MURRAY VALLEY ENCEPHALITIS AND AUSTRALIAN X DISEASE

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(With Plates 14 and 15 and 2 Figures in the Text)

INTRODUCTION

Of the outbreaks of severe human encephalitis which have been recorded in Australia, five conformed to one clinical and epidemiological type. The disease was designated Australian X disease in 1917, 1918, 1922 and 1925 and Murray Valley encephalitis (MVE) in 1951. The causal virus was transmitted to laboratory animals during 1917, 1918 and 1925, but was not retained for later comparison with other viruses. The virus was isolated again during 1951 and identified as a new member of the arthropod-borne encephalitides, closely related to, but distinct from, Japanese B encephalitis virus.

Infection with the virus of Murray Valley encephalitis was common in humans, and domestic and wild fauna during 1951. The occurrence of the epizootic in the Murray Valley was probably related to climatic factors; its distribution in Eastern Australia was related to the geography of the country.

Throughout the relevant laboratory procedures, great use was made of the chorioallantoic membrane of the developing hen egg. This technique was employed for the original isolation of the virus, the titration of the virus, the preparation of complement-fixing antigen, and for the detection and titration of virus in vertebrate blood and in emulsions of mosquitoes. It is believed this was the first time the chorioallantois had been successfully employed in this fashion for a virus of this group.

THE RELEVANT GEOGRAPHY OF AUSTRALIA

Eastern Australia may be divided broadly into three areas—the large central arid area, very thinly populated by humans, the pastoral hinterland and the coastal belt, where the great majority of the population of 8,380,000 lived in 1951. The Great Dividing Range lies between the latter two areas. This range is continuous with the mountains of northern Queensland from which it runs south, more or less parallel to the coastline, until it reaches the Alps in eastern Victoria. The range continues to follow the Victorian coastline by turning west, finally to disappear into the low hills of central and western Victoria (Text-fig. 1.).

West of the Great Divide, a system of rivers flows south and west through New South Wales, and, as the Darling River, joins the Murray River near Mildura in north-western Victoria. The Murray River, rising in the Alps, forms the northern boundary of Victoria. It receives tributaries from northern Victoria and southern New South Wales.

Most of northern Victoria and western and central New South Wales is pastoral plain country, but the central arid region of Australia includes both the far west of New South Wales and the north-western corner of Victoria. Towns which have figured in the investigation of encephalitis are Broken Hill and Mildura. Broken Hill is a mining centre in the arid area of western New South Wales; Mildura is a vine-growing and citrus region, established by artificial irrigation, on the banks of the Murray River in north-western Victoria.

AUSTRALIAN X DISEASE, 1917 AND 1918—CLINICAL STUDIES

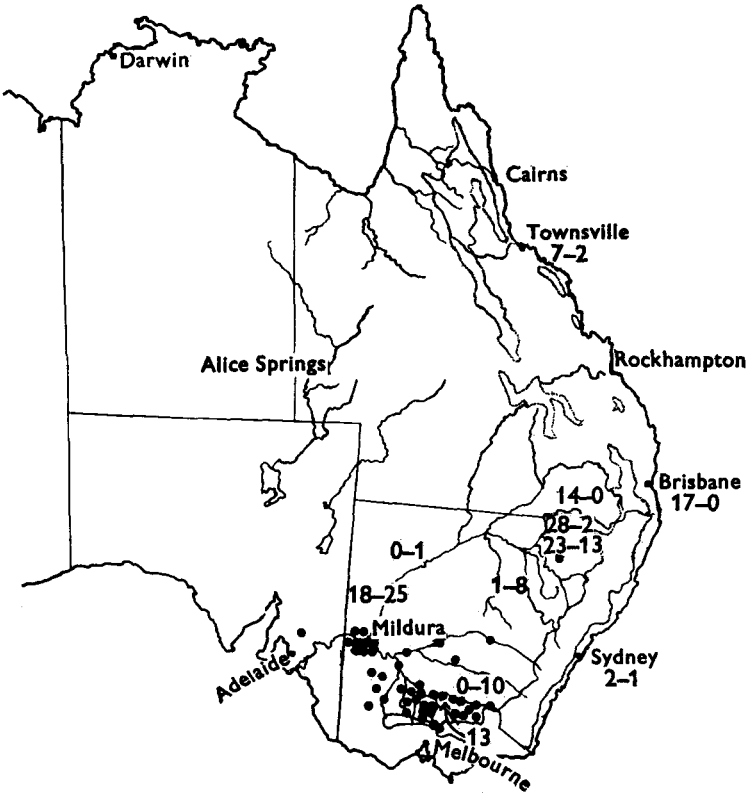
The cases of X disease in New South Wales were reported in detail by Burnell (1917), and by Cleland, Campbell & Bradley (1918); seventy-two patients were listed in 1917 and sixty in 1918. Of this total of 132 patients, ninety-four (71 %) died. Of the 124 patients whose ages were stated, ninety-two (74 %) were aged less than 15 years, and the oldest was 68 years of age. Of the 121 patients whose sex was recorded, eighty-five (70 %) were male. The month of onset was recorded for 116 patients. During 1916–17 there was one case in October 1916, one in January 1917, eleven in February, thirty-six in March and fifteen in April, making a total of sixty-four. During 1917–18 there was one case in December, six in January, thirty in February, thirteen in March and two in April, making a total of fifty-two.

The Victorian cases were referred to by Cleland *et al.* (1918), but not discussed. There were apparently thirteen so diagnosed during 1918, all in the Goulburn Valley in north central Victoria.

The Queensland cases which occurred in 1917 and 1918 were described in three groups. Breinl (1917, 1918) saw seven patients in Townsville early in 1917; the first of these fell sick on or just before 4 March; there were two more cases during March, two during April, one during May and one on 3 June. The ages ranged from 20 months to 19 years. Six were male. Five of the seven patients died. Breinl studied a further patient during December 1917, and another during January 1918. Both died. Mathewson & Latham (1917) studied seventeen patients at the Children's Hospital, Brisbane, all diagnosed as cases of X disease, between 31 March and the beginning of May 1917. The ages ranged from 21 months to 9 years. Fourteen were male. Eleven of the seventeen patients died, the deaths occurring between $3\frac{1}{2}$ and 23 days after onset. Anderson (1917) described fourteen cases of X disease in Goondiwindi, 200 miles west of Brisbane, during 1917. He saw six during February, five during March, two during April and one during May. Thirteen were aged less than 11 years, one was 38 years old. Six were male, seven female; in one case the sex was not recorded. Six died.

The geographical distribution of the cases occurring during 1917 and 1918 is shown in Text-fig. 1 (the first number of each pair indicates the number of cases during 1916–17; the second number applies to 1917–18). As Cleland *et al.* (1918) remarked regarding the cases in New South Wales, the disease was concentrated around the railway lines, but this was to be expected in view of the distribution of population in relation to this means of communication in western New South Wales. Only three cases, all doubtful as to diagnosis, were apparently infected

along the coastal belt of New South Wales, Victoria or South Australia, although the majority of the population of the three states was living in the coastal belt at that time. On this basis, Cleland *et al.* (1918) concluded that in the case of a man developing the disease while visiting Adelaide (outside the affected area) the incubation period must have been at least 2 weeks. They presumed that the geographical distribution of the disease depended in some way on the climate of the area of country affected, but as they assumed that the disease was transmitted by



Text-fig. 1. Map of eastern Australia. Each dot represents one case of Murray Valley encephalitis occurring during 1951. The pairs of figures represent the incidence of Australian X Disease, the first figure the number of cases during 1917 and the second figure the number during 1918.

