CENTRAL NERVOUS ACTIVITY IN GUINEA PIG ANAPHYLAXIS

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IN RECENT years there has been a renewal of interest in the relation of the central nervous system to anaphylaxis. Szentivanyi and his associates[1, 2, 3] reported that bilateral focal lesions of the tuberal region of the hypothalamus produced inhibition of generalized anaphylaxis, local anaphylaxis (Schultz-Dale test), and antibody production in both rabbits and guinea pigs. Freedman and Fenichel[4] reported inhibition of anaphylaxis in the guinea pig with bilaterally symmetrical lesions of the midbrain reticulum at the level of the superior colliculus.

Observation of electrical activity in the central nervous system during anaphylaxis has been limited to studies of surface recordings by Gozzano and Colombati,[5] and by Brandon.[6] The former reported cortical bursts of high potential spikes, "similar to those seen in epilepsy," during generalized anaphylactic shock in rabbits immunized to horse serum and challenged by the intravenous route. They also reported similar activity limited to the local site of application when challenge was by topical application of horse serum to the exposed brain. Brandon[6] observed a variety of electrical phenomena recorded over the cortex and spinal cord of guinea pigs sensitized to horse serum and challenged by either the intravenous or intraperitoneal routes. In the former, he observed high potential spikes followed by depression of activity and exitus, while in the latter the spike activity was followed by periods of slow wave desynchronization intermixed with periods of generalized depression of electrical activity.

As there have been no reports of the electrical activity of subcortical centers to our knowledge, the present study was undertaken to determine if specific electrical phenomena in the brain areas implicated by the extirpation studies cited above could be observed in anaphylaxis.

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This work was supported in part by United States Public Health Service Grants, B-610 and B-1883.

The contents of this artical are the personal views of the Air Force author and are not to be construed as statements of official Air Force policy or as Air Force indorsement of any commercial product described.

Received for publication March 2, 1960.

MATERIAL AND METHODS

Thirty-five young adult albino guinea pigs of both sexes and of an inbred strain,* weighing 500 to 700 grams, were used in these experiments. Eight were passively immunized by the intravenous injection of 0.1 ml. per 100 grams of body weight of a high titer (approximately 4 mg. precipitin Ab protein/c.c.) rabbit anti-BSA (bovine serum albumin) serum. They were challenged twenty-four hours after sensitization by either the intravenous injection of 5 mg. of bovine serum albumin in a 1 per cent aqueous solution or by the intraperitoneal injection of 250 mg. of bovine serum albumin in a 10 per cent aqueous solution.

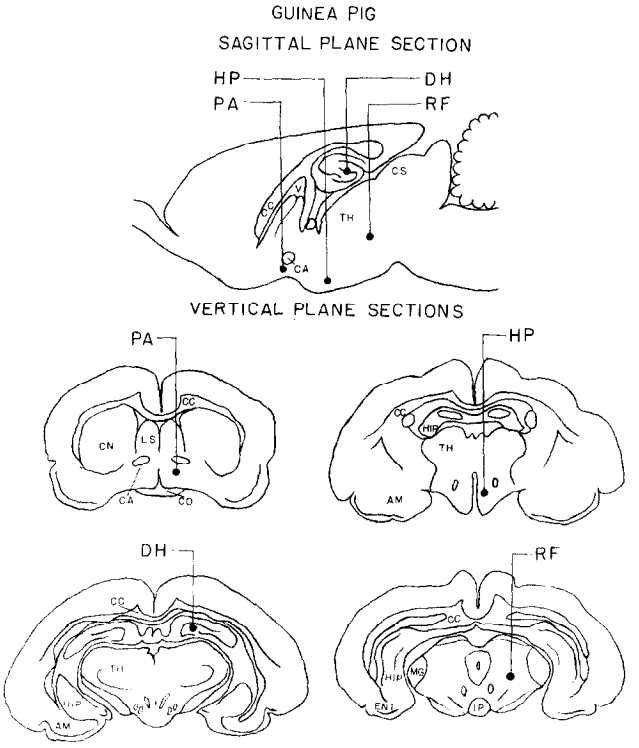

Fig. 1.—Diagrammatic representation of electrode placements in the guinea pig brain. *HP* = Hypothalamus. *DH* = Dorsal hippocampus. *RF* = Reticular formation. *PA* = Preoptic area. *CN* = Caudate nucleus. *CA* = Anterior commissure. *CO* = Optic chiasma. *TH* = Thalamus. *HIP* = Hippocampus. *AM* = Amygdala. *LS* = Lateral septal nuclei. *ENT* = Entorhinal area. *MG* = Medial geniculate. *IP* = Interpeduncular nucleus.

