MC2343 was tested and found to be inactive, at a dose of 0.06 mg per egg via the yolk sac against about 100 $LD_{50}$ doses of the agent of meningopneumonitis and against about 100 $LD_{50}$ doses of the vole rickettsia (Baker). At 40 mg/kg, using the same treatment schedules as for the vaccinia experiments in mice, MC2343 was inactive against influenza virus (Shope swine influenza strain V15) in mice infected intranasally.

*Summary.* *p*-Aminobenzaldehyde, 3-thiosemicarbazone (MC2343) caused a significant delay in death and survival of a small percentage of chick embryos and mice infected with vaccinia virus.

At the concentrations tested this compound was inactive against the agent of meningopneumonitis, the vole rickettsia (Baker) and swine influenza virus.

Preliminary results indicated that the *p*-acetamido analogue (Myvizone) was also active against vaccinia virus.

Received January 20, 1950. P.S.E.B.M., 1950, v73.

## Excretion of Poliomyelitis Virus by Monkeys Used for Testing Material of Human Origin.* (17653)

ALEX J. STEIGMAN† AND ALBERT B. SABIN.

*From The Children's Hospital Research Foundation, Department of Pediatrics, University of Cincinnati, College of Medicine.*

The variables which determine excretion of poliomyelitis virus in the stools of infected animals cannot as yet be clearly defined.(1) It was deemed desirable, therefore, to collect additional data on the excretion of virus by monkeys which develop paralysis following inoculation with material of human origin.

*Methods.* As part of another investigation,(2) there were available rhesus and cynomolgus monkeys which had developed paralysis following inoculation with material from a considerable number of individual patients with poliomyelitis. Each monkey was inoculated intracerebrally with the extract of throat swabs, intraabdominally with ether-treated stool extract, and intranasally with untreated stool suspension of the same patient. During the course of the nasal instillations, it was obvious that most of the 1 ml of stool suspension, which was put into each nostril each day, was being swallowed. The presence of typical poliomyelitis lesions in the olfactory bulbs of the monkeys which became paralyzed indicated that the stool suspensions which were being instilled probably contained active virus. Such animals could, therefore, be regarded as having received active virus by the oral route, the nasal route, the intraabdominal route and possibly, also, the intracerebral route. The monkeys which received the human material were killed by chloroform anesthesia on the first day of paralysis. The colon was excised aseptically and its entire contents were removed without disturbing the mucosa and stored in the frozen state in a dry ice box. In only one instance were the contents from 2 monkeys, which received material from the same patient, pooled for testing. Otherwise, the colon contents from each monkey were tested individually. After thawing the frozen material at 37°C, it was ground in a mortar with sufficient distilled water to make approximately a 10% suspension. This suspension was allowed to sediment in long tubes for 1 hour in the refrigerator; the sediment was retained for subsequent nasal instillation in the recipient monkeys, while the supernatant liquid was treated with anesthetic ether, which was added in sufficient quantity to yield a final concentration of 20% by volume. After

* Aided by a grant from The National Foundation for Infantile Paralysis, Inc.

† Senior Fellow in Pediatrics, National Research Council.

1. Sabin, A. B., *Ann. Int. Med.*, 1949, v30, 40.

2. Steigman, A. J., and Sabin, A. B., *J. Exp. Med.*, 1949, v90, 349.

TABLE I.
Test for Virus in Colon Contents of Monkeys on First Day of Paralysis Following Intracerebral, Intraperitoneal, and Intranasal Inoculation with Material from Patients with Poliomyelitis.

| | Data on paralyzed monkeys whose colon contents were tested | | | | | | |
|---|---|---|---|---|---|---|---|
| Species | Interval between last nasal instillation and onset of paralysis, days | Monkey No. | Strain | No. of days nasal instillation given | Amt I.P. ml | Polio lesions in olfactory bulbs | Result of test for virus in colon contents* |
| *M. mulatta* (rhesus) | 3 | 2 | FRO | 6 | 55 | + | 6; 0; (2) |
| | | 13-41 | | | | | |
| | | 14 | RIS. | 6 | 80 | n. e. | 0; 0 |
| | 4 | 62 | KNI. | 6 | 40 | + | 9; (1) |
| | | 87 | SIC. | 7 | 40 | + | 0; 0 |
| | | 1 | RIC. | 6 | 57 | n. e. | 0; (14) |
| | | 13-88 | RIS. | 6 | 80 | n. e. | 0; (16) |
| | 5 | 37 | HOF. | 6 | 60 | + | 0; 0 |
| | | 89 | OBE. | 6 | 25 | + | 0; (8) |
| | 6 | 16-66 | MOH. | 6 | 40 | 0 (+)† | 0; 0 |
| | 7 | 14-76 | YEA. | 10 | 0 | + | 0; 0 |
| | | 36 | HOF. | 6 | 60 | + | 0; (9) |
| | | 11 | SMI. | 6 | 30 | n. e. (+)† | 0; 0 |
| | 8 | 34 | VAU. | 6 | 60 | + | 0; 0 |
| | | 38 | FAL. | 6 | 20 | + | 0; 0 |
| | | 70 | PET. | 6 | 40 | + | 0; (16) |
| | 10 | 4 | HOPP. | 6 | 72 | + | 0; 0 |
| | 18 | 8-18 | SMI. | 6 | 30 | + | 0; (4) |
| *M. cynomolgus* | 1 | 82 | WAL. | 6 | 20 | + | 7; 7 |
| | 2 | 60 | FAL. | 6 | 20 | + | 26; 0 |
| | | 71 | PET. | 6 | 40 | + | 16; (11) |
| | 4 | 57 | COL. | 6 | 20 | + | 20; (11) |
| | | 88 | OBE. | 6 | 25 | + | 10; (5) |