'silent' human carriers, they were unable to delineate the role of climatic factors in the ecology of the disease. They did not think that an insect vector was concerned.

These authors noted as the earliest symptoms and signs, headache, drowsiness, lassitude, malaise, irritability, mental confusion, limb pains, pains in the neck, and weakness of the limbs. Headache and drowsiness were the commonest symptoms. Vomiting was a common presenting feature in children. The onset was sudden and the clinical progress rapid. The following features distinguished the full clinical picture: fever, nuchal rigidity, convulsions or localized purposeless movements in a comatose or semicomatose subject. In eight patients there were reports of paresis of limb or intercostal muscles. There was a pleocytosis in the cerebrospinal fluid in

those cases in which the appropriate test was made; nearly half the patients examined exhibited a leucocytosis, with an increase of both polymorphonuclear leucocytes and large lymphocytes. Three patients were described with a rash, but this sign was of uncertain relation to the disease.

Death or recovery was described usually during the first week of the illness. The majority of the fatal cases died between 4 and 6 days after onset and an occasional patient died as early as 24 hr. after onset. The latest death occurred on the 12th day of illness. Limb paralysis remained as a sequel in three of thirty-eight survivors in Cleland's series, and in two patients there was residual mental deterioration.

As far as can be judged from the published descriptions, the Queensland cases closely resembled those just described. There seems no doubt that the cases in Queensland, New South Wales and Victoria comprised one epidemic. While we know of 110 cases in 1917 and seventy-five in 1918, there must have been at that time a number of other severe cases whose records have not been published.

LABORATORY INVESTIGATIONS

Cleland *et al.* (1918) examined histological sections of the brains and spinal cords of sixteen human patients who died of Australian X disease. They described vascular congestion, mainly venous, microscopic haemorrhages and perivascular cuffing with small round cells. These changes were present in the pia mater and also throughout the grey and white matter of the brain and in the grey matter of the cord. They described also two other features; first small areas of necrosis in the cortex which they attributed to infarction, and secondly small collections of mononuclear cells in the grey matter and apparently unrelated to vessels. They stated that they could find little evidence of neuronophagia in any of the sections and that there was no alteration of the Purkinje cells of the cerebellum.

Perdrau (1936) re-examined Cleland's sections from six human cases and from seven animals experimentally infected with X disease virus. He extended the previous pathological observations by describing the presence of obvious neuronophagia seen frequently in basal ganglia, pons and medulla, and occasionally in the spinal cord; but perhaps Perdrau's chief contribution was his description of the cerebellum. In Bielchowsky preparations he demonstrated the presence of many distorted and empty 'baskets' from which Purkinje cells had obviously disappeared during life. The absence of Purkinje cells was most pronounced near the tops of the folia, a situation where they are normally closely packed in the human cerebellum. Adjacent to the empty 'baskets' there were small areas of cellular reaction in the molecular layer.

Perdrau pointed out that a similar histological picture is seen in animals following infection by louping ill virus. He concluded that he could not distinguish the histological picture of Australian X disease from that of Louping Ill, but he carefully refrained from claiming that they were the same disease.

Breinl (1918) described the histology of the brains and cords of five of his seven cases which occurred in Townsville early in 1917, and of each of his two cases in the same town in 1917–18. The vascular congestion, haemorrhages and cellular infiltration followed Cleland's description of the New South Wales cases, but in

addition Breinl emphasized the occurrence of neuronophagia and the presence of neurones in all degrees of degeneration. The lesions were present through most of the brain and cord but he stated that nothing abnormal could be seen in the cerebellum.

In 1917, Cleland & Bradley inoculated one monkey by the intraperitoneal route with material from one human brain, and monkeys, guinea-pigs and rabbits with cerebrospinal fluid from other human cases. No lesion developed in the animals.

At this time Breinl inoculated human cerebrospinal fluid, and crushed spinal cord suspensions intracerebrally into monkeys (*Cercopithecus*? sp.) and so produced an infection of the animals which was fatal in 16–18 days. Histological sections of central nervous system tissue of the monkeys showed a picture similar to that described for the human.

The following year Cleland *et al.* (1918) in New South Wales similarly inoculated monkeys intracerebrally with human brain material, and repeated Breinl's isolation of the virus of Australian X disease. On three separate occasions, from three human cases, an agent pathogenic for monkeys was isolated, and in one instance this agent was maintained through a series of five monkey passages. The human material which was used to initiate these three infections in monkeys was obtained from patients dying 2, 3 and 5 days respectively after onset of clinical symptoms. The agent derived from monkey brain was found to be pathogenic by the cerebral route for a proportion of the sheep into which it was injected, for a calf and for a foal.

The clinical picture in these animals was that of a severe encephalitis. In view of the wide geographical distribution of this disease and so the possibility of a naturally acquired active immunity in sheep, it is of interest that Cleland found approximately one-third of his sheep to be resistant to experimental infection.

On the basis of the monkey infections he had produced in 1917 and from a study of the histology of his human patients, Breinl (1918) claimed that Australian X disease was Heine-Medin disease (anterior poliomyelitis). In making this claim he admitted that the human clinical picture was not quite typical of poliomyelitis, but he believed that he was dealing with an encephalitic form of the disease.

On the evidence available in 1918 Cleland *et al.* disagreed with Breinl, and rightly felt confident that the agent they had isolated was distinct from the virus of poliomyelitis. They based their contention on the wider host range of the new agent which they had demonstrated to be pathogenic for sheep, a calf and a foal, the clinical picture including the high mortality rate among human cases, and the virtual absence of typical cases of poliomyelitis from the same region at the same period.

AUSTRALIAN X DISEASE IN 1922 AND 1925

Cases resembling Australian X disease occurred in Queensland during 1922 and 1925 and in Broken Hill during 1925, but descriptions of the outbreaks, particularly of that in 1922, are not recorded in great detail.

At a medical meeting in Brisbane on 25 May 1922 it was reported that twenty-three patients with X disease had been diagnosed in and near Ipswich, closely adjacent to Brisbane, during 1922. At the same meeting Telford (1922) stated that

in response to a request by the Commissioner of Public Health, Queensland, sixty cases had been notified in that state during 1922. Moore (1922) stated that during 1922 in Queensland there were records of seventy-five patients with X disease, of whom forty-nine died.

The clinical picture recorded in these publications resembles that of X disease but is described too briefly to allow a definite opinion to be formed of the diagnosis. However, Mathewson (1922), who had published descriptions of cases of X disease during 1917, believed that the patients he saw in Brisbane during 1922 were suffering from X disease.

We conclude that there were a number of cases of X disease throughout Queensland during 1922, and that Brisbane and Ipswich were two centres of the outbreak.

Baldwin & Heydon (1925) described an outbreak of at least eleven cases near Townsville during April and May 1925. All the eleven patients were children under 8 years of age, ten being male, and all but one died. The summary they present of the clinical picture, and the histology of the brains of the three patients examined, are entirely consistent with the diagnosis of X disease which these authors applied to the cases.

Kneebone & Cleland (1925) recorded ten cases in Broken Hill with onset between 11 January and 22 April 1925. Nine were presumably infected locally, one came from Tibooburra, 200 miles to the north. Six died. Seven were children under 9 years, three were aged between 17 and 26 years. The clinical and histological records adequately support the authors' contention that these cases were due to X disease. The virus was transmitted directly to sheep by intracerebral inoculation of human brain, and was maintained through three, but not four intracerebral passages in sheep.

