CORRELATION OF CLINICAL AND PATHOLOGICAL FINDINGS OF AVIAN ENCEPHALOMYELITIS VIRUS IN YOUNG CHICKS

by

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INTRODUCTION

A new disease entity affecting the central nervous system of young chicks was investigated and reported by Jones (9) as "an encephalomyelitis of chickens". After study of experimentally produced infection and other naturally occurring infections she proposed the term "epidemic tremor".

Etiological studies by Jones (9), (10), and later confirmed by other workers (30), showed the causative agent to be infectious and filterable. Filtration and antigenic studies showed that the size of the virus is within the range characteristic of the encephalitidies group, and that it is antigenically distinct from Eastern and Western equine encephalomyelitis viruses. The agent is quite resistant, having been stored for as long as 836 days with little loss of virulence. The brain and spinal cord of affected birds was the best source of the virus (10), (30). Suspensions of selected visceral organs and fecal material have been used with variable results in producing either symptoms and lesions, or both (14).

Reports differ as to the economic importance of avian encephalomyelitis (AE). Later reports (4), (25) in general express more concern as to the seriousness of the threat of this disease. A recent survey (4) showed that AE is wide spread, and is on the increase in many areas in the United States. It has also been reported in Australia (8) and

Canada.[1, 2] The average mortality of AE is approximately ten per cent (20) but may reach 65 per cent or more in some outbreaks. Infection has not been considered to influence potential production, however recovered birds should not be retained for breeding purposes. Taylor, et al. (27) observed an outbreak in a breeding flock, in which there was a temporary reduction or a pause in egg production. The hatchability of the eggs was appreciably reduced during this time.

Natural outbreaks have been reported in chickens and pheasants (18). Experimental transmission has been successful in the chicken, pheasant, turkey poult, young pigeon, young guinea fowl, and duckling (20). Laboratory mammals have proven refractory to experimental infection (8), (20).

The onset of the disease usually occurs during the first three weeks of life, although Taylor, et al. (27) and Schaaf and Lamoreux (25) have reported the incidence in producing flocks. Natural outbreaks are seasonal in nature, a major peak of incidence occurring in the spring and a minor one in the fall. The outstanding symptoms observed are ataxia and tremor, especially of the head and neck. Either or both symptoms may be present in the individual bird. Symptoms exhibited in experimentally infected birds are similar to those observed in field cases except the incubation period and tremor symptoms are reduced,

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1.Dr. J. F. Crawley, Head Veterinary Section, Connaught Medical Research Laboratories, University of Toronto, Toronto 4, Canada, personal communication.

2.Dr. J. S. Glover, Veterinary Extension Officer, Ontario Veterinary College, Guelph, Canada, personal communication.

while infectivity, mortality and atactic symptoms are progressively increased by repeated passage. Recent observations (25), (27) indicate that a subclinical outbreak may occur in laying flocks. This symptom, which may be overlooked unless careful production records are kept, is a brief period in which birds stop laying or produce at a reduced rate.

Transmission experiments led Jones (10) to state that the intracerebral route of inoculation was the most effective for experimental infection. Experimental transmission trials by the peripheral routes has given variable results (14), (20). Observations and experimental evidence point toward the probability of dam to chick infection through the egg. Experiments to determine the possibility of contact infection, and observation of field outbreaks, have prompted some workers to conclude that contact infection does not occur or that the possibility is "very limited" (10), (14). Other workers, (18), (25), (30) however, suspect contact to be an important factor in the spread of the disease.

Lesions are microscopic in nature and consist of inflammatory, degenerative and proliferative changes in the central nervous system (CNS) and focal hyperplasia of lymphoid elements in organs other than the CNS (14).

A tentative laboratory diagnosis of AE is routinely made from history, symptoms and general absence of gross lesions. Diagnosis is verified by the demonstration of characteristic histopathological changes in the selected specific organs and

or/and the intracerebral inoculation of young chicks with brain suspension prepared from affected fowl. Serum neutralization tests have been suggested as an aid in making a definite diagnosis (20).

Most workers report little success for managing an outbreak in a given flock, other than improving sanitary conditions, removing and nursing affected birds to prevent starvation, and selecting strains resistant to the virus. Taylor, et al. (27) indicated that affected hens were capable of passing the virus to offspring for a very brief period and that discarding eggs for hatching during this period may be a safeguard against outbreaks in chicks. Application of this control measure would be possible only when careful production records are available. One worker (25) reported success in vaccinating pullets before their first laying season, and adults during their first molt period.

The present study was initiated to determine whether the various symptoms exhibited in AE infection could be correlated with the microscopic tissue changes produced. To facilitate the development of a serological diagnostic procedure, experiments were directed toward the adaptation of the virus of AE to laboratory animals.

REVIEW OF LITERATURE

History

Jones (9) reported the results of her examination of young

chickens from four outbreaks of a malady which she termed "an encephalomyelitis in chickens". These first outbreaks occurred in Massachusetts. Later she (10) investigated epizootics in four New England states and designated the disease "epidemic tremor". Van Roekel, et al. (30) preferred the trinomial name "infectious avian encephalomyelitis". In their experience the occurrence of tremor was low. The Committee on Poultry Disease Nomenclature of the American Veterinary Medical Association (26) adopted the binomial form "avian encephalomyelitis". In 1939 Doyle (3) reported the disease in Indiana. An outbreak was reported in Australia in 1940 (8). By 1942 outbreaks of AE were recorded in New York, New Jersey, Delaware, Colorado, Georgia, Tennessee, and Florida (14). A survey by Feible, et al. (4) in 1951 showed that of the 43 states reporting, 36 had diagnosed AE. A check of literature failed to reveal the incidence of AE in Canada. Correspondence from Dr. J. F. Crawley[1] stated that it is on the increase in Canada and that it is now one of the more important virus infections of baby chicks. Dr. J. S. Glover[2] indicated the disease was quite common in Ontario during the fall of 1955.

