EXPERIMENTAL DISSEMINATED ENCEPHALOMYELITIS IN WHITE MICE

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Disseminated demyelinating encephalomyelitis occurs not infrequently as a sequel to, or during convalescence from, clinically apparent infection with a number of different viruses, and rarely after vaccination against smallpox, rabies, and other viral diseases. A similar pathological picture characterizes several neurological maladies, for example, multiple and diffuse sclerosis, Schilder's disease, leucoencephalitis, neuromyelitis optica, and a score of other encephalitides, each regarded by the one who first named it as a nosological entity. Several investigators, however, hold that several or all the latter demyelinating affections belong in the same groups of essentially similar histopathological processes (Ferraro (1); Putnam (2); Roizin, Helfand, and Moore (3); and others).

Although the problem of the etiology of the demyelinating diseases has been studied during the past century and considerable experimental research has been carried on, there is still no solution. A new impetus, nevertheless, has been the successful experimental production in laboratory animals of neurological syndromes accompanied by histopathological changes in the central nervous system similar to those found in the acute demyelinating affections.

The earliest reports of successes were those in 1933–35 of Rivers, Sprunt, and Berry (4) and Rivers and Schwentker (5) who injected monkeys repeatedly over a period of months with brain tissue obtained from apparently normal rabbits. The first paper recorded that of eight animals receiving 14 to 93 intramuscular injections thrice weekly, two reacted with demyelinating encephalomyelitis, one after the 52nd and the other after the 84th inoculation. The second report showed that of eight monkeys similarly treated 46 to 85 times, seven gave positive results. This finding was promptly confirmed by Ferraro and Jervis (6) who introduced rabbit brain into monkeys 29 to 103 times over a period of 112 to 405 days; the animals first showed neurological signs after 3 to 13 months. Ferraro (1) in 1944, concluded that the reaction in the nervous system is of an allergic nature—a view which found active support so that commonly the experimental disease, and occasionally the human acute demyelinating

affections are now called "allergic (or isoallergic) encephalomyelitis."[1] In other words, the brain tissue used for producing the encephalomyelitis is considered to be a sensitizing antigen.

Another advance was gained by the addition to brain tissue of what is now known as the "Freund adjuvant" for the purpose of bringing about experimental disease more rapidly and regularly. Freund and McDermott (8) demonstrated that a mixture of lanolin-like substances, paraffin oil, and killed tubercle bacilli, induced a prompt and high antibody response when added to a wide variety of antigens, and Kopeloff and Kopeloff (9) showed later that by use of the adjuvant antibrain antibody could also be elicited, for example, in the blood of monkeys receiving injections of sheep brain. Morgan (10) and Kabat, Wolf, and Bezer (11) at the same time and independently, adding the adjuvant to homologous and heterologous cerebral tissue, induced encephalomyelitis in monkeys by only a few injections, even a single one, and neurological signs appeared in days instead of months. Later Ferraro and Cazzullo (12) demonstrated that chronic types of encephalomyelitis could be brought on in monkeys given reduced amounts of the antigen. Such types displayed histopathological changes similar in many respects to the lesions noted in acute human demyelinating diseases (12).

A technical advance was the finding that rabbits (13, 14) and guinea pigs (14–16) could be utilized instead of monkeys. The experimental production of neurological signs and lesions in any species, whether monkey, rabbit, or guinea pig, was found, however, to be unpredictable. Now and again half the injected number, sometimes less than half, reacted positively. Furthermore, in those animals which gave positive results the number and spacing of injections, and the length of time after exposure before the onset of the syndrome varied within extraordinarily wide limits.

Disseminated encephalomyelitis of the type now under discussion has not as yet been induced in albino mice, despite several attempts (11, 14, 15). If mice could be proved susceptible, especially, if they reacted with some regularity and to nearly the same degree, an advantage would be gained for the study of the experimental disease. The results of the present investigation[2] show that acute, subacute, and possibly, chronic disseminated encephalomyelitis can be readily produced in mice merely by intramuscular and subcutaneous injection of homologous brain tissue mixed with an adjuvant modified in a way to be described. Moreover at least one of the causes for the failures of others to produce encephalomyelitis in white mice was revealed in the fact that different strains of mice differ markedly in susceptibility.

Methods and Materials

Mice.—Albino mice of the Swiss and the Rockefeller Institute strains, aged 6 or 7 weeks were selected from apparently healthy stocks. Mice of the Swiss strain proved to be much more responsive to injections of brain tissue and hence the experiments were mainly carried out with this strain.

[1] For a discussion and review of literature on allergy in the nervous system reference should be made to Ferraro (1), Stevenson and Alvord (7), and Hurst (22).

[2] A preliminary report of these investigations has already been published (17).

