Symposium on Hepatitis
Chairman: H J GOLDSMITH, Liverpool, United Kingdom

There comes a time in the development of most branches of science and medicine when, through the advent of a new theory, a new laboratory finding or a new technique, instead of accumulating knowledge haltingly, our understanding advances suddenly.

The discovery of the Australia antigen and the subsequent realisation of its close relationship to Serum Hepatitis initiated such an advance in relation to our knowledge of this disease.

As we have heard earlier during this congress, Serum Hepatitis constitutes a serious and continuing risk to the work of all dialysis and transplant units. You will not need reminding that in the United Kingdom alone, six members of staff have died from hepatitis contracted whilst working with renal patients.

The time is therefore overdue to give this subject a serious airing and we are fortunate in having speakers who are experts on various aspects of the disease.

Dr Ogg has the distinction of being physician to the renal unit at Guy's Hospital, London, which to date has produced the largest epidemic of dialysis-related hepatitis in the United Kingdom. In spite of this, he remains cheerful and quite determined that nothing as small as a virus will ever get him down! Dr Ogg will now describe the outbreak at Guy's Hospital which, whilst mainly non-immune staff are available to look after infected patients, continues to claim the occasional staff victim.
Hepatitis at Guy's Hospital
C S OGG, M BEWICK, J S CAMERON, F G ELLIS
Guy's Hospital, London, UK

During the last three years there has been an epidemic of hepatitis in the
dialysis and transplant units at Guy's Hospital. The two units, which were
set up in 1966 and 1967, are closely integrated and, before the onset of the
epidemic, they were trying to provide a comprehensive service for the treat-
ment of patients with terminal renal failure. Patients were accepted initially
for hospital dialysis but were later offered transplantation or home dialysis.
Dialysis was conducted using standard techniques with Kil dialysers and
Dylade proportionating systems. Transplantation was largely from cadaveric
donors, the recipients being selected according to matching within the ABO
and HLA systems. By the beginning of 1968 a substantial number of patients
had been accepted who were relatively unsuitable for home dialysis and who
had proved difficult to match with a suitable graft. As a result the unit be-
came increasingly crowded, the ten bed stations were stretched to twelve,
overnight dialysis was carried out on four nights a week and day dialysis on
five days a week.

THE EPIDEMIC
In March, 1969 a nurse, who had been working in the unit for nine months
developed an acute illness with hepatitis. Her blood was not tested for
Australia Antigen and there were some atypical features about her illness,
however when the blood of our patients was screened in July positive tests
were obtained in three cases. At the same time a doctor and another nurse
became ill. By the end of the year eight more patients and seven previously
healthy subjects had developed evidence of infection, and it was clear that
an epidemic was established.

The origin of the infection has never been determined; a plasma sample
taken before the start of dialysis was available for Australia Antigen testing
in only one of the first three positive patients. This gave a negative result;
the other two patients had only started dialysis in February 1969 and it seems
improbable that the nurse who became ill in March had contracted her infection from this source. However, as far as it is known this nurse had not been exposed to infection from any other source. No symptomless carriers were identified amongst the staff but all of the patients had received blood from untested donors. When screening was introduced to the hospital’s small blood donor panel, 4 out of 750 symptomless subjects gave positive tests, but it has not been possible to demonstrate a link between these donors and our patients.

New cases continued to occur amongst the patients until August, 1971 by which time a total of 34 had become infected (Figure 1). Since then no more patients have developed signs of infection despite the fact that we have, once again, started to admit new patients.

![New cases of Hepatitis among patients in the dialysis and transplant units at Guy's Hospital](image)

**Figure 1**

The incidence of new cases amongst the staff (Figure 2) shows a similar pattern with the exception that we are still seeing sporadic cases. So far we know of a total of 44 cases. The vast majority have occurred amongst the dialysis nurses and at one time it seemed that a nurse working in the unit for more than a week or two was almost certain to become infected. Infection was largely introduced by direct inoculation of infected blood via a contaminated needle. Most nurses recalled such incidents, but infection also occurred via other routes; two nurses who were on the ward for one and seven days respectively never treated a patient. All of the medical staff were involved with shunt care and had declotted the shunts of infected patients. In addition six had been involved with operations on these patients. Infection amongst laboratory personnel partly reflected overcrowded accommodation and partly clumsy laboratory techniques. Thus one technician ingested infected blood while mouth pipetting a blood sample. Only two of these cases, who were involved in the enhancement research
programme, could have been infected by direct inoculation of blood.

The 18 secondary cases are of special interest; 16 occurred amongst contacts of patients, and 2 amongst contacts of infected members of staff. Amongst the patients’ contacts 9 were actively involved in the home dialysis routine but the remainder were not, and it looks as if at least 9 of these secondary cases were infected by a route other than direct inoculation of infective material. Thus there is evidence to suggest that something like 20% of our cases were not infected in this way.

THE ILLNESS

It has been difficult to calculate the incubation period accurately. Amongst the staff, at least, it would seem to be about three months and it is certainly not as short as the two month period found in Edinburgh. The patterns of illness amongst the patients and the previously healthy subjects were quite different.

Healthy subjects

The majority of the staff had a prodromal illness with an urticarial rash and pains in the joints – usually of the hands. After a week this was usually followed by an acute illness with anorexia, jaundice and pain in the epigastrium and right hypochondrium. This was associated with gross elevation of serum bilirubin and enzyme levels. This acute clinical illness usually lasted 3-4 weeks, although enzyme levels often remained raised for six or eight weeks. Following the acute illness there was almost invariably a prolonged period measured in months during which the patient remained irritable and
easily tired. Most stayed off work for 3-4 months although this varied from six weeks to nine months. No deaths occurred, although one nurse developed hepatic precoma and was treated with two exchange transfusions. No staff member had two acute attacks. However, one nurse who had a typical acute illness with a positive test for Au antigen had had, eighteen months earlier, a prolonged low grade illness with minor enzyme changes and a negative antigen test. Blood was tested for Au antigen using a gel diffusion technique at the onset of the illness in 35 of the previously healthy subjects. Positive results were obtained in 25. No positive tests were obtained more than eight weeks after the start of the illness.

