CUTANEOUS INFECTIVITY IN EXPERIMENTAL POLIOMYELITIS

Increased Susceptibility after Neurosurgical Procedures*

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Present knowledge of experimental poliomyelitis is founded largely upon experience with a few strains of virus highly adapted to the monkey. Valuable as these data are, it is not unlikely that interesting findings would follow the study of a greater variety of strains and, in particular, fresh strains. For example, a comparison of fresh strains, in spite of certain unavoidable irregularities, indicates that the effective routes of inoculation are not identical for all strains (1). Such variations may be responsible for the reports of infectivity by the gastrointestinal (2) and cutaneous (3–6) routes. Intracutaneous inoculation with certain strains has proven infective with relatively small amounts of virus (4, 5). With one of these strains (3) in hand, the present study was undertaken in an effort to determine the pathways of neurotropic propagation of the virus from the skin to the spinal cord in monkeys.

The experiments were begun on the hypothesis that propagation of virus from an intracutaneous site of inoculation to the spinal cord could be prevented by previous division of appropriate neural connections. The denervations were done by the following methods: (*a*) anterior and posterior rhizotomy; (*b*) production of an isolated skin graft by a two stage flap method; (*c*) complete isolation of a limb from its nerve supply. In addition, a few observations were made upon animals after bilateral olfactory neurectomy.

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It was soon evident that these procedures were ineffective in preventing the appearance of typical experimental poliomyelitis. In fact, the susceptibility of the denervated animals to cutaneous infectivity was enhanced over that observed in the normal controls. This became obvious because the cutaneous infectivity of the strain diminished in the controls during the study.

Materials and Methods

Description of Strains.—The Wfd. strain was recovered from cord and medulla of a child dead of bulbar poliomyelitis in Los Angeles, California, in the epidemic of the summer of 1934. The cutaneous infectivity of this strain in its early passages and some of its other properties have been described (1, 3, 7). From the 3rd to the 7th passage this strain showed a high degree of infectivity when inoculated into the skin; 7 of 10 monkeys so inoculated developed the experimental disease. In later passages this property diminished.

Five other established strains, previously compared with the Wfd. strain (1, 7), were used in one experiment, and Experiment 7 was done with the fresh McL. strain.[1] It was obtained as glycerolated human cord in September, 1937, from the epidemic in Toronto, Ontario (8), and was first used here without prior animal passage. The cutaneous infectivity of this strain has been noted (4).

The strains were kept in 50 per cent glycerol and distilled water at 4°C. On the afternoon of use they were freshly prepared as 10 per cent suspensions of spinal cord by grinding with sand and cold saline, but in the last experiment, in September, 1937, the grinding was done with powdered pyrex glass and distilled water. The suspensions were centrifuged at 1000 R.P.M. for 5 minutes. The usual dose was 2 cc. of the cloudy supernatant fluid. Intracutaneously, it was given in 10 piqûres of 0.2 cc. each. Intravenously, 2 to 3 minutes were used for inoculation; when it was given rapidly several animals died before recovering from the anesthesia. Intracerebral inoculations were made into the left frontal lobe. For titrations, tenfold dilutions were made in saline and 0.5 cc. volumes were inoculated intracerebrally. All inoculations were done under full anesthesia (ether or nembutal or dial).

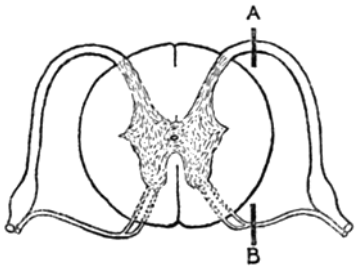
Apparently healthy *Macacus rhesus* monkeys of 2 to 3 kilos were used, but some had tuberculosis. None had been used previously except 4 convalescents, included to test the virus; 3 were convalescent from a poliomyelitic infection induced by the Flexner (7) strain and 1 from a new strain (Rl.). Daily rectal temperatures were recorded for 4 weeks following inoculation, unless death or the development of poliomyelitis terminated the experiment. At autopsy, sec-

[1] For this material we are indebted to Dr. F. F. Tisdall and Dr. L. N. Silverthorne of the Hospital for Sick Children and the University of Toronto, Toronto, Canada.

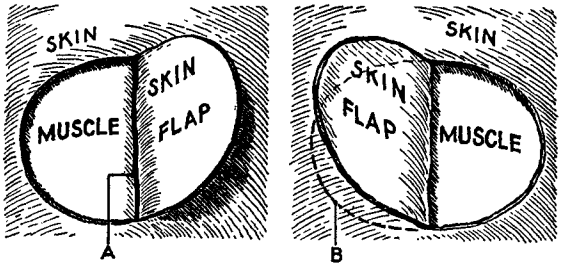
tions of medulla and cervical, dorsal, and lumbar cord were taken for histological examination and the rest of the cord was saved in 50 per cent glycerol.

Surgical Procedures.—All operations were carried out under nembutal anesthesia (35 mg. per kilo intraperitoneally).

1. Rhizotomy (Section of Anterior and Posterior Spinal Nerve Roots).—Anterior and posterior rhizotomies were done through a lower thoracic and upper lumbar laminectomy. After opening the dura, the motor and sensory roots, usually of the 7th thoracic to the 1st lumbar segments, inclusive, were divided between the



TEXT-FIG. 1. Rhizotomy. A and B, section of posterior and anterior spinal nerve roots.

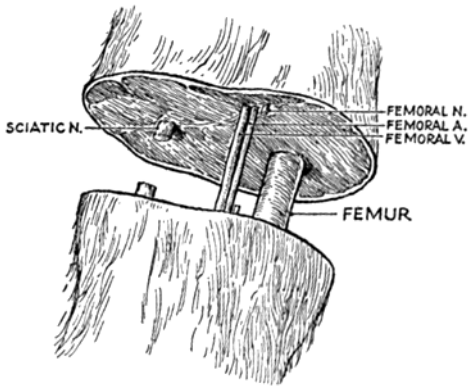


TEXT-FIG. 2. Elevation of skin flap in two stages. A, first stage showing thread marker. B, second stage showing line of healed first incision.

spinal cord and the intervertebral foramina, on the right side. An extradural rhizotomy was done on animal 6-50, Experiment 2, dividing the roots with an electrosurgical cautery. The dura, muscles, fascia, and skin were then closed in separate layers with silk sutures. Inoculations were made into the denervated skin of the flank.

