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EXPERIMENTAL ALLERGIC ENCEPHALITIS IN ANIMALS, AND ITS BEARING UPON THE ETIOLOGY OF NEUROPARALYTIC ACCIDENTS FOLLOWING ANTIRABIES TREATMENT IN MAN

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SYNOPSIS

In an attempt to throw light upon the etiology of neuroparalytic accidents following antirabies treatment in man, the author analyses the process and effects of experimentally induced allergic encephalitis in various animals. He concludes that the two conditions are apparently identical, and suggests that the solution to the problem of neuroparalytic complications be sought in either the elimination of the encephalitogenic factor or the use of an antirabies vaccine which does not contain brain tissue.

The purpose of this presentation is to summarize the present status of knowledge on the nature of the neuroparalytic accidents occurring in antirabies treatment as it appears from the study of experimental allergic encephalitis.

The problem of allergic encephalitis in animals has been extensively investigated from two separate points of view—(*a*) in the hope of obtaining information on the pathogenesis of demyelinating diseases, especially multiple sclerosis; and (*b*) in an attempt to produce an effective vaccine against poliomyelitis. Curiously enough, neither of these aims has been accomplished but, as a by-product, a new approach was opened to the problem of the pathogenesis of neuroparalytic accidents of antirabies treatment.

Experimental allergic encephalomyelitis may be produced with repeated subcutaneous injections of brain material in various animals, including monkeys,[9, 25, 26, 41, 42, 51] guinea-pigs,[2, 7, 15, 23, 33] rabbits,[43] dogs,[18, 55] sheep,[21] rats,[37] and mice.[46, 48] Some strains of guinea-pig [36] and mouse [45] appear to be more susceptible than others. Heterologous or homologous brain tissue is similarly effective. Human, monkey, rabbit, guinea-pig, rat, and mouse brain has been successfully used.[1, 27, 34, 38] Fish and frog brains are ineffective.[27] A monkey infected with its own brain substance may develop encephalitis.[28]

The use of adjuvants [14] incorporated into the brain material has considerably simplified the procedure, yielding more consistent results in a shorter

period of time. The standard technique of preparation of the brain-tissue suspension is as follows : 9 parts of freshly removed brain are mixed for 3 minutes in a Waring blendor with 10 parts of isotonic sodium chloride; 10 parts of melted Aquaphor and 20 parts of liquid petrolatum containing 2.5 mg of dead tubercle bacilli per ml are added; the ingredients are mixed again for 3 minutes and then heated at 60° C for one hour. The mixture is injected subcutaneously at weekly intervals at the dose of 1-3 ml. Variations of the technique are the intraperitoneal injection of guinea-pigs [7] and the intracutaneous injection in rabbits,[43] guinea-pigs,[48] and rats.[37] Instead of tubercle bacilli, other acid-fast organisms may be used.[13]

Clinical manifestations of involvement of the central nervous system vary in the time of occurrence and severity from species to species. In monkeys, they appear rather suddenly after from one to ten injections, and the course is usually rapid.[9, 25, 26, 41, 42, 51] Paralysis of limbs, incoordination, impairment of ocular muscles, and convulsions betray the multiple distribution of the pathological lesion. A more chronic disorder can be obtained by diminishing the dose of brain substance and tubercle bacilli.[8] In the guinea-pig, the clinical symptomatology is less protean, paralysis being the dominating manifestation. The disease may show remission and lapses, and new symptoms may appear upon re-injection of brain substance.[2] Guinea-pigs which show resistance to one series of injection are also resistant to successive courses.[38] In dogs, after repeated injections, a severe disease often develops suddenly.[55] A chronic condition may be obtained by substituting for *Mycobacterium tuberculosis*, *Mycobacterium butyricum* grown without glycerol to reduce its acid-fast properties.[13] In the mouse, a chronic relapsing type of disease is observed.

