inhibit lipid peroxidation abolished the increase in both TBA-reactive material and tetraols and lowered the *anti/syn* ratio to 0.5. The addition of EDTA also appeared to partially inhibit the low level of epoxidation by the mixed-function oxidases. The data from a typical ascorbate-dependent reaction are included in Table 1. Also included is the *anti/syn* ratio of 2.5 that we previously measured for the epoxidation of BP-7,8-diol by hematin and unsaturated fatty acid hydroperoxide (ROOH) (*8*). The data provide strong evidence that BP-7,8-diol is epoxidized during NADPH- and ascorbate-dependent lipid peroxidation in rat liver microsomes.

The results in Table 1 were obtained with racemic BP-7,8-diol. We carried out similar experiments with the resolved (+) enantiomer of BP-7,8-diol. Since mixed-function oxidases convert this enantiomer to only the (+)-*syn*-diol epoxide, tetraols derived from the (+)-*anti*-diol epoxide should be formed only by lipid peroxidation. Incubation of 40 μM (+)-BP-7,8-diol with NADPH and liver microsomes yields 0.4 μM tetraols derived from the *syn*-diol epoxide and 0.1 μM from the *anti*-diol epoxide. Inclusion of Fe^{3+}-ADP, and Fe^{2+}-EDTA generates 1.2 μM *syn*-diol epoxide–derived and 4.0 μM *anti*-diol epoxide–derived tetraols. The *anti/syn* ratio is 0.3 in the absence of metal complexes and 3.5 in their presence. The dramatic increase in metabolism and in the *anti/syn* ratio in these experiments forces the conclusion that BP-7,8-diol is epoxidized as a result of NADPH-dependent lipid peroxidation.

Lipid peroxidation is a consequence of the evolution of organisms to oxygen utilization. Some of the products of unsaturated fatty acid degradation are toxic and mutagenic (*21*), and the disruption of membrane integrity can lead to cell lysis (*22*). The generation of potent oxidizing agents during lipid peroxidation can cause the selective inactivation of heme-containing proteins such as cytochrome P-450 (*23*). This has been implicated as a principal factor in the alteration of hepatic xenobiotic metabolism after glutathione depletion in rats (*24*). Our report indicates that oxidizing agents generated during lipid peroxidation can epoxidize xenobiotics and can, in the case of polycyclic aromatic hydrocarbons, trigger the formation of their ultimate carcinogenic forms.

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Calicivirus Isolation and Persistence in a Pygmy Chimpanzee (*Pan paniscus*)

Abstract. *What may be the first calicivirus isolate from any primate species, including man, was recovered from a herpesvirus-like lip lesion on a pygmy chimpanzee and then, 6 months later, from the throat of the same animal. The infected individual and its cage mates had circulating antibodies that were type-specific for this calicivirus. The agent was antigenically different from 30 other calicivirus serotypes and is tentatively designated primate calicivirus* Pan paniscus *type 1 (PCV-Pan 1).*

We have isolated from a rare and endangered primate, the pygmy chimpanzee (*Pan paniscus*), a new calicivirus tentatively designated primate calicivirus *Pan paniscus* type 1 (PCV-Pan 1). In the naturally infected chimpanzee this agent persists and is shed for up to 6 months. The virus was first isolated from a vesicular lip lesion (Fig. 1). Although the pathogenesis in pygmy chimpanzees has not been established, inferences from infections in other species suggest an array of possible disease effects. One group of caliciviruses or calicivirus-like agents that has not yet been isolated in vitro causes enteritis in calves, piglets, and humans (*1–3*). This group includes the Norwalk agent and other closely related agents that have been associated with outbreaks of gastroenteritis among children and adults on several continents (*4–7*). Other caliciviruses isolated from swine, cats, and pinnipeds cause systemic disease resulting in pneumonia, abortion, myocarditis, and encephalitis. Most notably, however, they cause oral vesicular lesions or epidermal erosions (*8–11*).