2. Skin Flap.—Isolated skin grafts were constructed by a two stage flap procedure. At the first stage, a flap composed of skin and subcutaneous tissue, was elevated from the underlying muscle in the flank for a distance of about 6 cm., with its pedicle attached anteriorly. A black silk thread marker was then placed

across the anterior extremity of the undercut area and the flap replaced on its original bed. The U-shaped incision was then closed with silk sutures, but the flap was not anchored to its bed. At the second stage procedure, 9 to 14 days later, an inverted U-shaped incision was made, connecting the open ends of the original U. The skin and subcutaneous tissue were elevated from the underlying muscle until the pedicle of the original flap was completely undercut. The site was readily identified by the black silk marker, inserted at the first stage, the marker being exposed and removed at the second stage. The flap, now attached at its posterior aspect to the original flap, was replaced and sutured as in the first stage procedure. Thus, an area of skin and subcutaneous tissue was completely isolated from the surrounding structures by a two stage procedure. Intra-



TEXT-FIG. 3. Denervation of limb, showing partial section in mid-thigh.

cutaneous inoculations were then made into the isolated area. The interval between operations was sufficient for establishment of a new blood supply (9), but it is extremely unlikely that nerve regeneration had progressed to any significant extent (10).

3. Denervation of a Limb.—This was accomplished by a circular incision around the mid-thigh, entirely dividing the skin, subcutaneous tissue, nerves, muscles, and fascia. Only the femur, femoral artery, and vein were not divided. The artery and vein were stripped completely of all covering, including the adventitia of the artery with its periarterial sympathetic fibers, over a distance of about 1.5 cm. The periosteum was stripped from the femur over a similar distance. The muscles and skin were approximated with silk sutures. Intracutaneous inoculations were then carried out in the denervated area below the knee. The possibility of intact neural communication between the site of inoculation and the cerebrospinal axis would appear remote.

4. Bilateral Olfactory Neurectomy.—This was done through a right frontal osteoplastic bone flap. The dura was opened and the frontal lobe elevated,

exposing both olfactory nerves on the floor of the anterior fossa. With the aid of a nerve hook the olfactory bulbs and tracts were separated from the overlying frontal lobes and from 1 to 2.5 cm. of nerve removed. The anterior stump of the bulb was not dislodged from the cribriform plate on the left side. In this manner neural continuity was interrupted without disturbing possible vascular or lymphatic communications.

EXPERIMENTAL

Experiment 1. Cutaneous Inoculation in an Area Denervated by Rhizotomy (Section of Anterior and Posterior Roots of Spinal Nerves).— Following a preliminary experiment, Experiment 1 was devised to

TABLE I

Experiment 1. Cutaneous Inoculation in Denervated and Normal Areas
Dec. 22, 1936.

Monkey		Inoculation			Result	
No.	Preparation	Time	Dose	Intracutaneously	Incubation period	Paralysis
		p.m.	*cc.*		*days*	
6-30	None	3.33	2	Face and head	—	Remained well, no paralysis
6-29	"	3.40	2	Tail	—	" "
6-27	Rhizotomy right, Dec. 22, 4.30 p.m.	5.50	2	Right flank	6	Severe
6-28	Rhizotomy right, Dec. 22, 2.15 p.m.	6.03	2	" "	11	"
6-32	None	6.06	2	" "	—	Fever only

Virus: 10 per cent No. 4-53; Wfd. strain; generation IX.

compare the infectivity of the virus in denervated and normal areas of skin. The preparatory operations (rhizotomies) were done the day of the inoculations and 3 normal monkeys were included to compare inoculations into skin of head, flank, and tail. Experiment 1 is presented in Table I and the protocols are in the Appendix. The results show that severe poliomyelitis followed intracutaneous inoculations in areas of skin deprived of spinal nerve supply. The ineffectiveness of inoculations into head and tail in Nos. 6-29 and 6-30 and in the flank in 6-32 suggests that the virus had ceased to infect readily in normal skin. It seems, therefore, that the rhizotomies might have

led to an increase in susceptibility of Nos. 6-27 and 6-28. Accordingly, it was decided to collect more data on the effect of denervation by rhizotomy and by other methods.

Experiment 2. Intracutaneous Inoculation after Various Operations.—Skin flaps were elevated in two stages at such intervals that the blood supply would be maintained and yet functional nerve supply would not be reestablished. In other animals olfactory nerves were sectioned; once, this was combined with rhizotomy. The preparatory operations were done during January, 1937, and the inoculations on the 27th of that month. The source of virus was the cord of No. 6-27 from Experiment 1.

The results of Experiment 2 are shown in Table II, and it is obvious that the experience of Experiment 1 was corroborated and extended. In other words, well marked experimental poliomyelitis followed intracutaneous inoculations placed in areas of skin deprived of spinal nerve supply by rhizotomy, or deprived of total nerve supply by elevation of skin flaps. Well marked experimental poliomyelitis followed intracutaneous inoculation in 3 monkeys prepared by sectioning both olfactory nerves. In 2 of these 3 monkeys, postmortem examination revealed that both olfactory nerves had been completely severed. In the third (6-47) a filamentous connection was found between the olfactory bulb and tract on the left side. Most likely this was fibrous tissue but further study was not made to learn its true nature.

The control monkey, No. 6-53, failed to develop experimental poliomyelitis; and 2 others also failed: No. 6-52, sick with tuberculosis, had been prepared by rhizotomy and inoculated in the denervated area, and 6-36, sick with tuberculosis, prepared by rhizotomy and inoculated in the contralateral flank. The comparison of control, No. 6-53, with prepared animals again suggests that the operations might have increased susceptibility to poliomyelitis.

Experiment 3. Intravenous Inoculation; Intracutaneous Inoculation in Denervated Limb; Test of Purity of Virus.—Since the virus had passed sectioned nerves it became desirable to test intravenous inoculations. In the experiment a new type of preparatory operation was included: No. 7-22 was prepared by dividing all structures in the left mid-thigh except femur, femoral artery, and femoral vein, as described

under methods. The skin and muscles were sewed together and the virus inoculated into the skin of the denervated calf.

