It is interesting to note that the maternal requirement for pteroylglutamic acid appears to be very much greater during the period of lactation than during pregnancy. This is supported by the fact that the birth weights of all the newborn were found to be about the same (5.5 ± 0.8 g) regardless of the maternal intake of pteroylglutamic acid.

Summary. The inclusion of succinyl sulfathiazole in the diet of maternal rats during pregnancy depresses lactation. Relatively high levels of pteroylglutamic acid are required to maintain lactation. Higher levels of pteroylglutamic acid appear to be required during lactation than are required during pregnancy.

[6] Nelson, M. M., and Evans, H. M., *Arch. Biochem.*, 1947, **13**, 265; 1948, **18**, 153.

[7] Sica, A. J., Allgeier, A. M., and Cerecedo, L. R., *Arch. Biochem.*, 1948, **18**, 119.

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Acetone-Ether Extracted Antigens for Complement Fixation with Certain Neurotropic Viruses.

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A method is described for the preparation of antigens for complement-fixation tests with certain neurotropic viruses. This antigen has been found to be practical, nonanticomplementary, specific and reliable. It is furthermore, easily prepared with ordinary laboratory equipment and can be made available, if necessary, within 6 hours from the time when the tissue is harvested, thus being useful for field work. Since the preparation depends on the elimination of the acetone-ether soluble fraction of the brain tissue with considerable reduction of lipid, it has the advantage of doing away with nonspecific reactions with Wassermann-positive human sera.[1,2]

Method of preparation. Brain tissue from mice infected intracerebrally with the desired virus is harvested; preliminary bleeding of the mice[2] is advisable in order to reduce the amount of hemoglobin present in the final product. The weighed tissue with 20 volumes of acetone is placed in a Waring blendor and allowed to run for 2 minutes. The blendor used for this purpose is surrounded by a metallic jacket which is filled with ice and water to maintain a low temperature; less preferably, the extraction can be carried out in the absence of this device. The suspension is then poured into a 250 ml bottle and centrifuged at 1500 rpm for 1 minute; the supernate is discarded. All the foregoing procedures should be rapidly performed. To the sediment is added 20 volumes (referred to the initial weight of the wet-brain tissue) of fresh acetone; the bottle is closed with a cork stopper, shaken by hand at intervals and kept at room temperature for 20 minutes. The preparation is then centrifuged as before, the supernate which is slightly opalescent, as are the subsequent supernates, is discarded. To the sediment is added 20 volumes of a mixture of equal parts of acetone and anhydrous ethyl ether. Again the suspension is kept at room temperature for 20 minutes, shaken occasionally by hand, centrifuged at 1500 rpm for 1 minute and the supernate discarded. The sediment is washed twice in succession with 20 volumes of ethyl ether each time and held for 20 minutes at room temperature. After the last ether extraction and centrifugation, the sediment is dried by connecting the centrifuge bottle with a vacuum oil pump; in a short time, from 15 minutes to 1 hour depending on the amount involved, the residual ether is evaporated and the sediment remains as a

[1] DeBoer, C. J., and Cox, H. R., *J. Bact.*, 1946, **51**, 613; *J. Immunol.*, 1947, **55**, 193.

[2] Espana, C., and Hammon, W. D., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 101.

TABLE I.
Protein and Lipid Contents of Complement-fixing Antigens.

Antigen	Method of preparation	Diluent/original brain tissue	Protein, mg/ml	Lipid, mg/ml
Japanese B	Acetone-ether extraction	3:1	10.7	0.18
Normal brain		1:1	38.8	1.33
" "		5:1	4.5	0.37
Japanese B	No extraction with acetone-ether; frozen and thawed	10:1	1.2	0.32
Normal brain		10:1	2.0	0.30

dry, fine powder. It is necessary to set a trap preferably containing cotton soaked in heavy oil between the bottle containing the material and the pump, owing to the fact that the evaporation proceeds rapidly and some of the fine powder may be drawn into the pump. After drying, the material always kept in the same bottle is resuspended in physiological saline solution; the amount of the latter can vary between 2 and 5 times the weight of the initial brain tissue, according to the concentration desired; ordinarily, 3 volumes of saline suffice. The suspension is placed in the refrigerator overnight; almost equally good results are, however, obtained if it is kept at room temperature for 30 minutes. The suspension is centrifuged in the angle-head centrifuge at 10,000 rpm for 1 hour; the supernate is pipetted off, Merthiolate in final concentration of 1:10,000 added and it is then stored in a glass-stoppered bottle at 2°C. This extract constitutes the antigen.

