diethylesters had no effect on the growth of the cells in concentrations as high as 1.5×10^{-4} M. The inhibitions of cell growth obtained with malathion and mercaptosuccinate were first evident 24 hours after the compounds were added to the cultures. The implications of these results with respect to the actions of insecticides on cells and tissues are discussed.

The authors wish to acknowledge the assistance of Miss J. B. Hirshberg, Mrs. M. L. Penner, Mr. D. M. Fry, Mr. H. O. Stinnett and Mr. T. K. Nelson.

1. Aldridge, W. N., Biochem. J., 1953, v54, 442.
2. O'Brien, R. D., Toxic Phosphorus Esters, Academic Press, New York, 1960.
3. Aldridge, W. N., Davison, A. N., Biochem. J., 1953, v55, 763.
4. Gabliks, J., Friedman, L., Proc. Soc. Exp. Biol. and Med., 1965, v120, 163.
5. Gabliks, J., ibid., 1965, v120, 168.
6. ———, ibid., 1965, v120, 172.
7. Harris, M., Cell Culture and Somatic Variation, Holt, Rinehart & Winston, New York, 1965, p306.
8. Wilson, B. W., Peterson, D. W., Stinnett, H. O., Nelson, T. K., Hamilton, W. H., Proc. Soc. Exp. Biol. and Med., submitted for publication.
9. Wilson, B. W., Lau, T. L., ibid., 1963, v114, 649.
10. Morgan, J. F., Morton, H. J., Parker, R. C., ibid., 1950, v73, 1.
11. Harris, M., Cancer Res., 1959, v19, 1020.
12. Stecher, P. G., The Merck Index of Chemicals and Drugs, 7th ed., Merck & Co., Inc., Rahway, N. J., 1960, p631.
13. Freedland, R. A., McFarland, L. Z., Life Sci., 1965, v4, 1735.

Received January 10, 1966. P.S.E.B.M., 1966, v121.

Attempt at Immunization by Oral Feeding of Live Rhinoviruses in Enteric-Coated Capsules. (31024)

C. C. MASCOLI, M. B. LEAGUS, R. E. WEIBEL, J. STOKES, JR., H. REINHART AND M. R. HILLEMAN

Division of Virus and Cell Biology Research, Merck Institute for Therapeutic Research, West Point, Pa., Department of Pediatrics, School of Medicine, University of Pennsylvania, Philadelphia and Vineland State School, Vineland, N. J.

The extreme antigenic diversity(1) of rhinoviruses renders it important to devise simplified means for inducing immunity if reasonable control of infections caused by these agents is ever to be achieved. Present evidence indicates that rhinoviruses do not ordinarily infect the gut and the virus does not appear in the feces(2). The rapid inactivation of rhinoviruses at pH 3 to 5(3,4) suggests that in natural infections, the agents are destroyed during passage through the stomach. The possibility remains that infection of the intestines might be achieved if the acidity of the stomach could be bypassed. Accordingly, live preparations of rhinovirus types 32 and 44 were prepared in enteric-coated capsules and fed to human volunteers. The fate of the capsules and the serologic findings in human subjects fed the virus are described here.

Materials and methods. Vaccines and placebo. Rhinovirus strains 1955M (type 32) (1) and 1863H (type 44)(1) were isolated from throat washings from cases of common cold (upper respiratory illness) in university students and were propagated in serial passage in human diploid cell strain WI-38(5) which was monitored for cell alterations according to the proposed Minimum Requirements(6). Both viruses used to prepare the capsules were in fourth passage in cell culture. The WI-38 strain of diploid cells used to cultivate the viruses was in passages 18 to 29. Preparation of the vaccine viruses and tests for identity, freedom from extraneous agents, sterility and safety, whenever applicable, were consistent with contemporary regulations of the Division of Biologics Standards, National Institutes of Health, U. S. Public Health Service. To prepare vaccine,

0.025 ml of the virus was added to powdered sucrose in gelatin capsules and these were enteric-coated by ordinary procedures using a mixture of cellulose acetate phthalate and diethyl phthalate. Capsules of identical size filled with barium sulfate and enteric-coated in the same manner were used for control purpose. *Clinical.* Following approval by proper authorities, clinical tests were carried out in human subjects at Vineland State School, Vineland, N. J. This institution cares for the mentally retarded. Details relating to the protocol followed and the composition of the population tested are given in the text. *Laboratory.* Titrations for rhinovirus infectivity and serum neutralization tests were carried out as described previously(1,3). All sera were tested undiluted and in serial dilutions from 1:2.5 to 1:320.

