was passaged through eight consecutive passages in cynomolgus renal epithelium cells grown in tissue culture. It would seem, therefore, that the attenuation of SM virus might well be considered as directly attributable to the outgrowth, in the course of passages through PRI mice, of selective mutants avirulent for monkeys.

Summary. A mixture of 2 strains of Type I poliomyelitis virus was adapted to the PRI strain of mice and to cotton rats. The virus could be propagated in certain sublines when either the intraspinal or intracerebral route of inoculation was used. The infectivity of different sublines for mice and cotton rats varied, but the cytopathic titer in tissue culture was of similar magnitude. The passages in PRI mice resulted in a subline completely avirulent for rhesus and cynomolgus monkeys.

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Administration of an Attenuated Type I Poliomyelitis Virus to Human Subjects. (21062)

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The preceding paper(1) describes the adaptation of a Type I strain of poliomyelitis virus to mice and cotton rats, and presents evidence of attenuation of the virus for monkeys injected intracerebrally. It has been previously reported that a Type II rodent adapted strain of similarly attenuated properties, when administered orally to 85 nonimmune human subjects, produces persistent immune response(2-4). This report describes the results of feeding Type I mouse adapted strain to 2 human subjects, and a mixture of Type I and Type II strains to one subject. These 3 individuals were affected with fatal neoplastic disease, and none had antibodies against the viruses fed.

Materials and methods. Virus. Two pools of infectious material were used for individuals who received only Type I virus: one represented the 22nd passage of the Swiss mouse subline of the combined Sickle and Mahoney strains(1), and the other represented the 22nd + one tissue culture + one mouse passage of the PRI mouse passage line. As stated elsewhere(1), both preparations had been found to have little or no pathogenicity for monkeys injected intracerebrally. Each feeding dose consisted of one ml of a 20% suspension of infected mouse spinal cord tissue diluted in one ounce of milk. The third individual received 4 ml of a virus suspension consisting of equal parts of the SM strain (Type I) and the TN strain (Type II). The SM strain represented the 28th PRI mouse + TC + one mouse brain passage of the virus(1), and the TN strain the 8th mouse brain passage of the virus(2).

Clinical observations, collections of blood and stool specimens. These were conducted according to methods described in detail before(2,3).

Method of virus assay. For determination of the presence of virus in stools or blood, 6 tissue culture (cynomolgus monkey renal epithelium) tubes were used for each specimen according to the method described by Horstmann(5). If all 6 tubes showed no cytopathic effect, 2 blind passages were made

TABLE I. Occurrence of Intestinal Carriage, Absence of Viremia, and Serological Evidence of Immunity in 3 Human Subjects after Oral Administration of Type I (SM strain) and Type II (TN strain) Poliomyelitis Virus.

Human subject No.	Inoculum: Virus	Inoculum: TD_{50} titer	Stools collected and processed (Days after feeding)	Virus isolated from stools	Viremia: Blood tested (Days after feeding)	Viremia: Virus isolated from blood	Antibody response: Virus type	Antibody response: 50% serum titer, Days after feeding: 0	8	15	20	33
1	SM*	4.50	4, 7, 10, 15, 25, 36	4, 7, 10, 15, 25	4, 5, 6, 7, 8	None	I	<1:2	>1:2	1:32	—	1:22
2	SM†	5.20	5, 7, 13, 28, 46, 70	5, 7, 13, 28, 46	1, 2, 3, 4, 5, 6, 7, 8	"	I	<1:2	<1:2	1:2	1:32	1:32
3	SM + TN‡	3.20 / 1.50	§3, 11, 17, 37	3,‖ 11,‖ 17§	1, 2, 3, 4, 5, 6, 7, 8	"	I / II	<1:2 / <1:2	1:8 / <1:2	1:22 / 1:8	1:11 / 1:64	1:365¶ / 1:64

* Received 22nd Swiss mouse passage of the virus.
† " " PRI + 1 TC + 1 mouse passage of the virus. } See preceding paper(1).
‡ Received 4 ml of suspension containing equal parts of: (1) SM (Type I) virus, representing 28 PRI + 1 TC + 1 mouse passage of the strain and (2) TN (Type II) virus, representing 8th mouse brain passage of the strain.
§ Type II isolated only.
‖ Both Type I and Type II viruses isolated from the stool suspension.
¶ Observed on the 63rd day.

before a specimen was considered negative. For titration of virus suspensions, 3 tubes were used for each dilution. The general technic was the same as described in the preceding paper(1).