Characteristics of the Virus

Filtration and bacteriological studies indicated that the causative agent was a virus possessing neurotropic properties. Physical and nutritional stress factors exerted minimal in-

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1 Crawley, op. cit.
2 Glover, op. cit.

fluence (10). Similar results of nutritional influence and effect were reported by Jungherr and Minard (14). The results of filtration, centrifugation and serological tests indicated that the virus particle was in the range of 20 to 30 millimicrons, was not sedimented at 12,000 rpm for one hour and was not antigenically related to Eastern and Western strains of equine encephalomyelitis. Van Roekel, et al. (30) agreed that the agent was a filterable virus but noted that filtration reduced the infectivity of brain suspensions.

Symptomatology

According to Jones (9) characteristic symptoms of the disease were tremor of head and neck followed by ataxia. One or both symptoms frequently occurred in an individual chick. Tremor was usually the first symptom. Van Roekel, et al. (30) agreed that tremor and ataxia were frequent symptoms but observed that the tremor symptoms were less common than and usually followed ataxia. A summary of the syndrome of symptoms described by them is as follows: (1) Symptoms sometimes developed within 24-48 hours after hatching but usually appeared during the first and second week. (2) A dull expression of the eye accompanied by incoordination and progressive ataxia, was usually the first noticeable symptom. (3) Birds sat on their haunches. (4) Some birds exhibited head and neck tremor, the frequency and duration of which was variable. (5) Incoordination and ataxia usually, but not always, preceded tremor symptoms. (6) The course was often prolonged, but birds were often normal one day

and became semicomatose the next. Jungherr and Minard (14) reported that there were "clonic" and "atactic" or "paretic" forms which appeared with the following frequency in their studies: 20.2 per cent tremors only, 40.8 per cent paresis only, and 39 per cent showed both tremor and ataxia. As symptoms progressed complete leg paralysis often occurred, producing an attitude of lateral recumbency. Death was often due to starvation. Taylor, et al. (27) reported an outbreak in a laying flock in which over 75 per cent of the birds decreased in rate of lay, or paused, for a short period. Personal records (22) revealed a similar history in three field outbreaks. AE was diagnosed in young chicks which originated from one hatchery. The history of these outbreaks revealed a noticeable drop in egg production which coincided with the hatches exhibiting AE symptoms. Information received from the hatchery management indicated that the hatchability was low during this period.

Pathology

Jones (9) stated that the microscopic lesions from field cases of AE were found throughout the central nervous system (CNS) and viscera. She considered the focal collection of glial cells throughout the brain and spinal cord to be characteristic. Macroglia and oligodendroglia with occasional microglia cells were the constituents of these foci. Numerous mitotic figures indicated the proliferous nature of these lesions. These lesions were less prevalent in the cerebellum. Jungherr (12) made a study of these focal processes by special metalic impreg-

nation and reported that most of the infiltrating cells failed to exhibit neuroglial processes. Small accumulations of glial cells, which were more prevalent in chronic cases, were reported by Olitsky (19). Tyzzer and Sellards (28) considered the regular occurrence of collections of glial cells of various types to be of diagnostic importance.

Perivascular cuffing was noted in the original description of the disease by Jones (9). Jungherr and Minard (14) observed that even in small, recognizable lesions lymphoid infiltration occurred in the walls and Virchow-Robin spaces of the capillaries with no special tendency for the lesions to remain confined to the latter. Extraordinary development of the perivascular lesions were noted in brain but not in the spinal cord. Vascular changes were not well developed.

Degeneration of the nerve cells of the CNS was observed by Jones (9), (10), both in natural and experimental AE cases. Neuronal degeneration throughout the CNS was listed by Olitsky (19) as being the most striking and consistent lesion. Changes were most prominent in the brain stem, medulla, and the anterior horn cells of the spinal cord, especially in the lumbo-sacral region. The neuron and its nucleus were first swollen, followed by various stages of nuclear displacement. A clearing process began by the formation of a Nissl body halo around the periphery of the perikaryon. As the granules continued to disappear the cells assumed eosinophylic properties until finally they appeared as a pink or red mass, or even disappeared entirely. Reports differed as to Purkinje cell changes but Olitsky and Van Roekel (20)

stated that the extent of damage to these cells is dependent upon the duration of the infection. Minor non-specific lesions have been reported to occur in the peripheral nervous system. Occasional development of demyelinization of the sciatic nerve was reported by Jungherr (12). Olitsky (19) did not observe this condition but did detect occasional neuronal degeneration in the spinal ganglia. Mathey (18) reported an outbreak of AE in pheasants in which the brain lesions consisted of perivascular infiltration and neuronal degeneration.

Visceral lesions, consisting of regular and irregular areas of infiltration, were reported by Jones (9). The infiltrating cells appeared to be lymphoblasts with many mitotic figures and pyknotic nuclei. Lesions were more prevalent in the pancreas and heart but were also present in the liver, spleen, kidney, testis, and other organs. Olitsky (19) described the visceral lesions as small, hyperplastic, lymphoid islands composed of lymphocytes with a few monocytes and myelocytes, and cellular debris. Neither elementary nor inclusion bodies were found. Spontaneous cases reported by Jungherr (12) exhibited lesions consisting of nodular and diffuse accumulations of lymphoblast-like cells, many of which presented mitotic figures. These foci resembled normal lymphoid follicles but were increased in number. The presence of these lesions in the ventriculus were considered diagnostic, but were not significant in the myocardium unless they were of the compact circumscribed type because loose extramedullary myelopoietic foci are commonly found in the normal heart. Jungherr and Minard (14) stressed the fact that normally

occurring lymphoid follicles found throughout avian tissue should not be confused with the pathological state. Changes in these follicles resulting from AE infection were of two types; they either had an irregular outline with no definite boundary, or they were oval or circular in shape and were surrounded by capillarized membrane. In order of decreasing diagnostic importance, the affected organs were ventriculus, pro ventriculus, pancreas, heart, striated muscle, spleen, liver, kidney, adrenal, gonads and intestines.