Results. Two experiments were performed. As shown in Table I, Experiment 66A persons were 12 to 18 years of age and were fed in the morning of May 26, 1965. Experiment 66B persons were 5 to 7 years of age and were fed in the morning of August 4, 1965. In Exp. 66A, 5 persons were fed 2 capsules of virus type 32 and 5 were fed 2 capsules of type 44. Additionally, 5 persons were given 2 capsules of $BaSO_4$ placebo. The subjects had no breakfast and were given a glass of milk at the time the capsules were taken. Recipients of the $BaSO_4$ were examined hourly by one of us (H.R.) by X-ray for 7 hours or until such time that the position of opening of each capsule was established. An additional X-ray was made at 24 hours if the capsules had not disintegrated by 7 hours. Exp. 66B was performed in a similar manner. In Exp. 66A, at least one capsule disintegrated in the intestines of 3 of 5 of the controls. In Exp. 66B, at least 1 capsule opened in the intestines of 3 of 4 of the placebo group. There was no vomiting detected in any of the test groups.

Sera collected from the vaccinated persons and from the placebo controls prior to feeding and again 1 month and 3 months later were tested for both homologous and heterologous antibody titer. Table II shows that none of the individuals developed homologous or heterologous antibody during the time period of the study and none of the placebo controls presented evidence of spontaneous antibody increase. None of the patients developed a cold or more than 1.4°F elevation of temperature during the month following feeding of virus.

Table III presents data which indicate that the virus was probably infectious at the time of capsule disintegration in the intestinal tract based on tests of capsules which had been appropriately treated, emptied, and the contents dissolved in Eagle's minimal essential medium. There was no significant loss of infectiousness of the virus on storage in the capsules at 4°C for 11.5 weeks, 2 days longer than the second feeding experiment. There was no significant drop in titer when exposed to 37°C temperature for 8 hours but $10^{1.5}$ infectious doses ($10^{4.0}$ to $10^{2.5}$ and $10^{3.25}$ to $10^{1.75}$) were lost in the interval between 8 and 24 hours.

Discussion. The rhinoviruses are a common cause for the common cold in adults and frequently cause febrile disease with lower respiratory tract involvement (bronchitis, bronchopneumonia) as well as common cold in children(7,8). Presently, there are 53 numbered serotypes of rhinovirus(1) and there may be hundreds or even thousands. Almost all patients with rhinovirus infections develop specific neutralizing antibody during convalescence(1,3) and this antibody persists for long periods. Additionally, such antibody is correlated with resistance to reinfection(9).

Formalin-killed and live rhinovirus vaccines given parenterally are reported(9-13) to stimulate antibody and to afford some protection against infection with homologous but not with heterologous serotype virus. Live type 1 (strain PK) virus vaccine given nasally induced antibody but also caused respiratory illness(13). Given orally in milk, there were no symptoms and no antibody responses, indicating failure to establish infection.

Rhinoviruses are extremely sensitive to acid pH(3,4) and may lose $10^{5.5}$ TCD_{50} or more of their infectivity titer on exposure to pH 3 for 3-4 hours at room temperature(3). They have not been recovered from the feces (2,14) in cases of respiratory infection with

these agents and it appears that the virus would be destroyed in most instances during passage through the stomach.

In the present study, at least 1 of the 2 capsules disintegrated in the intestines of 6 of 9 of the controls who were given $BaSO_4$ placebo in capsules. If this ratio were the same for those given virus capsules, then at least 12 persons who were fed virus were satisfactorily inoculated. None of the seronegative persons developed detectable antibody and none of the 4 persons who had antibody initially showed an increase in titer in response to feeding with homologous or heterologous virus. This indicated that in the amount of virus used and under the condi-

TABLE I. Age Composition of Human Study Groups and Approximate Position of $BaSO_4$ (Placebo) Capsule Disintegration in Control Groups.

