# THE POLIOMYELITIS GROUP OF VIRUSES

by

CHARLES G. HENRIQUES

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# I. HUMAN POLIOMYELITIS VIRUS, PAST AND PRESENT

## First Isolation

The now accepted etiologic agent of human poliomyelitis was first demonstrated in 1909 by Landsteiner and Popper (1). These workers succeeded in producing a febrile illness characterized by flaccid paralysis, in a rhesus monkey inoculated intraperitoneally with a spinal cord emulsion prepared from a fatal case of poliomyelitis. Histopathological studies of the brain and spinal cord of this animal, and also of those of asymptomatic monkeys sacrificed on the nineteenth day after inoculation presented a picture essentially the same as that encountered in human cases.

The next important step in the establishment of etiology was made by Flexner and Lewis (2) in 1910. These workers showed that the transmissible agent of Landsteiner and Popper was filtrable, thus establishing its virus nature. In the same series of experiments it was demonstrated that the monkeys could be infected by a variety of routes.

## Other Suggested Etiologic Agents

As in the case of many other diseases, there is a minority of workers not in agreement with the concept of virus etiology. Foremost amongst the dissenters is Rosenow (3), who advanced the theory that the filtrable form of a plemorphic alpha hemolytic streptococcus was the responsible agent. Along similar lines, several workers (4 - 10) observed the presence of "globoid bodies" in enriched cultures of material from poliomyelitis cases. These observations were thoroughly investigated by Logrippo (11), who demonstrated that such particles could be produced in the uninoculated media by the establishment of an

electric field, and that they consisted of tissue lipoids. This evidence, together with the progressively increasing knowledge of the poliomyelitis virus make any concept other than that of virus etiology untenable.

Physico-chemical Studies

The size and shape of the virus have been studied by means of filtration, sedimentation and diffusion constants, and with the electron microscope. As a result of these studies, two different concepts have been put forward. Gard (12) working with virus isolated from fatal human cases in 1945 viewed it as a filamentous particle 12.5 x 580 mu. Elford (17) and his co-workers concluded, on the basis of filtration studies also using human virus, that the particles had a minimum diameter of the order of 8 - 12 mu. More recently Loring and his group (13 - 16) using the electron microscope have shown that the Lansing and MV strains of human poliomyelitis virus are slightly asymmetrical with an average diameter of 25 mu, whilst the Theiler's and SK strains of mouse encephalomyelitis virus appear to be filamentous. These latter studies were of purified virus samples.

The virus is very susceptible to heat, being destroyed by exposure to 45° - 50° C. It is also killed rapidly by ultra-violet light (18). The list of agents to which the virus is resistant includes freezing, drying, Roentgen rays, sonic vibration, and numerous protoplasmic poisons (18). The virus also withstands one percent phenol and fifteen percent ether for several months at 0° C., the latter property being a useful tool for isolation of the virus (19, 20). The virus may be preserved in fifty percent glycerol for four to six years (21), and is relatively stable in the range of pH 4 - 10 (15). Among the most successful poliomyelitis virucidal agents are mercuric chloride and the

oxidizing agents, such as potassium permanganate (18, 22).

Host-Range Studies

Only primates are susceptible to the human strains of poliomyelitis for purposes of original isolation (23, 56). Several strains of monkey-passage virus, such as the Lansing and $MEF_1$, have also been adapted to cotton rats and mice.

Routes of Infection

There is much confirmed information on the site of entry, route of spread, and portal of discharge of the poliomyelitis virus. Conflicting results have suggested different theories of human infection. The two most popular of these implicate the respiratory and the alimentary routes, with the bulk of evidence and present opinion in favor of the latter (23). Insect-borne infection has been suspected, but is improbable as the virus has never been demonstrated in the blood-stream. To the contrary, virus has been recovered from flies trapped in epidemic areas, a finding which is easily explained by contact with feces and sewage (23).

Localization and Pathologic Changes ascribed to the Virus

The major site of localization of the virus in asymptomatic carriers and in abortive cases of poliomyelitis is the alimentary tract. Paralytic cases, in addition, show invasion of the central nervous system in varying degree. Hypertrophy of Peyer's patches and degenerative changes in the myenteric plexuses have been described, but their relationship to the disease is not clear at present. There are no characteristic findings except in the central nervous system. Lesions are found wherever neurons have been destroyed by the virus. The main reacting cells are lymphocytes, plasma cells and macrophages. There is almost always peri-vascular cuffing

of the neighboring arterioles and venules, and focal areas of inflammation, marking the site of neuronal injury. Edema is not a prominent part of the inflammatory process (24). Bodian and Howe (25) have shown that the virus is highly neuronotropic, not reacting with any other elements of nervous tissue, so all the above mentioned changes are due to neuronal damage. Neurons which are attacked, but not destroyed, undergo a reversible degeneration of their Nissl substance. Such neurons occasionally show acidophilic intranuclear inclusions, but there are none pathognomonic of poliomyelitis. Due to its extreme selectivity, the virus attacks certain nerve centers and their connections more than others. Thus, terminally, the following characteristic location of lesions (26) and of virus (27) is found. The main involvement is in the anterior gray columns of the spinal cord, and to a lesser extent in the intermediolateral and posterior gray columns. There is also an extensive encephalitis with so characteristic a distribution that it is more useful in differential pathological diagnosis than the cord lesions. The sites of cortical involvement are confined sharply to the motor and premotor areas, only very rarely extending into the somaesthetic area. In the brain stem, the motor cells of the reticular formation, and the vestibular nuclei and their cerebellar connections, are affected.