Jungherr (11), (12) observed that the presence of mononuclear infiltrations in the liver, pancreas, cerebellum and cerebrum, but not in the sciatic nerve, helped differentiate AE from a number of other conditions causing paralysis in chickens. Olitsky and Van Roekel (20) stated that the malady may pass clinical recognition, or on the other hand, may be confused with other conditions producing nervous symptoms. Differentiation from equine encephalomyelitis is chiefly by animal inoculation and serum neutralization (SN) tests. AE may be distinguished from the nervous form of avian pneumoencephalitis by SN tests and by the characteristic changes in nervous tissue and lymphoid tissue hyperplasia noted in AE, which in turn does not produce respiratory lesions. Tissue changes reported in nutritional disturbances such as rickets and nutritional encephalomalacia assist in making a differential diagnosis. Salmonellosis of chickens and coccidiosis can be determined by bacteriological study. Transmission studies were useful in eliminating a condition called cage paralysis. In differentiating AE from lymphomatosis galli-

narum it should be remembered that the former occurs in the absence of tumor formation, enlargement of the dorsal root ganglia, peripheral neuritis, iritis, and involvement of the intestinal tract. Diagnostic histological signs were reviewed but it was emphasized that specific diagnosis depended upon isolation of the virus and specific SN tests. Feibel, et al. (4) was able to produce both gross and microscopic iridocyclitis in chicks by intraoccular inoculation of AE virus.

Reports by West (32) indicated that a variety of CNS symptoms were associated with bacterial and mycotic osteomyelitis of the spongy portions of the bones enclosing the brain of chickens and turkeys. There was a striking similarity of symptoms observed in chicks suffering from this condition and those affected with AE. Diagnosis was made on the basis of lesions produced and isolation and identification of the various organisms incriminated. Diagnosis of these cases was complicated due to the fact that tests showed some of the birds were concurrently infected with AE or New Castle disease. Diagnosis was made on the basis of lesions produced and isolation and identification of the various organisms incriminated. Gessellchen (5) mentioned observing an occasional case of fowl cholera which involved the spongy bone about the brain. The symptoms produced were similar to the nervous form of New Castle disease.

Immunological Studies

Immunization studies by Olitsky (19) led him to conclude that an attack of the experimentally induced disease led to the devel-

opment of resistance to reinoculation and of antibodies in the serum. In their neutralization studies Jungherr and Minard (14) found that normal serum failed to neutralize decimal virus dilutions of from 10^{-2} to 10^{-4}. Neutralizing antibodies for virus dilutions of 10^{-4} to 10^{-5} were demonstrated in the serums of natural and experimental convalescent chicks 50 to 60 days old. They stated that although serum from convalescent chicks possessed weak specific neutralizing antibodies the SN test in the present form was not suitable for detecting the carrier state. Schaaf and Lamoreux (25) reported the following observations and experimental results concerning the immunological aspects of the AE virus:

> During an outbreak of avian encephalomyelitis (AE) in 1949-1950, it was observed that high mortality occurred only among the progeny of the youngest generation of breeding hens. This suggested that the older hens had had the disease previously, had developed immunity to it and, therefore, were incapable of transmitting AE through their eggs to the chicks.
>
> Since that time, there has been no serious recurrence of the disease among baby chicks under conditions where:
>
> 1) the survivors of the 1949-1950 outbreak of AE have been retained as breeders,
>
> 2) the live virus of AE has been systematically distributed to young chickens before they reach maturity and to older ones at the time of their annual molt,
>
> 3) the disease is known to be present in active form as shown by the demonstration of clinical signs by a few of the inoculated chickens and by recovery of AE virus from them, and
>
> 4) eggs have been hatched every week of each year so that no important recurrence of AE could have passed unnoticed.
>
> In two different experiments, young chicks inoculated in the wing web with 25 chicken lethal dose $_{50}$ (c.l.d.)

quantities of AE virus later suffered less than half the mortality observed among controls following intracerebral challenge with 250 c.l.d.$_{50}$ quantities of the virus.

These results indicate that AE can be satisfactorily controlled by the vaccination of immature chickens with the live virus which causes the disease, thereby enabling them to develop a useful degree of immunity to the disease before their eggs are used for hatching.

MATERIALS AND METHODS

Sources of Virus

Brain was aseptically removed from chicks sent to the laboratory from various areas of Kansas and surrounding states for diagnosis. History and symptoms were compatible with that of AE. The brain material was triturated in physiological saline by "shaker bottle" technique (4), (15), as described on page 14, to produce a ten per cent suspension. Day old chicks, obtained from a local hatchery, were inoculated intracerebrally with 0.03 milliliter of brain suspension. Due to the relatively low attack rate and the prolonged incubation period, which are frequently encountered in recent virus isolates, further passages were not attempted. Highly pathogenic strains were obtained from Dr. M. C. Morrissette[1] and Dr. Kermit Schaaf[2] for the remainder of these studies.

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1 Dr. M. C. Morrissette, Department of Physiology and Pharmacology, School of Veterinary Medicine, Oklahoma Agricultural and Mechanical College, Stillwater, Oklahoma. This virus suspension was labeled: "A37020, our 15th passage of AE virus (Van Roekel strain) 10^{-1} dilution of chick brain; spun down after being thawed." Passages of this virus used in this experiment were prefixed by the letters VR.

2 Dr. Kermit Schaaf. This virus was labeled "Avian Encephalomyelitis Virus Pass. 35. Nov. '55. Kimber Farms, Inc., Niles, California."

Preparation of Suspensions

A "shaker bottle" technique similar to that described by Kehnke (15) and Feibel, et al. (4), was used to prepare the stock suspensions. To a screw top flint glass bottle containing beads, the brain material and physiological saline were added in quantities to produce a one to ten brain-saline dilution on a weight-volume basis. This mixture was shaken manually until the brain material was triturated. This trituration process was facilitated by freezing after a short period of shaking and then re-triturating the frozen suspension. The suspension was then centrifuged for 15 minutes at 3,000 rpm, the supernatant removed and stored in a commercial freezer at -4° C until needed. Suspensions were checked for the presence of bacterial contamination by inoculating blood ager plates and thioglycollate broth with 0.5 milliliter of the suspension.

Sources of Experimental Animals

Day old Leghorn-white rock cross chicks, obtained from a local hatchery, were used for the two series in which symptomatology and histopathology were compared, and chicks from this source were also used for part of the mouse-chick series. White Leghorn cockerels hatched by the Kansas State College poultry farm were used for the remaining mouse-chick series. Adult white rock hens, which had been used as controls in a previous nutritional experiment, were used for production of hyper-immune serum and adult susceptibility studies. A colony of white mice was established in the laboratory to supply young mice for mouse

adaptation studies. Breeding stock for this colony were obtained from the department. A latent virus infection was not demonstrated in either the breeding stock or experimental mice.

