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A COMPREHENSIVE STUDY OF VIRAL HEPATITIS

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A B S T R A C T

1. Preparing Institution: Department of Pathology, Johns Hopkins University School of Medicine, Baltimore 5, Maryland.

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Investigations carried out under this contract have included the following:

I. A study of viral agents associated with infectious hepatitis in man.

A. Study of candidate agents. A major effort has been the study of the virus tissue culture cell system supplied by Parke, Davis & Co. Research Laboratories. And repeated attempts under specified conditions utilizing several cloned lines of D6y cells, infective plasma, and cultures of representative agents, cytopathic effects were obtained only sporatically and unpredictably. Although it was concluded that the Parke,Davis test system is not a useful laboratory tool at the present time, a commitment has been made by Parke, Davis to supply acute phase sera from experimentally inoculated human volunteers for independent isolation attempts in the future. The San Carlos 8 agent has been found to multiply readily in a variety of human cell cultures. The observation of others that this agent types as an adenovirus type 16 was confirmed but an antiserum produced in this laboratory against the San Carlos agent appeared to show a one-way (prime) relationship rather than a relationship of identity. This will have to be checked by a complete box neutralization test. CF tests with 10-paired sera from confirmed cases of Korean hepatitis against the San Carlos agent showed positive reactions of 1 in 8 or greater and only one-half the cases and none showed a significant rise in titer. A working agreement has been reached with the Cutter Laboratories for release for two additional candidate agents for study in this laboratory.

B. Through the courtesy of Dr. Riopelle of the Yerkes Laboratories in Orange Park, 3 young chimpanzees were inoculated, 2 with the San Carlos 8 agent and 1 with serum obtained from a confirmed case of Korean hepatitis. The clinical course of these animals is still being followed but all, beginning on Day 29, should arise in SGOT values and serial biopsies have been taken for histologic examination. The San Carlos agent has been isolated from all 3 of the chimpanzees and probably indicates a cross infection. Efforts to isolate additional candidate agents from the material brought back from Korea by Major Conrad are continuing.

II. The histopathologic aspects of viral hepatitis. Descriptions of the histologic findings in liver needle biopsies on 100 chimpanzees of various ages and sexes, on the animals inoculated as described above, on liver needle biopsies from chimpanzees with so-called

"contact viral hepatitis", and of liver biopsies from human volunteers inoculated with the Parke, Davis agents are described in considerable detail in the main report.

III. Further Others.

A. The histologic comparison of cases of neonatal hepatitis in biliary atresia has been carried out and is to be published.

B. Electron microscopic studies of various stages of liver injury produced by the murine hepatitis virus has been continued. Additionally a study is underway of the serial changes in mice infected with the cytomegalovirus. Continuing attempts to obtain the propagation of parenchymal murine liver cells on a collagen base are underway. Finally, a comprehensive review of spontaneous liver lesions in experimental animals is in preparation.

NOTE: Qualified requestors may obtain copies of this report from the Armed Services Technical Information Agency.

PROGRESS REPORT

Introductory Statement. Investigations supported by this contract during 1962-63 have been carried out in several laboratories at Johns Hopkins in Baltimore, at the Armed Forces Institute of Pathology in Washington, and at the Yerkes Farm in Orange Park, Florida. Under the various headings below, the names of professional personnel responsible for each phase of the study are given. Incidentally, studies with Dr. Riopelle of the Yerkes Laboratory will be removed to the Tulane Primate Laboratory in Covington, Louisiana in the future in accordance with Dr. Riopelle's recent change in location and affiliation.

I. STUDY OF VIRAL AGENTS ASSOCIATED WITH INFECTIOUS HEPATITIS IN MAN (Drs. Berge, Douglas, and Felsenfield).

A. Study of Candidate Agents. A major effort was made to study the virus-tissue culture cell system supplied by the Parke, Davis and Company Research Laboratories. Materials supplied included several cloned lines of Detroit 6 (D6y) cells, infective plasma and tissue culture preparations of representative agents, and pre-tested culture media and unfiltered fetal bovine serum for growth and maintenance studies. In repeated attempts under specified conditions, clear-cut cytopathic effects were obtained only sporadically and unpredictably, and were frequently difficult to distinguish from similar effects encountered in non-inoculated cell passage controls. An interferon-like effect was suspected but not proved. Attempts to develop a viral interference test to demonstrate infection of D6 cells in the absence of CPE, employing a number of ECHO viruses (1-4, 5-9, inclusive) as challenge agents, were unrewarding. No CPE was found in other human cell cultures (Davis continuous embryonic lung, primary embryonic kidney) in blind-passage attempts. It was concluded that the PD test system would not provide a useful laboratory tool for purposes of the present study until cultural conditions could be better defined.