Complement-fixing antigens have been prepared by this means with the following viruses: Japanese B, St. Louis, Western equine, and Russian Far East encephalitis. No attempt has been made to lyophilize the preparations; in liquid form they can be kept at least 4 months at 2°C. It should be borne in mind that the antigens contain active virus immediately after preparation; it may be possible to inactivate it by formolization or ultraviolet radiation. The writer, however, employs the material without the inactivating treatment.

The general procedure for carrying out the complement-fixation test and for preparation of animal immune sera has been described elsewhere.[3] Unless otherwise indicated the acetone-ether antigens used in the following experiments had been prepared by resuspending the final material in 3 volumes of diluent, referred to the initial wet-brain tissue weight.

Protein and lipid content. Although the precise chemical nature of the antigens is unknown, it was observed that the titer of samples deriving from the same lot increased with the concentration of protein in solution. When lipids were not extracted, increased protein concentration in the antigen resulted in increased lipid in suspension, thus yielding anticomplementary and nonspecific effects. When antigens were prepared by the present method, the concentration of protein could be increased considerably, with resulting higher titer, and lipids, although not wholly removed, were present in such small amount as to give no anticomplementary reaction. Table I shows the result of determinations of protein and lipid in antigens prepared by the present, as well as in antigens prepared by a previously described method,[4] without lipid extraction.*

It can be seen that the ratio of protein to lipid which was 7 to 1 or less in the nonextracted antigens, was 60 to 1 in some of the acetone-ether treated antigens. Furthermore, the concentration of protein could be increased in the latter to a value far beyond that shown by the nonextracted antigens, with no indication of anticomplementary effect.

Wassermann-positive sera. An investigation of the possible nonspecific reaction with Wassermann-positive human sera† revealed that under present conditions of inactivation

[3] Casals, J., *J. Immunol.*, 1947, **56**, 337.

[4] Casals, J., and Palacios, R., *J. Exp. Med.*, 1941, **74**, 409.

* Protein was estimated by quantitative biuret test. Lipid determinations were made by Dr. E. H. Ahrens, Jr., of the Rockefeller Institute Hospital, to whom many thanks are due.

TABLE II. Complement-fixation Reaction with Wassermann-positive Human Sera Inactivated at 63°C for 3 Minutes and Acetone-ether Extracted, and Nonextracted Antigens.

Serum dilutions / Serum No.	Acetone-ether extracted antigen									Nonextracted, frozen and thawed antigen										No antigen, saline	
	West. equine				Japanese B					Japanese B				St. Louis							
	1:2	1:4	1:8	1:16	1:2	1:4	1:8	1:16		1:2	1:4	1:8	1:16	1:32	1:2	1:4	1:8	1:16	1:32	1:2	1:4
1	0	0	0	0	0	0	0	0		4	4	3	±	0						0	0
2	0	0	0	0	0	0	0	0		3	2	1	0	0						0	0
3	0	0	0	0	0	0	0	0		2	1	0	0	0						0	0
4	2	0	0	0	3	2	0	0		4	4	3	±	0						0	0
5	0	0	0	0	0	0	0	0		4	3	±	0	0						0	0
6	0	0	0	0	0	0	0	0		3	3	3	±	0						0	0
7	0	0	0	0	0	0	0	0							0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0							4	4	0	0	0	0	0
9	0	0	0	0	0	0	0	0							0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0							3	3	±	0	0	0	0
11	0	0	0	0	0	0	0	0							2	±	0	0	0	0	0
12	2	0	0	0	2	0	0	0							4	4	1	±	0	2	0

Degree of fixation is expressed from 0 indicating no fixation to 4 indicating complete fixation.