The characteristic flaccid paralysis and subsequent disuse atrophy are thought by an overwhelming majority of workers to be due entirely to the destruction of somatic motor nerve supplies (24). This concept is opposed by Sister Kenny who believes that both muscles and nerves are primary sites of damage. Her idea has received some experimental support from the observations of Carey (28) and his co-workers, who studied three fatal cases of bulbar poliomyelitis and observed that the respiratory

muscles were denervated at the neuromyal junction within thirty-six hours after the onset of the illness.

In the bulbar form death may be due either to respiratory or to vasomotor center paralysis. Asphyxia, caused by laryngeal paralysis and tracheal obstruction, produces the usual non-specific pulmonary changes varying from edema and congestion to definite pneumonia and death (29, 30). Therapy is directed towards alleviating shock, supporting respiration or relieving obstruction.

Attempts at Prophylaxis and Specific Treatment

Treatment with random pooled sera, convalescent poliomyelitis sera, and gamma-globulin have all been tried with disappointing results (31). Chemoprophylaxis by means of coating the nasal mucosa with solutions of alum, picric acid, or zinc sulphate produced striking protection in monkeys, but human trial has again been unsuccessful. Not only was there no prophylactic effect, but there was also considerable damage done by extensive coagulation necrosis of the olfactory apparatus of the patients so treated (32, 33). The production of immunity in monkeys by vaccination with inactivated virus has been the subject of many studies (34 - 38). Sodium ricinoleate, aluminum hydroxide, ultra-violet light, and formalin have been used as agents for the preparation of vaccines. Because comparatively few animals were used, and immunity was determined by challenge with a minimal dose of virus, the validity of these experiments is open to question. Furthermore such vaccines still contained active virus and have caused poliomyelitis in monkeys and in children (39). More recently Morgan (62) has reported that virus inactivated with formalin at 4° C., and injected by the intramuscular route, will regularly produce immunity against challenge dosage up to 6000 $LD_{50}$, without ever causing any symptoms of poliomyelitis in a large series of monkeys.

In Vitro Cultivation

There have been numerous attempts to cultivate the virus of poliomyelitis in vitro. The apparently successful attempts have been those of Sabin and Olitsky (41) who grew the virus in the presence of living nervous tissue removed from human embryos, and of Enders and his co-workers (42) who recently reported propagation of the virus on non-nervous tissues derived from the extremities of human fetuses. In both experiments the Lansing mouse-adapted strain was used and in the latter case typical poliomyelitis was produced in monkeys by the injection of sub-culture material.

Attempts at Laboratory Tests

There are at present no simple diagnostic procedures for detecting infection with the poliomyelitis virus. Hopes were raised by the proposed "interference" test of Lepine (43), only to be destroyed by this worker's later retraction of his former claims (44). At the present time, the neutralization test with the Lansing or $MEF_1$ mouse-adapted strains seems to be the nearest approach to a useful tool, but it is greatly limited by several factors, amongst which are the low titer in mice, strain differences, the presence of antibodies in a high percentage of normals, and the relatively long time intervals and large numbers of mice required for performance of the tests.

The very recently proposed protamine adsorption flocculation test of Roberts (57) shows a great deal of promise, appearing to be reasonably specific. However, the results are of a preliminary nature and have not yet been confirmed.

## II. THE MULTIPLICITY OF STRAINS OF HUMAN AND ANIMAL ENCEPHALOMYELITIS

One of the greatest obstacles in the path of poliomyelitis research has been the lack of knowledge concerning the differences and relationships among the many strains of virus isolated. As early as 1910, Flexner and Lewis (2) observed differences between strains of poliomyelitis virus, recognizing two varieties, one too weak to incite any effects in monkeys, and the other strong enough to produce paralysis. Similar findings were made by Levaditi (45), Warburgh (46), and others.

The first immunological reports of strain variation using neutralization and cross-protection tests in monkeys were made by Burnet and Macnamara (47) in 1931. These workers were able to demonstrate definite differences between a virus recovered in Melbourne, Australia, and the standard Rockefeller MV strain. Further confirmation of the existence of strain differences, using similar methods, and a greater variety of strains came from Meyer (48), Paul and Trask (49, 50), Howitt (51), Stimpert and Kessel (52), Kessel, Moore and Pait (53), Lennette and Gordon (54), and Trask, Paul, Beebe, and German (55). Paul and Trask noted that there was a slight degree of cross-protection among certain strains with which they worked, suggesting the existence of a group relationship. These studies are all subject to the criticism of too few animals, and the performance of homologous challenge with minimal effective doses of virus. The later studies of Kessel and Pait (56, 57) and those of Bodian, Morgan and Howe (58), using the same tests as before, but with greatly expanded scope and numbers of animals, have precluded criticism and furnished a classification of the poliomyelitis viruses into three groups. Some workers maintain

that such strain difference is the result of monkey passage, but the results of Kessel and Stimpert (56) in 1940 would appear to disprove this theory.

Loring, Marton and Schwerdt (14) using the electron microscope demonstrated morphological differences between the Lansing and MV strains, which were spherical, and the Theilers and SK strains of mouse encephalomyelitis virus, which were filamentous. Purified virus samples were used throughout the study.

