

## Cerebral Reactions during Intrauterine Asphyxia in the Sheep. II. Evoked Electroencephalogram Responses

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### *Extract*

Somatosensory evoked electroencephalogram (EEG) responses (SER) were recorded in 19 fetal lambs with gestational ages varying from 66 to 140 days. The ewes were anesthetized with chloralose and the fetuses were exteriorized with intact umbilical circulation. Periods of hypoxia at different pH levels were induced by ventilating the ewe with gas mixtures including 8-15% oxygen, either alone or combined with a 10% CO<sub>2</sub> mixture, or with an intravenous infusion of bicarbonate to the fetus.

The reaction of the SER during hypoxia followed two main patterns, one seen in the immature fetus and one in the more mature fetus. In the immature type of response (seen in all fetuses at or less than 110 days of gestational age) the primary response of the SER is more sensitive to oxygen lack than the secondary components, whereas in the mature type of response (seen at or more than 115 days of gestational age) the reverse is true.

At moderate degrees of hypoxia, Pa<sub>O<sub>2</sub></sub> between 15 and 20 mm Hg, the SER were moderately affected provided that the pH of the blood was within normal limits. At lower Pa<sub>O<sub>2</sub></sub> values, and when moderate hypoxia was combined with acidosis, the SER were seriously affected or completely abolished. At Pa<sub>O<sub>2</sub></sub> values above 20 mm Hg an acidosis had no effect on the SER.

### *Speculation*

The function of the fetal brain is seriously jeopardized by the combination of hypoxia and acidosis. Therefore, both hypoxia and acidosis should be counteracted during asphyxia to prevent brain damage.

### *Introduction*

In certain conditions one of the few methods available for studies of brain function is that of evoked EEG responses (ER). Some promising results have already been presented. For example, Brierley *et al.* and associates [3, 9] found in rhesus monkeys with experimental profound arterial hypotension that somatosensory ER

changes correlated closely with the degree of brain damage and the severity of the metabolic deficit.

The same authors demonstrated the superiority of ER recordings over recordings of spontaneous EEG for the evaluation of the functional state of the brain in some situations which involve brain damage.

Using dogs, Lee *et al.* [8] elicited a consistent and gradual increase in latency and amplitude of certain

components of visual ER by a decrease of serum sodium, chloride, and osmolality levels.

The influence of hypoxia and anoxia on ER has been studied in different animal species (cat, monkey). In all experiments the primary component of the ER exhibited the greatest resistance to anoxia, whereas the later components were more vulnerable [1, 2, 11].

It must be emphasized that all the animals studied in the above investigations were adult. Our experiences with human neonates during hypoxia (idiopathic respiratory distress syndrome) and after asphyxia yielded the opposite result: the primary components of visual as well as of somatosensory ER were less resistant to hypoxia than the secondary components [5].

Evoked responses develop pre- and postnatally in all species in a characteristic manner. The development of somatosensory ER in fetal lambs was described by Molliver [10]. As in other species, there is increasing complexity and decreasing latency of the response with increasing maturity.

The present study was undertaken to use the ER technique in the fetal lamb in order to test whether this aspect of brain function would be affected at a certain reproducible degree of hypoxia, and whether other environmental influences, such as acidosis, would modify the cerebral response to hypoxia.

#### Methods

The experiments were conducted on 15 ewes of mixed breed with 19 fetuses ranging from 66 to 140 days of estimated gestational age. Complete experiments with both blood gas determinations and recordings of somatosensory evoked responses were obtained on 15 fetuses, between 94 and 140 days of gestational age. The gestational age was estimated from fetal weight and crown-rump length using standard curves [6].

The ewes were anesthetized with pentothal (5 mg/kg body wt) followed by chloralose (30 mg/kg body wt). The ewes were tracheotomized and ventilated with known gas mixtures, using an open circuit respirator. Maternal blood pressure was recorded through a catheter in the medial plantar artery of one foreleg. The same catheter was used for arterial blood sampling. The fetus was delivered onto a heated small table with intact umbilical circulation. Fetal arterial blood pressure and heart rate were recorded from a catheter in the right brachial artery. The same catheter was used for blood sampling.