A young adult pygmy chimpanzee (Loretta) showing signs of upper respiratory disease had a small lip lesion like those induced by Herpes hominis type 1. The lesion was scraped and swabbed, and the sample was placed in 2 ml of tissue culture medium with 10 percent

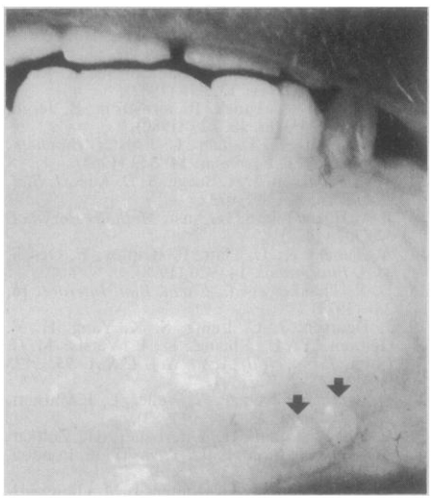

Fig. 1. Small herpesvirus-like vesicular lesions on the inner lip of a pygmy chimpanzee. Within 24 hours of first observation, these progressed to shallow erosions.

fetal bovine serum. The sample was clarified by centrifugation at 3500*g* for 10 minutes, and 0.2 ml of the supernatant was adsorbed to monolayers of PK(15) cells (from kidneys of pigs) and Vero cells (from kidneys of the African green monkey, *Cercopithecus aethiops*) for 60 minutes at 37°C. The cells were rinsed and refed with L-15 medium containing gentamicin (100 μg/ml) and 5 percent fetal bovine serum and then incubated on a roller drum (*12*).

A virus was isolated on the second passage of the sample in both Vero and PK(15) cells and was then plaque-purified (*13*). Nucleic acid content was determined with 5-fluoro-2-deoxyuridine (*14*). Ether sensitivity, *p*H stability, heat lability, and divalent cation (0.1*M* $MgCl^{2+}$) effects were tested (*14–17*). Stock virus passed six times in Vero cells was grown to a titer of $10^{7.5}$ median tissue culture infective doses ($TCID_{50}$'s) per milliliter, and typing serum was prepared in rabbits (*18*). The physicochemical characteristics of the isolate were those of a naked RNA virus. The detrimental effect of divalent cations in the presence of heat and the typical morphology as seen with negative staining and electron microscopy (*19*) were indicative of a calicivirus (Fig. 2). Eighteen of a probable 31 other different serotypes of calicivirus antigen and 30 of the typing sera for these were available to us (*20*). The 18 viruses were not neutralized by PCV-Pan 1 typing serum with 100 $TCID_{50}$'s of virus and 20 antibody units of serum (*21*). In the reciprocal neutralization tests, the PCV-Pan 1 agent was inhibited only by its homologous serum. For these reasons we find PCV-Pan 1 to be a new calicivirus serotype.

The animal yielding the isolate had serum neutralizing titers of 1:20 against the agent, as did its cage contacts, and this level of activity did not change over a 6-month period. Culture of a routine throat swab sample from the pygmy chimpanzee that had the lip lesion led to the isolation of a second calicivirus 6 months after the first, and this second isolate was fully neutralized by PCV-Pan 1 typing serum. It appears that the PCV-Pan 1 calicivirus infection was maintained in the chimpanzee in the presence of serum neutralizing antibodies and that this organism was shed from the throat either intermittently or continuously for at least 6 months. Cage mates were not examined for virus shedding, so we cannot address their state of infection except to reiterate that virus-neutralizing activity was present in their serum.

Using established electron microscopy procedures (*22*), we found that antigen-antibody complexes were formed between the PCV-Pan 1 virus and the convalescent chimpanzee's serum (Fig. 3). The same procedures showed that the PCV-Pan 1 virus was not reactive with antisera to 25 of the 26 known calicivirus types (all copies of one serotype of vesicular exanthem of swine virus, 101B, have apparently been lost) and to the five other putative calicivirus serotypes isolated from dolphins, walruses, minks, calves, and rattlesnakes (*20, 23*). Antiserum to a calicivirus-like enteric agent of humans (the Norwalk agent) did not form aggregates with the chimpanzee virus. Some of these enteric calicivirus-like agents are pathogenic in humans, and evidence suggests that the calicivirus types associated with systemic disease and vesicular lesions in animals may also have a pathogenic potential in humans. For example, the calicivirus reported here is, to our knowledge, the first ever isolated from primates, yet 12 serotypes have been isolated from species as divergent as fish (*24*), pinnipeds, and cattle by using the Vero cell line, and all that have been thoroughly tested readily replicate in some human cell lines (*25*). Nearly all of the remaining 13 known serotypes (12 originally isolated from swine and one from cats) replicate in primate cell lines. Humans unknowingly exposed to these viruses have developed serum neutralizing activity against several serotypes, and the three virus types that have been tested in monkeys readily produce infection, with lesions appearing at the site of injection (*26*).

