PRODUCTION OF EXPERIMENTAL ENCEPHALOMYELITIS
WITH CALCIUM ACETATE COMPOUND EXTRACTED
FROM BRAIN TISSUE*†

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In previous communications we have reported the experimental production of encephalomyelitis in guinea pigs with some brain lipid extracts and brain residue (after lipid extraction with cold acetone, ether and alcohol), composed mostly of proteins (1). In the present report we shall record our observations on the production of experimental encephalomyelitis in guinea pigs with a protein calcium acetate compound, extracted from brain tissue.

EXPERIMENTAL PROCEDURES

Twenty five guinea pigs, used in these studies, were subdivided into two groups. The first group consisted of young guinea pigs, weighing between 250 and 300 Gm. After preliminary observation of approximately 5 days, the animals received an intramuscular injection into the nuchal muscles of 3 cc. of calcium acetate emulsion, prepared according to Freund’s technic (2). The calcium acetate compound was obtained through the courtesy of Dr. Bell and was prepared from brain tissue vaccines as described by Habel, Bell, and Wright (3, 4). It consisted of a 20 per cent infected brain suspension (rabbit brain vaccine) in distilled water which was dried from a frozen state. To the dry powder, sufficient benzene, equal to twice the original volume, was added. The suspension was vigorously agitated to break clumps of tissue, poured into a screw-top bottle, and placed in a water bath at the desired temperature (for the inactivation of rabies virus, as specified by Habel, Bell, and Wright (3, 4)). After an appropriate period of time the benzene was removed by suction from filtrate through a sintered glass filter of “M” porosity. As suction was turned off, clean benzene was added to the residue and the filter. Resuspension was accomplished by stirring with a sterile applicator stick, and again suction was applied to remove the benzene. The residue on the filter was resuspended in one volume of ether (the ether was allowed to filter without suction). A second resuspension in ether was prepared and the ether was removed by suction. The entire residue and filtrate were put in a vacuum chamber for a period of 30 minutes for the purpose of removing the small amount of ether from the residue. The dry powder was then added to a volume of distilled water, equivalent to the original volume. Hydration and mixing was done in a Waring blender. Sufficient solution of calcium acetate was added to make a final concentration of M/10 calcium acetate, and the suspension was permitted to stand in the cool (frigidaire) for an hour or two. The calcium acetate solution was then removed by centrifugation or filtration (sintered glass filter of “M” porosity) and brought to dryness in a vacuum chamber.

The second group of animals consisted of 15 adult guinea pigs weighing between 600 and 750 Gm. These animals were treated in the same manner as those in Group 1 with the exception that the calcium acetate compound for the preparation of the emulsion was obtained from sheep brain and prepared in our laboratory. The time extraction with cold acetone, benzene and ether respectively, as well as the hydration time and treatment with

* This investigation has been supported by a grant from the United States Public Health Service, 6 B-5 (MH-129).
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The production of experimental encephalomyelitis was prolonged to 24 hours in each instance. The final disease producing emulsion was prepared as indicated in Table I.

**CLINICAL OBSERVATIONS**

The animals were examined daily following the intramuscular injection. The weight was recorded 3 times a week. The majority of the animals disclosed from 10 days to 2 weeks after the injection, signs of a general reaction, indicated by hypersensitivity to touch or pressure, sluggishness, easy fatigability, and loss of weight. Table II illustrates variations in body weight of 24 observed guinea pigs during a period of 90 days. For the purpose of simplification, 5 average curves were selected, each curve is a composite one. The first curve (from above downwards) illustrates the average fluctuation in weight of 12 guinea pigs; the second, third and fourth curves, 2 guinea pigs each; and the fifth curve, the average of 6 guinea pigs.

Neurologic manifestations were generally observed between 14 and 30 days following the intramuscular injection. Hypotonia of the posterior or the anterior extremities was the most common early neurologic sign. Hypotonia was generally followed by paresis and paralysis. Usually, the posterior extremities showed the highest incidence and the severest type of motor disorders. Only a few animals have had convulsions and sphincter involvement. Table III illustrates in more detail the onset and duration of both general and neurologic disorders in the 25 studied animals.

