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TECHNICAL MANUSCRIPT 46

HOST INFLUENCE ON THE LIPID
CONTENT OF VENEZUELAN EQUINE
ENCEPHALITIS VIRUS

JUNE 1963

UNITED STATES ARMY
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ENCEPHALITIS VIRUS

The work reported here was performed under Project 4X99-26-001, "Basic Research in Life Sciences, Task -02, Basic Research-Microbiology. The expenditure order was 2039. This material was originally submitted as manuscript 5078.

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Project 1A012501B02802

June 1963

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ABSTRACT

Ada *et al* reported that the relative proportions of lipid, phospholipid, and cholesterol in the Murray Valley virus propagated in mouse brain (10:0.7:0.9) are different from those in seven-day normal mouse brain (10:7.1:1.2). In contrast, the ratio of phospholipid, cholesterol, and triglyceride in influenza virus is similar to that in the host chick embryo chorioallantoic membrane (Frommhagen *et al*). Analyses of Venezuelan equine encephalitis virus from two host materials (suckling mouse brain and chick embryo), purified by treatment with Celite and protamine, followed by sucrose gradient centrifugation, indicate that the total lipid content of virus from both sources is approximately the same (22 per cent). Moreover, the proportions of lipid, phospholipid, and cholesterol are the same for virus from either host (10:2.7:2.7), whereas these proportions for four-day normal mouse brain and 11-day normal chick embryo are 10:6.2:3.6 and 10:4.1:3.6, respectively. These results suggest that the arboviruses differ from the myxoviruses in the relationship of viral lipid composition to that of the normal host.

HOST INFLUENCE ON THE LIPID CONTENT OF VENEZUELAN EQUINE ENCEPHALITIS VIRUS

In an earlier report, Wachter and Johnson¹ showed by density gradient centrifugation that the lipid content of Venezuelan equine encephalitis (VEE) virus, a Group A arbovirus, was less than half the lipid content of this virus purified without gradient centrifugation, and also less than half that reported earlier by Taylor *et al*² for Eastern and Western equine encephalitis viruses. It was felt that the gradient-purified virus contained lipid that was an integral part of the virus structure. Since this work was mainly concentrated on virus derived from one host, it was of interest to determine, first, whether VEE virus propagated in different hosts would have the same lipid content and, secondly, whether the lipid composition of the host would influence the lipid composition of the virus. From the work of Frommhagen and others³ it is generally accepted that the lipid content ratios of the myxoviruses coincide with those of the host. Recently, Ada and co-workers⁴ reported that the lipid content ratios of Murray Valley virus (a Group B arbovirus) propagated in baby mouse brain were markedly different from those of seven-day normal mouse brain.*

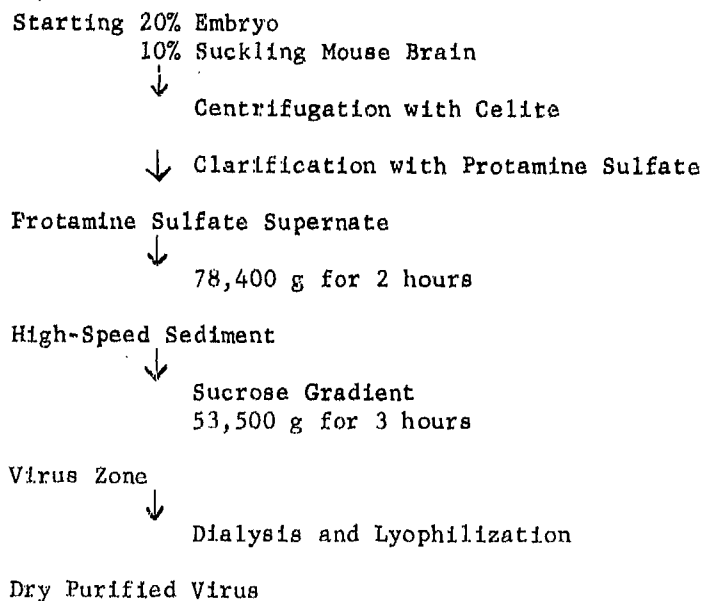
* In conducting the research reported herein, the investigator(s) adhered to "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

In this report, the lipid, phospholipid, and cholesterol ratios of VEE virus purified by gradient centrifugation from two host materials, suckling mouse brain and chick embryo, will be compared.

The procedure employed for the isolation of VEE virus from 11-day chick embryo and 4-day mouse brain suspensions is outlined in Table I.

Following treatment with Celite and clarification with protamine sulfate, the virus was concentrated by high-speed centrifugation before being placed on a continuous sucrose gradient. Centrifugation at 53,500 g for three hours in a Spinco SW-25 swinging bucket rotor resulted in the formation of discrete virus zones. The specific bands were removed by puncturing the side of the gradient tube. Purified virus suspensions from a series of identical trials were pooled, dialyzed against distilled water, lyophilized, and dried to constant weight.