Patients

The clinical picture was identical in the dialysis and transplant patients. Only three out of 34 patients had obvious clinical attacks with jaundice. The remainder had either no illness at all, or a mild illness with general fatigue. The majority were simply found to be Au antigen positive and showed either no biochemical abnormalities or minor elevations of serum enzyme levels. Two dialysis patients were suspected on clinical grounds of having hepatitis, but had negative tests for Au antigen. One was a child, and may have had infectious hepatitis while the nature of the illness in the other remains obscure, although for administrative reasons he is regarded as having had hepatitis. Positive tests for Au antigen have tended to persist. Only 4 out of 22 dialysis patients and none of the transplant patients have reverted to negative during periods of observation ranging from 1-36 months. At present 14 patients who have been involved in our epidemic are alive and on dialysis. Ten of these have positive tests for Au antigen; and in addition we have nine patients with functioning renal transplants and persistent positive tests for Au antigen. No patient has died as a direct result of hepatitis; none have so far sustained permanent liver damage although, because of the risk of spreading the infection, this assessment has necessarily been superficial. We have not, for example, done liver biopsies.

CONTROL OF THE EPIDEMIC

Since the start of the epidemic numerous attempts have been made to control the spread of infection. Initially, because of overcrowding in the unit, there were insufficient dialysers for each patient to have his own. Even after this was rectified, new cases continued to develop at the same rate, possibly due to contamination of dialysers during breakdown and assembly and possibly to the use of a small pool of spare dialysers for use in the event of failure of a patient's own dialysers. Only disposable dialysers have been used in the hospital since August 1970, and it seems likely that this policy change has played a major part in the control of the infection. In view of the unit's role
as a home dialysis training centre, flat bed dialysers rather than coil
dialysers were selected so that transfer to the Kiil in the home would be
facilitated.

The second major factor in the control of the epidemic has been the pro-
vision of separate dialysis facilities for infected and non-infected patients.
Until August 1971 this consisted simply of allocation of three Dylade machines
at one end of the unit for the exclusive use of infected patients. However,
for the last nine months we have been able to dialyse the two groups of
patients in separate parts of the hospital. Since then we have had no further
cases of hepatitis amongst the patients, although two cases have occurred
amongst the staff.

A number of other measures may have helped. Initially, we attempted
to transplant all the infected patients, but abandoned this policy because of
infections amongst the surgical team, ward and theatre nursing staff, and
laboratory technical staff. All patients were therefore transferred to home
dialysis, the majority using arterio-venous fistulae. Unfortunately, many
of these patients are our original hospital dialysis patients and are least
willing and able to use their home units. Furthermore, their fistulae, con-
structed usually in limbs that have previously carried many shunts, are not
always as satisfactory as we might have hoped. Therefore few weeks pass
when we are not dialysing the occasional infected patient in hospital.

Laboratory investigations are restricted to an absolute minimum and
amongst the infected dialysis patients are virtually restricted to measure-
ments of the haemoglobin level. Transplant patients, however, require
much more extensive supervision. Dialysis patients are hardly ever trans-
fused, and then only with screened blood. Again, however, transplantation
destroys this policy – especially when things go wrong. All patients and
staff working with them are screened regularly for the Au antigen. Although
administratively attractive, it is impossible to provide separate medical,
nursing and technical staff for the care of clean and infected patients. Staff
change clothes completely after attending an infected patient, and, where
possible, a nurse does not dialyse a clean patient within 24 hours of dialys-
ing an infected one. This policy can be applied most of the time but breaks
down sometimes at night, at weekends and especially during staff shortages.
It seems likely that there will continue to be new cases amongst the staff
and that this will impose a permanent hazard to the clean patients. However,
there are grounds for hoping that this risk is relatively small; most of the
staff treat other patients who are not on the regular dialysis programme
and who may be suffering from acute renal failure, or a variety of other
renal or non-renal disorders. So far none of these patients has been known
to have contracted hepatitis, nor have dialysis patients from our home pro-
gramme or from other dialysis units who have been admitted to hospital
for treatment of incidental illness or for renal transplantation.

AVOIDANCE OF A FURTHER EPIDEMIC

Clearly the clean unit will remain indefinitely at risk of re-infection both from the infected unit and from the usual sources. It seems essential therefore to modify our routine so that an explosive epidemic cannot start again.

Avoidance of overcrowding is of fundamental importance and we have decided not to accept patients for haemodialysis unless there is a reasonable prospect that they can be established on home dialysis within the incubation period of the disease (ie within about 10 weeks). This means that home modifications are being undertaken while the patient is still being managed conservatively or while he is being held on peritoneal dialysis. Transplantation will generally be carried out on patients established on home dialysis, but if the patient does not have a suitable home he will be held on peritoneal dialysis until a graft is obtained. This solution is obviously far from ideal as these patients seem to tolerate the complications of transplantation particularly badly.

We will continue to use disposable dialysers in hospital and aim to retain a facility for dialysis of infected patients indefinitely. We are particularly worried about transmission of infection occurring via an infected venous pressure monitor and now use isolators on the tube between the bubble catcher and the alarm.

ETHICAL PROBLEMS

One of the factors which has probably contributed to the control of this epidemic has been the restriction of entry of patients to the dialysis programme. During 1968 and 1969 we accepted a total of 62 patients, but during 1970 and 1971 we admitted only 9 patients. Since the beginning of this year we have admitted 7 patients. This is at a time when only a fraction of the patients in the UK who require RDT are being offered it. Due to the collaboration of our colleagues in other London units no ideal patients are known to have been rejected for treatment. However, many of our own suitable but relatively less perfect patients were not accepted, and we suspect that the squeeze was applied indirectly in areas of the UK that are not as well serviced as the area around London. It is therefore probably true to say that although no-one has dies as a direct result of our epidemic, perhaps as many as 50 or 100 patients have died as an indirect result. We have also been concerned about the morality of applying pressure to infected patients to transfer from hospital dialysis to home dialysis with the implication that it is better for them to infect their relatives than infect other patients and hospital staff. Our justification has been that near relatives would probably contract the infection wherever the patient was dialysed; this may well be
true as cases have occurred amongst the assistants of about half our infected home dialysis patients and amongst the families of a similar proportion of the infected transplant patients.

The position with regard to the insurance of staff is a major problem. Provided sufficient national insurance contributions have been paid, staff may receive industrial accident benefit in addition to sickness benefit. However, there has often been considerable delay in the payment of industrial accident benefit and in any case the total sum is only about half what a nurse normally receives. In the past, the hospital has made up this difference to those nurses employed by the hospital, but has not done so to nurses employed via nursing agencies. Several of these girls have not had private sickness insurance and have been many hundreds of pounds out of pocket. This problem has been particularly severe with nurses who had recently arrived in the UK from overseas and had not paid sufficient national insurance contributions to qualify for state assistance.