Pathological lesions differ somewhat from species to species but the type is fundamentally the same. In monkeys [9, 25, 26, 41, 42, 51, 59] the typical initial lesion consists of a perivascular area of infiltration made up of lymphocytes, leukocytes, plasma cells, and microgliacytes. Demyelination of the areas follows, with formation of scavenging cells. The lesions are multiple and may coalesce to form large areas of demyelination. Macroglia reaction is inconspicuous. Haemorrhages are not uncommon. These lesions are disseminated throughout the central nervous system and involve the white more than the grey matter. Often the midbrain, cerebellum, pons, medulla, and spinal cord seem to be more affected than the cerebral hemisphere. In guinea-pigs, the initial lesions are identical but demyelination is much less conspicuous. Nerve roots may be affected [15] and meningeal reaction is often present.[2] Lesions in mice and rats are quite similar to those observed in guinea-pigs. More conspicuous demyelination with some macroglia reaction is observed in chronic forms of the condition in monkeys [8] and guinea-pigs.[48]

Lesions at the site of injection consist of a granulomatous reaction surrounding the fatty material inoculated subcutaneously. Proliferated fibroblasts, plasma cells, polymorphonuclear leukocytes, among which there are many eosinophilic elements and lymphocytes, make up the cellular reaction. Multinuclear giant cells may be observed. The granuloma is richly vascularized.

The etiopathogenesis of allergic encephalitis is still incompletely understood. The almost general consensus of opinion is that the condition is due to an antigen-antibody reaction. In favour of this hypothesis, besides the negative findings of the absence of micro-organisms or viruses, are the following positive points of evidence :

(1) The delayed development of lesions following the injection of brain substance (" incubation period ").

(2) The enhancing action of " adjuvants ".

(3) The depressive action of adrenocorticotrophic hormone [44] upon the development of the condition in guinea-pigs, and of cortisone in monkeys.[29] It is pertinent to note that their depressive effect is probably mediated through a direct action upon the development of the granuloma at the site of infection.

(4) The depressive action of salicylate and *p*-aminobenzoic acid,[44] substances which are known to diminish hypersensitivity reaction.

(5) The occurrence of skin reaction to rabbit brain in guinea-pigs inoculated with rabbit brain,[33] and to homologous brain in rabbits inoculated with rabbit brain.[56] In the latter instance, the dermal reaction correlates fairly well with the encephalitic process in both degree of severity and time of occurrence.

(6) The development of an Arthus' phenomenon in guinea-pigs sensitized to homologous brain by the subcutaneous route and successfully inoculated intracerebrally with the same brain suspension.[34]

(7) The presence of circulating antibrain antibodies.

It should be noted, however, that attempts to transfer the hypersensitivity state have failed; with intravenous, intraperitoneal, or intracisternal injection of massive doses of blood of inoculated animals into normal animals, no encephalitis developed,[15, 19, 27, 42] nor has the transfer succeeded by intraperitoneal or intramuscular injection of peritoneal exudate, spleen, or lymphatic tissue from encephalitic animals.[19, 27]

If one accepts the hypothesis that the reaction is allergic in character, it becomes essential to investigate further the nature of the antigen and of the antibodies.

Little is known of the antibodies. Complement-fixing antibodies, similar in nature to those which are known to develop following injection of brain substance,[32] have been repeatedly demonstrated in the sera of

inoculated animals.[19, 34, 40, 55] However, no correlation exists between the presence or the titre of these antibodies and the development or the severity of the encephalitic process.[2, 19, 40, 55] It is possible, therefore, that the serum antibodies' development may be purely coincidental and not relevant to the encephalomyelitic process. In other words, encephalitogenic factor, and antigen responsible for stimulating complement-fixing antibodies, may be two distinct substances.

The results of numerous investigations on the presumable antigen are incomplete. It seems fairly well established that the antigen is somehow connected with myelin. In fact, foetal brain which contains no myelin is not encephalitogenic,[2, 27] and white matter of the hemisphere is more effective than grey cortex. Other tissues—heart,[1, 15] kidney, [15, 38, 39, 56] placenta, [39] liver, [33, 38, 39] testis,[1, 33, 39] and pancreas [1] are not encephalitogenic. Autoclaving and boiling,[2, 25, 27, 39] as well as fixation in formalin,[27, 39, 43] fail to destroy the antigenic property of the brain.

