SECTION ON MICROBIOLOGY

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Viremia in Poliomyelitis†

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A basic problem in the pathogenesis of poliomyelitis is whether the virus is a strictly neurotropic agent, or whether there is a primary non-neural development, and possibly a blood phase. The search for virus in the blood of patients with the disease has yielded largely negative results during the past forty years, viremia having been detected on one occasion only, in blood drawn several hours after onset of an abortive attack.[1] However, when it became known recently that antibodies are already present at the time of onset of CNS signs, it seemed likely that, if viremia occurs in the human infection, it does so early, in the incubation period, or in the minor illness phase; the negative results with human blood may thus have been due to the fact that the search was made too late in the course of infection. The problem was therefore attacked experimentally, in cynomolgus monkeys (*M. cynomolgus*) and chimpanzees (*Pan satyrus*) following infection by a natural route,—i.e., orally.[2] Animals were fed 5-15 ml. of 10-20 per cent virus suspensions of Type 1 (Y-SK) or Type 2 (Egypt strain), and beginning twenty-four hours later, they were bled daily from the femoral vessels, for five to seven days in the earlier experiments, and for an average of twelve to fifteen days in the later ones. The blood specimens were heparinized, centrifuged, and frozen until they were tested for the presence of poliomyelitis virus, either (a) by inoculation of whole blood or plasma intracerebrally into rhesus monkeys (*M. mulatta*), or (b) in tissue culture roller tubes.

The results of the first experiment in cynomolgus monkeys, in which four of thirteen animals which had been fed the Y-SK strain of virus became paralyzed, indicated that one of the four paralyzed animals had viremia: its blood, drawn the fifth day after virus ingestion and six days before it showed any neurological signs, produced typical paralytic poliomyelitis on i-c inoculation into a rhesus monkey. In the second experiment, only one of twenty-six animals which were fed the Y-SK strain became paralyzed, in spite of the fact that a number of the animals were given daily cortisone injections (25 mg.) for twelve days following poliomyelitis virus feeding, and some were fed coincidentally with the latter a pool of Coxsackie viruses. The paralyzed animal had viremia on the fourth and fifth days after virus ingestion, and developed neurological signs on the eleventh day. Two animals which failed to develop any signs of poliomyelitis were also found to have viremia on days four and five; both of these

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subsequently developed specific neutralizing antibodies, in contrast to nine other monkeys which showed no signs of illness, did not have viremia, and failed to develop antibodies. In a third experiment, ten of fifteen cynomolgus monkeys became paralyzed after ingestion of the Egypt strain (Type 1), with incubation periods ranging from eight to twelve days. Nine of the ten paralyzed animals had viremia on the fourth, fifth, or sixth days after virus ingestion, some of them on all three of these days. One animal also had virus in the blood at twenty-four and forty-eight hours after its ingestion.

Several experiments were carried out with chimpanzees. Of four animals fed Type 2 Y-SK strain as their first exposure to poliomyelitis virus, three were shown to have viremia on the fourth and fifth days; all four developed antibodies. As is usual with chimpanzees infected orally, all four animals remained well throughout. These results are similar to those reported by Bodian[3] who also has found viremia and early antibody development in orally infected chimpanzees. Two months after their first virus exposure, our same four animals were fed the heterologous Type 1, Egypt strain. Again, three of the four had viremia on the third to sixth days and one had virus in the blood twenty-four hours after it was fed. All four developed antibodies rapidly: in one animal they were detected as early as the eighth day after virus was fed, and in all four by the twelfth day at which time serum dilutions of 1:10 and 1:100 neutralized 100 tissue culture doses of virus. The same chimpanzees were thus shown to have viremia and to develop antibodies on two separate occasions with two different types of virus. They remained asymptomatic throughout both of the experiments.

That viremia occurs following another peripheral route of inoculation, viz., the intracutaneous one, was shown in an experiment in which four animals, paralyzed after receiving small amounts of Type 1 Hartford strain in the skin, all had circulating virus during the incubation period. Viremia was present as early as three days after inoculation, and continued in some animals over a period of four days. At the time of paralysis, all four had antibodies.