Twenty-three animals were actively immunized by the subcutaneous injection of 2.5 mg. of crystalline egg albumin in 0.5 c.c. of physiologic saline emulsified with 0.5 c.c. of Arlacel-Baylol mixture, after the manner of Freund.[7] These animals were challenged three weeks after immunization, six by the intraperitoneal injection of 500 mg. of egg albumin in a 25 per cent aqueous solution, thirteen by the intravenous injection of 2.5 mg. of egg albumin in a 1 per cent aqueous solution. In this group of animals it was found expedient to give, as a

*Kindly furnished by Dr. Dan H. Campbell, California Institute of Technology, Pasadena, Calif.

"supportive" measure, 2 mg. of Benadryl intravenously fifteen minutes prior to challenge. This dose does not inhibit the development of anaphylaxis, but obviates early death and prolongs the shock state.

Two normal animals were given intravenous injections of histamine phosphate (250 μg histamine base/c.c.) at a dose of 50 μg per kilogram of body weight.

Two normal animals were subjected to asphyxia by suspension of artificial respiration.

Light ether anesthesia was used during tracheal cannulation and exposure of the brain. Operative sites and pressure points were infiltrated with 1 per cent procaine hydrochloride. Immobilization was secured with a single intraperitoneal injection of D-tubocurarine at a dose of 0.4 mg. per kilogram. Artificial respiration was carried on throughout the remainder of the experiment.

Prior to the investigation, a stereotaxic atlas of the guinea pig brain was prepared by one of us (C. W. D.). Using a slightly modified Johnson stereotaxic instrument, precise localization of concentric 1 mm. diameter steel electrodes in the brain was obtained. Bipolar records of central nervous activity were recorded, using a Grass Model IV electroencephalographic instrument. At the conclusion of all experiments, the brain was removed and precise disposition of the electrode tracks subsequently determined from serial Nissl sections, cut frozen at a thickness of 50 microns.

The regions studied included the cerebral cortex, hypothalamus, preoptic area, dorsal hippocampus, and reticular formation (Fig. 1). A unipolar electrocardiogram (ECG) was recorded concomitantly.

RESULTS

Benadryl caused a transient increase in the excitability of all centers studied without significant change in the ECG. Within a maximum of ten minutes, the electroencephalogram (EEG) returned to normal and nervous activity resembled that observed in the control records.

In all animals tested, a marked biological variation in the nervous response during anaphylaxis was observed. The most common finding after intravenous injection of antigen was the development, within three to six minutes, of high amplitude spike activity in the dorsal hippocampus and in the preoptic and hypothalamic areas, with a concomitant flattening of the background activity in all regions, especially in the dorsal hippocampus (Fig. 2, *B*). For the next five to ten minutes the background activity became further depressed and the incidence of spiking more random (Fig. 2, *C*). No significant ECG changes were observed during this time. After this period, a gradual return of nervous activity was recorded, but recovery was never complete (Fig. 2, *D*). It was at this time that ECG changes became pronounced, beginning at first with flattening of the T wave and changes in the S-T segment. These changes were followed by prolongation of the P-R interval, progressed into various types of heart block and conduction defects, and finally to complete disorganization of the ECG pattern. As the ECG pattern became more chaotic, spiking was again

seen in the dorsal hippocampus (Fig. 2, *E*), and to a lesser extent in the reticular formation. Quite often spiking was also observed in the hypothalamic, preoptic, and cerebral cortical regions. In some experiments, the EEG and ECG changes described above would be repeated, and if Adrenalin (0.05 c.c. of a 1:1,000 solution) was administered intravenously before irreversible cardiac deterioration, a normal ECG would result and at the same time nervous activity would assume a near normal pattern.