**TABLE I**

<table>
<thead>
<tr>
<th>MATERIAL</th>
<th>TOTAL AMOUNT</th>
<th>AMOUNT PER 1 CC</th>
<th>PROPORTIONS</th>
<th>REMARKS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain protein calcium acetate</td>
<td>.190 Gm.</td>
<td>2.17 mg.</td>
<td></td>
<td>Vacuum dried powder.</td>
</tr>
<tr>
<td>Saline</td>
<td>35.0 ml.</td>
<td>0.4 ml.</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Falba</td>
<td>17.5 ml.</td>
<td>0.2 ml.</td>
<td>1</td>
<td>Culture: agar blood: negative.</td>
</tr>
<tr>
<td>Bayol F</td>
<td>35.0 ml.</td>
<td>0.4 ml.</td>
<td>2</td>
<td>Preserved in deep freeze.</td>
</tr>
<tr>
<td>Heat Killed T.B.</td>
<td>29 mg.</td>
<td>0.33 mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Mixture.</td>
<td>87.5 ml.</td>
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On closer examination, it appeared that in a large number of cases, the signs of general reaction preceded the development of the neurological symptoms (animals $\approx 1, 2, 4, 8, 9, 10, 11, 12, 14, 16, 20, 21$). However, in some animals the general reaction lasted a long period of time (table III), and was not followed by neurologic symptomatology (animals $\approx 3, 5, 6, 13, 17, 19, 22, 25$). Others, after having exhibited general and neurologic symptomatology showed improvement and, in a few instances, made an apparently full recovery. In a few instances relapses were observed without precipitating injections having been used. To 7 guinea pigs (animals $\approx 17, 19, 20, 21, 22, 24, 25$) of the second group a second injection was given to see whether it would modify the clinical symptomatology as indicated in Table III. Of these animals, however, only 2 ($\approx 21$ and 19) developed neurologic symptoms after the second injection, whereas the others ($\approx 17, 20, 22, 24, 25$) continued to exhibit the same symptomatology as indicated previously.

**HISTOPATHOLOGICAL OBSERVATIONS**

The material selected for histopathological studies was in part fixed in 5 per cent isotonic neutral formalin, and in part, in 80 per cent alcohol.

The local tissue (areas of injection) changes and the visceral tissue reactions are the subject of a separate histopathologic study, and will be reported at a later date.
TABLE II

Variations in Body Weight of 24 Studied Guinea Pigs during a Period of 90 Days as Described in the Text.

End of curves indicate completion of the experimental observations due to the death or sacrifice of the studied animals.
Indicates the time of the second injection as described in the text.

Dotted spaces indicate the recorded clinical symptomatology and the free spaces indicate no clinically detectable abnormalities.

<table>
<thead>
<tr>
<th>Type of Symptomatology</th>
<th>Onset and duration of symptoms (in days) following the intramuscular injection of brain calcium acetate emulsion.</th>
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<tbody>
<tr>
<td>General Reaction</td>
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<tr>
<td>Fatigability or Asthenia</td>
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<tr>
<td>Hypotonia</td>
<td></td>
</tr>
<tr>
<td>Paralysis</td>
<td></td>
</tr>
<tr>
<td>Convulsions</td>
<td></td>
</tr>
<tr>
<td>Sphincter involvement</td>
<td></td>
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</tbody>
</table>
Fig 1. (a) and (b): Predominance of inflammatory exudate in the meninges: (a) Cerebellum, and (b) spinal cord. Nissl stain. Low power magnification.
Fig. 2. (a) and (b): Predominantly meningo-encephalomyelitic type of distribution of the lesions. (a) Brain cortex and (b) spinal cord. Nissl stain. Low power magnification.
The neurohistologic technics used included Nissl and hematoxylin and eosin stains for cytologic studies; Hortega's silver carbonate method (Globus-Penfield modification) for micro- and oligodendrogial cells; Cajal's gold sublimate (Globus-Penfield modification) for astrocytes; Roizin's combined method for myelin and lipid products of disintegration; Bielschowsky's silver impregnation for nerve fibers, and Holzer's stain for astrocytic glia fibers.

