Certain Properties of the Causal Agent of Experimental Disseminated Encephalomyelitis in Mice. (19155)

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Acute disseminated encephalomyelitis develops in albino mice after introduction of homologous brain tissue mixed with the Freund adjuvant(1). Not all strains or stocks of mice are, however, susceptible: some are highly resistant, *e.g.*, the Rockefeller Institute strain, and difference in susceptibility exists even between two lines of mice ultimately deriving from the same parent stock, the B-line of W-Swiss strain being relatively resistant while the H-line of the same strain, highly susceptible(2). The availability of such highly susceptible experimental animals offers a proper standard for comparison, and a point of reference for statistical interpretation of the results of the studies to be presented in the present article. The results define properties of the active agent in addition to those already described(1,2), and relate to (a) titration of its concentration in the suspensions as ordinarily prepared; (b) effects of centrifugation; (c) susceptibility of species other than mice to the agent, as well as (d) whether heterologous tissues can also induce the characteristic encephalomyelitis in mice.

Methods and materials. The preparation of materials and the methods used have already been given(1,2). During the course of the present tests the composition of the brain-adjuvant mixture was: Normal brain tissue, 10 g; heavy mineral oil, 50 ml; autoclaved tubercle bacilli, H37Rv type, 250 mg; merthiolate, 20 mg (1:5000 final dilution), and 0.85% NaCl solution, 50 ml, or proportional amounts of this formula as needed. The merthiolate was added to prevent microbic contamination of the mixture which was kept at 4°C; a final dilution of 1:5000 was found satisfactory and in the maximum concentration since 1:500 dilution proved to be toxic for mice (Table I). 0.3 ml of the homogenized material was introduced subcutaneously into H-line Swiss mice, 1 to 3 months old. The standard course of injections in the present series of tests consisted of an inoculation once a week for 6 weeks or until neurological signs appeared. Mice were observed for more than 2 months after the initial exposure. The signs and the pathological picture accompanying the malady have already been described (1).

Titration of the active agent. From an initial suspension of mouse brain in 10^{-1} dilution, further dilutions in the Freund adjuvant were prepared and each was tested in the manner shown in Table I, which also points out the effect of adding merthiolate to the brain-adjuvant mixture. It is clear from this table that when the standard 10^{-1} dilution of mouse brain, with or without merthiolate, is introduced into mice, a uniform reaction is induced. The response is also regular since practically all animals showed characteristic neurological signs or histopathological lesions, or both, after the third inoculation or within a month after the initial exposure. It will also be noted that even after a dilution of 1:1000 was introduced, 5 or 6 mice of 15 developed the experimental disease. Finally, in accord with prior experience(2), an animal exhibited now and again the pathognomic lesions in its central nervous system without any visible sign of neurological disturbance.

Centrifugation tests. 10.5 g mouse brain were homogenized in 42.5 ml saline solution and held at 4°C for 12 hours. The suspension was then centrifuged at 8000 G for one hour. The supernate was removed and kept, and the sediment after resuspension in 10 ml saline solution was recentrifuged. The supernate was added to that of the first centrifugation. The sediment was similarly resuspended and washed 3 times. The wash-fluids were dis-

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TABLE I. Titration of Activity of Normal Mouse Brain Suspensions to Which 1:5000 Merthiolate Was Added.

Dilution	No. of mice inj.	No. showing neurological signs	No. showing lesions	Total No. positive	
10^{-1}	35	32	34	34	No merthiolate
10^{-1}	35	35	35	35	Merthiolate 1:5000 added to all others
2×10^{-2}	15	15	15	15	
10^{-2}	15	14	15*	15	
10^{-3}	15	6†	5	5 or 6	
10^{-4}	15	0	0	0	

10^{-5} and 10^{-6} dilutions, like 10^{-4}, completely inactive.
* One mouse showed characteristic lesions but no signs of illness.
† One mouse died, histopathological examination not possible due to postmortem changes.

