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Preparation of Measles Antiserum in Horses. (27553)

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The need arose in our laboratory to prepare a large volume of measles immune serum for research purpose. This was accomplished simply and effectively by immunizing horses with measles virus emulsified in Freund's incomplete Arlacel A-mineral oil adjuvant. The present report summarizes the technics used and the findings.

Materials and methods. Virus. The Edmonston(1) strain of measles virus was obtained from Dr. John F. Enders and was received in the 29th passage in primary cell cultures of human amnionic membrane. The virus was carried in our laboratories in the Fogh and Lund(2) and in the Wistar Institute WISH(3) line cell cultures of human amnion. Virus which had been serially passed 10 times in our laboratory was used for immunization purpose. The infectivity titer of the virus used was $10^{-4.5}$ per 0.1 ml and the complement-fixing (CF) titer was 1:16. *Emulsification.* Equal volumes of the live measles virus and of the Arlacel-mineral oil mixture* were emulsified by ordinary force-mixing procedures. The Arlacel and mineral oil components were tested for toxicity by usual methods† prior to use. The emulsified vaccine was free of bacterial and mold contamination and was stored at 4°C in the refrigerator until used up to 4 weeks later. *Immunization.* The horses were ordinary draft animals of 11 to 17 years age and weighed 1000 to 1200 lb. Pre- and post-vaccination bleedings were in 4 liter amounts and were drawn into evacuated flasks *via* a trocar inserted into the jugular vein. Injections of aqueous vaccine were of 10-ml volume and were given into the lateral cervical muscles of the neck. The first dose of adjuvant vaccine given into the neck muscles caused stiffness and, thereafter, injections were made into the gluteal muscles. The dose of adjuvant vaccine consisted of two 10-ml injections given

* 10% Arlacel A, Atlas Powder Co., Wilmington and 90% Drakeol 6 VR, Pennsylvania Refining Co., Butler, Pa.

† These were described in "The Tentative Draft, Minimum Requirements: Influenza Virus Vaccine, Emulsified in Mineral Oil," U. S. Dept. of H.E.W., Nat. Inst. Health, Jan. 30, 1956.

TABLE I. Antibody Response in Horses to Aqueous and to Adjuvant Measles Virus Vaccines.

Day of vaccination	Vaccine regimen		Neutralizing antibody titer *vs* Measles virus				RS virus	
			Adjuvant		Aqueous		Adjuvant	Aqueous
	Adjuvant	Aqueous	Horse 1	Horse 2	Horse 3	Horse 4	Horses 1 & 2	Horses 3 & 4
0	2 doses*, 10 ml ea.	1 dose, 10 ml	< 1	< 1	< 1	< 1	< 4	< 4
7	—	"	—	—	—	—		
14	2 doses, 10 ml ea.	"	4	4	< 4	< 4	—	—
28	"	"	128	128	2	8	—	—
41			256	256	8	16	—	< 4
52			256	512	—	—	< 4	—
75			512	512	—	—	< 4	—

* Each dose, 10 ml of 50% aqueous vaccine and 50% Arlacel-mineral oil. All vaccine was given intramuscularly.

into separate sites. *Procedures* for conduct of the neutralization and infectivity titrations have been described(4).

Results. Four horses were immunized according to the regimen and with the findings shown in Table I. It is seen that a high titer of measles neutralizing antibody was achieved following a course of 3 injections of the adjuvant antigen. The response was gradual and maximal titers of 1:256 to 1:512 were achieved by the sixth to seventh week following the first dose. It is likely that fewer doses of vaccine would have achieved the same effect and it is possible that the titers might have risen to higher levels had later blood samples been tested. In contrast to adjuvant vaccine, the response to aqueous vaccine was poor and the sera had reached titers of only 1:8 or 1:16 by the sixth week, at a time when the serum titers of animals given adjuvant vaccine had reached a level of 1:256.

The sera obtained from animals given adjuvant vaccine were not suitable for complement-fixation (CF) tests with measles virus antigen prepared either in grivet renal kidney or in human amnion cells. The sera were often anticomplementary and, as might be expected, contained high titers of antibody against the normal tissues. These anti-tissue antibodies did not, however, affect the specificity of the measles antibody in neutralization tests. Thus, as shown in Table I, respiratory syncytial virus was not neutralized in tests carried out in a manner similar to that for measles antibody.