An effort was made to determine the quantities of circulating virus, and also with which fraction of the blood the virus is associated,—i.e., serum, plasma, washed RBC, or "whole blood," the latter meaning here equal parts of plasma and red cells and the white cells, from 6-7 ml. of blood. Four cynomolgus monkeys were fed Type 1 virus, Egypt strain, and bled daily for fifteen days. One of the animals developed mild paralytic poliomyelitis on the tenth day. Its blood contained virus on the fifth, sixth, seventh, and eighth days after virus feeding, in titers of 10^{-1} to $10^{-2.4}$; maximum titers were found on days 6 and 7. There was no significant difference in the titers in serum, plasma, or "whole blood," but no virus was found in the washed red cell fraction at any time.

With these experimental results as a background, the problem of viremia in *human* poliomyelitis was reinvestigated. Dr. Robert McCollum, of the Section of Preventive Medicine, Yale University, collaborated in this phase of the work. Efforts were concentrated on obtaining blood from *contacts* of cases, who might be in the incubation period, and from children with the minor illness syndrome in the midst of an epidemic. To achieve this, blood specimens, rectal swabs, and throat swabs were collected from 120 such individuals during an epidemic in Ohio, in June and July, 1952. These were subsequently tested for the presence of poliomyelitis virus, using tissue culture roller tubes, and for the blood specimens, in addition, intracerebral inoculation of rhesus monkeys. To date fifteen individuals have been suspected of being infected with Type 1 (Brunhilde) poliomyelitis virus as evidenced by the isolation of virus from throat and/or rectal swabs. Four of these fifteen were found to have virus in the blood, whereas eleven of the blood specimens were negative, but six of the negative specimens had antibodies to Type 1 poliomyelitis virus. The four positive results were obtained with blood specimens from four children in the same family, the W family, which was seen during an outbreak of minor illnesses which affected three of the four children. The three older children, aged ten, four, and three, had slight fever, head-

ache, sore throat, anorexia, and vomiting in various combinations. Type 1 poliomyelitis virus was isolated from the blood, throat and rectal swabs of all four children,—from the asymptomatic child as well as from the three with symptoms.

Viremia has thus been demonstrated to occur in *human* as well as *experimental* poliomyelitis. It has not been found at the time of CNS signs and symptoms, but early in the course, i.e., either during the prodromal period, with the minor illness, or during inapparent infection, presumably a few days after exposure. None of the children whose blood contained virus went on to develop signs of the *major illness* either paralytic or nonparalytic, so that actually it is not known whether viremia occurs in such cases.

Turning to the implications of viremia in terms of the pathogenesis of poliomyelitis: the significance of the presence of virus in the blood is not entirely clear. That viremia occurs regularly in experimental infections, and that it also occurs in human ones seems definite, but is it an essential feature of the chain of events leading to invasion of the CNS? Faber and his co-workers[4] believe that it is not important; they adhere to the interpretation that poliomyelitis virus is strictly neurotropic, and multiplies and spreads only in nervous tissue. Basing their conclusions on experiments in cynomolgus monkeys infected orally, they feel that the virus in the blood results from a primary multiplication in peripheral ganglia. Bodian,[5] on the other hand, believes that in all probability there is a primary visceral phase of virus multiplication in the mucosa of the intestinal tract, followed by viremia, i.e., a vascular phase, and finally invasion of the CNS from the blood, and a neural phase.

In the experiments reported above, viremia appeared in several animals within twenty-four hours after virus ingestion. It then disappeared from the blood, and reappeared several days later. This suggests the following possible course of events: Virus is absorbed from the oropharynx soon after exposure, either directly into the blood, or indirectly via the lymphatics. It is then distributed to susceptible cells in the intestinal mucosa, perhaps the lymph nodes, spleen, kidney, etc. Following primary multiplication in these organs it is discharged into the blood in considerable amounts and over a period of days, and by this route reaches the nervous system—both central and peripheral. In considering means of testing this hypothesis, the thought occurred that in animals inoculated intracerebrally, no primary peripheral multiplication would be necessary, since the virus would be in direct contact with susceptible CNS cells at once, and therefore one would not expect to find a vascular phase. Accordingly sixteen cynomolgus monkeys were inoculated with Type 1 or Type 2 virus, with large and small doses. All were bled daily after injection, through the period of paralysis. It was a surprise to find that ten of the fifteen animals which became paralyzed had viremia during the incubation period. Furthermore, in contrast to the results of Sabin and Olitsky,[6] and Morgan,[7] who used rhesus monkeys, and von Magnus[8] who used cynomolgus in experiments demonstrating a long delay in the appearance of antibodies after intracerebral inoculation, antibodies appeared early in our intracerebrally inoculated animals having viremia, being already present at the time of paralysis. Those animals in which no viremia was demonstrated, did not develop antibodies. The explanation of the viremia is not easy. If it were simply a spill over from multiplication in the CNS, it should occur in all the animals, which was not the case. One possibility is that in some animals virus was absorbed directly into the blood from the site of inoculation, distributed to the same susceptible tissues outside the CNS to which it may be distributed after oral infection; here multiplication may have occurred, followed by discharge of virus into the blood, viremia for several days, and finally disappearance of the viremia with the appearance of antibodies. In other animals on the other hand, perhaps the virus did not get into the blood at any time, no peripheral multiplication occurred, there was no viremia, and antibodies did not develop. The fact that antibodies appeared in the animals with viremia supports the theory that virus multiplication was occurring outside the nervous system (as well