Certain animals were included also to test the purity of the virus. This was desirable because the "takes" following denervations raised

TABLE II

Experiment 2. Intracutaneous Inoculation after Various Operations
Jan. 27, 1937.

Monkey		Inoculation			Result	
No.	Preparation	Time	Dose	Route	Incubation period	Paralysis
		p.m.	*cc.*		*days*	
6-40	Skin flap, Jan. 12 and 21	3.25	2	Skin flap	12	Severe*
6-39	" " " " " "	3.28	2	" "	5	Severe
6-50	Extradural rhizotomy right, Jan. 25	3.43	2	Skin right flank	5	"
6-47	Olfactory neurectomy, Jan. 20; rhizotomy right, Jan. 26	3.49	2	" " "	6	Mild
6-54	None, intracerebral control	3.57	0.5	Brain	9	Moderate
6-55	" " "	4.08	0.05	"	11	Severe*
6-44	Olfactory neurectomy, Jan. 15	4.39	2	Skin right flank	7	Severe
6-37	Laminectomy mid-line, Jan. 7	4.49	2	" " "	8	"
6-36	Rhizotomy right, Jan. 6	4.50	2	Skin left flank	—	None*
6-38	Rhizotomy right, Jan. 8	5.05	2	Skin right flank	7	Severe
6-33	Rhizotomy right, Jan. 5	5.09	2	" " "	12	"
6-52	Rhizotomy right, Jan. 27	5.20	2	" " "	—	None. Killed, 24th day*
6-43	Olfactory neurectomy, Jan. 14	5.23	2	" " "	8	Severe
6-53	None, control	5.31	2	" " "	—	Remained well, no paralysis

Virus: 10 per cent No. 6-27 Wfd. strain; generation X; harvested Jan. 4, 1937. (See Experiment 1.)

* Tuberculosis also.

the possibility of accidental contamination of our stock virus. For these tests 3 convalescent monkeys, paralyzed by 2 heterologous poliomyelitic strains, were inoculated intracerebrally together with

appropriate controls for dosage. 3 rabbits and 2 guinea pigs were inoculated into brain, eye, skin, and peritoneal cavity, and 5 Swiss mice were inoculated intracerebrally. The result of the tests for purity gave no evidence of a contamination. The animals were observed for 4 weeks and 1 of the 3 convalescent monkeys remained well, 1 developed fever and no paralysis, and 1 had fever and slight

TABLE III

Experiment 3. Intravenous Inoculation; Intracutaneous Inoculation in Denervated Limb

Mar. 23, 1937.

Monkey		Inoculation				Result	
No.	Preparation	Time	Dose		Route	Incubation period	Paralysis
		p.m.	*cc.*	*per cent*		*days*	
7-04	None	3.44	2	10	Vein	5	Severe*
7-15	"	3.49	2	10	"	11	Mild
7-12	"	3.57	0.5	0.05	Brain	6	Moderate
7-01	"	3.58	0.5	0.05	"	11	"
7-03	"	4.05	2	10	"	4	Severe, purulent meningitis, died
7-14	"	4.08	2	10	"	4	Moderate
7-17	"	4.15	2	10	Skin of flank	—	Remained well, no paralysis
7-06	"	4.17	2	10	" " "	—	" "
7-22	Partial section thigh	4.34	2	10	Skin of calf	11	Severe
7-10	None	4.37	2	10	" " "	4	None, fever only

Virus: 10 per cent No. 6-39; Wfd. strain; generation XI; harvested Feb. 3, 1937. (See Experiment 2.)

* Tuberculosis also.

paralysis. The 3 rabbits and 2 guinea pigs remained well. 4 of the 5 mice remained well and 1 died on the 23rd day. We gave little weight to this mouse because all of 6 Swiss mice survived another intracerebral test with the Wfd. strain. (See Appendix, Experiment A.)

The rest of the experiment is presented in Table III, where it may be seen that the intracerebral infectivity of the virus was considerable. The 2 cc. dose of 10 per cent cord was infective by vein in each of 2

animals, and this dose failed to induce paralysis by the intracutaneous route in all 3 normal monkeys, although 1 of them (7-10) had fever on the 5th to 8th days. However, in No. 7-22, prepared by partial section of the thigh, intracutaneous inoculation in the denervated calf led to severe poliomyelitis. The notes for 7-22 follow.

No. 7-22. *Preparation*: Mar. 23, 1937, 3.00 to 4.30 p.m. Under nembutal anesthesia all the skin, fascia, and muscles were divided in the left mid-thigh down to the femur. The sciatic, femoral, and all other nerves were divided. Only femur, femoral artery, and vein were left intact. All tissue about the femoral artery and vein was stripped clear for about 1.5 cm. The periosteum was divided and scraped from the bone for about 1 cm., completely circumscribing the femur. The muscles were approximated by interrupted mattress suture of silk. The fascia was closed by continuous silk, and a continuous stitch of silk to the skin completed the closure. *Inoculation*: Mar. 23, 4.34 p.m., 2 cc. dose intracutaneously in 10 piqûres in calf of left leg. *Result*: Apr. 3, fever. Apr. 4, tremor, no fever, paralysis of both legs. Apr. 7, prostrate, temperature 95.6°F. Apr. 9, cold, killed. *Autopsy*: Extensive lesions in medulla and moderate lesions in cord. Subcutaneous staphylococcal abscess of site of operation. *Diagnosis*: Severe poliomyelitis.[2]

Experiment 4. Intravenous Inoculation after Olfactory Neurectomy; Intracutaneous Inoculation in Skin Flap; Intracutaneous Inoculation in Denervated Leg; Test of Virus in Convalescent Monkey.—Experiment 4 was planned to repeat some of the previous ones (partial section of thigh and skin flap), and to see if bilateral olfactory neurectomy would prevent infection following intravenous inoculation.

Experiment 4 is shown in Table IV and the protocols are in the Appendix. Two controls, Nos. 7-42 and 7-44, remained well after intravenous inoculation. Thus the intravenous infectivity of the strain appeared to be less than in Experiment 3. A similar decrease in normal intracutaneous infectivity from that originally described (3), was noted in Experiments 1, 2, and 3.

