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VIRAL HEPATITIS

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Introduction.

The physicians of the 8th century A.D. recognised an infectious jaundiced disease, and from that time epidemics of jaundice have occurred, especially in time of war. It was not until 1855 when 181 people out of 1291, vaccinated against smallpox, developed a condition which Bamberger described as catarhal jaundice that a serious attempt was made to identify the disease and determine the pathology. It was suggested that a mucous plug secreted by an inflammatory condition in the duodenum blocked the outflow of the common bile duct.

Viral

In 1918 the virus was suggested as the agent causing jaundice, but no more certain description of it was given. Clinical cases were often confused with Weil's disease, and not until the 1940s was a clear difference in aetiology demonstrated. Volunteers either ingested faecal material or were given serum injections from patients with the disease and they developed a similar condition, although no agent could be isolated.

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This very brief historical account helps to show why the term 'Viral' hepatitis is of recent origin. Until the virus could be isolated, it was still only conjecture that the causative organism of a particular clinical condition was a virus, however strong the epidemiological or pathological grounds for the statement. Isolation was claimed by Richtsel and his fellow-workers in 1956, but even up to the present the virus, or as thought nowadays viruses, have not been precisely described.

In this essay, it is proposed to discuss briefly the nature of the viruses, the pathology, clinical features, diagnosis, treatment and prophylaxis and spend proportionately more space in discussing the epidemiological and serological aspects of viral hepatitis. Two separate conditions are included under this heading, infectious hepatitis and serum hepatitis. Their differentiation is on mode of infection, incubation period and epidemiological pattern, rather than a clear difference of causative agent.

Aetiological Agents

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In 1956 Richtsel and his co-workers isolated a virus-like agent from the faeces of patients with infectious hepatitis which produced cytopathic effects in the Detroit 6 line of human cancer cells. There was evidence of vacuolation with distorted degenerating mitochondria. The agent was viable for 30 minutes at 60°C. Under the electron microscope the size was determined as 12 - 18 mμ in diameter.

Three serotypes could be identified, of which two gave rise to infectious hepatitis in volunteers, after parenteral injection. Despite attempts to grow the virus in other cell media, in chick eggs or in sucking mice, the virus has still not been grown. Its size is still unconfirmed, but its resistance to 10% ether for 24 hours and to long periods of freezing has been established.

Even less is known about the agent causing serum hepatitis. It is less than 26 mμ as determined by filtration. It has a greater resistance to ether and phenol and heat than the infectious hepatitis virus, but it has not been isolated yet.

Pathology

Although two separate diseases are included under the title of viral hepatitis, the pathology and most of the clinical features are similar. The route of entry differs, being oral in infectious hepatitis and parenteral in serum hepatitis. The main lesions are in the liver despite the systemic nature of both diseases. In fatal cases there are acute inflammatory lesions in the gastro-intestinal tract, pancreas and heart, often with ascites.

(wounded)
(True?)

Fatality is not the usual course, and for this reason it was only when individuals with the disease met their deaths from other sources, as in war, that a hepatitis was found to be the cause of the clinically obvious jaundice. A needle biopsy will also provide the microscopic evidence.

The extent of the lesions varies considerably in

individuals. Initially a ballooning of the parenchymal cells is observed with eventual coagulative necrosis in a centrilobular distribution. Special stains show that the reticulin framework of the lobules is preserved, a feature which allows rapid regeneration. Occasionally, acidophil inclusion bodies can be seen. There is no evidence of fatty change.

The swollen parenchymal cells, together with the lymphocytic infiltrate, tend to block the biliary tracts. Dilatation of the intralobular canaliculi and deposition of bile thrombi take place. The infiltrate is heaviest around the portal tracts and centrilobular zone. This process results in an obstructive type of jaundice being superimposed on the hepatocellular form, the biochemical features of which are reflected in the rise of conjugated as well as unconjugated bilirubin in the serum and urine, the pale stools and low level of urobilinogen in the urine.

This pattern is by no means characteristic as in some cases the obstructive element is not sufficient to prevent secretion of bile down the biliary tree and clinical jaundice may never develop. Also, in children, a different pattern is sometimes seen in the biopsy specimen. There is less necrosis, but giant cells with 30-40 nuclei appear.

