These considerations support the proposition that nephritis produced by NTG administration cannot be compared directly with human glomerular nephritis and lipoid nephrosis; the possibility that a study of NTG nephritis may lead to generalizations which are relevant to the study of human nephropathies cannot be denied. Further, the prompt deaths that follow NTG administration in the rat cannot be considered to result from the nephrotoxic action of NTG, but are the result of extrarenal effects.

Summary. Administration of nephrotoxic globulin (NTG) to the rat produces death in less than 12 hours if the amount given is sufficient. Bilateral nephrectomy usually does not lead to death in less than 24 hours, when the animals are maintained on a stock diet containing 17% protein. The prompt death is related principally to anti-rat-tissue antibodies other than anti-rat-kidney. Anti-rat-erythrocyte is of definite but minor significance in the production of prompt death. Cortisone and tripelennamine exert no protection against the prompt death described. It is evident that nephrotoxic globulin (NTG) has important extrarenal effects which must be considered in comparing NTG nephritis with other similar diseases of the kidney, such as those of natural occurrence in man.

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The Chemical Nature of Encephalitogenic Proteolipide A and B. (19951)

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The brain proteolipide A and B (PL, AB), a new type of tissue lipoprotein recently discovered by Folch and Lees(1), deriving from the mouse, was found to be capable of inducing acute disseminated encephalomyelitis in mice indistinguishable from that brought about by the injection of whole brain(2,3). It was also shown that PL. AB possessed a degree of encephalitogenic power similar to that of whole brain and that the latter, after this constituent had been extracted, was inactive. It was therefore assumed that all of the encephalitogenic agent was present in the PL, AB fraction. An attempt was then made to test for activity the remaining fractions of the extract; to break up the PL into smaller units and to determine whether the active agent could be identified with any one of these components. The present paper describes these investigations.

Materials and methods. The PL, AB was prepared from mouse brain and the H-line W-Swiss mice(4) were employed as experimental animals. All fractions were mixed with the Freund-type adjuvant and inoculated into mice subcutaneously, 6 times or less, at weekly intervals. The methods of preparation, inoculation and observation of reactions in animals have already been described(2-5). Proteolipide C (PL, C) was obtained following closely the technic of Folch and Lees(1):

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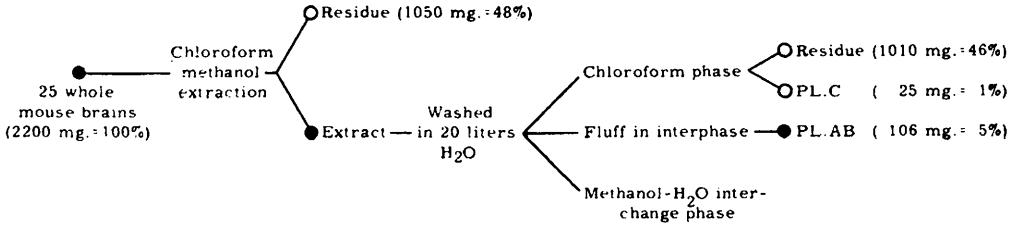

FIG. 1. Encephalitogenic activity of various fractions of mouse brain. ○ = negative; ● = positive reaction of experimental encephalitis in mice; PL = proteolipide; mg = dry wt.

the yield from 53 g dry (240 g wet) weight, mouse brain was 600 mg or about 1%. PL, C supernates were collected, pooled and concentrated by drying in a vacuum (residue of chloroform phase in Fig. 1). Fractionation of PL, AB into lipid and protein moieties followed the method of Folch and Lees(1,6): 60 g (150 fresh mouse brains) yielded 636 mg dry weight of fluff which contains PL, AB. The frozen fluff, collected on filter paper, was dried for 6 hours in a $CaCl_2$ desiccator *in vacuo* and then stored at −10°C overnight. To the material resuspended in 6 ml distilled water in a suction flask, 100 ml chloroform were added. Methanol was added to the resulting milky suspension until one phase was obtained then the solvent was removed by vacuum distillation. 100 ml of hot chloroform-methanol (2:1 by volume) were added and the precipitate filtered through fat-free filter paper. The extraction was repeated and the yield of precipitated protein was 200 mg, about 31%. The supernate was evaporated to dryness and yielded 400 mg lipid, about 63%. Attention should be called to the fact that Folch and Lees(1) found that dissolving the PL, AB in chloroform-methanol-water mixture and removing the solvents by vacuum distillation, thus drying it, splits the bond between protein and lipid and consequently separates them. After the separation the protein is rendered insoluble in the chloroform-methanol-water mixture, the amount of the insoluble protein can then be a measure of the extent of the splitting of the PL, AB complex.

