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# Emerging Concept of Poliomyelitis Infection

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The discovery that polio virus can be isolated from the blood stream of infected human beings and of experimental nonhuman primates in the preparalytic period (*1–4*) emphasized the unsatisfactory state of our knowledge of the events of infection during this critical phase of the disease. It also underlined the fact that data on virus distribution in the body obtained from fatal human cases can tell little about the primary sites of viral multiplication, although they give insight into the tissues that are eventually capable of supporting virus growth. It has been impossible thus far to determine the distribution of virus in the tissues of human beings in the preparalytic period; information concerning virus in this period is limited to that obtained from excretions and accessible body fluids. Therefore, it seemed desirable to obtain such information from an anthropoid ape like the chimpanzee, which closely resembles human beings in terms of susceptibility to poliomyelitis infection and in every essential characteristic of infection so far studied in the two species (*5*).

The experiments with chimpanzees described in this preliminary report (*6*) were, therefore, designed to determine which tissues after virus feeding contain virus in high enough concentration to indicate that primary viral multiplication was taking place. This, of course, can be ascertained in animals only prior to invasion of the blood stream by virus and its subsequent hemal spread to secondary sites of viral multiplication, or "target organs." This study was facilitated by the experience in a previous study of 18 chimpanzees inoculated by virus feeding with a single poliomyelitis strain, which was used in two of the three chimpanzees described here.

In the earlier experiments information was available concerning the time course of virus excretion from the alimentary tract in relation to the time of occurrence of viremia and of the subsequent antibody response in the serum. Subsequently, it was found that the recently developed tissue-culture methods (*7–9*) could be used to determine accurately the viral concentration in excretions and in tissues of chimpanzees by means of direct titration of clarified tissue suspensions in tissue cultures of trypsinized monkey kidney epithelium. The relatively high concentrations of virus demonstrated in certain tissue suspensions indicated that this quantitative assay system is highly satisfactory and, indeed, greatly superior to the older methods of subinoculation in monkeys.

Two chimpanzees were sacrificed 10 days after simple feeding with 50,000 tissue-culture doses of the Wallingford strain of polio virus (type II), a strain with a relatively long incubation period in chimpanzees by this route (*5*). A third chimpanzee was sacrificed 4 days after virus feeding with a similar quantity of the Mahoney virus (type I), a strain with a relatively short incubation period after inoculation by peripheral routes (*10*). The three chimpanzees at the time of sacrifice had not yet experienced fever or other symptoms of illness. Fecal specimens, throat swabs around the tonsillopharyngeal area, and blood specimens were taken daily. At the time of sacrifice, the animals were exsanguinated, and various tissues were removed, with aseptic precautions and with separate instruments to prevent virus contamination of one tissue by another. In this manner a sample was obtained of most of the principal viscera in which virus might multiply during the presymptomatic period.

The tissue suspensions in some cases were toxic to the tissue cultures in dilutions up to 1 to 8; hence, titrations were done in two-fold steps, with two cultures per dilution, beginning with a dilution of 1 to 16. A negative finding, therefore, indicates that viral titer was less than 1 to 16 per gram of tissue. Each virus isolated from a sample of tissue, serum, or excretions was subsequently tested in tissue cultures with type specific serums from hyperimmunized monkeys to determine its serotype and, therefore, to confirm that the virus isolated was the same as the virus introduced into the chimpanzee rather than a latent virus present in the animal prior to inoculation.

Table 1 summarizes the distribution and titers of polio virus in the tissues of these chimpanzees and in the feces and throat swabs collected at daily intervals. The titers shown are for the day with highest virus concentration. In these animals and in three other chimpanzees sacrificed after onset of viremia, no virus was isolated from throat swabs on the day after virus feeding, so that it is clear that throat-swab virus was the result of viral proliferation and was not the residue of the virus given by mouth. It is also clear that, before hemal invasion and before virus can be detected in most of the internal organs, virus is present in relatively high concentration in two superficial sites in the alimentary tract. These are the tonsils and the Peyer's patches of the ileum. The latter specialized lymphoid structures in the mucosa of the ileum were positive in an animal in which no virus could be isolated from the surrounding wall of the ileum. This also was the case in three additional chimpanzees studied after onset of viremia. These structures, as well as all other portions of the alimentary tract, were thoroughly washed with sterile saline at the time of removal of the tissue in order to eliminate the possibility of contamination with the contents of the alimentary tract. Evidence that this procedure was effective came from the fact that the contents of the jejunum, ileum, and ascending colon were regularly found to contain virus, whereas suspensions of the washed wall of these segments of the gut, except the Peyer's patches, were consistently free of virus.