The most recent evidence for strain difference in the poliomyelitis group of viruses is derived from the flocculation test of Roberts (60). This worker using eight monkey passage strains found that the Lansing and $MEF_1$ viruses were very closely related, whilst six other strains were unrelated to these. Cross reactions suggested the existence of sub-groups.

## The Strains of Human Poliomyelitis Virus

Following the original work of Landsteiner and Popper (1), there have been many such successful isolations of strains of the poliomyelitis virus from typical as well as from abortive cases. In most instances, the validity of such strains and their differentiation from murine encephalomyelitis viruses has been established using the following criteria suggested by the Committee on Nomenclature of the National Foundation for Infantile Paralysis (61).

CRITERIA FOR THE DIFFERENTIATION OF HUMAN AND MURINE STRAINS OF ENCEPHALOMYELITIS VIRUS

| Criteria | Human Strains | Murine Strains |
|---|---|---|
| Source | Must be isolated from human material | From murine material |
| Host Range | Limited to primates for original isolation - Some strains later adapted to rodents | Limited to rodents and embryonated eggs - will not "take" in monkeys |
| Clinical Manifestations | Paralysis in man, monkeys and rodents (there may be abortive cases) | Paralysis in rodents only. |
| Pathological Manifestations | Specifically distributed brain and cord lesions | Brain and cord lesions not yet standardized |
| Immunological Relations, Physicochemical Properties | Useful adjuncts, but neither is at present sufficiently developed to exclude strains which satisfy the first four criteria. | |

The committee has also proposed a temporary system of nomenclature. The designation "Smith-Hartford-1942-$M_5$ Strain" is illustrative of this nomenclature and tells the reader at a glance that the strain was isolated from patient Smith in Hartford, in 1942, and that it had been through

five passages in rhesus monkeys. This method of designation, although admittedly unwieldly, would serve to clarify the literature and will probably be adopted by most workers.

Classification of the Polio-encephalomyelitis Group of Viruses

An attempt will be made herein to summarise in tabular form the essential information on the relations of the members of the poliomyelitis group of viruses. The bases for these tables are the neutralization tests of Kessel and Pait (56, 57), the cross-protection studies of Bodian, Morgan and Howe (58), and the flocculation tests of Roberts (60).

A CLASSIFICATION OF THE HUMAN OR POLIOMYELITIS STRAINS OF THE ENCEPHALOMYELITIS VIRUSES

| Group I | | Group II | | Group III | |
|---|---|---|---|---|---|
| Baltimore | (Brunhilde<br>(Frederick<br>(Sudeck<br>(Seich | Mid-West | (Lansing | California | (Leon |
| | | East Coast | (YSK<br>(Philadelphia<br>(MV | | |
| West Va. | (Per | | | | |
| | | California | (Wallingford | | |
| Mid-West U.S. | (Kotter<br>(Riley<br>(Minneapolis | Egypt | ($MEF_1$ | | |
| Egypt | ($MEF_2$ | | | | |
| California | (Brockman<br>(McL<br>(Ca<br>(Gu<br>(Op | | | | |
| | MV * | | | | |

* Indicates partial relationship.

A CLASSIFICATION OF THE MURINE STRAINS OF ENCEPHALOMYELITIS VIRUSES

| | Theiler's Viruses | |
|---|---|---|
| Group I (M)** | Group II (M) | Group III (M) |
| MM | TO | FA |
| Columbia SK | | GD VII |
| Schultz Variant | | |
| Powell-Jamieson Variant | | |
| | The WP, NY65, NY Pool II, and CO strains of Jungeblut and Dalldorf appear to belong to these groups. | |

From the foregoing table it will be seen that all human strains studied to date fall into three immunologically distinct groups. This grouping does not seem to be related to the geographical area of primary isolation. Most of the strains belong to Group I, of which the Brunhilde virus is the prototype. Group II strains appear to be characterized by the fact that they can be adapted to rodents, a property not shared by the other groups. The prototype of this group is the classic Lansing strain. Group III is represented by a single member which differs sufficiently from the others to warrant its separation (56 - 58).

The murine strains also fall into three classes. Goup I (M) includes those which are highly virulent for many rodents including rats, mice, guinea pigs and hamsters. Theiler's viruses, which are all of low virulence, fall into Groups II (M) and III (M). The one strain in II (M) was originally isolated from the feces of normal mice, whilst those of III (M) have only been isolated from the central nervous system tissues of apparently normal mice.

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** Refers to Murine groups.

ORIGIN OF HUMAN POLIOMYELITIS AND MURINE ENCEPHALOMYELITIS STRAINS

A. Group I Human Strains: These are presented in tabular form, based on the work of Bodian, Morgan and Howe (58), supplemented by that of Kessel and Pait (56, 57).

| Strain | Source | Where Isolated | When Isolated | Mean days for Paralysis | Severity | 1st and Severest Paralysis |
|---|---|---|---|---|---|---|
| Brunhilde | Stool Pool | Baltimore | 1939 | 8 | Sev. | Legs |
| Kotter | Stool | Illinois | 1942 | 10 | Mild | Arms or Legs |
| Frederick | Throat swabs (abortive) | Baltimore | 1944 | 10 | Mild | Arms or Legs |
| Minneapolis | CNS | Minneapolis | 1946 | 8 | Sev. | Legs |
| Per | CNS | W.Virginia | 1940 | 9 | Mod. | Arms |
| Riley | Stool | Chicago | 1943 | 9 | Sev. | Legs |
| Sudeck | Stool | Baltimore | 1941 | 11 | Mild | Legs |
| Beich | Stool | Baltimore | 1941 | 11 | Mod. | Legs |
| $MEF_2$ | CNS | Cairo, Egypt | 1942 | 8 | Sev. | Arms |
| Brockman | CNS | Los Angeles | 1936 | -* | Mild | - |
| McK | CNS | Los Angeles | 1935 | - | Mod. | - |
| Ca | - | Los Angeles | - | - | - | - |
| Cp | - | Los Angeles | - | - | - | - |
| Cu | - | Los Angeles | - | - | - | - |