Adjuvant.—As originally devised by Freund (8) the adjuvant consisted of killed tubercle bacilli and paraffin oil in a water-oil emulsion. To secure proper emulsification, various agents were used, chief among them "falba" and "aquaphor," both of them proprietary materials, the former an adsorption base derived from lanolin, the latter an ointment base, from a mixture of wool fat and hydrocarbons. They have been employed by earlier investigators for the purpose of enhancing the experimental production of encephalomyelitis.

In the present studies, the preparation of the adjuvant was modified. No special emulsifier was added to the autoclaved tubercle bacilli, and for light paraffin oil, that of heavy type (liquid petrolatum, soconal)[3] was substituted. This was used not only as an emulsifier but also for its possible enhancing action on the antigenicity (8, 15) of the mouse brain tissue. The tubercle bacilli were of human type, virulent strain, H37Rv (18), stored acetone-dried, and autoclaved for 15 minutes at 15 pounds pressure before use.[4] The mixture of materials was homogenized in a small sized Waring blendor, the end product being a thick emulsion which could be stored for several weeks at 4–5°C. without evident deterioration.

The proportions of the materials in the mixtures were changed as time went on and the antigen finally was made up as follows: Mouse brains derived from normal appearing Rockefeller Institute or Swiss strain mice, 10 gm.; autoclaved tubercle bacilli, 20 mg.; liquid petrolatum, heavy type (soconal), 50 ml.; and 0.85 per cent saline solution, 50 ml.

Dosage.—Of the mouse brain–adjuvant mixture, 0.3 ml. was injected intramuscularly into the thigh. When more than two or three intramuscular injections were given, the later ones were introduced subcutaneously because the nodules which developed at the site of inoculation prevented a reinoculation of the same area. The way in which the experiments were conducted made it possible to select for the larger number of exposures to the antigen the more resistant animals of a given group. For example, the reactors to one injection did not receive any further inoculations; the non-reactors were given a second dose, and so on, those animals which showed no effects being inoculated up to 5 or 6 times, that is to say until they responded or consistently resisted, as happened in a few instances.

Histopathological Studies.—60 to 100 sections obtained from various parts of the brain and cord of each injected mouse were studied for changes. Some of each series of sections from the same fragment of tissue were stained by the erythrosin-Azur I, hematoxylin-eosin, and Loyez' myelin methods respectively; also by the Bodian silver impregnation method and sometimes by the Ranson.

EXPERIMENTAL

The syndrome of disseminated encephalomyelitis accompanied by characteristic histopathological changes, which will be described in the next sections of this paper, was induced in Swiss strain mice by means of peripheral (intramuscular and subcutaneous) injections of normal mouse brain mixed with adjuvant, as just described.

Symptom Complex

Local Reaction.—In every instance in which the adjuvant, heavy liquid petrolatum by itself or together with killed tubercle bacilli, was injected, a nodule promptly formed at the site of inoculation. The nodule was absorbed

[3] Dr. J. Freund (21) in a repetition of some of the tests here described succeeded in producing the characteristic encephalomyelitis by substituting light for heavy liquid petrolatum.

[4] The writers are indebted to Dr. G. Middlebrook, Dr. C. Pierce, and Dr. M. W. Chase for these materials.

with difficulty and hence persisted for several weeks. It contained grumous and fatty material which was probably unabsorbed inoculum. Generally speaking, these nodular masses can be looked upon as sterile or "cold" abscesses. Since they were of considerable size, and were situated in the thigh muscles, they often affected the movements of the animal—a fact which should be reckoned with in estimating the neurological significance of a peculiar gait.

Constitutional Reaction.—Dyspnea, occasionally accompanied by wheezing respiration, was commonly a manifestation of the disorder. The type of breathing was similar to that in allergic bronchial asthma. The mice showed this difficulty intermittently, periods of a few hours to several days intervening before the attack was renewed. Sneezing and pawing of the nose also accompanied the stridor.

In an exceptional case, two mice receiving one injection of brain tissue–adjuvant mixture showed on the 2nd day dyspnea accompanied by wheezing respiration and no other sign except ruffled fur. This endured for 2 and 3 days, after which the animals recovered their former apparently normal, state. On the 8th and 9th day, respectively, both lapsed into the characteristic neurological state, soon to be described.

