tempt was successful might be attributed to the initial low titer of virus in the tissues used or that the virus may have been dissipated while being processed. Specific LCR CF antigen is consistently produced by the technic described. While it was difficult to process consistently satisfactory CF antigens from Brunhilde infected cords, the comparative success with Wisconsin virus has caused us to concentrate our efforts on the latter virus. It is possible that another virus might be superior even to the latter.

We have prepared specific Lansing CF antigens by the acetone-ether technic previously described(6,7) from infected brains of mature cotton rats. Thus far, similar attempts with Leon and Brunhilde-infected monkey tissues have not yielded CF antigens when tested with suitable antiserums.

The adjuvant "Pendil" as used by us did not enhance the CF antibody response of monkeys to Brunhilde and Leon viruses as did the Arlacel A and Bayol F of Salk *et al.*(9). Why "Pendil" worked with LCR and not with Brunhilde and Leon antigens is as yet beyond explanation. The advantage of the depot-producing agent with LCR is apparent and explains the CF difficulties in past experiences with antiserums prepared in monkeys. This is a good example of how suitable CF antigens might be discarded because of unsuitable antiserum, the latter presumably being produced by sound technics.

We have demonstrated specific CF with the three prototype viruses of poliomyelitis. Whether this will have a practical application will depend on future development with the virus and on the serological pattern demonstrated in human serums. Thus far the CF antigens processed from Leon and Brunhilde-type infected tissues were neither easily prepared nor potent when finally attained. We still need a better source of virus than that used for this study.

Summary. Specific complement-fixing antigens were prepared from Lansing-, Brunhilde-, and Leon-infected tissues by sedimentation of the viruses in the high speed centrifuge and using the resuspended sediment as antigen. Specific complement fixing antiserums were produced more rapidly in Lansing immunized monkeys when the virus inoculum was incorporated into a depot-producing adjuvant. It appears that the adjuvant treatment as recommended by Salk is needed for production of Brunhilde and Leon complement fixing antiserum.

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Acute Disseminated Encephalomyelitis Produced in Mice by Brain Proteolipide (Folch-Lees).* (19269)

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The isolation and identification of the encephalitogenic agent present in brain tissue has been sought since experimental disseminated (allergic or isoallergic) encephalomyelitis was first definitely produced(1). Various fractions deriving from extracts of cerebral tissue by lipid or other solvents—substances considered by some investigators to be leci-

thins, cephalins, cerebrosides, phosphatides, or protein, and by others, to be lecithin- or lipid- or protein-free residues—were reported now and again as encephalitogenic. Monkeys, rabbits and guinea pigs chiefly were the experimental animals but few of them were studied, and consistent results were not always had, and positive findings were, therefore, generally reported with caution(2). Recently, Ferraro and Roizin(3) investigated the effects of a calcium-acetate soluble compound obtained from benzene-ether extracted rabbit or sheep brain prepared by themselves or by Habel, Bell and Wright(4,5). This compound was designated(3) as being predominantly protein and induced either neurological symptoms of encephalomyelitis in 12 of 25 guinea pigs, or in 5 others showing no such signs, characteristic lesions in the CNS. Ferraro and Roizin concluded that while this protein calcium acetate fraction was more actively encephalitogenic than others heretofore reported, it was still not as potent as whole brain; they suggested, consequently, that either more than one substance in brain tissue might be active or the lipid fractions hitherto described as producing the affection might have been contaminated by a more potent protein fraction. Finally, Vogel(6) showed by both histologic and cytochemical means that a lipolytic enzyme was found in affected guinea pigs in the cytoplasm of a large proportion of the reactive histiocytes in the granulomatous tissue at the site of injection, and in the inflamed regional lymph nodes. He suggested, therefore, that a lipolytic enzyme may be a factor in the pathogenesis of the experimental affection and its accompanying demyelinization. No uniform opinion exists, therefore, on the identity or nature of the encephalitogenic agent in brain tissue.

