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Gas Chromatographic Detection of In Vivo Activity of Equine Infectious Anaemia Virus

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By making use of the marked response of the electron capture detector, it has been possible to detect the presence of extremely small numbers of bacteria in culture media by gas chromatography (B. M. Mitruka and M. Alexander, Appl. Microbiol. **16**:636, 1968). The same technique has been employed for the detection of canine viruses in vitro and in vivo (B. M. Mitruka et al., Science **160**:309, 1968). It has now been observed that electron capture-gas chromatographic techniques can be applied to the rapid detection of the in vivo activity of equine infectious anaemia virus. Latent infections caused by this virus can only be shown by the inoculation of infective blood into susceptible horses.

Serum samples from five healthy experimental horses were taken at occasional intervals for a 2- to 3-month period and at 2-day intervals during the 2 weeks immediately prior to infection. The animals were then inoculated intravenously with 5.0 ml of infective serum from clinical sources. For chromatographic analysis, 2.0 ml of serum was treated with 0.10 ml of 5 N HCl and 1.0 ml of 0.2 M HCl-KCl buffer (*p*H 2.0); the samples were centrifuged for 15 min at 3,000 × *g* and the resultant supernatant fluid was extracted three times with 10 ml of ether. A 3.0-μliter portion of the combined extract, or of the combined extract after concentration, was injected into the instrument. The chromatographic techniques were essentially the same as previously described (Y. Henis et al., Appl. Microbiol. **14**:513, 1966; B. M. Mitruka and M. Alexander, Appl. Microbiol. **16**:636, 1968). The animals were kept under observation for temperature elevation and changes in hematocrit readings and precipitin tests. A rise in temperature of 2 to 3 C was noted in the animals at 2 to about 4 weeks after inoculation. Animals showing illness had hematocrit readings of 20% or less as determined by packed cell volume, whereas the healthy animals showed readings of 30 to 40%.

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Gas chromatograms of ether extracts derived from both the healthy and inoculated animals usually contained compounds with retention times of 25, 45, 60, 70, 85, and 100 sec. Peaks

TABLE 1. *Appearance of two compounds in sera of horses inoculated with equine infectious anaemia virus*

Case	Time after incubation (days)	Area of peak (mm^2)		Period of fever (days)
		H	I	
1	4	0	0	
	6	120	110	
	8	270	260	17 to 30
	9	320	300	
	15	140	100	
2	4	2,250	280	
	8	3,170	1,950	15 to 20
	9	2,000	450	
	15	600	300	34 to 37
	17	0	0	
	21	0	0	
	25	0	600	
	33	0	750	
3	4	20	210	
	6	2,250	1,750	15 to 20
	8	3,000	1,900	
	9	1,000	<10	
	15	<10	0	34 to 44
	25	0	20	
	33	0	80	
4	4	0	0	
	15	0	0	31 to 36
	25	900	1,050	
	33	0	0	
	40	350	110	48 to 55
	44	108	64	
5	8	1,260	1,010	
	9	480	240	19 to 23
	15	<10	0	
	25	0	0	
	40	20	180	54 to 59
	44	250	60	
	50	0	0	

with retention times of about 115, 160, 200, 340, 600, and 1,485 sec were observed in extracts derived from several horses prior to inoculation. In some instances, one or more of these peaks were absent at the time of inoculation, but appeared subsequently in one or several animals. Such compounds probably have little or no diagnostic value.

Serum taken from the inoculated animals before the appearance of clinical symptoms, i.e., fever and changes in hematocrit readings, contained compounds with retention times of 140 and 150 sec, designated H and I. At no time prior to infection were these products, which were easily distinguishable, observed. Representative data for the first 7 weeks are presented in Table 1. The tabulated times of observation are in days after inoculation, and the peak areas listed are the areas calculated for the entire serum sample rather than the sample injected into the instrument.

The two compounds appeared 4 to 25 days after inoculation and 6 to 11 days before clinical symptoms were evident. The symptoms recurred in all but one case 5 to 8 weeks after inoculation. Peaks H and I usually disappeared before the first fever developed and one or both reappeared 1 to 2 weeks prior to the recurrence of the disease. After the last day recorded in the table, serum constituents with retention times of 140 and 150 sec were no longer detectable, although analyses were performed for a 10- to 15-week period.

The findings, that two substances appeared in all the animals after inoculation but were consistently absent before infection and that these became evident before the fever developed, are of particular interest in attempts to design methods for the early diagnosis of this viral disease. These studies on equine infectious anaemia, although preliminary, are in accord with the observation of B. M. Mitruka et al. (Science **160**:309, 1968) on canine viruses, that extremely sensitive gas chromatographic methods can be used for establishing quickly the activity of viral agents in vivo.

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