the colored sheath cells take origin from the stained neural crest as do the ganglia or whether they originate from the dorsal part of the definitive cord *per se,* which also possessed some blue stain. If the sheath cells take origin from the ventral portion of the cord and migrate out by way of the ventral roots, then sheath cells possessing the natural unstained brownish pigment should be seen. Their absence in such preparations is evidence that they do not originate from this source in early embryos. That they could not have migrated out by way of the dorsal roots is also obvious from the fact that the embryos were studied prior to the development of sensory roots.

In spite of the want of definite proof there are some indications of a common origin (neural crest) for spinal ganglion cells and the early sheath cells. This is based on evidence of a lateral and ventral migration of stained crest cells across the mid-dorsal line indicating that spinal ganglion cells and the sheath cells on one side may have their origin, at least in part, from the contralateral stained crest. It is hoped that experiments which are in progress will yield more decisive results. The evidence so far obtained by this method does not lend any support to the view that the early migrating sheath cells take origin from the ventral or lateral portions of the spinal cord. Further, it does not disprove that the sheath cells in later stages may come from this source.

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Virus Isolated from Nasal Washings during Acute Poliomyelitis in New York City in 1935.*

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The virus of poliomyelitis has been infrequently detected in the nasal secretions of patients during and following an attack of poliomyelitis. We have reviewed the literature and considered that until the summer of 1935 when this study was begun, the virus had been isolated from human nasopharyngeal secretions only 11 times.[1-7]

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1 Kling, C., Pettersson, A., and Wernstedt, W., *Communications de l'Institut Medical de l'État a Stockholm,* Tome III, 1912.

The criteria which we used to determine the presence of virus were: (a) the transmission of the clinical disease to monkeys, *viz.*, fever, tremor, ataxia and paralysis, (b) the typical histopathological changes in the spinal cord and medulla, *viz.*, perivascular and interstitial infiltration and neuronophagia, particularly of the gray matter in the anterior horns, and (c) the serial passage of the disease to other monkeys. The above requisites were present in all isolations except those of Kling[1] in which 2 of the 3 requirements were met. Since then, Kramer[8] has recorded the isolation of the virus from 2 additional cases. Our report describes a fourteenth instance (Table I.)

TABLE I.
Summary of Isolations of Virus from Human Nasopharyngeal Secretions in Poliomyelitis.

Investigators	Type of Case	Day of Illness Virus Isolated
Kling and co-workers[1]	Case 10 paralytic	4
	" 16 "	4
	" 22 "	4
Dubois and co-workers[2]	Non-paralytic	17
Taylor and Amoss[3]	Abortive	4
	Paralytic	5 days before onset of paralysis
Lucas and Osgood[4]	"	4 months after onset of second attack
Levaditi and Willemin[5]	"	8
Paul and Trask[6]	Abortive	1
	"	1
Paul and co-workers[7]	"	1
Kramer and co-workers[8]	Paralytic	16
	"	13
Stillerman and Brodie	"	9

An opportunity to test nasopharyngeal secretions for the virus presented itself at the Willard Parker Hospital during the 1935 New York City poliomyelitis outbreak. With a view of determining how long the virus survived in the nasopharynx, serial nasal washings from 15 patients, 9 paralytics and 6 non-paralytics, were

2 Dubois, P. L., Neal, J. B., and Zingher, A., *J. A. M. A.*, 1914, **62**, 19.

3 Taylor, E., and Amoss, H. L., *J. Exp. Med.*, 1917, **26**, 745.

4 Lucas, W. P., and Osgood, R. B., *J. A. M. A.*, 1930, **60**, 1611.

5 Levaditi, C., and Willemin, L., *Ann. Inst. Past.*, 1931, **46**, 233.

6 Paul, J. R., and Trask, J. D., *J. Exp. Med.*, 1932, **56**, 319.

7 Paul, J. R., Trask, J. D., and Webster, L. T., *J. Exp. Med.*, 1935, **62**, 245.

8 Kramer, S. D., Sobel, A. E., Grossman, L. H., and Hoskwith, B., *J. Exp. Med.*, 1936, **64**, 173.

tested. The washings were obtained at weekly intervals up to the 3rd, and in some cases during the 4th and 5th weeks of the disease. Although several nasal washings were tested from each of these 15 patients, the virus was demonstrated in only one specimen.

The nasal washings were obtained with the patient lying on one side or in the sitting position with the head lowered. A soft rubber bulb syringe was inserted into one nostril and the superior portion of the nasal passage irrigated with 60-75 cc. of sterile distilled water or saline. The drippings were caught in a basin and then used to flush the other nostril. This was repeated several times. The washings were kept frozen until filtered. A Seitz filter with a single pad was used. The filtrate was concentrated *in vacuo* at 35°-38°C. until the volume was reduced to 2-4 cc. This required 3-5 hours. The sterility of the filtrate was tested by culture and by inoculation into mice.

Each concentrated filtrate was inoculated into a *Macacus rhesus* monkey, 1 cc. intracerebrally and the remainder intraperitoneally. These animals were examined daily and the temperature recorded. Whenever symptoms and signs indicative of poliomyelitis were manifest, the animal was sacrificed for histopathological study and animal passage.

