n-butanol (IS) was obtained within 5 min. The chromatogram of the water in the plasma has a pattern similar to that of figure 3. The calibration curve for water is linear[10]. The average value and standard error (SE) of the 6 mean water contents in red cells obtained from one individual in triplicate determinations are 71.39 ± 0.28%. The mean SE of the values determined in triplicate is 0.25%. Thus, the data show excellent reproducibility.

The mean water content in fresh red cells of 14 adults, who ranged in age from 22 to 45 years, was 71.26 ± 0.31%. The water content of red cells obtained from blood supplemented with ACD is shown in the table. It has a tendency to increase as the amount of ACD increases. The water content of red cells with the addition of 150 µl ACD to 1 ml blood increased from 71.22 ± 0.22% (original content) to 73.36 ± 0.28%.

A similar tendency is shown in the content of trapped water. The volume increased 6.8 ± 0.5 to 7.4 ± 0.4% in the packed red cells before and after the addition of 3 volumes ACD to 20 volumes of the blood.

In contrast, physiological saline in the same ratio did not increase the intra and inter-cellular water content.

Discussion. The previous data on water content in red cells according to the weighing method are as follows: 72.2% (v/v) reported by Hald et al.[1], 71.7% (v/v) by Savitz et al.[4], 71.0% (v/v) by Murphy[5] and 66% [(w/w) about 70% (v/v)] by Kuroda[3]. The values are considered to differ from each other as a result of the use of nonstandardized equipment different temperatures and times for the drying procedures, and neglect of other volatile components in the red cell.

Our result (71.26%) agrees well with the value found by Savitz et al. and that found by Murphy, and also 71.73% found previously by Kageyama[8].

The average increase in the water content of red cells following the addition of ACD is 2.93% of the original content when the ratio of blood to ACD was 20:3 (as used to store blood for transfusion), in spite of the fact that the pH (7.4) and osmotic pressure (291 mosmol) of the mixture did not change. The increase is not due to dilution of the blood supplemented with the ACD, because the same concentration of physiological saline does not cause an increase in water content. The mechanism of the phenomenon cannot yet be clarified but is now being studied.

We have also developed a method for measuring water content of brain tissue by GLC (Yamagata et al., unpublished). The new rapid and precise method for measuring water content will be useful for many studies, using various tissues or cells.

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Electrocorticographic activity induced by gamma hydroxybutyrate in the rat during ontogenesis

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Summary. GHB at a dose of 200 mg · kg^{-1} i.p. elicited groups of slow waves with a frequency of 4–5 Hz in both frontal and occipital ECoG leads in adult rats. In 25- and 18-day-old rats similar slow wave activity became continuous and exhibited a clear-cut maximum in the frontal regions. In 15- and 12-day-old animals slow wave activity was also registered in the frontal region but it was organized into short groups of unstable frequency. No ECoG effects of GHB could be found in 9-day-old rats.

Gammahydroxybutyrate (GHB) which was originally proposed to be a hypnotic and anaesthetic agent[1] has been shown to be a useful drug in experimental epilepsy. Godschalk and collaborators[2,3] found that GHB at a subhypnotic dose of 200 mg · kg^{-1} injected i.p. elicited 'hypersynchronous activity' in rats. The bursts of large slow waves with a frequency of 3–5 Hz were accompanied by a sudden arrest of ongoing motor activity; this immobility lasted for the duration of the burst. These bursts could be suppressed selectively by anticonvulsants effective against petit mal epilepsy[2]. Because this type of epilepsy is predominant in children of the preschool and school age[4], we studied the ECoG effects of GHB during ontogenesis[5].

Methods. Rats aged 90 (i.e. adult animals), 25, 18, 15, 12 and 9 days were used. Surgical preparation (trephine openings, tracheal cannula) was performed under ether anaesthesia, then the wounds were carefully infiltrated with procaine, the anaesthesia was disrupted and the animals were immobilized by d-tubocurarine. Artificial ventilation as well as body temperature were maintained.

Electrocorticograms were recorded by means of silver ball electrodes from sensorimotor (frontal) and visual (occipital) areas of both hemispheres, whereas an indifferent electrode was placed on the nasal bone. The electrocardiogram served for monitoring the state of the animals. At the beginning of the experiment, a 5-min period of spontaneous ECoG was recorded, then gammahydroxybutyrate sodium was administered i.p. at a dose of 200 mg · kg^{-1} and ECoG was continuously recorded for at least 30 min. In adult, 25- and 15-days-old rats driving was also studied. 15-sec periods of rhythmic photostimulation with frequencies of 3 and 5 Hz were applied before and 30 min after the injection of GHB.

Results. Adult rats (n = 8, fig. 1): Development of 5-6,5 Hz rhythm was visible after the 2nd to 3rd min after GHB administration; during the next minutes the incidence of

rhythmic sections increased, the frequency of this activity slowed down to 4–5 Hz and its amplitude increased, specially in frontal regions, and occasional spikes appeared. Around the 10th min the pattern was formed by 2- to 4-sec sections of large slow waves (sometimes with intermingled spikes) in all leads with shorter sections of desynchronized activity separating these rhythmic sections. This pattern was preserved until the end of registration, sustained spike-and-wave rhythm was never recorded.