Periods of hypoxia were induced by ventilating the ewe with gas mixtures with 8–15% oxygen. Different

pH levels were achieved by adding extra CO<sub>2</sub> to the gas mixture or by giving an intravenous infusion of bicarbonate to the fetus.

A more detailed description of the preparation and the procedures is given in the accompanying paper [7].

Evoked EEG responses were recorded from the surface of the intact scalp. Cup electrodes were fixed over the part corresponding to the suprasylvian gyrus. The indifferent electrode was fixed at the posterior part of the head. At the beginning of the experimental series it was confirmed that the responses recorded from the scalp were similar to those recorded directly from the exposed cortex. The procedure of registration from the intact head precluded the damage to the brain that might otherwise occur in the course of the experiments, with a duration of several hours. The EEG was monitored on a Grass polygraph *model 7B* [13], with an ink recorder. The data were also stored on a Philips FM tape recorder [14]. For averaging, a PDP 12 digital computer [15] was used. Electric stimulation was carried out with a battery-operated stimulator, with lead stimulation electrodes, placed 5 mm apart on the muzzle close to the nostrils. The stimulus was a rectangular pulse of 1-ms duration and of about 10 V.

#### Results

The maturity of the fetal lamb determines the shape, the complexity, and the latency period of the SER. Figure 1 shows a few representative examples of the development of the SER at increasing degrees of maturity. It should be noted that the complete response

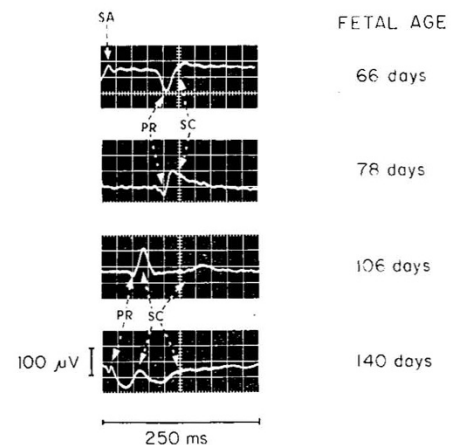


Fig. 1. The development of somatosensory evoked electrocephalogram responses in fetal lambs from 66 to 140 days of gestational age. Primary responses (PR) and secondary components (SC) are marked. SA: stimulation artifact.

covers a long time period, so that a recording time of 250–500 ms is often necessary to register the complete set of slow, secondary components. As is apparent from Figure 1 increasing maturity leads to a shorter latency period and an increased complexity, with a higher frequency pattern of the response.

The different components of the SER were separated according to several criteria, one of the most important being the localization. The primary, specific response was in every experiment most distinctly recorded in the corresponding ipsilateral projection area, and in several instances present in the ipsilateral area exclusively. The secondary, nonspecific components were recorded from both hemispheres.

Hypoxia affected the SER profoundly when the oxygen tension of the fetal arterial blood was reduced below 20 mm Hg. The first reaction of the SER was a decrease in amplitude. In the most severe hypoxia, the whole SER was abolished. When the hypoxia was discontinued the SER returned gradually during the following 30–60 min, except for those fetuses in which

circulatory collapse occurred during the hypoxic period.

Hypoxia also affected the spontaneous EEG pattern recorded. However, this will be left out of the analysis since in several cases, particularly in the more immature fetuses, distinct SER continued to be recorded even though no spontaneous electrical activity, or only an amorphously irregular activity, was observed. Furthermore, it was repeatedly observed that, in cases with a distinct spontaneous electrical activity, this was preserved long after the SER demonstrated marked alterations.

The reaction of the SER during hypoxia followed two main patterns, one seen in the immature fetus and one in the more mature fetus.