The constitutional disorder was notable for (*a*) wide divergence in time before the onset of illness; (*b*) varied symptomatology; and (*c*) repeated relapses arising in the course of an enduring yet ordinarily non-fatal affection. The earliest indications of disease were ruffled fur; considerable loss of weight; slowness of movements and weakness of the extremities. Within a few days intensification of the neurological symptoms was noted, accompanied by generalized coarse tremors; ataxia; excited movements in some mice and apathy in others. Paresis of the limbs occurred often but paralysis, usually spastic in type was infrequently seen, as was paralysis of the rectum and bladder. Certain animals exhibited alternating periods of excitement and somnolence or circling; others developed a long enduring catatonic-like posture in which the mouse stood partially erect, resting on the pads of the hind feet with forepaws flexed on the chest. Still others walked with arched or hunched backs on tiptoes, a mincing gait. Generalized convulsive movements were observed but rarely. A single mouse might exhibit all, or only one or more, of the described patterns of behavior during an attack. Clearly, the marked diversity of reaction depended for its expression on the area of the brain and cord which was damaged. During relapses the signs were not always identical or even resembled those seen in the attack immediately preceding. Thus the main sign of one attack might be wheezy breathing; of a relapse, paralyses of the limbs. The usual course of the experimental disease tended, therefore, toward a state of chronicity marked incidentally by relapses of variable duration and terminated usually by recovery, and infrequently by persistent paralysis of one or more limbs. Death supervened in about 5 per cent of inoculated animals and then, as a rule, during the early stages of the first attack. [5]

Histopathological Picture.—The earliest histopathological indications consisted of vascular changes; a relatively slight degree of intramural and perivascular infiltration extending out into the parenchyma. Such lesions were scattered here and there throughout the brain, especially the caudal part, and the cord. The infiltrating elements were chiefly mononuclear and polymorphonuclear leucocytes including some eosinophiles.

[5] The possible production of encephalomyelitis by means of mixtures of killed tubercle bacilli, petrolatum, and various fractions of mouse brain tissue is under investigation; the lipid material was the first fraction to be tested.

The picture seen in the fully developed encephalomyelitis revealed a greater degree of vascular reaction in respect both to the number of vessels involved and to the cellular reaction in and around them (Figs. 1, 2, 6, 10–12). The infiltrating cells in and about the vessels and especially in the parenchyma were not only leucocytes and plasma cells but also microglia, including rod and compound granular (gitter) cells (Figs. 3 and 8). No giant cells could be detected. Associated with the vascular reaction, which apparently was the essential pathological process, were disseminated petechial hemorrhages (Fig. 4), and the formation of thrombi of hyaline and leucocytic types (Fig. 2). The changes were more prominent in the white matter than in the gray, and in the mesencephalon, cerebellum, and spinal cord more than elsewhere in the central nervous system. Degeneration of nerve cells (Figs. 3 and 7) and of Purkinje cells (Fig. 12) was found in scattered areas in these regions but neuronal necrosis was rare or absent. In addition, areas of agglomerations mostly of mononuclear, less of microglial cells, and still less of polymorphonuclear leucocytes and plasma cells (Fig. 9) were seen, as were disseminated areas of diffuse glial cell infiltrations (Fig. 8).

In animals in which the signs of active illness endured for 3 or 4 weeks demyelination was met with more often than in the acute cases of shorter duration. Myelinolytic areas were found more commonly in the parenchyma (Figs. 5 and 7) than perivascularly (Fig. 6) and involved the myelin sheaths of fibres with or without destruction of the axons themselves. One could also see in certain animals irregularly outlined coalescing areas of demyelination. The scattered glioses or glial scars so prominent in chronic multiple sclerosis were not in evidence in the present study.

In recovered mice killed for the purpose of histopathological examination—the possibility should be considered of an impending relapse had the animal been allowed to survive—the brain and cord still showed evidence of inflammatory reaction. Here and there a mild degree of the vascular reaction could be seen and in some areas a slight, diffuse glial infiltration.

The leptomeninges were not always involved; when positive they showed a characteristically spotty infiltration with mononuclear and polymorphonuclear leucocytes predominating. In such discrete areas, blood vessels were also damaged in the characteristic way as described. The choroid plexus rarely exhibited mild changes of similar types of infiltration. The subependymal areas were at times the sites of massive infiltrations (Fig. 2) which also involved the ependymal lining.

In the mice which reacted only with respiratory symptoms no changes were found in the central nervous system. The lungs observed at the height of a dyspneic attack were noted as pale, partially collapsed, and having an increased consistency—a picture which was similar to that seen in anaphylactic reactions of mice (19).

The persistent nodules which developed locally after intramuscular or subcutaneous injection were found to contain a mass of cells of the mononuclear series, polymorphonuclear leucocytes, epithelioid cells, and an occasional multinucleated giant cell, along with amorphous material and broken down cells. This entire mass was held together in a firm encapsulated structure honeycombed by strands of newly formed fibrous tissue.

