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MEG.

STUDIES ON THE NEUROTROPIC VIRUS OF HORSESICKNESS IV. THE PATHOGENESIS IN HORSES.

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The progressive attentuation which accompanies neurotropic adaptation of the virus of horsesickness in mice and guinea pigs was recognised first by the author (1933).[1] The possibility of utilizing that attenuation for the development of a suitable method of immunization was appreciated immediately, and as will be seen from preliminary reports on the subject the method has found application in the field today on a practical and economic basis.[2] The development of immunity has been described and discussed in a previous publication[3] but some interesting features of the pathogenesis of the neurotropic virus in equines merit attention. Unfortunately suitable horses were not available in sufficient numbers to approximate the detailed study devoted to the analagous problem in Yellow Fever, notably by Lloyd and Penna (1933).[4] However, many observations have been made during the course of the experimental work on immunization, and several points have been confirmed by the critical experiments to be described.

Subcutaneous injection of horses.

From time to time blood has been drawn promiscuously from horses before, during and after the reaction produced by the subcutaneous injection of neurotropic virus. After defibrination this blood, usually diluted 1 : 3 in saline, has been injected intracerebrally into mice but only on comparatively rare occasions have any succumbed to a specific horsesickness encephalitis. Every mouse which survived was shown subsequently to be fully susceptible. It was then

decided to determine by systematic investigation whether circulating virus could be detected regularly in the blood of reacting animals.

Separate suspensions in 10% serum saline were prepared from the brains of mice destroyed in extremis after injection with each of four strains of virus known to have attained an adequate level of attenuation. Sufficient of each suspension was then added to a known volume of diluent to produce a 1 : 1000 dilution and of the mixture 10 c.c. was injected subcutaneously into two susceptible horses (20915 and 20919). The febrile reaction produced is shown graphically in charts I and II. At no time was there any clinical deviation from normal health in either animal.

Commencing from the third day after injection defebrinated blood from each horse was diluted aa with saline and 0.05 c.c. was injected intracerebrally into white mice. The results are given in Table I.

Table I. Fate of Mice injected with Defrinated Blood

Interval days.	Horse 20915 Mild reactor	Horse 20919 More severe Reactor.
3	0,0,0	0,0,0
4	7,8,0	0,0,0
5	0,0,0	6,7,0
6	0,0,0	5,5,6
7	7,0,0	4,4,5
9	0,0,0	5,5,7
10	0,0,0	6,6,7
11	7,0,0	5,5,7
12	0,0,0	8,0,0
13	0,0,0	10,0,0
14	0,0,0	0,0,0
16	0,0,0	0,0,0

Note: The numerals indicate day after injection on which mouse died. 0 = survival for 10 days. Thus 7,8,0 means three mice injected of which one died on day 7, one died on day 8 and one survived.

It is seen that in the one horse (20915) which reacted comparatively more mildly to the immunizing injection, as indicated by the rise of the morning temperature to a point above normal on only two days, little or no virus could be demonstrated in the peripheral circulation. Only on the fourth day did two of the three injected mice die, while there is recorded the deaths of one out of three mice which received blood drawn on the seventh and eleventh days respectively.

The second horse (20919) showed a more severe febrile reaction and circulating virus could be demonstrated from the fifth to the twelfth day.

In previous work on the determination of the infective titre of virus suspensions it was shown that the injection of material on the borderline of infectivity results in a well marked prolongation of the incubation period in mice, so that, within limits, the course of the disease may be taken as a rough index of the concentration of the virus. If this is accepted it becomes apparent that in the first horse (20915) when virus was present in the blood it was in low concentration since the mice did not die until the seventh day; in the second horse (20919) the concentration slowly increased until it reached a maximum on the seventh day, after which it gradually decreased, virus being demonstrable no longer on the 14th day.

It must be pointed out that the virus after its undoubted multiplication in the horse retained its neurotropic character since the disease produced in mice differed in no respect clinically from that seen in passage experiments and on five occasions the mortality was 100%, the course of the

disease being no longer than that which would have been anticipated from the use of minimal quantities of mouse adapted virus.