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Table 1. Distribution and concentration of polio virus in tissues of chimpanzees after virus feeding and before onset of viremia or of antibody response. Titers are expressed as the number of tissue-culture infective doses per gram. All positive isolations of virus were verified as to serotype. No virus was isolated from feces or throat swabs on the day following virus feeding; titers shown are for day with highest value.

| Tissues assayed for virus | Chimpanzee N-147 ♂ type II—10 days | Chimpanzee N-149 ♀ type II—10 days | Chimpanzee N-939 ♂ type I—4 days |
|---|---|---|---|
| Feces | 10 | 5000 | 2,500 |
| Throat swab | | | 40,000 |
| Blood serum (daily) | 0 | 0 | 0 |
| *Lymphoid tissues* | | | |
| Tonsils | 10 | 3000 | 2,000 |
| Peyer's patches | | | 16,000 |
| Deep cervical nodes | | 2000 | 250 |
| Mesenteric nodes | 100 | | 0 |
| Axillary, inguinal nodes, thymus, spleen, bone marrow | 0 | 0 | 0 |
| *Alimentary tract* | | | |
| Tongue, salivary glands, lung, duodenum, jejunum, ileum, appendix, ascending colon, pancreas, liver | 0 | 0 | 0 |
| *Neuromuscular tissues* | | | |
| Trigeminal and coeliac ganglia, biceps brachii, diaphragm, heart muscle | 0 | 0 | 0 |
| *Other* | | | |
| Bladder, kidney, adrenals, uterus, ovary, testis, brown fat | 0 | 0 | 0 |

Also shown to contain relatively high concentrations of virus were the lymph nodes that drain the tonsillar area and the Peyer's patches, namely, the deep cervical and the mesenteric lymph nodes, respectively.

These results appear to clarify appreciably two of the important aspects of the mechanism of infection after virus feeding. First, they offer a logical explanation for the source of virus that is found in the secretions of the upper alimentary tract in the region of the tonsillo-pharyngeal area as well as virus that is found in the contents of the lower alimentary tract. Second, these sites of primary virus proliferation—the tonsils and Peyer's patches—contain so much virus in the period just prior to blood stream invasion, when virus is absent from the many other tissues examined, that it must be presumed that viral invasion of the blood stream stems from these sources of viral proliferation by way of lymphatic pathways and lymphatic structures that drain them.

Since the throat swabs in chimpanzee N-939 contained no virus on the day after virus feeding and since the concentration then rose to the remarkably high concentration of 40,000 tissue-culture doses per gram by the fourth day, it seems possible that some of this virus produced in the tonsillar area could have made its way through the alimentary tract to infect secondarily the Peyer's patches, which then became an additional site of viral multiplication in the alimentary mucosa. An important aspect of these findings in such an early period of the infectious process is that as early as 4 days after virus feeding virus has become widespread by means of the alimentary and lymphatic routes.

It is clear from other animals now being studied that, within the next few days, further spread by the hemal route involves such diverse susceptible structures as the somatic lymph nodes, the axillary and suprasternal brown fat, and the central nervous system. The brown fat has previously been shown by Shwartzman and his colleagues (*11*) to be one of the susceptible tissues in cynomolgus monkeys and in chimpanzees, and our own work not only confirms this point of view but also indicates that this interesting tissue is merely a "target organ" that is invaded only after hemal invasion by virus. Whether it contributes significantly to the viremia that apparently can be developed by early viral multiplication in lymphatic tissues is not clear at the present time.

