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Behavioral and Electroencephalographic Studies of Beagles with an Eck's Fistula: Suitability as a Model of Hepatic Encephalopathy

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WATANABE, M., T. OZAKI, T. MUSHIROI, Y. UKAI, F. UEDA, K. KIMURA, M. KATOH, A. MATSUMOTO, E. KOTANI, S. ITOH, K. YAMAGUCHI AND K. KYUKI. *Behavioral and electroencephalographic studies of beagles with an Eck's fistula: suitability as a model of hepatic encephalopathy.* PHARMACOL BIOCHEM BEHAV **57**(1/2) 367–375, 1997.—Behavioral manifestations, electroencephalograms (EEGs) and visually evoked potentials (VEPs) were studied in beagles with Eck's fistula (portacaval shunt [PCS]), an established model of hyperammonemia, to determine whether they developed CNS disorders characteristic of hepatic encephalopathy. After PCS, behavioral changes occurred in the form of listlessness, sluggishness (altered gait, snapping and transient catatonia-like symptoms) and apparent blindness, which appeared in that order and progressed to coma and death in some animals. The EEGs from the frontal cortex showed a gradual decrease in voltage and frequency. Development of snapping and catatonia-like symptoms coincided with the occurrence of high voltage fast waves in the EEGs from the occipital cortex. In comatose Eck's fistula dogs, flattening of the EEGs was recorded from the frontal cortex and a lowered voltage was noted in the EEGs from the occipital cortex. After PCS, the latencies and amplitudes of the components of VEP were increased. The snapping and catatonia-like symptoms were markedly ameliorated by carbamazepine and the coma by flumazenil and thyrotropin-releasing hormone. These findings indicate that Eck's fistula dogs provide a useful model of hepatic encephalopathy. © 1997 Elsevier Science Inc.

Eck's fistula dogs Behavior EEG Hepatic encephalopathy Carbamazepine Flumazenil TRH

HEPATIC encephalopathy is a neuropsychiatric syndrome associated with hepatic disease that progresses to impaired consciousness. Ammonia, some amines, and fatty acids containing 4 to 8 carbons in the molecule have been implicated in the pathogenesis of hepatic encephalopathy (19,23,24) although the entire mechanism has not yet been clarified. Among these compounds, ammonia is thought to be the most important etiologic factor, because it impairs CNS neurotransmission (27) as well as energy (7) and monoamine (9) metabolism directly or indirectly. Hepatic encephalopathy is associated with acute or chronic hepatic failure, e.g., fulminant hepatitis and liver cirrhosis. Patients with hepatic encephalopathy have various symptoms and signs, including emotional changes, memory disorders, disorientation, hallucination, illusion, motor ataxia, behavioral abnormalities, excitation, delirium, confusion and coma. Rats with a portacaval shunt (PCS) have been shown to develop an increase in low voltage slow waves in electroencephalograms (EEGs) and to become comatose in response to exogenously administered ammonia (8,12). However, this model is not suitable for long-term observation,

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TABLE 1

RATING SCALE FOR CONSCIOUSNESS IN ECK'S FISTULA DOGS

Grade	Behavioral Criteria
0	Normally active
I	Listless; no abnormal activity
II	Sluggish; inconstant abnormal behavior (altered gait, inappropriate barking or snapping, transient catatonia, ataxia)
III	Constant abnormal behavior; apparent blindness; convulsions
IV	Comatose

because it only shows acute symptoms induced by exogenous ammonia. Thus, in the present study, Eck's fistula dogs, an established experimental model of hyperammonemia, were prepared by a surgical procedure similar to that used in rats and observed for 15 weeks following PCS to assess changes in their general condition, behavior, EEGs and visually evoked potentials (VEPs). We also examined the effects of three agents including antiepileptic carbamazepine, analeptic thyrotropin-releasing hormone (TRH) and benzodiazepine antagonist flumazenil on behaviors and EEG patterns in the animals which showed snapping and transient catatonia-like symptoms, or coma at 15 weeks or more after PCS.

METHODS

Animals

Five to six months-old beagle dogs, weighing 7.6 to 10.8 kg, were purchased from Nihon Nosan Kogyo (Yokohama, Japan). Following an acclimatization, 14 healthy and well-acclimatized animals were selected for the study when they were 6 to 7.5 months old. Throughout the acclimatization and experimental periods, animals were housed individually in a room maintained at 20–26°C and 40–70% humidity and ventilated 12 times or more per h. The room was artificially illuminated with a 12 h daily period of light. They were given daily 200 g of a pellet diet (TC-1; Maruha Pet Food, Tokyo, Japan) and tap water ad libitum. TC-1 dog food contains 26% crude protein, 11% fat and 50% carbohydrate. Proteins are derived from corn, wheat flour, corn gluten meal, soybean and meat meal.