Attempts at isolating the encephalitogenic factor in the various lipid fractions have been thus far inconclusive. Using Bloor's [6] extraction method for lipid, the factor is found in the phosphatide fraction and not in the cholesterol, cerebroside, sphingomyelin, or protein fractions.[1, 2] Using Klenk's method of separating lipid,[31] the factor is found in both the ether and the methanol-chloroform fractions.[31, 39] Using Folch's method,[12] it has been claimed [47, 53, 54] that a mixture of proteolipid A and B contains the factor responsible for the production of encephalitis in mice. However, in guinea-pigs this finding was not confirmed (our own unpublished data), and the factor could also be found in the ether extract which contains no protein.[16] In rabbits, the proteolipid fraction was found to be less active than the ether-soluble fraction which contains no proteolipids.[57] It should be noted that, with few exceptions,[47, 53, 54] the factor isolated in the various fractions was less active than that present in the whole brain.[11] A calcium-acetate protein compound obtained from the brain has been claimed to contain the encephalitogenic factor,[5, 20] and to produce the disease in guinea-pigs.[10] These experiments could not be duplicated in our laboratory.

Using Warren's technique of protamine treatment,[58] it is possible to precipitate the encephalitogenic factor from a suspension of brain material,[50] as shown in the following tabulation, in which the results of experiments by Paterson et al.[50] and by the author are combined.

Incidence of encephalomyelitis in guinea-pigs following injection of crude and protamine-treated rabbit-brain suspension

Crude suspension			*Protamine suspension*	
Uncentrifuged	Supernatant	Sediment	Supernatant	Sediment
42/60	13/42	17/23	0/87	50/72

It would appear, therefore, that this method may be useful in separating the antigen, but no evidence is obtained as to the chemical nature of the substance.

A more precise identification of the encephalitogenic factor is indispensable to the understanding of the process of allergic encephalitis. At present one may only tentatively state that the injection of antigenic substance, probably connected with myelin, results in the formation of anti-brain antibodies. These antibodies react upon the brain, and more particularly the myelin, producing extensive and multiple lesions within the central nervous system. The role of adjuvants is still little understood. It is likely that the presence of fatty substances decreases the absorption-rate of the antigen, and the tubercle bacilli incite a rich granulomatous reaction in which cellular elements, active in the formation of antibodies, predominate.

The present status of knowledge concerning allergic encephalitis having been reviewed, one may ask what type of evidence this knowledge offers to a better understanding of the neurological complications occurring during antirabies vaccine treatment.

It is obvious that the procedure of antirabies vaccination in the human, consisting of repeated subcutaneous injections of heterologous brain tissue, is strikingly similar to the procedure in the experimental situation. It may be noted, in addition, that the more numerous the injections, the higher the percentage of neuroparalytic accidents.[3] The absence of adjuvants in the antirabies vaccine may account for the discrepancy in the incidence-rate of neurological manifestation between animal and man. The incidence in man is, to be sure, higher than was previously believed.[3, 49, 52]

The clinical manifestations in man include neuritis, myelitis, and encephalitis. It is relevant to note that symptoms develop after an incubation period not unlike that seen in the experimental allergic encephalomyelitis.[3] The onset of Landry's type of ascending myelitis and of encephalitis is abrupt, as noted in animals, and symptoms and signs are very similar. Neuritis, the most common neurological complication, has no counterpart in animal experimentation, probably because it is masked by the overwhelming cerebral symptoms. In guinea-pigs, however, involvement of the spinal roots is a feature of the pathological picture.[2, 15, 23]

Pathological findings in cases of neuroparalytic accident in man are few in number and often inadequately described. It is unquestionable that the lesions in the central nervous system [4] cannot be differentiated histologically from those observed in allergic encephalomyelitis. Perivascular cuffings, with early demyelination disseminated throughout the central nervous system, and preservation of neuron cells, are the outstanding characteristics of both conditions.

