TABLE II. Hemagglutinin Loss by the Nelson and Levaditi Viruses during Serial Passage in the 2X and 4X Tumors.

Virus	Passage in 2X tumor	HA titer*	Virus titer‡	Passage in 4X tumor	HA titer	Virus titer
Nelson	10	160	4.5	8	40	3.9
	11	160	5.0	9	20	4.7
	12	80	4.1	10	10	4.4
	13	0†	3.9	11	0	4.0
Levaditi	7	80	4.7	4	20	4.3
	8	40	5.0	5	20	4.1
	9	10	5.5	6	10	4.6
	10	0	5.7	7	0	4.2

* Hemagglutinin titer of a 20% suspension prepared from chorioallantoic membranes bearing confluent lesions as a result of inoculation with virus from the respective passage.
† The designation 0 indicates the titer was less than 1:10.
‡ Log of the pock-producing units per 0.05 ml of tumor on 6th day after virus inoculation.

2X and 4X tumors. We could not demonstrate this earlier, because only recently have we succeeded in adapting these originally very refractory viruses to the tumors.

These results provide supporting evidence for a role of the hemagglutinin inhibitor in bringing about the hemagglutinin loss in vaccinia virus. In an effort to establish a better control in our experiments, various methods, including heat treatment and exposure to antibody, have been tested for removing inhibitor from the tumor fluid without altering other constituents of the fluid. No method has proved to be satisfactory. We have concluded that the best evidence for a role of the inhibitor in hemagglutinin loss would be through addition of chemically pure inhibitor to a system such as the L cell in medium 199, and examination of this system for its effectiveness in bringing about hemagglutinin loss. Inhibitor purification studies are now being carried out in another laboratory.

Summary. The present study strengthens the evidence for the role of a hemagglutinin inhibitor in the loss of hemagglutinin by vaccinia virus, and answers a criticism of a previous study. It has also been demonstrated that on serial passage in the Ehrlich ascites tumor loss of hemagglutinin is not limited to the IHD strain of vaccinia virus, but occurs also with the Nelson and Levaditi strains.

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Comparison of the Lipids in Maternal and Cord Blood and of Human Amniotic Fluid.* (27434)

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Preliminary studies by Boyd and Wilson (1) indicated that cephalins were disproportionately low in umbilical cord blood. Mod-

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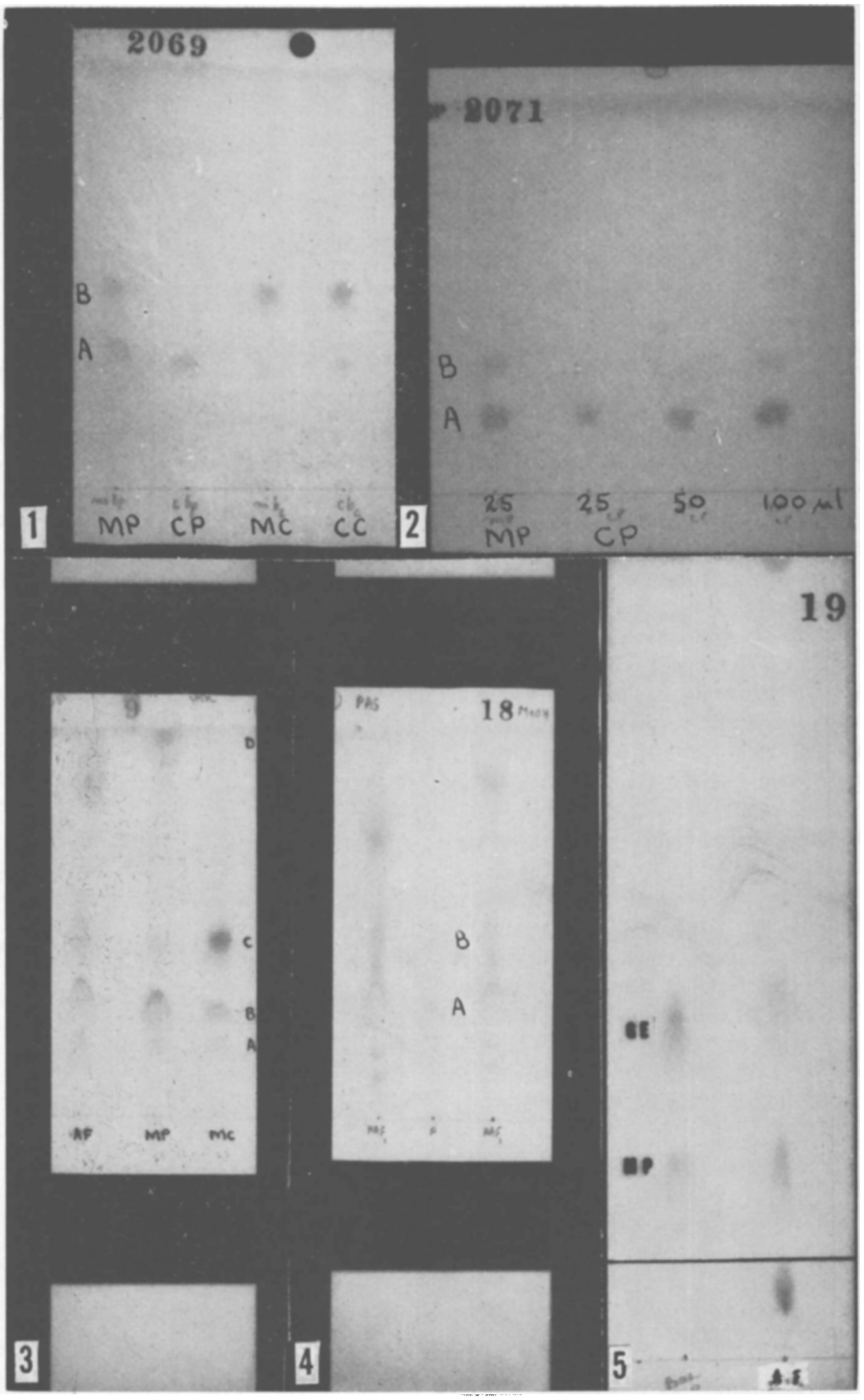