Methods of Inoculation

Stock brain suspension was removed from freezer and rapidly thawed previous to use. Using a one-half milliliter Luer syringe fitted with a one-half inch 27 gauge needle, 0.03-0.05 milliliter dose of the inoculum was injected intracerebrally into young chicks. A large number of the birds showed symptoms of shock for a few hours after inoculation, but mortality was less than one per cent. The size of the dose appeared not to be a factor in the severity of the temporary ill effects produced. The chicks were placed in wire cages and isolated as well as facilities permitted during the experimental period. Controls were injected intracerebrally with 0.05 milliliter of physiological saline.

Attempts to produce hyperimmune serum by injections of one milliliter doses of brain suspension in the wing vein of adult chickens produced anaphylactoid symptoms and death in all of the three birds injected by this route. Intraperitoneal injection of the same suspension at the above dosage produced no immediate observable symptoms.

Procedure similar to that described for chick inoculation was followed in the mouse studies. Litters of one to seven day-old mice were used. The virus dosage varied from 0.03-0.05 milliliter. One series of these mice was also injected intra-

muscularly, at the time of inoculation, with 35-75 micrograms of cortisone acetate to investigate the influence of the steroid upon the virus adaptation and pathogenicity. The cortisone acetate treatment was altered in a second series by administering intramuscularly a 75 microgram dose 12 hours previous to inoculation, followed by a 35 microgram dose 12 hours after inoculation. When size of litter permitted, controls were maintained, half of which received intramuscularly the above dosage of cortisone acetate and intracerebrally .05 milliliter of physiological saline. The other half received the above dosage of saline only. Four serial mouse to mouse passages and three zigzag mouse to chick passages have been completed.

Tissue Preparation

Studies were conducted to determine the possibility of correlating the symptomatology with the microscopic changes produced in experimentally infected birds and a comparison of the histopathology of experimental with that of naturally infected chicks. Two groups of day-old chicks, consisting of 25 chicks per group were inoculated intracerebrally as described above. Ten controls were maintained for each series. Tissue for section was collected from all inoculated chicks and from two normal controls from each series. Corresponding tissue sections were also obtained from selected field cases submitted to the diagnostic laboratory. Brain, spinal cord, eye, pancreas and liver were routinely collected for microscopic study. These tissues were identified and fixed in 10 per cent buffered form-

alin. After a minimum fixation period of 24 hours the tissue was removed and areas of each organ prepared for histopathological study. The brain was sectioned at four different levels. (See photograph in Fig. 1.) Level "a" was a transverse section through the middle of the anterior quarter of the cerebrum. Level "b" transversed the middle of the posterior quarter of the cerebral lobe dorsally and the floor of the third ventricle just posterior to the infundibulum ventrally. Level "c" was a segment through the middle of the anterior half of the cerebellum, the posterior portion of the optic lobe, and the anterior part of the pons-medulla. Level "d" transversed the posterior portion of the cerebellum and that part of the medulla oblongata just anterior to the origin of the vagus nerve. Transverse sections were made of the spinal cord at three different levels. One section was taken near the center of the cervical region between the sixth and eighth cervical vertebrae. A thoracic section was taken between the fifth and sixth vertebrae. A third section was made through the lumbar region between the first and the third vertebrae just anterior to the sinus in the lumbar cord. The site selected for the examination of the eye was a plane transversing the middle of the lens anteriorly and the middle of the optic nerve posteriorly. The portion of the pancreas selected for histological studies was a cross section of both lobes near their proximal portions. Apical areas of suitable lobes of the liver were also selected for examination.

Tissues were dehydrated in alcohol, cleared in xylol and embedded in paraffin.

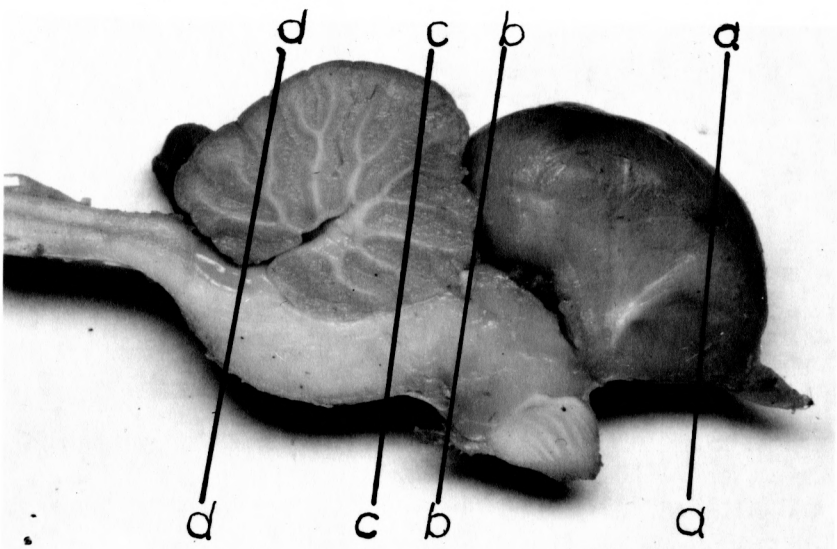

Fig. 1. Medial section of a chick brain showing levels from which histological sections were taken.

Sections from all tissues were cut at six microns and stained by the hemotoxylin-eosin and azure-eosin (17) methods. In addition, 12 micron sections of the brain and spinal cord were stained with the thionin (4) and gallocyanin techniques (6).

RESULTS AND DISCUSSION

Symptomatology

Following inoculation birds of the VR series were observed several times daily for the appearance of symptoms. Each bird was placed in an individual cage when the first observable symptom was manifested. The bird was checked several times each day for changes in symptomatology.

The incubation period ranged from 11 to 34 days. The first symptoms in 19 birds appeared in the following order: three birds on the 11th day, two on the 12th, eight on the 15th, and two on the 16th. Of the remaining four birds one exhibited symptoms on the 22nd day, one on the 24th, one on the 28th and one on the 34th day. Three of the asymptomatic birds were retained for continued observation. Two were sacrificed and tissue used for histological studies. Symptomatology was summarized in Table 1.

