sions the distal sodium concentrations remained identical, *i.e.* the distal tubule was not capable of further lowering concentration even when allowed more time.

The effect of aldosterone on the adrenalectomized dogs is shown in Table I. In all dogs, distal sodium concentrations were normally lowered during occlusions performed approximately 2 hours after completion of control occlusion and intravenous injection of aldosterone. This ability of aldosterone to increase distal sodium reabsorption may have one of several explanations. As sodium is actively transported out of the distal tubule there is no doubt some passive diffusion with the concentration gradient back into the tubule. Aldosterone may either activate the enzyme systems responsible for active sodium transport outwards or, less likely, decrease passive diffusion inwards of sodium through changes in permeability characteristics of the tubular cells or membrane.

Proximal sodium reabsorption. As described above, sodium concentrations of the best proximal stop flow samples are equal to their respective free flow values in both the normal and adrenalectomized dogs. This indicates that during occlusion the proximal tubule could not change sodium concentration from its free flow value in the proximal tubule(3,4). As calculated by methods described previously(4), sodium concentration of proximal reabsorbate is equal to that of plasma in both normal and adrenalectomized animals, and is not changed detectably by administration of aldosterone. However, the data collected thus far do not conclusively indicate that the volume of proximal sodium and water reabsorbed is not affected by adrenalectomy and aldosterone. Such experiments are now in progress.

Summary. The technic of stop-flow analysis has been used to localize the site of renal sodium reabsorption which is affected by adrenalectomy and aldosterone. Following adrenalectomy, the distal tubule was not able to reduce sodium concentration to the low value achieved during stop-flow in normal dogs. Following aldosterone administration this distal reabsorptive capacity was restored. No conclusion could as yet be made regarding the effects of aldosterone on the proximal tubule.

1. Barger, A. C., Berlin, R. D., Tulenko, J. F., *Endocrin.*, 1958, v62, 804.

2. Malvin, R. L., Wilde, W. S., Sullivan, L. P., *Am. J. Physiol.*, 1958, v194, 135.

3. Wilde, W. S., Malvin, R. L., *ibid.*, 1958, v195, 153.

4. Malvin, R. L., Wilde, W. S., Vander, A. J., Sullivan, L. P., *ibid.*, in press.

Received July 31, 1958. P.S.E.B.M., 1958, v99.

Contact Transmission of Poliomyelitis Virus Among Monkeys.* (24339)

DONALD E. CRAIG AND THOMAS FRANCIS, JR.
Department of Epidemiology, Virus Laboratory, School of Public Health, The University of Michigan, Ann Arbor

The problem of transmitting poliovirus from infected to normal animals is of interest and importance to humans because of its value as a model in the study of the natural transmission of the disease. As early as 1912, Levaditi and Danulesco(1) reported that normal Rhesus monkeys housed with infected monkeys did not develop poliomyelitis. Sabin and Winsser(2) were not able to demonstrate any evidence of poliovirus in normal monkeys associated with other animals receiving type 2 virus orally. On the positive side, Faber and his colleagues(3) were able to detect lesions typical of poliomyelitis in two of the cranial nerve ganglia of uninoculated monkeys housed in the same room with infected animals. Howe and Bodian(4) isolated poliovirus from the stools of two chimpanzees which were

* This study was aided by grant from Nat. Foundation for Infantile Paralysis.

housed for six months in the same room with Rhesus monkeys receiving human stool inoculations intranasally. In 1953 we reported the results of several experiments in which normal cynomolgus monkeys were housed with monkeys that had been orally infected with cord suspensions of active virus, but were unable to demonstrate any transmission of poliovirus under these conditions. In the current study, high titered tissue culture fluids were employed for the oral inoculation of monkeys and tissue culture systems were used for the detection and typing of virus as well as for antibody studies.

Methods. Monkeys. Cynomolgus monkeys were employed because of their greater susceptibility to infection with the agent of poliomyelitis(5). *Tissue Cultures.* Trypsin dispersed monkey kidney cells were prepared according to the methods described by Youngner(6), and grown in his medium D consisting of:

95% mixture 199(7) in Hank's balanced salt solution
2% calf serum
3.5% sodium bicarbonate (2.8%)
100 units/ml each of penicillin, streptomycin and mycostatin.