B. Group II Human Strains

The Lansing Strain was isolated in monkeys by Armstrong from the brain and cord of a fatal case of bulbar poliomyelitis in 1937. The fourth monkey

* No data available.

passage material was successfully passed to eastern cotton rats (63), and later to mice, in both cases retaining the ability to cause paralytic symptoms and characteristic pathology in monkeys. This strain, because it is transmissible in rodents, has been the one most used in experimental poliomyelitis up to the present time. As previously mentioned (page 6) there have been two unconfirmed claims of in vitro cultivation. Successful passage to chick-embryos has been claimed by Enright and Schultz (64) and Powell and Jamieson (65), but in both cases the recovered viruses have subsequently been found to be immunologically unrelated to the original strains used (65 and this paper) and closely related to natural murine encephalomyelitis viruses, and so must be regarded as contaminants arising from latent viruses in the mice used for passage.

The $MEF_1$ Strain was isolated by van Rooyen in monkeys from a fatal case of poliomyelitis (66). The strain was studied and later adapted to cotton rats and mice by Schlesinger, Morgan and Olitsky (69).

The Y-SK Strain (Yale SK) was originally isolated in monkeys from the feces of an abortive case of poliomyelitis by Trask, Vignec and Paul. Later, Jungeblut and Sanders claimed to have adapted it to cotton rats and mice, but the rodent passage strain was no longer virulent for monkeys. Recent evidence has indicated that here again, the material was contaminated by a natural murine encephalomyelitis virus during passage and the original YSK strain lost. Additional evidence for this conclusion is furnished by the recent successful adaptation of the YSK strain to cotton rats and mice. In this case, the recovered strain proved to be serologically identical to the original YSK strain and unrelated to the so-called SK strain of Jungeblut and Sanders (66).

The MV (Mixed Virus, "Rockefeller Mixed") Strain. Flexner and Lewis isolated two strains in 1909. One was designated as the MA or weak strain, and the other as the K or strong strain. These strains were later pooled and the mixture redesignated as the MV strain (52), which has since been extensively used in experimental work despite the fact that there would appear to be no certainty that it is a pure strain. Kessel, Stimpert and Fisk (67) suggest that since the strain now produces consistently severe symptoms, the MA strain may have been lost in passage, leaving the pure K strain.

In 1943, Toomey, Frohring and Takacs reported the adaptation of the MV strain to the cotton rat (68).

The Wfd. (Wallingford) Strain. This strain was recovered from the medulla and spinal cord of a fatal case of poliomyelitis in Los Angeles in 1934 by Trask and Paul (55). Severe paralysis is produced about the eighth day and occurs in both the upper and lower extremities.

C. Group III Human Strain: The Leon Strain was recovered from the central nervous system of a fatal case of poliomyelitis in Los Angeles in 1937. A very severe paralysis is produced in the arms about the sixth day (58).

D. Unclassified Human Strains:

The AY (Aycock) Strain: This strain was isolated by Aycock in 1921 from a case of poliomyelitis in Vermont. It has since been passed many times, and extensively studied (55).

The WE Strain was isolated in 1931 from the nasopharynx of an abortive case of poliomyelitis in New Haven by Paul and Trask, and has been through many passages (49).

The New York 1931 (Flexner) Strain was isolated from the medulla and spinal cord of a fatal case in the New York 1931 epidemic.

The MoC Strain was recovered from the nasopharynx of an abortive case of poliomyelitis in Los Angeles by Paul, Trask and Webster in 1939 (55).

E. Group I (M) Strains (Murine Encephalomyelitis)

The MM Virus was recovered by Jungeblut and Dalldorf from the brain of a hamster inoculated with medulla and spinal cord suspension from a human case of poliomyelitis. This hamster material, in contradistinction to the original human material, showed the ability to produce paralysis in cotton rats, mice, guinea pigs, and hamsters, but not in rabbits or monkeys. This virus reaches the very high titer of $10^{-11}$ in mice by the intracerebral route, and is also effective intraperitoneally to a dilution of $10^{-9}$. The oral route is also successful (61). More recently this virus has been adapted to chick-embryos (64).

The Columbia SK Virus is the previously mentioned (page 13) SK virus of Jungeblut and Sanders, now known to bear no relation to the Y-SK strain of poliomyelitis virus. This virus has also been successfully adapted to chick-embryos (64).

The Powell-Jamieson Variant was isolated by Powell and Jamieson during an attempt to propagate the Lansing strain in embryonated eggs (65).

The Schultz Variant was recovered by Enright and Schultz, in a similar attempt to propagate the Lansing virus (64).

F. Theiler's Viruses: This group of viruses was originally discovered by Max Theiler in 1934 in the mouse stock at the Rockefeller Institute, New York. He designated them very descriptively as spontaneous mouse

encephalomyelitis viruses. TO (Theiler's Original) was recovered from the feces of normal stock animals and from the brain and spinal cords of mice showing spontaneous paralysis of the extremities. Some strains are pathogenic for cotton rats, but no strain has so far been found which will infect monkeys.