The statistical survey of the results of dialysis and transplantation in Europe given by Dr Brunner make it clear that the incidence of hepatitis in dialysis units is increasing steadily. Nearly half the units contributing to the survey have had cases and it seems almost inevitable that many others will soon become involved. Probably the most important factor contributing to the spread of the epidemic was our initial failure to isolate the infected cases because of a lack of a separate dialysis facility. The infection was not controlled until the patients were separated and it seems clear that isolation facilities must be available before they are needed, so that once an infected patient is recognised he can be isolated immediately.

REFERENCE


GOLDSMITH: Thank you, Dr Ogg. All of us will sympathise with your difficulties, many of us from personal experience.

Dr Cossart works as a Virologist at the Central Public Health Laboratory, Colindale, London. The Public Health Service keeps a watchful eye on infective diseases in England, and through its regional laboratories can give early warning of new epidemiological trends. Dr Cossart has made a special study of the immunological, electronmicroscopic and diagnostic aspects of hepatitis and its related antigen. I am sure that we will find her paper of great interest.
Australia Antigen and Hepatitis in Renal Units

YVONNE E COSSART
Central Public Health Laboratory, London, United Kingdom

The factors which may cause liver damage in individual renal dialysis and transplant patients are multitudinous and range from the toxicity of drugs or of chemicals leached from dialysis tubing (Neergaard et al, 1971) to infection with the hepatitis viruses, or with other agents such as cytomegalovirus or EB virus. However, most of the outbreaks of hepatitis in renal units which have involved such large numbers of patients and staff in many countries have been due to serum hepatitis (Polakoff et al, 1972).

The presence of Australia antigen in the serum has proved to be a very useful marker of the viraemic phase of this disease and other contributors to this symposium have described its use, both in the diagnosis of infection and in the prevention of outbreaks. The fundamental nature of Australia antigen remains obscure, but as more becomes known about its properties and its relation to the pathogenesis of hepatitis it may become possible to adopt a more active approach to the problem in renal units.

Australia antigen consists mainly, if not entirely, of protein (Gerin et al, 1969) and has a specific gravity of about 1.2 g/cm$^3$ (Gerin et al, 1971) and an electrophoretic mobility in the $\alpha_2$ globulin range (Kim & Tilles, 1971). Its most striking feature is its particulate morphology (Bayer et al, 1968) which is sufficiently characteristic to be useful as a diagnostic test. Three particle types are recognised. The most plentiful are approximately spherical and about 16–22 nm diameter. In most instances long forms are also present. These also have a diameter of about 20 nm but vary greatly in length. Some have expanded ends, some are bent and many exhibit cross striations which have a periodicity of 3.5 nm (Almeida et al, 1969). The third particle type is the double shelled or 'doughnut' form which is about 45 nm in diameter (Dane et al, 1970). These are present in about one-third of sera examined (Cossart & Field, 1970). When a specific antiserum is added to a preparation containing all three particle types all are included in the resulting complexes (Almeida et al, 1969). This suggests that they are all composed of the same material, and recently it has been shown that antisera raised against preparations of the individual particle types also reacts with the others (Bond & Hall, 1972).

This immune complexing of particles provides evidence for identifying Australia antigen by the electronmicroscope, and incidentally makes mechanical searching of the preparations much easier.
Immune complexes of Australia antigen and antibody sometimes occur in vivo and it has been suggested (Almeida & Waterson, 1969) that they play a role in causing liver damage both in acute hepatitis and in chronic liver disease. These clumps which may also be found in asymptomatic carriers (Field, 1971) may be hard to detect by serological tests if they are too large to migrate through the gels in diffusion and electrophoresis tests. They may also give rise to anticomplementary reactions in the complement fixation test, although this is inconstant (Cossart et al, 1971).

Although the Australia antigen particles are about the same diameter as that expected for serum hepatitis virus from filtration studies, and their appearance is very 'virus-like', they are unlikely to be the agent itself because their density of about 1.2 g/cm³ is considerably less than would be expected for a nucleic acid-containing virus of these dimensions. The variation in size is also rather too large for a true icosahedral virus. Thirdly, the concentration of Australia antigen particles in the serum may reach $10^{13}$ particles per ml and this far exceeds that known to occur in any vireaemia. Infectivity titres of such sera for man are of the order of $10^6$ I.D. 50 per ml. At present it seems probable that Australia antigen is a reconstitution product of excess viral protein around polyanions such as phospholipids in the serum (Cossart, 1972). Particles showing all the morphological features of Australia antigen have been constructed in vitro from the protein subunits of some plant viruses (Bancraft et al, 1969) whose infective particles are spherical and about 27 nm diameter. Recently it has been shown that the double-shelled particles of Australia antigen can be disrupted by mild detergent treatment to release the internal component component (Almeida et al, 1971) and it is claimed that these 'cores' are agglutinated specifically by the serum of patients who have recovered from serum hepatitis. So far this reaction has only been observed in the electronmicroscope, and stable preparations of the core antigen reactive in conventional serological tests must be made before this reaction can be applied on a clinically useful scale.

Thin sections of the liver in serum hepatitis and in asymptomatic Australia antigen carriers frequently show masses of small (24 nm diameter) spherical virus-like particles packing the nuclei of parenchymal cells (Scotto et al, 1971; Huang, 1971) and it seems very likely that these are indeed the virus particles. Their situation in the nucleus indicates that they probably contain DNA. It is hoped that this technique may provide evidence of the propagation of serum hepatitis in tissue culture, but so far there has been little progress in this field.

The pathogenesis of serum hepatitis is also poorly understood. In 'normal' persons, inoculation or oral ingestion of ieterogenic serum is followed by an incubation period which may be as short as 4 weeks or as long as 6 months. The length does not seem closely correlated either with dose or with virus
'strain'. The first symptoms are often allergic in type, e.g. joint pains and rash. These and the constitutional disturbance tend to disappear as jaundice develops. Recovery after an illness of several weeks is the rule, but a very small proportion (probably far less than 1%) develop chronic liver disease.

Australia antigen and infectivity appear in the serum before symptoms appear, the interval is usually only a few days, but it may be many weeks.

The natural route of virus excretion is unknown and studies of urine (Apostolov et al., 1971) and faeces (Grob & Jemelka, 1971; Gust et al., 1971) for the presence of Australia antigen have been inconclusive. However, there is considerable epidemiological evidence of spread of serum hepatitis in the community unassociated with parenteral inoculation (Prince et al., 1969; Cossart & Vahrman, 1970; Hersche et al., 1971).

The titre of Australia antigen in the serum reaches a peak at about the time of onset of clinical illness and declines fairly quickly during convalescence (Prince, 1968). The persistence of antigaeminaemia is probably related to continuing activity of infection (Johnston et al., 1971).