For possible continued study, a commitment has been made by Parke, Davis to supply acute phase sera from experimentally inoculated human volunteers for independent isolation attempts. Specific immune sera or convalescent sera from volunteers experimentally infected with the PD agents are to be made available at a later date for trials with immunofluorescent techniques.

One San Carlos agent (San Carlos 8) was received from Dr. Eldon Davis in mid-december 1962. This agent was found to multiply readily in a variety of human cell cultures (Davis continuous embryonic lung, D6y, diploid fetal kidney, WI-26, primary embryonic kidney, primary embryonic lung). Cytopathic effects were also produced in continuous chimpanzee liver, primary fetal lamb lung, primary guinea pig embryo heart cells, and to a limited degree in African green monkey kidney cells. The CPE was characteristic of that shown by Adenovirus.

It was reported to us verbally by Dr. Davis that the San Carlos agent had been typed as an Adenovirus Type 16. In tests with prototype Adeno 16 antiserum obtained from Dr. Wallace Rowe, Dr. Janet Hartley and from Microbiological Associates, this relationship was confirmed. However, San Carlos antiserum produced in our laboratory appeared to show a one-way (prime) relationship rather

than a relationship of identity. Approximately 32 units of San Carlos antiserum were required to protect against 16-160 TCD_{50} of Adeno 16 virus; while 8 units of serum neutralized 1,600 TCD_{50} of the homologous S.C. virus. This typing is being re-confirmed in a complete box neutralization test.

Screening tests for neutralizing antibody against the San Carlos agent with a limited number of paired sera from chimpanzee-associated hepatitis cases at Holloman AFB, supplied by Dr. William Hillis, and from the well-documented cases of infectious hepatitis occurring in American military personnel in Korea, provided by Dr. Marcel Conrad, have shown interesting results. Both acute-phase and convalescent sera showed presence of neutralizing antibody of still undetermined titer in all specimens tested. The significance of these preliminary findings has not yet been established.

Complement-fixation tests with 10 paired sera from the Korean series against 2 units of the San Carlos agent showed positive reactions of 1 in 8 or greater in only one-half of the cases, with none showing a significant rise in titer. Whether these reactions represent adenovirus group-reactive antibody has not yet been determined. Similar tests have not been carried out with sera from other types of patients or from normal individuals.

A working agreement has just been reached with the Cutter Laboratories for release for our examination of two additional candidate agents isolated by Dr. Karol Hok. One strain was said to have been recovered repeatedly in a Cutter line of FL cells (FL-RR) from a hepatitis stool pool, while a second agent was recovered from a non-irradiated fibrinogen preparation. These agents are said not to be identifiable in relation to any previously recognized enterovirus either in the laboratory of Dr. Hok or of Dr. Lennette. FL-RR cells, viral isolates and specific immune rabbit serum are to be supplied for study.

B. Chimpanzee Study. Three young chimpanzees born and raised under semi-isolation conditions at the Yerkes Laboratories in Orange Park were made available for study by Dr. Riopelle. Consideration was first given to testing the Parke, Davis material in these animals, but a decision was made against it in view of our failure to manipulate the test system adequately in the laboratory, with consequent doubt that we could recover the agent or perform meaningful serologic studies following infection.

The San Carlos 8 agent was selected for test. Baseline liver biopsies, serum and stool specimens were obtained on the three animals prior to inoculation. Twin 31-month old female chimpanzees received SC-8 infected tissue culture fluid containing 107.5 TCD_{50} per ml. Martha was inoculated intramuscularly with 2.0 ml while Mary received 0.5 ml dropped onto one eye after superficial corneal scarification. For comparison, a 27-month old male animal (Ball) was given an intramuscular inoculation with 2.0 ml of plasma obtained 24 hrs. prior to onset of clinically and histologically confirmed infectious hepatitis (Case No. 11 of Conrad's Korean series). All animals were observed daily for clinical signs of disease. Blood and stool specimens were collected at frequent intervals during the critical period of the study, and serial liver biopsies were obtained for histologic and electron microscopic examination.

