Studies in Equine Encephalomyelitis

Susceptibility of Some Mammals and Birds

By Chas. A. Mitchell and R. V. L. Walker*

THE VIRUS of encephalomyelitis appears to be harboured transiently in animals, such as horse and man, in which a well marked infection is provoked. It has not been isolated subsequently to the disappearance of clinical symptoms in those which recover; in those which die not infrequently the virus has disappeared from the tissues or has been neutralized by the rising antiviral content of the blood. In a word, the causative agent of encephalomyelitis persists in the tissues of infected animals for a very short time.

The epizoology of the disease shows that it is seasonal, lasting for two or three months, during the warm period of the year. This suggests that a vector plays a part in distribution. Assuming that such is the case then the vector must act as a host for the entire year or obtain the virus from some mammal or bird which plays host. There are points in the epizoology which suggest the latter. If this should prove true it is self-evident that the virus must be contained in some tissue such as blood to which the vector has access. The examination of mammals, birds and insects which may be hosts to the virus is likely to prove a tedious time-consuming problem. It is perhaps pertinent to note that the presence of antiviral substance is no indication that the particular animal containing it plays host. It simply means that this animal, like a recovered horse or man, had been infected.

Experiments which likely will lead to a determination of the host or hosts may be roughly divided into two groups, those which aim at surveying thoroughly, by inoculating susceptible animals with tissues taken from different and representative members of the animal kingdom in affected districts and secondly, those based upon the artificial infection of animals, birds and perhaps insects in the hope of discovering hosts among them.

In a former paper[1] mention was made of attempts to determine the susceptibility of some mammals and birds and the results would appear sufficiently interesting to place on record.

Strain of virus used.—The strain was obtained in 1937 from the brain of a 16-year old horse which had become infected in the Rainy River District of Ontario. It belongs to the Western type and had been carried in serial order in guinea pigs and chick embryos. This virus has proved capable of infecting guinea pigs in a very high dilution and therefore it is assumed to be one of considerable virulence.

The dose of virus used for each was not in ratio to the body weight of various animals, nor constant for species. Each inoculation however, represented a considerable number of infecting guinea pig doses and it is felt that sufficient virus was given to infect all susceptible animals under trial. The inoculated animals were not destroyed in a fixed number of days after inoculation.

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Tests for virus and neutralizing antibodies

Brain and blood were the tissues chosen in which to search for virus. (In some, common tissues from members of the group were pooled rather than examined individually). The brain was macerated finely in a mortar, agitated in a bottle with glass beads and sufficient buffered glycerine added to make a 10 per cent suspension. The blood was added to buffered glycerine to make a 10 per cent mixture. These were left standing for four or five days at 36° F., centrifuged a few minutes, diluted with an equal amount of physiological saline and inoculated intracranially into guinea pigs.

When examining for antiviral substance the test virus was titrated carefully on guinea pigs to determine the number of infecting doses in a given amount. Equal volumes of serum and diluted virus, computed so that 0.2 cc. of the mixture would give the test guinea pig 10 infecting doses of virus, were incubated together for one hour then inoculated intracranially.

For purposes of clarity the inoculations and results are tabulated below (Table A).

The condition which occurred in the two geese is of special interest. Two days after inoculation both showed evidence of illness characterized by inability to stand, loss of appetite, rapid dehydration from which they gradually recovered except for a stunted growth. Suddenly 46 days after inoculation *goose 1* showed symptoms suggestive of central nervous system involvement. The bird was at first weak, then unable to stand, the tone of voice altered, later became hoarse and finally inaudible. Progressive weakness was evident until even the neck seemed unable to support the head, the beak resting on the ground (*Photo 1.*) Finally the creature fell over on its side and remained in this position for several hours before death, which took place two days after symptoms appeared. *(Photo 2.)*

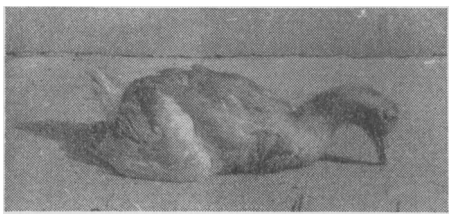

Photo I

Photo II

During this time *goose II* remained apparently healthy, then suddenly on the 54th day after inoculation it too showed the exact symptoms of its mate, passed through a similar course and died in two days.

Due to the surprising course of events great care was taken to secure tissues under favourable conditions and inoculate a sufficiency of guinea pigs. Brain and blood were prepared in buffer and each inoculated intracranially into guinea pigs.

Table B — gives the inoculations made and results.

Goose No. 1

Of the twelve guinea pigs inoculated with brain of goose No. 1 ,4 died within a few hours. A post-mortem examination revealed extensive haem-

Table A
SUSCEPTIBILITY OF VARIOUS ANIMALS.