To summarize: The histopathological picture in murine experimental disseminated encephalomyelitis was not essentially different from that seen earlier in monkeys (20), rabbits (13), and guinea pigs (15, 16). The local lesion at the site of inoculation was characteristic of a cold abscess, or a foreign body cutaneous reaction in which one found epithelioid and giant cells. The changes in the brain and cord related to a primary vascular reaction followed by productive inflammation involving mesodermal-glial elements and in later cases, a certain but not a marked degree of gliosis and of demyelination. It remains for further

study of chronic cases of the experimental disease—those of many months' duration and more sustained exposure to the antigen—to determine whether

TABLE I

An Experiment Illustrating the Response of Swiss Strain Mice to Injection of Normal Mouse Brain Plus Adjuvants

Mouse No.	No. and route of injection (0.3 ml. each)	Time of onset	Time after first injection			Signs		Lesions in CNS	Remarks
			Killed	Died	Survived	Neurological	Respiratory		
		days	*days*	*days*					
1	3 IM; 1 SC	17	46			P	P	+++	Three relapses
2	" "	33			Yes	P	A		Two "
3	" "	33			"	P	A		Two "
4	3 IM	16	17			P	P	++	Lesions shown in Fig. 10
5	" "	18	33			P	P	+++	Paralyzed 15 days
6	" "	18		43		P	P	++	Six relapses
7	" "	18		20		P	P	+++	Lesions shown in Fig. 4
8	" "		21			A	A	++	Observed for lesions before signs
9	" "		21			A	A	+++	" "
10	" "	21	42			P	P	+++	Ill 21 consecutive days
11	" "	21	54			P	A	+	Three relapses, then recovery 7 days; rare vascular lesion only
12	" "	21	54			P	P	++	Two relapses
13	3 IM; 1 SC	22			Yes	P	A		Three relapses; recovery 30+ days
14	" "	25	54			P	P	++	Ill 29 consecutive days
15	" "	27	54			P	P	None	Recovery 27 days
16	3 IM; 2 SC	42		79		P	P	n.h.	Ill 37 consecutive days
17	" "	42			Yes	P	A		Ill 33 consecutive days, recovery 7+ days
18	" "	54			"	P	A		Two relapses
19	" "	112		113		P	A		Ill only 1 day
20	" "				Yes				No signs (120+ days' observation)

IM, intramuscular; SC, subcutaneous; +, ++, +++, arbitrary units denoting degree of involvement of central nervous system (CNS); A, no physical signs detected; P, signs present; 30+ days, animal still ill or apparently well up to the time of writing; n.h., no histopathologic study made.

glioses, glial scarring, and demyelination take place, such as are the indicators of demyelinating diseases as they occur in nature (*cf.* Ferraro and Cazzullo (12)).

Enumeration of Results.—The results set down in Table I were obtained in a single experiment in which normal mouse brain plus liquid petrolatum and tubercle bacilli as adjuvant comprised the antigen. The object of this tabulation is to give an informative picture of the response to the test. There

were additional experiments, not tabulated, which included 30 Swiss strain mice. The results of them all will be briefly set forth.

Encephalomyelitis was produced after one to six injections of the antigen. Of fifty mice, two gave positive results after only one inoculation of antigen and five after two. The largest number, namely eighteen, was positive after three injections; the next largest number, thirteen, after four. Only three mice responded to five doses and two to six. Three animals failed to show any visible effect even though five or six inoculations were given. Four animals were killed from 13 to 21 days after receiving two or three doses of antigen, before any clear indication of illness, and three of them exhibited histopathological lesions characteristic of disseminated encephalomyelitis.

The earliest period of time noted before onset of definite neurological signs was 9 days after the first treatment or 2 days after the second, and the longest, a rare instance, 112 days after the first, and 7 days after the sixth. The earliest development of respiratory signs of sneezing and wheezing respiration which later was followed by a definite neurological syndrome was first observed 2 days after a single injection of antigen. The majority of the mice recovered, only to relapse after a variable time. Quiescent periods lasted from 1 to 15 or 20 days. The number of such relapses over a period of 133 days was found to be six or less. The duration of illness, whether a primary attack or a relapse, varied from 1 to more than 30 days. Since most of the mice were killed during the active stage of the syndrome it is difficult to measure precisely the total number of days in the longest period of continuous illness.

To sum up the results on Swiss strain mice, it appears that disseminated encephalomyelitis could as a rule, be set up in them in a shorter time and with fewer exposures than in the other species of animals hitherto reported upon. Thus some mice exhibited signs of dyspnea and wheezy respiration within 2 days after a single inoculation; the majority showed a definite neurological syndrome within 9 to 21 days after two or three injections and only three of fifty animals failed to respond to the injections. A well defined individual resistance existed among the Swiss strain mice; now and again one reacted positively after one or two exposures to brain tissue-adjuvant mixture whereas others did so only after several injections given over a period of 2 or 3 months.