In contrast to the negative results in normal animals (7-42 and 7-44), Nos. 6-69 and 6-81, prepared by bilateral olfactory neurectomy, developed mild poliomyelitis after intravenous inoculation. Severe

[2] Lesions mean: destruction of ganglion cells, focal accumulations of glial cells, and perivascular cuffing with mononuclear cells.

The estimation of severity of the experimental disease was based on a summation of paralysis, postcritical drop of temperature, and histological findings.

poliomyelitis developed in Nos. 6-97 and 7-21, inoculated in the prepared skin flap, and in 7-45, inoculated intracutaneously in the denervated leg. The immunity of the old convalescent, No. 4-64, is another indication that we were using the virus of poliomyelitis.

The results of intracutaneous and intravenous inoculations in Experiments 1 to 4, together with the results of one preliminary experiment, are summarized in Table V. Of the 19 animals prepared

TABLE IV

Experiment 4. Intravenous Inoculation after Olfactory Neurectomy; Intracutaneous Inoculation in Skin Flap; Intracutaneous Inoculation in Denervated Leg; Test of Virus in Convalescent Monkey

Apr. 14, 1937.

Monkey		Inoculation				Result	
No.	Preparation	Time	Dose		Route	Incubation period	Paralysis
		p.m.	*cc.*	*per cent*		*days*	
6-81	Olfactory neurectomy	3.40	2	10	Vein	4	Mild*
6-69	" "	3.43	2	10	"	5	Mild
7-42	None	3.46	2	10	"	—	Remained well, no paralysis
7-44	"	3.53	2	10	"	—	" "
7-21	Skin flap	4.04	2	10	Skin flap	5	Severe
6-97	" "	4.07	2	10	" "	5	"
7-45	Partial section thigh	4.10	2	10	Skin calf	17	"
7-41	None	4.15	0.5	0.05	Brain	9	Moderate
4-64	Convalescent Flexner 9 mos.	4.17	0.5	0.05	"	—	None

Virus: 10 per cent No. 7-04; Wfd. strain; generation XII; harvested Mar. 30, 1937. (See Experiment 3.)

* Tuberculosis also.

by some form of denervation, 17 developed paralysis after intracutaneous inoculation, while none of the 7 normal controls showed paralysis and only 2 had fever. Accordingly, Experiment 5 was planned to see if denervation by the skin flap method would be effective with other strains of poliomyelitic virus.

Experiment 5. Other Strains of Virus in Skin Flaps.—Skin flaps were elevated in two stages in 10 monkeys and they were used in pairs

for intracutaneous inoculation with 5 other strains (McC.; We.; Flexner; Park; and Aycock) previously compared with the Wfd. strain (1, 7). Fever without paralysis and without lesions in cord or medulla was seen 4 times. A definite positive result was obtained but once; in 1 of 2 monkeys inoculated in the skin flap with the McC. strain (11). This was the first time this strain had been infective by the skin although tests had been made 3 times in previous passages in normal skin. 5 control monkeys inoculated into normal skin with these 5 strains respectively remained well, and the 5 controls inoculated intracerebrally developed poliomyelitis. The scant success just

TABLE V

Summary

Preparation	Total	Paralysis	Fever	Negative
A. Intracutaneous Inoculation				
None	7		2	5
Laminectomy	1	1		
Rhizotomy	9	7		2
Skin flap	4	4		
Bilateral olfactory neurectomy	2	2		
Bilateral olfactory neurectomy and rhizotomy	1	1		
Partial section thigh	2	2		
B. Intravenous Inoculation				
None	4	2		2
Bilateral olfactory neurectomy	2	2		

described with the more extended use of the skin flap induced us to simplify the procedure.

Experiment 6. Recovery of Virus (McL.) from Human Cord by Intracutaneous Inoculation.—In Experiment 6, 2 monkeys were prepared by elevating the skin flap in one stage, and virus which had not yet been subjected to animal passage was used as the inoculum. The operation consisted of merely the first stage procedure for isolation of an area of skin. The source of virus was a 10 per cent suspension of glycerolated cord from a child (McL.) dead on the 5th day of bulbar poliomyelitis in Toronto, Ontario, in August, 1937. The experiment is shown in Table VI. 5 monkeys were used: 1 for intracerebral and intraperitoneal inoculations and 4 for intracutaneous inoculations. Among the

last, 2 were inoculated into normal skin and 2 into skin flaps. The experimental disease was mild, but it was most marked in No. 8-07, which had a skin flap. From the spinal cord of this monkey the strain was successfully passed to its fourth generation by intracerebral and intraperitoneal inoculations.

It is to be noted that there was a failure in one of the prepared animals, No. 8-06. The explanation for this is not clear, although this skin flap was swollen and fluctuant.

TABLE VI

Experiment 6. Recovery of Virus (McL.) from Human Cord by Intracutaneous Inoculation in Monkey

Sept. 13, 1937.

Generation	Monkey		Inoculation			Result	
	No.	Preparation	Time	Dose	Route	Incubation period	Paralysis
			p.m.	*cc.*		*days*	
I	8-10	None	3.14	2	Skin	13	Mild
	8-09	"	3.23	7	Brain and abdomen	7	Moderate
	8-08	"	3.26	2	Skin	12	Mild
	8-06	Skin flap, Sept. 13	3.40	2	Skin flap	—	Remained well, no poliomyelitis
	8-07	" " " "	3.43	2	" "	7	Moderate
			Subsequent Passages				
II	8-07	→ 8-16			Typical poliomyelitis		
III	8-16	→ 8-23			" "		
IV	8-23	→ 8-29			" "		

Virus: Generation I, 10 per cent human cord; later generations, 10 per cent monkey cord inoculated intracerebrally and intraperitoneally.

DISCUSSION

The denervation procedures not only failed to prevent the development of typical experimental poliomyelitis following intracutaneous inoculation into the denervated area, but, in most cases, resulted in an increased susceptibility. Thus, the results must be considered both from the negative and positive aspects. The former relates to the question of strict neurotropism or axonal spread while the latter may be considered in terms of altered neural resistance to the virus.