Other preparations for test of their encephalitogenic activity consisted of: 1) PL, AB dried, but not extracted with hot chloroform-methanol; *i.e.*, the substance was dried over $CaCl_2$ *in vacuo* over night at room temperature. 2) This process of drying was repeated after resuspension in the solvent mixture. 3) To dried PL, AB hot chloroform-methanol (2:1 by volume) was added and the solvent removed by evaporation without separation of component protein or lipid. 4) To fresh, not dried PL, AB in water suspension hot chloroform-methanol was added; it was kept then in a water bath at 100°C until all the solvent was evaporated, the residue consisting of the treated PL, AB in a water suspension. In Fig. 1 and Tables I and II will be found a summary of the results of tests on these various preparations of PL, AB and its components.

Experimental. The fact that all of the encephalitogenic activity of brain tissue is contained in the PL, AB fraction has already been shown(2,3) and results of tests with the fractions given in Fig. 1 and Table I support this finding: of the 4 fractions of brain, obtained by extraction with chloroform-methanol and washing, only PL, AB was regularly active. That is, of 2200 mg dry weight of brain, 106 mg PL, AB were secured which represents 5% of the solids of the original brain, and all mice inoculated with this material developed encephalomyelitis. The PL, AB-free residue amounted to 1050 mg, or 48%, and was inactive; so was the 25 mg, or 1% fraction PL, C (Fig. 1). Only 1 of 34 mice in 2 tests developed the experimental affection after inoculation of the residue of the chloroform phase which weighed 1010 mg or 46% and represented substances other than PL, C soluble in the chloroform. It is not certain whether this single positive animal might not have received an inoculum contaminated slightly with PL, AB. The residue of the whole-brain; PL, AB; PL, C; and the residue of the chloroform phase (Fig. 1) accounted for 100% of the solids in the original brain tissue;

TABLE I. Encephalitogenic Activity in Mice of Fractions of Mouse Brain.

Fraction	No. of mice	Dry wt, mg/ml	Total mg dry wt in 6 inj. in 1.8 ml	No. pos./neg.	% pos.	Brain wt equiv., mg/ml Wet	Dry
Whole brain (control)	20	22	39.6	20/0	100	100	22
PL. AB*	20	1.06	1.91	20/0	100	100	22
PL. C	20	6	10.8	0/20	0	2400	528
Residue of PL. C	14	121	217.8	0/14	0	1200	264
	20	20.2	36.36	1/19	5	200	44

* PL. AB = Proteolipide A and B.

TABLE II. Encephalitogenic Activity of Proteolipide (PL.) Fractions.

PL. fraction	No. of mice	Dry wt, mg/ml	Total mg dry wt in 6 inj.	No. pos./neg.	% pos.	Protein insol. in chloroform-methanol, mg/ml
PL. AB (control)	30	1.06	1.908	30/30	100	0
Protein	20	1.20	2.16	0/20	0	—
Lipid	20	2.09	3.762	0/20	0	—
Desiccated PL. AB	20	1.49	2.682	7/20	35	.435
Desiccation repeated PL. AB	20	2.50	4.55	0/20	0	.750
Treated,* desiccated PL. AB	40	2.50	4.55	0/40	0	.750
Treated, fresh PL. AB	20	2.50	4.55	13/20	65	.742

* Treated with hot chloroform-methanol mixture.

the only possible remaining fraction of this system was that of the upper water phase, above the fluff.