25-day-old rats (n = 7, fig. 1): GHB led to a marked increase of occipital 4.5–6 Hz rhythm during the first 2–3 min. During further recording frontal activity sharply increased by the appearance of 1- to 2-sec groups of large slow waves (with occasional spikes at a frequency of 4–4.5 Hz). This frontal activity progressively increased; between the 7th and 10th min it became continuous and clearly predominant over the occipital ECoG. Concomitantly, its frequency spectrum broadened towards slow frequencies and the variability of individual waves increased (frequencies from 3.5 to 5 Hz were present). Continuous slow wave activity in frontal regions lasted until the 25th min when very short sections of low amplitude began to separate groups of slow waves lasting some sec.

18-day-old rats (n = 5): Results were identical with those in 25-day-old animals until the 15th min when further slowing of rhythms appeared so that delta waves with a frequency of 1–2 Hz appeared quite frequently.

15-day-old rats (n = 7, fig. 1: GHB increased the number of groups of slow waves in both frontal regions. These groups (lasting 3–4 sec) usually started with a very slow wave followed by a group of waves of lower amplitude and of increasing frequency. Such activity became nearly continuous between the 7th and 15th min; short sections of depressed activity then appeared.

12-day-old rats (n = 7, fig. 1): Since the 3rd to 5th min GHB elicited in frontal regions short periods of slow waves with a frequency of 2–3 Hz and of amplitude higher than that of spontaneous activity. The incidence of these periods increased until the 10th min: some of them are identical to groups described in 15-day-old rats. Towards the end of recording the number of groups decreased again.