The neuropathologic findings in both groups of guinea pigs were essentially of the same character. Therefore they will be discussed together.

Although topographically no part of the central nervous system was spared, some individual variations were noticed. In some instances, for example, the meningitic (fig. 1), or the meningo-encephalo-myelitic (fig. 2), or meningo-radiculo-myelitic (fig. 3) involvement represented the most prominent feature; in other cases the encephalomyelitic, or the polio-myelitic varieties were predominant. In association with these histopathologic varieties frequent involvement of the choroid plexuses was observed (fig. 4). The cerebellum and brain stem were often affected.

Generally the lesions were circumscribed and perivascular in distribution (fig. 5a). Although they were mostly perivenous in character, the arteries or the arterioles appeared, at times, surrounded by inflammatory cuffings. In some instances peri- or panarteritis, leading to vascular thrombosis was observed. In contrast to circumscribed lesions in some circumstances, the distribution of lesions here was more diffuse and widespread in character (fig. 5b). Occasionally the lesions assumed the character of granulomatous formations (fig. 6a and b), scattered in the gray or white matter of the brain, brain stem, and spinal cord.
Fig. 4. (a) and (b): Inflammatory exudate within the stroma of the choroid plexuses and the ventricular cavity. (a) Nissl stain: (b) Hematoxylin-eosin stain. Medium power magnification.
Fig. 5. (a) and (b): (a) Predominantly perivascular lesions scattered mostly in the brain cortex. (b) Diffuse and widespread inflammatory reaction in the brain stem. Nissl stain. Medium power magnification.

The exudate appeared in most instances to bear a close relationship to the development and the course of the disease process: in the acute phases of the inflammatory reaction, the polymorphonuclear elements were predominant (fig. 7), whereas the other mononuclear
Fig. 6. (a) and (b): Granulomatous formations in (a) the gray and (b) the white matter. Nissl stain. Medium power magnification.

Fig. 7. Polymorphonuclear leucocytes among various hematogenous and histogenous elements in the acute inflammatory phase. Nissl stain. High power magnification.
hematogenous cells, histiocytes and glial elements were present in a much lower ratio. In the subacute and chronic phases, instead, the mononuclear hematogenous and histogenous cells of histiocytic or glial origin were predominating. Of interest is the frequent appearance of plasma cells which generally were mixed with the various elements of the inflammatory exudate (fig. 8). No hemorrhages or polynucleated giant cells were encountered in this material.

Hortega’s silver preparations disclosed frequent reactive glial proliferation within, or in the vicinity of, the lesions. Cajal’s gold impregnation was not very successful. Holzer’s stain did not reveal definite glial fiber reaction. The myelin sheath stain revealed, at times, rarefaction or various degrees of swelling and disintegration of myelin, mostly perivascular in location. Fat products of disintegration were only occasionally detected. Silver impregnation preparations for axis cylinders showed only limited rarefaction or various degrees of nerve fiber alterations, limited mostly to scattered circumscribed areas, and mostly around or in the vicinity of blood vessels.

![Fig. 8. High ratio of plasma cells (especially left upper side of the microscopic field) mingled with various elements of the inflammatory exudate. Nissl stain. High power magnification.](image)

**COMMENT**

A detailed analysis of the clinical observations in the studied 25 guinea pigs indicates that the majority of the animals exhibited a general reaction which consisted usually in the loss of weight, hypersensibility, fatigability, sluggishness and asthenia. These general symptoms usually appeared during the second and third week, following the injection of the precipitating encephalomyelitic calcium acetate emulsion, and lasted for various periods of time as indicated in Table III. At times, some animals showed remissions and regained weight (table II). However, more frequently these general reactions were followed by neurologic signs consisting of hypotonia, paresis or paralysis involving the various extremities at different periods of time or simultaneously. These signs were, at times, associated with: (a) tremors or ataxia of the involved extremities, head, or trunk; (b) convulsions or myoclonic twichings; or (c) sphincter involve-
ment (generally incontinence of urine and feces). The neurologic symptoms appeared toward the end of the second or the beginning of the third week, following the precipitating injection with the highest incidence between the third and fifth week.

