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Viral Agents in Hepatitis

A Review

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An annotated bibliography of investigations concerned with the isolation and characterization of viral agents from human cases of infectious hepatitis has been assembled. Documentation of the host systems, which included both conventional and nonconventional laboratory animals and tissue cultures, utilized in the recovery of 23 classified viruses and six or more strains of unclassified viral agents has been presented. In addition to those reports categorized under various host systems used in isolation, supplementary documentation of published reports by various investigators from a number of foreign countries has been presented to emphasize the international and universal problem of ascertaining etiologic agents for this disease and to provide a means for correlation of the isolated agents. The classified viruses, unclassified viral agents, and host systems employed have been tabulated. The bibliography includes one hundred and sixty-five references.

Additional key words: Infectious disease, Tissue culture, Viral isolation.

This paper represents an attempt to bring together an annotated bibliography of studies on the isolation and characterization of etiologic agents of infectious hepatitis. In addition, certain thoughts which occurred to the authors during their review of the literature are presented to suggest ways in which the present state of the art might be viewed and possibly improved. There have been numerous reviews which have appeared in the literature.[25, 30, 56, 93, 110, 113, 114, 142, 164, 166] Several of these reviews did not include many of the recent tissue culture studies or significant proportions of the foreign literature. The present report has attempted to incorporate the more recent tissue culture isolation studies and to include more of the foreign references in an extensive review of the literature. Although it is possible and even likely that references have been overlooked, no references were intentionally omitted since the purpose of this report was to provide an extensive list of references from around the world, and thus provide an opportunity for a more detailed study of the individual papers, rather than as a critical evaluation.

Two summary tables have been presented in which a number of isolations have been tabulated. Known viral agents which were either initially or later identified as such and which were isolated in connection with hepatitis are listed in Table 1. Table 2 has been prepared to characterize and bring together, in a broad classification, those as yet unidentified agents that have been isolated in conjunction with cases of hepatitis and have received rather extensive study. This list was not intended to be all-inclusive, as there are in existence numerous other cases most of which involved single publications with no corroborative studies.

Much of the literature which described early isolation attempts referred to the use of more than one test system or species of animal. Such papers have been included under one test system and are not repeated. A cross-reference listing has been prepared and is included as Table 3. In many of the cases cited, secondary passages were performed in test systems different from those used for primary isolations. The studies on nonhuman primates have been separated and have been dealt with individually because of their special interest.

ATTEMPTS AT PROPAGATION IN THE CHICK EMBRYO

There have been a number of attempts to isolate the etiologic agents of hepatitis in embryonated eggs, with almost all of the investigators reporting some degree of success in isolation of an agent. In most cases, however, the subsequent investigations on the isolated agents have not been extensive enough to evaluate the relationship of the recovered agents to the clinical syndrome. Among the earlier reports which have appeared in the literature are those of Siede and Meding,[153] Dresel, Meding, and Weineck,[29] Dohmen,[27] Pendl,[139] Henle *et al.*,[63] and Drake *et al.*[28]

Both Siede and Meding[153] and Dresel *et al.*[29] noted death of embryonated eggs within 3 to 4 days when the chorioallantoic sac was inoculated with duodenal juice. No consistent pathologic findings were observed; however, the lethal factor was reported to have been transmitted for two to eight passages. Urine and blood were seldom found to infect chick embryos.

Pendl[139] performed experiments with 7-day-old embryonated eggs by using both filtered duodenal juice from patients and liver juice of experimentally in-

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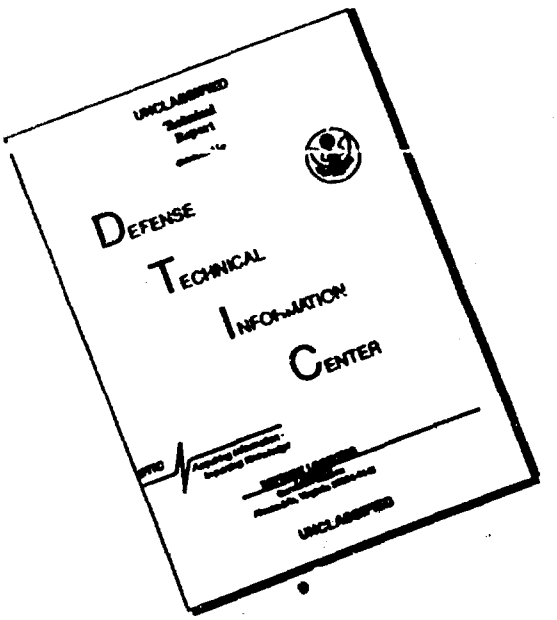

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fected pigs. Both filtrates caused death of the embryo and incomplete growth of the allantoic membrane. These results were transmitted in 17 serial passages in eggs. Virulence was decreased with succeeding passages. The agent was reported as filterable. Dohmen[27] attempted cultivation of virus on the chorioallantoic membrane in embryonated eggs, and the inoculation of fourth passage material into mice produced illness.

Beginning in 1945, Henle and associates[63] attempted the isolation of etiologic agents of infectious hepatitis. These workers reported the isolation of two viral agents from separate outbreaks of infectious hepatitis. Original inoculation was performed with tissue cultures of rabbit liver cells in roller tubes and of minced chick embryos, followed by passage into the amniotic cavity of the chick. In the follow-up study to Henle's first paper, Drake *et al.*[28] reported on the development of hepatic disease resulting from human volunteer inoculations with these agents. Inoculated subjects were anicteric but showed other symptoms suggestive of natural infection. These investigators also found that an antigen prepared from infected amniotic fluid and inactivated with ultraviolet light gave a positive skin test in all convalescent patients, whether infection was experimental or natural.

Henle[62] later published a review of various attempts to adapt the virus for propagation in the chick embryo. Sengoku[140] reported serial passage of six strains of an agent through chick embryos and then into cultures of Ehrlich's ascites tumor cells, HeLa cells, cells from human amniotic membrane, and L cells. Cells from the human amniotic membrane showed a high susceptibility to the virus. Segagni, Ansaldi, and Nigro[145] utilized sera from 30 children at an early stage of jaundice. The sera were inoculated on the chorioallantoic membrane of embryonated eggs and in tissue cultures of chick embryo cells. These workers reported the development of chorioallantoic and hepatic lesions, both macro- and microscopic. Essen, Lembke, and Schlecht[39] reported that allantoic inoculations resulted in the formation of necrotic areas, or in death of the embryo; however, these manifestations were insufficient for titration of infectivity. Morzycki, Taylor, and Juskiewicz[126] reported passage of a virus of infectious hepatitis, strain MMG, in embryonated eggs. Electron micrographs from infected and dead embryos demonstrated spherical, irregular bodies approximately 180 $m\mu$ in diameter. Similar particles were seen in tissue cultures inoculated directly with blood of sick individuals. The tissue culture preparation used was not described. Essen and his colleagues have published a number of papers[35-38] dealing with their isolation attempts in eggs, and more recently in 1963, on the use of a skin test with material grown in embryonated eggs.[41] At least three other papers[28, 63, 75] have appeared in which skin test antigens prepared in embryonated eggs have been developed and employed with some reported degree of success. These are discussed in greater detail under the original isolation attempts connected with them.

