cells separated by this method, clear-cut information can be obtained in this way about the directions of age-related changes in the erythrocytes. Moreover, valid comparisons can be made between various sets of experiments performed under standard conditions of cell fractionation.

The levels of the main cations in different density fractions of bovine erythrocytes are shown in the table. As in other mammalian species[1-6], the K^+ content decreased gradually with increasing cell density and the Na^+ content showed an increase, though of smaller magnitude than the K^+ loss. In contrast to results obtained for human erythrocytes fractionated according to Murphy[8] (but not by other means[10]) the Mg^{2+} content was found to decrease progressively. The K^+ loss shown by these data is apparently reminiscent of the phenomenon of K^+ loss induced by oxidative stress[11], and may be due either to an increased passive K^+ permeability of the erythrocyte membrane, or the cell age-related modifications in ATPase properties.

The changes in cation content between the heaviest and lightest cell fractions [6]-[1] were: -2.2 moles/moles hemoglobin for K^+, -0.09 moles/moles hemoglobin for Mg^{2+} and $+0.7$ moles/moles hemoglobin for Na^+, equivalent to a net cation loss of 1.6 moles/moles hemoglobin. This cation loss should be accompanied by an equivalent anion loss to secure electroneutrality of the cell interior. Therefore the net decrease in the electrolyte content, approximated by changes in the concentrations of 3 main cell cations from fraction [1] to [6]: $((K^+ + Na^+ + Mg^{2+})_{[1]} - (K^+ + Na^+ + Mg^{2+})_{[6]})/(K^+ + Na^+ + Mg^{2+})_{[1]}$ would be $5.5 \pm 0.9\%$ of the cation content of fraction 1. In this set of experiments, increase in the mean cell hemoglobin concentration between fractions 1 and 6 was $6.2 \pm 1.3\%$. It thus seems that the shrinking of senescent bovine erythrocytes can be accounted for by the decrease in the electrolyte content. On the other hand, if one were to assume that the increase in microviscosity of the bovine erythrocytes interior, estimated[7] with the Tempamine spin probe, from fraction [1] to [6], is due only to concentration changes of intracellular solutes, the increase should be about 8.7%. (The separation efficiency did not differ between both sets of experiments, as judged from the magnitude of increase in the mean cell hemoglobin concentration.) Therefore, other factors apart from the electrolyte loss must contribute to the increased microviscosity of senescent erythrocytes. This is understandable, taking into account the progressive elevation in the microviscosity of the cell interior during incubation of extravasated erythrocytes which is not accompanied by significant changes in the electrolyte content.

Relative cation content of different density (age) fractions of bovine erythrocytes (percent of values found in the fraction of lightest cells)

Fraction No.	K^+	Na^+	Mg^{2+}
1	100	100	100
2	$89.5 \pm 6.8\%$	$101.1 \pm 1.5\%$	$90.6 \pm 5.5\%$
3	$83.5 \pm 6.9\%$	$102.0 \pm 1.6\%$	$87.0 \pm 7.0\%$
4	$76.9 \pm 8.8\%$	$101.8 \pm 1.1\%$	$82.8 \pm 5.8\%$
5	$74.9 \pm 7.3\%$	$103.0 \pm 3.5\%$	$77.4 \pm 7.1\%$
6	$67.5 \pm 8.8\%$	$103.2 \pm 1.3\%$	$73.3 \pm 7.4\%$

Absolute values for fraction 1: $K^+ - 6.7 \pm 0.6$; $Na^+ - 22.1 \pm 1.6$; $Mg^{2+} - 0.35 \pm 0.09$ moles/moles hemoglobin (mean $\pm$ SD; $n = 6$).

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A follow-up electrophysiological study of rats with poor intrauterine fetal growth: the development of visual evoked responses (VERs)[1]

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Summary. The development of some electrophysiological activities of the visual system (VERs) was compared in control rats and in young rats with poor intrauterine fetal growth caused by an electrolytic lesion of the placenta. Treated rats showed a delayed development of the electrophysiological functions considered, thus confirming the postnatal effect of poor intrauterine fetal growth.

