the serum of the fasting rat than in the fed rat and the increased serum enzyme activity of the fasted rat supports this type reaction. In man the serum enzyme forms principally cholesterol linoleate and the proportion of cholesterol arachidonate in the synthesized ester mixture is lower than is found in the unsaturated fatty mixture (2-position) of phosphatidyl choline (2, 6). This points up the marked differences in the specificity of the human and rat serum enzyme systems. The possibility also exists that specific phosphatidyl cholines of specific lipoproteins (4) are the principal reactants involved in ester formation by the serum enzyme of these two species.

Summary. The serum cholesterol ester transferase enzyme is more active in the serum of fasted than fed rats. This increase in activity is associated with principally one cholesterol ester, namely, cholesterol arachidonate. The serum cholesterol esters and phosphatidyl choline of fasted rats contain a higher proportion of arachidonic acid and less linoleic acid than those of fed animals. The findings indicate that the types of cholesterol esters synthesized by the serum enzyme are dependent, in part, on the nutritional state of the animal.

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Serologic Characterization of Adenoviruses Isolated from Chimpanzees Associated with Viral Hepatitis* (33322)

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Chimpanzees and certain other nonhuman primates have been implicated in recent years in the transmission of viral hepatitis to their human caretakers and to other contacts (1–6). Mounting epidemiologic evidence for such transmission is documented by more than 100 human cases of the disease. This evidence supports the postulate that certain nonhuman primates act as carriers of the etiologic agent or agents of human viral hepatitis or of a disease so closely related as to be

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indistinguishable from it by clinical, biochemical, and histologic findings.

Liver lesions compatible with those of viral hepatitis have been found in several such "carrier" animals and also in other primates spontaneously or experimentally ill with the disease but not implicated in human transmission (4, 7). More recently, inoculation of infective material from human patients with viral hepatitis into certain species of small New World primates has produced a serially transmissible disease highly suggestive of hepatitis (8).

Hillis (4) reported the isolation of viral agents from a group of 25 chimpanzees, 9 of which were specifically associated with either spontaneous or experimental hepatitis. The investigation here reported was conducted in an attempt to relate the agents isolated from these nine hepatitis-associated primates to known viruses. The study presents evidence that seven of the agents are members of the adenovirus group, each of which is serologically related to a specific serologic type of human adenovirus.

Methods. Viral agents, after 2–3 serial tissue-culture passages in primary chimpanzee kidney cells (ChKC) were inoculated into monolayer tube cultures of primary human embryonic kidney (HEK) and rhesus monkey kidney cells (MKC) a 1:10 final dilutions of the previous ChKC passage in maintenance medium (MM). The latter consisted of Medium 199 with 10% fetal bovine serum and standard tissue-culture dosages of penicillin, streptomycin, and amphoterocin B. Cells were alternately frozen and thawed, together with MM, for three cycles after viral destructive effects upon the cells became maximal, followed by centrifugation to free the viral harvests of cellular debris. Viral titrations were accomplished by inoculations of tenfold dilutions of the resulting viral harvests onto HEK cells in tube cultures, utilizing 3–4 tubes per dilution in duplicate titrations.

Standard serum neutralization (SN) tests, employing approximately 100 tissue-culture 50%-infective dosages ($TCID_{50}$) of each virus, were performed with dilutions of type-specific antisera to human adenoviruses, types 1 through 28, and to chimpanzee adenovirus type C-1 (9). The rabbit antisera initially used for these tests were commercially obtained. Serologic results were later confirmed in two laboratories with reference rabbit and monkey antisera obtained from the Communicable Disease Center, Atlanta, Georgia, and from the Research Reference Reagents Branch, National Institutes of Health, Bethesda, Maryland.

TABLE I. Mean Yields of Chimpanzee Virus Strains Isolated in Chimpanzee Kidney Cells on One Subsequent Passage in Various Primary Cells.[a]

Virus strain	Mean titer ($-\log TCID_{50}$/ml) of virus after passage in		
	ChKC[b]	HEK[c]	MKC[d]
Y-15	6.0	6.0	4.3
Y-34	6.3	7.8	5.3
Y-37	2.3	4.0	1
Y-43	2.3	4.3	1.3
Y-76	6.8	5.0	4.3
Y-173	3.3	3.3	1
Y-195	3.3	4.0	1
Y-213	4.3	5.3	2.0

[a] Duplicate titrations performed in human embryonic kidney cells.
[b] Chimpanzee kidney cells.
[c] Human embryonic kidney cells.
[d] Rhesus monkey kidney cells.

Hemagglutination-inhibition (HaI) tests were conducted by the microtechnique of Sever (10), Utilizing kaolin- and erythrocyte-absorbed dilutions of the above antisera with 2–4 hemagglutinating units of the appropriate adenovirus antigens prepared from tissue-culture harvests and a 0.5% suspension of selected Sprague-Dawley rat erythrocytes containing a 1:50 dilution of heterotypic immune serum prepared against human adenovirus type 5, having an homologous HaI titer of 1:320 (11).

Results. Eight of the nine chimpanzees specified above to be associated with viral hepatitis yielded isolates in ChKC that readily propagated in HEK cells, achieving the titers indicated in Table I. The agents, indicated in the table by their original "Y" strain designations, were derived from fecal specimens of animals as previously reported (4).