An example of the response of the immature fetus is shown in Figure 2, *left panel*. The primary part of the SER proves to be more sensitive to lack of oxygen and decreases in amplitude and vanishes earlier than the secondary components. When, after the complete response is abolished, the hypoxia is discontinued, the

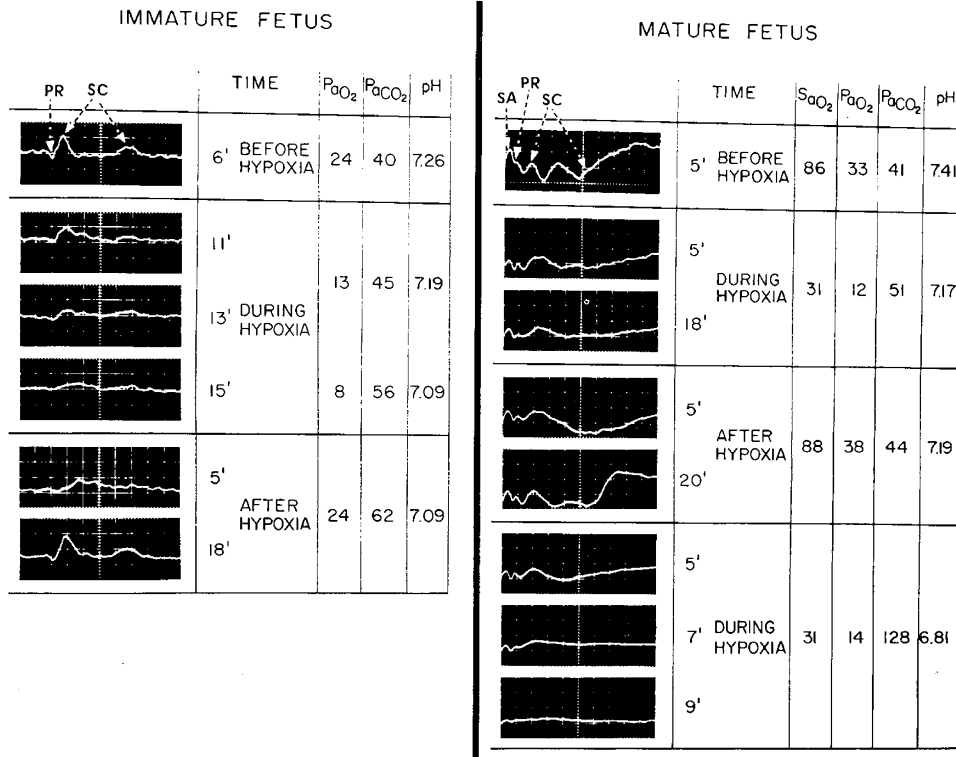


Fig. 2. The reactions of somatosensory evoked electroencephalogram responses (SER) to hypoxia. The *left panel* shows from an immature fetal lamb with an estimated gestational age of 106 days. Note that the primary response (PR) is more sensitive to hypoxia than the secondary components (SC). The *right panel* shows the reactions to two periods of hypoxia of the SER from a mature fetal lamb with an estimated gestational age of 125 days. Note that in this example the primary response is more resistant to hypoxia than the secondary complex. Calibration is as in Figure 1. In the responses from the mature fetus a stimulation artifact (SA) is present.

components of the SER return in the reverse order: first the secondary components (at 5 min) and later the primary response (at 18 min).

In the mature lamb fetuses the reverse type of response was observed. Figure 2, *right panel*, gives an example of a fetus of 125 days of gestational age. Here the primary response is more resistant to hypoxia and is affected first when the secondary components have already been extinguished. When the hypoxia is relieved the primary response reappears earlier than the secondary components.

In 14 experiments, the hypoxia was severe enough to extinguish the complete SER. In these experiments the sensitivity of the different components of the response was compared. Figure 3 gives a schematic comparison between the sensitivity of the primary and the secondary components to hypoxia for different degrees of maturity. Note that all instances at or under 110 days of gestational age demonstrate the immature type of response, whereas fetuses over 115 days show the mature response.