On the tenth day after injection, at a time when virus was shown to have been present in the peripheral circulation of the donor, 2,500 c.c. of blood was transfused into a susceptible horse (20920). The subsequent temperature record is shown in chart III. After an interval of 24 hours a well defined febrile reaction commenced, of which the cause is unknown since virus could not be demonstrated in the blood by intracerebral subinoculation into mice, and no symptoms could be established to support a clinical diagnosis. Just when it appeared that the reaction would terminate by the usual lysis a second reaction commenced which lasted for nearly three weeks. This reaction is shown in the temperature chart for a particular purpose, but it is believed to have no connection with horsesickness, since a clinical diagnosis of broncho-pneumonia was made. An enzootic of this affection due to a virulent streptococcus had passed through the stables during the previous month.

After an interval of 63 days the horse was given an immunity test of 5 c.c. of O virus to which it proved to be solidly immune. This virus was selected for the immunity test because probably it is the most virulent strain available and in the original immunizing injection probably it was present in the lowest concentration.

Conclusions. From this experiment several interesting points emerge :-

1. Virus may be demonstrated in the blood of animals during the reaction produced by the subcutaneous injection of neurotropic virus. The amount of circulating virus is roughly proportionate to the severity of the reaction being

present in minimal quantities during very mild reactions, and abundant during severe reactions. Virus makes its appearance at the commencement of the febrile reaction, during which it may be present, and disappears when the temperature returns to normal.

2. After injection the neurotropic virus undoubtedly multiplies in the body of a susceptible animal. At least in the first generation the neurotropic character is retained as seen from the results of subinoculation into mice.

3. In the first generation in horses the attenuation is retained since transfusion of as large a quantity as 2,500 c.c. of infective blood produced a mild reaction followed by the development of a solid immunity.

4. The immunizing reaction appears to lower the resistance of horses to other conditions notably affections of the respiratory system. This is illustrated by the incidence of catarrhal broncho pneumonia in the case described (2080) and is supported by clinical observations in batches of animals which it would be superfluous to detail.

In this connection it may be mentioned in passing that an extensive outbreak of a specific broncho-pneumonia - jaagsiekte - occurred at Onderstepoort this year amongst horses immunized by the neurotropic virus, the serum virus and the formalized spleen virus methods. The problem is being investigated but preliminary work indicates that it cannot be associated with horsesickness or horsesickness immunisation.

Intracerebral injection of horses.

From the point of view of economic vaccine production it became of the utmost importance to determine whether the metamorphosis to neurotropism attendant upon serial passage

through the brains of mice would produce in the virus an affinity for the nervous tissue of horses. Such an affinity might be followed by unpleasant sequelae in any scheme of mass immunization. To clear up this point susceptible horses have been given intracerebral injections of mouse-virus at different levels of fixation.

I. On 27 April 1933 a horse (20576) received an intracerebral injection of 1 c.c. of a 2% emulsion of infective mouse brain representing generation 38, strain 20449. The operation was carried out under full chloral hydrate anaesthesia. After trephining the ~~frontal~~ parietal bone slightly to one side of the sagittal line, the injection was made at an angle into the substance of one hemisphere using a long fine needle (B.D. 205). After great care had been taken to prevent contact of virus with blood or muscular tissue by thorough plugging of the surgical wound.

A febrile reaction followed which is shown graphically in Chart IV. During the reaction the horse showed distinct signs of nervous derangement. It became exceedingly difficult to handle and clinical investigations could be made only with the greatest difficulty. On the 7th day after injection slight incoordination of movement was noted, the horse walking with a high stepping action and swaying gait. In the loose box these developed a tendency for aimless walking in a circle always to the left, the feet being lifted unsteadily to an exaggerated height. Soon impairment of vision was apparent, the animal being unable to drink from the trough without bumping its muzzle against the side and severe abrasions of the supraorbital crest resulted from banging into the wall. These symptoms gradually disappeared with the return of the body temperature to normal by lysis and when a dikkop (oedema of the supraorbital fossa) commenced to appear on the 18th

day a normal habitus had been regained, except that there persisted a slight defect in vision which could not be accounted for by clinical examination of the eyes.