Host Specificity.—As already stated two inbred strains of mice, the Swiss and the Rockefeller Institute strains, were tested for their relative susceptibilities to development of encephalomyelitis following injections of mixtures of mouse brain and adjuvant.

Of fifteen Rockefeller Institute strain mice three received two intramuscular and one a subcutaneous injection of active material containing normal mouse brain mixed with adjuvant, and twelve were given four intramuscular and three subcutaneous inoculations. Of the fifteen animals only one, and that one of the first group, reacted with characteristic signs, on the 30th day after the first of three injections. This mouse was killed and typical lesions were found in its brain and cord (Fig. 7). In contrast, the same inoculum induced characteristic disseminated encephalomyelitis in nineteen of twenty Swiss strain mice after three intramuscular injections in nine mice and three intramuscular with additional one to two subcutaneous ones in the remaining ten; the malady appearing from 16 to 112 days after the first inoculation (Table I).

A wide difference was found, therefore, in the susceptibility of the two strains of mice; the Swiss strain were highly susceptible while the Rockefeller Institute

ones were relatively resistant. Not only was there this variation in strain but, as revealed in Table I, there were also differences among animals of the same Swiss strain some succumbing promptly upon a few inoculations whereas others did not respond at all or only after many weeks and after several exposures to the antigen.

Transmissibility of the Agent in Series.—The agent responsible for the experimental encephalomyelitis was found not to be serially transmissible in normal animals; it appeared not to be an infective, multiplying agent, as the results of the following tests showed.

The central nervous tissues of each of three mice killed at the height of the encephalomelitic reaction were suspended in a 10^{-1} dilution in physiological saline solution. 0.03 ml. of the preparations was injected intracerebrally into each of ten Swiss strain mice for each sample, or into thirty for all. None of the mice exhibited signs of illness.

Though no infective agent was thus demonstrated in Swiss strain mice an additional test was made with mice of the Rockefeller Institute strain, animals employed in this laboratory at at the time for experimental work on various encephalitis viruses. Each of fifteen normal animals was given 0.03 ml. intracerebrally of the active mouse brain–adjuvant mixture, and fifothers received 0.3 ml. peripherally (intramuscularly). No untoward effect was noted nor were any significant lesions produced except for the familiar nodules at the site of inoculation.

A third series of experiments involved the use of an acetone-ether "lipid" fraction obtained from normal mouse brain having a concentration of lipid of 20 mg./ml.[5] The aim here was an activation of a possible latent virus or other type of infective agent which might be present in the stock Swiss strain mice. Twenty animals were injected intramuscularly with 0.3 ml. of the lipid. The same animals received a second series of inoculations containing this material emulsified with equal parts of heavy liquid petrolatum. Each mouse was inoculated eight times but no observable signs or lesions could be discerned in the central nervous system during 139 or more days of observation. A second group of twenty mice received six intramuscular inoculations of the lipid fraction mixed with the adjuvant containing petrolatum and killed tubercle bacilli, and none of these showed signs of illness.

Repeated Exposure to the Active Agent.—A single injection of the mixture containing mouse brain–adjuvant failed, as a rule, to give a positive result; repeated exposure to this material was found to be essential.

Twenty Swiss strain mice received a single dose of 0.3 ml. of active antigen intramuscularly. Although the familiar nodules were formed at the site of inoculation, only one animal became ill, on the 54th day, showing signs of encephalomyelitis. On the other hand, positive results in nineteen of twenty mice, were obtained upon repeated injections of the same material (Table I).

Rôle of Killed Tubercle Bacilli as Adjuvant.—The enhancement of sensitization by an antigen through the adjuvant action of killed tubercle bacilli (Freund (8)) has been regarded hitherto as essential for producing disseminated encephalomyelitis readily. Killed tubercle bacilli were also found necessary to the affection produced in mice, as the following experiment makes clear.

Nineteen Swiss strain mice were given six intramuscular injections of an emulsion containing 25 ml. of heavy liquid petrolatum and 5 gm. normal mouse brain in 25 ml. of 0.85 per cent

saline solution; tubercle bacilli had not been added. No sign of illness was noticed; yet the emulsion used contained all the ingredients, except for the bacilli, which were present in mixtures producing positive results.

It is of interest that the liquid petrolatum which was added to normal brain tissue in the foregoing test did not suffice of itself to confer encephalitogenic power, even though it is known to promote antibody formation or antigenicity (Freund (8)). What may have been required was a higher degree of sensitization —if this indeed was the process involved—resulting from the presence in the inoculum of either killed tubercle bacilli as such or paraffin oil in addition to the bacilli.