In addition to hemal spread of virus to somatic lymphoid structures, viral invasion of previously uninfected portions of the tonsils or of Peyer's patches probably also can occur from the blood, so as to increase the sources of virus excretion. In a chimpanzee inoculated intramuscularly with the Mahoney strain of polio virus, with careful subsequent sterilization of the skin at the site of inoculation, nonsymptomatic infection with fecal virus excretion resulted. The only reasonable source of alimentary infection was the blood stream, possibly by way of regional lymph nodes draining the site of viral inoculation.

These findings also suggest that, in the chimpanzee after virus feeding, the trigeminal nerve and the sympathetic pathways via the celiac plexus cannot play a significant role in the spread of virus from the alimentary tract to the central nervous system. The trigeminal and celiac ganglia are not only consistently negative in attempts to demonstrate virus or pathological lesions (*12*), in the paralytic period of infections in chimpanzees and in human beings, but are obviously not involved in chimpanzees in the preparalytic period, when the infectious process is rapidly developing in other tissues. Our findings in the earliest stages of development of the infection in chimpanzees are consistent also with the terminal distribution of virus in fatal human cases, as well as in chimpanzees in the postviremic period. Apparently, the "target organs" susceptible to viral invasion by the hemal route are quite limited in number in these species. This fact, rather than the previously tenable idea that viral proliferation in some organs could have become masked by the development of antibody or other damping factors, may explain the limited terminal distribution of virus in fatal cases.

The results of this study of the early phases of poliomyelitis infection are, moreover, of considerable interest in suggesting that similar analysis of other intestinal virus infections might yield evidence regarding the sites of initial virus implantation, the routes of spread, and the sources of virus that is excreted either into the alimentary tract lumen or into the blood stream. Figure 1 is an attempt to summarize the present status of our information concerning the sites of virus multiplication and the pathways of spread of virus after virus feeding in chimpanzees. It seems likely that this schema could apply to the human infection as well, since the isolation of the virus from fatal human cases (*13–15*) fits in most particulars with the sequence of pathogenetic events that has been discovered in chimpanzee experiments.

These results are also of interest in relation to the terminal distribution of virulent strains of virus in cynomolgus monkeys and in infant rhesus monkeys, as is described by Sabin and Ward (*16*) and by Horstmann *et al.* (*17*). Their results in retrospect suggest that those Old World monkeys that are susceptible by virus feeding have a pathogenetic sequence similar to that of the chimpanzee and of human beings, except for the possibility that a few more susceptible "tar-

get organs" may be available in the cynomolgus monkey.

The similarity of pathogenesis in the Philippine cynomolgus monkey (*Macaca syrichta*) is of special interest, since I have presented conclusive evidence that invasion of the central nervous system from the blood stream takes place in this species (*18*). In a recent publication (*19*) confirming our findings in this respect one of the strongest proponents of the exclusive neural multiplication of virus and of neural spread to the central nervous system has also reconsidered his former position, so that there now appears to be general agreement that the pathogenesis of poliomyelitis infections may well be fundamentally similar to that observed for the arthropod-borne viruses and for other viruses that reach their susceptible "target organs" by the hemal route.

The principal difference between the pathogenic capacity of the neurotropic viruses and that of other viruses appears to reside in the ability of the neurotropic viruses to multiply readily within the nervous system and to migrate along nerve fiber pathways within it. In the period preceding the neural phase of infection, in poliomyelitis at least, the infectious process may, however, be disseminated within the body by spread of virus within the lumen of the alimentary tract and within the lymphatic and hemal systems. Moreover, it is during these early phases of viral multiplication in lymphatic tissues and of early viral spread that the serum antibody response develops (*1, 20, 21*). Although the serum antibody clears the virus from the blood and probably from the throat secretions, it develops too late to affect materially the infection in the central nervous system and in other "target organs" after virus has become implanted there and has little, if any, effect on virus excretion in the feces.