* Figures refer to day after inoculation paralysis first noted in individual monkeys.
0 = Neither clinical nor histological evidence of poliomyelitis; n. e. = not examined.
(1) = Encircled figure indicates day of death of monkey exhibiting neither clinical nor histological evidence of poliomyelitis.
† (+) = Evidence obtained in tests on other monkeys indicated that the stool specimens had active virus.

shaking for 10 minutes, the lightly stoppered flask was left in the refrigerator overnight during which time most of the ether evaporated. The suspensions were then submitted to 2 cycles of centrifugation, one for 15 minutes at 2,000 r.p.m. on the horizontal machine, and the supernatant liquid at 13,000 r.p.m. in an angle centrifuge for 1 hour in the cold room. After cultures on blood agar had shown it to be free of bacteria, the final supernatant liquid was inoculated into monkeys. Each specimen was tested in 2 rhesus monkeys. The untreated sediment was given intranasally, 1 ml in each nostril daily until the material was exhausted, which was usually in about 6 days. The centrifuged, etherized, supernatant liquid was injected intracerebrally in 1 ml amounts and intraperitoneally in 10 to 20 ml amounts for 1 to 2 days, depending on the amount available. Approximately 1 ml of the centrifuged, etherized suspension was saved, frozen in dry ice, and used for a second intracerebral inoculation in all monkeys which exhibited no paralysis 7 days after inoculation. Each pair of monkeys was kept in a separate cage. They were not permitted to exercise in a common run with other monkeys in order to avoid contamination with the feces of other animals. It is perhaps noteworthy that only 5 of the 45 monkeys inoculated in this manner died of toxic or septic complications resulting from the intracerebral injection of the stool extract. All monkeys which failed to show paralysis were sacrificed 28 to 35 days after inoculation and examined histologically. It may be noted that all the positive results,

TABLE II.

Summary of Data on Presence of Virus in Colon Contents on First Day of Paralysis in Monkeys Inoculated Intranasally and Intraperitoneally with Human Stools of Proved* Virus Content.

| Species | Interval between last nasal instillation of human stool and onset of paralysis, days | No. of monkeys received human stool of proved virus content | No. whose colon contents yielded poliomyelitis virus |
|---|---|---|---|
| *M. mulatta* (rhesus) | 3 and 4 | 4 | 2 (or 3)† |
| | 5 to 18 | 11 | 0 |
| *M. cynomolgus* | 1, 2, and 4 | 5 | 5 |

* Monkeys No. 14, 1 and 13-88, listed in Table I, are excluded because their olfactory bulbs were not examined, and there is no way of knowing whether the stool suspensions, inoculated extraneurally, or the extract of the throat swabs, inoculated intracerebrally, contained the virus.

† One of the positive tests was obtained with a pool of the colon contents of 2 rhesus monkeys, which developed paralysis at the same time after the last nasal instillation of the same human stool suspension.

indicated in Table I, are based upon the development of typical paralytic poliomyelitis, confirmed histologically, and that there were no instances of nonparalytic infection in these animals.