Neutralization tests. Aliquots of serial 4-fold dilutions of test serum were mixed in equal amounts with dilutions of virus containing 20-60 TD_{50} of Mahoney strain (Type I) and, in the case of combined feeding, an equal dosage of TN (Type II) strain. Also in the instance of dual feeding, the stool isolate was not only tested against each specific serum, but also against a mixture of antisera of the 2 types. After incubation at room temperature for one hour, the suspensions were inoculated into tissue culture tubes which were observed for cytopathic effect. If each antiserum tested separately failed to neutralize the cytopathic effect of the virus, but the mixture of the 2 sera did prevent cytopathic effect, the conclusion was reached that 2 types of virus were present simultaneously.

Results. None of the 3 individuals showed any apparent signs or symptoms of illness which could be attributed to the ingestion of the virus. No virus was isolated from blood specimens of the 3 subjects collected daily during the 8 days following ingestion of the virus (Table I). Intestinal carriage was established in all 3 individuals, as indicated by the isolation of Type I virus from stool samples of the 2 subjects who received the SM strain alone, and by the isolation of Type I and Type II virus from the stools of the person who received the viral mixture.

Homologous neutralizing antibodies were observed in the serum of individual No. 1 as early as the 8th day after ingestion of the virus, and a rise was noted in blood collected 14 days after feeding of the virus. The serum of subject No. 2 had no demonstrable antibodies on the 8th day after administration of the virus, but antibodies appeared in the blood between the 14th and 21st day. The serum of subject No. 3 obtained on the 8th day after feeding contained antibodies against Type I, but not against Type II. Seven days later, the serum of this subject was found to neutralize both Type I and Type II viruses and

TABLE II. Comparative Pathogenicity of SM Strain of Poliomyelitis Virus for Monkeys before and after Passage through Human Intestinal Tract.

Subline*	Virus origin: Passage	Human sub.	Material	TD_{50} titer	PD_{50} titer in mice	Paralytic ratio of monkeys injected IC with dilutions of virus: 10x†	1.0	2.0	3.0	4.0	5.0	6.0
Swiss mouse	6 CR + 22 Swiss mouse	—	Mouse cord	4.5	1.00	—	2/4	0/3	0/2	0/2	0/2	0/2
	6 CR + 22 Swiss mouse + 1 human	No. 1	Human stool	2.5	<1.00	—	0/4	0/4	0/4	0/4	0/4	—
PRI mouse	22 PRI + 1 TC + 1 mouse	—	Mouse cord	5.2	2.00	—	0/4	0/4	0/4	0/4	0/2	—
	22 PRI + 1 TC + 1 human	No. 2	Human stool concentrate	3.8	<1.00	2/2	0/4	0/4	0/4	0/4	0/4	—

* See preceding paper(1).
† 10x concentrated by differential ultracentrifugation.
CR = Cotton rat passage. TC = Tissue culture passage.

the antibody titer against Type I became higher on the 63rd day.