Serological studies in a few cases investigated [30, 35] showed increased brain-antibody titres, as determined by complement-fixing methods. Antibodies were also found in 50% of the individuals who had received a 14-day course of treatment. As in animals, no correlation could be established between severity of neurological manifestations and antibody titres.

It may be noted, in conclusion, that experimental allergic encephalomyelitis in animals, and neuroparalytic accidents occurring in man during antirabies vaccination, are apparently identical conditions. The observation of a disease similar to allergic encephalitis following antirabies vaccination in dogs,[22] and in mice,[24] adds considerable weight to this statement.

Elimination of the encephalitogenic factor would obviously eliminate the danger of neuroparalytic accidents, but attempts thus far have been unsuccessful. The alternative would be the use of an antirabies vaccine that does not contain brain tissue.

RÉSUMÉ

L'auteur résume les connaissances actuelles sur la nature et le mécanisme des accidents paralytiques consécutifs à la vaccination antirabique, que les études de l'encéphalite allergique expérimentale, chez l'animal, ont permis de mieux comprendre.

L'encéphalite allergique peut être produite par injections sous-cutanées répétées de substance cérébrale à des singes, cobayes, moutons, lapins, rats et souris. Le tissu cérébral paraît également actif, qu'il soit homologue ou hétérologue. L'adjonction d'adjuvants, tels que des bacilles acido-résistants, par exemple, a permis d'obtenir des résultats plus nets, en un laps de temps plus court. Les manifestations cliniques (chez les singes : paralysie des membres, incoordination, convulsions, atteinte des muscles oculaires) révèlent des lésions disséminées dans le système nerveux central. Les caractères histopathologiques, bien que différant quelque peu d'un animal à l'autre, sont, dans l'ensemble les mêmes : zone d'infiltration périvasculaire constituée par des lymphocytes, des leucocytes, des plasmocytes et microgliocytes. Les lésions peuvent confluer et donner de larges zones de démyélinisation. La racine des nerfs peut être affectée et les réactions méningées sont fréquentes. Ces lésions sont disséminées dans le système nerveux central et s'observent plus souvent dans la substance blanche que dans la substance grise.

La pathogénèse de l'encéphalite allergique est encore incomplètement connue. On s'accorde à admettre, de façon générale, qu'il s'agit d'une réaction antigène-anticorps. Toutefois, bien que plusieurs des caractères de la maladie témoignent en faveur de cette hypothèse, tous les essais tendant à transmettre cette hypersensibilité d'un animal à l'autre par injection de doses massives de sang, d'exsudat péritonéal, de rate ou de tissu lympathique, ont échoué. On sait peu de chose des anticorps. La présence d'anticorps fixateurs du complément dans le sérum des animaux, à la suite d'injections de substance cérébrale, a été démontrée à plusieurs reprises. Toutefois, il n'existe aucun rapport entre le titre des anticorps et la gravité de l'encéphalite. Il se peut donc que l'antigène qui stimule la formation d'anticorps et le facteur responsable de l'encéphalite soient deux substances distinctes. L'antigène semble avoir quelque rapport avec la myéline, car les tissus encéphalitogènes en contiennent. Le facteur pathogène semble se trouver dans la fraction phosphatidique de cette substance. En résumé, on suppose que l'injection d'un antigène — probablement en relation avec la myéline — provoque la formation d'anticorps anti-substance cérébrale. Ces anticorps agissent sur la substance cérébrale — en particulier la myéline — produisant des lésions nombreuses et étendues dans le système nerveux central.