MURRAY VALLEY ENCEPHALITIS 1951

The next recorded outbreak of a similar type of encephalitis was in the Murray Valley during the late summer of 1950–51 (Special article, 1951; Miles, Fowler & Howes, 1951; Anderson, 1952; Garven, Margolis & French, 1952). The disease was named Murray Valley encephalitis. There were forty-five reported severe cases, thirty-four infected in northern Victoria, ten in western New South Wales, three of these being recorded for the first time in this communication, and one in South Australia (Text-fig. 1). In addition there were probably unreported cases in the same region during the same period. Of the forty-five reported cases, the first commenced during December 1950 in south-western New South Wales. There were thirteen cases during January 1951, twenty-seven during February, two during March and two during April. The ages ranged from 7 weeks to 69 years, twenty-eight (62 %) being aged less than 15 years. Thirty-one (69 %) of the forty-five were male. Nineteen (42 %) of the group died.

CLINICAL STUDIES

As with Australian X disease, so also with Murray Valley encephalitis, the diagnosis of individual cases was based primarily on the clinical picture. Laboratory investigations were undertaken in relation to thirty-eight of the forty-five cases

(Anderson, Donnelley, Stevenson, Caldwell & Eagle, 1952; French, 1952). The clinical diagnosis was confirmed in seven instances, the serology was consistent with the diagnosis in twenty-nine cases, one case did not develop specific antibody to the virus. In one case no serological examination was made, but post-mortem histological examination confirmed the diagnosis.

The clinical picture was of a severe acute viral encephalitis, without a distinctive feature which would allow of a more exact etiological diagnosis. The disease began suddenly with malaise, anorexia, headache, fever, lethargy and drowsiness, irritability, vomiting, giddiness and nuchal rigidity (Robertson & McLorinan, 1952). Progress of the disease was rapid and by the time a patient reached hospital he was frequently semicomatose and exhibited involuntary purposeless movements. Transient and patchy paresis of upper or lower motor neurone type was frequent, and often led to difficulty in swallowing or breathing. Inability to initiate or control movement of voluntary muscles was a typical finding. Death of the patient or recovery occurred usually within 2 weeks of onset but occasionally was considerably protracted. Recovery was not always complete; occasional paralytic or mental sequelae were observed.

During the acute stage there was a moderate increase in the leucocyte count of the cerebrospinal fluid to between 20 and 760 cells per cu.mm. and usually a rise in protein content of the fluid. A high polymorphonuclear leucocyte count was common, the highest figure noted being 29,100 white cells per cu.mm. on the 5th day of the disease.

The clinical manifestations varied slightly according to the age of the patient. In infants the course was more rapid than in adults and convulsions were common. Two of the older children developed a macular erythematous rash, a finding which recalls the report of a rash seen in three patients during 1917 and 1918.

During the epidemic of Murray Valley encephalitis, many adults in the Murray Valley presented with mild 'neurological' symptoms. It was impossible to decide from the clinical evidence whether the mild cases were in fact due to infection by the virus of Murray Valley encephalitis. Serological evidence (Anderson *et al.* 1952) demonstrated that many such mild clinical cases had not been infected by the virus. Two groups of individuals who complained of very mild symptoms—headache, giddiness, vomiting, malaise and muscular tremors—were examined for the presence of complement-fixing antibody to the virus of Murray Valley encephalitis. In north-eastern Victoria where the healthy population showed a serological infection rate of 4·5%, only three of thirty-one 'mild cases' carried antibody. Similarly, in Mildura, where there was a serological infection rate of 20% in healthy persons, only nine of nineteen 'mild cases' carried antibody. It was concluded that a few of the 'mild cases' may have been due to Murray Valley encephalitis infection but that the majority were due to other causes.

Concident with the Victorian epidemic of 1951, a case of Murray Valley encephalitis occurred in a country district 75 miles north of Adelaide (Miles, Fowler & Howes, 1951).

The histological description of the brain closely resembled that of the Victorian cases; a virus was isolated by the inoculation of suckling and weaned mice and

was passed intracerebrally in suckling mice. The virus was shown to be pathogenic for monkeys by the intracerebral route, and was established in fertile hen eggs; the virus was serologically identical with those strains isolated from patients in Victoria and New South Wales.

MORBID HISTOLOGY

Robertson (1952) made a histological examination of eight brains and two spinal cords of patients dying of Murray Valley encephalitis during 1951. Although the description is published as a preliminary report, the evidence presented is sufficient to link the disease with Australian X disease. The salient features described in the acute cases were patchy infiltration of the pia-arachnoid with inflammatory cells, focal accumulation of reactive cells through the grey matter of the nervous system and disappearance of Purkinje cells from the folia of the cerebellum. There was also a moderate degree of perivascular cuffing particularly in the grey matter, but also in the white matter and the meninges.

There was generalized involvement of neurones which appeared in various stages of damage, but in Robertson's series no instance of neuronophagia was described. His figure IIIA suggests, however, that the phenomenon may have occurred occasionally. Later stages of the infection were also described from autopsy material. At the 34th day, loss of neurones, microglial proliferation, moderate cuffing of vessels and astrocytic proliferation were described in the grey matter; foci of degeneration were seen in the basal ganglia and pons.

Sixty days after the onset, the brain of a child then 5 months of age showed a further stage of glial proliferation and macrophage activity. There was deposition of calcium salts in relation to areas of degeneration in the basal nuclei.

In the late specimens gross shrinkage of the cerebellar folia was obvious, with widening of the sulci, and there were macroscopic areas of degeneration in the thalamus.

The most northerly fatal case of Murray Valley encephalitis recorded during 1951 was a boy of 2 years of age, described by Garven *et al.* (1952), who was infected at Narrabri on a tributary of the Darling River in northern New South Wales. He developed a cold on 13 April, typical neurological symptoms and signs appeared on 16 April, and he died on 18 April.

The clinical and histological features were basically similar to those of the Victorian cases; but in addition neuronophagia was prominent in the spinal cord, and was present to a less extent in the occipital cortex and in relation to the Purkinje cells of the cerebellum. The Narrabri case was examined histologically only 2 days after onset of neurological symptoms—considerably earlier than any of the Victorian cases described by Robertson. Garven *et al.* believed this to be the reason for the presence of neuronophagia, which they imply is limited to early stages of infection with Murray Valley encephalitis virus. However, we have the fairly well-documented evidence from earlier years that neuronophagia was also prominent in the central nervous system of a number of patients dying of Australian X disease, after illnesses lasting many days.

The diagnosis of Murray Valley encephalitis infection was confirmed in the

Narrabri case by the isolation of a serologically typical virus from separate specimens of cerebral cortex, basal ganglia, pons and cerebellum. No virus could be recovered from the medulla in mice, but it was recovered in eggs (Garven *et al.* 1952).

ISOLATION AND IDENTIFICATION OF THE VIRUS

The causal virus of Murray Valley encephalitis was isolated from each of four human brains. French (1952), in Melbourne, inoculated human material directly on to the chorioallantoic membrane of the developing hen egg, and found the virus had produced small focal lesions by the end of 3 days. In later passages the virus required only 2 days for the formation of pocks and the production of other lesions in the embryo. The chorioallantoic technique was satisfactory in attempts at isolation from three human patients, including the boy from Narrabri, and was probably a more sensitive technique than the intraperitoneal or intracerebral inoculation of suckling mice.