The various methods of denervation were developed during the course of the experiments in an attempt to establish, if possible, a completely denervated area of skin for intracutaneous inoculation. The failure of the early rhizotomies to prevent infection suggested that the virus might be propagated over the autonomic system from skin to spinal cord. Denervation by means of an isolated area of skin (two stage skin flap) was, therefore, employed. When severe infection occurred with this method, the possibility of autonomic fibers, carried into the isolated area by proliferating blood vessels, was considered. The denervated limb experiments were designed to eliminate this factor. A critical analysis of this procedure leaves no doubt that the somatic nerves were divided; the perivascular sympathectomy was as complete as possible, but the chance retention of one or two intact filaments cannot be disproven. However, this point is probably not as important as might appear on preliminary consideration. In the first place, it is extremely unlikely that the perivascular sympathetic fibers at the mid-thigh level have any neural connection with the site of inoculation in the skin of the calf (12). Secondly, it is evident that intact neural communications, if not completely absent, were at least decreased to an infinitesimal fraction of their normal number. Finally, in striking contrast to the severe experimental disease in the denervated animals, the controls, inoculated in the same site, remained well. Further evidence in support of this thesis is found in the results of intravenous inoculations, which demonstrated the possibility of hematogenous transport of virus; so that from any locus with blood supply the virus could readily reach intact nerves.

However, a certain neural pattern of paralyses indicated a considerable neurotropic tendency of the virus. In 10 of 11 instances where the point could be determined in intracutaneous inoculations, the first limb affected was on the side of the inoculation. The simultaneous onset of fever and paralysis in 4 of 6 monkeys, prepared by skin flaps, is reminiscent of Hurst's (14) results with sciatic inoculations. The failure of bilateral olfactory neurectomy to prevent infection after intracutaneous inoculation is not directly contradictory to the findings of Brodie and Elvidge (15) or Schultz and Gebhardt (16), who employed intranasal inoculations. The successful intravenous inoculations after olfactory neurectomy (Experiment 4) are partially at variance with the experience of Lennette and Hudson

(17), using another strain. However, their failure to infect on intravenous inoculation was preceded by an intranasal (perhaps immunizing) test.

The positive factor, enhanced susceptibility after denervation procedures, was a striking, if rather surprising, result in 17 of the 19 animals tested with the Wfd. strain. The interpretation of this phenomenon rests, at present, chiefly upon a theoretical basis. Alteration of neural resistance probably occurs at the site of nerve injury and as far centrally as the corresponding ganglion cells in the cord. Thus, Webster (18) found that rabies may be localized in the medulla by a mere prick of the tongue in mice. In addition, an abnormal neurovascular relationship is certainly present during the early period at the site of nerve section. Peripheral vascular dilatation in the denervated area undoubtedly resulted from all denervation operations (13) except the olfactory neurectomies. This factor may be of importance when considered in the light of the intravenous infectivity of this strain of virus. Finally, certain of the procedures might disturb the blood brain barrier (19).

Another factor was hypothermia which followed some of the operations and which might have been of importance twice. In view of this and the fact that Wolf (20) found that experimental poliomyelitis could be aborted by hyperthermia, one might question the interpretation of Dalldorf, Douglass, and Robinson (21) when they discount the rôle of fever in the sparing action of dog distemper in experimental poliomyelitis.

Other investigators, notably Flexner and Clark (22), Hurst (14), and Toomey (23), have described methods of increasing susceptibility to experimental poliomyelitis. The denervations acted in this rôle. The degree of increased susceptibility may be appreciated from the fact that on several occasions equal or smaller doses of virus led to a more severe disease on intracutaneous than on intracerebral inoculation.

The skin flaps had the obvious advantage of simplicity and were effective in all of 4 trials with the Wfd. strain. However, with the 6 other strains the method was effective only twice in 12 trials, and this raises considerable doubt concerning the general usefulness of the procedure. Nevertheless, the results of the whole series of operations

show that susceptibility to cutaneous infection can be enhanced. This observation is of considerable interest in view of its possible relation to poliomyelitis in man following tonsillectomy (24) and following subcutaneous inoculations of the virus (25).

CONCLUSIONS

1. Bilateral olfactory neurectomy did not prevent experimental poliomyelitis on intravenous or intracutaneous inoculation.

2. Various operative procedures increased the susceptibility of monkeys to infection with experimental poliomyelitis.

APPENDIX

Preliminary Experiments

Experiment A.—Nov. 13, 1936. *Source of virus*: 10 per cent Wfd. No. 5-92, generation X, harvested Oct. 30, 1936.

Monkey 4-92. *Preparation*: May 8, 1936. Laminectomy; anterior and posterior rhizotomy of spinal roots dorsal 8 to lumbar 2, right. *Inoculation*: Nov. 13, 1936, 2.58 p.m., 2 cc. dose intracutaneously in 10 piqûres in skin of normal (left) flank. *Result*: Nov. 23, fever. Nov. 25, hobbles, weakness of four limbs. Nov. 30, better, killed. *Autopsy*: Moderate lesions in medulla and cord. Rhizotomy verified. *Diagnosis*: Mild poliomyelitis.

No. 6-12. *Preparation*: None. *Inoculation*: Nov. 13, 1936, 3.04 p.m., 2 cc. dose intracerebrally and 9.5 cc. dose intraperitoneally. Nov. 19, fresh supply of virus made up and dose repeated, 2 cc. intracerebrally and 14 cc. intraperitoneally. To test the purity of the virus intracranial inoculation of 6 Swiss mice with 0.03 cc. was done. *Result in No. 6-12*: Nov. 21, fever, Nov. 25, weakness of four limbs, Nov. 30, recovering, killed. *Autopsy*: Moderate lesions in medulla, cervical and lumbar levels of cord; dorsal cord negative. *Diagnosis*: Mild poliomyelitis. *Result in Swiss mice*: All remained well 4 weeks and were discarded.

Experiment B.—Dec. 15, 1936. Nembutal anesthesia. *Source of virus*: 10 per cent Wfd. No. 4-53, generation IX, harvested Apr. 11, 1936.

No. 4-85. *Preparation*: Apr. 24, 1936. Laminectomy; anterior and posterior rhizotomy D. 8 to L. 2, right. *Inoculation*: Dec. 15, 2.44 p.m., 2 cc. dose intracutaneously in 10 piqûres in right flank supposedly in denervated area. *Result*: Temperature 96–97°F. during incubation period. Dec. 20, fever, Dec. 21, paralysis right arm, Dec. 23, prostrate, temperature 98°F., killed. *Autopsy*: Extensive lesions in medulla and cord. Rhizotomy verified. 2 or 3 piqûres of virus had been placed above denervated zone. *Diagnosis*: Severe poliomyelitis.