Cells were grown in 15 ml screw-capped tubes for use in virus isolation and identification, and in five liter bottles for the preparation of large virus pools. HeLa cells were maintained and prepared for use according to the methods of Scherer, Syverton and Gey(8). *Virus.* The type 1, Mahoney strain, virus was used as a seed to prepare large pools of virus in monkey kidney tissue cultures. The pools contained 10^6 infectious doses/ml when titrated in the same tissue cultures, and in the experiments to be described caused paralysis in 17 of 20 monkeys that received the virus orally. The virus inoculum was administered in two equal doses of five ml of undiluted tissue culture fluid on consecutive days by means of a short catheter tube attached to a syringe and placed in the animal's mouth while it was held open. *Collection of specimens.* Stools were obtained by removing the animals from the cage an hour after feeding time and applying pressure in the abdominal region. Anal swabs were obtained by inserting a sterile cotton-tipped applicator stick into the anal orifice; the pharyngeal area of the monkeys was also swabbed. Two swabs were obtained at each site on each collection so that two tissue cultures could be inoculated. Swabs were placed in a small amount of tissue culture medium containing twice the usual concentration of antibiotics in order to reduce contamination by bacteria and molds. Bloods to be tested for antibody levels or the presence of virus were collected by cardiac puncture without the use of anesthesia. All specimens were stored at −20°C until tested. *Testing of Specimens.* Anal and pharyngeal swabs were placed directly into monkey kidney tissue culture tubes, the fluid expressed, and the swab discarded. 0.25 ml of blood serum from some of the monkeys was placed in tissue culture tubes to test for the presence of virus. Stool specimens were triturated in a mortar and pestle with alundum, and tissue culture medium with twice the usual antibiotic concentration was added to make a 20% suspension by weight. Clarification was accomplished by centrifugation, first at low speed for 10 minutes, then at 5000 rpm in a Sorvall angle-head centrifuge (35,000 × G) for one hour. The supernate from the last centrifugation was diluted 1:1 (10%) and 0.25 ml was added to each of two tissue culture tubes. The inoculated cultures were examined microscopically every other day for 7 days for evidence of cytopathology. Fluids from tubes with obvious tissue destruction were retested in the presence of homologous type 1 hyperimmune monkey serum in order to prove the presence of poliovirus. Fluids from 24 or 48 hr cultures showing contamination due to bacteria and molds were centrifuged at 35,000 × G for one hour and the supernate treated with 1000 units/ml of the antibiotics listed previously, before retesting in tissue cultures. Serum antibody levels were determined in the cytopathogenic test as described by Brown (9) employing HeLa cells.

Plan of experiments. In each of 4 experiments 5 monkeys were infected by feeding virus. They were then held for 8 hours in separate cages to reduce the possibility of contaminating their uninfected cagemates with excess inoculum, then placed in a large me-

TABLE I. Fate of 20 Monkeys Infected Orally with Type 1 (Mahoney) Strain of Poliomyelitis Virus.

Exp.	Monkey No.	Day of paralysis	Day of death	Days excreting virus: Anus	Throat	Blood	Day antibody first detected*
1	1	7	8	None			None
	2	7	14	2, 7, 9			"
	3	8	11	4			"
	4	14	31	None			21
	5	8	12	2			9
2	11	None	31	3, 30			21
	12	8	10	None			None
	13	7	9	7			"
	14	11	15	3, 9			"
	15	12	12	3, 7, 9			"
3	21	8	11	2, 7	4	3, 4	7
	22	8	8	2, 7	2, 4, 7	2, 3, 4	None
	23	10	10	None	4, 7, 9	3, 4	7
	24	10	11	7	4, 7, 9	None	None
	25	None		4	2, 4, 9, 11	3, 4	21
4	31	5	5	2, 4	None	4, 5	None
	32	8	8	7	5, 7	None	"
	33	9	11	4, 6, 7, 8, 9	4, 5, 6, 7, 9	5, 6, 7, 8, 9	"
	34	18	22	2, 4, 7	5, 7, 8, 9, 11, 18	11	18
	35	None		4, 8	4, 5, 6, 7, 8, 11, 18	None	8

* All animals were negative initially.

tabolism cage measuring 2 × 2 × 3 feet, together with 5 normal uninoculated animals (contact monkeys). In each of the experiments specimens were collected on all monkeys prior to the start and daily thereafter for 14 days in Experiments 1, 2, and 4; specimens were collected on alternate days for 2 weeks in Experiment 3. In all of the experiments additional specimens were collected once or twice a week from the 14th to 30th day.