The most commonly detected first symptom was ataxia. It varied in degree from mild paresis to complete paralysis of the legs. Ataxia developed in all of the clinically affected birds, either as the only sign or in conjunction with other symptoms. Tremor of the head and neck occurred in four cases in this series. Three of the atactic birds were tremorous until shortly before

Table 1. Major symptoms exhibited and date first symptoms appeared.

Case number	Date of first symptom	Autopsy date	Symptoms exhibited
VR1	3-1-56	3-1-56	Respiratory symptoms
VR2	3-5-56	3-9-56	Tremor, progressive paralysis
VR3	3-5-56	3-5-56	Progressive paralysis
VR4	3-5-56	3-6-56	Progressive paralysis
VR5	3-6-56	3-9-56	Tremor, progressive paralysis
VR6	3-6-56	3-9-56	Tremor, progressive paralysis
VR7	3-9-56	3-11-56	Progressive paralysis
VR8	3-9-56	3-9-56	Progressive paralysis
VR9	3-9-56	3-9-56	Progressive paralysis
VR10	3-9-56	3-9-56	Progressive paralysis
VR11	3-9-56	3-10-56	Progressive paralysis
VR12	3-9-56	3-10-56	Progressive paralysis
VR13	3-9-56	3-10-56	Progressive paralysis
VR14	3-9-56	3-10-56	Progressive paralysis
VR15	3-10-56	3-15-56	Progressive paralysis
VR16	3-10-56	3-18-56	Progressive paralysis
VR17	3-16-56	3-28-56	Tremor, progressive paralysis
VR18	3-18-56	3-19-56	Progressive paralysis
VR19	3-22-56	3-26-56	Progressive paralysis
VR20	3-28-56	3-28-56	Progressive paralysis
VR21			Asymptomatic
VR22			Asymptomatic
VR23			Asymptomatic
VR24		3-29-56	
VR25		3-29-56	

1. These chicks were inoculated 2-23-56.

death or until they were sacrificed. One became comatose and failed to show tremor symptoms after the first day. The lowered incidence of tremor, as compared to that in some reports can be partially explained by the fact that the virus used had been artificially passed a number of times previous to this experiment.

Somnolence and depression were occasionally the first symptoms observed, and were constantly present in advanced stages of the disease. Frequent periods of hoarse chirping and restlessness were characteristic of advanced paralytic cases.

The appetite remained good and attempts to eat and drink were made by all birds until they became comatose. Tremor symptoms did not handicap the birds in their efforts at feeding and drinking.

It was impossible to obtain complete data concerning the course of the disease in this series but the eight which were allowed to die did so within a period of one to five days. In the remainder of the series birds were affected for as long as 12 days before they were sacrificed. All of the latter group were exhibiting advanced symptoms when sacrificed.

The frequency of occurrence of the major classes of symptoms were as follows: ataxia 64 per cent, ataxia and tremor 16 per cent, and asymptomatic 20 per cent. As mentioned above this series failed to produce cases in which tremor was the only outstanding symptom.

Histological Changes

Lesions in the VR series were microscopic in character except

that a definite hydrocephalus was detected in 44 per cent of the birds. When the calvaria of chick VR4 was opened an excess of fluid was detected in the cavity. Further investigation revealed that the peripheral wall of the lateral ventricles was thin and that it had ruptured. It appeared to have been greatly distended previous to rupture. Careful removal of the calvaria of the remaining cases revealed that a total of 11 birds exhibited either a gross external or internal hydrocephalus or both. Nine of these brain showed considerable distention of the lateral ventricle and an excess of fluid under the meninges. In two cases there was a detectable increase in fluid under the meninges without noticeable distention of the lateral ventricles. When the lateral ventricles were affected it appeared as a fragile membraneous fluid filled pouch protruding from the posterior extremity of the cerebral lobe. Bilateral involvement was more common, but unilateral distention was found in two cases. Where external hydrocephalus was pronounced the gyra of the cerebellum became flattened and indistinct.

The extent of microscopic changes was determined by examining sections from seven levels of the CNS, the eye, liver and pancreas. A description of the levels and preparation methods was given under materials and methods. It became evident after detailed study of the sections that a system of recording and classifying results was necessary. The system shown in Table 2 was sufficiently wide in range to include all prominent lesions observed. To prevent misinterpretation the lesions listed were limited to commonly recognized classes (7). It was impossible

to include histological data on all cases in this series. Three birds were retained for extended observation. Tissues from five cases were judged unsatisfactory for histological study and were discarded. Details concerning the various classes of histological changes found in the CNS of 17 birds are recorded in Table 2.

Vascular changes of various degrees were most pronounced and constant in the cerebral lobes and the cerebellum. The extent of these lesions varied with the cases and with the different levels of the brain and cord in the individual case. Reports (14) concerning vascular changes in the spinal cord indicate that they were either mild or absent. In this study the vascular lesions were mild to extensive and observed in one or more levels of the spinal cord in all cases. These lesions were constant and extensive in the lumbar region.

Neuronal degeneration of advanced stages were manifested constantly in the spinal cord. Changes were slightly more advanced in the lumbar region than at other levels. Degeneration was only slightly less advanced in the medulla and the pons. Changes were also found in other areas of the brain in decreasing degree as follows: optic lobe, cerebellum and thalmus, and cerebrum.

Gliosis surrounding perivascular accumulations of lymphoid elements was a constant finding. Infiltration of glial and microglial cells in areas of neuronal degeneration were highly variable as to incidence and extent.

Sections of the visceral organs in this series revealed no lesions comparable to those described as being characteristic in

Table 2. Histopathology exhibited by individual birds at various levels of the central nervous system.