FA and GD VII Strains were recovered from brains and cords of mice during the course of work with other viruses, but have not so far been recovered from the intestinal contents of mice. These strains differ from the TO strain in that they give rise to encephalytic as well as paralytic signs, show shorter incubation periods, higher titer, and greater infectivity by peripheral routes. They are immunologically unrelated to the TO strain. The WP, NY 65, NY Pool II, and CC strains recovered from mice by Jungeblut and Dalldorf would appear to belong to this spontaneous mouse encephalomyelitis group (61).

G. The Virus of Teschen's Disease of Swine has been proposed for admission to this group of viruses by Gard (12), on the grounds that it shows certain similarities to both Theiler's viruses and the human poliomyelitis virus. However, it would appear that the evidence is far too incomplete to warrant its inclusion at the present time.

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## STUDIES OF THE SUPPOSED LANSING VARIANT ADAPTED TO CHICK-EMBRYO PASSAGE BY SCHULTZ

### HISTORICAL

This virus was isolated by Dr. Edwin W. Schultz in 1946 - 1947. Prior to egg-passage, the virus had been a typical low-titer, murine-adapted Lansing strain, and had been passed in mice for a period of over four years. Sometime in 1946, before egg-passage was attempted, an unusual increase in titer to $10^{-7}$ occurred. Believing that the virus had undergone mutation, Enright and Schultz (64) attempted and obtained propagation in embryonated eggs. Subsequent pathological and serological studies by these workers supported the concept that the egg-passage material was a Lansing variant.

Original egg-passage was made using a Chamberland $L_3$ filtrate of a one percent suspension of infected mouse brain-cord tissue in half-strength Martin's peptone solution. The $LD_{50}$ of this filtrate was $10^{-5}$ and not less than $10^{-7}$ for the unfiltered material. Initial passage was made using 0.1 ml of this filtrate via the chorioallantoic route. Subsequent passages were made using the ground heads and necks of the embryos prepared in one percent solution of the same diluent. After the fifth passage, the entire embryos were harvested. Of a total of seventy-eight embryos inoculated in making fifteen passages, seventy-six percent died between the second and sixth days.

Whilst the material from the egg-passages retained its high titer in mice, its infectivity for monkeys was diminished. Of six monkeys, four of which were inoculated intracerebrally with an undiluted $L_3$ filtrate of a one percent suspension of fifth egg-passage material, and two of which were inoculated by the same route with undiluted tenth egg-passage material,

none developed frank paralysis, or well-defined cord lesions, although all the animals developed a definite awkwardness of movement. One of the three sacrificed in the first group showed peri-vascular cuffing in the brain, but the fourth animal of this group recovered without any residual manifestations. During this time, control titrations of the $L_3$ filtrate were made using mice and cotton rats and the $LD_{50}$ was found to be $10^{-5}$ in the former, and $10^{-4}$ in the latter group. Neutralization tests performed by Enright and Schultz showed that when used in a $10^{-4}$ dilution, the filtrate from the tenth egg-passage was neutralized by an equal volume of anti-Lansing rabbit serum (final serum dilution 1:5), but not by antiserums against the Columbia SK strain and Theiler's GD VII strain. In a recent personal communication these workers expressed a change of opinion. They now feel that the variant is more closely related to the Columbia SK than to the original Lansing strain.

The virus was received in this laboratory in February 1948, and was successfully passed undiluted to mice and eggs. The material obtained from the first egg-passage made here was called Schultz I, and that from subsequent egg-passages was designated Schultz II, Schultz III and so on.

## EXPERIMENTAL

### Comparison of Infectivity of the Virus by Various Routes in the Swiss Mouse.

#### Methods

It was first ascertained, using Schultz II diluted 1:10 with extract broth, that the virus would produce paralysis in one hundred percent of

three to four weeks old Swiss mice by the intracerebral, intranasal, intraperitoneal, subcutaneous, and intravenous routes. The oral route was also tried, using food mixed with the 1:10 virus suspension instead of water, with sufficient suspension used to make the dosage approximately 1.0cc. The mice were previously starved and then observed to see that all the food was eaten within twenty minutes after the mixture was made. Of a total of sixteen mice so inoculated only one developed paralysis, and four died at irregular intervals without having become paralysed. No subsequent use was made of this route since it was felt that the one mouse probably became paralysed as a result of regurgitation of virus into the nasal passages. Since the virus infected by the subcutaneous route, further work by the intravenous route was not done, because there was no certainty that the former was not the effective route when injection of the tail vein was attempted.

In the above experiments, and in all subsequent titrations in this study the volumes of inoculum used were as follows:

TABLE I

Dosage by Various Routes

| | |
|---|---|
| Intracerebral ..... | 0.03 ml |
| Intraperitoneal ..... | 0.30 ml |
| Intravenous ..... | 0.10 ml |
| Intranasal ..... | 0.03 ml |
| Oral ..... | approximately 1.00 ml |

First, a series of preliminary titrations was made, using dilutions from $10^{-1}$ to $10^{-5}$, to determine the approximate titer-range in each case.

Next, the final titrations were set up in the ranges indicated by the results of the preliminary tests, and the combined results were used to calculate the titers and the $LD_{50}$ in each case, according to the method of Reed and Muench (70) as indicated in Table II.