Miles *et al.* (1951), in Adelaide, isolated a virus from the only proven case of Murray Valley encephalitis reported from South Australia. They inoculated human brain into suckling and weaned mice by the intracerebral route.

The four strains, two from Victorian cases, one from the patient from Narrabri and one from South Australia, were compared independently by French (1952), and Garven *et al.* (1952), and by Miles & Howes (1952). From the results of complement-fixation titrations and neutralization tests in suckling and weaned mice, it was apparent that the strains comprised only one type of virus. Serologically, this type was closely related to, but not identical with, Japanese B encephalitis virus. Later work by Paterson *et al.* (1952) established more fully the close relation of Murray Valley encephalitis, Japanese B, St Louis, and West Nile viruses, and emphasized the fact that Murray Valley encephalitis virus is a new and distinct member of the group. Sabin (1953) discussed the relation of the more classical members of this group to dengue and yellow fever and to Russian spring summer encephalitis and louping ill viruses. He sets the equine encephalitides in a separate, although probably related, group. The clinical and histological descriptions of human infection with Murray Valley encephalitis are in complete accord with the overseas records of clinical cases of Japanese B and St Louis encephalitis.

THE RELATION BETWEEN AUSTRALIAN X DISEASE AND MURRAY VALLEY ENCEPHALITIS

From the recorded descriptions of the earlier Australian outbreaks, Australian X disease is indistinguishable from Murray Valley encephalitis. In particular the following features are strictly comparable: the clinical picture, including the high mortality, the morbid anatomy and histology, the age incidence, the sex distribution, the seasonal incidence, the broad geographical boundaries to the epidemic areas and the host range of the viruses isolated. In the absence of other evidence to the contrary, we feel justified in accepting the identity of the two diseases.

During 1953 McLean & Stevenson (1954) examined the sera of Broken Hill residents for the presence of neutralizing antibody to Murray Valley encephalitis

virus. A higher proportion of residents born before 1919 (19 of 92) carried such antibody than did people under 34 years of age (2 of 69). This finding is entirely consistent with the identity of the two diseases.

The infection of monkeys with the virus of Australian X disease by Breinl in 1917, and by Cleland *et al.* in 1918, were thus the first instances of the purposive transmission of a member of the arthropod-borne encephalitides to laboratory animals.

HOST RANGE OF THE VIRUS AND OTHER PROPERTIES

The host range of Murray Valley encephalitis virus does not differ significantly from the spectrum so far established for the other arthropod-borne encephalitides. French (1952) found Murray Valley encephalitis virus to be lethal by the intracerebral route for suckling and weaned mice, monkeys, young chickens and a sheep. Guinea-pigs developed a pyrexia from which they recovered with production of antibody. Rabbits were unaffected clinically but developed antibody following intracerebral inoculation. Subcutaneous or intraperitoneal injection of minimal doses of virus produced a fatal infection in suckling mice, but in weaned mice a much larger dose by these routes was necessary to kill.

Coincident early studies by Miles (1952) of weaned and suckling mice, monkeys and young chickens, gave findings similar to those of French. Miles found intracerebral inoculation of virus to produce fatal infection in one of two domestic ducks, two silver gulls (*Larus novaehollandiae*), rats 3–4 days old and four sheep 48 hr. old. The following animals survived intracerebral inoculation of virus with subsequent production of circulating neutralizing antibody: cockerels, guinea-pigs, rabbits, homing pigeons, one old mare, and rats 21–28 days old. A foal 9 months old survived an intradermal inoculation. There was no clinical or good serological evidence of infection in sheep 3 and 17 days old, in three brush-tailed opossums (*Trichosurus vulpecula*) and a kangaroo, experimentally inoculated with the virus, but it seems unwise on this evidence to assume that such species are not able to be infected. Indeed Anderson *et al.* (1952) found complement-fixing antibody to Murray Valley encephalitis in wild brush-tailed opossums late in 1951.

McLean's (1953*a*) more recent work suggested that chickens developed antibody following subcutaneous injection of virus; they were rarely or never clinically affected by this procedure, although Miles stated that intramuscular inoculation would produce clinical disease and death.

Miles (1952) determined the thermal death point of the virus to be between 51° C. for 30 min. and 56° C. for 15 min. From filtration experiments he suggested that the virus might be of particle size 20–50 mμ.

Considerable attention has been given to the behaviour of Murray Valley encephalitis virus in the chick embryo (French, 1952). The primary isolation was successful on the chorioallantois, and after two passages the virus regularly produced small discrete foci suitable for titration of the virus. However, neutralization of the virus by specific antisera was not clearly demonstrable on the chorioallantoic membrane; for this reason antibody could not be titrated by a pock-counting technique.

A simple saline extract of ground infected membrane provided a satisfactory specific complement-fixing virus antigen (French, 1952; Donnelley & French, 1953), an antigen which has been widely used in the serological surveys summarized later. Infection of the egg by Murray Valley encephalitis virus produces a characteristic picture of a small embryo with haemorrhages in the skin, particularly in the feather follicles, in the brain and at the extremities of the toes. The embryo dies about 48 hr. after inoculation.

Following the work of Sabin & Buescher (1950) and Sabin (1951) on haemagglutination by Japanese B, St Louis and West Nile viruses, Macdonald (1952*a*) found that Murray Valley encephalitis virus would agglutinate red cells derived from 1-day-old chicks. The strict conditions of pH (6·5–6·8) and salt concentration (0·01 M phosphate buffer in 0·9 % saline) desirable for the reaction with Japanese B encephalitis virus were also suitable in the case of Murray Valley encephalitis virus. In an extension of this work Macdonald found that Murray Valley encephalitis virus would agglutinate adult pigeon cells, and later, examining Japanese B virus, found that this too agglutinated adult pigeon cells.

Macdonald (1952*b*) determined the LD_{50} of the virus for suckling and weaned mice. Olitsky, Sabin & Cox (1936) and Lennette & Koprowski (1944) had previously observed the general phenomenon of development of non-specific resistance by older mice to intraperitoneal or subcutaneous inoculation of several neurotropic viruses including those of Japanese B, St Louis and West Nile encephalitis. A similar situation holds in the case of Murray Valley encephalitis virus.

VIRAEMIA IN MICE AND CHICKENS

Three laboratory studies have been concerned with the development of viraemia following inoculation of Murray Valley encephalitis virus. Miles (1952) described a viraemia following intracerebral inoculation of virus in weaned mice, chickens 14 days old, older cockerels, a pigeon, two silver gulls and two domestic ducks, and also following intramuscular inoculation of 14-day-old chickens.

Macdonald (1952*c*), working with mice 5–10 days old, noted a viraemia commencing between 24 and 48 hr. after intramuscular inoculation of about 200 baby mouse LD_{50} of virus. At 24 hr. little virus was detected either at the site of inoculation or in the blood, but at 48 hr. virus was abundant in both these sites, although it was not found in uninoculated muscle, nor in central nervous system tissue. The viraemia had disappeared at 120 hr., but virus was then recovered in large amounts from inoculated and uninoculated muscle, and from central nervous system tissue.

McLean (1953*a*), working with chickens between 2 and 28 days old, found that a viraemia followed subcutaneous inoculation of minute amounts of virus. The viraemia developed usually within 24 hr. of inoculation, and in 2-day-old chicks persisted till the 5th day or occasionally longer. The concentration of virus in the spleen ran parallel to the concentration in the blood, both rising to about 10^6 infective particles per ml. or per g. on the 3rd and 4th days. Viraemia always appeared in chicks infected after 28 days of age, but was at a lower titre than in younger birds. No clinical evidence of infection was observed in chickens infected

between 2 and 28 days of age, but each bird, when examined later, had developed circulating specific neutralizing antibody. McLean failed to find evidence of persistence of virus in fowls after antibody had appeared in the circulation.