No. 6-26. *Preparation*: None. *Inoculation*: Dec. 15, 2.51 p.m., 2 cc. dose intracerebrally and 2 cc. dose intraperitoneally. Dec. 22, 2 cc. dose intracere-

brally repeated. *Result*: Remained well for 4 weeks, discarded. *Diagnosis*: No poliomyelitis.

No. 6-25. *Preparation*: None. *Inoculation*: Dec. 15, 2.54 p.m., 2 cc. dose intracerebrally and 2 cc. dose intraperitoneally. Dec. 22, 2 cc. dose intracerebrally. *Result*: Dec. 21, fever, Dec. 22, paralysis right arm, Dec. 23, prostrate, temperature 96.1°F., killed. *Autopsy*: Extensive lesions in medulla and cord. *Diagnosis*: Severe poliomyelitis.

Experiment 1.—Dec. 22, 1936. Nembutal anesthesia. *Source of virus*: 10 per cent Wfd. No. 4-53, generation IX, harvested Apr. 11, 1936.

No. 6-30. *Preparation:* None. *Inoculation*: Dec. 22, 3.33 p.m., 2 cc. dose intracutaneously in 10 piqûres in face and head. *Result:* Remained well.

No. 6-29. *Preparation*: None. *Inoculation*: Dec. 22, 3.40 p.m., 2 cc. dose intracutaneously in 10 piqûres in tail. *Result*: Remained well.

No. 6-27. *Preparation*: Dec. 22, 4.30 p.m. Laminectomy; anterior and posterior rhizotomy D. 9 to L. 2, right. *Inoculation*: Dec. 22, 5.50 p.m., 2 cc. dose intracutaneously in 10 piqûres in denervated area of right flank. *Result*: Dec. 28, fever. Jan. 3, 1937, limbs weak. Jan. 4, prostrate, temperature 93.1°F., killed. *Autopsy*: Extensive lesions in medulla and cord. Rhizotomy verified. *Diagnosis*: Severe poliomyelitis.

No. 6-28. *Preparation*: Dec. 22, 2.15 p.m. Laminectomy; anterior and posterior rhizotomy D. 9 to L. 2, right. *Inoculation*: Dec. 22, 6.03 p.m., 2 cc. dose intracutaneously in denervated area of right flank. *Result*: Jan. 2, 1937, fever. Jan. 3, tremor. Jan. 4, four limbs weak. Jan. 5, prostrate, temperature 93.5°F., killed. *Autopsy*: Extensive lesions in medulla and cord. Rhizotomy verified. *Diagnosis*: Severe poliomyelitis.

No. 6-32. *Preparation*: None. *Inoculation*: Dec. 22, 6.06 p.m., 2 cc. dose intracutaneously in right flank. *Result*: Dec. 29 to Jan. 4, fever without other signs. Jan. 19, killed. *Autopsy*: No lesions. *Diagnosis*: Questionable abortive poliomyelitis.

Experiment 2.—Nembutal anesthesia. *Source of virus*: 10 per cent Wfd. No. 6-27, generation X, harvested Jan. 4, 1937. (See Experiment 1.)

No. 6-40. *Preparation*: Jan. 12 and 21. Two stage elevation of skin flap in right flank over 8th to 10th ribs. *Inoculation*: Jan. 27, 3.25 p.m., 2 cc. dose intracutaneously in 10 piqûres in the skin flap. *Result*: Feb. 8, fever. Feb. 9, paralysis right arm. Feb. 11, prostrate, 94°F., killed. *Autopsy*: Moderate lesions in medulla and cord. Caseous tubercles in lungs, liver, and spleen. *Diagnosis*: Severe poliomyelitis; tuberculosis.

No. 6-39. *Preparation*: Jan. 12 and 21. Two stage elevation of skin flap in right flank, over 8th to 10th ribs. *Inoculation*: Jan. 27, 3.28 p.m., 2 cc. dose intracutaneously in 10 piqûres in the skin flap. *Result*: Feb. 1, fever, paralysis of right arm and leg, back weak. Feb. 3, prostrate, temperature below 92°F.,

killed. *Autopsy*: Extensive lesions in medulla and cord. *Diagnosis*: Severe poliomyelitis.

No. 6-50. *Preparation*: Jan. 25, laminectomy; extradural anterior and posterior rhizotomy, D. 7 to L. 1, right. *Inoculation*: Jan. 27, 3.43 p.m., 2 cc. dose intracutaneously in 10 piqûres in skin of denervated area of right flank. Feb. 1, fever ? (temperature has been irregular), paralysis of legs. Feb. 2, four extremities weak. Feb. 3, prostrate, temperature below 92°F. Feb. 4, found dead. *Autopsy*: Extensive lesions in medulla and cord. Rhizotomy verified. *Diagnosis*: Severe poliomyelitis, fatal.

No. 6-47. *Preparation:* Olfactory neurectomy and rhizotomy. Jan. 20, bilateral olfactory neurectomy. Jan. 26, laminectomy, intradural anterior and posterior rhizotomy, D. 7 to L. 1, right. *Inoculation*: Jan. 27, 3.49 p.m., 2 cc. dose intracutaneously in 10 piqûres in denervated area of right flank. *Result*: Irregular fever since first operation. Feb. 2, agitation, tremor. Feb. 3, paralysis of right arm. Feb. 4, prostrate, temperature below 92°F. Feb. 6, found dead. *Autopsy*: Mild lesions in medulla and cervical cord, lumbar cord negative. Antemortem rupture of esophagus with gastric contents in abdominal and thoracic cavities. Rhizotomy verified. One tiny filament of left olfactory nerve intact. *Diagnosis*: Mild poliomyelitis; rupture of esophagus.