Poliomyelitis virus was recovered from only one patient. This was a 9-year-old girl, who for one week had had a cold which did not improve. On October 5, 1935, she developed headache, vomiting, and fever. The following day when she was admitted to the hospital, her temperature was 103.2°F. and a weakness of the right side of her face was found, which persisted until her discharge from the hospital 19 days later. Blood-tinged spinal fluid (traumatic) was obtained. A diagnosis of bulbar poliomyelitis was made. The nasal passages were irrigated 4, 9, 15, and 20 days, and 5 weeks after the onset of her illness.

The specimen obtained from this bulbar case on the 9th day of illness, produced poliomyelitis in a monkey. The animal developed the typical experimental disease with flaccid paralysis which progressed to a complete quadriplegia 8 days after inoculation. An emulsion made from the spinal cord of this monkey, produced paralysis on the 6th day in a second animal. In the 3rd passage, 10 of 11 monkeys developed extensive paralysis in from 6 to 14 days. The histological sections of the first passage were lost but those obtained from monkeys of the 2nd and 3rd passages revealed the characteristic lesions of experimental poliomyelitis.

The virus isolated from this patient appeared to be highly viru-

lent, for 1 cc. of a 5% suspension of the spinal cord obtained in the second passage, diluted 200 times, produced infection. Neutralization tests with this virus and a passage virus (FL strain) were done on sera obtained from patients during the acute and convalescent stages of the disease in 1935. In these experiments, both strains of virus reacted similarly to 25 sera, 6 neutralizing and 19 failing to neutralize each strain.[9] These results differed from those of Howitt[10] and Paul and Trask,[11] who found that human convalescent sera more often neutralized a recently isolated human strain than a passage strain. The above mentioned investigators may have obtained neutralization more often against their recently isolated strains because they were less virulent than the passage strains. An attempt to reinfect 6 monkeys having residual paralysis caused by the FL strain, using our 1935 strain, resulted in one definite and one questionable reinfection.[9]

The virus was not isolated from any nasal washing other than that obtained on the 9th day of illness from this patient. Whether it was absent in the other specimens, or was inactivated by the natural neutralizing property of nasal secretions,[12] or was not demonstrated because of the technical difficulties inherent in the method employed, is difficult to state. Some of the virus might have been lost during the dilution of the nasal secretions by irrigation, and the sterilization of the nasal washings by filtration. The use of a single monkey for testing each specimen may be inadequate. It is interesting to note that the virus has never been isolated more than once from the same case of poliomyelitis by any investigator.† Flexner and Amoss[13] demonstrated the presence of the virus in tonsils and pharyngeal tissue of 5 of 10 human beings that died of poliomyelitis during the first 7 days of their infection, but were unable to detect it in 4 fatal cases at later periods of the disease.

Washings obtained from 2 paralytic cases on the 4th day of illness, and a non-paralytic on the 2nd day, were followed by what appeared to be experimental poliomyelitis with flaccid paralysis. However, in neither instance was the diagnosis substantiated by histo-pathological examination or monkey passage.

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9 Brodie, M., Fischer, A. E., and Stillerman, M., *J. Clin. Invest.*, 1937, **16**, 447.

10 Howitt, B., *J. Infect. Dis.*, 1933, **53**, 145.

11 Paul, J. R., and Trask, J. D., *J. Exp. Med.*, 1933, **58**, 513.

12 Amoss, H. L., and Taylor, E., *J. Exp. Med.*, 1917, **25**, 507.

† The one exception is Kling and co-workers,[1] whose findings were inconclusive because the pathological changes they describe in the central nervous system of inoculated monkeys do not appear to be characteristic of poliomyelitis.

13 Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1919, **29**, 379.

Conclusion. Poliomyelitis virus was isolated from the nasopharyngeal secretions of a bulbar case on the 9th day of illness.

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9581 P

The Culture and Division Rate of *Dileptus gigas.*

S. RUDIN. (Introduced by J. A. Dawson.)

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Although pedigree isolation cultures of protozoa have been carried on during the last 50 years, relatively few investigators have experimented with carnivorous forms. Woodruff and Spencer[1] and Woodruff and Moore[2] kept *Spathidium spathula* alive for long periods without degeneration, conjugation or endomixis on a diet of *Colpidium colpoda.* Beers,[3] feeding *Didinium nasutum* on *Paramecium caudatum* succeeded in attaining 1384 generations in about a year's time, without any degeneration or internal reorganization of any kind. With these results in mind it was decided to attempt to find a suitable diet for the carnivorous ciliate, *Dileptus gigas,* and to ascertain its division rate on such diet in isolation pedigree cultures.

In 1936 a number of individuals of *Dileptus gigas* were isolated from some of the writer's stock cultures. These cultures were originally collected from Van Cortlandt Park Pond, New York. By preliminary feeding experiments it was soon ascertained that the large, blue *Stentor coeruleus* made an excellent food basis for *Dileptus* and this readily cultured organism[4] was therefore selected as the standard food supply for the experiment. Visscher[5] has described the interesting and unusual manner in which *Dileptus* manages to attack, paralyze, and cytolyze by means of the powerful trichocysts (toxicysts), stentors much larger than itself and feast upon its prey. It is interesting to add that *Stentor,* itself predominantly car-

1 Woodruff, L. L., and Spencer, H., *J. Exp. Zool.*, 1924, **39**, 133.

2 Woodruff, L. L., and Moore, E. C., *Proc. Nat. Acad. Sci.*, 1924, **10**, 183.

3 Beers, C. D., *Am. Nat.*, 1929, **43**, 125.

4 Gerstein, J., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 210.

5 Visscher, J. P., *Biol. Bull.*, 1923, **45**.