	Vaccine		Persons fed			Disintegration of $BaSO_4$ capsule		
Exp No.	Date fed	Lot No.	Virus type	Age range (yr)	Average (yr)	Case No.	Capsule No.	Time (hr)	Approximate position
66A	5/26/65	169	32	12–16	14	66- 1 to 5	--	—	—
		170	44	12–17	15	66- 6 to 10	—	—	—
		197	Placebo	—		66- 11	1	—	Vomited
						"	2	—	"
		"	"			" 12	1	7	Stomach (obstruction)
						"	2	7	"
		"	"			" 13	1	3	Upper jejunum
						"	2	5	Terminal ileum
		"	"			" 14	1	2	Stomach
						"	2	3	Mid jejunum
		"	"			" 15	1	3	Mid jejunum
						"	2	3	" "
		"	Entire placebo group	14–18	17	—	—	—	—
66B	8/ 4/65	169	32	5–7	6	66–22 to 25	—	—	—
		170	44	5–7	6	66–27, 29 to 31	—	—	—
		197	Placebo	—		66- 18	1	7–24	Intestines
						"	2	"	"
		"	"			" 19	1	4	Small intestine
						"	2	"	"
		"	"			" 20	1	2	Stomach
						"	2	7	"
		"	"			" 21	1	1	Stomach
						"	2	4	Mid duodenum
		"	Entire placebo group	5–7	6	—	—	—	—

The capsules were prepared on 5/24/65.

TABLE II. Results of Tests for Neutralizing Antibody Among Persons Fed Live Rhinovirus Vaccines.

Exp No.	Vaccine virus: Lot No.	Vaccine virus: Virus type	Patient No.	Reciprocal of neutralizing antibody titer against viruses at time period indicated: Type 32: 0	Type 32: 1 mo	Type 32: 3 mo	Type 44: 0	Type 44: 1 mo	Type 44: 3 mo
66A	169	32	66–1 to 3	0*	0	0	0	0	0
	"	"	66–4	2	—	1	0	0	0
	"	"	66–5	5	5	0	0	0	0
	170	44	66–7, 8, 10	0	0	0	0	0	0
	"	"	66–6	20	20	20	0	0	0
	"	"	66–9	5	5	—	0	0	0
	197	Placebo	66–11 to 15	0	0	0	0	0	0
66B	169	32	66–22 to 25	0	0	0	0	0	0
	170	44	66–27, 30, 31	0	0	0	0	0	0
	"	"	66–29	0	0	0	5	5	5
	197	Placebo	66–18 to 21	0	0	0	0	0	0

* Zero titer equals less than 1:1.

TABLE III. Stability Testing and Time Relationships in Oral Feeding of Live Rhinovirus Vaccines.

Lot No.	Virus serotype and strain	Vaccine testing: Date	Vaccine testing: Storage temp	Vaccine testing: Stage	Infectivity titer, neg $\log_{10}$/0.2 ml
169 (C-4128)	32 (1955M)	5/17/65	4°C	Aqueous*	4.25
		5/26	"	Capsule	Fed to Exp 66A persons
		5/27	"	"	3.75
		8/ 4	"	"	Fed to Exp 66B persons
		8/ 6	"	"	3.5
	Stability test	7/21 + 0 hr	37°C	"	3.5
	" "	" + 8 "	"	"	4.0
	" "	" + 24 "	"	"	2.5
170 (C-4129)	44 (1863H)	5/17/65	4°C	Aqueous*	4.0
		5/26	"	Capsule	Fed to Exp 66A persons
		5/27	"	"	4.5
		8/ 4	"	"	Fed to Exp 66B persons
		8/ 6	"	"	3.5
	Stability test	7/21 + 0 hr	37°C	"	3.25
	" "	" + 8 "	"	"	3.25
	" "	" + 24 "	"	"	1.75

* Capsules filled on 5/24/65.

tions of test, infection was not established. Such failure was not due to virus inactivation since the viruses in the capsules were stable for at least 8 hours at 37°C and still showed infectious virus at 24 hours. None of the volunteers were febrile at the time of feeding and none developed respiratory illness in the observation period after feeding. It is reasonable to conclude that rhinovirus types 32 and 44 failed to infect the intestinal tract and induce antibody in the amount given and under the conditions of the test. The possibility of alteration or attenuation of virus with loss of infectiousness for the gastrointestinal tract in the course of passage in cell culture cannot be ruled out.