On peut faire les remarques suivantes, concernant les rapports entre l'encéphalite allergique expérimentale et les accidents paralytiques consécutifs à la vaccination antirabique : les manifestations cliniques de la paralysie postvaccinale chez l'homme comportent de la névrite, de la myélite, de l'encéphalite. Le temps d'incubation est à peu près le même que dans l'encéphalite expérimentale. La paralysie ascendante, type Landry, et

l'encéphalite expérimentale apparaissent toutes deux brusquement. L'anatomie pathologique des lésions humaines n'a été que rarement décrite. Il est certain que l'histopathologie du système nerveux central, chez l'homme, est semblable à celle de l'animal d'expérience. Les zones d'infiltration avec démyélinisation sont les caractères principaux des deux maladies. Le titre des anticorps anti-substance cérébrale (déterminé par le test de fixation du complément) s'élève aussi dans les cas d'accidents paralytiques. On a observé pareil accroissement chez 50 % des personnes ayant reçu un traitement antirabique comportant 14 injections. Comme dans l'encéphalite expérimentale, on n'a pu établir aucune relation entre le titre des anticorps et l'intensité des manifestations neurologiques.

L'encéphalite allergique expérimentale et les accidents paralytiques consécutifs à la vaccination antirabique semblent être des maladies analogues. Cette similitude est confirmée par le fait que des phénomènes pathologiques semblables à ceux de l'encéphalite expérimentale ont été observés chez des chiens et des souris à la suite de la vaccination antirabique.

REFERENCES

1. Alvord, E. C. (1948) *Proc. Soc. exp. Biol. (N.Y.)* **67**, 459
2. Alvord, E. C. (1949) *J. Immunol.* **61**, 355
3. Appelbaum, E., Greenberg, M. & Nelson, J. (1953) *J. Amer. med. Ass.* **151**, 188
4. Bassoe, P. & Grinker, R. R. (1930) *Arch. Neurol. Psychiat. (Chicago)*, **23**, 1138
5. Bell, J. F., Wright, J. T. & Habel, K. (1949) *Proc. Soc. exp. Biol. (N.Y.)* **70**, 457
6. Bloor, W. R. (1943) *Biochemistry of the fatty acids and their compounds, the lipids*, New York
7. Cazzullo, C. L. & Ferraro, A. (1949) *Arch. Path. (Chicago)*, **48**, 316
8. Ferraro, A. & Cazzullo, C. L. (1948) *J. Neuropath.* **7**, 235
9. Ferraro, A. & Jervis, G. A. (1940) *Arch. Neurol. Psychiat. (Chicago)*, **43**, 195
10. Ferraro, A. & Roizin, L. (1951) *J. Neuropath.* **10**, 394
11. Ferraro, A. Roizin, L. & Cazzullo, C. L. (1950) *J. Neuropath.* **9**, 18
12. Folch, J., Ascoli, T., Lees, M., Meath, J. A. & LeBaron, F. N. (1951) *J. biol. Chem.* **191**, 833
13. Freund, J., Lipton, M. M. & Morrison, L. R. (1950) *Arch. Path. (Chicago)*, **50**, 108
14. Freund, J. & McDermott, K. (1942) *Proc. Soc. exp. Biol. (N.Y.)* **49**, 548
15. Freund, J., Stern, E. R. & Pisani, T. M. (1947) *J. Immunol.* **57**, 179
16. Goldstein, M. P., Kolb, L. C., Mason, H. L., Sayre, G. P. & Karlson, A. G. (1953) *Neurology*, **3**, 609
17. Good, R. A., Campbell, B. & Good, T. A. (1949) *Proc. Soc. exp. Biol. (N.Y.)* **72**, 341
18. Halpern, B. M., Bertrand, I. & Lhermitte, F. (1950) *Presse méd.* **58**, 684
19. Hill, K. R. (1949) *Johns Hopk. Hosp. Bull.* **84**, 302
20. Hottle, G. A., Medzel, G. A., Wright, J. T. & Bell, J. F. (1949) *Proc. Soc. exp. Biol. (N.Y.)* **72**, 289
21. Innes, J. R. M. (1951) *J. comp. Path.* **61**, 241
22. Jervis, G. A., Burkhart, R. L. & Koprowski, H. (1949) *Amer. J. Hyg.* **50**, 14
23. Jervis, G. A. & Koprowski, H. (1948) *J. Neuropath.* **7**, 309
24. Jervis, G. A. & Koprowski, H. (1949) *Canad. J. comp. Med.* **6**, 116
25. Kabat, E. A., Wolf, A. & Bezer, A. E. (1946) *Science*, **104**, 362
26. Kabat, E. A., Wolf, A. & Bezer, A. E. (1947) *J. exp. Med.* **85**, 117
27. Kabat, E. A., Wolf, A. & Bezer, A. C. (1948) *J. exp. Med.* **88**, 417
28. Kabat, E. A., Wolf, A. & Bezer, A. E. (1949) *J. exp. Med.* **89**, 395
29. Kabat, E. A., Wolf, A. & Bezer, A. E. (1951) *Fed. Proc.* **10**, 412
30. Kirk, R. C. & Ecker, E. E. (1949) *Proc. Soc. exp. Biol. (N.Y.)* **70**, 734
31. Klenk, E. (1939) *Hoppe-Seyl. Z. physiol. Chem.* **262**, 128