In individuals with impaired immunity the duration of all three phases of infection may be greatly prolonged, but the clinical severity of the illness tends to be very much less than in normal persons. Moreover, the titre of Australia antigen and probably also of infectivity in the blood tends to remain very high for months or years. In regular dialysis patients Australia antigen has been found in the urine during transplant rejection (Blaney et al., 1971) and in pleural and peritoneal (Kaboth & Mietzsch, 1971) exudate. These fluids are then presumably infective.

Neither in normal or in immunodeficient persons is antibody to Australia antigen regularly produced after infection, although immunity to reinfection is produced (Havens et al., 1945). This seems remarkable in view of the amounts of 'foreign' protein which circulate during the acute phase of illness and has not been unexplained. Anti-Australia antibody is, however, found in a proportion of individuals who have received many blood transfusions and also in a very small number of healthy persons. It has been claimed that more sensitive tests such as passive haemagglutination (Vyas & Shulman, 1970) and radioimmunoassay (Walsh et al., 1970) do demonstrate the production of anti-Australia antibody during convalescence from serum hepatitis (Lander et al., 1971a) and in about 15% of healthy adults (Lander et al., 1971b). Unfortunately, complete confirmation of these claims is not yet forthcoming. In our hands passive haemagglutination has not proved useful either in diagnosis of infection or in prediction of susceptibility in an outbreak (Gibson & Cossart, unpublished).

The timing and the techniques of screening for Australia antigen in renal unit patients and staff must be chosen on the basis of our rather inadequate knowledge of the properties of the antigen and the pathogenesis of serum
<table>
<thead>
<tr>
<th>Test</th>
<th>Laboratory Feature</th>
<th>Suitable for mass production</th>
<th>Speed</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gel</td>
<td>simple and economical of reagents</td>
<td>Yes</td>
<td>Several days</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Electrophoresis</td>
<td>simple but needs special equipment. High reagent use</td>
<td>Yes</td>
<td>Several hours</td>
<td>Medium</td>
<td>Medium</td>
</tr>
<tr>
<td>CFT</td>
<td>Standard test but needs skill. High reagent use</td>
<td>?Yes</td>
<td>2 days</td>
<td>High</td>
<td>Medium</td>
</tr>
<tr>
<td>EM</td>
<td>Highly specialised operation and expensive machines (microscope and ultracentrifuge)</td>
<td>No</td>
<td>Several hours</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Latex</td>
<td>messy but standard test. Reagents expensive, best obtained commercially and shelf life unknown</td>
<td>Not in present form</td>
<td>Several minutes</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Passive HAA</td>
<td>Difficult. Reagents difficult to prepare and needs expensive machinery (ultracentrifuge). Reagent life short</td>
<td>Not in present form</td>
<td>1 day</td>
<td>High</td>
<td>Not known</td>
</tr>
<tr>
<td>Radioimmuno assay</td>
<td>Difficult. Reagents as for HAA and test needs expensive scintillation counter</td>
<td>Not in present form</td>
<td>2 days</td>
<td>v. high</td>
<td>Not known</td>
</tr>
</tbody>
</table>

hepatitis. Each of the tests available has special features, making it more suitable in some situations than in others (Table I).

The original description of Australia antigen (Blumberg et al, 1965) and much subsequent work has used the gel diffusion and the conventional medium is 1% agarose in 'tris' buffered saline. The test is simple to perform and the most economical of reagents. Its greatest advantage is that serological identity is demonstrated with the controls but a considerable amount of antigen is needed to produce a visible precipitin line and the test is relatively insensitive. Precipitation is also rather slow and weak reactions may take several days to develop.

In countercurrent (or 'immune') electrophoresis, the antigen and antibody are forced into contact and precipitation occurs more rapidly. Results are obtained in about 2 hours, and visible lines are formed with lower concentrations of antigen. A 4 to 8-fold increase in sensitivity results, but more reagents are used, and because of the electrophoretic heterogeneity of Australia antigen it is not easy to obtain proof of serological identity.
The complement fixation test is 10 to 40 times as sensitive as the gel diffusion test. It uses about the same amount of antiserum as electrophoresis but overnight fixation is necessary to obtain maximum sensitivity. It does not afford a ready means of checking serological identity. Jaundiced sera are frequently anticomplementary and the possibility that immune complexes may be present in the sample must then be eliminated. Prozone may occur in both the CFT and electrophoresis tests when high concentrations of antigen are present and can produce erroneous negative results. It does not occur in the gel diffusion test.

Electronmicroscopy has proved extremely useful in confirming doubtful serological results. Its sensitivity is approximately equal to that of the CFT but its value resides mainly in its independence of the serological reagents. The expense and complexity of the machine and the time taken to perform a single test limit its application to research projects and checking unexpected serological results.

Recently various commercial firms have introduced latex preparations to which anti-Australia antibody has been coupled (e.g. Leach & Ruck, 1971). Agglutination rapidly occurs in the presence of antigen, but the usefulness of these tests is greatly limited by the large number of false positive results encountered. There is considerable variation between different preparations even from the same source, and little is known about the stability of the latex suspension. The sensitivity of the latex test is also about the same as that of the CFT. So far at the Virus Reference Laboratory, 17 specimens have been examined by electronmicroscopy which were latex positive but negative by gel and electrophoresis. Only one contained Australia antigen particles.

Both the passive haemagglutination test and radioimmunoassay methods can be adapted to measure Australia antigen rather than antibody. Both are more sensitive than the CFT (Hollinger et al, 1971) or electronmicroscopy, but apart from their expense and complexity these tests produce 'positive' results which cannot be checked by alternate methods and will be difficult to interpret until much more experience has accumulated.

Taking all these technical factors into account the choice of test for routine screening lies between electrophoresis and the CFT as both offer reasonable speed and sensitivity. However, both these tests may miss very high concentrations of Australia antigen, as well as very low ones, so in my laboratory all specimens are also tested by gel diffusion. The high levels tend to occur around the time of onset of acute hepatitis, but they are also not uncommon in immunodeficient carriers. Low levels occur during both the prodromal and convalescent stages of acute hepatitis, but in these circumstances the clinical course suggests its presence. Much more rarely antigen may persist at very low level for many months in the serum of a healthy person or an immunosuppressed patient, only to be found because of
a special search when contacts become infected. The amount of antigen present tends to fluctuate and frequent testing, which may chance on a time when the concentration is detectable by the screening test, seems to be the best method of picking up these individuals.

Our dependence on the electron microscope to demonstrate the morphology of Australia antigen particles in difficult cases is likely to decrease as the antigenic structure is worked out. Heterogeneity has been recognised for some time (Le Bouvier, 1971) but it has been believed that the presence of a common antigen in all the subtypes made antigenic variation relatively unimportant in laboratory diagnosis.