Class of lesion	Specific lesions at each level examined: Cerebrum "a"	Cerebrum "b"	Thalmus	Cerebellum "c"	Optic Lobe	Pons	Cerebellum "d"	Medulla	Lumbar cord	Thoracic cord	Cervical cord
Chick VR2											
Vascular	H,VCL&G3	H,VCL&G3	H,VCL&G3	VCL&G3	VCL&G3	VCL&G3	H,VCL&G3	VCL&G3	VCL&G3	H,VCL&G2	H,VCL&G3
Neuronal	N1,2,3,4,5,6	N1,2,3,4,5,6	N1,2,3,4,5,6	P1,2,3,4,5,6	N1,2,3,4,5,6	N1,2,3,4,5,6	P1,2	N1,2,3,4,5,6	N1,2,3,4,5,6	N1,2,3,4,5,6	N1,2,3,4,5,6
Glial	G2,3	G2,3	G2,3	G3	G2,3	G2,3	G2,3	G2,3,4	G2,3	G2,3	G2,3
Chick VR4											
Vascular	H,VCL&G3	H,VCL&G3	H	VCL&G3	VCL&G3	VCL&G3	VCL&G3	VCL&G3	H,VCL&G1	H,VCL&G1	H
Neuronal	N1,2	N1,2	N1,2	P1,2	N1,2	N1,2	P1,2	N1,6	N1,2,3,4,5,6	N1,2,3,4,5,6	N1,2,6
Glial	G3	G3	G3	G3	G2,3	G2,3	G3	G3	G2,3	G2,3	G2,3
Chick VR6											
Vascular	EHI VCL&G2	EHI VCL&G2	EHI VCL&G2	AH VCL&G2	VCL&G1	VCL&G1		H	H	H	H
Neuronal	N1,2,6	N1,2,6	N1,2,6	P2,6	N1,2,6	N1,2,6	P1,2	N1,2,5,6	N1,2	N1,2	N1,2
Glial	G3	G3	G3	G3	G3	G1,2		G3			
Chick VR7											
Vascular	VCL&G3	VCL&G3	VCL&G3	VCL&G3	VCL&G3	VCL&G3	VCL&G2	VCL&G3	H,VCL&G1	H	
Neuronal	N1,2	N1,2	N1,2	P1,2,5,6	N1,2,5,6	N1,2,3,4,5,6	P1,2,5,6	N1,2,3,4,5,6	N1,2,3,4,5,6	N1,2,3,4,5,6	N1,2,3,4,5,6
Glial	G3	G3	G3	G2,3	G1,3	G2,3	G2,3	G2,3	G2,3	G2,3	G2,3
Chick VR8											
Vascular	EHI,AHI							EH			H
Neuronal	N1			P1	N1	N1,2	P1	N1,2,5,6	N1,2,3,4,5,6	N1,2,3,4,5,6	N1,2
Glial								G1	G1,2,3	G1	G1
Chick VR9											
Vascular	H,VCL&G3	VCL&G3	VCL&G3	H	AH,EH, VCL&G3	AH,EH, VCL&G3	VCL&G3	VCL&G1			EH, VCL&G2
Neuronal	N1,2	N1,2	N1,2	P1,2	N1,2,6	N1,2,6	P1,2,6	N1,2,3,4,5,6	N1,2,3,4,5,6	N1,2,3,4,5,6	N1,2,3,4,5,6
Glial	G3	G3	G3	G3	G3	G3	G3	G3	G1,2,3,	G1,2,3	G1,3
Chick VR11											
Vascular	H,EH, VCL&G2	H,EHI, VCL&G3	H,EHI, VCL&G3	VCL&G2	VCL&G3	VCL&G3	VCL&G3	VCL&G2	VCL&G2	VCL&G3	VCL&G1
Neuronal	N1,2,6	N1,2,6	N1,2,3,4,5,6	P1,2,3,4,5,6	N1,2,3,4,5,6	N1,2,3,4,5,6	P1,2,3,4,5,6	N1,2,3,4,5,6	N1,2,3,4,5,6	N1,2,3,4,5,6	N1,2,3,4,5,6
Glial	G3	G1,2,3	G1,2,3	G3	G1,2,3	G1,2,3	G1,2,3	G1,2,3	G1,2,3	G1,2,3	G1,2,3

Table 2. (cont.)