The influence of nutrition on the development of the brain in the rat before and after birth has been studied by quite a large number of researchers. What we propose in the present paper is an investigation of this problem performed in young rats born after inducing placental insufficiency in dams by an electrolytic lesion.

Other authors have reported data about the changes in VERs of developing rats following starvation and other restricted conditions; this technique appeared to be suitable for the investigation of the effects of poor intrauterine fetal growth on the development of the visual system[3-5]. When investigating the visual system, most studies are on VERs to low frequency stimulation (transient VER), fewer deal with high frequency stimulation (steady-state VER).

The transient VER, by averaging, supplies some basic data which are the latency of the response and the main features of the response itself. Both are age-related. The latency decreases in the first 5-6 weeks and the shape of the response becomes more and more complete in the 1st month of life. These changes take place at fixed ages in Sprague-Dawley rats. The development of the transient VER, moreover, has been demonstrated to be related to the critical periods of intense protein and DNA synthesis and largely dependent on myelination[6].

When the sinusoidal response to high frequency stimuli (steady-state VER) is investigated by frequency analysis according to recent biophysical assessments[7], one may obtain interesting data on the development of the evoked activity in the range of the after-discharge (8–12 Hz). This rhythmic activity is possibly related to the number of synapses set up, as shown in tissue culture[8].

We used both the above mentioned techniques to give an exhaustive picture of the development of VERs in rats with poor intrauterine fetal growth.

Materials and methods. 45 Sprague-Dawley albino rats with poor intrauterine fetal growth were studied at different ages (see Table) in acute experiments. The control group consisted of 44 rats of the same stock. Poor intrauterine fetal growth was obtained by electrolytic lesion of the placenta performed on the 14th day of pregnancy[9]. All animals received light Nembutal anaesthesia (35 mg/kg, i.p.). White binocular flashes were supplied by an OTE stroboscope (10 µsec duration, intensity 0.7 J) placed at 40 cm from the rat's eyes. Frequency of stimulation was increased in steps of two. Band-pass filter was 1–70 Hz. Transient response was recorded up to 5–6 Hz. EEG and EKG monitoring were performed by a Grass Polygraph AC model P5. Data were stored on a HP 3960 magnetic tape recorder and analyzed by a Laben 701 computer. Visual evoked responses to low-frequency stimulation were averaged by a HP 5480. Sampling for transient responses was 128. The age of appearance of each component and their latencies were considered.

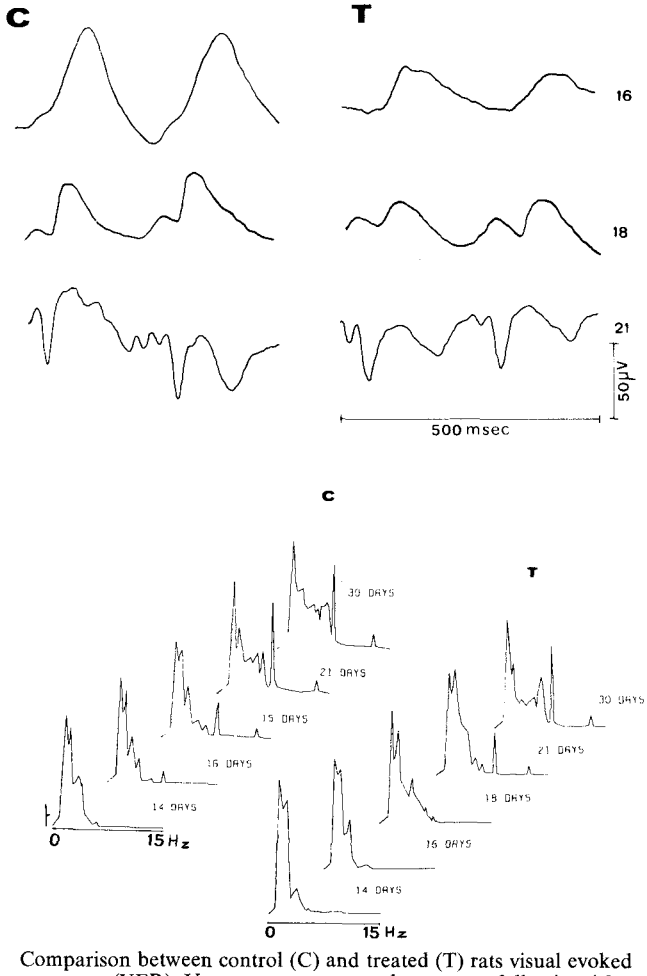
The power spectra were evaluated by frequency analysis with fast fourier transform for standard EEG and steady-state responses to high frequency stimulation (fig. 1). Power spectra were displayed as amplitude peaks of each frequency band. Sampling rate was 50 Hz (Njquist frequency 25 Hz), evaluated periods were 60 sec, segments 5.12 sec.