All 3 animals developed loose stools 5 days after inoculation; this was considered to be unrelated to the injections. Treatment with bismuth pectinate and paregoric relieved the condition within 3 days. Mary showed no clinical evidence of illness over a 66-day period (to 15 April). No conjunctivitis was found.

Martha became unusually lethargic on day 40 after inoculation but continued eating. This condition was noted for the next 2 weeks before more activity was displayed. During this period she developed hepatomegaly which reached a maximum about 52 days after inoculation at which time the margin of the liver was 4 fingers below the costal arch.

Ball developed anorexia and lethargy on day 43 after inoculation. Two days later diarrhea and abdominal distention due to flatulence became evident. Antibiotic therapy was instituted 4 days after diarrhea began, and a marked response to achromycin (250 mg/day) and chloramphenicol (250 mg/day) was noted within 2 days. During this period, Ball's liver also became palpable two fingers below the costal arch.

Serum transaminase determinations performed at the Yerkes Laboratories first showed elevated SGOT values on day 29 after inoculation in all 3 animals with peak levels attained on day 35. At this tire SGPT values also increased. Results of blood chemistry determinations are shown in Table I. The values observed appear to be quite comparable to those recently reported in chimpanzees by Deinhardt's group (Amer. J. Hyg. 75 (3): 311, 1962 (May).

Histologic examinations of liver biopsies were performed by Dr. Smetana and Dr. Peterson as well as by Dr. Wharton in Jacksonville and are described in detail in a later section of this report (See II-E below). Essentially the earliest changes were seen in Ball's 29-day specimen with a significant increase in the 35-day biopsy, diminishing in the 46-day post injection specimen. Similar but milder changes were found in the 26-day biopsy from Martha; subsequent examinations have not been reported. Biopsies from Mary showed no significant changes.

Attempts to recover virus from the infected chimpanzees are currently in progress, employing WI-38 diploid, Davis continuous human embryonic lung and primary human embryonic kidney tissue culture (HEK). Results with the first 2 cell lines have been negative to date. No isolations have been made from serum specimens thus far. An agent showing adenovirus-like CPE has been isolated and established in HEK from a 29-day post-injection throat swab from Ball. Adenovirus-like agents were recovered from a 15-day stool specimen of Ball. This agent also has not been identified, but shows a CPE distinctly different from the adeno-type.

The adenovirus recovered from the throat swab of Ball taken on day 29 post-inoculation has been identified as the San Carlos agent (Adenovirus type 16'), and probably indicates a cross-infection from Mary or Martha, mediated by common animal handlers. Other isolates have not yet been typed.

C. Virus Isolation Studies in Man. Because of the relative paucity of candidate agents which had been obtainable for study in this laboratory by the end of 1962, a decision was made to initiate independent virus isolation studies. A factor in this decision was the

availability of excellent study material collected by Major Conrad from well documented cases of infectious hepatitis occurring in American military personnel in Korea. From all cases, serial blood, urine and stool specimens and serial liver biopsies were obtained at various stages of disease, frozen promptly and stored in liquid nitrogen until time of study.

Twelve acute-phase sera were arbitrarily selected for intensive isolation trials, including eleven from the Korean series of Conrad and one from a severe IH case at Andrews AFB. Urine specimens from most of these cases were also worked up. Cell systems employed included the Davis continuous human embryonic lung, Detroit-6y, Wistar WI-26 and WI-38, primary embryonic kidney and lung, and African green monkey kidney tube cultures. Diploid human fetal kidney and lung were employed less extensively.

Agents showing CPE characteristic of an adenovirus were recovered from serum of 3 of the 12 cases tested and from urine of a fourth case. The serum isolates appeared to be more closely related to the prototype Adenovirus 16 than to Adeno 16' (San Carlos) in tissue culture neutralization tests. Homologous antisera have not yet been prepared for reciprocal tests. The adenovirus-like agent recovered from urine was not neutralized by either Adeno 16 or Adeno 16' antiserum (32 units tested against less than 10 TCD_{50} of virus), and has not yet been identified. Re-isolations have not been accomplished for confirmation.