Of the 25 studied guinea pigs, only 12 showed definite neurologic symptoms (table III), 7 of which belonged to the first group (in which the original calcium acetate prepared by Dr. Bell from rabbit brain vaccine was used as precipitating emulsion), and the other 5 to the second group (in which the used calcium acetate compound was prepared from sheep brain in our own laboratory).

The neurologic symptomatology in the majority of cases appeared progressive in character, ending in the death of the animal within a few days or a few weeks.1

In a few instances, however, some animals disclosed various degrees of improvement and occasionally even apparent clinical recovery while in others, spontaneous relapses occurred. Some paraplegic animals, which survived for a long period of time, disclosed also trophic changes of the hair, skin, and various degrees of muscular atrophy.

Although histopathologic studies on the guinea pigs, which during life presented definite neurologic symptomatology, revealed the types of lesions, illustrated in Figures 1–8, some discrepancies were also observed. For instance, of 4 guinea pigs (***5, 6, 13, 17) which exhibited clinically only a general reaction and no clear-cut neurologic signs (table III), 2 (***5, and 13) showed a mild form of meningitis and a mild encephalomyelitic reaction, whereas the other 2 (***6, and 17) presented perivascular inflammatory cuffings and small granulomatous formations, scattered throughout the brain substance (fig. 6a and b). Finally, the histopathologic study of 1 guinea pig (**7), which died suddenly on the 6th day following the precipitating injection without presenting any visible clinical signs of generalized or neurological symptomatology, revealed a mild, but definite, encephalomyelitic process predominantly in the brain stem (fig. 9), cerebellum, and to a lesser extent, in the spinal cord.

On the basis of the aforementioned findings it is evident that the clinical and neuropathologic manifestations of the meningo-encephalo-myelitic process, induced by the brain-calcium acetate compound plus adjuvants, are essentially similar to those produced when the whole brain plus adjuvants is used as antigen (5–10). Moreover, on comparing the clinicopathologic observations following the use of various lipid fractions and brain residue as reported by other investigators (9, 10, 11) and ourselves (1) it seems that the brain calcium acetate compound has a more definite and stronger antigenic property than other brain fractions.

In view of the fact that some of the lipid fractions have also antigenic properties, one may speculate that: (a) there might be more than one substance in the central nervous system possessing antigenic properties which might be responsible for the induction of the meningo-encephalo-myelitic process, or (b) the lipid fractions might have been contaminated during the process of lipid preparation.

1 Some animals, however, died following only some general reaction and, occasionally, even without any previous clinical abnormality in their behavior or physical appearance.
extraction with some substances of a more potent and predominantly protein origin.

The number of animals used for the study of the antigenic properties of the brain calcium acetate compound is still rather small for statistical comparisons with that following the use of the whole brain. However, the data, available at present, indicate that the whole brain is still the most potent antigen thus far available for the production of the allergic experimental encephalomyelitis.

Fig. 9. Distribution of the inflammatory reactions in the brain stem: some of which are around vessels; others have no apparent relationship to the vascular pattern. Nissl stain. Low power magnification.

SUMMARY AND CONCLUSIONS

A total of 25 guinea pigs, subdivided into two groups, were used for the induction of experimental allergic encephalomyelitis with a calcium acetate compound extracted from the brain tissue.

Histologic studies disclosed circumscribed or diffuse (to a lesser extent) inflammatory reactions involving the meninges and the parenchyma at various levels of the central nervous system. The inflammatory exudate was more frequently perivascular in character and consisted of both hematogenous and histogenous elements which varied in percentage in relation with different stages of the inflammatory process. Frequently, plasma cells appeared mixed with the various elements of the exudate. The process of demyelination was rather light and mostly perivascular in location.

Both the clinical and the neuropathologic findings were essentially similar to those previously reported in allergic encephalomyelitis in which the whole brain plus Freund's adjuvants was used as antigen.
It is still too early to attribute specific antigenic properties to the various lipid or protein fractions of the brain. However, there is indication that some of the chemical components of the brain, when used separately, possess some antigenic properties capable of inducing an encephalomyelitic process. Of these, the calcium acetate fraction which is predominantly a protein compound appears as the most potent one.

REFERENCES