FIG. 1 (Chromatogram No. 2069). Comparison of maternal (M) and cord (C), plasma (P) and cells (C). Plasmalogen stain showing choline plasmalogen (A), ethanolamine plasmalogen (B).

ern methods of lipid analysis are capable of providing much more precise data; this is especially true of paper chromatographic technics where only μM amounts of lipid are required for a very adequate analysis. In this study, a direct comparison was made between maternal and umbilical cord whole blood, plasma and cells. In addition, the lipid pattern of human amniotic fluid was examined and compared with the above blood fractions.

Materials and methods. Five to 10 ml of blood were drawn from normal mothers just prior to delivery and from the umbilical vein at delivery. To facilitate collection of both cells and plasma, a mixture of potassium and ammonium oxalate was used as anticoagulant. One ml of whole blood, plasma or washed cell suspension was precipitated by adding to 5 ml of methanol. To complete extraction of the lipid 5 ml of chloroform and 5 ml of 0.73% NaCl was added with mixing; the chloroform was separated from the methanol-water by centrifugation at 600 *g* for 10 min. Twenty-five μl of this extract was sufficient for chromatographic analysis by the technic described earlier(2).

The amniotic fluid was obtained at Caesarian section through the cooperation of Dr. Conrad Collins. Ten to 20 ml aliquots of the whole fluid were promptly freeze-dried and extracted with one ml of chloroform:methanol (2:1) per ml of amniotic fluid. Ten to 12 ml of the whole fluid were centrifuged at 600 *g* for 10 minutes and the resulting precipitate and supernate collected, freeze-dried and extracted as above. These chloroform: methanol extracts were separated into a chloroform phase and a methanol-water phase, as for blood, by addition of 0.73% NaCl. The chloroform extract was concentrated to 1/10th its volume in order for 25 μl to contain sufficient lipid for chromatographic analysis.

Results. Blood. As illustrated in Fig. 1, plasma and cells contain both ethanolamine and choline plasmalogens; however, there was a marked difference in the relative amounts of these 2 phosphatides; phosphatidyl ethanolamine (and ethanolamine plasmalogen) characterized cells and phosphatidyl choline (and choline plasmalogen) characterized plasma. It is also clear from this figure that the ethanolamine phosphatide of cord plasma was much reduced by comparison with maternal plasma (Fig. 2), whereas the concentration of lecithin and sphingomyelin (as seen in Rhodamin stained chromatograms) more nearly resembles maternal plasma. The cord plasma neutral lipid was also reduced. There were no marked differences between the corresponding cells or whole blood extracts. The above data were obtained after complete spot-test analysis(3), requiring several chromatograms for each extract; only the plasmalogen-rhodamin chromatograms are shown because they were the easiest to photograph. No evidence for glycolipid was obtained when the PAS reaction was used. Although not shown in the figure, appropriate chromatograms showed that phosphatidyl serine was present only in cells (both maternal and cord) and here only in trace amounts.