As the supply of human 'hyperimmune' antiserum diminishes due to the effectiveness of antigen screening in blood banks, more work is being done with animal sera and human sera from healthy blood donors. Both may exhibit restricted specificity and very much larger and better defined panels of antigens will be needed in future to test reagents intended for screening programmes.

The length of the incubation period of serum hepatitis means that screening of patients and staff on entry to a dialysis unit is inadequate and a system of regular testing must be adopted if antigen positive individuals are to be detected and isolated before infection of other patients can occur.

This policy can greatly reduce the problem of hepatitis in renal units, but only if it is applied systematically to cover all patients and staff as well as all the possible routes of entry of infection (Polakoff et al, 1972). Even if this system is perfectly applied, some patients will become infected by the natural routes operating in the general community, and chronic carriers will continue to be the source of infection in renal units. As yet there is no treatment which will influence the duration of antigen carriage. The injection of anti-Australia antibody might be considered were it not for the danger of producing severe or even fatal reaction because of the production of circulating immune complexes.

Passive immunoprophylaxis of serum hepatitis has been attempted using pooled normal human immunoglobulin in dialysis units and in other situations (Polakoff et al, 1972; Drake et al, 1953; Co-operative study, 1970). It seems to be ineffective.

More recently 'specific' immunoglobulin has been produced from blood donations found to contain anti-Australia antibody. Early reports (Holland et al, 1969; Prince et al, 1971) suggested that it was more likely to be effective against infection following accidental inoculation of a small amount of serum than against transfusion of a unit of antigen positive blood. Soulier et al (1972) have, however, had encouraging results even in this situation.

We have seven patients under observation who have been given specific immunoglobulin after accidental inoculation of Australia antigen positive
material. So far none has developed hepatitis or antigenemia, whereas probably about half would be expected to be affected if no treatment had been given. A recent paper (Alter et al., 1972) has pointed out that there is a danger that specific immunoglobulin treatment may suppress clinical serum hepatitis, but allow chronic hepatitis to develop. For this reason it seems unwise to attempt a trial of regular administration of antibody to patients or staff even where sufficient supplies of specific immunoglobulin are available. The risk of producing 'complex disease' in an antigen positive recipient of the material means that an individual should be tested and the immunoglobulin administered with the minimum of delay after inoculation or ingestion of antigen positive serum.

Active immunisation against serum hepatitis is even more experimental. Krugman et al. (1970, 1971) have shown that heat-treated (98°C for 1 min) Australia antigen positive serum is greatly reduced in infectivity and produced considerable resistance to infection following subsequent injection of the same serum which had not been heated.

Soulier et al. (1972) have shown that serum heated to 60°C for 10 hours also has greatly reduced infectivity and injection is followed by a measurable antibody response. No tests of protection have been made by this group. It is to be hoped that these findings may lead to the development of an inactivated vaccine, but until a non-human assay of infectivity becomes available the difficulties are formidable.

REFERENCES

Alter, H. J., Holland, P. V., Schmidt, P. J. and Plotz, P. H. (1972) Lancet, 1, 1110
Bond, H. E. and Hall, W. T. (1972) Journal of Infectious Diseases, 125, 263
Cossart, Y. E. and Field, A. M. (1970) Lancet, 1, 848
Cossart, Y. E. and Vahrman, J. (1970) British Medical Journal, 1, 403
Huang, S. (1971) American Journal of Pathology, 64, 483
Johnston, D. J., Powell, N. and Gitnick, G. (1971) California Medicine, 114, 14
Kaboth, U. and Mietzsch, G. (1971) German Medical Monthly, 1, 139
Le Bouvier, G. L. (1971) Journal of Infectious Diseases, 123, 671
Prince, A. M. (1968) Proceedings of the National Academy of Sciences of the United States of America, 60, 814

GOLDSMITH: Thank you Dr Cossart. Your studies illustrate that we may soon hope to achieve greater diagnostic accuracy and a better definition of immunity against this disease.

Dr Carbonara works as immuno-geneticist in the Department of Genetics at the University of Turin. For many years he has made serological studies of immunological pleomorphism in relatively isolated communities. Thus he had available, for further study, sera dating back to 1964. Using these and other sera, he has been able to formulate a genetic theory of the hepatitis carrier state, confirming one put forward earlier by Blumberg. He will now tell us about his work.

242
Influence of Environment and Genetic Factors on Carriage of the Australia Antigen

A O CARBONARA
Istituto di Genetica Medica, Dell ' Universita di Torino, Italy

In these few minutes I would like to summarize data concerning the influence of environmental factors and genetic factors in relation to the presence of hepatitis associated antigen, also called Australia (Au) antigen, in different categories of disease (Blumberg et al, 1970).

Table I shows how different clinical conditions share the presence of Au antigen in the serum; I have classified them into four categories on the basis of clinical and serological data.

<table>
<thead>
<tr>
<th>Group</th>
<th>Transfusion of Au</th>
<th>Persistence of Au, %</th>
<th>Frequency SGPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Acute hepatitis:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-transfusion</td>
<td>+</td>
<td>-</td>
<td>58 +++</td>
</tr>
<tr>
<td>Infections</td>
<td>-</td>
<td>-</td>
<td>38 +++</td>
</tr>
<tr>
<td>II Chronic active hepatitis</td>
<td>±</td>
<td>+</td>
<td>10 ++</td>
</tr>
<tr>
<td>III Chronic anicteric hepatitis:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Down's syndrome</td>
<td>-</td>
<td>+</td>
<td>30 +</td>
</tr>
<tr>
<td>Leukemia</td>
<td>±</td>
<td>+</td>
<td>10 +</td>
</tr>
<tr>
<td>Lepromatous leprosy</td>
<td>-</td>
<td>+</td>
<td>20 +</td>
</tr>
<tr>
<td>Chronic renal dialysis</td>
<td>±</td>
<td>+</td>
<td>10 +</td>
</tr>
<tr>
<td>IV Normal Carriers:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apparently normal people</td>
<td>-</td>
<td>+</td>
<td>1-20 +</td>
</tr>
<tr>
<td>(Cebu, Japan, Rougelap, Sardinia)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. The first group consists of classical acute hepatitis following infection or transfusion. In this case the antigen appears in the blood within a few days of infection and in most cases will disappear as the disease subsides.
2. The second group is represented by persistent clinical viral hepatitis; in
these cases the antigen may persist in the blood for months or even years; and only the clinical evolution will allow a diagnosis of chronic aggressive or chronic persistent hepatitis. 3. The third category consists of patients affected by unrelated diseases in which a common pattern is the occurrence of the antigen in the serum for a long period. However, these patients do not show clinical and laboratory data suggesting impairment of liver function. The group includes Down's syndrome, lymphocytic leukaemia, Hodgkin's disease, lepromatous leprosy and chronic renal failure treated by regular haemodialysis.