DISCUSSION

Swiss strain mice, repeatedly injected intramuscularly and subcutaneously with a mixture of normal mouse brain and adjuvant developed symptoms and histopathological lesions of a disseminated encephalomyelitis like those of the encephalopathies induced by similar methods in monkeys, rabbits, and guinea pigs. Fewer injections were needed and of a simpler antigen, than in the case of the other animals mentioned; the experimental disease revealed itself in a shorter time; acute, subacute, or chronic forms could be readily produced; a larger proportion of inoculated mice became affected within a relatively brief time; and finally, the lesions induced were as marked, at least, as those found in the other species, from all of which it would appear that the mouse as experimental animal has certain advantages, apart from more convenient handling, lesser cost, and greater availability. It should be pointed out, however, that demyelination although met with occasionally in the mouse, now perivascularly, now parenchymally, and then in both sites, was not a prominent lesion. Demyelination has not always been seen in guinea pigs (15), although when it occurred in this species it was more often found, as in the mouse, in the later stages of the experimental affection (21). A study is planned of mice which have been kept under the influence of the active encephalitogenic mixture for many months, to determine whether more extensive demyelination and glial scarring develop.

The opinion has been expressed that the histopathological changes found in the various diseases included in the group of human demyelinating encephalopathies are essentially similar (1–3, 20, 22); and several investigators (1–7, 11–13, 20) have compared the lesions induced experimentally in various animal species with those of the human acute demyelinating diseases. There is good agreement on the resemblance of the pathological picture seen in man to that in the lower animals. No uniformity of opinion exists, however, as to whether the experimentally induced lesions resemble those of the human chronic encephalitides, multiple and diffuse sclerosis for example. In this relation, Wolf, Kabat, and Bezer (20) and Ferraro and Cazzullo (12) and others,

have stressed how individual is the reaction of an animal, the differences resulting from the duration of active illness and the number of relapses. The lesions in the mouse as disclosed by the present experiments resemble those in the species hitherto employed, and since the picture in the latter is similar to that observed in the natural disease, it would appear that study of the mouse can help in elucidation of some of the problems of the encephalopathies. The demonstration of strain differences as well as of individual resistance to the development of disseminated encephalomyelitis has interest because of the known differences in the susceptibility of human beings to certain demyelinating diseases (Hurst (22)).

The mouse may conceivably prove useful in the study of allergy in the nervous system. The present findings support the view that the experimental affection is an allergic, or isoallergic reaction to the injected material. The dyspneic, wheezy respiration and sneezing and pawing at the nose, occurring at the height of the reaction, are not unlike the signs of anaphylaxis in mice (19, 23). The fact that repeated injection with brain tissue–adjuvant mixture was necessary, a single inoculation being generally without effect, suggests sensitization (7). Furthermore, the development of encephalomyelitis was favored by the use of material which is known to enhance antibody formation. Whether the mixtures used excite an actual increase of antibrain antibody in the brain of mice is a problem awaiting further study as is that of a possible correlation between relapses and a recurring rise and fall of the level of this antibody. It may be that mice can be employed for investigations on the inhibition or destruction of the encephalitogenic factor present in normal brain.

CONCLUSIONS

Disseminated encephalomyelitis was readily induced in mice of the Swiss strain by means of repeated intramuscular and subcutaneous injections of apparently normal mouse brain mixed with an adjuvant. The latter consisted of autoclaved virulent tubercle bacilli and heavy liquid petrolatum, a modification of the Freund adjuvant.

The syndrome and the histopathological picture of the induced malady were essentially similar to those in monkeys, rabbits, and guinea pigs, previously reported by others. Certain exceptional characteristics of the affection, as occurring in mice, suggest that they may be the animals of choice for its study as well as for that of other encephalitides. Not only were the signs indicative of marked involvement of the central nervous system but also of the respiratory mechanism, and only a few injections of mouse brain–adjuvant mixture were required to evoke the neurological symptom complex in almost every animal.

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EXPLANATION OF PLATES

PLATE 19

FIG. 1. Mouse 2-5B, pons-medulla. Mural, perivascular, and extravascular infiltration. Hematoxylin-eosin stain. × 514. (Compare with Figs. 2, 3, 6, 10 and 11.)

FIG. 2. Mouse 7-5B, posterior colliculus. Extensive lesion chiefly perivascular, mural, and extravascular infiltration; diffuse glial infiltration of parenchyma and vascular thrombosis. Hematoxylin-eosin stain. × 135. (Compare with Figs. 10 and 11.)

FIG. 3. Mouse 2-5B, thalamus. Neuronal shrinkage and degeneration but no necrosis; also an area of vascular infiltration with polymorphonuclear and mononuclear leucocytes. Hematoxylin-eosin stain. × 308. (Compare with Fig. 7.)