Table 1. Identifiable Isolates Associated with Viral Hepatitis or Hepatic Involvement

Coxsackie virus	
Group A	
Type 4	Morris *et al.*[124 a]
Type 10	Embil *et al.*[32]
Types 13, 18	Jézéquel and Steiner[77]
Coxsackie virus	
Group B	
Type 4	Kibrick and Benirschke[84]
Type 5	O'Shaughnessey and Buechner[137]
Type 5	Siegel *et al.*[154]
Types 2, 4	Hosier and Newton[73]
Type 3	Sun and Smith[163]
Echoviruses	
Types 1, 8	Kiseleva[85]
Type 4	Karzon *et al.*[80]
Type 4	Malhaerbe, Harwin, and Smith[109]
Type 9	Sabin, Krumbiegel, and Wigand[145]
Type 6	Lapinleimu[100, 101]
Types 7, 12	Sharlai *et al.*[150]
Adenoviruses	
Types 1, 3	Hatch and Siem[59]
Type 3	Davis[24] (identification as an adenovirus cited by Deinhardt and Holmes[25] and Hatch and Siem[59])
Types 1, 5	Ananiev *et al.*[2]
Types 1, 4	Kerim-Zade[82]
Type 5	Hartwell *et al.*[57], Hillis[66]
N.T.[b]	Köhler *et al.*[89]
Reoviruses	
Type 1	Felsenfeld[48]
Type 2	Strutsovskaia[162] (cited in Russian editorial[30])
Types 1, 2	Joske *et al.*[78]
Type 1	McKee[111], Burlingham and McKee[19]
Enteroviruses	
Miscellaneous	Sharlai *et al.*[150]
Myxoviruses	
WB Virus	Giles, Liebhaber, Krugman, and Lattimere[50]
	Liebhaber, and associates[106, 107]
	Prose, Balk, Liebhaber, and Krugman[140]
DA Virus	Hsiung, Isaacson, and McCollum[74]
Mycoplasma	
M. Gallisepticum	O'Malley and associates[123, 124]

[a] Reference number, and worker or group of workers.
[b] N.T., Not yet typable.

ATTEMPTS AT ISOLATION IN EXPERIMENTAL ANIMALS

Andersen and Tulinius[5] employed the oral administration technique of bile to mice and pigs and obtained serial transmission of an infectious agent. Pendl[139] filtered duodenal juice from German soldiers and inoculated it into pigs, mice, and embryonated eggs. He noted a slowed rate of weight increase in inoculated pigs. In one of the pigs sacrificed after 63 days he observed changes in the liver, which subsequently could be induced by serial transmission of the liver tissue after three passages. Virulence decreased with passage. Intraperitoneal inoculation of mice with filtered duodenal juice induced liver lesions resembling those in man. The incubation period in mice was

TABLE 2. REPRESENTATIVE AGENTS, NOT YET IDENTIFIED AS KNOWN VIRUSES, ISOLATED FROM INFECTIOUS HEPATITIS PATIENTS

Common name	Original description and confirming references	Host Range	Serology and immunology[a]
Motol virus	Originally isolated and described by Kubelka *et al.*[97] further studies by Kubelka and associates[95, 98]; Chlap *et al.*[20]; Schon *et al.*[147]; Kowalczykowa *et al.*[91, 92]	Originally isolated in allantoic cavity of 9-day-old chick embryos; grown in monkey kidney, Chang's liver, and KB tissue cultures; after passage in MK and Chang's liver cells, it is pathogenic for mice.	Varies according to host system, but under various conditions has yielded + CF, + neutralization, and + hemagglutination; positive skin tests; hemagglutinating fraction is antigenically related to type 5 simian virus and DA myxovirus.
Laszlo agent	Isolated by Laszlo *et al.* between 1957 and 1964; Laszlo and associates.[102-105]	Grows and produces cytopathic effect in tissue cultures of embryonic human liver and Detroit-6 cells; induces hepatitis lesions in hamsters.	+ CF tests; + neutralization.
K3 virus	Isolated in 1960 from feces of patient with infectious hepatitis. Ananiev and associates[2-4]; Barinskiy and associates.[12, 13]	Grown in human renal cell culture and Detroit-6 cells; 50 passages in human kidney cells; does not grow in monkey kidney cells.	+ CF tests; + neutralization by patient sera; Cross-reacts with Mitroiu's agent from Rumania.[118]
Mitroiu virus	Viruses originally isolated from children with epidemic hepatitis; Mitroiu and associates.[117-121]	Grows equally well in KB, HeLa, HEp2, Detroit-6, and monkey heart cells; nonpathogenic for suckling mice and rabbits.	Neutralized by patient sera; cross-reacts with Moscow K3 strain (two of five strains from Rumania showed cross-neutralization); virus was neutralized by ECHO 7 antiserum after 20 passages, plus later ones; types 19 and 20 echovirus antiserum neutralized virus strain BF at 20th passage, but not after 36th one; original isolates did not react with echovirus antiserum.
Rightsel and McLean agents	Viral strains MR-1, AR-17, WW-55, RA-214, and LG-217 recovered from serum or plasma of patients with viral hepatitis in Detroit-6 (PD) cells; McLean *et al.*[113-114]; and Rightsel *et al.*[143, 144]	Limited to Detroit-6 (PD) cells; AR-17 produced clinical jaundice in volunteer subjects, Boggs *et al.*[14]; heat-stable at 60° C. for 30 min.; filterable through 03 Selas filter, Rightsel.[144]	+ Serum neutralization (7 years postdisease); − serum neutralization with viral recovery (3 months postdisease); + hemagglutination of rhesus erythrocytes only by patient serum not by viral agents, Rightsel.[144]

[a] +, Positive; −, negative; CF, complement fixation test.

TABLE 3. REFERENCE LIST TO HOST SYSTEMS USED FOR ISOLATION AND STUDY OF ETIOLOGIC AGENTS ASSOCIATED WITH VIRAL HEPATITIS

Host systems			
Embryonated eggs	Mice and or other nonprimate animals	Subhuman primates	Tissue cultures
6, 27, 28, 29, 35, 36, 39, 62,[a] 63, 79, 90, 97, 126, 131, 132, 139, 148, 149, 153, 164[a]	5, 6, 46, 51–54, 85, 91, 92, 95, 99, 104, 108,[a] 116,[a] 122, 130, 132, 136, 137, 139, 164[a]	26, 42,[a] 55,[a] 138, 156, 164[a]	2 4, 8 11, 15, 18, 19, 20, 21, 23, 24, 28, 31, 43 45, 49, 50, 57, 61, 63, 67, 68, 70, 74, 77, 79, 82, 86 88, 90, 93, 94, 97, 100 107, 110 112, 115, 117, 119, 124, 125 127, 133, 134, 137, 140, 141, 143, 144 146, 148, 149 152, 154, 158, 159, 160, 161, 163, 164,[a] 165

[a] Review articles.

variable, with the animals dying after 16 hours to 29 days. Passage of infected pig material has already been mentioned in connection with Pendl's work on inoculation into embryonated eggs. By means of intranasal instillation and intraperitoneal injections of bile from patients with infectious hepatitis into mice, Haagen and Wywiorski-Scheele[51] produced a liver pathology similar to that found in the patients; elementary bodies were seen in liver and lungs of mice, as well as in the human patients. The agent was filterable and could be maintained through serial passage.