After an interval of 70 days it was shown that a solid immunity to the homologous strain of virus had developed

II. On 15 January 1934 a horse (20871) was given an intracerebral injection of 1 c.c. of a 2% saline emulsion of infective mouse brain representing generation 100 of the same strain of virus (20449). Control mice injected with the same suspension died on the 4th and 5th day. The technique for this injection was modified slightly. Instead of removing a small plate of bone by trephining, a tiny hole was drilled to permit passage of the syringe needle into the brain for injection. The modification did not permit insertion of the needle at an angle and was not successful since it is believed that the major portion of the fluid escaped on withdrawing the needle.

On the 18th day after injection the temperature rose to 103°. From this time on it was quite impossible to take the temperature since the horse became uncontrollably wild and was dangerous to handle. Apart from this persistent 'madness' no clinical symptoms were noticed.

After an interval of 58 days 5 c.c. of homologous virus was given as an immunity test. Typical Dikkop Horse-sickness developed which proved fatal on the fourteenth day. On post mortem examination no macroscopic or microscopic brain lesion could be detected. The course of the disease was rather long but as this is a characteristic of the strain of virus used it is doubtful whether any immunity had developed

III. On 10 August 1934 a third horse (20578) was given an intracerebral injection of 1 c.c. of a 0.5 % saline suspension of infective mouse brain representing passage generation 158 of strain 20449, the original technique being used. A febrile reaction shown graphically in Chart V commenced on the sixth day. At no time did the horse appear ill. The appetite might have been decreased slightly and at the height of the temperature curve there was a tendency to a swaying gait with a high stepping action but this was never pronounced. Three weeks after injection the habitus was normal and no dikkop developed.

From the sixth to the fourteenth day defibrinated blood was injected intracranially into mice each day. The presence of circulating virus in the peripheral blood could not be established.

After an interval of 54 days the horse proved to be immune to the homologous strain of virus.

Conclusions. After 158 passages through the brains of mice the neurotropic virus had not developed an affinity for the central nervous system of horses. There is no evidence to show whether any multiplication of the virus took place within the nervous tissue but an active immunity develope

Discussion. The results of these experiments are of particular importance to the problem of the immunization of equines. For economic reasons the simultaneous use of high titre hyperimmune serum to block out the reaction due to the injection of neurotropic attenuated virus cannot be justified except under special circumstances where exceedingly valuable animals are being treated. This statement will be supported by data being prepared for publication, wherein

it is shown that after attenuation by at least 100 passages through mice no fatal reaction has occurred in over 3000 animals injected subcutaneously although some seven reactions have been reported. It has been shown that during the febrile phase particularly of severe reactions virus will circulate in the peripheral blood of the host for a period of several days, thus serving as a potential reservoir for the insect vector of horsesickness. Consequently promiscuous immunization cannot be advocated in isolated areas where, owing to absence of a reservoir for the infection of the vector, the disease does not occur, since there is at least a theoretical danger of introducing the virus. On the other hand it has been shown that for at least one generation in equines the neurotropic virus retains its attenuation and does not revert to virulent viscerotropism. Whether attenuation is permanent can be decided only when the investigation is carried to its logical conclusion after the mode of transmission of horsesickness under natural conditions has been worked out. However from an analogy with Yellow Fever, in which disease this aspect has been investigated thoroughly by Sawyer, Kitchen and Lloyd[5] and Lloyd and Penna,[6] it may be anticipated that it will not be possible to bring about a change in the virus back to viscerotropism either by passage through the vertebrate or invertebrate host. If this should prove to be the case in horsesickness then the animal reacting to immunization cannot be a potential danger to the susceptible equine population and immunization throughout the country should be carried out in an attempt gradually to replace in nature the virulent virus by an attenuated form, or alternately to eliminate the virus reservoir, should this prove to be confined to equines. At first sight the latter suggestion may appear to be over ambitious but when it is realised that according to the

agricultural census of 1930 the number of horses in the Union was only 867,614 the production and maintenance for some years of a completely immune population is definitely practicable.