carded and the sediment resuspended in 52.5 ml saline solution. To the supernate and the sediment respectively were added 50 ml of the Freund adjuvant. Supernate and sediment were homogenized separately in a Waring Blendor and both were injected into mice, 20 for each material. All 20 mice receiving the sediment exhibited characteristic signs of disseminated encephalomyelitis: 1 after the second weekly injection and 19 after the third. These animals also showed the familiar lesions of the experimental disease. All 20 mice receiving the supernate remained well, even after 6 weekly injections and no pathological changes were found in the brains from 2 to 3 months after the initial inoculation. Similar results were obtained on repetition when insignificant changes in the technic were made but always the encephalitogenic factor was found in the sediment, not in the supernate. In another test, a saline-solution homogenate of mouse brain was held for 5 minutes at 100°C in a water bath, and then prepared and centrifuged as above. In this instance the supernate was again inactive in 12 mice but the sediment induced the disorder in 3 of 12 mice; of the 3, two showed no visible neurological signs only the characteristic lesions in the brain.

Heterologous tissues in mice and mouse brain in guinea pigs. The question has not been answered hitherto whether cerebral tissues of other species of animals are active in mice and whether mouse brain is encephalitogenic in another species, for example, the guinea pig which had been shown previously to be highly susceptible to homologous tissue (3). The results would be significant since it is implied that the active agent in mouse brain is of the same nature as that present in other species; hence hetero-reactions, a generality of the latter, should also apply to mouse brain. Guinea-pig and sheep brain were prepared in 10^{-1} dilution in the Freund adjuvant and to the mixture was added 1:5000 merthiolate. The method of preparation was precisely similar to that already described for mouse brain. Guinea pigs received 0.25 ml of the mixture subcutaneously at weekly intervals; otherwise the treatment of the animals was as stated in another section of this paper.

It will be observed from Table II, which summarizes the experiment, that heterologous cerebral tissue was active in mice and conversely mouse brain was encephalitogenic in the guinea pig. The number of reactors and the intensity of effect was not as marked as among mice receiving homologous tissue. This is taken as an indication of a quantitative difference: less of the agent was present in the heterologous brain. The smaller amounts were then accompanied by a suppression of outward neurological signs of the disorder (v. line 3, Table II) at a time when characteristic lesions, sometimes extensive, could be found in the central nervous system.

Discussion. It is apparent from the foregoing results that the merthiolated, 10% suspension of mouse-brain tissue plus adjuvant, which brought about uniformly in mice acute disseminated encephalomyelitis, contained an excess of the active agent. The excess in an ordinary suspension over the

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TABLE II. Effect of Heterologous Brain in Mice and Mouse Brain in Heterologous Species.

Brain from	Injected 10^{-1} dilution into	No. inj.	No. showing neurological signs	No. with lesions	Total No. pos.
Guinea pig	Guinea pig (control)	6	4	4	4
	Mice	20	15	12*	15
		20	0†	13	13
Mouse	Mice (control)	20	19	20	20
	Guinea pig	3	1	1	1
Sheep	Mice	20	8	7*	8

* Others were not examined for lesions; their symptoms were, however, characteristic.
† Indefinite signs were observed such as ruffled fur and sometimes slowness, but no definite neurological signs such as tremor, ataxia, convulsive movements and paralysis.

minimal amount needed to induce the experimental affection was from 10- to 100-fold. Even a dilution of 1:1000 sufficed to produce the neurological disorder in about one-third the number of animals receiving it. In this latter regard Alvord(4) states that guinea-pig brain diluted 1:100 was active in 1 of 3 guinea pigs and 1:1000 inactive in 5, and Lumsden(5) reports that 0.03 g of such a tissue was encephalitogenic in guinea pigs. Thus mouse brain tested in mice offers here an advantage for experimental investigations. Another point of interest is the finding that centrifuged suspensions contained the encephalitogenic agent only in the sediment, not in the clarified supernate. The inference deriving therefrom is that (a) the factor is not soluble; or (b) in whatever form it may exist in nature, the agent needs some element, cerebral tissue itself or a component of it, to set up encephalomyelitis in inoculated animals. In additional experiments with 75 mice attempts were made to determine whether the encephalitogenic factor in mouse brain could be dialyzed(6) against distilled water through a bag of Visking seamless cellulose tubing for 10 days at 4°C. The dialysate, as such and lyophilized, was used. These attempts failed.