A few animals were quite resistant to anaphylaxis (Fig. 3). In these it was observed that the dorsal hippocampus appeared to be the most affected structure. Long trains of regular 4 to 6/second waves, often observed in the hippocampus under normal conditions, were replaced after fifteen to twenty minutes after intravenous challenge by much shorter trains of regular waves interspersed with desynchronized activity (Fig. 3, *B*). At this time the ECG showed early changes. This state of nervous activity was maintained for very long periods without any deterioration in the ECG. If a second injection of antigen was administered thirty to forty minutes after the initial challenge, the dorsal hippocampus activity became irregular and further changes in the ECG became evident shortly thereafter (Fig. 3, *C*). As the ECG became more and more markedly abnormal, the nervous activity became depressed with simultaneous spiking in the cerebral cortex, hypothalamus, and dorsal hippocampus (Fig. 3, *D* and *E*). As in all other experiments, the dorsal hippocampus was the first to become depressed.

Other signs of abnormal nervous activity observed in anaphylaxis were the appearance of high amplitude slow waves broken at random by spike formations in the cerebral cortex, dorsal hippocampus, and hypothalamic and preoptic areas. During the depressed state, regular 4/second slow waves were often observed in the dorsal hippocampus. These waves gradually lost amplitude as the period of complete depression of nervous activity approached. Throughout these studies, the reticular formation appeared to be the least affected of the regions studied.

The EEG and ECG effects noted above in actively sensitized animals during anaphylaxis were also observed in those passively sensitized.

The same sequence of events was observed when the intraperitoneal route was employed for challenge injections in either actively or passively sensitized animals. However, after intraperitoneal injection, anaphylactic signs did not develop for from forty to eighty minutes and the EEG and ECG effects described above were distributed over a much longer period. Due to the irregularity and delay in time of onset of anaphylaxis, this route was abandoned in favor of the intravenous route plus Benadryl support as the most expedient method of experimentation.

Since it is well established that histamine release plays a large role in the production of anaphylactic symptoms in the guinea pig and that many, if not all, of the symptoms and signs of anaphylaxis can be produced in this species by injection of histamine, the EEG and ECG effects of intravenous histamine were recorded in normal guinea pigs as additional observations. In both instances, three to five seconds after injection, marked ECG changes were observed (Fig. 4, *B*), although very little modification in nervous activity was observed