The Motol virus, described later in this review, has been reported, by several investigators,[91, 92, 97, 147] as infectious for mice. A brief review on the early use of rodents in isolation attempts was prepared in 1954 by Mirick.[116] Two other papers, by MacCallum[108] and Morris,[122] have appeared; these cover the period up to 1954. The report of MacCallum[108] provided a summary of studies in animals (cats, dogs, mice, ferrets, etc.) usually associated with experimental laboratory work, whereas the study by Morris[122] provided the results of studies involving a wide range of both domestic and wild animals, including both mammals and birds. A total of 23 mammalian and six avian species were tested, all with negative results. Dresel *et al.*,[29] Herzberg,[64, 65] and Onizuka[135, 136] have reported on their attempts to propagate the virus in canaries. Dresel *et al.*[29] tested not only canaries but crossbreeds of goldfinches with canaries. Filtered urine was inoculated into the breast muscles of birds. Disease symptoms appeared

within 1 to 2 days after injection and consisted of ruffled plumage, shortness of breath, listlessness, and accelerated heart rates. Death usually occurred on the fourth day. Crossbreeds were most resistant; eight specimens survived two or three passages and, in one case, nine passages.

Some of the early reports on the efforts of Japanese workers to isolate hepatitis virus in small animals have been reviewed by Arakawa *et al.*[6] A series of reports by Hara and his coworkers[52] are among the more extensive studies. These investigators[52] inoculated liver cells, obtained from a fatal case of hepatitis, intraperitoneally into mice, which died 4 and 10 to 12 days later. Passage was made serially at least 20 times, with subsequent death at 5 days. Histologic lesions in mice closely resembled those seen in human patients. Virus was neutralized by human convalescent sera in dilutions up to 10^{-6}. Electron microscopy demonstrated virus particles of 100 to 150 mμ. In later articles Hara and his associates[53, 54] reported the isolation of a similar agent from other patients, and described the preparation of a skin test antigen from infected mouse tissue.

A rather impressive list of investigators who have attempted isolation of the virus in subhuman primates could be included in this review. The literature prior to 1954 has been reviewed by Evans.[42] In order not to duplicate publications that have covered the subject rather extensively, reference has been made only to several of the more recent reviews.[25, 55, 69, 156] The review by Harasti[55] included a comprehensive table of attempts and results with an appropriate bibliography. The attempts to date, although promising, still provide no breakthrough in terms of a useful animal host for the routine isolation of the agent for viral hepatitis, with the possible exception of a recent report by Deinhardt, Holmes, Capps, and Popper[26] on marmosets. These authors reported that inoculations of human serum or plasma, obtained during the acute phase of viral hepatitis, induced chemical and morphologic hepatic disease in marmosets in two out of five experimental series. The disease was transmissible in series from marmoset to marmoset, and resulted in increasing virulence. Liver biopsy specimens examined under code exhibited some of the characteristics of human viral hepatitis and were readily distinguishable from nonspecific changes. No *in vitro* viral isolation has been reported, although biophysical and serologic characterization studies of the infective plasma from marmosets are in progress. Final proof that the hepatitis observed in these marmosets is caused by agents of human viral hepatitis is still lacking and must await further investigation. Parks, Voss, and Melnick[138] have recently reported on studies of the Barker strain, isolated by Dienhardt *et al.*[26] in marmosets, as well as two viruses they isolated in marmosets. The study of Parks *et al.*[138] on the Barker strain showed this agent to have characteristics unlike those characteristics for human hepatitis virus, in that the agent was sensitive to ether and could be destroyed by heating at 56° C. for 30 minutes. Neither human γ-globulin nor convalescent marmoset serum altered the infectivity of the virus in marmosets. This report[138] also included a statement to the effect that pooled sera of "normal" marmosets produced similar patterns of liver enzyme and histopathologic changes similar to those noted in animals infected with either the Barker strain or with their own clinical specimens. A more detailed report of these observations will undoubtedly be forthcoming.

TISSUE CULTURE

Tissue culture studies have been performed extensively over the past 15 to 20 years in attempts to isolate etiologic agents for infectious hepatitis. It should suffice, at this point, to cite several papers that have involved a wide variety of cell lines, *e.g.*, the studies of Bang and Warwick,[11] Henle,[61] McLean,[112] Ananiev, Kaverin, Narskiy, and Barinskiy,[3] and Cole, Danks, and Campbell.[22] In the McLean study,[112] 18 cell lines were tested, eight of which gave at least some positive results although the results with all but one (PD39T) have not been consistent or clear-cut. Among the cell lines showing some promise were HeLa, human amnion and the Detroit-6 (strain PD). Ananiev *et al.*[3] studied the effect of K3 virus in a wide range of primary tissue cultures and cell lines, and reported a cytopathic effect in both human kidney and aorta primary cultures as well as some cytopathic effect in Detroit-6, KV, SK human kidney and SOTs cell lines. Highest titers of the agent were obtained in human kidney and aorta primary tissue cultures, the highest reached being 10^{-8} $TCID_{50}$. Among the primary tissue cultures tried were those of monkey, pig, guinea pig, calf, cat, rabbit, mouse, sheep, chick embryo, and dog. Cell lines included, other than those showing positive cytopathic effect, were HeLa, M10, SPEV and P_8O. Cole *et al.*[22] employed primary tissue cultures of monkey kidney, human amnion and lung, and human embryonic kidney and intestine. The HEp2 and HeLa cell lines and the Fairfield lines of human epithelium and monkey embryonic tissues as well as Detroit-6 were tested for susceptibility to their agent; only the Detroit-6 tissue culture supported growth of this agent. Gard and Alin[49] attempted to cultivate the agent of infectious hepatitis in oyster tissue, but the results were doubtful, in light of the difficulty these authors reported in growing satisfactory tissue material.

A comparison of isolation successes in the various cell lines would be of little significance because of the wide variation of material and its source. Many of the agents reported to date, as will be noted below, have been identified as various prototypes of classified viruses that may or may not bear a direct relationship to infectious hepatitis. Some of the cell lines that have been employed both with and without success included chick embryo, HeLa, human epithelial KB, human embryonic lung and kidney, monkey and rabbit kidney, and the Detroit-6 line. Cell lines of the latter in variant forms, VA and PD[105], have also been tested. Both stationary and roller tube methods have been employed with reported success. Organ cultures of liver have also been used for isolation attempts.[10]

Hillis and Bang[70] employed roller tube cultures of human and mouse embryo liver, as well as adult monkey, organ cultures of human embryo liver, and tryp-

sinized human embryo liver cells, in attempts to cultivate agents of infectious hepatitis—all without success. No cellular destruction or pathologic effect of any recognizable type was obtained with 58 infectious hepatitis specimens. Examination of the inoculated trypsinized cells failed to show any detectable antigen in indirect fluorescent antibody tests. Eight of the specimens inoculated had been demonstrated, in previous human tests, to contain agents capable of producing typical disease with jaundice. Tissue culture techniques have provided an additional means for studying the isolated agents by fluorescence microscopy[8, 75, 103] and other immunologic tests.[3, 4, 79, 102, 103] Comparisons have been reported concerning the cytopathic effect of various isolates from different sources[20] and have suggested a supplemental method of identification, which might be employed if the results could be consistently repeated and confirmed by other investigators.