In late stages mitotic figures can be seen, an indication of the capacity of regeneration which the liver possesses. Only 2-3 months elapse after the icteric phase before the liver is fully regenerate and no sign of infection remains.

Recovery does not take place in a small proportion of cases. The inflammatory process continues to a stage of acute massive liver necrosis. Biopsy shows that fibrosis has taken place and at necropsy the surface of the liver is nodular. The size varies depending how much tissue has been absorbed. If the patient does not die, the liver shows post necrotic scarring.

Increasing fibrosis also is seen in livers of those who have had continual relapses or repeated attacks of jaundice. The pattern is of portal cirrhosis, and may be due either to the persistence of the virus or to an autoimmune cause. It has been suggested that the smaller intralobular ducts sustain damage in addition to the parenchymal cells.

Clinical features

The course of infectious hepatitis is very similar to that of serum hepatitis once the icteric phase has been reached. The incubation period for the former is 15 - 40 days and for the latter 40 - 160 days before the constitutional symptoms of the pre-icteric phase develop. These tend to be of abrupt onset in infectious hepatitis but more insidious in serum hepatitis, consisting of fever (101^oF.), anorexia, nausea, vomiting, perhaps diarrhoea, malaise and vague abdominal discomfort. The symptoms are more severe in serum hepatitis and there may be urticaria and itching as well. On examination, there is commonly cervical lymphadenopathy, the liver may be tender on deep palpation and occasionally there will be slight splenomegaly.

After 6 - 7 days the sclera and frenum of the tongue develop a yellowish tint which deepens in the next few days. The urine darkens as the bilirubin content rises, the stools become paler, looser and have an offensive odour and the constitutional symptoms disappear. This icteric phase lasts for 4 - 6 weeks, as measured by the raised bilirubin in the plasma, although the signs of hepatomegaly and clinically apparent jaundice subside much sooner. There is often a period of depression for some months after an attack of viral hepatitis. The fatality is low, only from 0.1 - 0.3 %, the majority being elderly.

The diseases can run different courses, but only 10 - 15 % are atypical. There may be the sub-clinical course which has no icteric phase, although the raised serum bilirubin, serum transaminases and change in plasma proteins gives biochemical evidence of altered liver function. In a typical course, the most sensitive test is that of the level of SGPT (Serum Glutamic Pyruvate Transaminase). At the end of the pre-icteric phase the level may have risen to 40 times the normal. The serum bilirubin level depends on the degree of biliary obstruction and the serum alkaline phosphatase shows a moderate rise, rarely exceeding 30 King Armstrong units per 100 mls. Plasma proteins show decreased albumen, an initially increased γ globulin and greatly decreased α_1 and α_2 fractions.

In the majority of those cases in which spontaneous recovery does not occur, liver biopsy shows a picture of little cell necrosis, but the biochemical tests suggest ^{an} obstructive pattern, as they are little disturbed apart from a high serum and urine bilirubin. This condition, cholestatic jaundice, has a long course but good prognosis.

Relapses tend to occur in those patients who have returned to full activity too soon, or who have consumed alcohol, a drug with hepatotoxic effects amongst others. Most recover, but a few proceed to liver cirrhosis.

Two forms lead to cerebral involvement from metabolites unremoved from the brain. Fulminating hepatitis with profuse vomiting will lead to coma and death in 10 days unless the course is altered by steroids. It tends to be found in the undernourished. Post-mortem examination reveals a very shrunken liver with massive necrosis. The other form is found mainly in women in the 35 - 50 age group. The clinical course is of fluctuating jaundice, hepatomegaly, splenomegaly and coma. Death takes place in 3 - 4 months.

Treatment ¹⁵

Until the aetiological agents for both types of viral hepatitis have been isolated, it is impossible to produce a specific antidote for either. Treatment must be symptomatic. Bed rest and a carefully balanced diet are essential to counter the lethargy and anorexia during the acute phase. Whilst the patient is convalescent, he must be advised not to exert himself at all and to take a rest in the middle of the day.

If ACTH is administered in the acute phase, there is a speedy return to a feeling of well-being. The bilirubin and SGPT levels return to normal in a short time, but there is the possibility of an early relapse as well as the unwanted side effects of steroid therapy, mooning, oedema, ascites and difficulty of stopping the therapy.