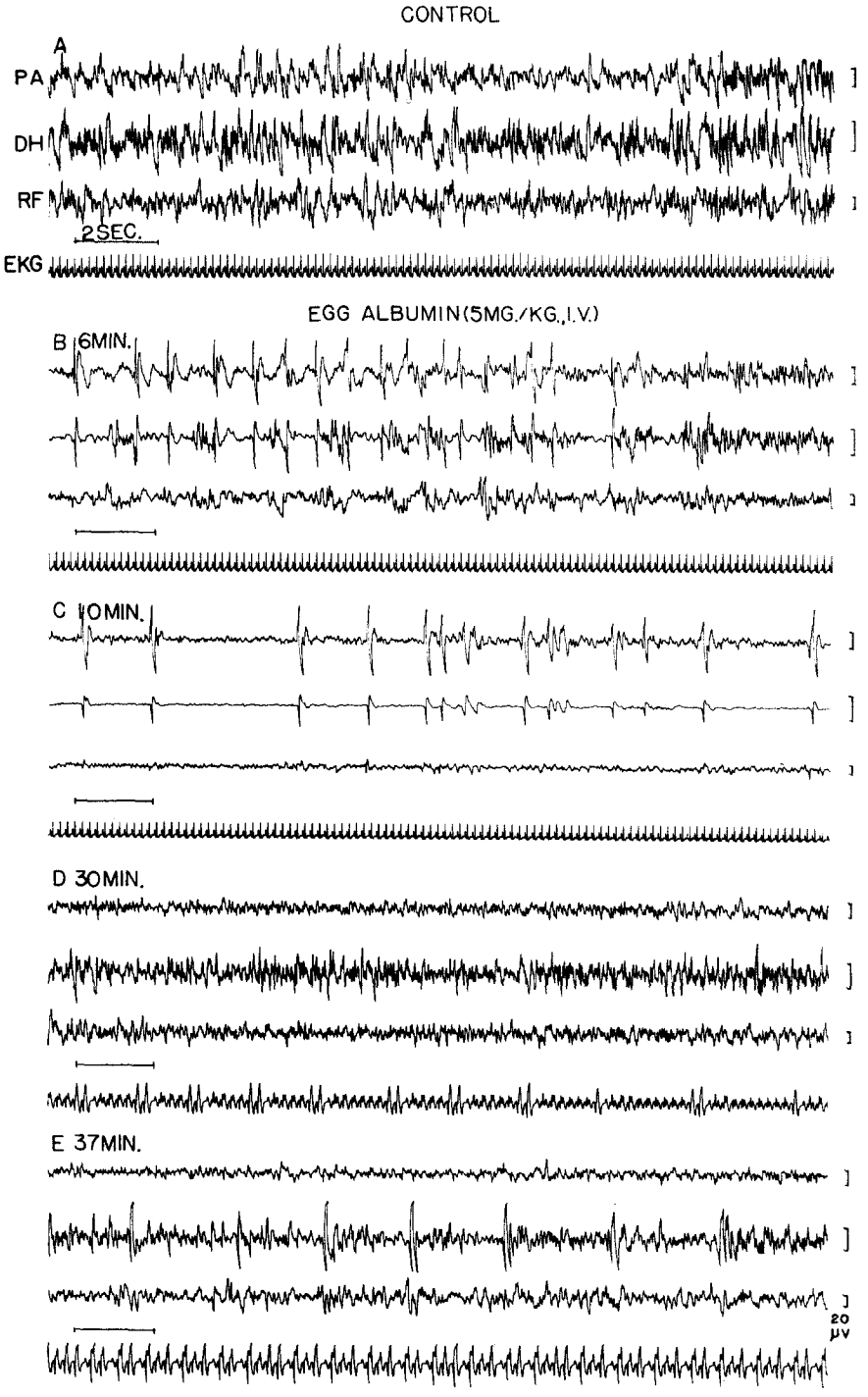

Fig. 2.—EEG and ECG recordings during typical anaphylaxis. *A*, control. *B*, *C*, *D*, *E*—6, 10, 30, and 37 min., respectively, after challenge. *PA* = Preoptic area. *DH* = Dorsal hippocampus. *RF* = Reticular formation.