A second type of agent, tentatively termed "Agent 2," has been recovered from serum of 5 cases, including 4 of the same cases from which adenoviruses were recovered. All agents have been passed from 3 to 5 times and appear to be established in tissue culture. The CPE shown by "Agent 2" differs markedly from that shown by the adenoviruses, including cell rounding, shrinking and disintegration. Some differences in host cell susceptibility have also been noted. Several cultures show CPE suggestive of a mixture of the two types of agents; attempts are in progress to purify these strains by agent separation by several techniques.

Re-isolation has been accomplished at least twice in the case of 3 of these agents; others have not yet been attempted. "Agent 2" has not been identified, nor animal pathogenicity tested. Two agents of this type have been found to differ from theusual enteroviruses in that they are inactivated by treatment for 4 hours in the cold with 50 per cent ether (7 log reduction in titer) and by 1:400 dilution of sodium desoxycholate.

Convalescent sera from cases from which these agents were recovered have not yet been tested for presence of homologous antibody. These tests will be carried out in the near future after virus mixtures have been separated. Relationship of these agents to infectious hepatitis has not yet been established.

D. Future Plans. Further viral isolation studies are planned after the present agent recoveries have been authenticated by re-isolation. Agent characterization will be pursued and precise identification made if possible. Attempts will be continued to obtain further candidate agents for study. Serologic tests will be expanded as suitable antigens can be prepared.

Further trials will be conducted in chimpanzees or monkeys (Erythrocebus patas) as feasible, employing the most promising candidate agents in attempts to reproduce the disease experimentally.

TABLE I.
BLOOD CHEMISTRY RESULTS
INOCULATION

MARTHA DATE:	2-8-63	2-23	3-9	3-12	3-15	3-21	3-26	4-2
S.C.-8,2 ml i.m.) DAY:	0	15	29	32	35	41	46	53
SGOT	16	5	110	110	160	128	94	35
SGPT	-	-	-	0	120	74	168	115
Thymol Turbidity	-	-	1.8	1.0	0.6	1.3	4.1	4.0
Serum Bilirubin								
Direct	-	-	-	2.6	-	-	-	-
Indirect	-	-	-	0.8	-	-	-	-
Total	-	-	-	3.4	-	-	-	-

MARY DATE:	2-8-63	2-23	3-9	3-12	3-15	3-21	3-26	4-2
(S.C.-8,0.5 ml on cornea) DAY:	0	15	29	32	35	41	46	53
SGOT	4	7	110	110	195	135	22	46
SGPT	-	-	-	0	100	45	32	38
Thymol Turbidity	-	-	1.0	1.0	2.7	2.0	1.8	2.0
Serum Bilirubin								
Direct	-	-	-	2.6	-	-	-	-
Indirect	-	-	-	0.8	-	-	-	-
Total	-	-	-	3.4	-	-	-	-

BALL DATE:	2-8-63	2-23	3-9	3-12	3-15	3-21	3-26	4-2
(Plasma No. 11, 2.0 ml i.m.) DAY:	0	15	29	32	35	41	46	53
SGOT	20	2	200	100	280	230	46	80
SGPT	-	-	-	0	240	134	44	95
Thymol Turbidity	-	-	3.0	3.0	5.2	3.4	2.7	2.6
Serum Bilirubin								
Direct	-	-	-	0.35	-	-	-	-
Indirect	-	-	-	0.12	-	-	-	-
Total	-	-	-	0.47	-	-	-	-

- Not done.

II. THE HISTOPATHOLOGIC ASPECTS OF VIRAL HEPATITIS (Drs. Smetana, Petersen, Ruebner, and Boitnott).

A. Histopathologic study of the liver in presumably normal chimpanzees: Observations were made on approximately 100 liver needle biopsies of chimpanzees of various ages and both sexes, performed at the Yerkes Laboratory, Orange Park, Fla. There had been no clinical evidence of disease of these animals although clinical laboratory investigations had shown evidence of helminthic infestation of various kinds in most of the animals. All the needle biopsies were performed with Menghini needle after anesthesia with Cl-400, and Cl-398.

Sections of these biopsies showed slight differences in the appearance of liver cells (perhaps related to food intake). Moderate cellular infiltrates were present in some of the portal canals in most of the animals but did not reach significant proportions. Some of the sections showed evidence of these and mild intralobular infiltrations were interpreted as an inflammatory reaction to parasites. However, no ova, larvae or adult parasites were visualized. Most of the special preparations such as reticulum stain, Masson and PAS showed no significant deviation from the expected normal but iron pigment was not infrequently recognized in Kupffer cells. The significance of this finding is not clear.

