onstrated that MBIII lymphoblasts grown *in vitro* can remove cholesterol esters from the medium and return free cholesterol to the medium. Bailey(19) later showed that the type of serum used, but not its lipid composition, influenced cholesterol uptake by these cells. Sterol synthesis by mouse fibroblasts grown *in vitro* has also been shown to be independent of the lipid composition of the medium (20).

Our findings indicate that the major changes in lipid compisition of L-5178Y cells observed when D_2O (30%) is present in the medium are due to *de novo* synthesis of lipid, principally fatty acid. There may be some exchange of cholesterol between the cells and the medium. Elucidation of the mechanisms by which deuterium oxide affects the lipid content of L-5178Y cells and the role that the lipids of the medium play in these changes must await further experiments involving labeled lipids and lipid precursors.

Summary. A comparison has been made of the relative lipid content of L-5178Y cells grown in conventional medium or in the same medium containing 30% D_2O. Cells grown in D_2O medium contain more total lipid. The major increases in the lipids of the D_2O grown cells are due to increases in the levels of triglycerides and sterol esters. There are very few differences in cellular levels of any of the other lipid fractions. Preliminary isotope experiments indicate that the increased lipid content in the D_2O grown cells is due to increased synthesis of lipid.

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Isolation of a Cytomegalovirus from African Green Monkey. (28115)

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The cytomegaloviruses, also called salivary gland, submaxillary gland, and cytoplasmic inclusion disease viruses, are a group of biologically related, but generally species-specific viruses(1). Infection with these agents is characterized by intranuclear and intracytoplasmic inclusions in greatly hypertrophied cells primarily in the salivary glands, but in other organs as well.

The human cytomegaloviruses have been

well characterized(2) as have the mouse(3,4) and guinea pig(5) representatives of the group. Serial transmission of the homologous virus with production of inclusion bodies in the salivary glands has been accomplished in the rat and hamster(6), and characteristic intranuclear inclusions have been found in the salivary glands of the mole(7), dog(8), and various rodents(9).

In primates other than man the only reported instance of spontaneous disseminated salivary gland virus disease has been that of an epidemic among chimpanzees(10). Although intranuclear inclusions have been described in the kidneys of rhesus(11) and Cercopithecus(12) monkeys, as well as in the salivary glands of 2 *Cebus fatuellus* monkeys (13), no virus has been recovered from the monkey. This report describes the isolation of viral strains from the salivary gland and from kidney tissue cultures of the African green monkey (*Cercopithecus aethiops*) which appear to be a monkey representative of the cytomegalovirus group.

Materials and methods. Tissue cultures. Cercopithecus monkey kidney (AGMK) tissue cultures were obtained from Microbiological Associates, Inc., Bethesda, Md., and Flow Laboratories, Arlington, Va. Cercopithecus monkeys used for tissue culture were captured in the Nairobi region of Kenya. There was no evidence of disease in the holding colony. At time of sacrifice, the monkeys were about 6 to 7 months old and weighed 4 to 7 pounds. The kidney cells were dispersed with trypsin, and tubes were planted with 150,000 to 200,000 cells per ml in a medium consisting of 97.5% Hanks' balanced salt solution (BSS), 0.5% lactalbumin hydrolysate, 2% calf serum, with 500 units penicillin and 500 μg streptomycin per ml. Three to 4 days later, the medium was removed, the sheet rinsed with BSS, and 0.7 ml of a solution containing 97.5% Earle's BSS, 0.5% lactalbumin hydrolysate, 2% calf serum, and antibiotics was added. After the seventh to ninth day, the monolayers were maintained with Eagle's basal medium (BME) with non-essential amino acids and antibiotics.

Human embryo fibroblast (MAF) monolayers, were obtained from Microbiological Associates, Inc., and were maintained on BME with 5% horse serum. Primary monkey testis cultures and human embryonic lung (WI-26), a semi-continuous diploid cell line, (obtained from Microbiological Associates, Inc.) were maintained on 49% BME, 49% medium 199, and 2% calf serum. Maintenance media contained 2 mM glutamine, 100 U penicillin, and 100 μg streptomycin per ml. Tubes were kept stationary and fluids were changed twice weekly. All sera were heated at 56°C for 30 min.

Complement fixation tests. The modified Bengtson procedure(14) was employed, using overnight ice-box fixation with 2 full units of complement. Sera were stored at −20°C and were heated at 56°C for 30 minutes before use in CF tests. Antigens consisted of cells plus fluid from human fibroblast cultures harvested when cytopathic effects (CPE) were essentially complete.

