

extracts from 10 normal plasmas were prepared and analyzed according to the method of Kirk, Page, and Van Slyke. The N:P ratios varied from 1.7 to 5.5, and averaged 3.4. The Amino N:P ratios varied from 0.6 to 1.7, and averaged 1.7. There was accordingly more of both total N and amino N present in the extract than could be combined in these phosphatides. In extracts from plasma of uremic patients the excess of N and NH_2 was still greater. The N:P ratio in 8 cases varied from 3 to 18, and averaged 9, while the Amino N:P ratio ranged from 0.9 to 4.7 and averaged 2.5. Part of both the amino and the non-amino nitrogen is removable from the petroleum ether by shaking with acidified water. The nature of the material is being studied further.

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Infection of Monkeys with the Virus of Poliomyelitis in Human Spinal Cords.*

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With 4 out of 5 human cords taken from acute cases of poliomyelitis at autopsy the virus was successfully transmitted to *Macacus rhesus* monkeys. All 5 individuals had died of respiratory paralysis in the acute stage of the disease. Histopathological study showed intense infiltration and considerable nerve cell destruction.

The first of these, strain B, was obtained from a child who died of bulbar paralysis, 5 days after the onset of paralysis, during an outbreak in Montreal, in 1931. More than 3 years later, during which interval the cord and tonsils were left in glycerine, at ice box temperature, a single intracerebral and intraperitoneal injection of each tissue was given to a monkey. The cord produced paralysis in 13 days and the animal was prostrate 4 days later, while the tonsil gave complete paralysis within 6 days.

The second strain, 1049, from a case occurring in New York in 1933, and had been in glycerine for a year when tested, was given in combined intracerebral and intraperitoneal inoculations over 3

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consecutive days and produced a paralysis 5 days later. Repeated intracerebral and intraperitoneal injections of liver, spleen, mesenteric and submaxillary glands, and kidneys failed to show the presence of virus.

The third strain, J, from a case who had died of bulbar involvement on the 6th day of progressive paralysis, had been in glycerine a year prior to inoculation. A combined intracerebral and intraperitoneal injection brought on paralysis in 8 days. Of special interest was the rapid rise of infectivity during 3 serial passages in monkeys and then its sudden drop.

In Table I, the M.C.P. (minimal completely paralyzing dose) of this strain during these monkey transmissions is compared with the infectivity of the fixed monkey strain.

TABLE I.

Passage No.	M.C.P. Dose	Result	M.C.P. dose of fixed monkey passage strain in cc. of 5% suspension
2	0.0006	Paralysis 14 days	0.0003
	0.0025	" 6 "	
3	0.0006	" 7 "	0.0006
4	0.0025	" 8 "	0.0025

It is interesting to note that after the first monkey passage the virus had reached an infectivity almost equal to that of the passage strain and that after 2 such passages it was just as infective. The fifth passage virus suspension had only $\frac{1}{4}$ the infectivity of the monkey passage strain and after 7 months of storage in the ice box, it failed to infect.

The cord of the fourth strain, H., was taken from a bulbar spinal case who died on the second day of paralysis. The spinal cord was preserved in glycerine for 15 months at ice box temperature. In this instance, the infectivity of the human cord was determined and it was found that 0.1 cc. of a 5% suspension gave an incomplete paralysis, whereas 0.2 cc. gave paralysis in 2 monkeys with the onset of paralysis in 7 and 10 days, respectively. The infectivity of the virus during the next 2 passages as shown in Table II did not rise as rapidly as in the previous cord.

No virus was demonstrable in the liver, spleen, mesenteric lymph

TABLE II.

Passage No.	M.C.P. Dose cc. of 5% suspension	Result	M.C.P. dose of passage virus in cc. of 5% suspension
1	0.2	Paralysis 7 days	0.0006
		" 7 "	
2	0.05	" 8 "	0.0006

nodes, or submaxillary glands using as the dose 1 cc. of a 20% suspension given intracerebrally and 5 to 10 cc. injected intraperitoneally. This injection was repeated in 10 to 11 days. On the other hand 1 cc. of a tonsil suspension inoculated intracerebrally and 3 cc. intraperitoneally gave paralysis in 8 days and the animal was prostrate the next day.