B. Study of the histopathologic alterations of the liver of chimpanzees suffering from conditions interpreted as "Contact Viral Hepatitis": Sections of liver needle biopsies (supplied by Dr. Hillis) of such animals occasionally showed mild infiltrations of the portal canals and rare intralobular focal necrosis accompanied by monocytic infiltrations about such foci. In one instance the biopsy of a chimp (Jiggs) revealed a few acidophilic bodies and rather coarse pigmented granules in some of the Kupffer cells which were identified as iron. Repeat biopsies of the same animal revealed portal infiltrates for a few weeks and iron pigment was present in even increased amounts. Eventually the acidophilic bodies disappeared and the general appearance of the lobules returned to normal.

Liver needle biopsies from two animal caretakers, who had come down with clinical hepatitis, revealed histologic evidence of acute viral hepatitis.

C. Study of liver biopsies from human volunteers inoculated with tissue culture cells infected with materials obtained from patients suffering from infectious hepatitis (Parke, Davis & Co.): Although the liver biopsies taken from such volunteers showed mild lesions characterized by focal necrosis and mild portal infiltrations, none of the alterations were considered diagnostic of changes seen in human viral hepatitis. It was thought that they might be the effect of an incidental virus. On inquiry it was learned that none of the volunteer cases with clinical viral hepatitis from which materials were taken for culture, had been unequivocally identified as viral hepatitis by liver needle biopsies.

D. Light and electron microscopy of liver biopsies of a small epidemic occurring among Army personnel stationed in Korea (1962) (Dr. Conrad): Serial biopsies were performed on 28 patients who were suffering from clinical viral hepatitis and who had significantly increased titers of serum transaminase (SGOT). Out of 28 patients, 25 showed histologic changes in liver needle biopsies that were interpreted as characteristic of a relatively mild viral hepatitis in the late

stage. In addition to balloon cells, acidophilic bodies, intralobular as well as periportal monocytic infiltrations, there were numerous Kupffer cells laden with abundant amounts of lipofuscin. Twenty-three of these patients showed marked reduction in the lesions 6 weeks after the first biopsy and practically normal liver lobules were seen in specimens taken 6 weeks later. None of the cases portrayed a virulent active phase of the disease histologically though all 25 were considered characteristic of viral hepatitis.

In addition to liver needle biopsies, each of the patients was subjected to a biopsy of the one kidney and a capsular biopsy of the intestine. The biopsies of the kidneys and intestine showed interesting alterations which, however, were difficult to relate to the underlying disease. Two of the patients had a relapse of clinical symptoms, following the second biopsy which in one case was accompanied recurrence of the histopathologic lesions to a degree greater than the alterations seen in the first biopsy. Three out of the 28 patients showed no evidence of liver disease in histopathologic examinations and were eliminated from this series.

E. Experimental transmission of human viral hepatitis to chimpanzees (See I-B above): Serum from a patient with infectious hepatitis obtained during the Korea outbreak of viral hepatitis was inoculated into chimpanzees and serial biopsies of these animals were studied. In one of the animals inflammatory lesions consisting of focal necrosis and monocytic infestations occurred about 2 weeks after inoculation of this material. The animal had shown clinical signs of weakness, anorexia and lethargy and clinical laboratory studies had indicated disturbed liver function tests of a significant degree. Recognizable lesions were present in the liver for about 4 weeks.

Similar but milder alterations of the hepatic parenchyma were seen in a second chimpanzee which appeared about 3 weeks after the inoculation, and persisted for about 3 weeks.

A third chimpanzee that was inoculated by scarification of the cornea did not develop hepatic lesions.

The hepatic changes were not exactly comparable to those seen in human beings. Although the animals were sick they did not exhibit clinical jaundice; however, no serum bilirubin determination had been made. The liver function tests indicate significant disturbances of the physiologic function of this organ and no other organ appears involved. Biopsy materials were also prepared for electron microscopic studies which will be reported later.

F. Attempts of experimental transfer of viral hepatitis to the red monkey, ERYTHROCEBUS PATAS PATAS: Preparations for a histopathologic and electron microscopic study of transmission of infectious hepatitis to the red monkey are being made. It is hoped that actual initial experiments can be performed early in May, 1963, at the Virus Laboratory of the West African Council for Medical Research in Yaba, Lagos, Nigeria, in collaboration with Dr. W. G. C Bearcroft, the Pathologist of this institution. It is planned to inoculate materials obtained from cases of infectious hepatitis from Korean cases into these animals.