AGENTS IDENTIFIED AS CLASSIFIED VIRUSES

A large number of viruses have been isolated and identified in association with cases of infectious hepatitis. Proof of a causative relationship to the disease has been difficult, and the status of these viruses, for the most part, has remained unclear. Many of the identified agents have been isolated from patients with symptoms not suggestive of infectious hepatitis, and the infectivity of the isolated agents and their ability to induce hepatitis have not been tested in man or experimental animals. Those agents which have been inoculated into man and test animals require further study and confirmation.

Kibrick[83] has recently reviewed the literature on the role of the Coxsackie viruses and echoviruses. He stated that "the relationship between Coxsackie B virus infection and hepatitis in newborns is clear," and that "there is suggestive evidence that Coxsackie A and B viruses may be related to hepatitis in patients beyond the newborn period." Although the number of references cited by Kibrick, both early and recent, would tend to support his assumption that these agents have been frequently isolated in connection with cases of hepatitis, a review of the cited papers identified only a few agents that were actually recovered from cases of infectious hepatitis. One such case is that of Morris, Elisberg, Pond, and Webb[124] who isolated a type 4, group A Coxsackie virus from the blood of a child with fever, rash, and a clinical picture of hepatitis. Both neutralizing and complement-fixing antibodies developed against the agent during the patient's convalescence. Whether the agent existed as a concurrent infection or actually produced the hepatitis symptoms cannot be ascertained. Embil, Van Rooyen, and Nagler[32] reported the isolation of a type 10, group A Coxsackie virus from the stools of 45 contacts in an outbreak of infectious hepatitis at a housing development. Four isolations were made from the blood of the contacts. Jézéquel and Steiner[77] recently reported the identification of the original Buchner and Shreeve[18] isolate, with which they have worked extensively,[75, 76] as cross-reacting with types 13 and 18, group A Coxsackie viral antisera. The original isolate was one of five agents recovered from the stools of five patients with infectious hepatitis. Isolates originally tested were found to be ether-resistant and heat-stable (in the presence of $MgCl_2$) up to temperatures of 60° C. for 30 to 40 minutes. These agents were noninfectious for suckling mice.

A report by Kibrick and Benirschke[84] involved two fatal cases of generalized infection in the neonatal period with isolation of type 4, group B Coxsackie virus. Isolation of the virus from the liver of one of the infants was reported. The report by O'Shaughnessey and Buechner[137] involved the isolation of a type 5, group B Coxsackie virus from a pregnant woman suffering from clinically detectable hepatitis. The paper by Siegel *et al.*[154] described the isolation of type 5, group B Coxsackie virus from young children during a 1961 outbreak of pleurodynia and pneumonia; at least 19 of the cases involved hepatomegaly. The clinical pattern in these cases makes a direct association with infectious hepatitis appear unlikely. The cases cited by Hosier and Newton[73] involving the isolation of various types of group B Coxsackie virus were characterized by severe focal hepatic necrosis without other evidence of infectious hepatitis. Sun and Smith[163] reported a case which suggested that infectious hepatitis may have occurred along the more classic symptoms of Coxsackie virus infections. The incidence of hepatitis associated with Coxsackie virus infection has been low, and the actual role of these viruses in infectious hepatitis has not been established.

The role of the echoviruses in hepatitis would appear to be more obscure. There have been reports[109, 145, 158] noting mild liver dysfunction or hepatomegaly associated with echovirus infections. Lapinleimu[100, 101] reported on the isolation, in 1959, of type 6 echovirus from two severe cases during a mild outbreak of hepatitis in Helsinki, Finland. Virus particles and antibody to echovirus type 6 were demonstrated. The author suggested that it might only have been a hepatotropic strain but that its close association with the disease made it a potential etiologic agent. Types 1 and 8 echovirus were reported by Kiseleva[86] to have been isolated from cases of infectious hepatitis in Turkmenia (U.S.S.R.). Neutralizing antibody against the isolated viruses was present in 63.3 per cent of the sera examined, with titers of 1/10 to 1 640—one case had a titer of 1 8000. In a parallel study with sera from 60 patients, type 1 echovirus was neutralized in 32 cases, and only two cases neutralized an adenovirus (unspecified type). The strains of echovirus isolated would appear to be closely associated with the described epidemic of hepatitis. Sharlai, Morozenko, and Talvik[150] have recently reported the isolation of diverse types of agents from patients suffering from infectious hepatitis which are associated with viruses of the intestinal and respiratory groups. These studies were performed on children with nonicteric forms of infectious hepatitis. Types 7 and 12 echovirus were involved in a number of the cited cases.

Types 1 and 2 reovirus have been isolated in associa-

tion with infectious hepatitis. The Brown and Buettner strains of a type 1-like reovirus were described by McKee[111] and by Burlingham and McKee,[16] and were isolated from the feces of patients with infectious hepatitis. Both virus strains could be propagated in chick embryos by either the chorioallantoic cavity or yolk sac route, and both produced a hemolysin, which was heat-stable at 121° C. for 21 minutes. The hemolysin was not firmly bound to the virion and could be separated by dialysis. The hemolysin did not require reovirus receptor sites to hemolyze erythrocytes, but the receptor sites appeared to be adjacent to the reovirus receptor. The role of these viruses in relation to viral hepatitis has not been clearly established. Strutsovskaia,[162] as reported in a Russian editorial,[30] recovered type 2 reovirus suspected as a possible etiologic agent in an outbreak of enteric infections with hepatitis in children. Joske *et al.*[78] reported on the isolation of type 2 reovirus from a number of patients, several of whom had clinical hepatitis. Although it was difficult to ascribe a definite connection between the reoviruses isolated and the children's illness, Joske and associates felt that, at least in the one fatal case, autopsy findings in the liver were typical of those observed in reovirus infections of infant mice. The occasional occurrence of encephalitis in infectious hepatitis indicated that, at least in some of their cases, a reovirus etiology could not be ruled out.