Animal	Route of Inoculation	Inoculum Virus contained in	No of Animals Inoculated	Number Infected	Clinical Symptons	Results	Virus Recovered from Brain	Virus Recovered from Blood	Neutralizing Anti-bodies Present in
Horses	Intracranially	Guinea pig bran	5	4	Yes	Killed in extremis 7-8—8-9 days other remained well	Yes	No	— Yes
Cattle	"	Guinea pig brain	2	0	No	Killed 8th day	No.	No.	—
	"	Chick embryo	3	1	Yes	Recovered Killed 71 days	No	No	Yes
Swine	"	Guinea pig brain	3	0	No	K-120 days after	No	No	No
	"	Mouse brain	2	0	No	K-112 days after	No	No	No
Sheep	"	Guinea pig	1	0	Yes	Died 15 days (brain abscess)	No	No	No
Goat	"	G. P. brain	1	0	No	—	—	No	No
Foxes	"	G. P. brain	12	0	No	K-15 to 34 days	No	—	—
Cats	"	G. P. brain	1	0	No	Killed 9 months after	No	No	—
	"	Chick embryo	1	0	No	" "	No	No	—
Chipmunk	"	Chick embyo	1	1	Yes	Died-100 hours	Yes	—	—
Groundhog (4)	Intracarnially	Chick embryo	1	1	Yes	K. in extremis 70 hrs.	Yes	No	—
"	"	Groundhog brain	1	1	Yes	" "	Yes	No	—
"	"	G. P. brain	1	1	Yes	" 6 days	Yes	No	
" (M)	"	"	1	0	No	Killed 23 days	No	No	—
Ferrets	Intranasal	Chick embryo	1	0	No	Killed 52 days	No	No	—
"	Intracrainally	"	2	0	No	Killed 52 days	No	—	—
Rabbits	"	"	6	0	No	Killed 2 to 4 weeks	No	—	—
White Rats	"	"	5	5	Yes	Died 5 and 6 days	Yes	No	—
Hamsters	"	"	6	5	Yes	Died 6 to 12 days	Yes	No	—
Guinea Pigs	"	Mouse brain	15	15	Yes	Died 4 to 7 days	Yes	No.	—
"	Intravenous	"	15	3	Yes	Died 6 to 8 days	Yes	—	—
"	Subcuta-neously	"	12	8	Yes	Died 6 to 8 days	Yes	No	—

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White Mice	Intracranially	Guinea pig brain	25	25	Yes	Died 3 to 6 days	Yes	No	—
Chickens (1 to 2 weeks old)	Subcutaneously	Chick embryo	6	6	Yes	Died 5 to 10 days	Yes	Yes	—
(3 wks. old)	"	"	3	0	No	Killed 217—460 days	No	No	—
(8 mths. old)	Intracranially	Guinea pig brain	1	0	No	Killed 670 days	No	No	—
(8 ms. old)	Subcutaneously	"	1	0	No	Killed 670 days	No	No	—
(8 mths. old)	Intracranially	Chick embryo	1	0	No	Killed 670 dsys	No	No	—
(8 mths. old)	Subcutaneously	"	1	0	No	Killed 670 days	No	No	—
Turkeys 3 wks. old	Subcutaneously	Chick embryo	2	2	Yes	K. in extremis 2 and 4th day	Yes	Yes	—
8 wks. old	"	"	4	4	No	Killed 39 to 177 days	No	No	
Pigeons (Mature)	"	"	1	0	No	Killed 150 days after	No	No	—
Ducks (2 wks. old)	Subcutaneously	Guinea pig brain	1	1	Yes	Died in 3rd day	Yes	Yes	—
(2 wks. old)	"	Chick embryo	1	1	Yes	K. in extremis 4th say	Yes	Yes	—
(4 wks. old)	"	"	1	0	No	K. in extremis 72 days	No	No	—
(6th wks. old	"	"	1	0	No	K. in extremis 445 dys	No	No	
Crows (2 wks. old)	Subcutaneously	Guinea pig brain	3	3	Yes	Died 35–40 hours after	Yes	Yes	—
(2 wks. old)	"	Chick embryo	1	1	Yes	K. in extremis 75 hrs.	Yes	Yes	—
Mature	"	"	1	0	No.	K. 50 days after	No	No	—
Robin 2 wks. old	Subcutaneously	Chick embryo	1	1	Yes	Died 35 hours after	Yes	Yes	—
Starlings (Young)	Subcutaneously	Chick Embryo	1	1	Yes	Died 48 hours after	Yes	Yes	—
Geese 4 wks. old	Subcutaneously	Chick embryo	1	1	Yes	Died 55 days after	—	—	—
6 wks. old	"	"	1	1	Yes	Died 48 days after	—	—	—