(63°C for 3 minutes) they reacted nonspecifically with antigens of the former type, but not generally with those acetone-ether treated (Table II).

Anticomplementary effect. Numerous titrations of the anticomplementary effect of acetone-ether extracted antigens have been carried out under test conditions, namely incubation of the mixture of antigen and complement for 18 hours at 2-4°C. Table III shows the result of the titrations of complement in 3 different tests, in the presence of extracted antigens and of saline. When the antigens were prepared by resuspending the extracted brain tissue in 2 or more volumes of diluent (referred to the initial brain weight), the titer of complement was the same whether antigen was present or not. The concentrations of protein and lipid in these antigens were of the order of 20 mg or less, and 0.6 mg or less, per ml, respectively. On the other hand, when 1 volume of diluent or less was used, the resulting antigens were somewhat anticomplementary and gave a degree of nonspecific cross-reaction.

Specificity of the Reaction. The specificity of the complement-fixation reaction with acetone-ether extracted antigens was investigated mainly with mouse hyperimmune sera, and in a few cases, with human convalescent sera. Serial twofold dilutions of serum beginning with dilution 1:2 were tested against several antigens, either undiluted or diluted as indicated. The high dilutions of serum served to indicate that high titers of antibody could be obtained with these antigens, whereas the low dilutions showed that no cross-reactions occurred between different viruses, except in those cases when they are known to exist naturally, as for instance, between Japanese and St. Louis encephalitis viruses.[5] Examples of such tests are given in Table IV, in which are noted the high titers and the specificity of the antigens.

Titer of Antigens. When the extracted

† Wassermann-positive sera were obtained through the courtesy of Miss J. Haber, New York Hospital, New York. When received these sera had already been inactivated by heating at 63°C for 3 minutes.

[5] Casals, J., *J. Exp. Med.*, 1944, **79**, 341.

TABLE III.
Titration of Anticomplementary Power of Acetone-ether Extracted Antigens.

Test No.	Antigen	Diluent/original brain tissue	Protein, mg/ml	Complement: diluted fresh guinea pig serum 1:30 Amount of complement in ml .16	.14	.12	.10	.08	.07	.06	.05	.04
1	None, saline			0	0	0	0	0	0	±	2	3
	Normal brain	2:1	16.0	0	0	0	0	0	0	±	2	3
	Japanese B	1:1	30.0	0	0	0	0	±	2	2	3	4
	West. equine	3:1	9.0	0	0	0	0	0	0	1	2	2
2	None, saline				0	0	0	0	±	1	2	
	Normal brain	3:1	10.9		0	0	0	0	0	1	2	
	Japanese B	3:1	11.7		0	0	0	0	±	1	3	
3	None, saline			0	0	0	0	1	2	2		
	Normal brain	0.9:1	45.0	0	0	2	3	4	4	4		
	Japanese B	3:1	11.7	0	0	0	0	1	2	3		

TABLE IV.
Complement-fixation Tests with Acetone-ether Extracted Antigens; Mouse Hyperimmune and Human Convalescent Sera.

Antigen Type	Protein, mg/ml	Dil.	Serum Species	Japanese B	Russian Far East	Western equine
Japanese B	9.7	1:4	Mouse	1:128*	0†	0
Russian Far East	8.5	1:4		0	1:64	0
West. equine	9.0	1:4		0	0	1:8
Japanese B	30.6	Undil.	Mouse	1:32		0
West. equine	7.9	"		0		1:8
Normal brain	17.5	"		0		0
			Human, A‡	1:512		
Japanese B	9.7	"	" B	1:256		
			" A	0		
West. equine	9.0	"	" B	0		

* Highest dilution of serum giving a 2+, or better, reaction. The first tube had in all cases serum dilution 1:2.