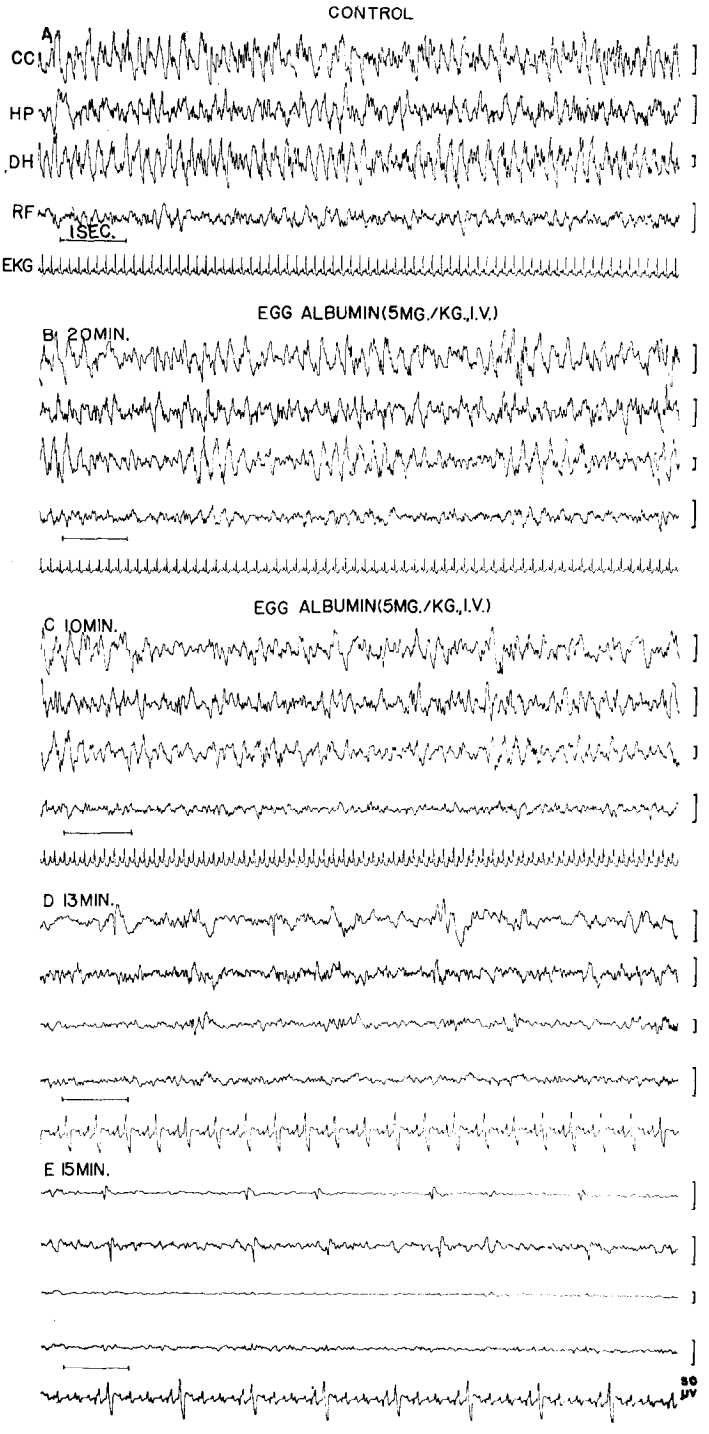

Fig. 3.—EEG and ECG recording during anaphylaxis in a resistant guinea pig. *A*, Control. *B*, 20 min. after initial challenge. *C*, *D*, *E*—10, 13, and 15 min., respectively, after a second injection given 30 minutes after the initial challenge. *CC* = Cerebral cortex. *HP* = Hypothalamus. *DH* = Dorsal hippocampus. *RF* = Reticular formation.