Results. The fate of the 20 monkeys which were infected by oral inoculation and the 20 contact animals is indicated below:

Treatment	No.	Virus	Paralysis
Infected	20	Pos 17	14
		Neg 3	3
Contact	20	Pos 11	1
		Neg 9	1

Infected animals. All infected animals had been tested for anal virus and 10 of them for virus in the pharynx and in the blood. The results are shown in Table I. Virus was recovered from a total of 17 of the infected animals. Sixteen of the 20 had virus in the intestinal tract, 9 of 10 monkeys tested for pharyngeal virus were positive and 7 of them had viremia. Fourteen of the animals excreting virus became paralyzed as did all 3 of the monkeys from whom virus was not recovered. Paralysis occurred in the majority of the animals between the 7th and 11th day after feeding virus, although one was paralyzed as early as the fifth day and another as late as the 18th. Excretion of virus was irregular regardless of the source tested, but when present, in all cases preceded onset of paralysis. Among the 10 animals tested for pharyngeal virus the agent was more frequently isolated from the throat than from the anus. With one exception viremia began 4 to 7 days prior to paralysis and usually ceased before the onset of paralysis. Viremia also occurred in 3 of 5 animals which became paralyzed without developing antibody.

Contact animals. All of the 20 contact monkeys were tested for anal virus and 10 of them for pharyngeal virus and for viremia. Table II shows that virus was recovered from 11 monkeys altogether; 9 of 20 excreted virus at the anus, 3 of 10 from the pharynx, and in none of the 10 was viremia detected. Paralysis occurred in 2 contact animals, in one of which no virus was detected in the excreta. Virus was found in the stool of one contact monkey as early as the second day and in an-

TABLE II. Fate of 20 Normal Monkeys That Were Cagemates with 20 Poliomyelitis Infected Monkeys.

Exp.	Monkey No.	Day of paralysis	Day excreting virus			Day antibody first detected
			Anus	Throat	Blood	
1	6	None	None			None
	7	"	4			"
	8	"	None			"
	9	"	"			"
	10	"	4, 7			"
2	16	None	30			None
	17	11	None			9
	18	None	9, 11			None
	19	"	3, 11			"
	20	14	7			9
3	26	None	4	7	None	None
	27	"	4	None	"	"
	28	"	None	"	"	"
	29	"	"	"	"	"
	30	"	"	"	"	"
4	36	None	None	8	None	None
	37	"	"	6	"	"
	38	"	2	None	"	"
	39	"	None	"	"	"
	40	"	"	"	"	"

other as late as the 30th day. The frequency of isolation from these animals was considerably less than from the monkeys given virus orally. In one experiment litter from the floor of the cage was collected during the first two weeks and tested for virus which was shown to be present during the first week but not subsequently. Since the litter consisted of stools as well as wood shavings and uneaten food and since both infected and contact animals were excreting virus freely during this period, the total amount found in the litter, representing approximately 1,000,000 tissue culture infectious units, was not surprising.

Antibody development. Only 8 of the 20 infected monkeys developed detectable antibodies during the course of the experiments. In three of these, antibodies were first observed immediately prior to or at the time of paralysis, an observation made by Bodian many years ago. In 2 animals antibodies were not detected until after paralysis, and the remaining 3 which developed antibodies were non-paralyzed animals. Eleven of the 12 animals which did not develop antibodies during the period of observation became paralyzed. The 2 contact monkeys which became paralyzed were the only 2 to develop antibodies. These appeared from 3 to 5 days prior to the onset of paralysis. In an effort to detect the presence of low level, unmeasurable antibodies in the other contact animals, one ml of poliomyelitis vaccine was injected intramuscularly on the 30th day of the test and they were bled again on the 45th day. Since no booster effect in the form of antibody production was observed it was concluded that these monkeys were truly negative.