*Results.* The colon contents of 18 paralyzed rhesus monkeys and 5 paralyzed cynomolgus monkeys were tested for virus. Insofar as these animals received material from 16 different patients, they may be regarded as representing the reactions to 16 different strains of poliomyelitis virus of human origin. Poliomyelitis lesions were present in the olfactory bulbs of all but one of the 19 donor monkeys which were examined, suggesting that the stool suspensions, which were administered intranasally, most probably contained active virus. Since the incubation period in the cynomolgus monkeys was relatively short, there was none with an interval longer than 4 days after the last nasal instillation of human stools, but the colon contents of all those tested had demonstrable virus by subinoculation. It is perhaps noteworthy that when the interval between the last instillation of human stool and the onset of paralysis in the cynomolgus monkeys was only 1 day, the colon contents produced paralysis in both recipient monkeys after an incubation period of 7 days; when the interval was 2 and 4 days, poliomyelitis did not appear in all the subinoculated monkeys and the incubation periods were considerably prolonged, suggesting that smaller amounts of virus may have been present. In the rhesus monkeys, the only positive results were obtained with the colon contents of the animals in which the interval between the last nasal instillation of human stool and the onset of paralysis was 3 and 4 days. The summary of the data shown in Table II indicates that when the interval between the administration of human stool and the development of paralysis was 5 to 18 days, no virus could be found in the colon contents of any of 11 rhesus monkeys which received stools of proved virus content. It would appear, therefore, that in the rhesus monkeys the detection of virus in the colon contents depended upon the interval since the last administration of human stool rather than on the development of paralytic poliomyelitis. This suggests that the virus recovered from the colon contents of the rhesus monkeys probably represented the original human material which was swallowed rather than any that might have resulted from proliferation of the virus in the monkey. The absence of detectable amounts of virus in the colon contents of the remaining 11 rhesus monkeys indicates that the 9 different strains of virus with which they were infected did not possess the capacity to give rise to infection of the alimentary tract, despite the fact that the virus was swallowed, inoculated intra-abdominally, and obviously multiplied in the central nervous system.

*Discussion.* The work of Clark, Roberts and Preston(3) on the passage of the "MV" poliomyelitis virus through the intestinal tract

3. Clark, P. F., Roberts, D. J., and Preston, W. S., Jr., *J. Prev. Med.*, 1932, v6, 47.

of rhesus monkeys indicated that virus can be recovered from the stools within 24 hours or less after administration. Their experiments did not indicate however, how long such excretion can persist. Faber, Silverberg and Dong(4) in reporting feeding experiments in cynomolgus monkeys suggested that 48 hours may be the period when "all ingested virus could reasonably be assumed to have left the alimentary tract." It would appear from the present study that the period of "passive" passage of virus through the alimentary tract can be as long as 4 days. The demonstration that the swallowing of small amounts of human stool (1 ml of 10% suspension per nostril per day) may lead to the excretion of poliomyelitis virus in detectable amounts, not only during the period of nasal instillation but also for about 4 days thereafter, is of some importance on several counts. First of all, it is evident that monkey stools might be a source of infection to the animal caretakers and laboratory workers, and it should be noted that 2 severe cases of poliomyelitis in laboratory workers, in all probability acquired in the laboratory, have been reported since 1941(5,6,7). Such excretion of virus may also be a source of infection to other animals. Howe and Bodian (8) have reported spontaneous infection in 2 chimpanzees housed in cages adjoining those of rhesus monkeys which were receiving nasal instillations of potent human stools, and recently Bodian(9) reported the appearance of paralytic poliomyelitis in an uninoculated adolescent rhesus monkey which was receiving desoxypyridoxine. In view of the results reported by Trask and Paul(10) and by Melnick(11) on the recovery of virus from the stools of various species of monkeys inoculated by extraneural, parenteral routes with strains of virus of recent human origin, it is noteworthy that in the present tests on rhesus monkeys, receiving large inocula of human stool extracts intraabdominally, there was no evidence of fecal excretion of virus.

*Summary.* Poliomyelitis virus was recovered from the colon contents of both rhesus and cynomolgus monkeys used in the routine testing of infectious human stools by means of nasal instillation, in addition to other routes of inoculation, when the interval between the last nasal instillation of infectious human stool and the first appearance of paralysis was 4 days or less. In none of 11 paralyzed rhesus monkeys in which this interval was from 5 to 18 days was virus found in the colon contents. The 4-day period following the last nasal instillation of infective human stools may be taken as the time required for elimination of virus swallowed by monkeys, when no local multiplication occurs. The present data indicate that monkeys, receiving human stool suspensions by the nasal or oral route, are a potential source of infection not only to their caretakers but also to other primates in the same animal quarters unless special precautions are taken to avoid fecal contamination. No evidence was obtained that poliomyelitis viruses of human origin can multiply in the alimentary tract of *rhesus* monkeys or be eliminated there by centrifugal spread from the central nervous system.

4. Faber, H. K., Silverberg, R. J., and Dong, L., *J. Exp. Med.*, 1948, v88, 65.

5. Sabin, A. B., and Ward, R., *Science*, 1941, v94, 113.

6. Sabin, A. B., *J. A. M. A.*, 1947, v134, 749.

7. Wenner, H. A., and Paul, J. R., *Am. J. Med. Sc.*, 1947, v213, 9.

8. Howe, H. A., and Bodian, D., *J. Exp. Med.*, 1944, v80, 383.

9. Bodian, D., *Am. J. Hyg.*, 1948, v48, 87.

10. Trask, J. D., and Paul, J. R., *Ann. Int. Med.*, 1942, v17, 975.

11. Melnick, J. L., *J. Immunol.*, 1946, v53, 277.

Received January 19, 1950. P.S.E.B.M., 1950, v73.