Some of these patients may have been infected by transfusion (as in the case of leukaemia and renal disease), but others have not received transfusion or needle injections at an appropriate time. 4. The fourth group consists of a large number of people who have persistent Australia antigen and no clinical evidence of disease. These individuals are also called 'carriers of Au antigen'. Blumberg et al (1966) originally described a high frequency of Au antigen carriers in tropical countries and other areas where sanitation is poor.

In Italy we have tested a large number of sera belonging to different populations (Table II). Our results show that antigen carriage is particularly high in Sardinians, where the frequency ranged from 3 to 12%. Those aged under 25 have remained carriers for 8 years. This high percentage has been confirmed in three independent surveys of the Sardinian population. These observations suggest that the finding is not linked to some epidemic episode nor limited to a small geographical area (Ceppellini et al, 1970).

<table>
<thead>
<tr>
<th>Table II. Frequency of the Au antigen in different 'normal' populations of Italy</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
</tr>
<tr>
<td>-----</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>1. Italian Army</td>
</tr>
<tr>
<td>2. Torino: blood donors</td>
</tr>
<tr>
<td>3. Ferrara</td>
</tr>
<tr>
<td>4. Sardinians:</td>
</tr>
<tr>
<td>(a) from Sardinia (1964-1967)</td>
</tr>
<tr>
<td>age 0-20</td>
</tr>
<tr>
<td>age &gt; 21</td>
</tr>
<tr>
<td>(b) from Torino (1969)</td>
</tr>
<tr>
<td>age 0-20</td>
</tr>
<tr>
<td>age &gt; 21</td>
</tr>
<tr>
<td>(c) Cagliari (1970)</td>
</tr>
</tbody>
</table>

The relationship between the Australia antigen and the hepatitis B virus is not completely clarified, as Dr Cossart has indicated; however, there is
a general agreement that presence of Australia antigen in the serum reflects previous contact with the same viral agent.

If this is correct we are dealing in our classification with acute hepatitis, chronic persistant hepatitis and a chronic anicteric carrier condition with clearcut host differences in response to a specific infectious agent.

In other words, the presence and the clinical evolution of the disease depend not only on the infecting agent, but also on the host and the interaction between them. Thus, when different people are infected with the same agent, some may get a severe form of the disease, some a mild form. In the RDT population for instance, this different host-virus interaction is particularly evident (London et al, 1969).

Due to environmental conditions, both the patients and the medical staff can be infected by the same viral agent. The clinical course of infection is in general very different. In the patients, when hepatitis develops the attack is mild, anicteric and often followed by persistence of Australia antigen in the serum.

In the medical staff, however, the clinical course is that of a typical acute hepatitis with rapid complete clearance of antigen from the blood.

What is the explanation for these different host-virus interactions? One hypothesis suggests the predominant importance of the cellular and humoral immun response (Dudley et al, 1972); in most cases the immunocompetent T lymphocytes react with the viral antigens present on the surface of liver cells leading both to necrosis of liver cells and to destruction of the infective agent. This basic mechanism could explain the clinical course and the serological data on classical acute hepatitis.

However, in individuals with an impairment of cellular immune response, the results will be completely different. Due to the absence of reaction between lymphocytes and viral antigens the liver cells will not be damaged and viral antigen could persist and proliferate in the organism. If the impairment of cellular immunity is very severe we can expect a clinical situation like that observed in the healthy carrier patients; if the impairment is only a quantitative one then we will have the clinical course of chronic hepatitis with a continuous process of liver cell damage and persistence of viral antigens.

This last situation could explain the chronic anicteric hepatitis often observed in the patients with Down's syndrome, with leukaemia or with chronic renal disease.

A clear impairment of the functions of immunocompetent thymus-derived lymphocytes has been established in all these patients by many tests, such as blast transformation following PHA stimulation, survival of skin homografts and skin tests to various antigens (Dammim et al, 1957; Wilson et al, 1965). In addition, the patients have also a decreased humoral antibody
response following immunisation with different antigens.

If this hypothesis is correct it would be very interesting to investigate the genetic basis of these individual immune response differences. The studies performed by Blumberg et al (1969) and our group (Ceppellini et al, 1970) on populations with a high frequency of (so called) 'healthy' Au antigen carriers clearly suggest that genetic factors must be relevant in determining the Au carrier condition.

Table III. Segregation of Au antigen in families

<table>
<thead>
<tr>
<th>Location</th>
<th>Type of mating</th>
<th>No of families</th>
<th>Recessive Obs.</th>
<th>Recessive Exp.</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cebu</td>
<td>Au(+) x Au(-)</td>
<td>7</td>
<td>12</td>
<td>12.189</td>
<td>0.010</td>
</tr>
<tr>
<td>Bougainville</td>
<td>&quot;</td>
<td>24</td>
<td>42</td>
<td>40.062</td>
<td>0.341</td>
</tr>
<tr>
<td>Sardinia</td>
<td>&quot;</td>
<td>7</td>
<td>14</td>
<td>14.570</td>
<td>0.048</td>
</tr>
<tr>
<td>Cebu</td>
<td>Au(-) x Au(-)</td>
<td>24</td>
<td>33</td>
<td>32.705</td>
<td>0.010</td>
</tr>
<tr>
<td>Bougainville</td>
<td>&quot;</td>
<td>41</td>
<td>56</td>
<td>53.815</td>
<td>0.412</td>
</tr>
<tr>
<td>Sardinia</td>
<td>&quot;</td>
<td>34</td>
<td>52</td>
<td>56.471</td>
<td>0.527</td>
</tr>
</tbody>
</table>

We have performed in the Sardinian population a classical genetic analysis (Table III); this means

(1) select the segregating families for Au carriage; that is family with at least a positive parent or a positive child.

(2) separate these segregating families into matings of positive x negative and negative x negative.

(3) test by appropriate statistical method if the expected and observed number of Au positive children fits with the hypothesis of a recessive trait. In other words, in mating positive x negative we expect to find 50% of positive children and in mating negative x negative we expect to find 25% of positive children.

Table III shows that in the three population studies the frequency of Au+ re children in both types of matings fits well with the frequency expected on the basis of a recessive monofactorial trait.