32. Kopeloff, L. M. & Kopeloff, N. (1944) *J. Immunol.* **48**, 297
33. Kopeloff, L. M. & Kopeloff, N. (1947) *J. Immunol.* **57**, 229
34. Koprowski, H. & Jervis, G. A. (1948) *Proc. Soc. exp. Biol. (N.Y.)* **69**, 472
35. Koprowski, H. & LeBell, I. (1950) *Amer. J. Hyg.* **51**, 292
36. Lipton, M. M. & Freund, J. (1953) *J. Immunol.* **70**, 326
37. Lipton, M. M. & Freund, J. (1953) *J. Immunol.* **71**, 98
38. Lumsden, C. E. (1949) *Brain*, **72**, 198
39. Lumsden, C. E. (1949) *Brain*, **72**, 517
40. Lumsden, C. E., Kabat, E. A., Wolf, A. & Bezer, A. E. (1950) *J. exp. Med.* **92**, 253
41. Morgan, I. M. (1946) *J. Bact.* **51**, 614
42. Morgan, I. M. (1947) *J. exp. Med.* **85**, 131
43. Morrison, L. R. (1947) *Arch. Neurol. Psychiat. (Chicago)*, **58**, 391
44. Moyer, A. W., Jervis, G. A., Black, J., Koprowski, H. & Cox, H. R. (1950) *Proc. Soc. exp. Biol. (N.Y.)* **75**, 387
45. Olitsky, P. K., Casals, J. & Tal, C. (1950) *Proc. Soc. exp. Biol. (N.Y.)* **75**, 276
46. Olitsky, P. K. & Tal, C. (1951) *Proc. Soc. exp. Biol. (N.Y.)* **78**, 607
47. Olitsky, P. K. & Tal, C. (1952) *Proc. Soc. exp. Biol. (N.Y.)* **79**, 50
48. Olitsky, P. K. & Yager, R. H. (1949) *J. exp. Med.* **90**, 213
49. Pait, C. F. & Pearson, H. E. (1949) *Amer. J. publ. Hlth*, **39**, 875
50. Paterson, P. Y., Pond, W. L., Warren, J. & Weil, M. L. (1953) *Proc. Soc. exp. Biol. (N.Y.)* **83**, 278
51. Rivers, T. M. & Schwentker, F. F. (1935) *J. exp. Med.* **61**, 689
52. Sellers, T. F. (1947) *J. med. Ass. Ga*, **36**, 30
53. Tal, C. & Olitsky, P. K. (1952) *Proc. Soc. exp. Biol. (N.Y.)* **81**, 590
54. Tal, C. & Olitsky, P. K. (1952) *Science*, **116**, 420
55. Thomas, L., Paterson, P. Y. & Smithwick, B. (1950) *J. exp. Med.* **92**, 133
56. Waksman, B. H. & Morrison, L. R. (1951) *J. Immunol.* **66**, 421
57. Washman, B. H., Porter, H., Adams, R. D. & Folch, J. (1953) *Fed. Proc.* **12**, 464
58. Warren, J. (1950) *Bact. Rev.* **14**, 200
59. Wolf, A., Kabat, E. A. & Bezer, A. E. (1948) *J. Neuropath.* **7**, 333