† 0 indicates that no fixation occurred in any tube.

‡ The 2 human sera in this test were derived from individuals convalescent from Japanese B encephalitis.

brain tissue was resuspended in 3 volumes of diluent, the titer of the antigens was between 1:16 and 1:128, depending on several factors, such as the type of virus, the particular lot of brain tissue, and the relative amounts of serum and antigen that were employed. With respect to the latter it should be noted that, unlike what happens with nonextracted antigens,[3] the titers of both serum and antigen depend on their dilutions; more so does this apply to serum of which the titer is closely correlated with the number of units of antigen (Table V). This observation confirms that of Espana and Hammon,[2] who observed a similar phenomenon with their benzene-extracted antigens. Thus if a "box" titration were performed, with dilutions of serum tested against dilutions of antigen, results as given in Table V could, as a rule, be found.

It can be seen in Table V that the titer of the serum varied from 1:32 to 1:128 depending on whether antigen was used undiluted or in dilution 1:16. Conversely, the titer of the antigen was 1:64 when serum was used in dilution 1:2 and 1:128 with serum in dilution 1:16. In order to secure the highest titer for a given serum it seems advisable to use, as recommended by Espana and Hammon,[2] from 8 to 16 units of antigen. One unit of antigen is the amount contained

TABLE V.
Complement fixation Test with Japanese B Encephalitis Virus Antigen and Mouse Hyperimmune Serum. Acetone-ether Extracted Antigens.

Antigen		Dilutions of serum							
Type	Dil.	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
Japanese B	1:1	4	4	4	4	3	0	0	0
	1:2	4	4	4	4	4	0	0	0
	1:4	4	4	4	4	4	2	0	0
	1:8	4	4	4	4	4	4	±	0
	1:16	4	4	4	4	4	4	2	0
	1:32	4	4	4	4	4	4	2	0
	1:64	3	3	4	4	4	3	1	0
	1:128	0	±	1	2	1	±	0	0
	1:256	0	0	0	0	0	0	0	0
West. equine	1:1	0	0						

in the highest dilution of antigen which in a box titration gives a 2+ or better reaction; in the example shown, dilution of antigen 1:128 equals one unit.

To conclude, reliable, high-titered antigens for complement-fixation tests with certain neurotropic viruses have been prepared by a simple method of extraction at room temperature of infected brain tissue with acetone and ethyl ether.

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Intravenous Infusions of a Combined Fat Emulsion into Human Subjects.*

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In previous papers from this laboratory, an emulsion of fat combined with protein and glucose was reported to have been administered intravenously into dogs with safety and with nutritional benefits.[1,2] The overall advantages of this intravenous emulsion were such that it warranted the present clinical investigation of its effects on human subjects. This however was not done without precedent for Holt had pioneered studies on intravenous fat in a series of pediatric patients whilst others reported sporadic attempts in adult humans.[3,4] The present report is devoted to the method of preparation of the combined fat emulsion and the effects of its intravenous infusion on human subjects.

Preparation of the Combined Fat Emulsion. The successful preparation of this emulsion is dependent upon adequate and thorough homogenization and emulsification which is accomplished in a specially constructed homogenizer adapted to medical usage. Basically the mechanism is that of a high pressure pump which forces the fluid mixture through a minute orifice against the resistance of a strong steel springed valve. The machine consists of three component parts: a reservoir tank with a high speed agitator incorporated

* Aided by grants from the Ben Lewis Fund in Experimental Surgery. Clinical investigation done at Goldwater Memorial Hospital (New York University, Third Surgical Division).

1 Shafiroff, B. G. P., and Frank, C., *Science*, 1947, **106**, 474.

2 Shafiroff, B. G. P., Baron, H. C., and Roth, E., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 387.

3 Holt, E., Jr., Tidwell, H. C., and Scott, T. F. McNair, *J. Pediat.*, 1936, **6**, 151.

4 Clark, D. E., and Brunschwig, A., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 329.