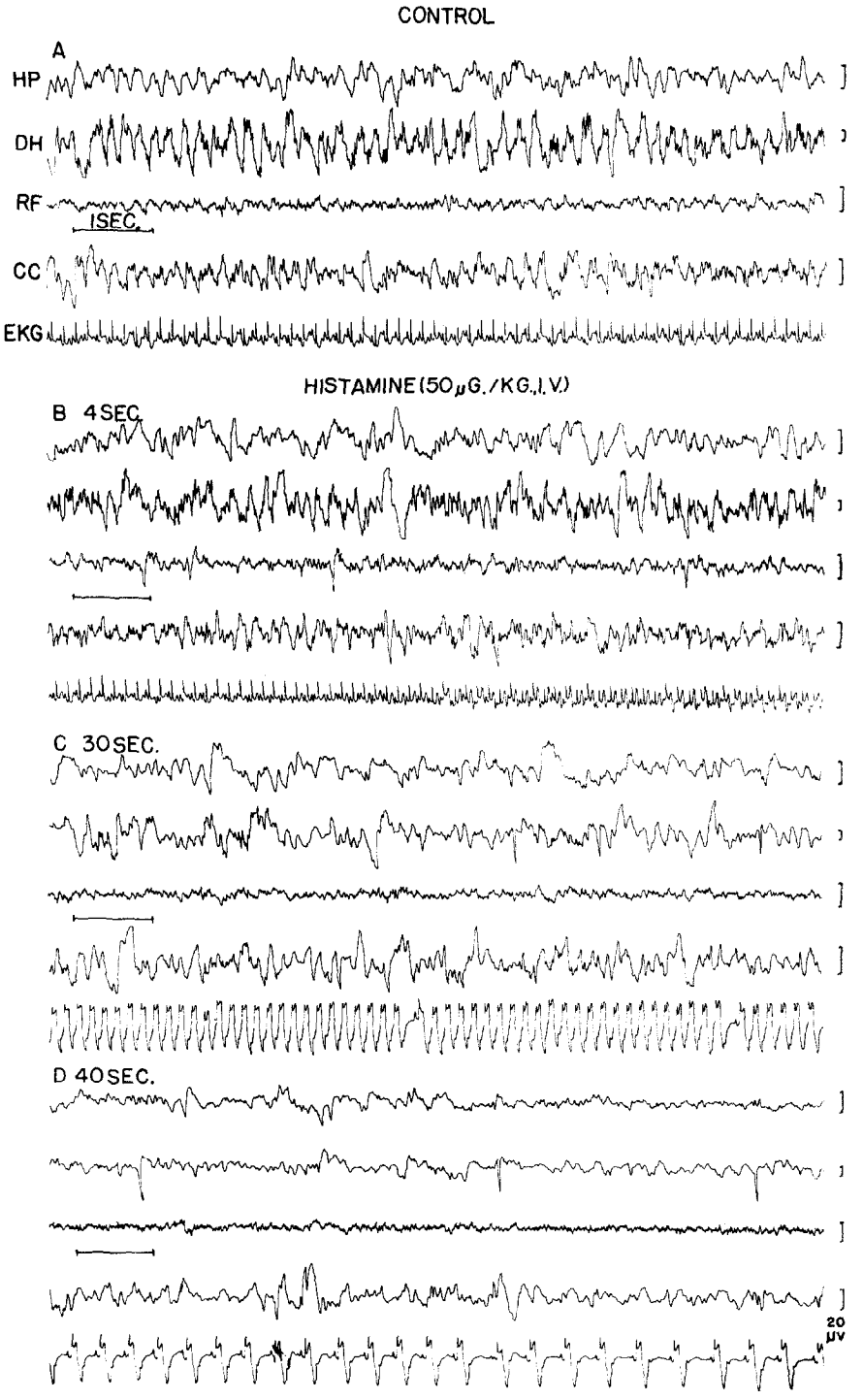

Fig. 4.—EEG and ECG recordings in a normal guinea pig after histamine injection. *A*. Control. *B*, *C*, *D*—4, 30, and 40 sec., respectively, after injection. *HP* = Hypothalamus. *DH* = Dorsal hippocampus. *RF* = Reticular formation. *CC* = Cerebral cortex.

until twenty-five to thirty seconds had elapsed (Fig. 4, *C*). At this time, the EEG started to become depressed with some spike activity evident in the hippocampus. The depressed state of the EEG was maintained for the next ten to twenty seconds (Fig. 4, *D*) and this was followed by gradual recovery with a return to the normal EEG and ECG occurring a short while later. With the exception that the ECG changes occurred before the changes in nervous activity and that the changes occurred more rapidly and with more rapid recovery, the results were very similar to those observed in anaphylaxis.

The marked bronchospasm produced by anaphylaxis and induced by histamine so readily in the guinea pig leads to ventilatory insufficiency and decreased blood oxygen saturation. As central nervous activity is readily modified by decreased oxygenation, the effects of simple asphyxia on nervous activity and the ECG were also observed. The changes recorded in both cases were very similar to those seen during histamine administration (Fig. 5) and (with the same exceptions noted above in the case of histamine) with those observed in anaphylaxis. As in the histamine experiments, ECG changes preceded those in the EEG. No modifications in nervous activity occurred until the heart rate had slowed to approximately 50 per cent of control values, at which time depression of nervous activity was observed (Fig. 5, *C*). Thereafter, both EEG and ECG activity deteriorated rapidly (Fig. 5, *D*).

In these four animals there was little or no biologic variability observed in the responses of the central nervous system.

