PHYSIOLOGICAL CHANGES IN THE NERVOUS SYSTEM AFTER SENSITIZATION WITH BRAIN TISSUE ANTIGEN

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In rabbits sensitized with homologous brain tissue antigen without the use of stimulators of immunogenesis considerable changes were found in the physiological parameters of the nervous system. They reached a maximum on the 15th-16th day of sensitization and were expressed as a decrease in the lability of the nervous centers, and changes in the thresholds of excitation and latent periods of reflex responses. Despite the continued injection of antigen and the increase in titer of brain antibodies in the blood, the parameters studied returned to normal on the 24th-32nd day of investigation.

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At the present time the best model available to simulate allergic lesions of the nervous system is experimental allergic encephalomyelitis [3, 4, 9, 12], produced by the use of antigen from brain tissue together with Freund's adjuvant. The results of a study of the stimulant effect of this adjuvant have indicated considerable changes in the tissues of the experimental animals receiving adjuvant alone [2, 15]. Some workers attribute the action of these stimulators directly to their cytotoxic effect [6, 10]. Still greater caution is aroused by investigations showing that changes can be produced with the characteristic picture of experimental alleric encephalomyelitis by administration of Fruend's adjuvant alone [7].

Repeated attempts have been made to produce lesions of the nervous system by sensitizing animals with heterologous brain tissue antigen without stimulators [8, 13], but to produce clinical and histological signs of the disease it was necessary to inject the antigen in long courses (up to 100 or more injections); even in this case the effect was inconstant.

The object of the investigation was to study the character of changes in the nervous system of animals sensitized by nerve tissue antigen but without the use of Freund's adjuvant. The method of electromyographic recording of reflex activity of the spinal cord was used, as is recommended when lesions of the nervous system are comparatively mild, and no definite clinical symptoms have yet appeared [5, 14].

EXPERIMENTAL METHOD

Long-term experiments with periods of observation of 35-40 days were carried out on 15 rabbits. The animals were sensitized with a saline extract of homologous nerve tissue (2 parts brain and 1 part spinal cord) preserved with 0.1% phenol solution [11]. Before use, sterility of the antigen was tested by seeding on blood agar. Four sensitizing injections, each of 10 mg antigen protein/kg body weight, were given. The antigen was injected into the plantar pad, and the intervals between injections were 6 days. The unanesthetized rabbit was fixed to a frame. A myotatic reflex was evoked by measured stretching of the limb at the ankle joint. The effects of 3 degrees of stretching the muscle were investigated: with weights of 50-100 g (weak), 300-400 g (moderately strong), and 800-1000 g (strong). Potentials were recorded by needle electrodes from the gastrocnemius muscle, with an interelectrode distance of 50 mm. The potentials were amplified by a type UBP1-01 apparatus, and the electromyogram (EMG) was recorded on film by means of a type MPO-2 loop oscillograph (amplification of the order of 5 μV/mm with a film winding speed of 50 mm/sec). Simultaneously, in the course of the investigation, the principal parameters of the EMG (frequency, integral amplitude/sec, and latent period) were determined by means of a modified EMB analyzer. At the beginning of each experiment the threshold of excitability of the proprioceptors was

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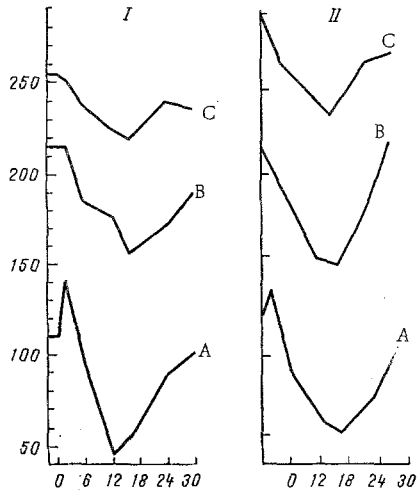

Fig. 1. Dynamics of changes in frequency (I) and integral amplitude (II) of muscle potentials after sensitization with brain tissue antigen. Abscissa, days of observation; ordinate: I) mean number of spikes/sec and II) absolute values of amplitude (in mV). A) Load of 50-100 g; B) load of 300-400 g; C) load of 800-1000 g.