Finally, it is timely to point out that, whether it is produced by artificial immunization or by previous infection, the effect of preexisting antibody on the mechanism of infection after oral virus exposure is clarified considerably by the schema presented. It does not appear surprising, for example, that low levels of serum antibody in experimental animals are capable of blocking invasion of the central nervous system by virus (*22*), and that a similar effect has been observed in human field trials of immunization procedures (*23, 24*). However, in experimental animals even preexisting serum antibody appears to be less able to block the implantation of virus in the primary sites of virus multiplication, or to prevent completely virus excretion in the feces (*5, 25*). Nevertheless, high levels of preexisting type specific serum antibody have been shown experimentally to be capable of greatly reducing the probability of virus excretion in the feces (*26, 27*). Information on this point in the human species is not yet available, but it is reasonable to suppose that preexisting serum antibody can prevent infection of tonsils and Peyer's patches from the hemal route, without being able to prevent their infection by way of ingested virus. This would explain the ability of serum antibody to limit virus excretion, without being able to prevent it completely.

It is interesting to speculate at this time on the significance of these findings in relation to the problem of the biological survival mechanism of this intestinal virus, in the face of mass immunization programs. Looking at the problem from the point of view of the survival of the virus species, it appears conceivable that, if mass immunization of a population included a large proportion of adults as well as children, even with inactivated virus vaccination plus booster doses, the amount of virus dissemination, from throat or feces, could become so reduced that the virus would disappear from large communities on a continental scale. We know that the survival of polio virus populations in smaller human communities may be so precarious even without immunization that the virus may disappear for many years until it is reintroduced from the outside.

Can this process be assisted in a large community by mass immunization? I believe the answer depends, as is so often the case, on quantitative relationships between the potency of vaccines, the degree of acceptance of vaccination, and the sociological factors that play an important role in deciding the immune status of nonvaccinated populations. Moreover, the prevention of reintroduction of the viruses of poliomyelitis into a community appears to be a much more formidable problem than the analogous problem in relation to smallpox.

On the other hand, if it is true that widespread vaccination with inactivated vaccines could conceivably reduce the amount of dissemination of virus in the population by fecal routes of contamination, as well as possibly reduce paralytic incidence, does it follow that an immunized population could be produced that progressively might become more susceptible in later life to epidemics of paralytic disease? One can only speculate regarding this point, but it appears to me, in view of what we know already, that first of all the populations in which epidemics of paralytic diseases are occurring are precisely those populations that already are presumably showing the effects of reduced prevalence and dissemination of excreted virus, so that they could hardly be said to be deprived of widespread virus exposure because of artificial immunization of any kind. It may be said, of course, that artificial immunization by virus feeding with avirulent strains not only could give such a population a primary immunological stimulus but also could produce to some extent the spread of excreted virus that would infect and immunize nonvaccinated individuals or reimmunize those whose immunity has waned after a previous vaccination experience. This, however, seems to be a rather contradictory hope, since it is already apparent that populations that would require immunization programs are able to reduce greatly the amount of virus in the environment, because of developments in sanitation and other habits of life, and

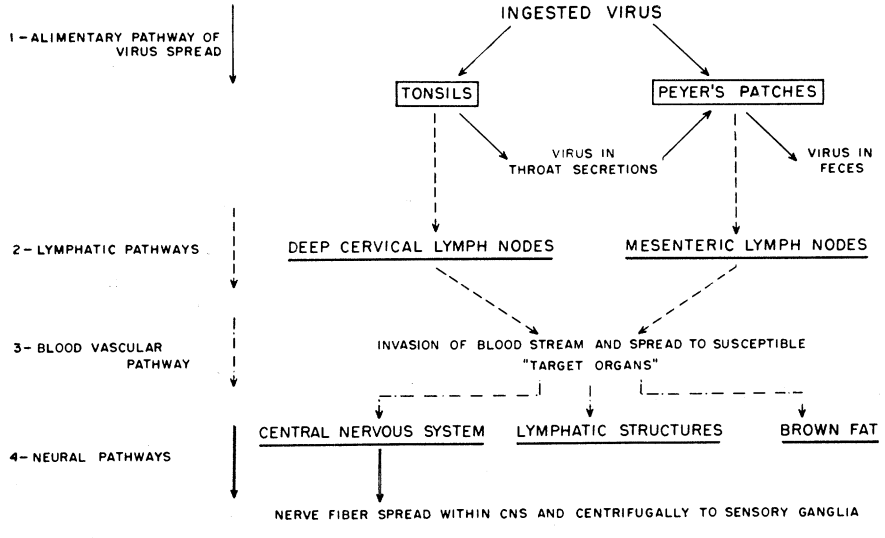


Fig. 1. Schema illustrating the primary sites of viral implantation and multiplication in chimpanzees after polio virus feeding (in boxes) and the pathways of subsequent viral spread in the body. Secondary and tertiary sites of viral multiplication are underlined. The schema summarizes data shown in Table 1 as well as data from other chimpanzees and from studies of the infection in human beings.

populations that scarcely need vaccination, because of a low incidence of paralysis, already have an abundance of poliomyelitis strains in the community that are actively immunizing children soon after they are born.