Felsenfeld[43] has reported repeated isolations of type 1 reovirus from stools of a psychology technician who handled a group of young chimpanzees, and also from the stools of the chimpanzees. Liver biopsies from both demonstrated changes suggestive of infectious hepatitis based on the Smetana[156] descriptions. A pseudoglandular transformation of parenchymal liver cells was apparently identical to that observed in epidemics of hepatitis in Delhi, India,[155] and in Accra, Ghana.[157]

The role of adenoviruses in association with infectious hepatitis has perhaps been even more interesting, although equally as unclear. Davis,[24] in 1961, isolated viral agents known as the San Carlos agents from the stools of young children hospitalized during an outbreak of hepatitis on an Indian reservation. Recently, Deinhardt and Holmes[25] and Hatch and Siem[59] reported identification of these agents as (predominantly) adenoviruses. Hatch and Siem[59] confirmed the identity of the Davis agents as types 1 and 3 adenovirus. In addition, an isolate identified as type 13 echovirus was found. The type 3 adenovirus differed from the prototype 3 adenovirus by hemagglutination-inhibition. By the use of the hemagglutination-inhibition technique, a relationship to type 16 adenovirus was indicated. Kerim-Zade[82] utilized HeLa cells for primary isolation and recovered 24 cytopathogenic agents from feces of 50 patients with infectious hepatitis. Of these agents, 23 were found to be antigenically and serologically related to types 1 and 4 adenovirus. The strains induced intracellular inclusions in monkey kidney, HeLa, and HEp2 cells. A significant proportion of paired sera tested showed an increase in complement fixation antibody. Hillis[67, 68] reported on viral agents isolated from sera of both human patients and chimpanzees with symptoms of infectious hepatitis, and these agents have been identified as similar to the type 5 adenovirus.[66]

Human experimental infections with at least one type of adenovirus have failed to induce hepatitis. Kasel, Loda, and Knight[81] utilized a type 16 adenovirus, recently isolated from children, in an attempt to infect volunteer subjects via the conjunctiva. Virus could be isolated from the inoculated eyes, the throat, and rectum, but no antibodies were demonstrable following injection. During the course of 7 months of observation, no jaundice or signs of liver dysfunction was noted. Hartwell, Love, and Eidenbock[57] reported the isolation of type 5 adenovirus from blood clots of patients in sporadic outbreaks of hepatitis in Arizona (U. S. A.), all in the vicinity of the city, Phoenix. To offset the possibility that adenovirus might be prevalent in the normal population, 70 blood samples were examined from normal patients of a similar age group, with only one isolation of a type 5 adenovirus.

During a 12-month period, Köhler, Apodaca, and Springer[89] examined 288 serum samples from clinical hepatitis patients and made 162 adenovirus isolations. During the same period, examination of serum specimens from 103 patients without evidence of hepatitis yielded only 10 isolations of adenovirus. Isolations were made in primary pig kidney cell cultures. No cytopathic effect was observed; however, identification was made by histologic examination of the cell monolayers. Repeated examinations were performed on 62 patients; 69 per cent of these cases gave repeated positives during the first 20 days after onset of icterus. None of the 40 isolates demonstrated any similarity with the 31 known types of adenoviruses. Neutralizing antibodies could not be demonstrated with homologous antisera. At least two diverse serotypes have been identified among the isolates. This report was a follow-up to the earlier report by Köhler and Apodaca[88] in 1966.

Although adenoviruses had been recovered from cases of infectious hepatitis, the etiologic basis for the disease was not clearly established for these agents. When a description of the adenovirus-associated, defective particles was reported by Hoggan and his associates[71, 72] and by Atchison, Casto, and Hammon,[7] their small size stimulated further research on a possible etiologic role in infectious hepatitis. The adenoassociated viral particles were dependent solely upon the presence of the adenovirus for propagation. They were approximately 220 to 240 Å in diameter as observed in negative contrast electron microscopy, and consisted of a deoxyribonucleic acid core. Haselkorn[58] considered them analogous to the satellite particles of the tobacco necrosis virus. Hoggan, Blacklow, Rapajko, and Rowe[72] described four or more serologic subtypes of the adenovirus-associated particles. As reported by Morris,[123] extensive studies have not been successful in the establishment of an etiologic relationship for either the adenoviruses or the subtypes of adenovirus-associated particles to infectious hepatitis.

Two abstracts of papers, presented at the XVIII

Scientific Session devoted to problems of infectious hepatitis held in Moscow (U.S.S.R.) during 1965, have recently appeared in the literature.[2, 90] In one of these, Korenblit and Panchenko[90] reported that viruses isolated from the blood of infectious hepatitis patients on a Detroit cell culture were neutralized with canine serum to type 1 adenovirus; however, in contradistinction to the general properties of the adenovirus group, the virus caused death of newborn and adult mice upon inoculation in the brain and abdominal cavity. The viral titers in the brain of the sick mice were 10^{-3} and 10^{-4} LD_{50}, and the virus retained the capacity to evoke typical cytopathic changes in tissue culture. In addition, the virus could be passed in 10- to 12-day chick embryos by inoculation into the allantoic and amniotic cavities. The properties mentioned are similar to those of the known virus strain called Motol, isolated by Kubelka, Slavik, and Sousek[97] in Czechoslovakia, and will be described later. The second paper by Ananiev *et al.*[2] was concerned with a study of the role of latent viruses in infectious hepatitis. Virologic studies were performed by using tissue cultures of Detroit-6 and trypsinized cultures of human embryonic kidney tissue, from which 89 strains of viruses were isolated in 1120 samples from 1023 patients with infectious hepatitis. The majority of the strains isolated were classified as belonging to the latent group of types 1 and 5 adenovirus and to the enterovirus group according to their biologic, physicochemical, and serologic properties.

Krugman and associates,[50, 104, 108] over a long period of time, studied cytopathic agents isolated from both inapparent and apparent infectious hepatitis and demonstrated the time the agents appeared in the blood and urine. Recently, this group[107, 140] has reported the identification of the Willowbrook (WB) virus (isolated from patients with inapparent and apparent hepatitis) as a myxovirus and a member of the parainfluenza group of viruses. This adds another previously known virus as a potential etiologic agent in infectious hepatitis. A relationship of WB virus to SV-5 is suggested by a reciprocal rise of SV-5 antibody titers in WB-immunized animals although homologous antibody titers were at least 8-fold higher than heterologous titers. The apparent relationship to SV-5 raises the possibility that WB virus might have been isolated as a contaminant from tissue cultures infected with a simian virus although it seems unlikely in this study, since (1) only WI-38 tissue cultures were used for primary isolations; (2) reisolations were performed in new stocks of WI-38 cultures; (3) negative specimens remained negative on repeated blind tissue culture passage; (4) isolation was made in at least one case in another laboratory directly from a serum specimen taken 33 days after inoculation, but not from the preinoculation specimen; and (5) each of the patients from whom virus was isolated developed a 4-fold rise in serum-neutralizing antibody.

In 1961, O'Malley, Meyer, and Smadel[134] isolated an agent designated as A-1 from a National Institutes of Health (NIH) plasma pool. The pool was previously used in volunteer studies and was known to contain the virus for serum hepatitis. Interestingly enough, serum specimens from 30 volunteers, recipients of the original pool of plasma, revealed rising levels of antibody against the A-1 agent, whereas serum specimens from patients with infectious hepatitis failed, for the most part, to reveal any antibodies against the A-1 agent. Only recently, O'Malley, McGee, Barile, and Barker[133] reported the identification of the A-1 agent as *Mycoplasma gallisepticum*, which would account for lack of neutralization by infectious hepatitis patient sera. Bolin, Alsever, Berger, and Jarvis[15] reported multiple isolations of viruses from the blood of volunteers inoculated with the NIH plasma pool 6. Further studies[16, 17] reported by this group will be discussed later in this paper. Some of the original isolates were infectious for mice and produced a cytopathic effect on a wide range of host cells.