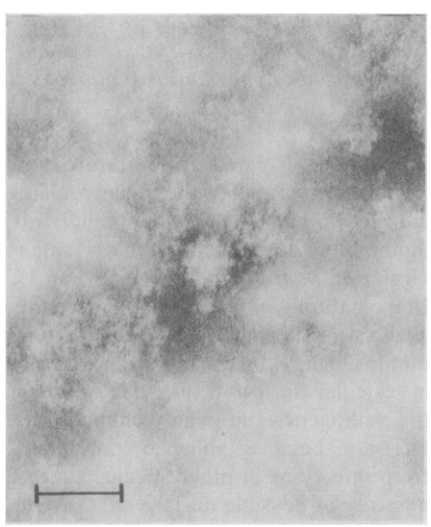

Fig. 2. Electron micrograph of a negatively stained particle 34 to 36 nm in diameter, showing the typical cupped surface morphology of a calicivirus. Scale bar, 50 nm.

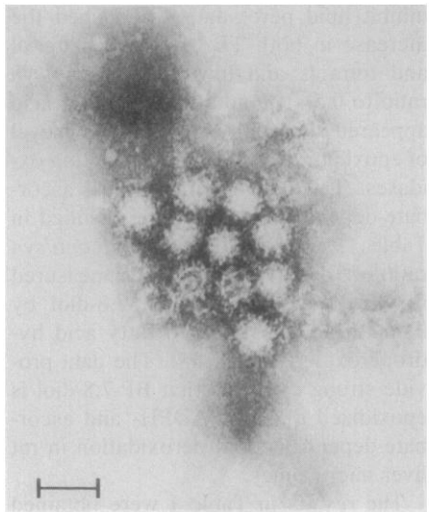

Fig. 3. Electron micrograph showing antigen-antibody aggregates between PCV-Pan 1 and serum from the convalescing chimpanzee. Scale bar, 50 nm.

Our finding of a calicivirus naturally infecting a chimpanzee, one of man's closest phylogenetic relatives, reinforces the likelihood that humans are now or may soon become established as a host species for the nonenteric caliciviruses.

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Sulfation Preceding Deiodination of Iodothyronines in Rat Hepatocytes

Abstract. *In man and animals iodothyronines are metabolized by deiodination and conjugation with glucuronic acid or sulfate. Until now these processes have been regarded as independent reactions. However, in the present study a close interaction of these pathways was observed in the hepatic metabolism of 3,3′-diiodothyronine and 3,3′,5-triiodothyronine. Studies with rat hepatocytes and liver microsomes indicated that sulfation of the phenolic hydroxyl group facilitates the deiodination of these compounds.*

The thyroid gland secretes mainly thyroxine (3,3′,5,5′-tetraiodothyronine, T_4), which is the precursor of the active form of thyroid hormone, 3,3′,5-triiodothyronine (T_3). Some 80 percent of the total T_3 production originates from outer ring deiodination of T_4 in peripheral tissues, especially the liver. Inner ring deiodination of T_4 yields 3,3′,5′-triiodothyronine (reverse T_3, rT_3), a biologically inactive compound. Both T_3 and rT_3 are further deiodinated to 3,3′-diiodothyronine (3,3′-T_2). A second important metabolic pathway for iodothyronines is conjugation with either glucuronic acid or sulfate. These principal pathways, deiodination and conjugation, have usually been regarded as functionally distinct processes. Here we present evidence for a close association of these processes in the hepatic metabolism of iodothyronines.