Class of lesion	Specific lesions at each level examined: Cerebrum "a"	Cerebrum "b"	Thalmus	Cerebellum "c"	Optic Lobe	Pons	Cerebellum "d"	Medulla	Lumbar cord	Thoracic cord	Cervical cord
Chick VR12											
Vascular	EHI,AHI, VCL&G1	EH, VCL&G3	EH,AH VCL&G1	EH,AH		VCL&G1	VCL&G1	H	H,EH	H,EH	H,EH
Neuronal	N1,2	N1,2	N1,2,6	P1,2,6	N1,2,3,4, 5,6	N1,2,3,4, 5,6	P1,2,6	N1,2,3,4, 5,6	N1,2,3,4, 5,6	N1,2,3,4, 5,6	N1,2,3,4, 5,6
Glial	G3	G3	G3	G3	G3	G3	G3	G1,2,3	G1,3	G1,3	G1,3
Chick VR13											
Vascular	EHI,AHI, H,VCL&G1	EHI,AHI, H,VCL&G1	EHI,AHI, H,VCL&G1	HVCL&G3	EHI, H,VCL&G3	EHI H,VCL&G3	H,VCL&G3	H,VCL&G3	H,VCL&G3	H	H
Neuronal	N3,4	N3,4	N3,4	P1,6	N1,2,3,4, 5,6	N1,2,3,4, 5,6	P1,2,3,4, 5,6	N1,2,3,4, 5,6	N1,2,3,4, 5,6	N1,2,3,4, 5,6	N1,2,3,4, 5,6
Glial	G1,2,3,4	G1,2,3,4	G1,2,3,4	G3	G3	G1,3	G3	G1,2,3	G1,4	G1,4	G1,4
Chick VR14											
Vascular	H,EHI, VCL&G1	H,EHI, VCL&G1	AHI, VCL&G1	VCL&G1	VCL&G1	VCL&G1	H,EHI, VCL&G3	H,EHI, VCL&G3	H	H	H
Neuronal	N1,2,3,4 5,6	N1,2,3,4, 5,6	N1,2,3,4, 5,6	P1,2,6	N1,2,3,4, 5,6	N1,2,3,4, 5,6	P1,2	N1,2,3,4, 5,6	N1,2,3,4, 5,6	N1,2,3,4, 5,6	N1,2,3,4, 5,6
Glial	G1,2,4	G1,2,4	G1,2,4	G3	G1,2,3	G1,2,3	G1,3	G1,2,3	G1,3	G1,3	G1,3
Chick VR15											
Vascular	H,VCL&G1	H,EH, VCL&G3	H,EH, VCL&G3	H,VCL&G1	H,VCL&G3	H,VCL&G2	H	H,VCL&G2	H	H,VCL&G3	H,VCL&G2
Neuronal	N1,2,6	N1,2,6	N1,2,3,6	P1	N1,2,6	N1,2,6	P1,2,6	N1,2,3,4, 5,6	N1,2,3,4, 5,6	N1,2,3,4, 5,6	N1,2,3,4, 5,6
Glial	G1	G1	G1,2		G1,3	G1,3	G3	G1,3	G1,3	G1,3	G3
Chick VR16											
Vascular	VCL&G3	VCL&G3		VCL&G2	EH	VCL&G2	VCL&G1	VCL&G1			
Neuronal	N1,2,6	N1,2,6	N1,2,6	P1,2,3,4, 5,6	N1,2,5,6	N1,2,3,4, 5,6	P1,2,6	N1,2,3,4 5,6	N1,2,3,4, 5,6	N1,2,3,4, 5,6	N1,2,3,4, 5,6
Glial	G3	G3	G1,3	G3	G3	G1,2,3	G1,3	G1,2,3	G1,2,3	G1,2,3	G1,3
Chick VR17											
Vascular	EH,VCL&G3	VCL&G3	VCL&G1	VCL&G3	VCL&G3	VCL&G3	VCL&G3	VCL&G3	VCL&G3	H	VCL&G1
Neuronal	N1,2,5,6	N1,2	N1,2	P1,2 3,4, 5,6	N1,2,3,4, 5,6	N1,2,3,4, 5,6	P1,2,3,4, 5,6	N1,2,3,4, 5,6	N1,2,3,4, 5,6	N1,2,3,4, 5,6	N1,2,3,4, 5,6
Glial	G3	G3	G3	G1,3	G3	G3	G1,3	G3	G1,2,3	G1,2,3	G1,2,3
Chick VR18											
Vascular	H,EHI,AHI, VCL&G3	H,EHI,AHI, VCL&G3	H,EHI,AHI, VCL&G3	VCL&G3	VCL&G3	EH,VCL&G3	VCL&G3	VCL&G3			
Neuronal	N1,2,6	N1,2,6	N1,2,6	P1,2,6	N1,2,3,4, 5,6	N1,2,3,4, 5,6	P1,2,3,4, 5,6	N1,2,3,4, 5,6	N1,2,3,4, 5,6	N1,2,3,4, 5,6	N1,2,3,4, 5,6
Glial	G1,3	G1,3	G1,3	G1,3	G1,2,3	G1,3	G1,3	G1,3	G3	G3	G3

Table 2. (concl.)

Class of lesion	Specific lesions at each level examined: Cerebrum "a"	Cerebrum "b"	Thalmus	Cerebellum "c"	Optic Lobe	Pons	Cerebellum "d"	Medulla	Lumbar cord	Thoracic cord	Cervical cord
Chick VR19											
Vascular	EH,VCL&G3	EH,VCL&G3			EH,VCL&G3	EH,VCL&G3	VCL&G3	VCL&G3	H,VCL&G1		VCL&G1
Neuronal			N1,2		N1,2	N1,2	P1,2,3,4,5,6	N1,2,3,4,5,6	N1,2,3,4,5,6	N1,2,3,4,5,6	N1,2,3,4,5,6
Glial	G1,2	G1,2			G3	G3	G3	G1,2,3	G3	G1,3	G1,3
Chick VR20											
Vascular	H,AHI VCL&G3	H,AHI,EHI, VCL&G1		VCL&G2	H	H,AH, VCL&G1	VCL&G2	H		H,EH	H,EH
Neuronal	N1	N1	N1	P1,2	N1,2,3,4,5,6	N1,2,3	P1,2	N1,2,3,4 5,6	N1,2,3,4,5,6	N1,2,3,4,5,6	N1,2,3,4,5,6
Glial					G1,3	G1,2,3		G1,2,3		G3	
Chick VR24											
Vascular	H,EHI,AHI, VCL&G3	H,EHI,AHI, VCL&G3	H				H,VCL&G1	EH,VCL&G2	VCL&G1	EHI	
Neuronal	N1,2,6	N1,2	N1,2,6				P1,2	N1,2,3,4,5,6	N1,2,3,4,5,6		N1,2,3
Glial	G3	G3	G1,3					G3	G1,2,3		G3

Vascular changes
- VCL..perivascular cuffing, lymphoid
- VCG..perivascular cuffing, glial
- EH...endothelial hyperplasia
- EHI..endothelial hyperplasia and infiltration of vascular wall
- AH...adventitial hyperplasia
- AHI..adventitial hyperplasia and infiltration of vascular wall
- H....hyperemia

Numerals 1, 2 or 3 following VC indicate mild, moderate or extensive degrees of vascular cuffing

Neuronal degeneration
- N1..swelling of cell body, known as acute disease of Nissl
- N2..swelling of cell with liquefaction, or acute cell disease of Nissl
- N3..Sclerosis of the cell body (cell shrinkage) indicating chronic stage of cell disease
- N4..axonal reaction, secondary to lesions of the axon
- N5..cell phantoms or shadows with only remnants of cell bodies remaining
- N6..vacuoled cells

Purkinje cell degeneration
- P1..swelling of cell body, known as acute disease of Nissl
- P2..swelling of cell with liquefaction, or acute cell disease of Nissl
- P3..Sclerosis of the cell body (cell shrinkage) indicating chronic stage of cell disease
- P4..axonal reaction, secondary to lesions of the axon
- P5..cell phantoms or shadows with only remnants of cell bodies remaining
- P6..vacuoled cells

Neuroglial changes
- G1..satellitosis without neurophagia
- G2..Neurophagia
- G3..Focal collections of glial and microglial not associated with neuronal degeneration and vascular lesions.
- G4..Diffuse infiltration of glial and microglial cells not associated with neuronal degeneration and vascular changes

field cases of AE. A few lymphoid follicles were detected in most sections of the liver and pancreas but the number and extent of individual follicles did not indicate a pathological state. These results agree with those of other workers (14) who report there are little or no histological changes in visceral organs in cases infected by intracerebral inoculation. The absence of eye lesions was consistent with other reports except those by Feibel (4) who produced an iridocyclitis by intraocular inoculation.