These results do not constitute definitive proof that the Au carrier condition is a monofactorial trait, like albinism or phenylketonuria. Many situations determined by contribution of multiple genes, (that is polyfactorial traits) can mimic a pattern of simple Mendelian inheritance. The only significant conclusion that can be derived is that the condition of healthy carriers of Australia antigen represents an individual mode of reaction, controlled to a significant extent by the gene type, perhaps through a genetically determined abnormal response of the immune system.
REFERENCES

Dammin, G. J., Couch, N. P. and Murray, J. E. (1957) Annals of the NY Academy of Science, 64, 967

Goldschmidt: Thank you Dr Carbonara for this stimulating paper. In the light of your findings, many of us will wish to look again at the pattern of secondary spread of hepatitis in the families of our patients.

Dr Polakoff works as epidemiologist at the Central Public Health Laboratory, Colindale, and for the past four-and-a-half years she has made a prospective study of hepatitis outbreaks in most of the British dialysis units, which will be published shortly. Today she will give us a summary of her findings.

The Epidemiology of Serum in Dialysis Units
SHEILA POLAKOFF
Central Public Health Laboratory, London, United Kingdom

The outbreak described by Dr Ogg was the largest ever noted in a dialysis unit in the United Kingdom. The information provided by the outbreak situation is, of course, of great epidemiological value but in order to have a balanced picture an account is also needed of the situation in dialysis units as a whole in any country.

I should like to give a very brief account of the results of studies made
since 1968 by consultants in charge of almost two-thirds of the dialysis units in the United Kingdom in collaboration with the British Public Health Laboratory Service and some hospital microbiologists.

In 1968, of 20 units only one had an outbreak. This had been in progress since 1966. In 1969, however, outbreaks began in two more of the 20 units and the unit at Guy's joined the survey after their outbreak began. Thus out of a total of 21 units, 4 were affected by outbreaks in 1969. Facilities for testing for Australia antigen (a laboratory marker of serum hepatitis) became available at the Central Public Health Laboratory in the summer of 1969. I shall not go into details of testing as my colleague Dr Cossart has dealt with this aspect.

Table I. Haemodialysis units with and without outbreaks or sporadic cases of hepatitis according to the results of gel diffusion tests for Australia antigen made in July 1969

<table>
<thead>
<tr>
<th>Units from which specimens were examined</th>
<th>Units in which Au/antigen was detected in one or more specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>With outbreaks beginning or continuing in 1969</td>
<td>4</td>
</tr>
<tr>
<td>With sporadic hepatitis at some time in 1969</td>
<td>4*</td>
</tr>
<tr>
<td>Without hepatitis throughout 1969</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
</tr>
</tbody>
</table>

*One unit had eight patients with abnormal results of liver function but only one had clinical hepatitis

In July 1969, 18 of the 21 survey units supplied sera for testing (Table I). The antigen was detected in the serum of one or more patients in each of four units. In these four units outbreaks of hepatitis were either in progress or began soon afterwards. The antigen was not detected in any samples from 13 units without outbreaks of hepatitis and it was not found in samples from a unit in which there had been an outbreak which was thought to be of infectious hepatitis. On this evidence it seemed that most outbreaks in dialysis units were antigen associated.

Hepatitis was neither epidemic nor endemic in most of the survey units in 1969 (Table II). Almost all the hepatitis was in the four units with antigen-associated outbreaks. Among the remaining 17 units almost all the cases were in one unit where there were eight patients with abnormal liver function tests. This was the unit with a previous outbreak thought to be infectious
Table II. Incidence of hepatitis in staff and patients in 1969 in four haemodialysis units with outbreaks and in 17 units without outbreaks

<table>
<thead>
<tr>
<th>Units in terms of outbreaks</th>
<th>Category</th>
<th>Number of persons* in unit during 1969</th>
<th>Number of cases hepatitis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Clinical</td>
</tr>
<tr>
<td>4 units beginning or continuing outbreaks</td>
<td>Patients</td>
<td>169</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>All unit staff</td>
<td>260</td>
<td>12</td>
</tr>
<tr>
<td>17 units not experiencing outbreaks</td>
<td>Patients</td>
<td>506</td>
<td>3†</td>
</tr>
<tr>
<td></td>
<td>All unit staff</td>
<td>698</td>
<td>1</td>
</tr>
</tbody>
</table>

* persons with at least one week of staff duty or consecutive dialyses
† one in Unit where infections not associated with Australia antigen
‡ all in Unit where infections not associated with Australia antigen

hepatitis. The hepatitis risk to staff in units without outbreaks seemed very small.

The questions in our minds in the second half of 1969 were:

1. How best the Au antigen test could be used to control the existing outbreaks and, more ambitiously,
2. if it could be used to prevent outbreaks. It was clear that for practical purposes reports of positive tests to clinicians were useless unless some effective action could be taken as a result of a report.

The measures needed to control the existing outbreaks seemed to be as follows:

1. Identification of asymptomatic infections by means of laboratory tests
2. Dialysis of infected patients in isolation outside the unit, either at home or in hospital isolation
3. Improvement of cross-infection precautionary measures.

Eventually in three of the four outbreaks these measures seemed successful. Even the outbreak which had been in progress from 1966 was brought under control in 1970. You have heard already about the one outbreak that was not controlled in 1970.

Nevertheless, although outbreaks may be controlled there are grave disadvantages in allowing them to begin. The chief of these are:

1. The risk of severe infections in staff and sometimes patients
2. The disruption of dialysis and transplantation programmes
3. The creation of a pool of long-term Australia antigen carrier patients — potential future sources of infection.

Most of the units in the survey were without outbreaks or any evidence of
hepatitis. An attempt to prevent infection being introduced to these units seemed at least of equal importance to the control of the existing outbreaks.

The basic ingredients of a prevention plan devised in the latter part of 1969 are as follows:

1. Australia antigen tests of blood for transfusion
2. Australia antigen tests of patients for admission
3. Australia antigen and liver function tests of patients at regular intervals
4. Facilities for dialysing any infected patient in isolation
5. Contingency plan of action when 'positive' tests reported
6. Stringent cross-infection precautionary measures at all times.

At this time the plan was not completely feasible; antiserum needed for tests was in short supply and few units had facilities for dialysis in isolation. Nevertheless, in January 1970 a prospective Antigen screening study was added to the original survey.

The results in 1970 show that the Antigen was introduced in to six units for the first time; four times by asymptomatic carrier patients, once probably by infected blood which had not been screened and in one unit the original source could not be determined. In all six units, two weekly testing of patients and monthly testing of staff was begun immediately the Antigen was detected and the affected patients were dialysed in isolation as soon as possible.