DISCUSSION

The EEG effects observed in these studies concur in the main with those observed by Brandon.[6] We cannot, however, unreservedly agree with his conclusion that these represent specific physiochemical changes in or on the nerve cell proper induced by the antigen-antibody reaction. Local anaphylaxis as demonstrated in isolated guinea pig gut or uterus in the Schultz-Dale technique has long been cited as evidence for a specific cellular response to an immunologic anaphylactic stimulus, unmediated by humoral factors or total generalized physiologic response. However, in the study of central nervous activity in the intact animal, many problems arise in the differentiation of primary neurocellular effects from secondary phenomena dependent on total body economy. Central nervous activity has been shown to be dependent on many homeostatic mechanisms including oxygen and carbon dioxide equilibrium, circulatory stability, electrolyte balance, etc. Anaphylaxis produces profound changes in these factors, and it is, therefore, difficult to ascribe the changes in central nervous activity described above to neural anaphylaxis per se.

No clear-cut differences in pattern appear to exist between the changes observed in anaphylaxis, histamine injection, and asphyxia, the differences being of degree, duration, and time of onset in relation to ECG effects. Although the EEG changes appear before those of the ECG in anaphylaxis, and afterward in histamine and asphyxia experiments, this need not imply a specific antigen-antibody effect on the nerve cell. Histamine, various enzymes, and other