Discussion. Although commonly used in the past to prepare antisera against bacteria or their products, the horse has been the "forgotten animal" where virus antisera are concerned. This is probably the result of past need for only small volumes by the individual laboratory and of the fulfillment of such requirement through utilization of small laboratory animals. The "explosion", however, in numbers of new viruses requiring identification and in the need for standardized reference sera for general typing purpose has made the use of small animals an uneconomic procedure.

The preparation of measles virus antiserum in horses utilizing Freund's adjuvant points the way to an inexpensive and efficient means for preparing these reagents. The measles sera were of high titer, were free of non-specific substances reactive in the neutralization test, and were non-toxic for cells in culture. The adjuvant vaccine given into the gluteal muscles of horses was without apparent harmful effect. Such horses could be bled once weekly for up to 4 liters of blood yielding, thereby, an approximate 8 liters of serum per month. The cost for such serum is but a small fraction of the cost when such sera are prepared in ordinary small animal hosts.

Summary. Horses immunized by injection of live Edmonston measles virus in Freund's Arlacel-mineral oil adjuvant produced measles antisera of high neutralizing antibody content and in large amount. The response to aqueous measles vaccine was very poor. The antiserum was devoid of bothersome non-specific substances and was not toxic for human cells in tissue culture. The economic and scientific utility of horses for preparing anti-

sera against viruses was discussed.

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Amino Acid Composition of Rat and Mouse Tumors.* (27554)

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It has been shown(1) that there is no significant difference in the total amino acid composition of 2 rat tumors, Sarcoma R-1 and Walker carcinosarcoma 256, grown in ethionine-treated or untreated, male or female, Wistar rats. It was also observed(1,2) that the composition of these tumors was in excellent agreement with that found for the U.C.L.A. fibrosarcoma(3) and for 4 other rat tumors studied by Sauberlich and Baumann (4). It was of interest, therefore, that the content of 6 amino acids (arginine, aspartic acid, lysine, phenylalanine, proline, and serine) reported by Mickelson and Barvick(5) for the Sarcoma 180 mouse tumor differed markedly from that observed for rat tumors (1-4). In all of these experiments amino acids were determined by microbiological assay.

Greenstein(6) and Roberts and Tanaka(7) have stressed the biochemical similarity of tumors and Dunn *et al.*(8) reported that there was no significant difference in total amino acid content of normal rat and mouse tissues. It appeared desirable, therefore, to analyze both types of tumor tissue by column chromatographic and paper chromatographic technics.

Materials and methods. Two samples of Walker carcinosarcoma 256 grown in rats, one sample of Sarcoma 180 grown in mice, and 2 samples of the Taylor mouse tumor, one grown in mice and one grown in incubating eggs, were treated for 3 minutes in boiling water, trimmed free of necrotic tissue and connective tissue, dried at 50-70° in a vacuum oven, extracted with ether-alcohol in a Soxlet apparatus, redried to constant weight, and hydrolyzed by refluxing in 6N HCl for 24 hours. After filtering and washing the residue, the samples were evaporated to dryness under reduced pressure, dissolved in a minimum of hot water, filtered, and made up to a known volume.

An additional sample of Sarcoma 180 was prepared as above with the exception that it was not treated with boiling water. This control sample was necessary since the treatment with boiling water was included in the procedure of Baginsky, Murphy and Dunn(1) but not in that of Mickelson and Barvick(5).

Two amino acids, phenylalanine and tyrosine, not included in the previous study(1), were determined microbiologically using *Leuconostoc mesenteroides* P-60 (8042) for the phenylalanine and *Lactobacillus casei* ϵ (7469) for the tyrosine assays. The general assay procedures were those described previously(1). The column chromatographic methods used were modifications of those of Moore and Stein(9), Ishii(10), and Chinard (11), and the paper chromatographic method was a modification of the procedures of McFarren and Mills(12) and Baker and Khan (13).

Results. Since no significant differences were observed between the results for the 2 samples of rat tumor, or the 4 samples of

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