In all work on mass immunization of horses experience has shown that the unpleasant sequelae to the efforts to produce a solid immunity are to be feared nearly as much as the disease itself. Some of these sequelae e.g. the inclusion of the virus of infectious anaemia in the hyperimmune serum used in the serum-virus succedaneous method can be avoided but up to the present the relation between equine staggers and immunization has not been determined. In extreme cases staggers has been responsible for a mortality of 45% in batches of immunized animals. By the neurotropic virus method the ultimate development of a polyvalent vaccine appears to be so simple that one subconsciously is on the look out for pit falls. Up to the present no cases of staggers have been reported amongst horses immunized by this method but there has always existed the unpleasant feeling that the virus might become neurotropic for horses as well as for mice, rats and guinea pigs. The experiments cited in which horses were given direct intracerebral injections of virus up to the 158th generation in mice indicate that by that time the virus had not acquired a tropisin for the central nervous system of horses. If a specific fatal inciphalitis does not occur as a result of direct intracerebral injection it is unreasonable to expect that it will result from subcutaneous injection, and consequently it would appear to be perfectly safe to incorporate in a vaccine virus attenuated by this number of passages. This finding is somewhat remarable in view of the analogous work with Yellow Fever since Lloyd and Penna (6) have

shown that inoculation of Berkefeld N. filtrates containing the mouse-brain-adapted yellow fever virus in M rhesus was followed by an incephalitis ------ accompanied by a wide spread distribution of the virus in the peripheral nervous system at the time of death. Consequently if future experience does bring to light some distressing sequel to immunization, it seems unlikely that such a sequel will bear any relation to a neurological disturbance.

Attention has been directed to a predisposition to pulmonary affections after immunization. It is not known whether this is dependent u on a general decrease to natural resistance against infections through the upper respiratory tract or whether a locus minoris resistance is produced in the lungs by the specific action of the virus. At all events the predisposition appears to be transitory and good hygiene should prove effective as a prophylactic measure.

Summary.

1. Virus may be detected in the peripheral blood of horses during the febrile reaction following the injection of mouse neurotropic virus.

2. The more severe the reaction the more likely is free virus to be encountered.

3. This circulating virus retains its neurotropic character and after at least one generation in equines retains its attenuation.

4. Intracerebral injection of horses with mouse brain adapted virus after 168 passages does not produce a specific incephalitis but does produce a normal mild reaction followed by the development of a solid immunity.

5. The febrile reaction produced by injection of attenuated virus appears to result in a temporary predisposition to pulmonary infections.

6. The significance of these findings is discussed.

Chart I

Horse 20915.

* 10cc subcut quadrivalent vaccine batch I

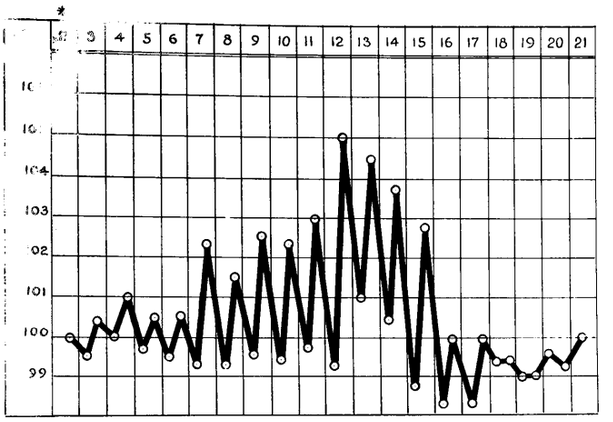
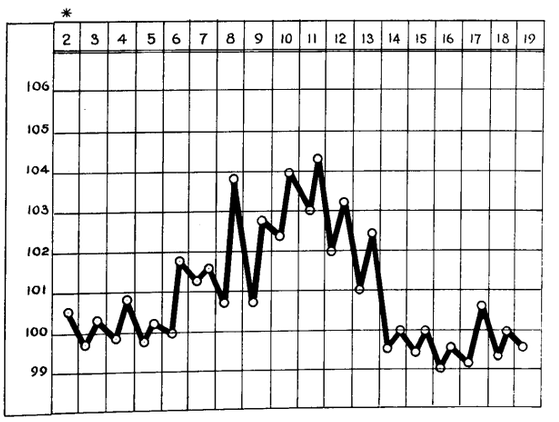