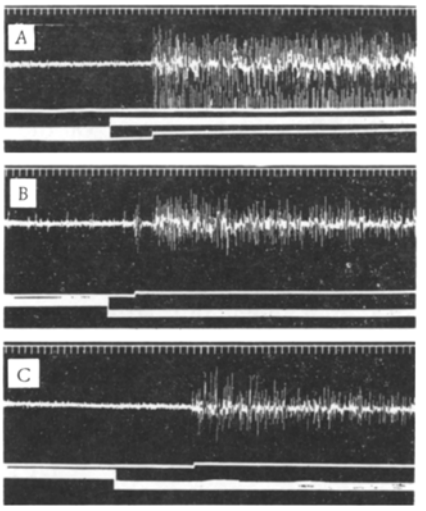

Fig. 2. Electromyograms of myotatic reflexes of rabbit No. 2 during stretching by 300 g. A) EMG before sensitization; B) EMG on 6th day of sensitization; C) on 16th of sensitization. From top to bottom: time marker, 20 msec; muscle potentials; marker of beginning of response; marker of stimulation.

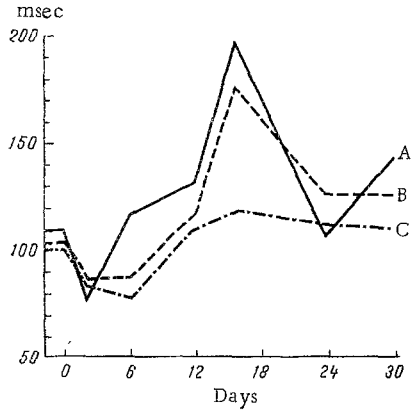

Fig. 3. Dynamics of latent periods of myotatic reflex during sensitization with brain tissue antigen. Abscissa, days of observation; ordinate, duration of latent periods (in msec). A) load of 50-100 g; B) load of 300-400 g; C) load of 800-1000 g.

determined. The threshold value was taken to be stretching causing the appearance of action potentials with amplitude exceeding 15-20 μV.

Circulating antibodies against nerve tissue were determined by Boyden's passive hemagglutination reaction as modified by Ado and Pol'ner [1]. Animals whose antibody titer before the beginning of the experiment did not exceed 1:8 were chosen.

EXPERIMENTAL RESULTS

Analysis of frequency-amplitude characteristics of the EMG of normal rabbits showed that the myotatic reflex is expressed as the appearance of potentials whose frequency and integral amplitude increased with an increase in the degree of stretching. Immunization had a marked effect on the character of the EMG of the experimental animals (Fig. 1). Immediately after a slight increase in the indices on the first days after primary injection of the antigen (principally in response to weak stretching) a marked decrease took place in both frequency and amplitude, reaching a minimum by the 15th or 16th day of the investigation. Toward the end of the observations the amplitude and frequency returned gradually to normal. The changes in response to stronger stretching were similar in character but lesser in degree.

With an increase in the degree of stretching the duration of the latent periods of the myotatic reflexes in normal rabbits was reduced.

Injection of brain tissue antigen shortened the latent periods of the reflexes in the first 3-6 days, and then lengthened them to reach a maximum on the 15th-16th day (Fig. 2). With an increase in the degree of stretching of the muscle, changes in the latent periods became less marked in character (Fig. 3).

Investigation of the threshold of excitability of the proprioceptors in normal animals showed that its value varied within the range 49±7.6 g. During sensitization with homologous brain antigen, considerable changes took place in excitability, expressed on the 6th day as a lowering of the thresholds to 37±5.6 g ($P < 0.05$), followed by an increase. On the 15th-16th day the level of excitability had fallen to 104±20.6 g, i.e., by half ($P < 0.01$). The level of excitability returned to its initial value by the 30th-32nd day of investigation.

Injection of the extracting solution caused no changes in these parameters on the 5th-6th day after injection.

In the passive hemagglutination reaction performed with sera of the experimental animals during sensitization, no correlation was found between the degree of increase in titer of the circulating brain antibodies and the maximal changes in physiological parameters of the nervous system. The highest titers appeared as a rule after the 4th injection of antigen and reached values of 1:32-1:64.

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