For this reason ACTH should only be used in severe progressive disease, when hepatic coma is present or imminent. Steroids in the form of prednisalone (15 - 20 mg/day) may help the resolution of a chronic condition.

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Prophyllaxis

As long ago as 1945 Havens and Paul had noted that serum from a patient convalescing from infectious hepatitis would confer immunity if injected into a volunteer who later was exposed to the disease. Doubt was expressed if the same was true of serum hepatitis once the two conditions were distinguished. Havens showed in 1946 that immunity was produced some 6 - 9 months after an infection, but that the degree of protection was not absolute in every case. His figure of 2 - 5% for reinfection in adults agreed with the figure of 4% recurrence over a period in a state hospital for mentally defectives.

An outbreak of infectious hepatitis of epidemic proportions in Des Moines, Iowa during 1960 - 1961 provided an opportunity to test the efficacy of cross immunity. γ globulin was offered to family contacts of children who had caught the disease, in a dose of 0.01 ml/lb body weight. Out of 133 contacts who did not receive γ globulin, there were 14 cases confirmed as having developed the disease more than 14 days after being offered the protection, an incidence of just under 10%. This contrasted with the 5 cases out of 1577, an incidence of 0.3%, who were given globulin. The majority showed no symptoms clinically, but it is possible that not only were they infected sub-clinically, but also they acted as carriers of the virus. 70% of all cases could not be traced as having direct contact with any of the known patients, suggesting that a major role in transmission was played by unrecognized or asymptomatic cases. The prophylactic use of γ globulin was established. (Moseley et al. 1963)¹³

Further work has shown that prophylactic γ globulin may be effective 6 days before the onset of symptoms and this protects for 3-6 months.

Comparable work with serum hepatitis has shown that infection is not prevented by globulin from a convalescent patient, and indeed the risk of transmission of the virus in the plasma is considerable as the virus has been found up to five years later in the blood. However it has been suggested that the icteric phase is reduced after 2 doses of 10 mls γ globulin at 30 days interval between doses.

Epidemiological Aspects

Virus hepatitis is the commonest of the acute diseases of the liver. Both infectious and serum hepatitis are endemic in the United Kingdom, but from time to time epidemics break out. The epidemiology of each will be dealt with separately.

Infectious hepatitis:

This is a disease primarily of children and young adults, although the majority of fatal cases occur in the older age groups. Fatality is probably linked with debility from other sources in these cases.

The mode of transmission was suggested as the faecal-oral route by Havens in 1945. Volunteer prisoners ingested food containing the agent, isolated from the faeces of patients with clinical hepatitis, and later developed the expected signs and symptoms. The agent was demonstrated in the faeces of these volunteers 3 - 4 days before the prodromal phase started.

It was not until the 2nd World War that opportunities arose to study the condition in the field. Troops provided the ideal closed community in which to investigate the spread. A great deal of valuable work was carried out by Cameron and his colleagues in the Middle East and Mediterranean theatres of war, where the natural incidence of infectious hepatitis was higher than in Great Britain. An early finding was that epidemics were often associated with carriage on food, evidence coming from comparative studies amongst officers and other ranks. The latter had a significantly lower incidence, almost certainly associated with the stringent supervision of their cookhouse orderlies' hygiene and preparation of food. There was less control over the officers' mess cookhouse. A further point noted was that whereas the officers used communal cutlery and crockery, the other ranks had their own cutlery. Cross infection from improperly washed cutlery was thus avoided.

Perhaps a clearer idea of the factors involved in such an epidemic in a community with all age groups comes from the results of an investigation by Moseley, Speers and Chin into two epidemics at Des Moines in the state of Iowa. Des Moines is a large urban area with surrounding suburbia whose population in 1960 - 1 was just over 10200.

The two epidemics were in 1952 - 3 and 1960 - 1, the difference allowing a comparison to be made in the incidence of cases related to poor sewage disposal. A feature of the first epidemic was the correspondence between the high rates in some areas with the lack of running water and use of the 'privvy'. Eight years later the majority of cases were again in these areas, but only where the 'Privvy' was still in use. A very much reduced incidence was found in those areas where major improvements in sewage disposal had taken place.