TABLE I. FLOW DIAGRAM OF PURIFICATION PROCEDURE FOR VEE VIRUS



Total lipid contents of purified virus, and of normal and infected chick embryo and mouse brain, were determined by direct weighing on an electrobalance after extraction with boiling methanol-ether (1:1 by volume) in a microextraction apparatus. Organic phosphorus was determined by the method of King,⁵ and phospholipid content was calculated by multiplying phosphorus values by a factor of 25. Cholesterol was determined by the method of Bowman and Wolf.⁶

It was observed that the buoyant densities in sucrose gradients of VEE virus from mouse brain and chick embryo sources were very much alike, which suggested that virus from the two hosts was of similar lipid content. Chemical analyses showed that the total lipid content of the VEE virus from each host was the same (Table II).

In addition to the lipid percentages, Table II also lists percentages of phospholipid and cholesterol for purified VEE virus. For comparison, the percentages of these components in normal and infected 11-day chick embryo and 4-day mouse brain are presented. It can be noted that VEE virus from each host contained approximately 22 per cent total lipid, 6 per cent phospholipid, and 6 per cent cholesterol. When compared with virus, host materials showed generally lower percentages of total lipid, similar percentages of cholesterol, but higher percentages of phospholipid. Although the amount of phospholipid present in normal and infected suckling mouse brain was twice that present in normal and infected embryo, virus from these two hosts contained the same amount of phospholipid.

TABLE II. COMPARISON OF THE PERCENTAGES OF LIPID COMPONENTS FOR VEE VIRUS AND HOST MATERIALS

Preparation	Total Lipid, %	Phospholipid, %	Cholesterol %
Chick Embryo Virus	22.5	5.8	5.9
Suckling Mouse Brain Virus	21.8	5.9	6.2
Normal 11-day Embryo	16.4	6.6	5.3
Infected 11-day Embryo	16.8	7.5	5.7
Normal 4-day Mouse Brain	21.6	13.2	7.8
Infected 4-day Mouse Brain	18.4	10.6	6.8

Table III shows the total lipid, phospholipid, and cholesterol contents of virus and host materials expressed as ratios. When these components are tabulated in this manner, it can be seen that the phospholipid portion represents about 60 per cent of the total mouse brain lipid, about 40 per cent of the total embryo lipid, but only 25 per cent of the total viral lipid. Cholesterol values generally ranged about 10 per cent higher in the host materials, but the marked difference observed in the phospholipid portion was not seen.

In summary, it can be stated that by chemical analyses the total lipid content of VEE virus from 11-day chick embryo and from 4-day suckling mouse brain was the same. These results, together with those of Ada⁴ for Murray Valley virus, suggest that the lipid composition of the arboviruses, unlike that of the myxoviruses, may be independent of the host background composition, and characteristic for the individual virus.

TABLE III. COMPARISON OF THE RATIOS OF LIPID COMPONENTS
FOR VEE VIRUS AND HOST MATERIALS

Preparation	Total Lipid	:	Phospholipid	:	Cholesterol
Chick Embryo Virus	10	:	2.7	:	2.8
Suckling Mouse Brain Virus	10	:	2.6	:	2.6
Normal 11-day Embryo	10	:	4.1	:	3.6
Infected 11-day Embryo	10	:	4.5	:	3.6
Normal 4-day Mouse Brain	10	:	6.2	:	3.6
Infected 4-day Mouse Brain	10	:	5.8	:	3.7

LITERATURE CITED

1. Wachter, Ralph F., and Johnson, Emil W. "Lipid content of the equine encephalitis viruses," *Federation Proc.* 21:461, 1962.
2. Taylor, A. R.; Sharp, D. G.; Beard, Dorothy; and Beard, J. W. "Isolation and Properties of the equine encephalomyelitis virus (Eastern strain)," *J. Infect. Diseases*, 72:31-41, 1943.
3. Frommhagen, L. H.; Freeman, N. K.; and Knight, C. A. "The lipid constituents of influenza virus, chick allantoic membrane, and sedimentable allantoic protein," *Virology*, 5:173-175, 1958.
4. Ada, G. L.; Abbot, A.; and Anderson, S. G. "Particle counts and some chemical properties of Murray Valley encephalitis virus," *J. Gen. Microbiol.* 29:165-170, 1962.
5. King, E. J. "The colorimetric determination of phosphorus," *Biochem. J.* 26:292-297, 1932.
6. Bowman, Robert E., and Wolf, Richard C. "A rapid and specific ultramicro method for total serum cholesterol," *Clin. Chem.* 8:302-309, 1962.