Results. Isolation of virus from monkey salivary gland tissue. Salivary gland tissue from an African green monkey dying of unknown cause in the N.I.H. monkey house was prepared in 10% suspension and inoculated into MAF tissue cultures. After 22 days in culture, foci of enlarged round to oval refractile cells in a linear arrangement appeared, which were strikingly similar to the changes produced by human cytomegaloviruses in this tissue culture system. Harvests of supernatant fluid or fluid plus cells obtained 32 to 46 days after infection produced identical focal changes on passage to both MAF and rhesus monkey testis fibroblast cultures with incubation periods ranging from 9 to 21 days. In early passages progression of cytopathic changes was very slow, requiring 6 weeks or longer for complete degeneration, but by the fourth passage in both types of tissue culture the incubation period to first cytopathic effect was reduced to 1 to 2 days with complete destruction in 10 to 14 days. Giemsa-stained cultures from the original isolation series contained acidophilic intranuclear inclusion bodies wholly consistent with those described for the cytomegalovirus group. MAF culture fluids from the tissue culture adapted line have infectivity titers of 10^4

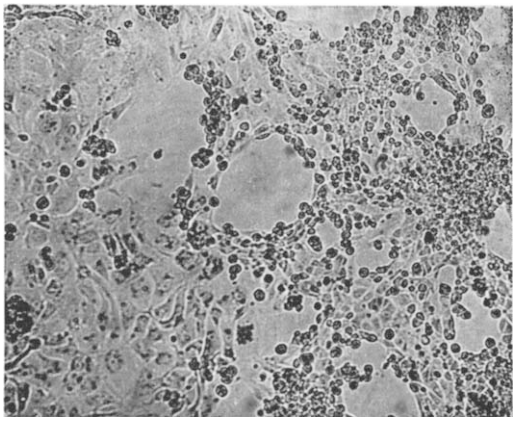

FIG. 1. Spontaneous CPE in Cercopithecus monkey kidney culture 5 days after first changes appeared. The tissue at the left is relatively unaffected. Unstained, 54 ×.

to 10^5 $TCID_{50}$ per 0.1 ml and fix complement in the presence of sera obtained from a number of African green monkeys.

This virus, designated CSG strain, has now been carried through 136 passages in MAF or WI-26 tissue cultures. The ability of the virus to grow in cultures of monkey tissue has been maintained, since occasional back passages to rhesus testis cultures (the latest after the 120th passage) have invariably resulted in rapid development of characteristic cytopathic changes. Conversely, the Ad. 169 strain of human salivary gland virus, tested at intervals during a long human fibroblast tissue culture passage series, has never produced cytopathic lesions in monkey testis cultures.

Isolation of strains from spontaneously degenerating monkey kidney epithelium. In 4 lots of Cercopithecus kidney cell cultures a unique spontaneous cytopathic effect was first observed on the 26th, 34th, 41st and 44th days of culture of the 4 kidneys. The cultures studied were derived from monkeys from 3 different shipments. In one series 23 of 72 tubes showed this effect; in another, 2 of 7 tubes, and in the remaining 2 instances only one tube each from series of 40 and 100 observed monolayers had the changes. Tubes showing the spontaneous degeneration included uninoculated controls as well as cultures which had been inoculated with various hamster tissue suspensions.

The spontaneous CPE consisted first of the appearance of large, dark, smoothly outlined, round cells in several loci. In 1-3 days lysis of tissue occurred in these areas, leaving holes lined with dark, round cells (Fig. 1). After 5 to 8 more days the holes enlarged in size and coalesced with other foci. In general, the progression of CPE was very slow and variable in extent of tissue involved; some culture tubes progressed to complete destruction within 2 weeks after first appearance of CPE while in others the CPE failed to progress beyond isolated small foci.

When the tissue was about half destroyed, the cells from one of the tubes were scraped off into the fluid, and the mixture was passed fresh to AGMK and MAF monolayers. The kidney epithelium cultures developed a small number of clumps of small, round, dark cells in 1-3 days; however, only a few foci similar to those appearing spontaneously were evident after 8 to 14 days, and these failed to enlarge. Only small amounts of virus were produced and the virus could not be passed in AGMK. The same result was encountered when fluids and cells of other spontaneously degenerated cultures and the tissue culture adapted strain were inoculated into AGMK. In MAF cultures, typical salivary-gland virus CPE occurred within 4 to 5 days, which progressed to involve the entire monolayer. The virus passed well in MAF and WI-26 human fibroblast cultures and produced cytopathogenic changes identical to those produced by the agent recovered from monkey salivary gland tissue. Now in the 12th passage in human fibroblast cultures, the agent produces CPE within 1 to 2 days, with complete destruction in 7 to 10 days. Eosinophilic intranuclear inclusions are produced. Like the CSG strain, this strain, called CK-1, also produces characteristic CPE in monkey testis cultures. This strain reacted in CF tests with a Cercopithecus monkey serum which had high titer CF antibody to the CSG strain.

The CSG and CK-1 strains are relatively labile, being completely inactivated by exposure to 20% ether for 2 hours at 4°C, as well as by heating at 56°C for 10 minutes. They are unaffected by broad-spectrum antibiotics

TABLE I. CF Reactivity of Cercopithecus and Human Sera with Cercopithecus and Human Cytomegalovirus Antigens.