To grade the influence of hypoxia on the SER, all responses were classified in terms of the decrease, from the control state of the amplitude of the most sensitive component.

At moderate degrees of hypoxia, at  $P_{aO_2}$  15–20 mm Hg, the SER were moderately affected provided the pH of the blood was within normal limits. At lower  $P_{aO_2}$  values, or when moderate hypoxia was combined with acidosis, the SER were seriously affected or completely abolished. Figure 4 is a graphic representation of the relation between  $P_{aO_2}$ , pH and the SER. At  $P_{aO_2}$  values above 20 mm Hg, acidosis had no effect on the SER.

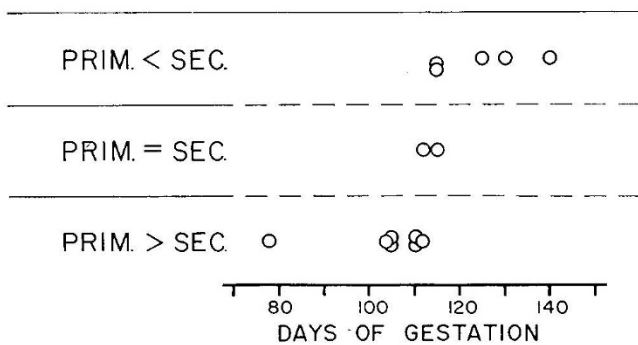


Fig. 3. A graphic demonstration of the relation between fetal age and the sensitivity to hypoxia of primary *versus* secondary complex of somatosensory evoked electroencephalogram responses. At a low gestational age the primary (*PRIM.*) response is more sensitive than the secondary (*SEC.*) response.

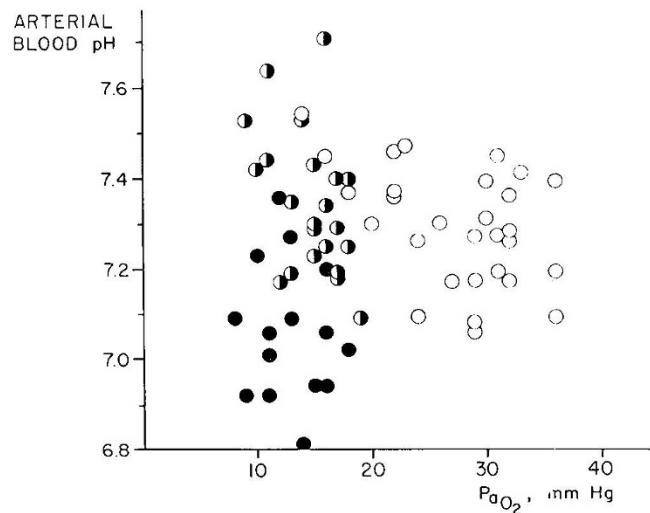


Fig. 4. The effect of varying arterial pH at different levels of hypoxia on somatosensory evoked electroencephalogram responses (SER). ○: SER not affected; ◐: SER distinctly affected (amplitude decreased to more than 50% of control); ●: SER completely extinguished.

Discussion

The development of SER in the fetal lamb during maturation has been described in detail by Molliver [10]. Our results demonstrate a close resemblance to his (Fig. 1), although the present results were obtained via scalp electrodes, fixed to the skin, and not by the direct cortical recording technique. Our results contain more information about the late components than Molliver's, since we used the longer recording period of 250 ms.

Our results corroborate earlier views of the advantage of using ER over spontaneous EEG [3, 9] in studies of this type. This is particularly true in the immature fetuses where spontaneous electric activity from the brain is sparse or absent.

The marked vulnerability of the primary component of the SER in comparison with secondary components in the immature fetus (Figs. 2 and 3) has not been described previously, but corresponds to the pattern seen in the preterm and full term human baby [5]. At 110–115 days of gestational age, corresponding to some 75% of the total fetal period, the response turns into the mature type (Figs. 2 and 3) described previously in adult animals of different species [1, 2, 11]. This finding is in agreement with the view of the newborn lamb as a neurologically mature creature.