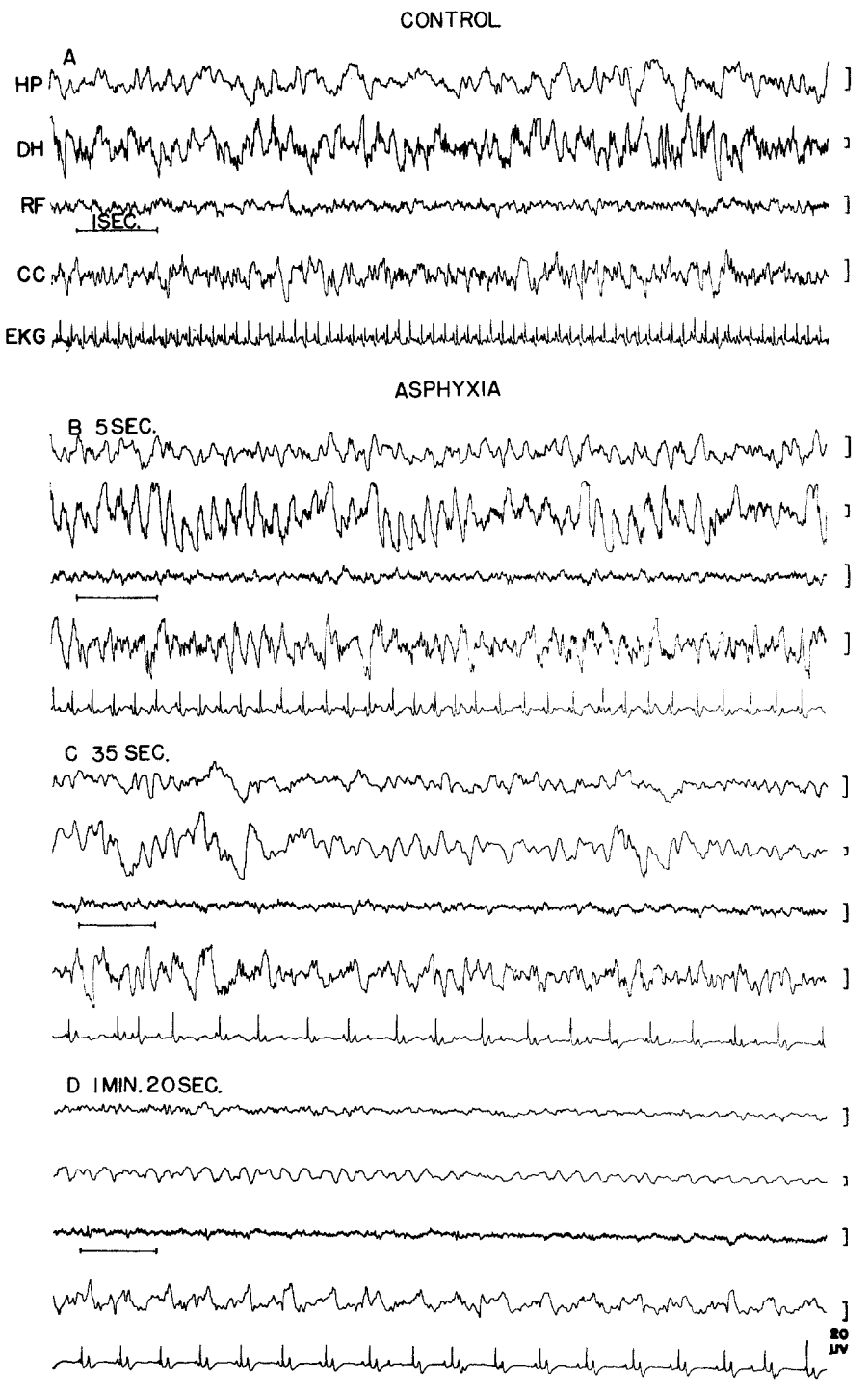

Fig. 5.—EEG and ECG recordings in normal guinea pig during asphyxia. *A*, Control. *B*, *C*, *D*—5, 35, and 80 sec., respectively, after suspension of artificial respiration. *HP* = Hypothalamus. *DH* = Dorsal hippocampus. *RF* = Reticular formation. *CC* = Cerebral cortex.

mediators are released, theoretically at all levels in the vascular tree during anaphylaxis. The early appearance of EEG changes may well represent central nervous reaction to release of these substances in the local blood supply, or to local vascular effects secondary to their release as conditioned by the high degree of sensitivity of neural tissue.

Additional studies will be needed to clarify the role of such physiologic changes as decreased oxygenation, decreased blood pressure, increased hematocrit and decreased blood volume, extracellular electrolyte shifts, and circulatory failure in the production of central nervous effects, with or without anaphylaxis. Pharmacologic studies of the effects of enzymes and mediators released in anaphylaxis on central nervous activity will also be needed to clarify the role of neuroanaphylaxis per se on the observed central nervous activity effects.

The area studied showing the highest reactivity in these studies, the dorsal hippocampus, is known to have an unstable blood supply and to be sensitive to factors affecting this supply.[8, 9] It exhibits high levels of reactivity and instability in general. The converse is true for the level showing the least reactivity, the midbrain reticulum, for this is known to be a very stable area.[10-14] Therefore, these regional differences in reactivity observed in anaphylaxis, histamine injection, and asphyxia appear to reflect pre-existing factors rather than any specific shift in reactivity related to the anaphylactic state itself.

In contradistinction to the extirpation experiments previously cited, we have been unable to demonstrate any areas of specific anaphylactic reactivity in the central nervous system, perhaps because the hypothalamus and the midbrain reticulum may not be primary centers of elaboration of anaphylactic response, but simply important afferent and efferent pathways for the complex system of neurovegetative and adaptive reflexes involved in those responses.

It should be emphasized that species specificities play an important role in neurophysiology in general and in anaphylaxis in particular. It would be of particular interest to corroborate the present EEG findings in a species, such as the rat, exhibiting less dramatic bronchospasm or disturbances of total body economy in anaphylaxis. Certainly caution is necessary in extrapolation of data in the guinea pig to other species.

SUMMARY

1. The changes in central nervous activity observed in the guinea pig during anaphylaxis, histamine injection, and asphyxia were characterized by high amplitude spike activity, flattening of background activity, desynchronization, and high amplitude slow waves.

2. Pronounced disturbances in electrical activity are recorded in the cerebral cortex, dorsal hippocampus, preoptic area, and hypothalamus. These occur to a markedly lesser extent in the midbrain reticulum.

3. These disturbances in electrical activity indicate that irritative mechanisms exist in the central nervous system during anaphylaxis.

4. The data suggest that these irritative mechanisms are not confined to specific regions in the central nervous system and may be the result of generalized phenomena.

The authors are indebted to Professor W. Ross Adey, M.D., Department of Anatomy, for facilities extended and critical review of the manuscript.

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