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Occurrence of Poliocidal Substances in Tears, Saliva and Cerebrospinal Fluid of Normal Individuals.*

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While the presence of poliocidal substances in the serum of normal individuals is generally known and this fact is tentatively put in correlation with the mechanism of resistance to the disease, there are but few data to indicate the extent of distribution of similar substances in other body fluids and secretions of man. Apart from the early work of Amoss and Taylor,[1] who reported neutralization of poliomyelitis virus by the nasal secretions of healthy persons and more recent observations on the inactivation of the virus by pregnancy urine, placenta extracts and various endocrine preparations,[2, 3] investigations in this field have been strangely neglected. Yet it is clear that the question whether or not virucidal substances may normally be found at the site of the presumable portal of entry or in the vicinity of the ultimate seat of the lesions, carries far greater significance than the successful demonstration of such substances in the circulating blood. In an attempt to bridge this gap in our knowledge, we have conducted a series of experiments in order to determine whether poliocidal substances are present in the tears, the saliva and the cerebrospinal fluid of normal adults.

Virucidal tests with normal human tears. Human tears were collected on the day of the experiment from a group of 5 or 6 adult persons after local irritation of the conjunctiva by mustard oil or onion juice. The individual specimens were combined and neutralization tests carried out with the pooled mixtures. The mixtures were used undiluted and in dilutions ranging from 1:2 to 1:20. Amounts of 0.8 cc. were combined with 0.2 cc. of a 10% suspension of poliomyelitis virus. After incubation of 1½ hours at 37°C., the tears-virus mixtures were allowed to stand in the ice-box for 24 hours and then injected intracerebrally into individual monkeys. A total of 11 tests were carried out, 2 with undiluted tears, 4 with

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

2 Jungeblut, C. W., Meyer, K., and Engle, E. T., *J. Immunol.*, 1934, **27**, 43.

3 McKhann, C. F., and Chu, F. T., *J. Inf. Dis.*, 1933, **52**, 268; *Am. J. Dis. Child.*, 1933, **45**, 475.

tears diluted 1:2, 4 with tears diluted 1:5 and 1 with tears diluted 1:20.

Both animals receiving the undiluted tears-virus mixtures survived without paralytic symptoms. Of those injected with the tears diluted 1:2 plus virus, 2 failed to develop paralysis while 2 came down with typical poliomyelitis. In the group of the 4 monkeys injected with the tears diluted 1:5 plus virus, 2 developed paralysis after greatly prolonged incubation periods, *i. e.,* 15 and 25 days respectively, while the remaining 2 animals developed typical poliomyelitis. The last monkey injected with tears diluted 1:20 plus virus showed the usual course of the disease as seen in controls. It appears, therefore, that undiluted tears possess definite poliocidal properties, but that the virucidal effect diminishes rapidly with dilution, the threshold concentration lying in the neighborhood of a 1:2 dilution.

Virucidal tests with normal human saliva. Fresh saliva was obtained from the same group of persons. Individual samples were combined and the pooled saliva was filtered through a Berkefeld candle. The sterile filtrate was tested undiluted and in various dilutions for poliocidal properties as indicated above. A total of 23 tests were performed, 4 with undiluted saliva, 15 with saliva diluted 1:2, 3 with saliva diluted 1:5 and 1 with saliva diluted 1:20.

In these experiments there was only one animal surviving without paralytic symptoms, *i. e.,* a monkey which had received a mixture of saliva diluted 1:2 plus virus, although 3 additional animals developed the disease after greatly prolonged incubation periods, ranging from 15 to 21 days. Of these latter animals 1 had been injected with undiluted saliva and virus and the other 2 with saliva diluted 1:2 and virus. All other monkeys came down with typical poliomyelitis so that it can be said that in the majority of the cases there was no evidence of the presence of neutralizing substances, even when the saliva was tested undiluted or in low dilutions.

Virucidal tests with normal human spinal fluid. These tests were carried out with 3 pools of human spinal fluid, each containing 10 individual samples. Two of these pools consisted of Wassermann-positive specimens, while the third contained only Wassermann-negative fluids. When tested for neutralizing property according to the standard technic, none inactivated the poliomyelitis virus, although in 2 instances (Wa+ pools) the monkeys receiving the spinal fluid-virus mixtures developed only very slight transient paralysis.