The evolution of changes in the SER at increasing degrees of hypoxia follows a reproducible pattern. At normal or increased pH levels, the SER is unaffected

down to a  $P_{O_2}$  in the arterial blood of 20 mm Hg, *i.e.* the lower limit of normality (Table I, Reference 7). Below this limit the SER are distinctly altered with a reduction of the amplitude to below 50% of the control value. When the  $P_{a_{O_2}}$  is reduced below a level of about 12–15 mm Hg the SER are completely abolished. If acidosis is added to the hypoxia the SER are affected at higher  $P_{O_2}$  values and are already extinguished at  $P_{a_{O_2}}$  as high as 18 mm Hg (Fig. 4). At normal oxygen tensions, on the other hand, a change of pH between 7.4 and 7.06 does not provoke any changes of the SER.

If the influence of hypoxia on the SER depends on the amount of oxygen delivered to the brain, rather than on the partial pressure of oxygen in the tissue, the combined effect of hypoxia and acidosis might be due to the shift of the hemoglobin dissociation curve with pH, the Bohr effect. The mature fetus in Figure 2 demonstrates the magnitude of the Bohr effect in one specific case. During the first period of hypoxia pH was only moderately reduced and the  $P_{O_2}$  of 12 mm Hg corresponded to a saturation of 31%. In the second hypoxic period an acidosis was added and exactly the same oxygen saturation was achieved, but now at a  $P_{O_2}$  of 14 mm Hg. Thus, the Bohr effect contributes to a moderate extent only in explaining the detrimental effect on the SER of the combination of hypoxia and acidosis. Instead, it appears reasonable to combine this item of information with the knowledge gained from studies on the metabolic rate of the brain in lamb fetuses [7]. Here, it was demonstrated that the oxygen consumption of the brain was reduced significantly more when hypoxia was combined with an acidosis than when hypoxia occurred at normal pH levels. Thus, the combination of acidosis and hypoxia might be deleterious to the electric activity of the brain by interfering with the energy metabolism. This view is supported by the findings in rats that the energy state of the tissue, "the energy charge potential," is reduced at significantly higher oxygen tensions when ischemic hypoxia is combined with a respiratory acidosis than when the ischemia is induced at normal pH levels [4].

The present findings are also of interest in the light of the report of Windle *et al.* [12] that the newborn monkey develops less morphologic signs of brain damage when experimental asphyxia is combined with alkalinizing treatment than when acidosis is allowed to develop in connection with the hypoxia.

Thus, various experimental series using different animal species and different techniques to evaluate the impact of asphyxia on the brain appear unanimous on

one point; the combination of hypoxia and acidosis is more dangerous for the brain than hypoxia alone.

#### Summary

In an attempt to analyze factors responsible for the brain damage during intrauterine asphyxia, SER were studied in the fetal lamb to test whether this aspect of cerebral function would be affected at a certain reproducible degree of hypoxia and whether other environmental influences, such as acidosis, would modify the cerebral response to hypoxia.

Nineteen fetal lambs, ranging from 66 to 140 days of gestational age, were studied in the acute, exteriorized preparation. Hypoxia was induced by ventilating the ewe with appropriate gas mixtures. Acidosis or alkalosis were produced by a  $CO_2$ -rich gas mixture to the ewe or by an infusion of alkali into the fetus.

The SER of immature lamb fetuses (less than 110 days of gestational age) responded to hypoxia in the same manner as the SER from human neonates; the primary response of the SER complex was more sensitive to lack of oxygen than were the secondary components. More mature lamb fetuses (over 115 days of gestational age) demonstrated the opposite response pattern, with the primary response more resistant to hypoxia than the secondary components, as is the case for adult animals of various species.

When  $P_{a_{O_2}}$  was diminished at normal or high pH levels, the SER were affected below 20 mm Hg but were abolished first at severe degrees of hypoxia. When an acidosis was superimposed, however, the SER were abolished at even moderate degrees of hypoxia.

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