All of these viruses, with the exception of three strains, also grew in MKC, yielding titers in the range of $10^{-1.3}$–$10^{-5.3}$. The ninth agent (Y-16) failed to replicate in either HEK or MKC and has not been further characterized.

Seven of the viruses, exclusive of strain Y-76, which grew in HEK, produced cytopathic effects *in vitro* typical of those seen with previously reported adenoviruses (12), including rounding, swelling, clumping, and eventually detachment of cells from the glass surfaces. Each of these agents was shown in standard complement-fixation (CF) tests with known positive human antiserum to adenovirus CF antigen (from Microbiological Associates, Inc., Bethesda, Maryland) to possess the common adenovirus group-reactive CF antigen. The remaining virus (strain Y-76), which grew in HEK cells, failed to produce adenovirus cytopathic changes and to react with known positive antiserum to the adenovirus CF antigen.

SN tests in HEK cells with type-specific rabbit or monkey antisera indicated serologic relationship of six of the seven adenoviruses with known human adenovirus types, as indicated in Table II. The results were confirmed in MKC SN tests with the four agents that passed in these cells.

Each of the adenovirus isolates was also tested by SN for serologic relationship with the single previously characterized chimpanzee adenovirus, type C-1, using specific rabbit anti–C-1 hyperimmune serum. Y-15 virus was neutralized at a serum dilution of 1:80, the same dilution at which an equal dosage of known C-1 virus was also neutralized. None of the remaining 6 adenoviruses was neutralized by anti–C-1 serum. Thus, Y-15 virus appeared to be identical with type C-1 chimpanzee adenovirus, showing a serologic cross-reaction with human adenovirus type 14. Such cross-reaction of C-1 adenovirus was previously reported (9).

Adenovirus Y-195, not neutralized by human adenovirus type-specific antisera, was tested with the same spectrum of reference antisera in HaI tests. Despite failure to demonstrate any serologic relationship to human adenovirus types 1 through 28 or with type C-1 virus by SN, HaI tests indicated that a reciprocal relationship existed between Y-195 and human adenovirus type 2, as shown in Table III. Thus, strain Y-195 appeared to be cross-reactive with human adenovirus type 2 by HaI but not by SN. HaI tests further indicated a serologic relationship of Y-195 to Y-213 virus and to still another chimpanzee adenovirus (strain D-3) isolated from a case of spontaneously occurring fatal hepatitis in a young chimpanzee (13).

TABLE II. Serologic Crosses by Serum Neutralization between Chimpanzee Strains and Human Adenovirus Serotypes.

Chimpanzee virus strain	Serotype of antiserum to human adenovirus causing neutralization[a]
Y-15	14
Y-34	14
Y-37	18
Y-43	5
Y-173	14
Y-195	None
Y-213	2

[a] Complete neutralization of approximately 100 $TCID_{50}$ of virus by serum dilutions of 1:10 or greater. All viruses tested with antisera to human serotypes 1 through 28.

Discussion. The finding of adenoviruses excreted in the feces of apes implicated as carriers of human hepatitis virus, or otherwise associated with spontaneous and experimental nonhuman primate hepatitis, does not necessarily indicate that such agents are etiologic of hepatitis. In fact, none of the adenoviruses derived from fecal specimens could be recovered from either the blood or liver of

TABLE III. Serologic Crosses between Human Adenovirus Type 2 and Chimpanzee Adenovirus Y-195 by Hemagglutination Inhibition.

Viral antigen	HaI[a] endpoint dilution of anti–human type 2 serum	HaI[a] endpoint dilution of anti–Y-195 serum
Human adenovirus type 2	1:320	1:10
Y-195	1:40	1:40

[a] Hemagglutination inhibition.

the respective animals, although each animal excreting a given virus in its feces was shown to have circulating serum neutralizing antibody to the homologous agent, ranging in titer from 1:20 to 1:80. However, hepatitis is caused by a specific adenovirus in at least one species, the dog (14), and human adenovirus type 5 has been shown to induce "hepatitis" in newborn hamsters (15). The findings reported here strengthen other evidence recently reported that adenoviruses are frequently associated with hepatitis and may at least play some ancillary role in the occurrence of the human disease (16–18).

Although the excretion of adenoviruses in such cases may be fortuitous or may represent activation of latent viruses by intercurrent infection with hepatitis virus, such agents may serve as "helper" viruses, aiding in the replication of the etiologic agent of hepatitis or otherwise assisting the latter in establishing disease in a susceptible host. Adenoviruses have recently been shown to be essential in the replication of certain DNA viruses and to aid in the replication of a number of other viruses, as reviewed by Rapp (19).

The serologic reactions of the chimpanzee adenovirus isolates with antisera to known human adenovirus types described here do not establish identity of the serologically related types of agents from the two species. The related viruses, in fact, differ from each other in several other biologic characteristics (Hillis, unpublished experiments). As has been shown for other simian adenoviruses related serologically to specific human adenovirus types (9), the relationships of the chimpanzee adenoviruses reported herein appear to be those of partial cross-reactions.

Although non-ape simian adenoviruses have been found to replicate poorly in cells of human origin (9), each of the above chimpanzee agents of this group has been shown to multiple readily and to relatively high titer in primary human epithelial cells. Further studies are being conducted in an attempt to clarify whether the chimpanzee isolates here described are of human or chimpanzee origin. The relationships of the seven agents to other adenoviruses recently isolated from various tissues of chimpanzees (20) are unknown.

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