Summary. Persons aged 5 to 18 were fed live type 32 or 44 rhinovirus in enteric-coated capsules with the hope of bypassing the acid pH of the stomach and of establishing infection in the intestinal tract. Simultaneous tests in persons fed similar capsules containing $BaSO_4$ and followed by X-ray revealed that at least 1 of the 2 capsules fed disintegrated in the intestinal tract of two-thirds of the subjects. None of the persons became ill

and none developed antibody against either virus. The findings in the experiments did not indicate that immunization against rhinoviruses may be achieved by the procedure used.

The authors are indebted to H. Allen, S. White, M. O'Neil and the nursing staff of the Vineland School for nursing assistance. Technical assistance was provided by B. B. Keech, D. M. Blaney and B. Atlee. The WI-38 diploid cell strains were monitored by Drs. A. A. Tytell and R. Zwickey and by E. Wasmuth. J. Bavitz carried out tests of the enteric coating.

1. Hamparian, V. V., Leagus, M. B., Hilleman, M. R., Proc. Soc. Exp. Biol. and Med., 1964, v116, 976.

2. Bloom, H. H., Forsythe, B. R., Johnson, K. M., Chanock, R. M., J.A.M.A., 1963, v186, 38.

3. Ketler, A., Hamparian, V. V., Hilleman, M. R., Proc. Soc. Exp. Biol. and Med., 1962, v110, 821.

4. Dimmock, N. J., Tyrrell, D. A J., Lancet, 1962, v2, 536.

5. Hayflick, L., Proceedings of the Symposium on the Characterization and Uses of Human Diploid Cell Strains. Permanent Section of Microbiologic Standardization of International Assn. of Microbiol. Soc., Opatija, Yugoslavia, 1963, 37.

6. Minimum requirements for the selection and use of human diploid cell strains on the production of virus vaccines, ibid., 1963, 709.

7. Reilly, C. M., Hoch, S. M., Stokes, J., Jr., McClelland, L., Hamparian, V. V., Ketler, A., Hilleman, M. R., Ann. Int. Med., 1962, 57, 515.

8. Tyrrell, D. A. J., Chanock, R. M., Science, 1963, v141, 152.

9. Scientific Committee on Common Cold Vaccines, Brit. Med. J., 1965, v1, 1344.

10. Price, W. H., Proc. Nat. Acad. Sci., 1957, v43, 790.

11. Mogabgab, W. J., Ann. Int. Med., 1962, v57, 526.

12. Mufson, M. A., Ludwig, W. M., James, H. D., Jr., Gauld, L. W., Rourke, J. A., Holper, J. C., Chanock, R. M., J.A.M.A., 1963, v186, 578.

13. Doggett, J. E., Bynoe, M. L., Tyrrell, D. A. J., Brit. Med. J., 1963, v1, 34.

14. Tyrrell, D. A. J., Bynoe, M. L., ibid., 1961, v1, 393.

Received January 10, 1966. P.S.E.B.M., 1966, v121.

Microneutralization Test for the Reoviruses. Application to Detection And Assay of Antibodies in Sera of Laboratory Animals.* (31025)

NATHALIE J. SCHMIDT, EDWIN H. LENNETTE AND MAUREEN F. HANAHOE
Viral and Rickettsial Disease Laboratory, California State Department of Public Health, Berkeley

Reoviruses are known to have a very wide natural host range, infections occurring in a variety of wild and domestic animals in addition to man(1,2,3). Type 3 reovirus has been shown to be indigenous in laboratory mice(4,5), and its presence in mouse colonies has caused increasing concern as attention has been focused upon the importance of defining the viral spectra of laboratory rodent colonies employed for virological research, particlularly for studies on viral oncogenesis (*e.g.*, 6,7). Detecting reovirus infections in laboratory animals is also important from the standpoint of choosing animals without pre-existing antibody for the preparation of viral immune sera and for production of guinea pig complement for use in viral serology.

Antibodies to the reoviruses are generally demonstrated in animal sera by hemagglutination inhibition (HI) tests using either kaolin-treated(8) or untreated(4,7) sera. The prevalence of low levels of HI activity for reovirus type 3 in murine sera has aroused speculation as to whether such activity is due to specific antibody or to nonspecific inhibitors(4,6,7). However, kaolin adsorption for removal of nonspecific inhibitors has not received wide acceptance for use with murine sera since it requires a relatively large volume of serum, and also tends to remove specific antibody(4).

When a monitoring program for rodent

* The work on which this paper is based was supported by Grant AI-01475-06 from Nat. Inst. of Allergy and Infect. Dis., Nat. Inst. Health, USPHS, HEW.