FIG. 4. Mouse 7-5E, floor of fourth ventricle. Numerous petechial hemorrhages. Erythrosin-Azur I stain. × 128.

FIG. 5. Mouse 7-5B, white matter upper cervical cord. Destroyed and demyelinated axons. Bodian silver impregnation. × 502. (Compare with Figs. 6 and 7.)

FIG. 6. Mouse 9-5E, medulla. Perivascular area of demyelination. Bodian silver impregnation. × 128.

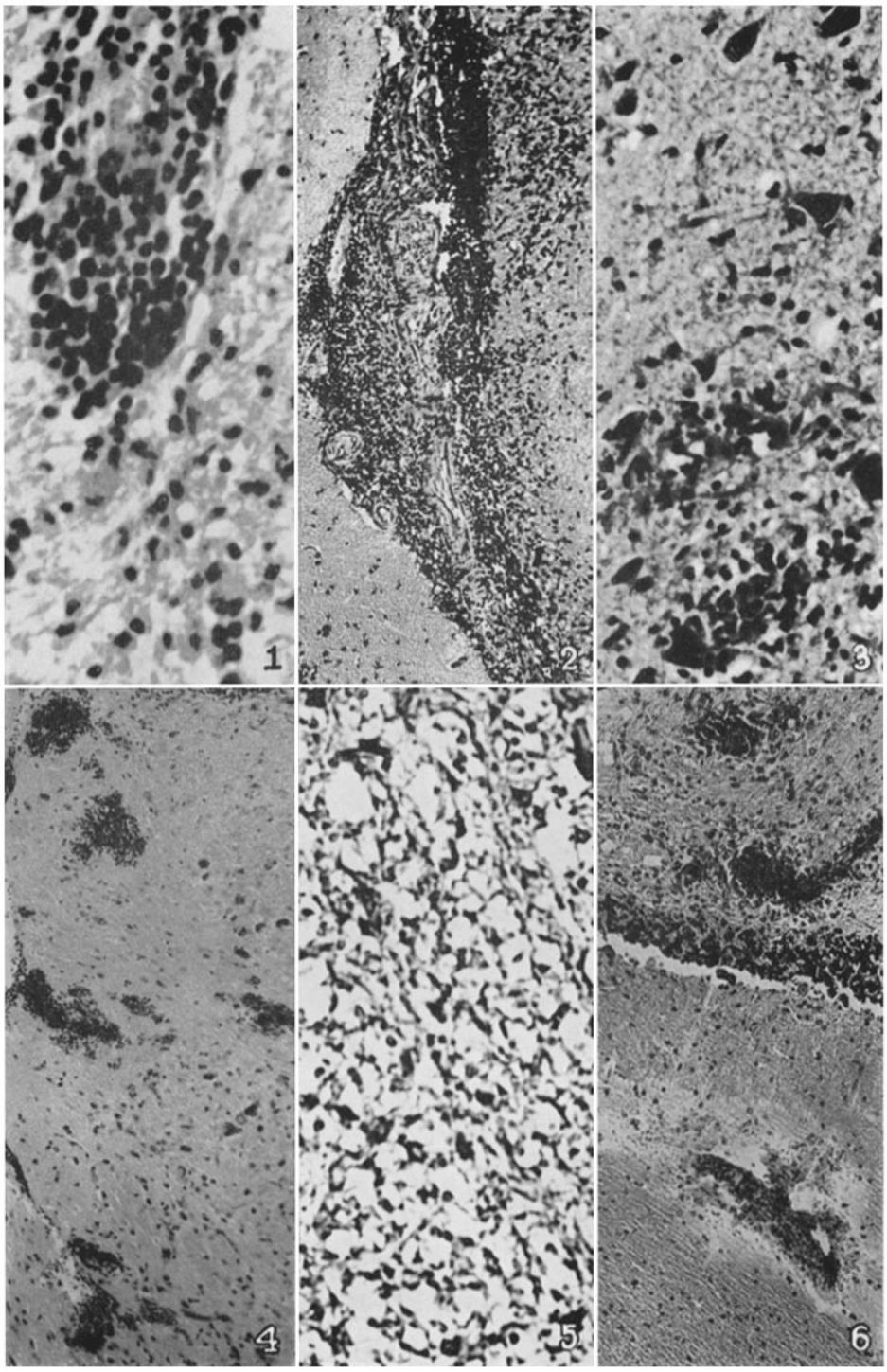

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PLATE 20

FIG. 7. Mouse 3-5D, cervical cord. Area of necrosis without surrounding zone of inflammation and demyelination in white matter; also shrunken and degenerated neurones. Hematoxylin-eosin stain. × 207.

