) and Heidelberg (Thamer, Gmelin & Kommerell, 1976).

Symptomless carriers of HBsAg

Among 190 symptomless HBsAg carriers tested, including 141 blood donors, e-antigen was detected in 7 (3·7%) and anti-e in 100 (53%). Of 187 for whom sex was recorded, 136 were males, 6 (4·4%) positive for e-antigen and 71 (52%) for anti-e; 51 were females, 1 (2%) positive for e-antigen and 28 (55%) for anti-e.

Of 68 donors in whom HBsAg titre was determined by reversed passive haemagglutination, 3 (4·4%) were e-antigen-positive all in the group of 12 with high titres of 1/64000 or greater. The incidence of anti-e did not however differ significantly in groups with high, moderate or low titres respectively (Table 2).

Follow-up tests were undertaken on 16 of these HBsAg-positive donors or other symptomless carriers at intervals after the initial test which varied from 6 months to 4 years. Of 3 who were e-antigen-positive, 2 had remained positive after 6 months and 2 years respectively but the other was negative for e-antigen and anti-e, although still strongly positive for HBsAg, when retested after 4 years. Of the 11 who were initially anti-e-positive, all had remained positive after intervals varying between 6 months and 3 years, and 2 who were initially negative for

e-antigen and anti-e had developed anti-e but without significant change in HBsAg titre.

Infection in a unit for mentally-subnormal

In this unit, between November 1973 and March 1975, 5 patients, aged between 10 and 19, developed acute HBsAg-positive hepatitis all of subtype *ad*; 2 of them were transiently e-antigen-positive including 1 tested during the incubation period but none became carriers of HBsAg. All were severely sub-normal but the causes were other than Down's syndrome. Tests on other patients in the unit in December 1973 and subsequently revealed a total of 5 who were HBsAg carriers, all of subtype *ad* and e-antigen-positive. These patients were all males aged between 9 and 16; the cause of subnormality was Down's syndrome in 4 and epilepsy in 1.

Haemodialysis-associated hepatitis

Of 12 members of the staff of a haemodialysis unit, or relatives of patients undergoing haemodialysis at home, who developed acute HBsAg-positive hepatitis, 1 was e-antigen-positive, the serum specimen having been collected 11 days before the onset of jaundice. The other 11 were all negative for e-antigen and anti-e when tested but from none of them was a specimen available that had been collected in the first week after onset. All but 1 recovered with loss of HBsAg and return to normal liver function tests. The exception was a man of 69 whose illness was prolonged despite treatment with hydrocortisone and prednisolone and who remained HBsAg-positive with abnormal liver function tests for 2 years. When tested at the start of his illness he was e-antigen-negative but he became e-antigen-positive 10 weeks after onset and remained positive for more than 14 months.

In this outbreak the source of infection was a group of patients, undergoing maintenance haemodialysis in hospital or at home, who were carriers of HBsAg, all of subtype *ay*. The HBsAg-positive staff and relatives were all of this subtype. All 10 of these patients who were HBsAg carriers were also e-antigen positive. In most of them the initial infection was symptomless and the first serum found to be HBsAg-positive was also e-antigen-positive. In 2 patients however there was a history of an attack of jaundice preceding the development of HBsAg carriage. In both these patients, e-antigen although presumably present in the incubation period was not detectable after the onset of jaundice but appeared respectively 3 months and 20 months later.

In the 10 patients on haemodialysis the chronic carrier state persisted indefinitely with very high HBsAg titres and positive tests for e-antigen with the exception of 2 in whom there was a fall in HBsAg titre accompanied by loss of e-antigen.

Hepatitis B in pregnant women and their infants

We investigated the outcome of pregnancy in 4 women with acute hepatitis and positive tests for HBsAg at term; e-antigen-positive reactions were not demonstrated. Of the 4, 2 gave birth to infants who both became positive for HBsAg and e-antigen at about 2 months of age. One of them was followed up until 2 years

old when she was still HBsAg-positive having been e-antigen-positive until about 1 year old; anti-e was not however demonstrated in later specimens. The offspring of the other 2 women were given anti-HBs immunoglobulin shortly after birth; the twins of one and the baby of the other were HBsAg-negative at 5 months and 4 months of age respectively.