9-day-old rats (n = 5, fig. 1): GHB did not elicit specific

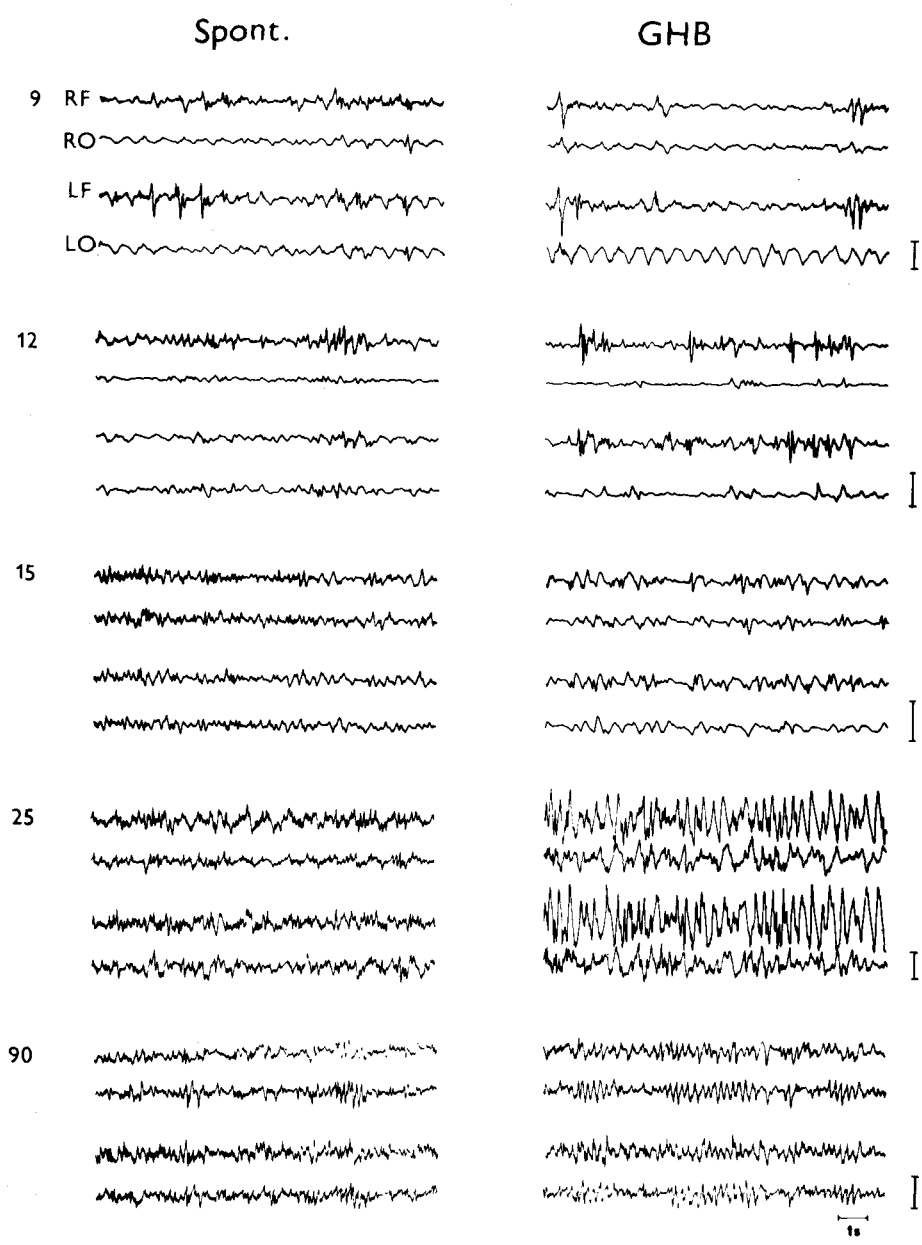

Figure 1. Electrocorticographic changes induced by GHB in rats. From top to bottom: rats aged 9, 12, 15, 25 and 90 days. ECoG activity was recorded from right frontal (RF), right occipital (RO), left frontal (LF) and left occipital (LO) areas in reference connections. Left column: spontaneous ECoG; right column: ECoG after GHB administration (time in min marked at the beginning of records; with the exception of 9-day-old rats the period of maximal GHB-induced changes was taken). Amplitude calibration 0.5 mV, time 1 sec.

changes in the discontinuous ECoG. Photic driving was substantially augmented by GHB in adult and 25-day-old rats (fig. 2); in 15-day-old animals it was absent before as well as after GHB injection.

Discussion. Pattern of the GHB-induced ECoG activity registered in adult rats in our acute experiments is identical with that described by Godschalk at al.[3] in rats with chronically implanted electrodes. This identity is important for the developmental study – it could be performed under conditions of acute experiments and we thus avoided the serious difficulties of chronic implantation of electrodes in immature rats.

The appearence of discernible GHB-induced activity between the 9th and 12th days is in agreement with the development of spike-and-wave cortical self-sustained after-discharge (SSAD) elicited by rhythmic stimulation of the thalamus. This type of SSAD could be evoked for the first time in 12-day-old rats and its shape matured until the 18th postnatal day[6].

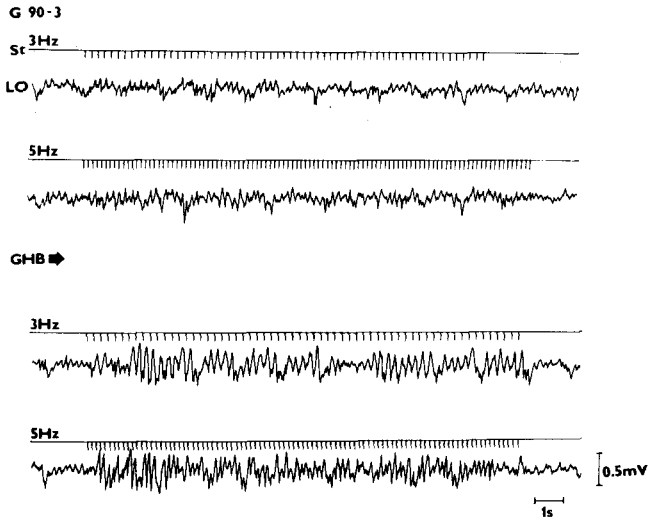

Figure 2. Photic driving elicited by rhythmic photostimulation of the right eye in adult rat. Upper part before, lower part 30 min after the administration of GHB. In each section St = marks of stimuli (frequencies 3 and/or 5 Hz), LO = monopolar recording from the left occipital, i.e. primary visual area. Other details as in figure 1.

Comparing adult rats with young ones the difference in localization of GHB-induced activity becomes obvious: diffuse incidence of this activity in adult rats is in contrast to clear-cut predominance of frontal activity in comparison with the occipital one in all immature rats. In animals aged 15 days or less the same holds true for spontaneous ECoG activity without drug pretreatment, but 18- and 25-day-old rats exhibit well-expressed spontaneous ECoG in all cortical regions[7]. This distribution strongly resembled a central maximum of spike-and-wave episodes in children with classical absences – the rat's frontal activity is formed by motor and somatosensory areas and is thus analogous to human central region.

The hypothesis that GHB-induced activity could be a model for petit mal epilepsy was formulated by Godschalk et al.[2,3] as well as by Snead[8]. Our results could be taken as additional evidence supporting this hypothesis – especially in relation to results of pharmacological studies in adult rats[2] and monkeys[8-10] as well as in 25-day-old rats[11,12]. An increased sensitivity to rhythmic photostimulation found in our experiments fits into the current concept of petit mal epilepsy[13].

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Specific metal binding sites on calcified concretions in epithelial cells of the clam kidney

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Summary. Calcified granules (concretions) in the kidneys of bivalve molluscs are able to absorb metals from solution and this ability is the result of the existence of saturable, medium affinity metal binding sites on the concretions. In addition different metals may compete for the same sites so that some metals will be accumulated preferentially over others.

Marine bivalve molluscs possess a remarkable ability to concentrate metals and other pollutants from seawater and there are currently extensive investigations into their properties as environmental monitoring organisms[1]. Until recently little was known about the process by which high tissue levels of metals were produced from environmental metal levels below the detection limit of analytical instruments. It has now been shown that certain organs, particularly the kidney have high concentrative capacity[2], and that this is due in part to metal binding proteins in this organ and in part to subcellular calcium-phosphorus concretions[3]. These concretions in the bivalve kidney contain such metals as zinc, manganese, copper, cadmium etc. but little is known of their formation or function. This study was