If the list of references associated with known agents seems lengthy, that of unidentified agents is even more impressive although many of those originally reported as unidentifiable have now been classified. These isolations have been reported from around the world. At present, there has been little or no opportunity for one laboratory to check and confirm the results of another. This interrelationship should increase as investigators are provided more opportunity to come together and discuss their observations. A recent example of this occurred in the IX International Congress for Microbiology, which devoted a focal topic session to infectious hepatitis.[1] The problem of evaluating viral isolations in infectious hepatitis has been compounded by the lack of good, reliable serologic tests, lack of a suitable animal host, and the difficulty involved in conducting studies in volunteer subjects.

In Canada, virologists have been active in this field, beginning with the report by Franklin and Sinclair,[45] who attempted the isolation of the agent of infectious hepatitis by using the Detroit-6 cell line. These results were negative or inconclusive. Buchner and Shreeve[18] reported the isolation of five agents from stools of patients with infectious hepatitis, two of which were studied in detail. The original studies indicated that the agents were not identified as known enteroviruses. Intermediary reports by Jézéquel and Steiner[75, 76] described further attempts to identify and characterize these agents. Electron microscopy revealed viral particles with diameters of 250 Å. A recent report by Jézéquel and Steiner[77] cited the serologic cross-relationship of one of the original isolates with two strains of Coxsackie virus. Embil *et al.*[32] have already been cited for their recent reported isolation of a type 10, group B Coxsackie virus, in connection with an outbreak of infectious hepatitis.

In Czechoslovakia in 1957, Kubelka *et al.*[97] reported the isolation of an agent known as Motol virus from pooled sera of two patients with hepatitis. Under certain conditions, the virus agglutinated erythrocytes and was infectious for mice. Georgiades and Zacek in personal communications to Kubelka[95] have suggested that it is probably a deoxyribonucleic acid virus, approximately

1000 Å in diameter. The C5 fraction of the Motol viral preparation, however, has been reported to have viral particles of approximately 200 Å with hexagonal form.[90] Kubelka, Schon, and Sulcova[98] recently reported that the hemagglutinating fraction was antigenically related to type 5 simian virus or the DA virus of Hsiung, Isacson, and McCollum,[74] and could possibly be a simian virus. Motol virus has been studied by other groups in Poland.[20, 91, 92] The infectious nature of Motol virus in mice has been amply described,[91, 92, 97, 147] and typical signs of acute hepatitis have been reported, with viral isolation from most organs and pathologic changes consistent with hepatitis.

Recent reports[100, 101] from Finland have indicated the possible role of type 6 echovirus in a hepatitis outbreak in Helsinki. A further discussion on this work appears earlier under the possible role of the echoviruses in the etiology of hepatitis.

German investigators have been active in hepatitis research for a long time. Much of their early work was described under the section involving egg and other animal isolation attempts.[27, 29, 139] Particularly productive over a long span of years has been the work from the laboratory of Essen and his colleagues.[34-41] These studies have involved isolation attempts, electron microscopy, and skin testing of hepatitis patients with the use of virus material propagated in embryonated chicken eggs. Recent reports have appeared by Kachani[79] and Spies[159-161] concerning the isolation of viral agents associated with infectious hepatitis. The work of Dr. Spies has centered primarily around the isolation and characterization of autointerfering viruses found in association with cases of infectious hepatitis. His first isolation was found in association with the Motol virus originally isolated by Kubelka *et al.*[97] discussed earlier. The isolation of cytopathogenic human autointerfering (CHAI) virus from 71 patients has been reported by Spies[160] with the use of high dilutions of patient material. Undiluted material or material diluted less than 10^{-3} did not show a cytopathic effect in the human epithelial liver cells of Chang or in swine and hamster kidney tissue cultures. The use of 20-methylcholanthrene appeared to enhance growth with the more concentrated viral inocula. Brief characterizations have been reported for the CHAI viruses, but to date they have not been identified more specifically.

In Italy, Segagni *et al.*[145] have reported their isolations in both tissue culture and embryonated eggs of agents found in the sera of 30 children with infectious hepatitis. Hepatic lesions were reported in the eggs 72 hours after inoculation. Babudieri *et al.*[9] reported tissue culture isolation attempts and the use of fluorescent antibody techniques in an endeavor to demonstrate both virus and antibody. Their results, however, are inconclusive although reported as encouraging by them.

Japanese workers have reported work on the etiologic agents of hepatitis for several years even though many of their reports have not been readily available in this country. Much of the status of the early work has been reviewed in a paper by Arakawa *et al.*[6] The work of this group involved isolation and fixation of infectious hepatitis virus in mouse brain by using, as inocula, the sera obtained directly from febrile hepatitis patients or obtained after serial propagation in embryonated chicken eggs. Positive complement fixation tests were obtained in 85 per cent of hepatitis cases, with the Mita strain as an antigen. Ultrafiltration tests performed by Arakawa and his group found the end point of filtration at 580 Å, and electron microscopy showed a diameter of 500 to 600 Å. Muraoka[128, 129] studied the biochemical and biophysical properties of the Mita strain and found positive complement fixation tests in all of the patients whose sera had been collected before the 20th day of their illness. In all other sera, collected after the 20th day of illness, only 60 per cent were positive for complement fixation antibody with the Mita strain of virus. Average neutralization indices of 320.5 and 207.6, respectively, were observed for these two groups of sera. Six cases of infectious hepatitis from the Medical College, Okayama, Japan had negative complement fixation tests but an average neutralization index of 21.7. Sera from five healthy individuals were negative for both tests. Complement fixation tests were negative in sera from five cases of serum hepatitis, but one individual had an equivocal serum-virus neutralization titer. Muraoka[130] also described histopathologic changes in liver tissues of mice that had received inoculations with the fixed virus. Fujiwara,[46-48] Kurauchi,[99] Ogasahara,[131, 132] and Onizuka[135, 136] reported extensive investigations of viral agents recovered from cases of infectious hepatitis in the Okayama prefecture. The viral strains were isolated from patient materials by successive transmission through mice and embryonated chicken eggs. According to Ogasahara[131, 132] the chick embryo was not very susceptible to the virus, but the mice showed pathologic findings on infection. Kurauchi[99] studied the course of chronic infection in the mouse with a virus recovered from the patients in the Okayama prefecture. Although a few of the mice died over this period (500 days) and had marked pathologic changes, most of the mice developed a chronic infection with degeneration and necrosis of the parenchymal cells and cellular infiltration. In the mouse lungs, the proliferation of round cells or mesenchymal cells around the bronchi and blood vessels was observed. Adaptation of the virus from the mouse to the chick embryo was established, suggesting that the virus had remained in the mouse organs for an extensive period of time with repeated viral multiplication and diminution. Fujiwara[46-48] described use of complement fixation and neutralization tests which employed a mouse liver homogenate as an antigen. Through a modified *Absättigungsversuch*, or cross-protection test, the results of the complement fixation and neutralization tests could be correlated and were found to elicit a common antigenicity among the isolated viral strains. The modified *Absättigungsversuch* was serologically significant with high titered sera but not with sera of a low titer against the viral agents.