In addition to the woman who was found to be a carrier when pregnant after an attack of acute hepatitis (*vide supra*) we investigated the outcome of pregnancy in 3 other women who were symptomless HBsAg carriers, all of subtype *ad*. One, a Chinese woman, was e-antigen-positive at the time of birth of each of her 2 children. The first was HBsAg-negative and e-antigen-negative at birth but HBsAg-positive and e-antigen-positive when next tested 2 years later; the second was not tested at birth but was HBsAg-positive and e-antigen-positive at 2 months of age. The other 2 women were both positive for anti-e. The child of one was HBsAg-negative but anti-e positive at birth and for about 20 weeks subsequently; she was still HBsAg-negative at 8 months when anti-e was not detectable.

The twins born to the other anti-e-positive carrier were negative for HBsAg at birth and for 10 months subsequently; anti-e was not detected in either child.

Apart from these serological findings, all the children born to these HBsAg-positive mothers appeared normal at birth and subsequently.

HBsAg carriers as sources of infection

In addition to our studies of transmission of hepatitis B virus infection among patients in a unit for mentally subnormal, from haemodialysis patients to their staff attendants and relatives and from pregnant women to their offspring we investigated 8 instances of acute HBsAg-positive hepatitis in which evidence pointed strongly to an HBsAg carrier of the same *ad/ay* subtype as the source of infection (Table 1). These included 6 patients with post-transfusion hepatitis between 1972 and 1974. In each of these incidents the HBsAg-positive donors were identified and in 2 were e-antigen-positive. Of the other 4, 1 was negative for e-antigen and anti-e; the other 3 were anti-e-positive but these tests were done on blood samples taken about 2 years after the incident.

We also investigated 2 hospital workers who developed HBsAg-positive hepatitis. One was a medical laboratory technician who carried out biochemical tests on the infant (*vide supra*) who developed HBsAg at 2 months of age and was e-antigen-positive for several months. The other was a male nurse who 5 months before he developed HBsAg-positive hepatitis had taken a sample of blood from a patient who was an e-antigen-positive carrier of HBsAg.

Thus of the 8 HBsAg carriers who were probable sources of infection at least 4 were e-antigen-positive at the time of the incident.

DISCUSSION

Long-term carriage of hepatitis B antigen (HBsAg) is associated in many subjects with detectability in the blood of either e-antigen or, more frequently its antibody, anti-e. In our study of 190 symptomless carriers of HBsAg, including

blood donors, in North West England and North Wales we detected e-antigen in only 3·7% but anti-e in 53%. Similar findings were reported recently from South West Scotland (Parker, Goudie & Shattock, 1977).

It is not known why some HBsAg carriers are e-antigen-positive. During the incubation period of hepatitis B, e-antigen can be demonstrated shortly after the first appearance of HBsAg; among the various groups that we investigated, the 5 patients who were tested during the incubation period were all e-antigen-positive at that stage. After the onset of jaundice, however, e-antigen is not often detected; of our 72 patients with acute hepatitis B, only 8 (11%) were e-antigen-positive after onset and these were all patients who were tested during the first week of illness. Of these, 7 were followed up and all became e-antigen-negative and subsequently HBsAg-negative. Among all the 36 patients in this group who were followed up, 3 became long-term carriers of HBsAg but without symptoms and with normal liver function tests. In none of these was e-antigen detected during the acute phase of the illness.

In one of the 3 patients, however, e-antigen reappeared after recovery from acute hepatitis. Although weakly HBsAg-positive and e-antigen-negative 3 months after the onset of illness, she was found 8 months later, when 7 months pregnant, to be strongly positive for HBsAg and e-antigen. We have observed a similar course of events in 4 other patients who after acute HBsAg-positive hepatitis developed chronic HBsAg carriage at first without detectable e-antigen but later with positive reactions for e-antigen. One was a 69 year-old man whose HBsAg-positive hepatitis was slow to resolve and he was given hydrocortisone and prednisolone.