TABLE II

CALCULATION OF THE FIFTY PERCENT LETHAL DOSE ($LD_{50}$) BY THE INTRACEREBRAL ROUTE

| Dilution | Dead | Survived | Accumulation Totals | | Fraction Dead |
|---|---|---|---|---|---|
| | | | Dead | Survived | |
| 1/1,000 | 6 | 1 | 18 | 1 | 18/19 |
| 1/5,000 | 4 | 1 | 12 | 2 | 12/14 |
| 1/7,500 | 3 | 2 | 8 | 4 | 8/12 |
| 1/10,000 | 4 | 6 | 5 | 10 | 5/15 |
| 1/20,000 | 1 | 7 | 1 | 17 | 1/18 |

Therefore the fifty percent LD occurred at a dilution slightly greater than 1/7,500, or $LD_{50} = 3.875$ (i.e. $1/7,500 = 10^{-3.875}$)

TABLE III

CALCULATION OF THE FIFTY PERCENT LETHAL DOSE BY THE INTRAPERITONEAL ROUTE

| Dilution | Dead | Survived | Accumulation Totals | | Fraction Dead |
|---|---|---|---|---|---|
| | | | Dead | Survived | |
| 1/5,000 | 7 | 1 | 20 | 1 | 20/21 |
| 1/7,500 | 7 | 1 | 13 | 2 | 13/15 |
| 1/10,000 | 3 | 8 | 6 | 10 | 6/16 |
| 1/20,000 | 3 | 2 | 3 | 12 | 3/15 |

Therefore the fifty percent LD occurred at a dilution slightly less than 1/10,000, or $LD_{50} = 4.0$.

TABLE IV

CALCULATION OF THE FIFTY PERCENT LETHAL DOSE BY THE INTRANASAL ROUTE

| Dilution | Dead | Survived | Accumulation Totals | | Fraction Dead |
|---|---|---|---|---|---|
| | | | Dead | Survived | |
| Full Strength | 5 | 0 | 13 | 0 | 13/13 |
| 1/10 | 4 | 1 | 8 | 1 | 8/9 |
| 1/50 | 2 | 5 | 4 | 4 | 4/8 |
| 1/100 | 2 | 6 | 2 | 10 | 2/12 |

Therefore the fifty percent LD occurred at a dilution of 1/50, or $LD_{50} = 1.699$.

Discussion

In comparing these titers, it should be taken into consideration that the intraperitoneal dosage was ten times that by the other two routes, so for more correct comparison the titer by this route should be multiplied by 10. Following is a table corrected in such manner:

TABLE V

COMPARISON OF THE EFFECTIVENESS OF THREE ROUTES OF INOCULATION

BASED ON A 0.03 ML. DOSE

| Route | Titer of 50% LD | $LD_{50}$ |
|---|---|---|
| Intracerebral | 1/7,500 | 3.875 |
| Intraperitoneal | 1/1,000 | 3.000 |
| Intranasal | 1/50 | 1.699 |

Hence, the intracerebral route is 7.5 times more effective than the intraperitoneal route and 150 times more effective than the intranasal

routes. This difference in titer may well be artificial, since if the virus is evenly distributed throughout the animal following intraperitoneal inoculation, then the amount reaching the brain would probably be much less than the total injected. Again the difference could be due to incomplete passage of the virus through the blood-brain barrier following peripheral administration. At the present time the latter mechanism is not sufficiently well understood to allow for any more than conjecture as to its operation in this situation.

The suprising finding that the Schultz variant would infect by peripheral routes, whereas the original Lansing strain is effective only by intracerebral injection, led to the suspicion that the virus had either undergone a marked variation, or that it had become contaminated with one of the murine encephalomyelitis viruses in transfer previous to egg-adaptation.

Neutralization tests with convalescent human poliomyelitis serums and hyperimmune monkey poliomyelitis serums

Despite the possibility that the Schultz variant was not related to the original Lansing strain, the efficacy and ease of the intraperitoneal route suggested the possibility of using it in a mouse neutralization test for poliomyelitis.

Methods

Neutralization tests were performed in the following manner. The virus was prepared so that 0.1 ml. contained 50 $LD_{50}$ as titrated by the intraperitoneal route. The convalescent serums were diluted either 1:5 or 1:10, and mixed with equal volumes of the virus

suspension. Extract broth was again used as the diluent. The mixtures were incubated in a 37° C. water-bath for two hours, and 0.2 ml. injected intraperitoneally into Swiss mice of an average age of three weeks. Control tests with anti-Schultz guinea pig serum, normal guinea pig serum, and in some cases normal monkey serum were included in each series. Four to six mice were used for each serum tested, and further studies were made wherever the results suggested the possibility of neutralization. During the course of the experiments, three separate lots of egg-passage material were utilized, each being previously standardized as to the $LD_{50}$. Animals dying within the first twenty-four hours following injection were discarded; death or paralysis after this period was taken as the end-point and usually occurred within a week. In all experiments the animals were observed for a period of two weeks after inoculation. The occasional failure to secure one hundred percent paralysis in the control animals was found to be due to the use of animals older than four weeks, rather than to deterioration of the virus, which was stored at -6° C.