Onizuka[135] described studies of fowl susceptibility to six varied strains of viruses (Ishihara, Kanamitsu, Noda, Ogawa, Morimoto, Aomori) which were isolated from

patients with infectious hepatitis in the Okayama prefecture. His criteria for infection, in eight species of fowl, were the specific pathologic changes in the liver and lung. With fixation of the viral strains achieved in five successive passages of the viral agents, pathologic alterations were increasingly severe. The most marked effects were observed at viral infectivity of 10^{-9} in *Serinus canaria*, and 10^{-10} in *Uroloncha domestica*, with successful reversion of the virus from fowl to mice in all cases. In his experiments of chronic infection in fowls, Onizuka[136] inoculated the Ishihara, Kanamitsu, and Noda strains of viruses into 37 canaries, 33 siskins (*Chloris sinica minor T. & S.*), and 20 lovebirds. Over a course of 6 months, chronic infections were judged to be present by alterations in the pathology of the liver and lung as well as in the white cell count and blood picture. A few lethal cases were observed; however, inapparent or chronic infections could usually be established with intrapectoral inoculation of suspensions prepared from mouse livers or embryonated eggs that had received the above three strains of viruses. Remarkable leukopenia was observed in the early stages of infection with a severe increase of atypical and vacuole-degenerated leukocytes. Liver alterations consisted of round cell infiltration in the periportal connective tissue with various types of cellular degeneration, such as nodules and necrotic changes, with localized necrosis scattered in the intermediate parts of the acini. The siskins were the most susceptible of the tested fowl, with many lethal cases in which very severe necrosis and cellular infiltration occurred in the liver. The changes in the lungs appeared later than those in the liver and persisted for 6 months, with mesodermal cellular infiltration and interalveolitis with hemorrhage around the bronchi, alveoli, and blood vessels.

Kimura and Hotta[55] reported the transmission of a viral agent recovered from patients during an epidemic of hepatitis occurring from autumn of 1945 to spring of 1946 in the Kyoto-Osaka-Kobe district of Japan. Heparinized blood, taken from patients in the febrile stage of illness, was inoculated via the intrahepatic route into white mice. An intracutaneous injection of a suspension of liver tissue from an infected mouse was given to a healthy adult man. After 23 days, a condition developed in the volunteer subject, which could not be distinguished from a case of nonicteric infectious hepatitis. Hayashi, Kawasoe, and Takahashi[60] reported more recently on the isolation of viral agents associated with hepatitis in Japan. The studies of Hara and his associates[52-54] were commented on earlier in this paper.

There have been reports from two Polish laboratories. Chlap, Georgiades, and Porwit-Bobrowa[20] reported on a comparative study of four groups of viral agents: (1) Motol virus, (2) 01, Severowa, (3) adenoviruses, and (4) a group of cytopathogenic viruses studied in association with their characteristic cytopathic effects in the KB line of tissue culture. By using the classification criteria of Enders,[33] they reported that the Motol virus produced a tissue culture change comparable to those described by Enders for Group I. The unspecified cytopathogenic group of viruses were described as producing morphologic changes similar to the second group of Enders. The 01, Severowa strain produced a cytopathic effect comparable to the third type of degeneration described by Enders. They concluded that sufficient morphologic differences occurred in the *in vitro* cultures for this type of information to provide a useful supplement to the identification of newly isolated viruses. The report by Morzycka and Taylor[125] pointed out that these authors were unable to isolate agents from blood and tissue specimens of hepatitis patients in the Detroit-6 strains of tissue culture even though virus isolations had been demonstrated in other host systems. The original isolations were made in human embryonic tissue cultures, as described by Morzycki *et al.*[126]

Rumanian workers have been very active in hepatitis research in at least two laboratories. Laszlo[103-105] and his associates have reported the isolation of viral agents in both human and embryonic chicken liver tissue culture. These studies included serologic testing as well as electron microscopic studies of the agents isolated. All of these agents measured in the range of 100 to 700 Å. More recently Laszlo *et al.*[102] have reported on a study of 21 strains isolated between 1957 and 1964. All of these were isolated in Detroit-6 cells. Both neutralization and complement fixation tests were employed to establish serologic relationships among the strains isolated from the patients. Since 1962, Mitroiu and his coworkers[117-121] have reported their studies of agents originally isolated from patients with epidemic hepatitis. These agents grew well in human epithelial, KB, HeLa, HEp2, Detroit-6, and monkey heart cell cultures. The viruses could not be identified serologically with any known enterovirus antisera although they were neutralized by the sera from convalescent patients. Included in more recent studies was the demonstration of a serologic relationship between the strain isolated by Mitroiu and associates and the Russian strain, K3, described by Ananiev *et al.*,[4] as well as changes in the isolates of Mitroiu *et al.* on adaptation to human embryonic liver cells in culture.

Russian workers have been quite active. Probably the most active have been Ananiev and his associates,[2-4, 12, 13] who have reported on Botkin's disease (a term frequently used in the Russian literature for infectious hepatitis). The strain principally associated with this disease is called K3, and the studies of Ananiev and his coworkers have involved descriptions of the viral properties in tissue cultures, as well as of the serologic behavior and an unidentified factor which seemed to enhance the susceptibility of cells to the agent. The relationship between this strain and the Rumanian isolates has already been indicated.[118] Klyachko and Galko[57] have reported the isolation (in Detroit-6 cell cultures) of viral agents from patients with infectious hepatitis. These viruses have been carried in serial passage after their isolation from blood specimens heated to 60° C. All six strains of virus appear to belong to a

homogeneous group and are not neutralized by immune sera against known viral agents. Sera from one-third of the patients neutralized the isolated agents in low titer. Further studies concerning their isolates were reported to be underway. Proskuryakova and Novikova[141] attempted viral isolations from 200 patients with infectious hepatitis by using primary human embryonic kidney and Detroit-6 cell cultures. More than 10 strains of cytopathic viruses were isolated, 20 of which were identified as type 3, group B Coxsackie viruses. Fourteen of the strains were not neutralized by various antisera from the enterovirus, herpes, and adenovirus groups. Of these strains two were studied in detail (strains 1-62 and 2-62). Both strains were nonpathogenic for suckling mice and did not multiply in chick embryo. They did not show any hemagglutinating activity against erythrocytes of man, horse, chicken, goose, sheep, monkey, or guinea pig. The viruses were stable in the presence of both ether and chloroform. A common antigen of strain 1-62 was noted with the K3 virus isolated in Moscow. The other 12 nontypable strains were similar to strains 1-62 and 2-62 in their cultured and cytopathic effects. Further studies on these agents and their relationship to hepatitis are being performed.

The report of Sharlai *et al.*[150] was mentioned earlier. Shevchenko, Danileichenko, and Metelitsa[152] isolated nine cytopathogenic agents from patients with infectious hepatitis by means of the Detroit-6 cell line. All agents withstood heating at 60° C. for 30 minutes and were not susceptible to the effects of ether. They did not possess hemagglutinating ability against any of the erythrocytes tested. The cytopathic effect of these agents in tissue culture was neutralized by the sera of the convalescent patients, but not by sera of known viral agents belonging to the enterovirus or adenovirus group.