Two others were patients on haemodialysis who developed acute hepatitis B, e-antigen-negative and subsequently HBsAg carriage with positive reactions for e-antigen. Another patient, not included in this investigation, was a drug addict in poor condition who developed anicteric hepatitis, HBsAg-positive but e-antigen-negative. His condition deteriorated and he died from haematemesis 6 months later. For the last 5 months of his illness, he was strongly positive for HBsAg and e-antigen.

Persistence of HBsAg in long-term carriers is believed to be the result of a defective cell-mediated immune reaction to hepatitis B virus infection. In these 5 patients there were evidently special factors such as pregnancy, advanced age, corticosteroid treatment, chronic renal failure and poor physical condition related to drug abuse which may have caused a depression of cell-mediated immunity leading not only to persistence of HBsAg but also to failure to eliminate e-antigen completely and consequently to its reappearance in detectable form. It is noteworthy that all the HBsAg carriers with Down's syndrome or chronic renal failure that we investigated were e-antigen-positive; both these conditions are known to be associated with a high incidence of HBsAg carriage presumably because of an associated immunological defect.

It has been suggested that HBsAg carriers who are also e-antigen-positive transmit hepatitis B virus more readily than those who are e-antigen-negative, (Alter *et al.* 1976; Grady, 1976) and our findings are consistent with this suggestion.

Thus in a mental subnormality unit in which there were several cases of acute hepatitis B within a limited period, the 5 HBsAg carriers who were possible sources of infection were all e-antigen-positive. In a maintenance haemodialysis unit in which there was an outbreak of hepatitis B among staff of the unit and relatives of patients on home dialysis the source of infection was evidently the patients on haemodialysis who were HBsAg-positive (Turner & White, 1969; Hawe, Goldsmith & Jones, 1971) and all were e-antigen-positive.

Among pregnant women who are HBsAg carriers there is a high rate of transmission of hepatitis B virus to offspring among those who are e-antigen-positive (Okada *et al.* 1976; Beasley *et al.* 1977). Two of the 4 pregnant HBsAg carriers that we investigated were e-antigen-positive and both, unlike the other 2, who were anti-e-positive, transmitted HBsAg to their children. Transmission of HBsAg to infants from mothers with acute hepatitis B at term however does not evidently depend on positive reactions for e-antigen at the time of delivery. We were not able to demonstrate e-antigen in any of the 4 mothers with acute hepatitis that we tested at term but the infants of 2 developed HBsAg; the infants of the 2 who did not were given anti-HBs immunoglobulin at birth.

In the 2 instances that we investigated of hospital staff who evidently acquired hepatitis B from the blood of HBsAg carriers, we demonstrated e-antigen in both carriers. We also identified 6 HBsAg-positive donors (Table 1) whose blood was given, before universal screening of donors, to patients who subsequently developed hepatitis B. Of these 6 donors, 2 were e-antigen-positive but, of the other 4, 3 who were not tested until 2 years after the original incident were then found to be anti-e-positive. Although the incriminated donations were not necessarily anti-e-positive, it evidently cannot be assumed in relation to blood transfusion practice, that anti-e-positive donors are not infective. It is clear, however, that any trials intended to assess the efficacy of passive or active immunization in hepatitis B should take into account the e-antigen/antibody status of identified sources of infection.

In symptomless carriers we found that positive tests for e-antigen were associated with strongly positive tests for HBsAg. Other workers have shown that e-antigen-positive sera contain relatively large numbers of Dane particles and detectable DNA polymerase (Nordenfelt & Kjellen, 1975; Imai *et al.* 1976).