Results

TABLE VI

Neutralization Tests with Serums from Convalescent Human Poliomyelitis Cases

| No. of test | Name of Patient | Time between onset of paralysis and drawing of serum | Animals dead / Animals used |
|---|---|---|---|
| 1 | Belcher | 1 mo. | 4/4 |
| 2 | Horton | Unknown | 4/4 |
| 3 | Burch | 2 wks. | 4/4 |
| 4 | Harris | 3 wks. | 4/4 |
| 5 | Warren | 2 wks. | 3/4 |
| 6 | Lemon | 3 wks. | 4/4 |
| 7 | Curry | Unknown | 4/4 |
| 8 | Herd | 1 mo. | 3/4 |
| 9 | Hammaker | Unknown | 3/4 |
| | Normal Controls | - | 4/4 |
| | Immune Controls | - | 0/4 |
| 10 | Smith | 5 mos. | 4/4 |
| 11 | Klein | 2 wks. | 4/4 |
| 12 | Owen | 1 mo. | 4/4 |
| | Normal Controls | - | 4/4 |
| | Immune Controls | - | 0/4 |
| 13 | Goalwell | 4 yrs. | 4/4 |
| 14 | Shelton | 4 yrs. | 4/4 |
| 15 | Pugh | 8 yrs. | 3/4 |
| 16 | Thissel | 11 yrs. | 4/4 |
| 17 | Fisher | 13 yrs. | 1/4 ** |
| 18 | Fabelquist | 5 yrs. | 3/4 |
| 19 | Germscheid | 9 yrs. | 4/4 |
| | Normal Controls | - | 3/4 |
| | Immune Controls | - | 0/4 |
| 20 | Allen | 9 yrs. | 3/4 |
| 21 | Phillips | 5 yrs. | 2/4 * |
| 22 | Kenworthy | Acute | 4/4 |
| 23 | Carsten | 2 wks. | 2/4 * |
| 24 | Eklund | 3 wks. | 3/4 |
| 25 | McGehr | 2 wks. | 2/4 * |
| 26 | Sisson | 3 wks. | 3/4 |
| 27 | Rose | 3 wks. | 3/4 |
| 28 | Riley | 2 mos. | 1/4 ** |
| | Normal Controls | - | 3/4 |
| | Immune Controls | - | 0/4 |

* Represents equivocal neutralization, ** Represents definite neutralization

TABLE VI (Contd.)

| No. of test | Name of Patient | Time between onset of paralysis and drawing of serum | Animals dead / Animals used |
|---|---|---|---|
| 29 | Benson | 2 mos. | 4/4 |
| 30 | Weston | 1 mo. | 3/4 |
| 31 | Harling | 5 yrs. | 3/4 |
| 32 | Turner | 1 yr. | 4/4 |
| 33 | Benoist | 7 yrs. | 3/4 |
| 34 | Pierce | Unknown | 2/4 * |
| 35 | Gonzales | 11 yrs. | 3/4 |
| 36 | Jackson | 3 yrs. | 3/4 |
| 37 | Bendickson | 10 yrs. | 2/4 * |
| 38 | Miller | 2 yrs. | 4/4 |
| 39 | Stillwell | 6 mos. | 2/4 * |
| 40 | Kerr | 5 yrs. | 2/4 * |
| | Normal Controls | - | 3/4 |
| | Immune Controls | - | 0/4 |
| 41 | Pederson | 6 mos. | 5/5 |
| 42 | Huger | 6 wks. | 4/5 |
| 43 | Knab | 6 wks. | 5/5 |
| 44 | Weiss | 6 wks. | 4/5 |
| 45 | Marasco | 6 wks. | 4/5 |
| 46 | Squelch | 6 mos. | 4/5 |
| | Normal Controls | - | 4/4 |
| | Immune Controls | - | 0/4 |
| 47 | Purser | 7 yrs. | 5/5 |
| 48 | Follet | 1 mo. | 5/5 |
| 49 | Brown, M. | 1 mo. | 5/5 |
| 50 | Price | 9 yrs. | 5/5 |
| 51 | Scott | 3 wks. | 5/5 |
| 52 | Brown, G. | 11 yrs. | 5/5 |
| 53 | Cole | 5 yrs. | 5/5 |
| 54 | Woodfield | 7 wks. | 5/5 |
| 55 | Koberstein | 4 mos. | 5/5 |
| 56 | Burke | 3 wks. | 5/5 |
| 57 | McPherson | 7 wks. | 5/5 |
| 58 | Voth | 3 wks. | 5/5 |
| 59 | Hedges | 3 mos. | 5/5 |
| | Normal Controls | - | 4/4 |
| | Immune Controls | - | 0/4 |

* Represents equivocal neutralization.

From Table VI it will be seen that only two of the fifty-nine serums tested showed definite neutralization as compared with the normal controls. These two serums, Fisher and Riley, were tested in dilutions of 1:5 and 1:10 on three occasions, and the preliminary results confirmed (Table VII). Seven of the above serums showed equivocal results, and two, Phillips and Garsten were selected at random for further study. Since neither showed any protection significantly different from the controls in these expanded tests (Table VII), no further work was done on the other five serums showing equivocal neutralization (Table VI).

TABLE VII

Further Neutralization Studies

| Name of Patient | Time between onset of paralysis and drawing of serum | Animals dead / Animals used | Normal Controls | Immune Controls |
|---|---|---|---|---|
| Fisher | 13 yrs. | 7/28 | 13/18 | 0/18 |
| Riley | 2 mos. | 4/20 | 10/14 | 0/14 |
| Phillips | 5 yrs. | 11/12 | 6/6 | 0/6 |
| Garsten | 2 wks. | 10/12 | 6/6 | 0/6 |

At this time, hyperimmune monkey serums against the Brockman *, Kotter ** (both Group I human poliomyelitis viruses) and Lansing strains were obtained and tested in the same manner as the human serums. The Kotter and Lansing antiserums gave no neutralization whatsoever, but in the first experiment the Brockman antiserum

* Obtained from Dr. J. F. Kessel, ** Obtained from Dr. I. M. Morgan.

showed apparent neutralization. Later titrations proved that this neutralization was not significantly greater than that produced by normal monkey serum (see controls, Table VIII).