The next step was an attempt to identify the encephalitogenic activity of PL, AB with either its protein or its lipid moiety, and to note the influence in this relation of breaking, by various manipulations, the bond between the two. For Folch and Lees(1) have stated that this bond can be split simply by drying the solutions as prepared. The results are summarized in Table II: in the last column, the amount of separated protein was determined by the quantity rendered insoluble in chloroform-methanol.

It will be observed that the protein and lipid constituents, separately, were inactive whereas the whole PL, AB, was encephalitogenic. On the other hand, a single desiccation over $CaCl_2$ at room temperature for 15 hours definitely reduced the encephalitogenic power of PL, AB; such desiccation when repeated destroyed this activity completely. In the latter instance, all of the protein moiety (30% by weight) was rendered insoluble in the chloroform-methanol-water. In another experiment shown in Table II, treatment of the fresh, not dried PL, AB with hot chloroform-methanol followed by evaporation of the solvent, a procedure which should not disturb the bond between its protein and lipid constituents, caused destruction of 35% of its encephalitogenic power. This treatment plus desiccation, however, produced a complete loss of activity of the proteolipide.

Discussion and Summary. An explanation of the difference in results obtained by treating PL, AB with hot chloroform-methanol, and with this solvent plus drying, may lie in the fact that during the treatment a certain amount of PL, AB at the surface desiccates at the same time that the solvent evaporates. Thus the loss of activity caused by partial drying corresponds with the surface area as well as with the original concentration of brain tissue, *i.e.*, with the amount of PL, AB exposed to desiccation. For example, a suspension of PL, AB treated the same way except that a container (wide flask) permitting much wider surface was employed, became completely inactive; whereas the same suspension in a container (test tube) having only a small surface exposed, revealed a loss of 35% of its activity.

The various fractions deriving from the extract of mouse brain secured during the procedure of isolation of PL, AB were tested in mice for their encephalitogenic activity. It was found that the PL, AB, which amounted to 5% of the original constituent solids, was at least as active as whole brain, but the residue obtained after extraction with a chloroform-methanol-water mixture; proteolipide C; and the residue of the chloroform phase, all of which aggregated the remaining 95% of the original solids, were inactive. Thus from the procedure here employed it appeared that the encephalitogenic power of brain tissue, as revealed by inoculation of mice, was possessed only by one of its constituents, namely, PL, AB.

The encephalitogenic activity of PL, AB was evinced only if the protein and lipid constituents remained in chemical combination. If the bond was split, even by the milder procedures, the resultant material was found to be inactive, as were the separated protein and lipid fractions. The possibility remained, however, that the methods of splitting the bond used in this study might have changed the chemical composition of the constituents. Further work on fractionation by other means would therefore appear to be indicated.

Conclusions. Of 4 fractions prepared by chloroform-methanol extraction of mouse brain, only one, proteolipide A and B, is encephalitogenic in mice. Its desiccation which brings about a split in the bond between its protein and lipid constituents results in a loss of its encephalitogenic power and the separated protein and lipid fractions are also inactive.

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Partial Purification of Bovine Intestinal Alkaline Phosphatase.* (19952)

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Hers(1). and Kallman(2) reported that intestinal alkaline phosphatase is concentrated in the microsome fraction of rabbit mucosal cells. We have confirmed this observation, and found that the same is true in the intestinal mucosa of the steer. It is the purpose of this report to demonstrate that the isolation of the microsome fraction may advantageously be made the initial step in the preparation of a soluble preparation, with reproducible properties, from steer mucosa. This method avoids the use of preliminary autolysis or tryptic digestion, heretofore considered essential to the purification procedure, and seems more likely to lead eventually to the isolation of a pure enzyme.

Materials and methods. The bulk separation of steer intestinal microsomes was carried out on material obtained within 30 minutes of slaughtering, kept at 2°C, and was performed as follows: the inside of the duodenum was rinsed with 0.25 M sucrose, and the mucosa stripped from it. The mucosa was suspended in 2 volumes of 0.25 M sucrose, the pH of which had been brought to 8.0 with $KHCO_3$. The suspension was homogenized in a Potter-Elvehjem homogenizer designed to

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