Socio-economic status and number in a household were investigated more fully in the second outbreak. In all there were 451 cases, 322 within the urban area, out of a population at risk of 10233. No fatalities were reported. In the upper socio-economic groups very few individuals contracted the disease, a different finding from the lower groups. These tended to occur in aggregates, sometimes based on a single street, both in the urban and suburban areas. Diagnosis was made on symptomatology and jaundice, or on a positive urine bilirubin level. The peak age group of those diagnosed was 5 - 9 years, with the 10 - 14 group next.

Correlation could also be found between the number in the house-hold and the degree of congestion in a room, only for the middle and lower income groups. This is what would be expected as the lower groups would be more likely to live in those areas with poor sewage disposal, to crowd the children into one room and to allow them to mix together whilst playing than would the higher socio-economic groups.

The finding that only 30% of cases could be related to a known contact, usually school friends, frequent playmates, members of the family of regular visitors, suggested that school was not a primary source of transmission, unless a high proportion of asymptomatic carriers existed. Poor personal hygiene combined with inadequate disposal of sewage seemed to be main factors, but as only 7 cases were in non-whites there may be a genetic predisposition or protection.

In 1956 a water borne spread of the virus caused some 30,000 cases, illustrating the role of a carriage medium in an epidemic.

Serum Hepatitis:

Diagnosis of serum hepatitis is made on the longer incubation period, the severer pre-icteric symptoms and the epidemiology. Whereas the virus for infectious hepatitis has been recovered from the faeces of patients, the same is not the case in serum hepatitis. Attempts to demonstrate a faecal-oral route have failed and instead it can be stated that the route of entry is by direct inoculation into the skin or mucous membrane. The age group at risk also differs from infectious hepatitis as all ages appear to be equally prone to serum hepatitis.

Members of the medical and nursing professions are frequently, albeit unwittingly, responsible for the inoculation of the virus. Upto very recent times no disposable syringes, needles and related pieces of equipment were available. The virus of serum hepatitis is blood borne and it only needed one small item not to be thoroughly cleaned and sterilised for cross-infection to take place. It has already been pointed out how resistant the virus is. Further the amount of infected material required to infect is incredibly small, 0.000002 ml being one estimate.

Outbreaks of serum hepatitis have been traced to the use of blood transfusion, pooled plasma (where the greater the number of samples pooled, the greater the risk of infecting a number of people) and unsterile syringes. Dougherty and Altman⁶ (1963) highlighted the risk of using disposable equipment for more than one person. They cited the case of a physician who used only 1000 infusion sets to give over 2500 infusions of amobarbital followed by saline. 41 of his patients developed hepatitis, 15 of whom died.

Another series of cases occurred before the resistance to ether was appreciated. Over 100 patients attending the diabetic clinic of the Royal Infirmary, Edinburgh in one day developed the condition. The instrument responsible was eventually pinpointed as the spring loaded stylet used to draw blood from the thumb for a rapid blood glucose level. This was dipped into ether after each patient and left to dry. The number of people affected again emphasises the minute quantity of inoculum needed to infect.

The growing number of young people who are turning to addictive drugs to satisfy their craving for new experiences, is bringing an increasing number of cases of hepatitis to light, no doubt caught from shared infected syringes or needles.

A rather more interesting outbreak took place in cross-country runners.¹⁶ The disease developed insidiously and an incubation period of 90 - 150 days was worked out retrospectively. 95% of those affected had scratches on their legs, obtained during their races. It was suggested that blood containing the virus was deposited on the twigs by one of the leading runners and that subsequent runners brushing against the same twigs picked it up. When adequate measures were taken to ensure protection, the incidence decreased and stopped, but it rose when the measures were relaxed.

These examples demonstrate what has also been shown experimentally, that the virus can be recovered from the blood for many months and years after the clinical effects of the disease have worn off. The longest recorded period is five years. To reduce the risk of transmission, the National Blood Transfusion Service will not accept the blood of anyone known to have had jaundice for transfusion purposes.

Serological Aspects

It would be convenient for the microbiologists either to have the viruses of hepatitis or to possess a specific antiserum to each form. If this were so the problems of diagnosis would be greatly simplified as agglutinating properties with each known cell system could be tested, haemagglutinating inhibition tests could be performed and the antigenic components of the viruses could be worked out.