TABLE VIII

Neutralization Tests on Hyperimmune Monkey Serums

| Antiserum | | Animals dead / Animals used | Normal Controls | Immune Controls |
|---|---|---|---|---|
| Kotter | | 18/22 | 12/17 | 0/17 |
| Lansing | | 16/18 | 9/13 | 0/13 |
| Brockman | First results | 0/4 | 3/4 | 0/4 |
| | Subsequent experiments | 30/42 | 24/28 | 0/30 |

Finally the MM strain of mouse encephalomyelitis virus was obtained * and tested with the Schultz variant hyperimmune serum. The results show that the two viruses are closely related:-

TABLE IX

Neutralization of MM Virus by Schultz Variant Anti-serum

| Animals dead / Animals used | |
|---|---|
| Test Mice | Normal Controls |
| 0/10 | 12/13 |

* From Dr. C. A. Evans.

Discussion

In the course of these experiments, the Schultz variant has been studied with regard to its use as a means of measuring antibody in serums from hyperimmune monkeys and convalescent human poliomyelitis cases. The virus did not prove to be of value for this purpose as it was neutralized by only two of the sixty-two serums tested, whereas it has long been known that about seventy-five percent of the random population carry antibodies against the Lansing virus (66). This observation, together with the results of the neutralization tests with the MM virus support the conclusions of Schultz * and of Powell and Jamieson (65) that the egg-adapted variant belongs to Group I (M), and probably was acquired as a contaminant during mouse passage previous to egg adaptation. In view of these conclusions, it is felt that further studies of this virus will not be useful for the elucidation of the properties of the human poliomyelitis virus.

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* Personal communication.

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## IV. OTHER EXPERIMENTAL STUDIES

### A. An Attempt to Induce Poliomyelitis in Mice by Means of Intraperitoneal Injection of the Lansing Virus.

In the first experiment four mice were inoculated intraperitoneally with 1.0 ml. of a five percent suspension of Lansing virus in physiological saline. Immediately following injection, they were exhausted by swimming in a tank of cold water. At the end of seven weeks, all animals were in apparently good health.

In the next trial, twelve and one-half percent glycerin was added to the virus suspension, and the following more intensive regime was adopted: Each mouse received 1.0 ml. of virus suspension twice daily for two days, each dosage being followed by exhaustion as before. Twelve days after the first injection, one mouse developed paralysis of one front leg and was sacrificed. Brain, spinal cord, liver, lung, spleen and gastro-intestinal tract and contents were all removed and stored at -6° C. No other mice showed any symptoms up to the fifth week at which time the experiment was terminated.

In a third experiment, a ten percent suspension of brain and cord from the paralysed mouse of the previous trial produced paralysis in five of six mice into which it was injected intracerebrally in a dosage of 0.03 ml., but showed no effect at the end of the fourth week in four mice inoculated subcutaneously with 0.5 ml., or in six mice inoculated similarly with 0.5 ml. of virus suspension containing five percent glycerin, followed by exhaustion as before. The liver, lung, spleen and gastro-intestinal tract (treated for twenty-four

hours with ether to remove bacterial flora) were all tested for virus content by intracerebral injection of 0.03 ml. of ten percent suspension in extract broth. Only one of four mice in the gastro-intestinal tract group developed paralysis, and subsequent passage of the brain and cord to six additional mice produced paralysis in all cases.

At this point, an unsuccessful attempt was made to repeat the original production of paralysis by intraperitoneal injection and exhaustion. This result led to the suspicion that we had been dealing with one of the spontaneous mouse encephalomyelitis viruses, so we next prepared an etherized suspension of stool from normal stock mice and injected this intracerebrally into four mice, one of which developed paralysis in twenty-four days, making it highly probable that our surmise as to the unmasking of a latent virus in our stock mice was correct.

B. An Attempt to Absorb the Lansing Strain on to the Erythrocytes of Various Species:

In this study, five percent virus suspension was mixed with equal volumes of washed human (Type IV), guinea pig, rabbit and mouse erythrocytes. The mixtures were incubated in duplicate at room temperature and at 8° C. Following incubation, all tubes were centrifugalized and the supernatant fluid withdrawn. Each of the sediments and each of the supernatant fluids was injected intracerebrally into four Swiss mice.

The results of this study showed that the virus remained in the supernatant fluid rather than the erythrocyte sediment, in all cases.

C. An Attempt to Inactivate the Lansing and $MEF_1$ Strains, and the Schultz Variant, with Human Erythrocytes.

Erythrocytes from two convalescent poliomyelitis patients, four patients with no history of the disease, and one guinea pig were washed and added to equal volumes of a five percent suspension of Lansing virus. The mixtures were incubated at room temperature for two hours and then mixed thoroughly and injected intracerebrally into Swiss mice. Some apparent inactivation was observed in two cases, but the controls were unsatisfactory and subsequent trials using the more reliable $MEF_1$ strain showed that none of the erythrocytes used exhibited any inactivation. The experiment was repeated using the Schultz variant under the assumption that it was a Lansing variant, but here again the results were entirely negative.

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