The several points of interest brought out in this study are as follows: 1. The virus isolated from the human was still infective after remaining in glycerine for more than 3 years, which observation is in keeping with that of others for the animal-fixed virus. 2. The virus was transferred with relative ease from the human to the monkey and produced a rapid and complete paralysis in a relatively short incubation period in 4 out of 5 animals. With one cord, as little as 0.1 cc. of a 5% suspension gave incomplete and 0.2 cc. gave complete paralysis. 3. The rapid fixation of the virus for the monkey with one cord specimen in which case, after the second serial passage, it became almost as virulent and, after the third, equally as virulent as the passage strain. This same strain was lost in the fifth passage after 7 months of storage on ice, although 3 months previously it was still infective. This is difficult to explain since the human material had been in glycerine for over a year. This sudden loss of infectivity after several passages has been described by others. 4. In 2 instances virus was not found outside of the central nervous system, although it was demonstrated in the cord. Likewise, Landsteiner, *et al.*,¹ and Webster² failed to demonstrate virus in the lymph nodes of human cases while Leiner and von Wiesner,³ Strauss and Huntoon,⁴ Flexner and Clark⁵ and others failed to find virus in the blood stream. Flexner and Lewis,⁶ on the other hand, demonstrated virus in the mesenteric lymph nodes on one occasion. Römer and Joseph⁷ and Leiner and von Wiesner⁸ found active virus in the mesenteric nodes of intracerebrally inoculated monkeys. They suggested that the virus had reached the glands from the cord. Our own studies give evidence for this belief, for it has been pointed out that the virus is probably in the

¹ Landsteiner, C., Levaditi, A., and Pasteur, *Compt. rend. Acad. de Sc.*, 1911, **152**, 1701.

² Webster, R., *Med. J. of Australia*, 1919, **6**, 21.

³ Leiner, C., and von Wiesner, R., *Wien Clin. Wchnschr.*, 1910, **23**, 91.

⁴ Strauss, I., and Huntoon, F. M., *New York Med. J.*, 1910, **91**, 64.

⁵ Flexner, S., and Clark, P. F., *J. Am. Med. Assn.*, 1911, **57**, 1685.

⁶ Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.*, 1910, **54**, 1140.

⁷ Römer and Joseph, *München Med. Wchnschr.*, **57**, 1059.

⁸ Liener und von Wiesner, *Wien. Clin. Wchnschr.*, 1910, **22**, 1698.

neurones, and combined immunological and histopathological studies carried out after the height of paralysis suggest its removal from the nerve cells to the perivascular spaces by means of phagocytic cells. Thus, the finding of virus outside of the central nervous system in the human on a single occasion does not imply a systemic disease, inasmuch as in the experimental animal, where the disease seems entirely neurotropic⁹ the virus may find its way out of the central nervous system. More important are the more frequent negative results obtained in attempts to isolate virus outside the central nervous system of humans, which fits in with the non-specific inconstant histopathological findings outside the central nervous system. In 7 acute human cases examined, slight changes were found in the lymphoid tissue. The mesenteric lymph nodes, Peyer's patches and tonsils showed slight catarrhal inflammation with swelling of the germinal centres, some necrosis of the reticular cells and disintegration of the lymphoid elements and slight infiltration with large mononuclear, plasma and polymorphonuclear leukocytes. The spleen showed swelling and disintegration of the reticular cells, while the thymus showed thickening and degenerative necrosis of Hassal's corpuscles, especially in the center, and slight infiltration with polylobed cells. These changes are certainly not significant for poliomyelitis infection, for they may be present in acute febrile conditions. These changes were not constant. The results of the histopathological studies together with the inability to isolate virus outside of the central nervous system, suggest the human disease to be entirely neurotropic. 5. It is interesting to note the relative ease with which virus was obtained from the tonsils in the 2 cases examined. Landsteiner, *et al.*,¹ Flexner and Clark⁵ and others have isolated the virus from the tonsils and inasmuch as the nasopharynx⁷ is probably the portal of entry for the virus, it is quite likely that the virus has entered due to contact with an infective source and has persisted there.

⁹ Brodie, M., and Elvidge, A. R., *Science*, 1934, **79**, 235.