Nos. 6-54 and 6-55. *Preparation*: None. Included for intracranial inoculation of 1/4th and 1/40th of intracutaneous dose. No. 6-54. *Inoculation*: Jan. 27, 3.57 p.m., 0.5 cc. dose into left cerebral hemisphere. *Result*: Jan. 30, fever. Feb. 5, agitation and tremor. Feb. 9, paralysis of left leg. Feb. 13, both legs weak, killed, lowest temperature 103°F. *Autopsy*: Mild lesions in medulla, cervical, and dorsal cord; moderate lesions in lumbar cord. *Diagnosis*: Moderate poliomyelitis. No. 6-55. *Inoculation*: Jan. 27, 4.08 p.m., 0.5 cc. dose 1 per cent virus into left cerebral hemisphere. *Result*: Jan. 30, onset of irregular fever. Feb. 7, tremor, paralysis of left face and right arm, weakness of left arm. Feb. 9, prostrate, temperature 98.5°F. Feb. 10, killed. *Autopsy*: Mild lesions in medulla and dorsal cord, extensive lesions in cervical and lumbar cord. Caseous tubercles in lungs, liver, spleen, and mesenteric lymph nodes. *Diagnosis*: Moderate to severe poliomyelitis; tuberculosis.

No. 6-44. *Preparation*: Jan. 15, bilateral olfactory neurectomy. Jan. 17, animal sick, incision infected. Jan. 21, better. *Inoculation*: Jan. 27, 4.39 p.m., 2 cc. dose intracutaneously in 10 piqûres in right flank. *Result*: Feb. 3, fever. Feb. 4, tremor, ataxia, right facial paralysis. Feb. 5, paralysis of left arm. Feb. 7, prostrate, temperature 96.2°F. Feb. 9, worse, killed. *Autopsy*: Extensive lesions in medulla and cord. Verified bilateral olfactory neurectomy. *Diagnosis*: Severe poliomyelitis.

No. 6-37. *Preparation*: Jan. 7, laminectomy. D. 7 to L. 1, dura opened but roots were not disturbed; incision in mid-line. *Inoculation*: Jan. 27, 4.49 p.m., 2 cc. dose intracutaneously in 10 piqûres in right flank. *Result*: Feb. 4, fever, agitation, tremor. Feb. 5, paralysis both legs. Feb. 6, prostrate, temperature

101°F. Feb. 11, same, temperature 99.3°F., killed. *Autopsy*: Moderate lesions in medulla, dorsal and lumbar cord; extensive lesions in cervical cord. Verified laminectomy. *Diagnosis*: Severe poliomyelitis.

No. 6-36. *Preparation*: Jan. 6, laminectomy and intradural rhizotomy of anterior and posterior roots, D. 7 to L. 1, right. *Inoculation*: Jan. 27, 4.50 p.m., 2 cc. dose intracutaneously in 10 piqûres in left (contralateral) flank. *Result*: Feb. 8 to 12 and on 22, irregular fever. No paralysis. Feb. 26, sick, killed. *Autopsy*: No lesions in medulla or cord. Many caseous tubercles in lungs, liver, and spleen. Verified rhizotomy. *Diagnosis*: No poliomyelitis; tuberculosis.

No. 6-38. *Preparation*: Jan. 8, laminectomy and intradural anterior and posterior rhizotomy, D. 6 to D. 12, right. *Inoculation*: Jan. 27, 5.05 p.m., 2 cc. dose intracutaneously in 10 piqûres in denervated area of right flank. *Result*: Feb. 3, fever, agitation, tremor, left facial paralysis. Feb. 4, legs weak. Feb. 6, prostrate. Feb. 10, temperature 97°F., killed. *Autopsy*: Moderate lesions in medulla and dorsal cord; extensive lesions in cervical and lumbar cord. Rhizotomy verified. *Diagnosis*: Severe poliomyelitis.

No. 6-33. *Preparation*: Jan. 5. Laminectomy and intradural anterior and posterior rhizotomy, D. 7 to L. 1, right. *Inoculation*: Jan. 27, 5.09 p.m., 2 cc. dose intracutaneously in 10 piqûres in denervated area of right flank. *Result*: Feb. 8, fever, right facial paralysis. Feb. 9, tremor. Feb. 10, paralysis of right leg, weakness of arms and left leg. Feb. 12, prostrate, temperature 101.3°F., killed. *Autopsy*: Extensive lesions in medulla and lumbar cord; moderate lesions in dorsal cord; cervical cord not saved. At site of laminectomy some attachments present at roots thought to have been divided. It was not clear whether the nerve roots had not been completely divided, or whether the attachments were mere fibrous adhesions. *Diagnosis*: Moderate to severe poliomyelitis.

No. 6-52. *Preparation*: Jan. 27, 2 p.m., laminectomy and intradural anterior and posterior rhizotomy, D. 7 to L. 1, right. *Inoculation*: Jan. 27, 5.20 p.m., 2 cc. dose intracutaneously in 10 piqûres in denervated area in right flank. *Result*: Jan. 31 to Feb. 12, fever 104.2–105.5°F. Feb. 19, emaciated, temperature less than 94°F., killed. *Autopsy*: No lesions in cord, extensive generalized caseous tubercles. *Diagnosis*: No poliomyelitis; tuberculosis. Rhizotomy verified.

No. 6-43. *Preparation*: Jan. 14, bilateral olfactory neurectomy. *Inoculation*: Jan. 27, 5.23 p.m., 2 cc. dose intracutaneously in 10 piqûres in right flank. *Result*: Feb. 4, fever. Feb. 5, left facial paralysis. Feb. 6, right arm weak. Feb. 8, prostrate, temperature 100°F., Feb. 9, worse, temperature 98°F., killed. *Autopsy*: Extensive lesions in medulla and cord; complete section of olfactory nerves verified. *Diagnosis*: Severe poliomyelitis.

No. 6-53. *Preparation*: None. *Inoculation*: Jan. 27, 5.31 p.m., 2 cc. dose intracutaneously in 10 piqûres in right flank. *Result*: Remained well. Mar. 1, killed for histological examination. *Autopsy*: No lesions in medulla or cord. *Diagnosis*: No poliomyelitis.

Experiment 3.—See text.

Experiment 4.—Apr. 14, 1937. Nembutal anesthesia. *Source of virus*: 10 per cent Wfd. No. 7-04, generation XII, harvested Mar. 30, 1937. (See Table III.)