LABORATORY CARRIAGE OF VIRUS BY MOSQUITOES

During the warmer months of 1952–3, McLean (1953*b*) conducted laboratory experiments with mosquitoes of twelve species known to be present in the Murray Valley during the summer. The individuals were both wild-caught adults and adults bred from larvae collected in the field. Adults and larvae were collected near Mildura, in the vicinity of Albury on the eastern Murray River, and in Melbourne and nearby countryside. The infected adults were maintained in an insectary at a temperature of 80° F. and a humidity of approximately 70%.

Adults were induced to feed on a suspension of egg membrane virus in a defibrinated blood medium. Engorged individuals were separated and examined for virus at various intervals after feeding, virus in the mosquito emulsion being titrated by pock-counting methods on the chorioallantoic membrane.

The following eleven species of culicine mosquito were studied; individuals of each species supported the growth of Murray Valley encephalitis virus, which was recovered at least 10 days after infection of the insect: *Culex annulirostris*, *C. fatigans*, *C. globocoxitus*, *C. pipiens australicus*, *C. pipiens molestus*, *Aedes camptorhynchus*, *A. occidentalis* (*A. queenslandis*), *A. notoscriptus*, *A. theobaldi*, *A. vigilax*, *A. vittiger*.

C. fatigans, *C. annulirostris*, *A. vigilax* and *A. vittiger* were studied in more detail; in each of these four species the virus was demonstrated to go through a biological cycle. Little or no virus was recovered from mosquitoes between 24 and 96 hr. after feeding but by the 10th or 14th day considerable quantities of virus had appeared in viable form in the mosquito. Thereafter the amount of virus per insect appeared to remain constant in the two species tested (*C. annulirostris* and *A. occidentalis*), and it was reasonably assumed that the mosquito would remain fully infective throughout the remainder of its life.

While this evidence strongly indicated that each of the eleven northern species could, and probably did, acquire and transmit Murray Valley encephalitis virus under natural conditions, it was desirable to demonstrate such a cycle in full. This was attempted with *C. annulirostris* and *A. vigilax*. Individuals of these two species bit a chicken during the stage of viraemia; 10–14 days later the mosquitoes were each fed on a separate healthy chicken; the latter was found to have developed a high-titre viraemia 2 days later. This is as far as laboratory demonstration could go in duplicating the suspected natural cycle of Murray Valley encephalitis virus.

Anopheles annulipes, the only anopheline mosquito known to occur in the Murry Valley, repeatedly failed to retain virus on which it had fed. Two days after feeding no virus was recoverable from emulsified mosquitoes, nor did virus reappear at any subsequent day up to 10 days. This almost certainly eliminates the very common anopheline mosquito from the range of possible natural vectors of Murray Valley encephalitis, but any or all of the eleven culicine species listed may play a part in natural transmission of the virus.

FIELD STUDIES—HUMAN AND ANIMAL SEROLOGY

During February and March 1951, even while the epidemic of Murray Valley encephalitis was current, it was obvious that the clinical disease was strictly confined to the area of eastern Australia bounded on the south and east by the Great Dividing Range. This excluded the coastal belt of New South Wales and Victoria (Text-fig. 1). Each recognized clinical case had at some very recent date been near one of the many flooded rivers in the region, and had been exposed to the hordes of mosquitoes breeding in such a situation.

At this time the causal virus of the disease had been isolated, and the host range of the new agent was being elucidated. This information, together with the epidemiological facts available, allowed a confident prediction (Special Article, 1951) that the epidemic was due to a virus closely related to Japanese B encephalitis virus. This working hypothesis, later completely confirmed, was the guiding principle in the planning of most of the subsequent field and laboratory investigations.

With the development of a complement-fixing antigen from infected chorioallantoic membrane, and the establishment by French (1952) of a neutralization test involving suckling mice, it became possible to conduct a serological survey of humans, animals and birds throughout Australia and New Guinea (Anderson *et al.* 1952; Anderson, 1953). It soon became clear that subclinical infection of humans and animals had been exceedingly heavy and widespread. Nevertheless the coastal belt of Victoria and New South Wales, which was free of clinical cases, was also virtually free of evidence of subclinical infections.

Of the 3483 Victorian adult sera gathered from healthy humans during 1951, 1524 were derived from residents of towns north of the Great Divide; of these 109 (7·2 %) carried Murray Valley encephalitis complement-fixing antibody. Of 1959 Victorian adult sera obtained south of the Great Divide, only fifteen (0·77 %) carried antibody. In view of the continuous movement of people north and south across the state, it was considered that the figures were compatible with the hypothesis that Murray Valley encephalitis virus had not moved into the southern coastal belt of Victoria. The proportion of subclinical infections among the population varied even throughout northern Victoria and along the course of the Murray River. In the neighbourhood of Shepparton, a river town in north central Victoria, eighteen of 398 adults (4·5 %) and nineteen of 407 children under 15 years (4·7 %) carried complement-fixing antibody, while in the Mildura area the corresponding figures were 66 of 330 adults (20 %) and 17 of 243 children (7 %).

In Queensland the spread of Murray Valley encephalitis virus apparently extended to the coast with the possible exception of the region around Brisbane, in the extreme south of the coastal belt. In Rockhampton six of thirty-one sera carried antibody, in Townsville four of twenty-six and in Cairns fourteen of sixty-two sera. Along the coastal belt of Queensland including the cities just mentioned but excluding Brisbane, there is a human population of about 350,000. Despite the high proportion of positive sera obtained from this area, no probable clinical case of Murray Valley encephalitis was recorded from the State during 1950–51. It is possible that few or no clinical cases occurred.

Particular attention was paid to an outbreak of a neurological disease among the natives of Mornington Island, which lies just off the coast of western Queensland near the southern shore of the Gulf of Carpentaria. It was finally concluded that the disease was not Murray Valley encephalitis, but the unpublished field investigations carried out by Dr M. J. Mackerras of Brisbane provided a valuable series of human sera, a large proportion of which carried Murray Valley encephalitis complement-fixing and neutralizing antibody.

Natives at Bulolo in New Guinea provided thirteen positive sera out of fifty-eight tested. Of a batch of eighty-five sera from Tasmania, all were negative except one with a very low titre of complement-fixing antibody, and a group of fifty-five sera from Western Australia similarly included only one which was positive, and that serum had a titre equally low.

Equine sera were examined during the same period, and they virtually confirmed the distribution of Murray Valley encephalitis virus already defined by the survey of human sera. Altogether 117 equine sera were received from the hinterland of New South Wales, and from Queensland. Thirteen (11 %) carried antibody. In northern Victoria thirty-one of sixty-two equine sera were positive with a reasonably high titre of antibody. From southern Victoria, four sera out of 191 carried low titre complement-fixing antibody and these four were shown to neutralize virus in a mouse protection test. Information was available regarding one of these latter four animals; it lived at Inverloch in southern Victoria, and had never been north of the Divide. We may have here a hint that there was an occasional infection in southern Victoria, but such an occurrence if it existed at all must have been rare. None of sixty-two sheep, twenty-one cattle and twenty four pigs from northern Victoria was positive in a complement-fixation test, but five of ten domestic dogs, five of nine wild foxes and two of three brush-tailed opossums (*Trichosurus vulpecula*) carried Murray Valley encephalitis complement-fixing antibody.