The subject of the identity of the encephalitogenic agent is being studied in this laboratory in Swiss-W mice(7), especially those of certain stocks, such as the H-line, which have been shown to be highly susceptible(7-9). Several attempts have been made during the past 3 years to secure an active material, chiefly from mouse brain, and in all instances, with the aid of the Freund type of adjuvant(7). Thus an acetone-ether "lipid" fraction already described(7) failed, as did also the residue, or non-lipid moiety. Negative results were also obtained with pyridine-precipitated or extracted brain tissue, also with dialyzed material after 2 repeated extractions by pyridine, or with dialysates obtained from brain homogenates carried out against distilled water through a Visking cellulose membrane (9). In addition, a fraction insoluble in chloroform, also in chloroform methyl alcohol, and dialysates of such extracted cerebral tissue were used, but without success. Finally, chemical substances, such as folic acid, galactose, acetylcholine, glutamic acid, and a protein-lipocarbohydrate complex derived from Shigella antigen, were likewise without noteworthy results. In the face of all these failures, the success achieved with brain "proteolipide A and B", a new type of tissue lipoproteins recently discovered by Folch and Lees(10) was striking, and is the subject of the present paper.

Methods and materials. The method of adding Freund-type adjuvant to materials to be injected into H-line Swiss-W mice; the nosography of the affection induced in them; the dosage; route of inoculation; period of observation, and age of animals were already fully described(6,8,9). It should be mentioned again, however, that the standard course of treatment comprised 6 injections given at weekly intervals or until neurological signs appeared when further inoculations were discontinued. The materials used were first, a sample of proteolipide A secured through the kindness of Dr. J. Folch-Pi, a preparation deriving from cattle cerebral white matter. The second material was made in this laboratory from whole brains of 150 uninoculated adult stock mice, equivalent to 60 g of wet tissue. The preparation followed closely the method of Folch and Lees(10) up to the steps of processing the fluff formed at the interphase between water and chloroform. The process was con-

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tinued to the point where the fluff was collected on filter paper, thus containing both proteolipide A and B. These proteolipides as defined by Folch and Lees are a group of lipoproteins consisting of lipid and protein components; they are soluble in chloroform-methanol-water mixtures but insoluble in water, thereby differing from the known lipoproteins, and thus forming a new distinct group. The A form is a mixture of proteolipides and of free lipids; the B, an isomorphic crystalline product probably of one or more proteolipides with cerebrosides. Neither A nor B types are regarded as pure substances(10). The A and B mixture was used in the present tests. Before use, however, the mixture was aerated to rid it of chloroform, of which an amount sufficient to be toxic for inoculated mice was present.

Experimental. For an experiment of orientation the proteolipide A fraction of cattle brain prepared by Folch and Lees was first studied. It was added to adjuvant in the following amounts: 0.5 g proteolipide A; 125 mg autoclaved tubercle bacilli; 10 mg merthiolate; 25 ml saline solution, and 25 ml heavy mineral oil, homogenized in a Waring Blendor. Thus the proteolipide was diluted about 1:100, and 20 mice were inoculated with it and of them only 12 received the full course of 6 weekly injections, and owing to lack of material, 8 were given only 4 injections. Of the 20 animals one showed paralysis of one posterior extremity after 3 injections; the others were apparently free from symptoms. Histopathological examination revealed that the paralyzed mouse, 4 others of the 12 receiving full dosage, and 2 of 8 after only 4 injections had lesions in the CNS characteristic of the experimental encephalomyelitis. Attention has already been called to the fact that in this experimental affection, mice can be symptom-free yet show a varying degree of specific lesions in the CNS(8). It appeared then that the proteolipide A of cattle brain was encephalitogenic in mice. Its activity was such that with 10^{-2} dilution, only 7 of 20 mice showed either signs of characteristic reaction or its specific pathologic picture or both. Reference has already been made to the fact that in mice heterologous tissue is less potent than homologous. The second experiment was therefore performed with proteolipide A and B prepared in this laboratory deriving from normal mouse brain as described in a foregoing paragraph (Methods and Materials).