Amniotic fluid. The 3 amniotic fluid fractions were found to be qualitatively similar; we therefore describe here only the data obtained from extracts made from the whole fluid. Fig. 3 illustrates the lipid pattern as

FIG. 2 (Chromatogram No. 2071). Demonstrating diminished concentration of ethanolamine phosphatide (B) in cord plasma (CP). 25 μl of maternal plasma (MP) contained more than was found in 100 μl of cord plasma extract. Maternal plasma showed about twice the concentration of lecithin and choline plasmalogen (A). Plasmalogen stain.

FIG. 3 (Chromatogram No. 9). Comparison of amniotic fluid (AF) with maternal plasma (MP) and cells (MC). A) Sphingomyelin, B) phosphatidyl choline, C) phosphatidyl ethanolamine, D) neutral lipid. Plasmalogen-rhodamin 6G stain. Fig. 1, 2, 3 were chromatographed in 2,6-dimethyl-4-heptanone, acetic acid, water system(2). 2-3 hr at room temperature.

FIG. 4 (Chromatogram No. 18). Amniotic fluid compared with plasma, PAS stain. The extra components are readily discernible, probably glycolipid and inositides. Developed in 2,6-dimethyl-4-heptanone, methanol, water solvent system(2). 2 hr at room temperature. *A* and *B* are lecithin and phosphatidyl ethanolamine respectively (and the corresponding plasmalogens).

FIG. 5 (Chromatogram No. 19). Acetone soluble fraction of amniotic fluid (AF) chromatographed on unimpregnated Whatman No. 1 filter paper with n-heptane: 2,6-dimethyl-4-heptanone 100:10, stained with PAS, compared with α-glycerylether (GE) and monopalmitin (MP).

compared with maternal plasma. Both ethanolamine and choline plasmalogens were present, their relative proportions resembling blood cells rather than plasma. Sphingomyelin and neutral lipid were also present. The PAS reaction (Fig. 4) visualized a number of additional substances, some of which were presumably glycolipid and inositide; the large PAS component near the solvent front resembled monoglyceride. This component was separable into 2 spots on chromatography in n-heptane: 2,6-dimethyl-4-heptanone 100:10 on unimpregnated Whatman No. 1 filter paper. Comparison with authentic samples of monopalmitin and batyl alcohol (α-glyceryl ether) (Fig. 5), showed very close similarities. The lecithin was proportionally much greater than in plasma and did not stain with OsO_4; it further resembled hydrolecithin in its hydrophobic properties.

Discussion. The marked reduction of ethanolamine phosphatide in cord plasma suggests that there may be an excessive demand by the fetus for this lipid. Indeed, in view of the recent demonstration(4) that the biosynthesis of lecithin can be mediated *via* the mono- and dimethyl derivatives of phosphatidyl ethanolamine (and perhaps the plasmalogen analogs as well), these observations are especially interesting.

To the best of our knowledge free glyceryl ethers have been observed on only one other occasion(5), although they have been isolated from the non-saponifiable material from bone marrow(6) and spleen(7) where they were presumably ester-linked to some other lipid component.

Although the phosphatide pattern of amniotic fluid showed a strong resemblance to plasma, the several marked differences imply that it most probably has a mixed origin; the monoglycerides, glycolipids and possibly the hydrolecithin may be contributions by the fetus or amniotic epithelium.

Summary. A paper chromatographic analysis of the lipids in maternal and cord blood and human amniotic fluid was made. The reduced lipid content of cord blood was confined to the plasma where phosphatidyl ethanolamine and the corresponding plasmalogen were disproportionately reduced. The amniotic fluid had a number of PAS positive components not observed in blood cells or plasma.

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Immune Agglutination and Lysis of Antigen Modified Erythrocytes from Various Animal Species.* (27435)

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During the past 15 years it has been shown that many bacterial polysaccharide antigens readily become attached to the surface of red blood cells. Erythrocytes thus endowed with

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