In four of the six units outbreaks did not follow. There are, of course, no controls in this study, but it is of interest that one unit acted inadvertently as a control by admitting an asymptomatic carrier without any tests. However, in this unit swift action, taken after the first secondary infection appeared, limited the outbreak to only two secondary cases. In the sixth unit the procedures of the screening study had not been completely observed and the original source of the outbreak has not been determined with any certainty. In this unit a large outbreak followed and still continues.

In the 20 units in the survey from 1968 the rising incidence of hepatitis noted from 1968 to 1969 appeared to be halted in 1970.

No-one would claim that the prevention programme outlined is infallible, but until research offers better means, it would seem worthwhile to pursue any programme which offers a reduction in the number of long-term carrier patients on dialysis, and which affords a chance of avoiding the sort of epidemic situation described by Dr Ogg.

GOLDSMITH: Thank you Dr Polakoff. Your study will help those of us who have not yet been afflicted to maintain that happy state.

Whilst we are now all convinced of the importance of transfused blood and plasma in the spread of serum hepatitis, we should not forget the possibility of other vectors, such as insects, which might be of special importance.
in tropical climates, where antigen carriage rates tend to be high.

Dr Ritz of Heidelberg University has studied hepatitis antigen carriage by Platta Americana, an insect which regrettably appears to be endemic in many hospitals, though I hasten to add that I have yet to see one in our dialysis unit.

Carriage of Australia Antigen by Cockroaches

E RITZ, H ZEBE, R SANWALD
University of Heidelberg Medical School, Heidelberg, German Federal Republic

When thinking about the mode of transmission of serum hepatitis in dialysis units, the possibility of insect borne transmission should be kept in mind.

This has been proposed by Blumberg et al (1970) to explain the high incidence of positive Au-antigen tests in natives in South-East Asia. However, this possibility has never been firmly established. Still, insect borne transmission is theoretically possible, since minute amounts of positive serum (as little as 0.04 μl) have been shown to be infectious (Drake et al, 1952).

Spurred by unhappy clinical observations we were induced to test this hypothesis experimentally using cockroaches (Periplaneta Americana) as an experimental model. Fortunately, this rather unsavoury insect is not found as a permanent guest in too many dialysis units. However, the conclusions drawn from the experiments are in a broader sense also applicable to other insect species, for example ants, mosquitos etc (Beatson, 1972).

The experiments were carried out in our unit by Dr Zebe, Dr Sanwald and Mrs Bartsch. Cockroaches, raised in captivity, were administered gentamycin in drinking water to eliminate unspecific complement fixation, which was probably due to bacterial contamination. 100 μl of Au-antigen positive serum, concentrated to a titre of approximately 1:100,000, were administered either by injection into the coelomic cavity or by feeding the animals through a micro PVC catheter. Control animals were treated similarly with non-infectious serum. As you may remember, insects have an open circulation so that the coelomic cavity — somewhat analogous to the peritoneal cavity in mammalian species — is contiguous with the vascular system.
At timed intervals after the application of Au-antigen, coelomic fluid was obtained by rinsing with saline through a tuberculin syringe without violation of the intestine. In addition, a peculiar fluid, that we called vomitus, was obtained by gently squeezing the insects with pincers. As part of a normal defensive reaction, upon irritation these animals spit a fluid that probably represents a complex mixture of secretions and gastric contents. This reaction might be an important mode of contamination. The coelomic washings and vomitus were collected on filter paper and eluted with saline. The elutes were applied to a Whatman column (Tripathis & Horst, 1971) (Sephadex 10, coated with anti-Au-antibodies by the bromocyanate method). After all protein had been washed out (disappearance of extinction at 280 nm) by phosphate buffer (0.15 M, pH 7.2), the column was eluted with acid glycine buffer (pH 1.5) to remove Au-antigen from its antibody. The eluate was ultrafiltered and dialysed; Au-antigen in the eluate was determined by microimmunodiffusion (Ouchterlony), counterimmunoelectrophoresis (CIE) and by complement fixation as described elsewhere (Zebe et al, 1972a, 1972b).

![Diagram](image)

Figure 1. Au-antigen after intra-coelomic infection of Periplaneta Americana

252
As you may see from Figure 1, after intracoelomic administration of Au-positive serum concentrate, Au-antigen was demonstrable both in the coelomic washings and in the vomitus for prolonged periods.

Similarly (Figure 2) after feeding the animals with Au-positive serum concentrate, not only the vomitus but also the coelomic washings were found positive for weeks. This is more surprising, since the linings of the intestine separate intestinal contents anatomically from the fluid in the coelomic cavity. Control animals were consistently negative. Confirmation of the presence of Au-antigen in the various fractions by electronmicroscopy is still lacking.

These data show that cockroaches, and probably insects in general, are not merely physically smearing infectious material all over the place — which would be bad enough — but must be regarded as true carriers for the infectious agent of serum hepatitis. In the absence of quantitative data it is impossible to know what happens to the virus in the insect; although titres rise progressively for weeks after infection, we have no positive evidence of virus replication.

From a practical point of view these results suggest that insect control may be an important measure to check the spreading of hepatitis, especially in the hospital environment.
REFERENCES

    New England Journal of Medicine, 283, 349
Drake, M. E., Hampel, B., Pennell, R. B., Spizzien, J., Henle, W. and
    Stokes, J. Jr. (1952) Proceedings of the Society for Experimental
    Biology and Medicine, 80, 310
Zebe, H., Sanwald, R. and Ritz, E. (1972a) Verhandlung der Deutschen
    Gesellschaft für Innere Medizin (in press)

GOLDSMITH: Thank you Dr Ritz. Your original observations will surely
    lead to further work on animal vectors.
    It would be pleasant to close this Symposium on a note of hope, particu-
    larly in relation to the preventive aspects of hepatitis.
    Dr Cossart has already touched on the work of Professor Krugman of
    New York University School of Medicine. Just before the Congress Professor
    Krugman kindly sent me his up-to-date results on active immunisation against
    serum hepatitis, using heat-inactivated hepatitis serum, which appears to
    have retained its antigenicity. Of 29 children, only 9 subsequently developed
    hepatitis when challenged with untreated hepatitis serum. Against this, of
    35 children similarly challenged without prior immunisation, 33 developed
    hepatitis. Passive immunisation with a specific immunoglobulin obtained
    from an antigen-positive haemophilic also appears able to prevent or modify
    hepatitis in a high proportion of susceptible subjects.
    The degree of success already attending these experimental attempts to
    procure active and passive protection against hepatitis allows us, without
    being unduly optimistic, to take the view that within two to three years, we
    need no longer fear the effects of this disease on our chosen field of work.