The metabolism of 3,3′-T_2 and T_3 was studied with monolayers of rat hepatocytes, prepared as described previously (*1*). Whereas hepatic T_3 metabolism is rather complex, 3,3′-T_2 metabolism is easier to follow as it is subject only to outer ring deiodination and sulfation (*2*). Initially, therefore, we focused on the metabolism of 3,3′-T_2 by incubating 3,[3′-^{125}I]T_2 with hepatocytes in monolayers. The medium was analyzed by chromatography on Sephadex LH-20. A good separation was obtained between $^{125}I^-$, produced by outer ring deiodination, and 3,3′-T_2 sulfate (T_2S) and unprocessed 3,3′-T_2. At substrate concentrations of 10 n*M* 3,3′-T_2 or less, iodide was the principal product observed (> 90 percent). At higher 3,3′-T_2 concentrations or during coincubation with 6-propyl-2-thiouracil (PTU), an inhibitor of deiodination, increasing amounts of T_2S accumulated in the medium at the expense of I^- formation (*2*). The rate of outer ring deiodination of 3,3′-T_2 in rat liver cells is similar to that of rT_3 (*3*). This is a surprising observation because 3,3′-T_2, in comparison to rT_3, is a poor substrate for outer ring deiodination by microsomal deiodinase activity (*4*). We, therefore, suspected that in intact hepatocytes deiodination of 3,3′-T_2 is preceded, and in effect accelerated, by sulfate conjugation. If indeed sulfation precedes deiodination of 3,3′-T_2, inhibition of the

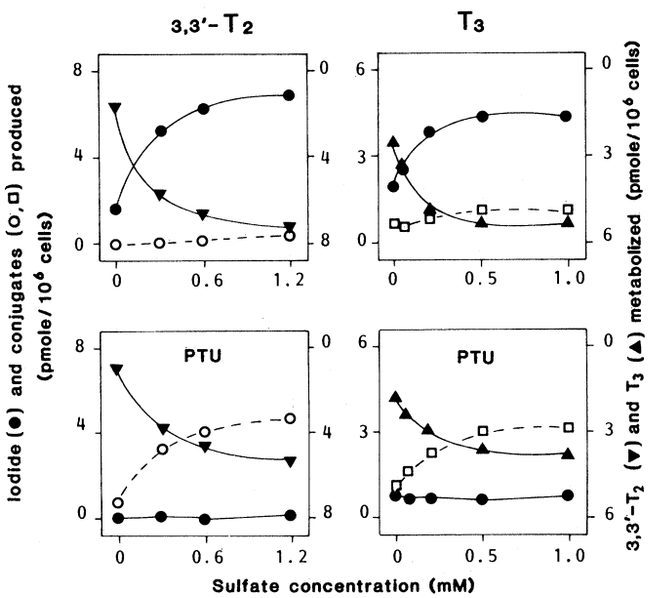

Fig. 1. Sulfate dependence of outer ring deiodination of 3,3′-T_2 and T_3 by monolayers of isolated rat hepatocytes. Approximately 2×10^6 hepatocytes were incubated for 60 minutes with sulfate- and protein-free Dulbecco's balanced salt solution to reduce the cellular sulfate content (*13*). Hereafter, incubations were performed with 4 ml of Dulbecco's medium containing 10 n*M* unlabeled plus 2 μCi of ^{125}I-labeled 3,3′-T_2 or T_3 and increasing sulfate concentrations. Identical incubations were conducted in the presence of 100 μ*M* PTU (lower panels) to inhibit deiodination. The 3,3′-T_2 incubations contained 0.5 percent bovine serum albumin. After 30 minutes for 3,3′-T_2 and 180 minutes for T_3, 100-μl samples were taken from the medium. After protein precipitation with ethanol, the supernatants were evaporated. The residues were dissolved in 0.1*N* HCl and chromatographed by subsequent 0.1*N* HCl and 0.1*N* NaOH elution on small (0.75 ml) Sephadex LH-20 columns. By this method a good separation was obtained between iodide and the conjugated and free iodothyronines, respectively. From the radioactivity in the various fractions, before and after incubation, the respective production and clearance rates were calculated. The results are expressed as picomoles produced or metabolized by 10^6 cells. The conjugates of 3,3′-T_2 and T_3 were identified by hydrolysis with sulfatase or β-glucuronidase (Sigma). Whereas 3,3′-T_2 was exclusively sulfated, T_3 was also glucuronidated. In the absence of PTU, T_3 glucuronide was the major conjugate. In the presence of PTU, the sulfate-dependent increment of T_3 conjugates represents accumulation of T_3S. Symbols: ●, iodide; ▼, 3,3′-T_2; ○, T_2S; ▲, T_3; □, T_3 conjugates.