Serum (1:8)	CF reaction* with human cytomegalovirus antigen (Ad 169) 4 units	CF reaction* Cercopithecus cytomegalovirus antigen (CSG) 4 units			
		Positive	Partial	Negative	Total
Monkey	Positive	11	0	0	11
	Partial	4	15	0	19
	Negative	8	23	17	48
	Total	23	38	17	78
Human	Positive	0	0	18	18
	Partial	0	0	1	1
	Negative	0	0	3	3
	Total	0	0	22	22

* Positive = 3 to 4 fixation; partial = 1 to 2+; negative = 0.

and may be stored at −65°C, but with some loss in infectivity.

Serology. In complement fixation tests of 78 sera obtained in 1958 from Cercopithecus monkeys in the N. I. H. colony, 23 were strongly positive (3 to 4+ fixation) when tested at a dilution of 1:10 against antigen prepared from MAF tissue cultures infected with the CSG strain (Table I). In contrast, no significant reaction was obtained in tests of 45 rhesus monkey sera obtained at time of capture.

Of particular interest in view of the usual strict species specificity of members of the salivary gland virus group was the demonstration of a one-directional CF cross-reaction between human and monkey salivary gland viruses (Table I). Antibody in the monkey serum was detected by both antigens, but more efficiently by the homologous; human sera reacted only with the homologous species antigen.

Discussion. The cytopathic and cytologic changes produced by the two strains in tissue culture are so typical of those noted with other species cytomegaloviruses as to be pathognomonic. The isolates also share other properties of cytomegaloviruses, being ether and heat sensitive and suffering substantial loss in infectivity titer from freezing and thawing. The cultural properties of the 2 Cercopithecus strains appear to be identical; cross serological tests have not been performed.

The long latent period before the spontaneous CPE became evident in kidney epithelium monolayers is also characteristic of this family of viruses, where benign and inapparent infection is the rule. The unmasking of this virus in tissue culture is analogous to the recovery of human cytomegaloviruses from spontaneously degenerating fibroblastic cells in cultures of adenoid tissue(15). The chronic subclinical nature of the infection is apparent from the seeming good health of the monkeys prior to sacrifice.

Another characteristic of this family of viruses has been the rigorous species specificity. Guinea pig, mouse, and human cytomegaloviruses grow only in homologous tissue, and no serologic cross reactivity had hitherto been observed in complement fixation or tissue culture neutralization tests(4). The ability of the monkey strains to multiply in both human and monkey tissue culture cells is the first exception to this specificity. The partial cross species reactivity of the human and monkey viruses is further revealed by the one way immunologic cross, *i.e.*, the reaction of certain Cercopithecus sera with both monkey and human complement-fixing antigens.

Little is known about the frequency of infection of monkeys in nature or in laboratory colonies. Cowdry and Scott(11) found kidney lesions with characteristic intranuclear inclusions in *Macacus rhesus* monkeys in 1 of 10 normal controls; in 12 of 16 receiving irradiated ergosterol, and in 18 of 107 animals in other experimental groups. The CF antibody studies reported here indicate that infection was quite common among one group of Cerco-

pithecus monkeys which had been held in captivity. In contrast, no definitely positive reactions were obtained in similar tests of 45 *M. rhesus* sera obtained at time of capture. That the rhesus monkey is susceptible to infection with this group of viruses is evident from the several reports describing typical inclusions in both salivary glands and kidney, and the susceptibility of rhesus testis cultures to the Cercopithecus isolates.

Since Cercopithecus kidney cultures are extensively used in some parts of the world for poliomyelitis vaccine production, and are being used increasingly for experimental work, description of indigenous agents in these cultures is of importance. The cytomegaloviruses described here may be of particular pertinence because of their ability to infect human cells, and the relative difficulty of their detection. The Cercopithecus cytomegaloviruses did not resemble the agents previously described in AGMK by Malherbe and Harwin(16).

Summary. Two similar strains of virus were recovered from Cercopithecus monkey salivary gland tissue and kidney tissue culture. The agents possessed the biological characteristics of cytomegaloviruses, and shared CF antigens with a human cytomegalovirus. They are unique among cytomegaloviruses in their ability to grow in heterologous (human) tissue cultures. Antibodies were common in Cercopithecus held in captivity but were not found in freshly captured rhesus monkeys.

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Vitamin A Ester and Alcohol of Rat Liver During Pregnancy.* (28116)

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The plasma level of Vit. A in humans(1) and rats(2) during pregnancy is less than that found in non-pregnant and puerperal animals. In addition, the concentration of Vit. A in the liver of pregnant animals is reported to be lower than non-pregnant values(2,3,4). Glover *et al.*(5) showed a proportional relationship between the Vit. A alcohol of liver and that found in plasma, but subsequent investigators (6,7,8) were unable to confirm this observation. Thus the present study was undertaken to determine if any relationship exists between the alcohol and esterified forms of Vit. A in liver and plasma Vit. A during pregnancy.

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