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# Citation Indexes for Science

## A New Dimension in Documentation through Association of Ideas


Eugene Garfield


"The uncritical citation of disputed data by a writer, whether it be deliberate or not, is a serious matter. Of course, knowingly propagandizing unsubstantiated claims is particularly abhorrent, but just as many naive students may be swayed by unfounded assertions presented by a writer who is unaware of the criticisms. Buried in scholarly journals, critical notes are increasingly likely to be overlooked with the passage of time, while the studies to which they pertain, having been reported more widely, are apt to be rediscovered." (*1*)

In this paper I propose a bibliographic system for science literature that can eliminate the uncritical citation of fraudulent, incomplete, or obsolete data by making it possible for the conscientious scholar to be aware of criticisms of earlier papers. It is too much to expect a research worker to spend an inordinate amount of time searching for the bibliographic descendants of antecedent papers. It would not be excessive to demand that the thorough scholar check all papers that have cited or criticized such papers, if they could be located quickly. The citation index makes this check practicable. Even if there were no other use for a citation index than that of minimizing the citation of poor data, the index would be well worth the effort required to compile it.

This paper considers the possible utility of a citation index that offers a new approach to subject control of the literature of science. By virtue of its different construction, it tends to bring together material that would never be collated by the usual subject indexing. It is best described as an association-of-ideas index, and it gives the reader as much leeway as he requires. Suggestiveness through association-of-ideas is offered by conventional subject indexes but only within the limits of a particular subject heading.

If one considers the book as the macro unit of thought and the periodical article the micro unit of thought, then the citation index in some respects deals in the submicro or molecular unit of thought. It is here that most indexes are inadequate, because the scientist is quite often concerned with a particular idea rather than with a complete concept. "Thought" indexes can be extremely useful if they are properly conceived and developed.

In the literature-searching process, indexes play only a small, although significant, part. Those who seek comprehensive indexes to the literature of science fail to point out that such indexes, although they may be desirable, will provide only a better *starting point* than the one provided in the selective indexes at present available. One of the basic difficulties is to build subject indexes that can anticipate the infinite number of possible approaches the scientist may require. Proponents of classified indexes may suggest that classification is the solution to this problem, but this is by no means the case. Classified indexes are also dependent upon a subject analysis of individual articles and, at best, offer us better consistency of indexing rather than greater specificity or multiplicity in the subject approach. Similarly, terminology is important, but even an ideal standardization of terminology and nomenclature will not solve the problem of subject analysis.

What seems to be needed, then, in addition to better and more comprehensive indexes, alphabetical and classified, are new types of bibliographic tools that can help to span the gap between the subject approach of those who create documents —that is, authors—and the subject approach of the scientist who seeks information.

Since 1873 the legal profession has been provided with an invaluable research tool known as *Shepard's Citations*, published by Shepard's Citations, Inc., Colorado Springs, Colo. (*2*). A citation index is published for court cases in the 48 states as well as for cases in Federal courts. Briefly, the Shepard citation system is a listing of individual American court cases, each case being followed by a complete history, written in a simple code. Under each case is given a record of the publications that have *referred* to the case, the other court decisions that have affected the case, and any other references that may be of value to the lawyer. This type of listing is particularly important to the lawyer, because, in law, much is based on precedent.

Citation indexes depend on a simple system of coding entries, one that requires minimum space and facilitates the gathering together of a great volume of material. However, a code is not absolutely necessary if one chooses to compile a systematic listing of individual cases or reports, with a complete bibliographic history of each of them. Thus, it would be possible to list all pertinent references under each case with sufficient com-


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