COMMENT: Although the transmission experiments of materials from viral hepatitis into primates indicate a certain response with disturbance of function of the liver and inflammatory reaction in this

organ, the changes are not entirely comparable to those seen in the liver in cases of viral hepatitis in human beings. It will be necessary to extend these studies to transfer of materials obtained from these experimental cases to other chimpanzees and primates as well as to attempt to recover the agent from these livers before being certain about the measuring of these results. For this reason the planned transfer of viral hepatitis to the red monkey might be very helpful to study the effect in this animal in comparison to chimps.

The outcome of the experiments in progress will undoubtedly influence the future course of the comprehensive study of viral hepatitis.

III. Other Studies (Drs. Ruebner and Miyai).

A. Cases of neonatal hepatitis and biliary atresia seen at Johns Hopkins Hospital have been reviewed (Ruebner and Miyai, Ann. N.Y. Acad. Sci., in press). Particular attention was paid to the presence of hematopoeisis and of hemosiderin deposition in neonatal hepatitis. While giant cells were frequently centrilobular, hemosiderin deposition tended to be peripheral. It was suggested that hemolysis is probably more frequent in neonatal hepatitis than is generally recognized.

B. Viral liver injury has been studied using murine hepatitis as a model (Miyai, Slusser and Ruebner, Exp. & Mol. Path., in press). Kupffer cell injury appeared to precede parenchymal cell injury. The chief changes in both types of cell were mitochondrial damage and dilatation of the endoplasmic reticulum. Parenchymal cells, in addition, showed focal necrosis, the development of aggregates of glycogen particles, and proliferation of the endoplasmic reticulum.

C. The mouse cytomegalovirus is immunologically distinct from the human cytomegalovirus but resembles it in many respects. Both viruses produce liver damage. The human type is a recognized cause of neonatal hepatitis. The murine virus produces focal hepatic necrosis as well as injury to other viscera. It was decided to employ this agent to reinvestigate the histopathology of viral liver injury with particular reference to the Kupffer cell changes (Ruebner, Miyai, Wedemeyer and Medearis, Fed. Proc. 1963, 22, 546). Electron microscopy showed Kupffer cell lesions during the first 48 hours when parenchymal cell damage was relatively mild. The sinusoidal lining cells were swollen, their mitochondria often had lost their cristae and occasionally seemed to develop into "cytolysosomes". In the later stages, the endoplasmic reticulum became prominent.

The most striking Kupffer cell change was the development of focal necrosis with masses of relatively dense material. This damage was thought to account in good measure for the acidophilic bodies seen in the sinusoids in viral liver injury. The nuclei showed chromatin margination with prominent nucleoli. Virus particles were rarely detected in these cells.

Parenchymal cell damage occurred later and was generally less severe. The cellular organelles showed changes similar to those of Kupffer cells. In addition there were bizarre mitochondria, some of which seemed to be undergoing fission. Many nuclei contained virus particles and there were also cytoplasmic virus inclusions associated with prominence of the Golgi apparatus. These inclusions

may represent areas of virus destruction.

It is proposed to continue work on liver injury produced by the cytomegalovirus. Enzyme histochemistry is to be employed, particularly to measure adenosine triphosphatase, acid phosphatase, and oxidative enzymes. The virus development is to be studied in mouse embryo tissue culture by fluorescent antibody and later by electron microscopic with a ferritin conjugated antibody.

D. Growth of parenchymal liver cells in tissue culture has been difficult to obtain. It is possible that growth of the hepatitis virus could be achieved of parenchymal cell growth could be regularly produced. So far the best method to achieve this seems to be the employment of a collagen base as suggested by Dr. Gey and Dr. Bang of the Johns Hopkins Medical School. It is proposed to study growing murine and human parenchymal cells by histochemistry and electron microscopy as a basis for later studies of the damages produced in these cultured cells by viruses. Acridine orange fluorescent staining for RNA and DNA is to be employed in this investigation.

E. Dr. Huebner is preparing a paper to be given at the New York Academy of Medicine in the fall on hepatitis and the spontaneous liver lesions in experimental animals.

Biopsies from babies with neonatal hepatitis and adults with hepatitis are being embedded in Araldite for future study with the electron microscope.

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