These systems have been used to produce a reliable specific test, but so far without success. One of the early erythrocyte systems used was that of the rhesus monkey. ~~Early~~ Acute and convalescent sera were titrated against the red cells. With the latter dilutions it was shown that 54.5% of individuals with clinically diagnosed infectious hepatitis gave a positive reading. However 50% of normal individuals with no history of this or related condition gave a similar reading. Chick erythrocytes gave an inconclusive result as well.

Havens extracted the serum samples with acetone and ether to remove non-specific agglutinins and inhibitors, added dilutions of these to 0.25% chick erythrocytes and noted a higher proportion of positive results. Using this technique, McCollum and his co-workers¹¹ found that 86% of convalescent patients gave positive results as compared with only 6% of normal people. The test was not specific because 28.6% positives were found in patients with portal cirrhosis of other aetiology, 25% of those with collagen diseases were positive but in a group with obstructive jaundice there were no positives. These workers suggested that there might be a correlation between the haemagglutinins and serum bilirubin levels, a surprising suggestion^{as} the level of serum bilirubin can be very high in obstructive jaundice.

Two years after he had demonstrated that the agglutinating factor was in the globulin fraction of the serum proteins, Havens⁸ (1960) showed that in the acute phase of infectious hepatitis the titre was high until the 10th - 12th day when it dropped, remaining low until well into convalescence. Using the same system, he found that 70% positive results could be obtained in the acute phase and 8.5% positives in chronic hepatic disorders. With volunteers he investigated the rise and fall of

the haemagglutinating titre. After 12 days the titre was falling, whereas the bilirubin level was only slightly or moderately raised. This was conclusive evidence that there was no relation between the two. At the same time Havens was unable to find a correlation between the titre and the serum enzymes, especially the transaminases, the titre and the severity of the disease as judged clinically and the titre and the onset of symptoms. In 7 out of 10 volunteers the titre had fallen to its low convalescent level before the clinical signs were apparent. Havens concluded that although this test might be useful in the differential diagnosis, it had to be performed early, often before the disease was manifest, and could not be thought to be in any way specific.

It is worth noting that at this time no cells supported by tissue culture media, no part of the chick egg and no living animal had been found which would support the growth of the viruses.

Hill¹⁰ reported the outbreak of infectious hepatitis in 1961 amongst staff connected with chimpanzees in a laboratory. He took a range of chimpanzee tissues and subjected these to acute human sera. Out of the 12 sera used, only two produced a cytopathic effect, both on kidney cells, which had been separated by trypsinisation. After 6 days slight damage was noted and this was complete on the 8th day. Fluorescent antibody and neutralisation tests demonstrated that the viruses, carried in the acute sera, were cross-reactive with serum from convalescent patients.

Also in 1961 Davis⁴ used a different medium for tissue culture. Whilst investigating an outbreak of infectious hepatitis in children between 20 months and 3 years on an Indian reservoir in eastern Arizona, he noted that a 2% faecal suspension from those children with deep scleral jaundice would produce a cytopathic effect in human embryo lung cells. This effect was consistent after serial passage, taking 5 days before the cells clumped together. 14 out of 22 suspensions produced this effect.

Davis⁴ also attempted to cultivate the agent in renal epithelial tissue of the macaque monkey, by intramuscular, intra-peritoneal and intracerebral inoculation of suckling mice and in the

amniotic and allantoic cavities of 7-day chick eggs, all without success. Equally unsuccessful were his attempts to relate the virus with known viruses by neutralising suspensions of Echoviruses 1 - 28, Coxsackie viruses A 1 - 9 and B 1 - 6, and the 3 polioviruses with sera from these children. Although these results were negative, they do help in the identification of the virus in that it would seem to be unrelated to these groups serologically and in a separate group of the small RNA viruses, assuming that its nucleic acid is found to be RNA.

These reports emphasise that as recently as 1961 no-one had any real knowledge of the nature or behaviour of the agents causing viral hepatitis. Occasional experiments had succeeded, but they were not able to be reproduced. However by 1965 some evidence was forthcoming from a series of trials by Cole. She used a line of human epithelioid cells from bone marrow, the Detroit 6 line. Sera from ~~four~~ patients with infectious hepatitis gave a comparable cytopathic effect to that produced by a viral agent which the Parke-Davis company supplied. Three blind trials were performed, the controls being a group of old men, a mixed group of old men and patients with obstructive jaundice and a group of people with similar age and sex distribution to the patients with infective hepatitis. In each case the infectious hepatitis patients gave a 100% positive cytopathic effect after three passages, whilst the controls gave a 100% negative reaction after three passages.