Virucidal tests with convalescent monkey spinal fluid. In order

to provide a basis of comparison with the mechanism of acquired immunity, a total of 6 specimens of convalescent monkey spinal fluid, obtained early as well as late after the onset of paralysis, were examined for poliocidal properties. The technic was the same as described above. Five specimens failed to give any evidence whatsoever of the presence of neutralizing substances. However, one monkey injected with the sixth sample, developed paralysis only after an incubation period of 28 days, suggesting a partial inactivation of the virus. This particular specimen was obtained during the acute stage of the infection.

All of these experiments were adequately controlled by the inclusion of 13 control monkeys injected with a mixture of 0.2 cc. 10% virus suspension and 0.8 cc. saline. All of these animals developed complete paralysis within from 6 to 10 days.

It would appear that, as far as our data go, the tears of normal adult persons contain regularly poliocidal substances of low titre. It seems logical to assume that the presence of such substances at the natural portal of entry signifies an important factor of local defense in protecting the nasal mucous membranes against the passage of poliomyelitis virus. The nature of these substances is obscure and we did not ascertain whether their presence coincides with the presence of poliocidal substances in the serum of the same individual. Human tears, however, are known to contain an enzymatic bactericidal principle (lysozyme) which occurs also in fresh egg white.† In human saliva, on the other hand, poliocidal substances seem to be normally absent. Occasional irregular or borderline results that were obtained in some neutralization tests may indicate the chance occurrence of minute amounts of the same substance. The possibility cannot be ruled out that their concentration might have been diminished by filtration. Normal human spinal fluid appears to be ordinarily devoid of poliocidal principles. Whether the light infections observed after injection of the Wa+ spinal fluid-virus mixtures were due to the presence of serum elements in these fluids is an attractive problem which merits further consideration. Similarly, the spinal fluid of convalescent monkeys, with rare exceptions, is evidently free from virucidal substances. In this respect our results agree with those previously reported in the literature.[4] It is rather remarkable that the spinal fluid of convalescent

† Preliminary experiments in which egg white was tested for poliocidal properties so far have given irregular results. The problem is being studied further at present and a complete report will be submitted at some later time.

[4] Poliomyelitis, Monograph of the International Committee for the Study of Infantile Paralysis, Williams & Wilkins, Baltimore, 1932.

monkeys should lack poliocidal properties even when the serum of the same animal can be shown to possess inactivating power, as was true in these experiments. This fact is in marked contrast to the distribution of immune bodies in some other infections of the central nervous system, such as neuro-syphilis, involvement of the brain and cord, whether latent or manifest, giving rise with great regularity to the appearance of the Wassermann reagin in the blood as well as in the cerebrospinal fluid. The reasons for this discrepancy seem to be worthy of further investigation.

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Effect of Adrenalectomy on Poliocidal Property of Serum of Normal Cebus and Convalescent Rhesus Monkeys.*

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In preliminary experiments it was reported that the natural poliocidal property of the serum of one Nicaraguan Cebus monkey was lost after subjecting the animal to bilateral adrenalectomy.[1] It was also demonstrated that this property could be restored by the addition of a small amount of cortical hormone, but not of adrenaline, to the deficient serum. In order to lend significance to this isolated observation it became necessary to repeat these experiments on a larger scale. Furthermore, it seemed of interest to extend the scope of this work to an investigation of the effect of adrenalectomy on the virucidal property of the serum of rhesus monkeys which had recovered from a paralyzing attack of poliomyelitis.

In conducting these experiments it was soon discovered that there exists a fundamental difference between black-faced Cebus monkeys from South America and white-faced Cebus monkeys from Central America with respect to the presence of natural poliocidal substances in their serum. Thus, in keeping with previous experience,[2] the normal serum of 3 Nicaraguan Cebus monkeys produced either complete or partial neutralization of poliomyelitis virus *in vitro*, 2

* Under a grant from the Rockefeller Foundation.

1 Jungeblut, C. W., *J. Bact.*, 1934, **27**, 81.

2 Jungeblut, C. W., and Engle, E. T., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 879.