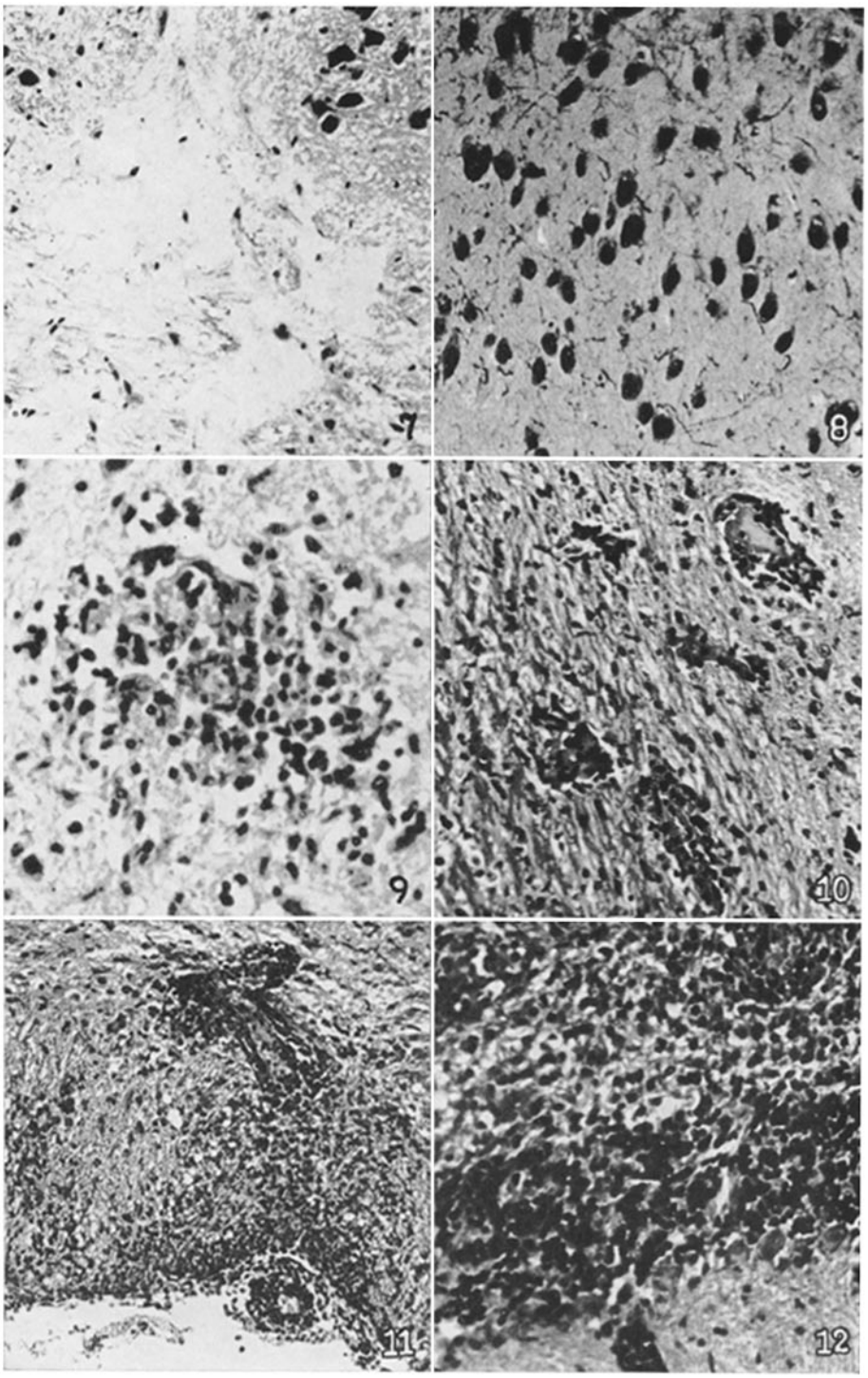
FIG. 8. Mouse 5-5B, thalamus. Glial infiltration of parenchyma (microglia and occasional gitter cells). Ranson's silver impregnation. × 394.

FIG. 9. Mouse 2-5B, thalamus. Nodular glial infiltration. Hematoxylin-eosin stain. × 406.

FIG. 10. Mouse 4-5E, neocortex. Vascular hyaline thrombus; also several vessels showing mural, perivascular, and extravascular infiltration. Hematoxylin-eosin stain. × 207.

FIG. 11. Mouse 7-5B, upper cervical cord, white matter. Massive lesion of vascular type, also diffuse glial infiltration of parenchyma and slight degree of demyelination at edge of lesion. Hematoxylin-eosin stain. × 165.

FIG. 12. Same mouse, cerebellum. Marked diffuse infiltration and vascular lesions in central white and granular laminae; Purkinje cells degenerated. Hematoxylin-eosin stain. × 287.



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