Liver biopsies show that, in e-antigen-positive patients, hepatocytes contain e-antigen in cytoplasm and hepatitis B core antigen in nuclei; these are not found in patients who are anti-e-positive (Trepo *et al.* 1976; Murphy *et al.* 1976; Hess *et al.* 1977). These findings suggest that there is more active replication of hepatitis B virus in HBsAg-positive carriers who are e-antigen-positive, and may explain not only the greater infectivity of these carriers but also the frequency with which e-antigen is found among patients with chronic aggressive hepatitis (Eleftheriou *et al.* 1975; El Sheikh *et al.* 1975; Nordenfelt & Andren-Sandberg, 1976).

It is not yet clear whether e-antigen is a component of the Dane particle or host protein formed in hepatocytes in which there is active replication of hepatitis B virus and liberated into the circulation independently of Dane particles. It has been suggested that it may be present in serum in both forms (Lam, Tong &

Rakela, 1977). In one of our patients a very early sample of blood taken in the incubation period was HBsAg-positive but e-antigen-negative. Electronmicroscopy revealed Dane particles in relatively large numbers. It is probably therefore an oversimplification to regard e-antigen as a marker for the presence of Dane particles: indeed Dane particles have been demonstrated in sera positive for anti-e (El Sheikh *et al.* 1975; Trepo *et al.* 1977).

It has been suggested that persistence of e-antigen may be related to the infecting strain of hepatitis B virus on the basis of contrasting results from 2 renal units (Gibson, 1977); or to the host response of which there was evidence in the report of different e-antigen reactions in husband and wife presumably infected with the same strain of virus (Perrillo *et al.* 1977). We conclude from our findings however that persistence or reappearance of e-antigen indicates a defective immune response to hepatitis B virus infection (which may perhaps be related to the strain of virus) of greater degree or of different nature to that responsible for persistence of HBsAg in subjects who are e-antigen-negative or anti-e-positive.

We thank members of staff of many pathology laboratories who referred blood samples to us for investigation and their clinical colleagues for information about patients; Dr D. A. Lehane and the staff of the Liverpool Regional Blood Transfusion Service for many samples from donors and much helpful co-operation, and Dr A. L. W. F. Eddleston and Dr N. El Sheikh of King's College Hospital, London for standard e-antigen-positive and anti-e-positive sera.

REFERENCES

Alter, H. J., Seeff, L. B., Kaplan, P. M., McAuliffe, V. J., Wright, E. C., Gerin, J. L., Purcell, R. H., Holland, P. V. & Zimmerman, H. J. (1976). Type B hepatitis: the infectivity of blood positive for e antigen and DNA polymerase after accidental needlestick exposure. *New England Journal of Medicine* **295**, 909.

Beasley, R. P., Trepo, C., Stevens, C. E. & Szmuness, W. (1977). The e antigen and vertical transmission of hepatitis B surface antigen. *American Journal of Epidemiology* **105**, 94.

Cayzer, I., Dane, D. S., Cameron, C. H. & Denning, J. V. (1974). A rapid haemagglutination test for hepatitis B antigen. *Lancet* i, 947.

Eleftheriou, N., Thomas, H. C., Heathcote, J. & Sherlock, S. (1975). Incidence and clinical significance of e antigen and antibody in acute and chronic liver disease. *Lancet* ii, 1171.

El Sheikh, N., Woolf, I. L., Galbraith, R. M., Eddleston, A. L. W. F., Dymock, I. W. & Williams, R. (1975). e antigen-antibody system as indicator of liver damage in patients with hepatitis-B antigen. *British Medical Journal* iv, 252.

Gibson, P. E. (1977). e antigen and antibody in outbreaks of hepatitis B in two renal dialysis units. *Journal of Clinical Pathology* **30**, 717.

Gibson, P. E. & Ruparelia, K. (1977). Occurrence of e antigen in acute hepatitis B. *Journal of Clinical Pathology* **30**, 925.

Grady, G. F. (1976). Relation of e antigen to infectivity of HBsAg-positive inoculations among medical personnel. *Lancet* ii, 492.

Green, H. T. & Turner, G. C. (1975). Subtypes of hepatitis B antigen among patients and symptomless carriers. *Journal of Hygiene* **74**, 123.