Chart II

Horse 20919.

* 10cc subcut quadrivalent vaccine batch I

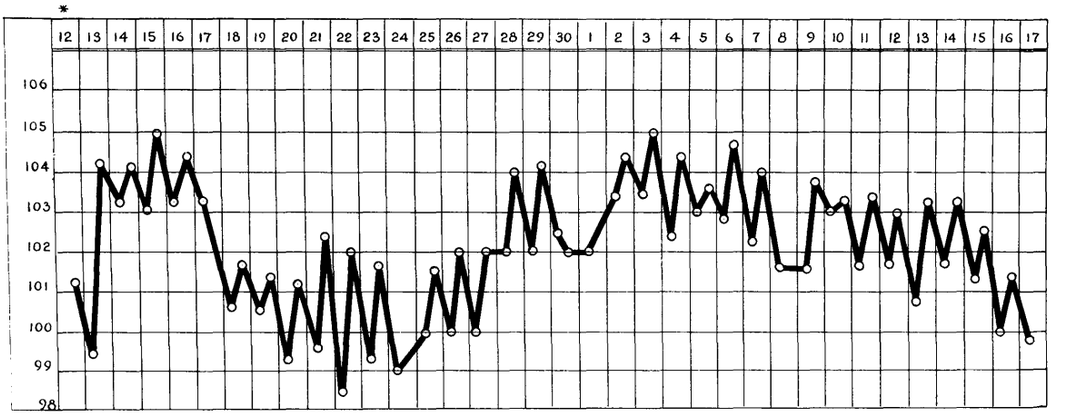


2,500 cc blood transfused into horse 20920 (chart III)

Chart III

Horse 20920.

* Transfered 2,500cc blood from 20919.



NB. for "transfered" read "transfused"