Although the agent had not been seen, isolated or identified, this report did seem to be conclusive proof that transmission could take place in the serum of patients in the acute phase. However attempts to develop a test for antibody or antigen were inconclusive. Ferris and Cole, at Fairfield Infectious Diseases Hospital, and Cross and Marmion at Monash University, both in Melbourne, carried out independent studies with sera from patients with infective hepatitis using as controls sera from patients with other unrelated diseases.

Ferris and Cole each examined a series of Deltoid 6 cell cultures seeded with sera coded by a member of the staff who took no further part in the study. Two passages of the agent were attempted

using the Parke-Davis virus as a positive control. Passages were carried out initially at 6 - 7 days and subsequently at 3 - 4 days. Of the five sera from patients with infectious hepatitis, all five showed positive cytopathic effects initially and after passage. Similar results were obtained for six out of the seven control sera, although the cytopathic effect was not of the typical pattern in every case. These findings did not support the earlier paper, but suggested that either that a viral agent had been in the inoculated medium, or that a latent virus in the Detroit 6 cells had been activated by nearly all the sera,

Cross and Marmion³ were more concerned to isolate or characterise the agent. They took acute phase sera from 30 patients with infectious hepatitis (3 being tested by Mrs. Cole and found to produce the typical cytopathic effect on Detroit 6 cells) and added these to cultures of the Bolin human lung epithelium line of cells. 12 produced a transmissible cytopathic effect, but rarely in a dilution of more than $10^1 - 10^2$. Two sera from seven month children with no evidence of hepatic disease also gave positive transmissible results.

Attempts to show immunofluorescence using convalescent sera on sheets of the cultures which gave positive cytopathic effects were unsuccessful. 28 other cell lines and strains were seeded with acute phase sera, the majority of which showed no cytopathic effect. No more successful were complement fixation tests, haemadsorption or haemagglutination reactions, nor even electron microscopic examination.

In a further double blind trial with the Detroit 6 cells, Cross and Marmion were unable to correlate the clinical state of the donor of the serum with the appearance of cytopathic effect. They suggested the possibility of the Detroit 6 cell line possessing a latent unstable virus.

These two studies, having independently reached similar conclusions that the Detroit 6 cell line was not unique in being able to support growth of infectious hepatitis, seemed to suggest that the enigma of the infectious hepatitis virus remained. However, late in 1967, Deinhardt and his colleagues⁵ reported the transmission of the clinical

and morphological disease from man to Marmoset monkeys in two cases from a series of five. Six passages from monkey to monkey were successfully carried out, with the disease increasing in severity with passage. The pathological picture was similar in many aspects to that of man, but as no rise in antibody could be demonstrated, it has yet to be proved that the agent transmitted was that of human infectious hepatitis. Nevertheless the possibility does exist, doubtless to be confirmed or discarded by further research.

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A similar claim that marmosets could be infected was made by Dr. F. Schaffner of Mount Sinai University, New York in a lecture at the Royal Free Hospital. He stated that particles of 100 Å had been observed by electron microscopic examination of liver cells from marmosets injected with blood from human patients with infectious hepatitis. These particles were to be found in the Endoplasmic reticulum and not found elsewhere nor in control specimens. On ultracentrifugation, an infective layer had been recovered. Dr. Schaffer conceded that there was the possibility of a marmoset disease producing this effect.

Conclusion

In the absence of a specific serological test as yet, it would seem that diagnosis of either of the types of viral hepatitis has to be made on clinical, epidemiological and supporting biochemical grounds. This is unsatisfactory from the physician's point of view as he must fall back on γ globulin as his means of prophylaxis. The priorities for further research in this field would seem to be the characterisation of the agents and isolation in order that a specific test, whether complement fixation, haemagglutination or haemagglutination inhibition, be devised and also so that a vaccine could be prepared by modifying the antigenic properties of the agents. As the most successful mode of investigation so far has seemed to be tissue culture, it would seem reasonable to combine this with the possible pathogenicity in the marmoset monkey by studying cell lines from this species, at least for infectious hepatitis. The virus of serum hepatitis, the more elusive, may reveal its secrets if examined in similar ways.

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