For animal inoculation, the undiluted proteolipide from 150 mouse brains was added to 100 ml of the Freund-type adjuvant and the dosage used for mice was the same as that of the cattle-brain proteolipide A. Four sets of animals were inoculated, of which 3 were controls. The first control consisted of 20 mice receiving untreated mouse brain plus adjuvant in similar proportions; this constituted a "positive" control to check the reactivity of the stock mice and the encephalitogenic power of their brain tissue. The second and third controls comprised 40 mice which received adjuvant and the residue of the tissue after the proteolipide had been extracted; the second series, 6 times the concentration of tissue as ordinarily used and as represented in the third set. The object here was to find out whether all of the encephalitogenic agent was removed from the tissue residue. The final series was the test itself with the proteolipide-adjuvant mixture, in which 28 mice were injected with it. Table I summarizes this experiment. Another series of 20 mice is not shown in the table. In this the animals received a single injection of proteolipide before its aeration to remove chloroform; 15 of the animals succumbed to chloroform poisoning within a few days; the remainder were unaffected. It is clear from the results that the mouse-brain proteolipides are actively encephalitogenic in mice, apparently to a greater degree than is whole mouse brain. Moreover, it would appear that most, if not all of the active agent had been extracted from brain tissue, for only 2 of 35 mice given the extracted residue developed experimental encephalomyelitis, the reactors having received the highly concentrated material.

Discussion and summary. Several conventional fractions of brain tissues and other chemical substances failed to produce encephalomyelitis in highly susceptible mice until a new group of lipoproteins, the proteolipides recently discovered by Folch and Lees were tried. Then the results were arresting: for not only were mouse-brain proteolipides as

TABLE I. Effect of Proteolipide A and B (PAB) in Mice.

Material inj. + adjuvant	No. of mice inj.	No. with signs	No. with CNS lesions; symptom-free	Total No. positive	Avg days to 1st sign
Normal mouse brain	20	19	0	19	25
Residue after PAB extracted	20	2	0	2	15
Residue diluted 1:6	15	0	0	0	—
PAB	28	24	3	27	14.6

actively encephalitogenic as whole brain, perhaps even more so, but all of the active agent could be practically extracted; the slight degree of activity shown by the residue (Table I) could have been ascribed to its possible contamination with proteolipides.

Two striking features of this new encephalitogenic material are worthy of note. The first is that since proteolipides are absent from unmyelinized brain, it is apparent that they are components of myelin (Folch and Lees) (10). It is well known that demyelinization is characteristic of the pathologic picture of disseminated encephalomyelitis. The second is the finding of Waksman and Morrison(11) who employing proteolipide A and B prepared by Folch and Lees showed weakly positive skin reactions with it in rabbits having experimental encephalomyelitis induced by injection of homologous spinal-cord tissue. The quantitative difference in the results obtained after inoculation of mice with Folch and Lees' own preparation of proteolipide A and the mouse-brain A and B substance prepared in this laboratory might be considered as an insufficiency of active agent in the A fraction by itself, or as a demonstration of the greater susceptibility of animals to homologous brain tissue. In view of the fact that the proteolipides are compounds constituted by lipid and protein moieties (Folch and Lees) further study is planned on possible purification of the active agent. Finally, with respect to the human demyelinating encephalitides to which the experimental affection has certain resemblance the problems are: What is the relationship of the proteolipide to their causation? Can proteolipide function as a test for diagnosis, perhaps in the way antigens are employed in allergic disorders?

Conclusion. Proteolipide A and B of Folch and Lees, a new group of lipoproteins, are capable of bringing about in mice acute disseminated encephalomyelitis indistinguishable from the affection induced by inoculation of whole brain tissue. The substance contains practically all of the encephalitogenic agent present in brain.

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