Hawe, B. J., Goldsmith, H. J. & Jones, P. O. (1971). Dialysis-associated hepatitis: prevention and control. *British Medical Journal* i, 540.

Hess, G., Nielsen, J. O., Arnold, W. & Meyer Zum Büschenfelde, K. H. (1977). e system and intrahepatocellular HBcAg and HBsAg in HBsAg-positive patients with liver diseases and healthy carriers. *Scandinavian Journal of Gastroenterology* **12**, 325.

Imai, M., Tachibana, F. C., Moritsugu, Y., Miyakawa, Y. & Mayumi, M. (1976). Hepatitis B antigen-associated deoxyribonucleic acid polymerase activity and e antigen/anti-e system. *Infection and Immunity* **14**, 631.

Lam, K. C., Tong, M. J. & Rakela, J. (1977). Release of e antigen from a Dane-particle-rich preparation of hepatitis B virus. *Infection and Immunity* **16**, 403.

Magnius, L. O. & Espmark, J. A. (1972). New specificities in Australia antigen positive sera distinct from the Le Bouvier determinants. *Journal of Immunology* **109**, 1017.

Murphy, B. L., Peterson, J. M., Smith, J. L., Gitnick, G. L., Auslander, M. O., Berquist, K. R., Maynard, J. E. & Purcell, R. H. (1976). Correlation between fluorescent antibody detection of hepatitis B core antigen in liver biopsies and presence of e antigen in serum. *Infection and Immunity* **13**, 296.

Nordenfelt, E. & Andren-Sandberg, M. (1976). Dane particle-associated DNA polymerase and e antigen: relation to chronic hepatitis among carriers of hepatitis B surface antigen. *Journal of Infectious Diseases* **134**, 85.

Nordenfelt, E. & Kjellen, L. (1975). Dane particles, DNA polymerase and e-antigen in two different categories of hepatitis B antigen carriers. *Intervirology* **5**, 225.

Okada, K., Kamiyama, I., Inomata, M., Imai, M., Miyakawa, Y. & Mayumi, M. (1976). e antigen and anti-e in the serum of asymptomatic carrier mothers as indicators of positive and negative transmission of hepatitis B virus to their infants. *New England Journal of Medicine* **294**, 746.

Parker, M. J., Goudie, R. B. & Shattock, A. G. (1977). Incidence of HBe antigen and HBe antibody in blood donors. *Lancet* ii, 770.

Perrillo, R. P., Gelb, L. D., Milligan, W. H., Wellinghof, W. & Aach, R. D. (1977). Discordant e antigen, DNA polymerase activity and DNA particle responses in two patients representing an index case-contact case pair with hepatitis B virus infection. *Journal of Infectious Diseases* **136**, 117.

Takahashi, K., Imai, M., Tsuda, F., Takahashi, T., Miyakawa, Y. & Mayumi, M. (1976). Association of Dane particles with e antigen in the serum of asymptomatic carriers of hepatitis B surface antigen. *Journal of Immunology* **117**, 102.

Thamer, G., Gmelin, K. & Kommerell, B. (1976). e antigen: prognostic marker in acute viral hepatitis B. *Lancet* ii, 577.

Trepo, C., Bird, R. G. & Zuckerman, A. J. (1977). Correlations between the detection of e antigen or antibody and electron microscopic pattern of hepatitis B surface antigen (HBsAg) associated particles in the serum of HBsAg carriers. *Journal of Clinical Pathology* **30**, 216.

Trepo, C., Vitvitski, L., Neurath, R., Hashimoto, N., Schaefer, R., Nemoz, G. & Prince, A. M. (1976). Detection of e antigen by immunofluorescence in cytoplasm of hepatocytes of HBsAg carriers. *Lancet* i, 486.

Turner, G. C. & White, G. B. B. (1969). S.H. antigen in haemodialysis-associated hepatitis. *Lancet* ii, 121.

White, G. B. B., Lasheen, R. M., Baillie, M. B. & Turner, G. C. (1971). Comparison of three serological methods for the detection of hepatitis-associated antigen. *Journal of Clinical Pathology* **24**, 8.