

Grass Sickness in Horses.

THE following report is an excerpt from the full memoir presented to the Highland and Agricultural Society of Scotland concerning researches into "grass sickness" in horses carried out by the Director of the Investigation, Dr. J. F. Tocher and his co-workers—Mr. W. Brown, Dr. J. W. Tocher and Mr. J. B. Buxton.*

* The report in this form was given by these investigators at a meeting of the Scottish Branch of the National Veterinary Medical Association, held at Perth, December 15, 1922, and subsequently published in *The Veterinary Record* of January 20, and February 3, 1923.

Dr. TOCHER said that in 1918 the belief was prevalent that "grass sickness" in horses was probably produced by alsike clover, and a thorough survey of the infected areas in Forfarshire and Perthshire was made, with the object of identifying toxic plants and clovers, but with negative results. Horses were fed on alsike clover for an entire season, but without ill-effects. Alcoholic extracts from that clover also proved non-injurious, and no toxic substances could be isolated from the extract.

In 1919 a complete tour of the whole of the infected area was made, and a series of *post mortem* examinations carried out. The results indicated the probability of the animals having died from acute toxæmia of bacterial origin, since vegetable toxins had been experimentally excluded. In the same year a large anaerobic bacillus was isolated by Drs. J. F. and J. W. Tocher from portions of the stomach and intestines collected in the course of the tour, and this bacillus was found to have the morphological characteristics and toxigenic properties of van Ermengen's *B. botulinus*, and it was then noticed that the clinical symptoms closely resembled those of botulism.

During 1920 the investigation was conducted on the assumption that *B. botulinus* was the probable cause of "grass sickness," and a report giving scientific evidence in support of this conclusion was sent to the Highland and Agricultural Society. As the result, funds were raised for continuing the research on bacteriological lines, and the association of the disease with botulism has been established.

Dr. Tocher also produced maps showing the distribution of "grass-sickness" since 1911, and its extension northwards from Forfarshire and Perthshire. In 1921 seven cases were recorded in the Elgin and Banff areas.

Mr. W. BROWN, in describing the symptoms and *post mortem* appearances of "grass sickness," pointed out that it was a seasonal disease which occurred among horses shortly after they are put on to grass, its incidence ranging from about May to September. Two distinct types of the disease were met with (*a*) the acute, and (*b*) the sub-acute, and there were distinct differences in the symptoms of those two types. The *post mortem* appearances were comparatively few but characteristic, and in the sub-acute cases, which might last for 2 or 3 weeks, there were certain features not observed in the acute cases. A full description of these and also of the diagnosis and treatment of the disease was given.

Dr. J. WILLIAMSON TOCHER, describing the bacteriological investigation, said that in acute cases the cerebro-spinal fluid was sterile, but that in sub-acute cases several organisms had been isolated. These included (1) staphylococci; (2) a diphtheroid-like organism; (3) streptococcus; (4) a strepto-bacillus or coccobacillus. Of these, the only organism pathogenic to laboratory animals was the streptococcus, but the horse did not react in any way to the sub-cutaneous injection of this organism. In all sub-acute cases of "grass sickness" there is a secondary meningitis of a mild type produced by organisms normally present in the naso-pharynx of the horse.

The method of isolating the causal anaerobic organism was to plunge the spleen in boiling water for 3 to 5 minutes and then to cut it, with aseptic precautions, into small pieces. These were placed in test tubes containing 2 per cent. glucose broth, heated for 20 minutes at 80° C., and, incubated anaerobically in McIntosh and Fildes' jars. In all cases slightly alkaline broth was used. The tubes were incubated for 48 hours, and were then examined for their reaction, and those showing formation of acid and gas selected for further tests. Tubes in which no reaction was shown after an additional 48 hours' incubation were rejected. It was noticed that there was an odour of butyric acid in the case of several of the tubes.

From the selected tubes sub-cultures of anaerobes were prepared in the

following manner: Two per cent. neutral glucose agar was poured in a thin layer on to the bottom of a Petri dish and allowed to cool. Thinly-seeded shake cultures of the suspected organisms were made either in 2 per cent. l  vulose agar or 2 per cent. lactose agar, the agar being faintly alkaline to neutral red. The tube of agar was then poured on to the surface of the glucose agar and allowed to solidify. When solid, a third layer of glucose agar was poured on to this second layer to prevent access of air, and good anaerobiosis was obtained with the resulting medium.

The chief contaminating organism in these spleen cultures was *B. sporogenes*. It was distinguished from *B. botulinus*, *B. Welchii*, and *V. septique* by not fermenting lactose, whereas l  vulose is fermented by *B. sporogenes*, but not by *B. botulinus*. The filtered cultures from *B. sporogenes* appeared to be non-toxic to laboratory animals. The organisms isolated in these cultivations agreed in morphological characteristics with *B. botulinus*, and the bacillus and its toxin produced in experimental animals and in horses lesions known to be associated with botulism, and identical in character with the lesions found in horses suffering from "grass sickness."

Mr. BUXTON gave a description of his diagnosis by serological and immunological methods. He said that when, in 1919, he had agreed to make a series of serological experiments to test the hypothesis of Drs. J. F. and J. W. Tocher that "grass sickness" was really botulism, he had told them quite frankly that he was unable to accept their hypothesis. At that time botulism was only known to be a primary intoxication, for the characteristic symptoms were only known to have been produced by direct administration of the toxin of *B. botulinus* which had been produced under abnormal conditions outside the animal body. He could not imagine the existence of suitable conditions for the production of the toxin in the field or during the process of preparing or handling horse-fodder.

Samples of the blood from acute and from chronic cases and from recovered animals were tested for the presence of botulinus antitoxin by means of toxins prepared from known strains of *B. botulinus*. These tests showed that botulinus antitoxin was present in the blood of horses which were suffering from the chronic form of "grass sickness," or had recovered from the disease, whilst the blood of a normal horse did not contain a similar anti-body. As was expected, the blood of acute cases did not contain antitoxin. The unstable nature of botulinus toxin was well known, and it was inconceivable that it could have been ingested with the pasture or taken in water. The only way of accounting for its presence in these animals was the assumption that in these cases one was dealing with an *infection* and not with a *primary* intoxication.

Toxin prepared from an organism isolated from a case of "grass sickness" was found to be neutralised by botulinus antitoxin, and this toxin, when injected subcutaneously into horses, reproduced the typical symptoms of an acute case of "grass sickness."

The active immunity produced by means of botulinus toxin-antitoxin mixtures appeared to have had some influence in decreasing the incidence of "grass sickness."

Dr. J. F. TOCHER pointed out that the prevential serum was not prepared from "grass sickness" cultures, but from known strains of the *B. botulinus* obtained from an external or non-grass-sickness source. The death rate among uninoculated horses amounted to 9.3 per cent., whilst the death rate among inoculated horses, on the same farms, was 2.8 per cent. There was, therefore, a reduction due to inoculation of 6.5 per cent. The odds in favour of inoculation reducing the death rate were 1,750,000 to 1. If grass sickness were not identical with botulism, this reduction in the death rate, by inoculation of a botulinus mixture from a non-grass-sickness source, could not have occurred.

These results were therefore conclusive as proving that grass sickness was botulism. Horses should therefore be protected by the use of the preventive serum prepared by Mr. Buxton. A supply was now available on a commercial basis (*cf.* ANALYST, 1923, 118).