Discussion. One of the purposes of the experimental approach used in the present study was to determine the feasibility of using monkeys as an epidemiological tool for the study of the spread of poliomyelitis in human beings. For several reasons this expectation was not realized. First, the duration of virus excretion in cynomolgus monkeys is considerably shorter than that which has been repeatedly observed in human beings. In addition, the percentage of normal contact monkeys excreting virus was less than that usually seen in susceptible humans residing under a comparable degree of close association with a case of the disease. The overall percentage of virus isolations from familial associates of cases reported in the literature from 1939 to 1952 has been summarized by Brown(10) and found to be 41% without respect to the state of susceptibility of the exposed persons.

Horstmann(11) has recently described the

spread of virus to associates of individuals fed attenuated virus in an institution. Although virus was being excreted, very little spread to those individuals that had naturally acquired antibody; however, most of the vaccinated associates became infected as evidenced by both virus excretion and antibody rise.

In the present study virus was recovered from 11 of the 20 contact monkeys, but ten of these remained non-paralytic and no evidence of antibody formation could be demonstrated, suggesting strongly that virus had merely passed through the animals without multiplying or stimulating the production of antibodies. Of the nine contact monkeys not excreting virus, one developed paralysis and no antibodies were detected in the others. In fact, the only contact monkeys shown to develop antibodies were those which became paralyzed. Unless virus excretion alone in the absence of subsequent antibody rise is accepted as evidence of sub-clinical disease, an unlikely conclusion, the infection rate in the contact animals was only 2 of 20 (10%). In contrast to these findings, 17 of the 20 monkeys which had been fed virus were shown to excrete the agent; 14 of these became paralyzed and the other three developed antibodies. Of the 3 which did not excrete virus all became paralyzed; therefore, the extent of infection in these animals was 100%.

Virus appears to spread among monkeys almost as effectively as among familial associates of a human case of poliomyelitis. However, in contrast to the expected event in the human, sub-clinical infection resulting from contact exposure in simians could not be demonstrated in the present study.

Summary. 1) Poliomyelitis virus was fed to 4 groups of 5 monkeys each which were subsequently caged with an equal number of normal animals. 2) All of those fed virus became infected as evidenced by either the development of paralysis or virus excretion accompanied by an antibody rise. 3) Two of the contact monkeys became paralyzed and although one of these and 10 others excreted virus briefly, antibodies developed and were demonstrated only in the 2 paralyzed animals suggesting that sub-clinical infection did not occur.

1. Levaditi, C., Danulesco, V., *Compt. Rend. Soc. Biol.*, 1912, v72, 543.

2. Sabin, A. B., Winsser, J., *Fed. Proc.*, 1943, v12, 456.

3. Faber, H. K., Silverberg, R. J., Doug, L., *J. Exp. Med.*, 1950, v91, 417.

4. Howe, H. A., Bodian, D., *ibid.*, 1944, v80, 383.

5. ———, *Am. J. Hyg.*, 1948, v48, 99.

6. Youngner, J. S., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 202.

7. Morgan, J. F., Morton, H. J., Parker, R. C., *ibid.*, 1950, v73, 1.

8. Scherer, W. F., Syverton, J. T., Gey, G. O., *J. Exp. Med.*, 1953, v97, 695.

9. Brown, G. C., *Univ. Mich. Med. Bull.*, 1955, v21, 186.

10. Brown, G. C., Rabson, A. S., Schieble, J. H., *J. Immunol.*, 1954, v73, 54.

11. Horstmann, D. M., *Yale J. Biol. and Med.*, 1957, v30, 81.

Received August 1, 1958. P.S.E.B.M., 1958, v99.

Antimitotic Action of Maleuric Acid.* (24340)

TADASHI OKADA AND EUGENE ROBERTS

Dept. of Biochemistry, Medical Research Inst., City of Hope Medical Center, Duarte, Calif.

Since the first report of the antimitotic effects of maleic acid(1), there has been considerable interest in this and related substances such as maleimide(2), N-ethylmaleimide(2), and maleic hydrazide(3). The antimitotic activity of these compounds has been attributed largely to their ability to form –SH adducts(2). Indeed, the ultraviolet spectral changes accompanying the interaction of –SH compounds with N-ethylmalei-

* This investigation was supported by research grant No. 2568 from Nat. Cancer Inst., U.S.P.H.S.