Several frequencies were tested but particular attention was paid to the 8–12-Hz range which is the rhythmic after discharge one and to the 16–20-Hz range. These 2 ranges of frequency have been shown to be good parameters in evaluating the development of VERs in the rat[11].

Recording electrodes were tungsten needles, uncoated for 2 mm, fixed on area 17 following Krieg[10]. Reference electrodes were put on the ear lobe.

Results. Mean weight showed remarkable differences during development (table) with a high statistical significance using Student's t-test ($p = 0.001$ up to 16 days, $p = 0.01$ up to 21 days). Spontaneous motor activity was tested every 2 days. No remarkable difference was found between control and treated young rats. Frequency analysis of basic EEG activity from the 12-day-old rat showed the appearance and the progressive development of the 3 frequency ranges (1–3 Hz, 3–4 Hz, 5–7 Hz), in agreement with the results obtained on hooded rats by other researchers[12,13]. No significant variation was found between control and treated rats.

The responses evoked by 2–6-Hz stimuli consisted, in the developed animal, of a triphasic complex negative-positive-negative (N_1, P_1, N_2). The 2nd negative wave appeared at 12 days in control rats. The positive wave (P_1) became evident (table) in treated rats later than in controls and significant differences of latency values were found up to 21 days (table) $p = 0.005$ at 16 days, $p = 0.05$ at 21 days). Responses to 4-Hz stimuli were difficult to compare up to 18 days, but showed significant latency differences in the P_1 wave at 21 days (fig.). The frequency analysis of EEG displayed on power spectra, only during high frequency of stimulation (above 6 Hz), an amplitude peak in the frequency band corresponding to the frequency of stimulation (fig.) parallel averaging of traces showed a sinusoidal response (steady-state). The response peak to the selected 8-Hz stimulation appeared later in treated rats (table), its presence in relation to age was shown to be an interesting comparative parameter, and its amplitude was always inferior to 1000 μV^2 up to 21 days in treated animals, while in control rats the amplitude value was around 1500 μV^2 already at 18 days. Responses to 10–20-Hz stimulation showed greater individual differences.



Comparison between control (C) and treated (T) rats visual evoked responses (VER). Upper traces: averaged responses following 4-Hz stimuli. On the right, ages of animals compared. Note the latency differences of positive waves at 21 days (negativity upwards). Lower traces: frequency analysis display of EEG traces during 8-Hz stimulation. Note the appearance of the response peak on the 8-Hz frequency at 16 days in control rats and at 21 days in treated animals. Horizontal scale: 15 Hz. Vertical bar on the left: 500 μV^2.

Comparison between control (C) and treated rats (T)

Age in days		7	12	14	16	18	21	30
No. of rats	C	5	5	6	6	8	8	6
	T	7	7	7	6	6	6	6
Body weight	C	13.6	18.4	20.7	26.4	29.3	31.1	49.3
(g)	T	9.6	12.2	16.5	19.5	23.9	27.8	47.2
P_1 latency	C	=	*	92.5	68.6	61.4	58.6	51.7
(msec)	T	=	=	*	88.5	74.3	66.2	54.4
8-Hz peak	C	=	=	33%	62%	87%	87%	100%
(presence)	T	=	=	=	33%	50%	66%	100%

Mean values: =, absent response; *, only negative waves are present.