Miles & Howes (1953) have described the distribution of human and equine antibody in South Australia and the Northern Territory, presumably in sera taken during 1951 and early 1952. Of fifty-three humans living within 20 miles of the Murray River, fifteen carried both complement-fixing and neutralizing antibody to Murray Valley encephalitis virus. Of twenty-nine horses in the same region, twenty carried both types of antibody. Although a small proportion of humans from other areas of South Australia carried antibody, such a concentration of recovered individuals was not found elsewhere than along the river. From the Northern Territory sera were examined from forty humans. Eleven were positive by both complement fixation and neutralization tests. Because the figures Miles & Howes quote concern sera with both types of antibody, their figures cannot be compared directly with those of Anderson *et al.* (1952) which were based only on an examination for complement-fixing antibody.

Beech, Howes & Miles (1953) have published figures of the incidence of both complement-fixing and neutralizing Murray Valley encephalitis antibody in sera obtained from 265 natives in ten centres in the Northern Territory. The sera were gathered during September and October 1952. The very high proportion

of positive sera found indicated that Murray Valley encephalitis virus had been present as far north and west as Darwin and Port Keats. There was a small proportion of positive results in sera obtained near Alice Springs in Central Australia.

INCUBATION PERIOD OF HUMAN INFECTIONS

The virtual absence of Murray Valley encephalitis virus from the part of Victoria which lies south of the Great Divide made it possible to estimate the incubation period of the disease in seven individuals (Anderson, 1952). In each instance the patient had visited northern Victoria during a limited period only; this visit comprised their 'short and only exposure' to the virus. In one case the last visit to northern Victoria terminated 59 days before onset of the disease, but in general the figures suggested a variable incubation period of between 1 and 3 weeks.

AVIAN SEROLOGY

From July 1951 to November 1951 Anderson (1953) studied sera from water birds in the Mildura area. These birds had found favourable and extensive breeding sites above the exceptional flood waters of the Murray River and its tributaries and backwaters. Large rookeries were found along the course of the Murray from Albury in the east to Mildura in the north-west, and beyond into South Australia.

A series of visits was paid to these sites near Mildura and in north central Victoria, and sera were obtained from adults and young of many species. Eggs were also taken. The sera were examined for neutralizing antibody in baby mice inoculated into the peritoneal cavity. Of ninety-nine waterbirds examined, forty carried antibody to Murray Valley encephalitis. The species providing positive birds were: native hen (*Tribonyx ventralis* (G.)), dusky moorhen (*Gallinula tenebrosa* G.), coot (*Fulica atra* L.), white-faced heron, (*Notophoyx novaehollandiae* (La.)), nankeen night heron (*Nycticorax caledonicus* (Gm.)), musk duck (*Biziura lobata* (Shaw)), black swan (*Cygnus atratus* (La.)), little pied cormorant (*Phalacrocorax melanoleucos* (Vieillot)), little black cormorant, (*P. sulcirostris* (Brandt)), big black cormorant (*P. carbo* (L.)), darter (*Anhinga rufa* (Daudin)), and wood duck (*Chenonetta jubata* (La.)). Four species comprising seventeen individuals did not provide a positive serum, but this does not allow the conclusion that these species cannot be infected.

Eleven of sixty land birds examined carried antibody to Murray Valley encephalitis virus. The species providing the positive birds were red-backed parrot (*Psephotus haematonotus* (G.)), white-plumed honeyeater (*Meliphaga penicillata* G.), willy wagtail (*Rhipidura leucophrys* (La.)), black faced wood-swallow (*Artamus cinereus* Vieillot), white-winged chough (*Corcorax melanorhamphus* (Vieillot)), grey shrike-thrush (*Colluricincla harmonica* (La.)), and whistling eagle (*Haliastur sphenurus* (Vieillot)). Seventeen species comprising thirty-three individuals did not provide a positive serum. In a parallel study Miles & Howes (1953) obtained sera from birds in South Australia. Twenty land birds taken north of Adelaide, but more than 20 miles from the Murray River, were free of neutralizing antibody. However, eleven of sixty water birds including ducks, swans, water hens and cormorants carried antibody. These birds were obtained within 20 miles of the banks of the Murray River.

It is of interest that Anderson, in Mildura, found no antibody in eleven hoary-headed grebes (*Podiceps poliocephalus* J. & S.). This bird is common on the billabongs beside the Murray; it hatches its young in floating nests and spends most of its existence swimming on the surface or diving to obtain food or to avoid danger. There was no opportunity to test the susceptibility of the species to laboratory infection.

Because of its abundance and wide distribution, the little pied cormorant was chosen for more detailed study. Neutralizing antibody was searched for in egg yolk and in the blood of fledglings from the day of hatching up to approximately 26 weeks of age. An inhibitor of virus was detected in the yolk of each of six eggs examined, and there is little doubt that this was maternal antibody. Specific neutralizing antibody, presumably of maternal origin, was present in twenty five of sixty-one fledglings under 15 weeks of age late in 1951. Six little pied cormorants about 6 months old were found during April 1952 to carry no antibody. The maternal antibody responsible for these results had evidently been formed by the mature birds following infection during the 1950–51 epizootic of Murray Valley encephalitis, and had passed to the fledglings through the egg.

A careful search of nine adults of this species late in 1951 and of twenty currently occupied nests failed to detect any blood sucking ectoparasite. The only ectoparasites found were Mallophaga. This is the only attempt so far made to study the possible role of arthropods other than mosquitoes in the natural history of Murray Valley encephalitis. The evidence available is clearly insufficient to allow of any conclusion.

During the later months of 1951 sera were obtained from fifty-six domestic fowls in the Mildura region. Of thirty-one sera gathered on the banks of the river and billabongs, twenty-two were positive; however, of twenty-five sera obtained more than a mile from the river and distant from large areas of natural water, only three were positive. It was concluded that domestic as well as wild birds shared in the epizootic of Murray Valley encephalitis, and it was suspected that the predominant vector was closely associated with large areas of natural water.

Hammon & Reeves (1945) concluded that *Culex tarsalis* was of primary importance as a vector of St Louis encephalitis virus in certain areas of U.S.A., and Hammon *et al.* (1949) believed that *C. tritaeniorhynchus* probably played a corresponding role in the case of Japanese B encephalitis in Japan. Earlier, Japanese workers had made a similar finding (Mitamura *et al.* 1938). The corresponding mosquito in the Murray Valley is *C. annulirostris*; it is mainly a river bottom mosquito, prevalent in New South Wales and northern Victoria during the summer, but it is also found less commonly in southern Victoria.

THE SEASONS OF 1951–52 AND 1952–53

By December 1951 when the above field observations had been virtually completed, there was considerable confidence that Murray Valley encephalitis was primarily a virus infection of birds, particularly water birds. The presumed pathway of infection was from a viraemic bird by way of a biting arthropod, almost certainly a mosquito, to a susceptible bird or animal. Infection of man was a biological

accident, of little survival value to the virus. The mosquito primarily suspected as a vector, on epidemiological grounds, was *C. annulirostris*. It was assumed that the virus was enzootic in the Murray Valley, flaring to epizootic proportions only occasionally. During epizootics there was a consierable spill-over to the human population and an epidemic was recognized. Only about one in 800 human infections was at a clinical level.

Two series of investigations were therefore carried out in the Mildura area during the summer of 1951–52.