Stool specimens collected from subject No. 1 on the 7th and 10th days after administration of the virus were pooled and made into a 10% suspension according to a process described previously(2). The same was done with stool specimens collected from subject No. 2 on the 7th and 13th days after ingestion of the virus, except that the virus content of the stool suspension was concentrated 10-fold by means of differential ultracentrifugation (6). Aliquots of 10-fold dilutions of the 2 stool suspensions (one in concentrated form) were then injected intracerebrally into rhesus monkeys. Table II shows the paralytic ratio of monkeys thus injected, as well as results of titration of the viral pools used in the original feeding of the individual(1). The results indicate that the SM virus, Swiss mouse passage line, after one passage through the human intestinal tract, failed to acquire pathogenic properties for rhesus monkeys injected intracerebrally. In these 18 animals there was no pathologic evidence of poliomyelitis. Fecal material obtained from individual No. 2 (PRI mouse passage line), concentrated 10 times, produced mild poliomyelitis in 2 monkeys which were injected intracerebrally with the undiluted concentrate. However, there was no clinical or pathologic evidence of involvement of the central nervous system in 16 monkeys which were injected intracerebrally with serial 10-fold dilution of the concentrate. Although the TD_{50} titer of the stool suspensions collected from the 2 subjects was lower than that of the original inoculum, this fact alone cannot account for the lack or low intracerebral pathogenicity of the material for rhesus monkeys, since in a study of another strain of poliomyelitis virus infected human stool suspensions with a TD_{50} titer of $10^{-1.50}$ were observed to be pathogenic for rhesus monkeys injected intracerebrally.

Summary. Two human subjects who had no antibodies against Type I poliomyelitis virus were fed a mouse adapted Type I strain, and one human subject who had no antibodies against Types I and II was fed a mixture of mouse adapted Type I and Type II strains. None of the individuals showed clinical signs

of illness as a result of ingestion of the virus. In the absence of viremia, all 3 excreted virus in the stools. Homologous neutralizing antibodies developed in the sera of each person. Virus passaged through the human intestinal tract maintained the very low pathogenicity for monkeys when injected intracerebrally.

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Chronic Toxicity Studies on Conidendrins and Conidendrols. (21063)

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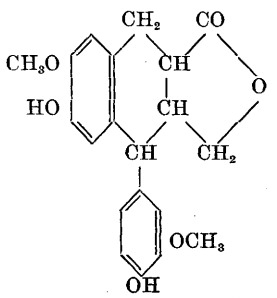
The importance of efficient, non-toxic antioxidants in foods subject to oxidative deterioration is unquestioned. Recently, it has been suggested(1-3) that α- and β-conidendrols could be used as effective antioxidants in edible fats, provided they cause no toxic reactions. This paper reports toxicity studies on these conidendrols, and on α- and β-conidendrin. α-Conidendrin is a partially methylated polyphenol occurring in a number of coniferous trees. (For a description of its occurrence, see Brauns(4)). It can be obtained from the sulfite liquor of the wood-pulp industry. Its chemical structure is:



By inversion at the carbon adjacent to the carbonyl group of the lactone ring, an isomer, β-conidendrin, is formed. Both of these compounds are mild antioxidants. α- and β-Conidendrol (also called α- and β-nonconidendrin) likewise are isomers, and are obtained by demethylation of α-conidendrin(5). They are stronger antioxidants than the conidendrins. Both conidendrols inhibit the rate of peroxide formation in fat when incorporated in the fat in concentrations of 0.01 to 0.1%. The β-isomer is the more effective as a fat antioxidant(3).

Reports on toxicity of these compounds seem to be limited to a short statement(6) on subacute toxicity following intraperitoneal administration to mice. Such administration for several days showed the maximum tolerated daily dose of α- and β-conidendrin to be 500 and 250 mg/kg, respectively, and of α- and β-conidendrol, 62.5 and 113.5 mg/kg, respectively. No statement has been found concerning the metabolism of these compounds in the mammal. However, there are reports that degradation of α-conidendrin can be accomplished by microorganisms(7), although identification of the metabolites has not yet been announced, and that a polyphenol oxidase of microbial origin can degrade conidendrin(8).

Experimental. The effect of continued feeding of diets containing these 4 compounds was studied on weanling albino rats.* The

* The conidendrins and conidendrols were kindly supplied by Drs. W. W. Moyer and W. M. Hearon, Crown Zellerbach Corp., Camas, Washington.