Discussion. The results show that the electrolytic lesion of the placenta on the 14th day of pregnancy in the rat induces poor fetal growth, whose effects influence body weight and brain electrical organization in post-natal life.

These data can easily be related to the well known time concordance between the development of VER in the rat and the periods of greater protein and DNA synthesis in the 1st month of life of this animal[12,14]. One can then speculate that the decrease in the supply of energy which follows placental insufficiency interferes with protein synthesis and leads to a delay of myelination as indicated by greater latencies of the low frequency evoked responses (transient VER). The differences between treated and control rats disappear in the 4th week of life. That is they do not exceed the time in which myelination of the optic pathways occurs in the rat.

In this sense the observed changes should be considered as reversible alterations in the maturation and development of the visual system. One should remember that alterations in the development of VERs have also been observed in undernutrition and other restricted conditions[2,4,5].

The 2nd, and perhaps the most interesting, part of our present experiment is that dealing with the investigation of the development of evoked responses in the range of the rhythmic after discharge (RAD) described by Anderson[15] in animals, that is about 8 Hz. The amplitude of the evoked response in this range of frequency is evaluated by power spectra after frequency analysis of EEG traces during stimulation. This range of frequency was selected as it showed a good display of power spectra and a regular development in amplitude during the 1st month of life. Moreover this kind of evoked response (steady-state VER) is possibly related to the onset of synapses in the visual cortex[16] and the organization which has been called by Regan[7] a frequency region addressed to the elaboration of luminance changes. On the basis of these last data one could conclude that poor fetal growth due to placental lesion not only results in a delay of myelination but also to a transitory alteration in the onset of some aspects of functional organization in the visual cortex.

The data presented are to be confirmed by parallel investigations in histology and biochemistry which are at present in progress.

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Hibernation at moderate temperatures: a continuation of slow wave sleep[1]

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Summary. Golden-mantled ground squirrels (*Citellus lateralis*) displayed virtually continuous electrophysiological states of sleep when hibernating at moderate ambient temperatures (22 °C). Rapid-eye-movement sleep progressively diminished with the fall in body temperature so that at a body temperature of 23 °C it was completely absent. At this temperature hibernation was characterized by slow wave sleep isomorphic with slow wave sleep episodes at non-hibernating (euthermic) body temperatures.

Recently, the adaptive value of sleep has been considered from an ecological perspective. Thus, sleep has been viewed as a process that reduces the energy requirements of endotherms[2,3] while also affording seclusion from predators[4,5]. Decreases in body temperature (T_b) and metabolism that accompany sleep are independent of, but normally superimposed upon the normal circadian variation of T_b[6]. Therefore, such declines in T_b are specific to sleep itself. Qualitatively similar, but much greater, declines in T_b occur during the entrance into hibernation[7]. We have proposed homology between sleep and hibernation based on electrophysiological and thermoregulatory continuities between these 2 processes[7,8]. Marmots and ground squirrels in a cold environment enter hibernation through sleep, but, as T_b decreases, the amplitude of electroencephalographic (EEG) activity progressively declines, becoming almost isoelectric at about 5 °C[9,10]. This EEG attenuation makes the records increasingly difficult to score for states of sleep and wakefulness at T_bs below approximately 25 °C[9,10]. Therefore, we studied complete bouts of hibernation in ground squirrels subjected to a moderate ambient temperature (T_a) of 22 °C, which prevented T_b from falling to low levels. Under these conditions, the hibernating ground squirrel showed electrophysiological patterns of sleep isomorphic with those occurring during sleep at non-hibernating (euthermic) T_bs.

8 adult golden-mantled ground squirrels (*Citellus lateralis*) were implanted under sodium pentobarbitol anesthesia with chronic cortical and hippocampal EEG, electrooculogram (EOG), and electromyogram (EMG) electrodes. The EMG electrodes were implanted in the dorsal neck muscles