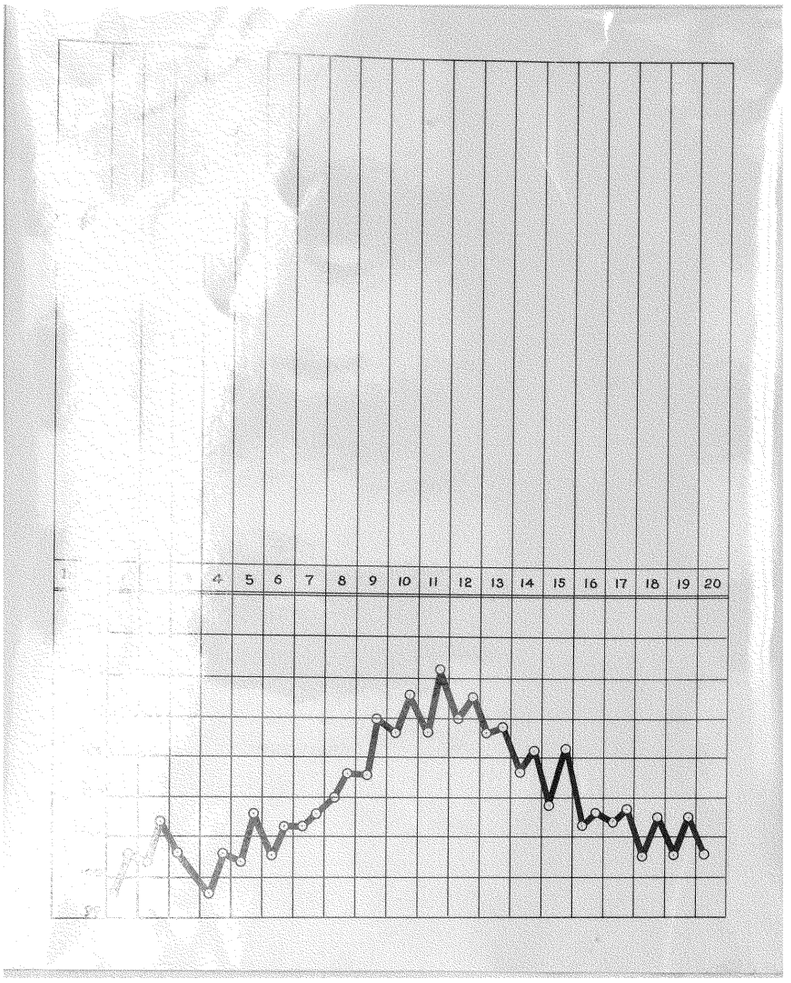


Chart IV

Horse 20576.

1cc brain emulsion intracerebrally = equivalent 38 Gram 20449 in mice.

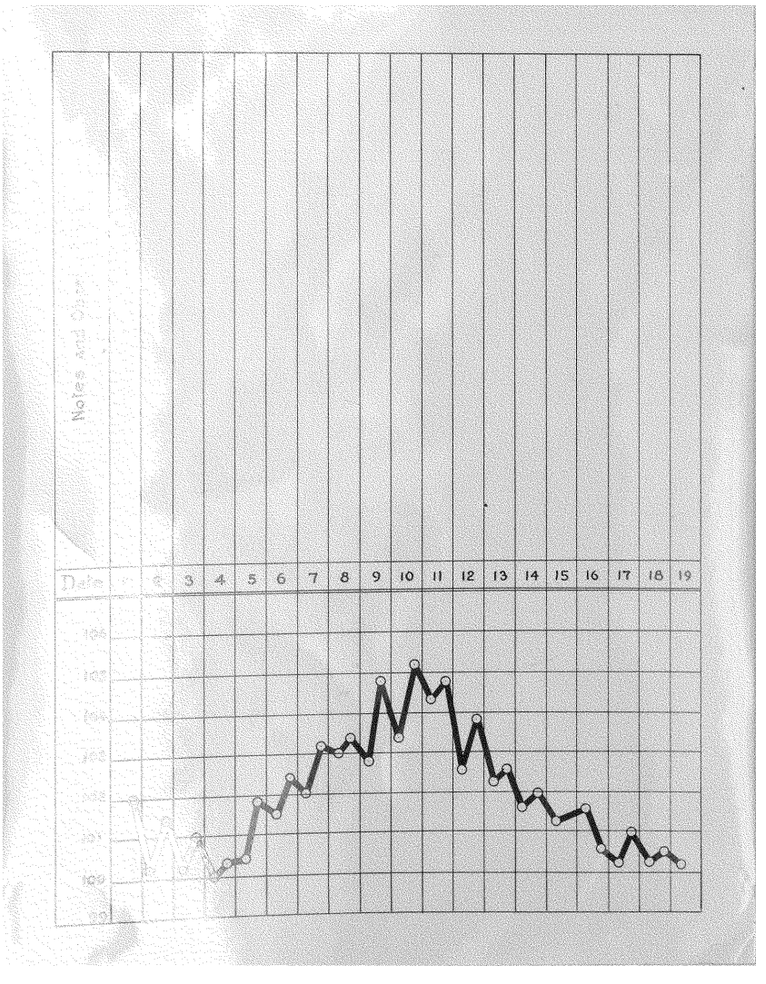


Chart V

Horse 20578

1cc brain emulsion intracerebrally = equivalent 158 Gram 20449 in mice.

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