Comparison of Symptomatology and Histological Changes

A detailed comparison of the symptomatology and the histopathology in the VR series of chicks was impossible because of high pathogenicity of the virus for this series. Comparison was further complicated due to the fact that individual birds failed to develop certain uncomplicated classes of symptoms. Since all of the clinically positive cases exhibited advanced neuronal changes in the lumbar cord, and the asymptomatic birds showed moderate changes in this region it could be concluded that sections from this level would be most desirable for histological diagnosis. It should be remembered, however, that with different virus strains and natural transmission it is possible that results would not be comparable.

Mouse Adaptation Results

Both serial mouse passage and zig-zag mouse-chick passage was attempted as an approach to adapting the VR strain of virus

to grow in mice. Favorable reports as to the adjuvant effect of cortisone acetate in adaptation of other viruses to grow in unnatural hosts made its use appear feasible in this study. A description concerning the administration of this drug was given in the section on materials and methods.

Serial passage was initiated by inoculating and treating a litter of 12 seven-day old mice, as described under section on materials and methods. This litter, which was designated as M1, was observed for a 14 day period. On the seventh day after inoculation one of the mice became depressed and died the following day. The brain was aseptically removed and a ten per cent brain-saline suspension prepared. A litter of six mice, designated as M2, was inoculated with this suspension and treated with cortisone acetate as described for litter M1 except that no controls were maintained. Ten day-old chicks, designated M1-a were also inoculated intracerebrally with 0.03 milliliter doses of the same suspension. Ten chicks from the same source were inoculated intracerebrally with 0.03 milliliter of physiological saline and designated M1-a (control).

One chick of the M1-a series showed symptoms of paresis and depression on the ninth day following inoculation. Progressive paralysis had developed by the following day when the bird was sacrificed. The brain was removed and a suspension prepared in the manner mentioned previously. Ten day-old chicks were inoculated intracerebrally with 0.03 milliliter doses of this suspension and controls injected intracerebrally with 0.03

milliliter of physiological saline. Neither the inoculated birds nor the controls exhibited any nervous symptoms for a period of 30 days. No further attempts have been made to infect chicks by inoculating with the refrigerated suspension prepared from the brain of the M1 mouse. Three additional blind passages of this material were made in mice without the appearance of nervous symptoms or death. Brain suspensions from each mouse passage was checked for the presence of virus by inoculating ten chicks with each suspension. These chicks showed no symptoms indicative of AE infection.

Three passages were completed in the zig-zag mouse-chick passage experiment. Seven-day old mice and one-day old chicks were treated and inoculated as described in the preceding experiment. Since the chicks and mice exhibited no symptoms of CNS involvment or paresis the brain of three individuals from each group were selected at random and pooled to prepare suspensions for future passage. This experiment was discontinued after the third passage failed to produce observable symptoms.

The limited trials in adapting a single strain of AE virus to mice only served to indicate the need for further experimental work. Success could conceivably result from use of other virus strains, shorter intervals between passages, and variation in dosage and type of adjuvant. A strain of AE virus adapted to grow in an unnatural host would be valuable in research procedures and could facilitate the development of a practical diagnostic test.

SUMMARY AND CONCLUSIONS

Twenty-five day-old chicks were inoculated intracerebrally with a suspension of a highly pathogenic strain of AE virus in an attempt to compare the symptomatology and the histopathology exhibited in experimental infection.

A comparison of the symptoms and microscopic lesions revealed a high degree of uniformity of symptoms and central nervous system lesions in this series. Lesions were not detected in sections from the eye, liver and pancreas. A comparison of the symptoms and lesions observed in this experiment with those produced by a strain of the virus possessing selective qualities should prove worthwhile.

A gross hydrocephalus, which has not been reported as a lesion associated with avian encephalomyelitis was encountered in 11 of the affected chicks. Progressive paralysis and coma were symptoms commonly found in cases exhibiting this lesion. Similar lesions and associated symptoms have been seen in field cases submitted to the diagnostic laboratory.

Using cortisone acetate as an adjuvant an attempt was made to adapt the VR strain of AE to grow in young mice. Chick inoculation tests indicated the possible recovery of the virus from one mouse in the first litter inoculated. Three additional serial passages failed to indicate that the virus had survived. Three alternate chick-mouse passages, in which the mice received cortisone acetate treatment, failed to demonstrate evidence of virus growth.

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CORRELATION OF CLINICAL AND PATHOLOGICAL FINDINGS OF AVIAN ENCEPHALOMYELITIS VIRUS IN YOUNG CHICKS

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In field outbreaks of avian encephalomyelitis in young chickens the most striking symptoms are ataxia or tremor of the head and neck or both. Subclinical cases, which are detected only by microscopic lesions or chick inoculation, are frequently encountered. In this experiment an attempt was made to correlate the symptomatology in intracerebrally inoculated chicks with the microscopic lesions produced. Twenty-five day-old chicks were inoculated. Nineteen of the chicks developed symptoms within periods ranging from 11 to 34 days. The course of the disease was short in most of the affected birds, ending in coma or death within 6 days or less in 17 of the 19 which exhibited symptoms.

Microscopic examination of four levels in the brain and at three levels in the spinal cord revealed a wide variety of lesions. These lesions consisted of vascular and perivascular changes, neuronal degeneration and gliosis. No pathological changes were detectable in sections from the liver, pancreas and eye.

A gross hydrocephalus, which has not been reported as a lesion associated with avian encephalomyelitis was encountered in 11 of the affected chicks. Progressive paralysis and coma were symptoms commonly found in cases exhibiting this lesion. Similar lesions and associated symptoms have been seen in field cases submitted to the diagnostic laboratory.

Using a strain of AE virus highly pathogenic for chicks, and cortisone acetate as an adjuvant, attempts were made to

serial-pass the agent in young mice. One mouse of a litter of 12 died on the 8th day following inoculation. Ten day-old chicks were inoculated intracerebrally with a bacterial free saline suspension of the brain of this mouse. One of these chicks developed paresis on the ninth day after inoculation. Attempts at further passage in both chicks and mice failed to produce nervous symptoms.