Since centrifugation served to localize the encephalitogenic factor in the sediment, thus making its detection more certain, a test was conducted with heated tissue. The result pointed to the active agent being relatively thermostable, a finding for mouse brain which agreed with that for monkey brain(7) and for guinea-pig tissue(5).

The assumption that the encephalitogenic factor present in mouse brain is of the same nature as that found in the cerebral tissue of other species(1) was strengthened by the fact that the mouse-brain agent was active in species other than mouse and heterologous brain was active in the mouse. In any event, the experimental disease produced by the encephalitogenic factor was found to be indistinguishable.

It was noted during the course of the present studies that when the experimental affection resulted from inoculation into more resistant individuals, or of smaller quantities of the active agent, the induced malady was characterized by an absence of any outward neurological signs such as tremors, ataxia, convulsive movements and paralysis, and the presence of more or less marked, typical reaction in the tissues of the central nervous system. Since investigators have pointed out certain resemblances between one or another feature of the induced encephalomyelitis and those of human demyelinating disease including multiple sclerosis(8,9), a comparison of the remission or absence of neurological symptoms in both the experimental affection and the human disease is noteworthy.

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Summary. The present study concerns itself with a titration of the encephalitogenic activity of mouse brain which induced acute disseminated encephalomyelitis in mice. In this regard the active agent revealed a potency higher than that hitherto described for the guinea pig receiving homologous tissue. The encephalitogenic factor was found not in the supernate but in the sediment after centrifugation at 8000 G; nor was it detected in a dialysate of mouse-brain tissue. Mouse brain was encephalitogenic in a heterologous species of animal and heterologous tissues were similarly encephalitogenic in the mouse; the disorder thus brought about by either was indistinguishable from that induced in mice by means of mouse brain.

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Observations of Neoprene Casts of Vascular Bed of the Kidney. (19156)

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Neoprene is an excellent material for the study of the structure of the renal vascular bed(1). A serious handicap to its use, however, is the fact that, injected *in vivo*, neoprene causes constriction of the vessels and prevents complete filling of the vascular system. Nevertheless, nearly complete casts of the vascular bed of the kidney can be obtained if the renal vessels are not constricted, but are dilated, and are empty of blood. In cases in which casts of the vascular system are obtained under such conditions the only consistently existing vessels which directly communicate between the renal artery and vein are situated in the hilum of the kidney.

We have obtained casts of the entire vascular bed of the renal cortex and a portion of the medulla by injections of neoprene into the aorta or renal artery *in vivo* provided that the renal vessels were rendered incapable of constriction, were dilated, and were emptied of blood by a brief period of ischemia. *In vivo* injections of neoprene or materials of a similar nature caused a marked renal vasoconstriction as indicated grossly by severe blanching of the kidney. Owing to this vasoconstriction, the filling of the vascular bed of the kidney with neoprene was incomplete. However, when the renal artery or the aorta above the origin of the renal arteries was clamped, after a few minutes the arterial bed of the renal cortex would no longer become constricted when neoprene was injected. Neoprene passed through the arterial and venous beds, and flowed out the renal vein. Surface vessels were filled with neoprene. The clamping procedure itself resulted in a transient cortical vasoconstriction which served to empty the cortical vessels of blood. This emptying of vessels was an additional aid in obtaining a more complete cast of the renal vasculature. Thus, although neoprene provides an excellent medium for the study of the structure of the renal vascular bed, it fails to provide accurate information regarding the intrarenal distribution of the circulating blood.

Especially noteworthy were the casts of relatively long vessels near the hilum, which communicated between the large branches of the renal artery and vein and probably were part of the vascular supply to hilar and pelvic tissues (Fig. 1). The exact caliber of the lumen of these vessels was not determined, but they looked small. These vessels had a greater tendency to form plexuses in the dog than in the rat. When the neoprene injected into the renal artery was of a different color than that injected into the vein, the two colors were found to be mixed in these vessels. These were the only direct communications between the renal artery and vein that were found to be constantly present. It was of interest that in several instances neoprene injected *in vivo*

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