virus. Monkeys immunized with egg-yolk suspension containing virus ($ID_{50} = 10^{-3.7}$) failed to develop neutralizing antibodies. In the latter monkeys, BEV antibodies were not found both *in ovo* and in mouse neutralization tests. The differences in the two antigens in regard to their capacities to form antibodies are being studied further.

Discussion. These observations on BEV are recorded because: (a) the virus has been adapted to new mammalian hosts, and (b) there appears to be an immunologic relationship between it and the Lansing strain of poliomyelitis virus. It should not be inferred from these data that BEV is caused by poliomyelitis virus. Further studies, now in progress, should clarify the nature of the viral agent. There is evidence(2,5) that the infectious agent of BEV may be a member of the psittacine group of viruses, since elementary bodies have been observed(2,6) in the exudates of calves sick with the disease, and in infected tissues derived from animals inoculated with BEV.

These observations are of additional interest, because they would explain heretofore perplexing observations regarding the occurrence of neutralizing antibodies for the Lansing strain of poliomyelitis virus in the body fluids of cattle(7) and other domestic birds and animals.

Summary. Two strains of bovine encephalitis virus have been adapted to mice, cotton rats, and *rhesus* monkeys causing meningoencephalomyelitis. Evidence was presented to show that the Lansing strain of poliomyelitis virus and the bovine encephalitis virus are related serologically.‖

5. Cox, H. R., and Wong, S. C., personal communication.

6. Wenner, H. A., unpublished data.

7. Hammon, W. McD., Mack, W. N., and Reeves, W. C., *J. Immunol.*, 1947, v57, 285.

‖ We have considered carefully the possibility of contamination of infected yolk suspension with the Lansing strain of poliomyelitis virus. Against this likelihood has been the fact that BEV has been passaged many times in chick embryonic tissues, that each of the strains studied caused encephalitis in hamsters and guinea pigs, and finally, that the egg-adapted strain was neutralized by Lansing serum as well as the mouse-adapted.

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Use of Trichloracetic Acid in Purification of Lipids.* (19174)

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Several methods have been developed for the removal of impurities, chiefly nitrogenous, from lipid extracts of animal tissues(1-3). That of McKibbin and Taylor(3) has been found in this laboratory to be the most satisfactory from the standpoint of general application and completeness of purification with minimal loss of lipid. The number of operations required in this method, however, reduces its desirability for use with micro quantities, and in work involving radioactive tracers. Attempts to find a suitable procedure indicated that removal of the acid-soluble material from a tissue with 10% trichloracetic acid (TCA) prior to extraction with lipid solvents may facilitate purification of the lipid extract. Swanson and Artom(4) reported the use of TCA in a similar manner, but they noted that when tissues were ex-

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2. Sinclair, R. G., *J. Biol. Chem.*, 1948, v174, 343.

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4. Swanson, M. A., and Artom, C., *J. Biol. Chem.*, 1950, v187, 281.

tracted with lipid solvents without previous use of TCA, higher values for the weight of lipid were obtained. It has been found that this discrepancy can be overcome by using 10% TCA containing 0.4 M $MgCl_2$.

The procedure that has been adopted, and which has been used in this laboratory for purifying lipids from samples of tissues weighing as little as 0.3 g, is as follows. The tissue is removed from the animal and immediately transferred to an all-glass homogenizer of the Potter-Elvehjem type. Cold 10% TCA containing 0.4 M $MgCl_2$ is added, about 5 ml being used for 0.5 g or less of tissue. The homogenizing tube is held in an ice bath, and the sample rapidly homogenized. It is centrifuged in the cold, and the residue is washed 3 times with cold 5% TCA containing 0.4 M $MgCl_2$, with centrifuging after each washing. The residue is then extracted in the usual manner with boiling alcohol, followed by 3 extractions with ethanol-ether (3:1). The extracts are pooled, and the solvent removed under nitrogen in a partial vacuum, keeping the temperature below 55°C. The near-dry residue is reextracted with boiling petroleum ether-chloroform (6:1), and the extract washed with water and dried.

Tissues treated in this manner have been digested with NaOH, followed by acidification and re-extraction with lipid solvents. The failure to recover additional lipid indicated that the original extraction was complete. This method has been compared with that of McKibbin and Taylor, and also with a method identical in all respects except that the acid-soluble material was not removed with TCA prior to extracting the lipids. In this study aliquot weights of the same tissue were used, and the lipids were compared on the basis of amount found (Bloor's oxidative procedure) (5), I_2 number, free fatty acid content, and N:P ratios. The present method and that of McKibbin and Taylor resulted in similar yields of total lipid (Table I). Lipids prepared by these two methods possessed N:P ratios that were similar, and considerably lower than those found in the unpurified samples. Furthermore, the ranges of the ratios were similar and much narrower in the

5. Bloor, W. R., *J. Biol. Chem.*, 1928, v77, 53.

TABLE I. Effect of Purification on Lipids Obtained from Various Tissues. (No. in parentheses indicate ranges).

Tissue	No. of exp.	Method A*			Method B*			Method C*		
		Total lipid†	I_2 No.	N:P	Total lipid†	I_2 No.	N:P	Total lipid†	I_2 No.	N:P
Liver	5	4.3 (4.2-4.5)	105 (101-108.2)	.54 (.47-.61)	4.2 (4.1-4.3)	102 (89-117)	.53 (.45-.63)	4.5 (4.2-4.7)	106 (98-112)	.69 (.56-.85)
Lung	5	3.5 (3.1-5.4)	79 (76-82)	.58 (.56-.63)	3.4 (3 -5.2)	74 (68-83)	.58 (.49-.68)	3.2 (2.6-5.9)	82 (77-95)	.75 (.62-.97)
Brain	5	7.8 (6.2-8.3)	79 (71-86)	.71 (.60-.76)	7.7 (6.4-8.1)	86 (80.2-94.2)	.68 (.58-.74)	8.5 (7.4-8.7)	84 (80-90)	.85 (.67-1)
Tumor	2	2 (1.9-2.1)	92 (91.8-92.6)	.50 (.47-.53)	2.1 (1.9-2.3)	90 (86-94)	.52‡	2.4‡	105‡	1.15 (.97-1.33)

* Method A: Described herein. Method B: That of McKibbin and Taylor(3); the $CHCl_3$ extracts were washed 6 times. Method C: Identical to method A except that acid-soluble materials were not removed with 10% TCA.
† Total lipid expressed as % of wet weight of tissue.
‡ Single observations.

case of the purified than in the unpurified samples. The difference is due mainly to decreased nitrogen content of the purified samples, as one might expect in view of the findings of others that the impurities present are largely those of nitrogenous nature(6-9). The lipids resulting from both purification processes may possess lowered I_2 numbers. The use of TCA did not result in measurable hydrolysis of the lipids, as indicated by a determination of free fatty acid content.

Summary. A method is described for the quantitative extraction and purification of small amounts of lipid with a minimum of manipulation. The acid-soluble material is removed from the tissue with cold 10% trichloracetic acid containing 0.4 M $MgCl_2$. The lipid is removed from the residue using ethanol-ether. This extract is taken to near-dryness, re-extracted with petroleum ether-chloroform, and the latter solution washed with water and dried. The lipid thus prepared has been found to compare favorably in several respects with lipid purified according to McKibbin and Taylor(3).

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