Reeves *et al.* (1954) captured more than 46,000 mosquitoes of the following species: *C. annulirostris*, *Anopheles annulipes*, *C. fatigans*, *A. theobaldi*, *Mansonia linealis*, *A. vittiger*, *A. camptorhynchus*, *A. alternans*, *M. uniformis*, *Tripteroides atripes*. No attempt was made at that time to separate the *C. fatigans* complex into its constituents.

The first three species mentioned were more abundant than the others. The mosquitoes were held in cages for 24 or 48 hr., then killed by freezing in pools of up to 100. They were stored at $-70°$ C. Attempts were made to isolate Murray Valley encephalitis virus by chorioallantoic inoculation of emulsions of mosquito pools. No Murray Valley encephalitis virus was isolated from any of the 17,833 mosquitoes so examined. This total comprised individuals from all the species mentioned.

During the same period, clinical and serological surveys by Anderson & Eagle (1953) confirmed the absence of Murray Valley encephalitis virus from the human and domestic bird populations. Between November 1951 and April 1952 sera were received from eighty-three patients throughout eastern Australia, suspected on clinical grounds to be suffering from some types of encephalitis, usually of a mild nature. None carried antibody to Murray Valley encephalitis virus. From a group chosen from the healthy adult population of Mildura and known to be negative in May 1951, eighty-five sera were examined in April 1952 without the discovery of Murray Valley encephalitis complement-fixing antibody. From northern Victoria, New South Wales and Queensland, 1017 sera were studied from healthy humans. One individual from Victoria, three from New South Wales and two from Queensland had low titre complement-fixing antibody, probably formed a year previously. None of fifty domestic fowl sera taken near the river in the region of Mildura during April carried neutralizing antibody.

Twelve months later, between November 1952 and April 1953 sera were obtained from northern Victoria by Anderson, White & McLean (1954). There was no serological evidence in 320 humans or 75 young domestic fowls that the virus of Murray Valley encephalitis had been in the Murray Valley during the summer of 1952–53.

These three studies strongly suggested that by the summer of 1951–52 the virus had completely disappeared from the region into which it had so widely infiltrated during 1950–51. It was then suggested by Anderson & Eagle (1953) and Miles & Howes (1953) that Murray Valley encephalitis virus is normally enzootic in the birds of northern Australia, that it does not over-winter in the Murray Valley, but that during occasional favourable years it spreads south and possibly east temporarily to invade and infect the fauna of the Murray Valley.

Epizootics of Murray Valley encephalitis represented the invasion of a clean area in the temperate zone by virus from a distant enzootic focus in the tropics. This Australian work was reviewed by Burnet (1952) who suggested that the principle exemplified by such pseudopodial epidemics had a wider application to overseas viruses of the same general type.

Lépine (1953), after considering the global distribution of the arthropod-borne encephalitides, brought forward a similar hypothesis. He favoured the view that the historical origin of this group of diseases was in the torrid zone, and he hinted that the immediate origin of many temperate zone epidemics might be in adjacent subtropical enzootic areas. A similar idea had been fully developed and illustrated by Taylor (1951) when discussing the behaviour of yellow fever virus in South America.

ENZOOTIC FOCI

The existence of enzootic foci of Murray Valley encephalitis virus implies a continual co-existence of a sufficient density of arthropods as vector reservoirs and non-immune vertebrates of susceptible species as temporary hosts. We must assume that such areas exist in the tropics but they have yet to be located. The northern fringe of Australia, the islands to the north of Australia, and the south-east corner of Asia are possible sites of these foci of Murray Valley encephalitis virus. The extent of each focus may vary from time to time, the limits presumably being set by climatic factors and the ecology of the local vectors and hosts.

The delineation of these enzootic foci is an obvious need. Several techniques might be used—the examination of wild caught mosquitoes for virus, the detection of clinical cases of encephalitis in humans, and the detection of newly formed complement-fixing antibody in humans or animals in the area. The fact that avian maternal antibody is present in egg yolk provides another very valuable technique. The short life of the domestic chicken and its generally reliable life history make this bird a very suitable sentinel animal. It should be possible to detect enzootic foci of Murray Valley encephalitis virus by finding neutralizing antibody in eggs from farms in a suspected area.

CLIMATIC FACTORS

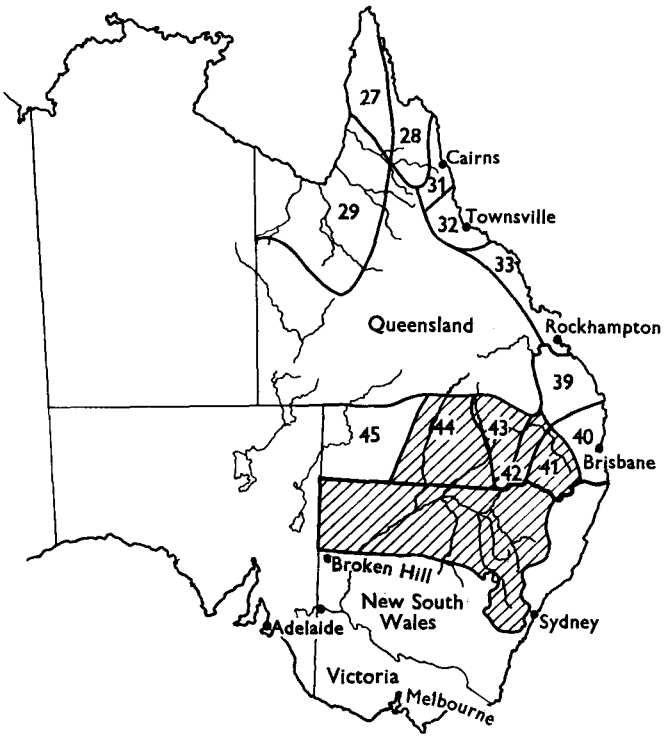
During 1952, Anderson and Miles both studied factors which induced the epizootic spread of Murray Valley encephalitis virus southward into the Murray Valley. Both authors accepted the probability that this disease was identical with Australian X disease.

During the period 1913 to 1952, the disease had been recognized in the valleys of the Murray River and its tributaries on only four occasions. These were during the latter months of the summers of 1916–17, 1917–18, 1924–25 and 1950–51. During the same 40 years there had been 5 years of grossly excessive spring rainfall in northern and eastern Australia. These were 1916, 1917, 1924 and 1950, all of which directly preceded outbreaks of encephalitis, and 1933. No encephalitis of this type was recorded during 1933–1934.

There was, however, no relation between *local* rainfall and the subsequent appearance of encephalitis. For example, although the majority of cases of Murray

Valley encephalitis during 1951 occurred in the Murray Valley, the 1950 spring rainfall in the north of Victoria, along the Murray Valley, was not greatly above normal.

Miles & Howes (1953) suggested that the occurrence of epidemics of encephalitis in New South Wales and Victoria had followed a rainfall greater than 110% of normal during spring and early summer, over a wide area of the Northern Territory, northern South Australia, and the whole or the greater part of Queensland. In particular, they mentioned rainfall in the meteorological district of Lower Carpentaria (no. 29) which extends around the southern shore of the Gulf of Carpentaria in Queensland (Text-fig. 2).



Text-fig. 2. Map of eastern Australia. The figures designate rainfall distribution as recorded by the Commonwealth Meteorological Bureau, Melbourne, The shaded portion includes the catchment area of the Darling River and its tributaries.