TABLE B

Inoculum No 1 Goose Brain				Inoculum No 1 Goose Blood			
G. Pig No.	Result-inoculation of Goose Brain	Challenged with	Result	G. Pig No.	Result	Challenged with	Result
56	Survived	Virus Encephalomyelitis Western Type	Survived	68	Survived	Virus Encephalomyelitis Western Type	Survived
*57	Died 8 days		—	69	Died 7 days		—
58	S		Died 6 days	70	D 10 days		—
59	S		S	71	D 9 days		—
60	S		D 8 days	72	S		S
*61	D 6 days		—	73	Killed 13 days		—
62	D 4 days		—	*74	D 8 days		—
63	S		D 6 days	75	S		S
64	D 1 day		—	76	D 7 days		—
65	D 1 day		—	77	D 3 days		—
66	D 1 day		—	78	S		Died 5 days
67	D 1 day		—	*79	K 4 days		—

*Subinoculations made from these (See Text).

orrhages which were believed brought about by traumatic injury. Three other members of the group had elevated temperatures, became paralyzed and finally died. However, the appearance of the sick animals was not the exact counterpart of encephalomyelitis. Motor paralysis was earlier evident and the saliva soaked chins so common in encephalomyelitis were absent.

The brain was removed from guinea pig No. 57, inaculated into an incubating egg and intracranially into two guinea pigs. The chick embryo survived and the guinea pigs died on the 6th day. Their brains were removed and inoculated intracranially into other guinea pigs. These remained well and died when subsequently challenged with virulent virus.

The brain was also removed from guinea pig No. 61, inoculated intracranially into two guinea pigs one of which died on the 4th day. Its brain was removed and seeded in chick embryo and inoculated intracranially and subcutaneously into healthy guinea pigs. The embryo remained healthy; the guinea pigs showed no evidence of illness and later died when challenged with active virus.

Blood also was taken from guinea pig No. 61 inoculated into two healthy guinea pigs. One of these died on the 9th day, and no evidence of virus could be found by chick embryo and guinea pig inoculations. The other guinea pigs remained well and when subsequently challenged with active virus were resistant.

The brain of guinea pig No. 74 was inoculated intracranially into two guinea pigs; these remained well and were found resistant when subsequently challenged with active virus.

The brain and blood of guinea pig No. 79 were inoculated into four guinea pigs, two died and subinoculations from these failed to infect. The two survivors were challenged and found resistant to encephalomyelitis.

Goose No. 2

This goose was bled from the wing vein, when first noticed ill, into a tube containing beads and buffer solution. The following day the defibrinated blood was inoculated subcutaneously and intracranially into six healthy guinea pigs. One of these died on the third day and we were unable to demonstrate the presence of virus in its tissues. Of the five remaining animals—two survived the challenge inoculation of virulent virus.

When the goose died brain and blood were each inoculated into three normal guinea pigs. Those inoculated with brain remained well and later were challenged with active virus, one survived. Those inoculated with blood also remained well and later two survived the challenge inoculation of active virus.

Results of Inoculation from Geese.—The results of inoculations made from the tissues of these geese are extremely puzzling. Blood and brain from each of these birds produced infection in a percentage of the guinea pigs inoculated. In a few instances infections were transferred to other guinea pigs but could not be maintained in serial order. Chick embryo was never infected.

A point of interest is the resistance which many survivors showed to a challenge inoculation. Needless to say this was well controlled and there can be no question regarding the pathogenicity of the virus used.

The inocula were checked from time to time and found bacteriologically sterile.

If the blood and brain of the affected geese contained encephalomyelitis virus it must have been in an altered form which though capable of infecting some was unable to permanently adapt itself to guinea pigs. A considerable number of those that survived were found immune when challenged and it is difficult to account for this unless the inoculation of goose brain and blood carried a virus capable of stimulating resistance.

Summary

(a) With the strain of encephalomyelitis virus western type used the following mammals were found quite susceptible — horses, guinea pigs, mice, white rats, hamsters and chipmunk.

(b) Cattle were only slightly susceptible.

(c) Swine, sheep, goats, foxes, cats, ferrets and rabbits were refractory.

(d) Groundhogs were susceptible when young.

(e) All young birds in which the virus was inoculated were found very susceptible; older birds were resistant. The borderline between the two was well defined. For instance, chickens were very susceptible until two weeks of age when they quickly became resistant.

(f) Virus was found only in the brains of mammals but in both brains and blood of birds which died from the infection. This should be qualified however by stating that the mammals in general were inoculated intracranially, the birds subcutaneously.

(g) No animal or bird studied became host to the virus except perhaps the goose which is fully discussed in the text.

Reference.—(1) Mitchell, Chas. A. — Can. Jour. of Comp. Med. Oct. 1940.