as in the CNS, into which it had been directly inoculated); it also seems possible that the peripheral sites of virus multiplication were responsible both for the viremia and for the production of antibodies. The results, taken along with the observations of others that antibody production may be delayed after intracerebral inoculation,[6-8] constitute additional evidence that viremia may be essential in the pathogenesis of the naturally occurring disease.

SUMMARY

Viremia has been demonstrated to occur regularly during the incubation period after oral infection in chimpanzees and cynomolgus monkeys after ingestion of Type 1 and Type 2 poliomyelitis virus. It has also been shown to occur after cutaneous and after intracerebral inoculation of cynomolgus monkeys. Whenever it occurs, it is followed by early antibody formation, often so early that maximum titers are reached in the first days of the ensuing paralysis.

In the human infection viremia has been demonstrated in four children in the same family, three of whom had the minor illness, and one of whom remained asymptomatic. None of the children went on to develop signs of the major illness, either paralytic or nonparalytic.

The significance of viremia in the pathogenesis of the infection has not been clearly delineated. However, the regular appearance of virus in the blood early, in the incubation period, and in the minor illness, suggests early and rapid multiplication outside the CNS, either in neural or non-neural tissues. It also introduces the possibility that viremia is essential to CNS invasion. The problem can only be resolved by demonstrating the early presence or absence of virus in various tissues, non-neural as well as neural ones, after different routes of virus administration.

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Aspermatogenesis in the Guinea Pig Induced by a Single Injection of Homologous Testicular Material Combined with Paraffin Oil and Killed Mycobacteria

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Injection into the dorsal skin of a suspension of guinea pig testis or spermia in a water-in-oil emulsion containing killed mycobacteria induces aspermatogenesis in the guinea pig. The injury begins with the inhibition of the maturation of spermia and proceeds through the degeneration and exfoliation of germinal cells. The process is *not* associated with inflammation. The Leydig cells are not affected. The seminal

vesicles and the prostate remain normal. Aspermatogenesis begins in two weeks and lasts for more than five months. It may cause atrophy of tubules and fibrosis. Animals receiving suspension of their own testis or spermia and adjuvants develop similar injury. Control experiments show that when a suspension of guinea pig liver or kidney is substituted for testicular material the testes of the injected guinea pigs remain normal. Suspension of rabbit or lamb testis plus adjuvants injected into the dorsal skin of guinea pigs is ineffective.

Antibody formation against homologous spermia and against homologous hyaluronidase has been demonstrated in the guinea pig with aspermatogenesis. The relationship of these antibodies to the production of injury to the germinal cells has not been elucidated. Under the conditions of the experiment, the presence of killed mycobacteria at the site of injection of antigen and paraffin oil is essential. It would appear that the aspermatogenesis induced is the result of an immunological process initiated by an autologous or homologous organ-specific antigen combined with paraffin oil and killed mycobacteria.

ANNOUNCEMENT

A completely revised and greatly enlarged fifth edition of NOMENCLATURE AND CRITERIA FOR DIAGNOSIS OF DISEASES OF THE HEART AND BLOOD VESSELS was published June 15, 1953.

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This edition includes new material in the sections on x-ray and electrocardiography to conform with recent advances, revised Charts of Functional Capacity and Therapeutic Classification and new information on injuries of the heart and great vessels. For the first time, a section on peripheral vascular diseases has been added.

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