Anderson & Eagle (1953) placed a greater emphasis on the rain falling in the watershed of the Darling River and its tributaries in southern Queensland and northern New South Wales. In this area during the five wet years noted above, the November rainfall was more than 200% of normal. This meteorological circumstance was believed to be the crucial factor in precipitating an epidemic some hundreds of miles farther south.

The outbreak of Australian X disease in Queensland during 1922 is distinct from the other outbreaks in that it was apparently confined to the one state. The rainfall figures for 1921 are therefore of some interest. First, the November rainfall in the catchment area of the Darling River was only 96% of normal and on Anderson & Eagle's hypothesis no southerly extension of virus would have been anticipated.

Secondly, the total Queensland rainfall during September 1921 was 236 % of normal, during October 161 % and during November 55 % of normal. There was a rainfall registering 172 % of normal during December 1921 in the combined coastal and southern inland areas of the State (rainfall districts numbered 27, 28, 31, 32, 33, 39, 40, 41, 42, 43, 44 and 45 by the Bureau of Meteorology, Melbourne; Text-fig. 2). It is tempting to suggest that these exceptionally heavy spring rains late in 1921 played a major role in the development of X disease in the State of Queensland early in 1922.

Although there may be some debate in detail as to the location and timing of rainfall which determines the southerly extension of Murray Valley encephalitis across New South Wales, the mechanism of the phenomenon seems clear. Flooding rains in northern or eastern Australia or both, provide large areas of surface water at a time and in a place where they will stimulate a southerly movement of water birds across southern Queensland and northern New South Wales. The flood waters would also allow extensive breeding of mosquitoes, which would act as local vectors of Murray Valley encephalitis virus between individuals in the southward moving flocks of birds. Movements of infected mosquitoes over long distances might also play a part in transmission of the virus. These circumstances would result in heavy seeding of the Murray Valley bird and mosquito populations with Murray Valley encephalitis virus. The disease would readily become epizootic within a few weeks. After several months two factors may terminate the epizootic, primarily the disappearance of those mosquito species most active in carrying and transmitting the virus within the Murray Valley and secondarily, the decrease in density of the local population of susceptible vertebrate hosts. Under these conditions the virus would completely die out in the temperate zone.

THE NORTHERN LIMITS OF MURRAY VALLEY ENCEPHALITIS VIRUS

The northern limits of spread of Murray Valley encephalitis virus are not known, nor is there evidence about the factors which control the northerly extension of the virus. Geography, animal and insect ecology, and climatic factors probably exercise this control, but we particularly wish to discuss the possibility that immunological factors also operate.

To the north of Australia, in a belt of country stretching from Japan to Malaya, Japanese B encephalitis virus has been isolated and identified. The area affected by Murray Valley encephalitis virus may well abut upon the territory affected by Japanese B virus. These two viruses are closely related antigenically, and it seems that any animal or bird actively immune to one of them will be resistant to the other. Territory bordering on enzootic centres of Japanese B virus will be frequently and heavily seeded by that virus. It will carry a high percentage of immune vertebrates, and will therefore be poor soil for the growth of Murray Valley virus. The occasional incursion of Murray Valley virus from more distant areas will not generally lead to persistence of the introduced virus. Somewhere between the territories 'occupied' by each of the viruses there will probably exist a common boundary zone, where the vertebrate population as a whole, though not each individual, will be infected by both viruses.

We suggest this phenomenon of immunological exclusion as one factor in limiting both the northerly extension of Murray Valley encephalitis virus, and the southerly spread of Japanese B virus to Australia. Perhaps the principle may be applicable to pairs of arthropod-borne viruses in other parts of the world, for example, to St Louis and Ilheus virus, to West Nile and Japanese B virus, and even to yellow fever and West Nile virus.

CONTROL OF OUTBREAKS OF MURRAY VALLEY ENCEPHALITIS

Control or elimination of the virus of Murray Valley encephalitis in any area will depend on control of mosquito vectors. This will demand a knowledge of the mosquitoes which carry the virus under natural conditions, the relative importance of each vector species, and the ecology of each species in the local terrain. These facts are not fully available, and will not be so until a fresh epidemic is studied.

It is not practical to eliminate Murray Valley encephalitis virus from endemic foci in tropical Australia or the islands to the north. What one can reasonably expect is that with greater knowledge of vector ecology it may be possible to control mosquitoes near such large towns as are encompassed by an epizootic of Murray Valley encephalitis, for example in the Murray Valley or on the eastern coast of Queensland. Mosquito abatement measures would be designed for the local situation. The population, particularly children, might be advised to avoid the times and places where vectors were abundant.

These measures would be applied more effectively and with greater confidence if each epidemic could be predicted several weeks in advance. We believe a study of the amount and distribution of spring rainfall will provide some factual basis for such a prediction.

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EXPLANATIONS OF PLATES

PLATE 14

Fig. 1. Collection of mosquitoes in a field near Mildura.

Fig. 2. Typical flooded country alongside the Murray River in north central Victoria.

PLATE 15

Fig. 3. Examination of cormorant and its nest for ectoparasites in a flooded cormorant rookery on the banks of the Murray River near Mildura.

Fig. 4. Young nestling darters over flood waters near Mildura.

(*MS. received for publication*, 7. IV. 54)

Fig. 1.

Fig. 2.

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Fig. 4.

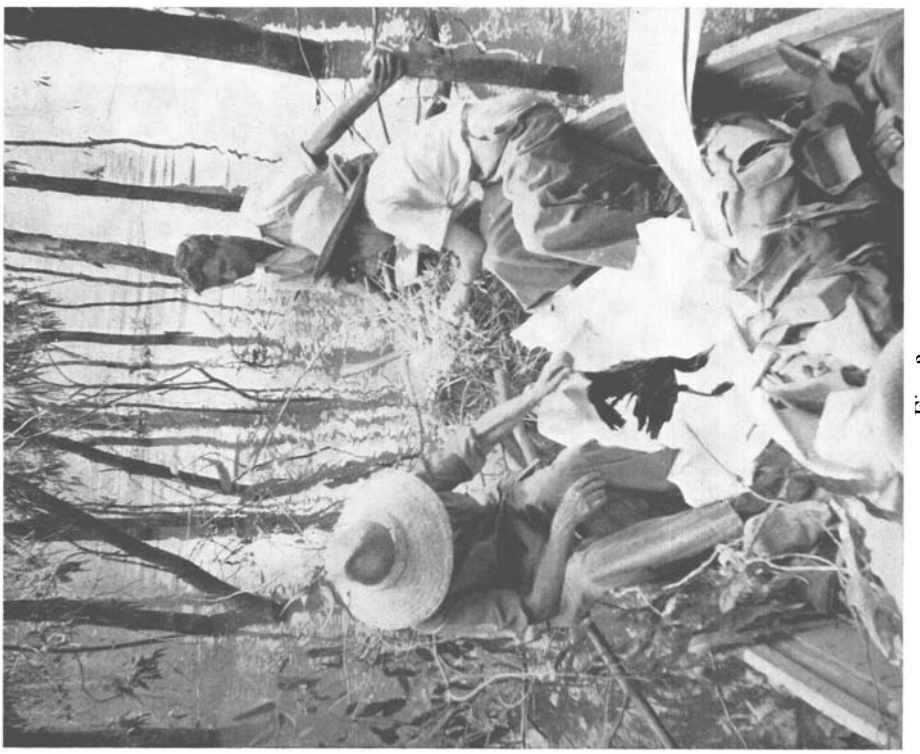

Fig. 3.

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