Much of the work performed by American investigators has already been described in studies under isolates identified as known viral agents. This is probably due to the extensive and continuing work in attempting to identify the isolates. However, there remain several United States isolation attempts for which agents have not been classified. Bolin *et al.*[15] reported the recovery of serum hepatitis isolates from volunteers inoculated with an NIH plasma pool. (This was the identical pool from which O'Malley *et al.*[134] recovered the A-1 agent, later described as a *Mycoplasma gallisepticum*.[133]) The viral agents were initially recovered in tissue culture of human lung cells, but after passage in tissue culture they grew in a wide spectrum of cell lines. In over 100 attempts, Bolin *et al.*[15] were not able to isolate an agent directly from the source of the A-1 agent. Later reports by Bolin, Brauninger, Pardee, and Alsever[16] and by Bolin, Chase, Alsever, and Mann[17] have been concerned with various serologic tests which were developed with the use of the isolates from Bolin's volunteer group as antigens. The first paper[16] was concerned with a complement fixation test and the second paper[17] with a latex coagulation test. The latter study suggested a relationship between infectious mononucleosis and the volunteer isolates used. In the absence of further reports from this group on the agents, an evaluation of the findings cannot be adequately made.

One of the most lengthy studies involving hepatitis isolates has been made by Rightsel, Keltsch, Tekushan, and McLean[144] who reported, in 1956, the isolation of numerous agents from sera and stools of hepatitis patients. Several reports were published by this group of investigators between 1956 and the present time.[14, 143, 165] The system they used in recent years involved the development of a cloned strain of Detroit-6 cells. These cells demonstrated consistent susceptibility in their laboratories and reasonably consistent susceptibility in those of other workers, although at least two early attempts by Morzycka and Taylor[125] and Franklin and Sinclair[45] were unsuccessful in the use of the cell line for isolation. The studies by Rightsel and his associates have provided considerable information, both in laboratory and clinical details, on the behavior of their isolates. There have been numerous reports of studies by former associates of the group including Schneider, Taylor, McCaughey, and Muirhead[146] and Muirhead and Schneider[127] who are still actively engaged in isolation attempts. More recently, Cole *et al.*[22] reported from Australia on the use of Detroit-6 cell line for viral isolation in neonatal liver disease, as well as in 28 sera of patients admitted to Fairfield Hospital, Victoria, Australia, with the diagnosis of infectious hepatitis.[21] Of the sera examined, 27 were reported as positive with this cell line. A recent report by Ferris and Cole[44] described experiments that used coded sera, and the Detroit-6 cells showed cytopathic effects almost as frequently in the control sera as in sera from patients with infectious hepatitis. Cross and Marmion[23] also reported a similar type of nonspecific cytopathic effect in cultures of Bolin's human lung epithelial cells. The exact cause of the cellular degeneration observed in both cell lines was not evident.

El-Alfi, Smith, and Biesele[41] have reported on the passage of an agent isolated from infectious hepatitis material in leukocyte cultures; infected cultures demonstrated chromosome breakage. No mention was made in the original report concerning the agent itself and its possible relationship to other isolates, or the patient's sera from whom the agent was recovered. Mella and Lang[115] have described the suppression of leukocyte mitosis both in patients suffering from infectious hepatitis and in normal leukocytes exposed to serum from patients with the disease. These reports, although they do not contribute appreciably to our knowledge of the etiologic agents, have provided partial insight into some of the possible effects of agents associated with infectious hepatitis.

After a retrospective review of the literature published to date, several facts have emerged from all of the studies reported. The first of these is that a wide variety of agents has been isolated from cases of infectious hepatitis ranging from the well known viruses to those not previously classified. Therefore, whether the cause of hepatitis, like the common cold, is protean

in nature or whether the etiologic agent is one or more of the specific agents, the hepatitis problem remains unsolved inasmuch as most work has failed to fulfill the classic Koch's postulates. Moreover, there have been few attempts to correlate viral agents and patients sera, obtained by different investigators, with one another. In spite of this difficulty, certain interrelationships have become apparent. For example, the recent reports by Liebhaber, Krugman, McGregor, and Giles[107] and by Prose, Balk, Liebhaber, and Krugman,[140] concerning an agent (WB) which had been previously isolated and described[106] and which has now been identified as a myxovirus, might have been of only passing interest. However, this particular agent was found to have an antigenic relationship to a type 5 simian virus. It should now be recalled that in an earlier report (Hsiung, Isacson, and McCollum[74]) in 1962, it was noted that an agent (DA) was recovered from a fatal case of infectious hepatitis. The DA agent also was immunologically related to type 5 simian virus. There was good evidence to show that the DA virus did not originate from the simian tissue cultures subsequently used in the studies of Hsiung *et al.* More recently, Kubelka *et al.*[98] reported that the hemagglutinating factors of the Motol virus were antigenically related to type 5 simian virus and to the DA virus. These reports, occurring independently on agents isolated and studied over a wide range of time, have demonstrated at least a certain antigen in common among these agents recovered in association with cases of human infectious hepatitis. Of course, the possibility of simian virus contaminants or the activation of latent simian viruses in the tissue cultures cannot be precluded in every case, but in two of these reports this would appear to be highly unlikely. Aulisio, Wong, and Morris,[8] on the other hand, have reported that examination of paired sera for type 5 simian virus-neutralizing antibody from 50 cases of hepatitis, contracted by United States troops in several geographic areas of the Far East, showed that 41 gave negative titer results in dilutions of 1/4. The remaining nine paired sera showed only titers from 1/8 to 1/32 in acute and convalescent sera. Aulisio *et al.*[8] concluded that on the basis of their data it was reasonable to assume that type 5 simian virus was not of etiologic importance in the hepatitis cases examined in their study. It is this type of collation of data from widely divergent sources that this review has attempted to stimulate by citing old, new, and extensive foreign entries so that those investigators most familiar with the field might reevaluate our present knowledge and discern previously unnoticed similarities.

Another common factor which has emerged from the work of numerous investigators has been the size of the particles associated with the unclassified agents that have now been recovered from human cases of infectious hepatitis. In review of the studies, which involved observation by electron microscopy of the isolated agents, at least six investigators have reported a virus-like particle with a size ranging from 180 to 200 Å in diameter. Their reports included the AR-17 isolate of Rightsel *et al.*,[144] the R, V9, V6, and 163S strains of Laszlo *et al.*,[102] the CHAI viruses of Spies,[160] the C5 fraction of the Motol virus of Kubelka,[98] the viral agent of Essen,[34] and the A-2 (CW) plaque virus initially recovered by the staff at the Armed Forces Institute of Pathology, Washington, D. C., and reported by Shaw and Banks.[151] Although the particle size might be similar to any number of viral agents, it has provided a certain uniform criterion of size for a number of isolates to date.

It has been an adamant requirement that each new viral agent, recovered in association with this disease, be thoroughly characterized and, if possible, classified. Much of the confusion that exists with regard to etiologic agents has existed in subsequent susceptibility studies in animals, in that such passages have possibly activated a latent virus from the animal host. The problem of relating the animal host to the originally isolated virus and to the human disease syndrome has been considerably difficult in resolution. The pursuit of the etiologic agent must be encouraged, not only under the standards and accepted principles and techniques of viral immunology but also within the framework of new concepts evolving from studies of molecular genetics, which have enlarged the comprehension and control of the mechanisms of viral and host cell interactions. Much work still remains to be done, in relating other properties of agents recovered in association with viral hepatitis, which the authors hope will soon be forthcoming.

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