No. 6-81. *Preparation*: Mar. 19, 1937. Bilateral olfactory neurectomy. *Inoculation*: Apr. 14, 3.40 p.m., 2 cc. dose intravenously. *Result*: Apr. 18, 106°F. Apr. 21, 106.5°F., agitation, tremor. Irregular fever continued to Apr. 29. Weak and sick thereafter. May 7, 103.5°F., killed. *Autopsy*: Mild lesions in medulla and lumbar cord, none in cervical or dorsal cord; caseous tubercles in lungs, spleen, and liver; bilateral olfactory neurectomy verified. *Diagnosis*: (*a*) Tuberculosis; (*b*) mild poliomyelitis.

No. 6-69. *Preparation*: Feb. 15, 1937, bilateral olfactory neurectomy. *Inoculation*: Apr. 14, 3.43 p.m., 2 cc. dose intravenously. *Result*: Apr. 19, fever, agitation, tremor. Apr. 24, fever down, weakness of legs. May 7, same, killed. *Autopsy*: Mild lesions in medulla, cervical and lumbar cord; none in dorsal cord. Bilateral olfactory neurectomy verified. *Diagnosis*: Mild poliomyelitis.

No. 7-42. *Preparation*: None. *Inoculation*: Apr. 14, 3.46 p.m., 2 cc. dose intravenously. *Result*: Remained well.

No. 7-44. *Preparation*: None. *Inoculation*: Apr. 14, 3.53 p.m., 2 cc. dose intravenously. *Result*: Remained well.

No. 7-21. *Preparation*: Two stage elevation of skin flap in left flank, Mar. 24 and Apr. 7. *Inoculation*: Apr. 14, 4.04 p.m., 2 cc. dose intracutaneously in 10 piqûres in skin flap. *Result*: Apr. 19, fever, paralysis left arm. Apr. 21, prostrate, temperature 99.3°F., killed. *Autopsy*: Extensive lesions in medulla and cord. *Diagnosis*: Severe poliomyelitis.

No. 6-97. *Preparation*: Two stage elevation of skin flap in left flank, Mar. 24 and Apr. 7. *Inoculation*: Apr. 14, 4.07 p.m., 2 cc. dose intracutaneously in 10 piqûres in skin flap. *Result*: Apr. 19, fever, tremor, paralysis of left arm. Apr. 22, prostrate, temperature 96.5°F. Apr. 26, dead. *Autopsy*: Extensive lesions in medulla and cord. *Diagnosis*: Severe poliomyelitis, fatal.

No. 7-45. *Preparation*: Apr. 14, 11 a.m., partial section of left leg at midthigh, as in No. 7-22. *Inoculation*: Apr. 14, 4.10 p.m., 2 cc. dose intracutaneously in 10 piqûres in calf of left leg. *Result*: May 1, fever, tremor. May 2, fever, paralysis of both legs. May 4, prostrate, cold. May 6, same, killed. *Autopsy*: Extensive lesions medulla and cord. *Diagnosis*: Severe poliomyelitis.

No. 7-41. *Preparation*: None. Inoculation: Apr. 14, 4.15 p.m., 0.5 cc. dose of 0.05 per cent virus intracerebrally, left. *Result*: Apr. 23, fever, tremor; Apr. 24, weakness of four legs. May 3, better. May 11, discharged. *Diagnosis*: Moderate poliomyelitis.

No. 4-64. *Preparation*: Poliomyelitis, June, 1936, following intracerebral inoculation of Flexner strain (7). *Inoculation*: Apr. 14, 4.17 p.m., 0.5 cc. dose of 0.05 per cent virus intracerebrally, left. *Result*: Remained well for 4 weeks,

later sick, died June 21. *Autopsy*: Old lesions, loss of ganglion cells in cervical and lumbar cord. No recent lesions; generalized caseous tubercles. *Diagnosis*: (*a*) Immune in test of Apr. 14; (*b*) tuberculosis.

Experiment 5.—See text.

Experiment 6.—Sept. 13, 1937. Nembutal anesthesia, except in No. 8-10. *Source of virus*: McL. strain; 10 per cent glycerolated human cord.

No. 8-10. *Preparation*: None. *Inoculation*: Sept. 13, 3.14 p.m., 2 cc. dose intracutaneously in 10 piqûres in right flank. Kept under dial Sept. 13 to 15. *Result*: Sept. 26, clumsy. Sept. 27, fever, paralysis right leg. Sept. 30, both legs weak. Oct. 4, same, lowest temperature 103°F., killed. *Autopsy*: Moderate lesions in medulla, cervical and lumbar cord; none seen in dorsal cord. *Diagnosis*: Mild poliomyelitis.

No. 8-09. *Preparation*: None. *Inoculation*: Sept. 13, 3.23 p.m., 2 cc. dose into left cerebral hemisphere and 5 cc. dose intraperitoneally. *Result*: Sept. 20, fever. Sept. 22, tremor. Sept. 27, right hand weak. Oct. 2, paralysis right hand, legs weak, killed. *Autopsy*: Extensive lesions in cervical cord; moderate lesions in medulla and dorsal cord; lumbar cord not saved. *Diagnosis*: Moderate poliomyelitis.

No. 8-08. *Preparation*: None. *Inoculation*: Sept. 13, 3.26 p.m., 2 cc. dose intracutaneously in 10 piqûres in right flank. Sept. 25, fever, tremor. Sept. 27, legs weak. Oct. 1, better; lowest temperature has been 103°F. Oct. 4, killed. *Autopsy*: Moderate lesions in cervical and lumbar cord; none in dorsal cord; medulla not saved. *Diagnosis*: Mild poliomyelitis.

No. 8-06. *Preparation*: Sept. 13, 2 p.m., skin flap elevated in right flank in one stage. *Inoculation*: Sept. 13, 3.40 p.m., 2 cc. dose intracutaneously in 10 piqûres in skin flap. *Result*: Sept. 14, flap puffy, fluctuant, and oozing. Remained well 4 weeks. *Diagnosis*: No poliomyelitis.

No. 8-07. *Preparation*: Sept. 13, 2.45 p.m., skin flap elevated in right flank in one stage. *Inoculation*: Sept. 13, 3.43 p.m., 2 cc. dose intracutaneously in 10 piqûres in skin flap. *Result*: Sept. 20, fever. Sept. 22, paralysis right leg. Sept. 23, paralysis both legs, weakness of arms and back; temperature 103°F., killed to harvest virus. *Autopsy*: Extensive lesions in cervical and lumbar cord; moderate lesions in medulla and dorsal cord. *Diagnosis*: Moderate poliomyelitis.

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