Experiments were carried out in accordance with the procedures outlined in the Guidelines for Animal Experiments of Discovery Research Laboratories of Nippon Shinyaku, Co., Ltd., which complies with the Guidelines for Animal Experimentation of the Japanese Association for Laboratory Animal Services.

Chemicals

Pure bulk carbamazepine was commercially obtained from Ciba-Geigy Japan (Tokyo, Japan) and each dose was administered in a gelatin capsule JP (1/2 oz). An injectable formulation of flumazenil (Anexate injection, 0.5 mg/5 ml; Yamanouchi, Tokyo, Japan) was administered intravenously (1 ml/kg body weight). A 6 mg/ml solution of TRH (Sigma, St. Louis, USA) was prepared in saline (Otsuka Pharmaceutical, Tokyo, Japan) and administered intravenously (0.5 ml/kg body weight). Other chemicals and reagents used in the study were as follows: distilled water for injection (Otsuka Pharmaceutical), sodium pentobarbital (Nembutal Injection; Dainippon Pharmaceutical, Osaka, Japan), benzylpenicillin procaine and benzylpenicillin benethamine injection (Doupen; Mallinckrodt Veterinary, Tokyo, Japan), nafamostat mesilate (Futhan; Torii Pharmaceutical, Tokyo, Japan), 5% glucose for injection (Otsuka Pharmaceutical), sodium ampicillin and sodium cloxacillin powder for injection (Viccillin-S 1000; Meiji Seika, Tokyo, Japan), and an ophthalmic solution containing 0.5% tropicamide hydrochloride and 0.5% phenylephrine hydrochloride (Mydrin-P; Santen Pharmaceutical, Osaka, Japan).

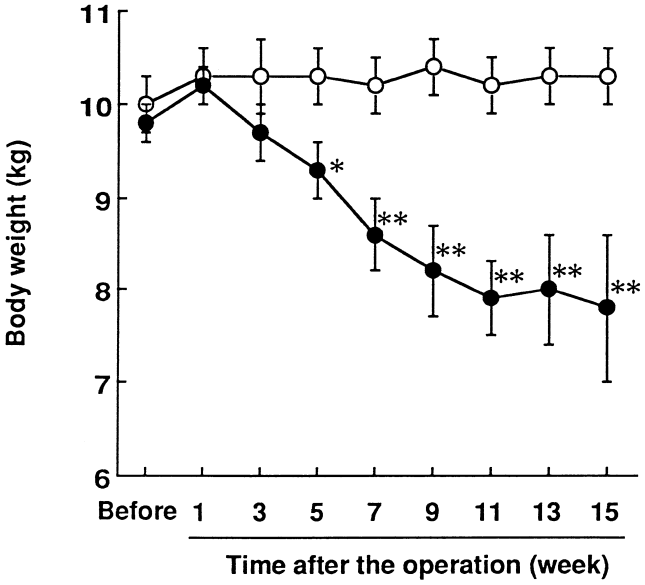

FIG. 1. Changes in body weight in Eck's fistula dogs and sham operated dogs.

Experimental Schedule

The animals were divided into two groups of equal size. All animals were implanted with electrodes for EEG and VEP recording. One week after the implantation, baseline EEG and VEP recordings were made, and after another week, one group underwent PCS (Eck's fistula group) and the other underwent transient portal ischemia only (sham-operated group) (week 0). EEG and VEP recordings were made at weeks 2, 5, 7, 9, 11, 13 and 15. The recordings were made at a fixed time each day (starting at 1300–1400 h) because of the circadian rhythm of EEG and VEP.

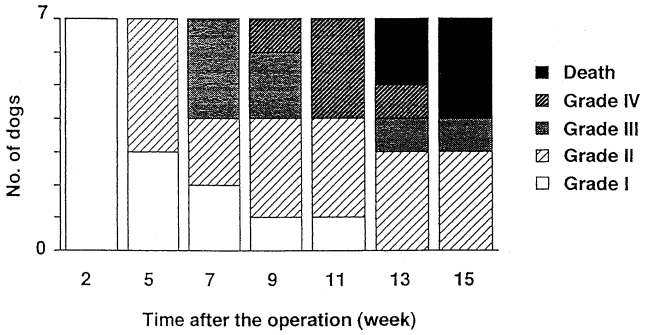

FIG. 2. Changes in gross behavior in Eck's fistula dogs.

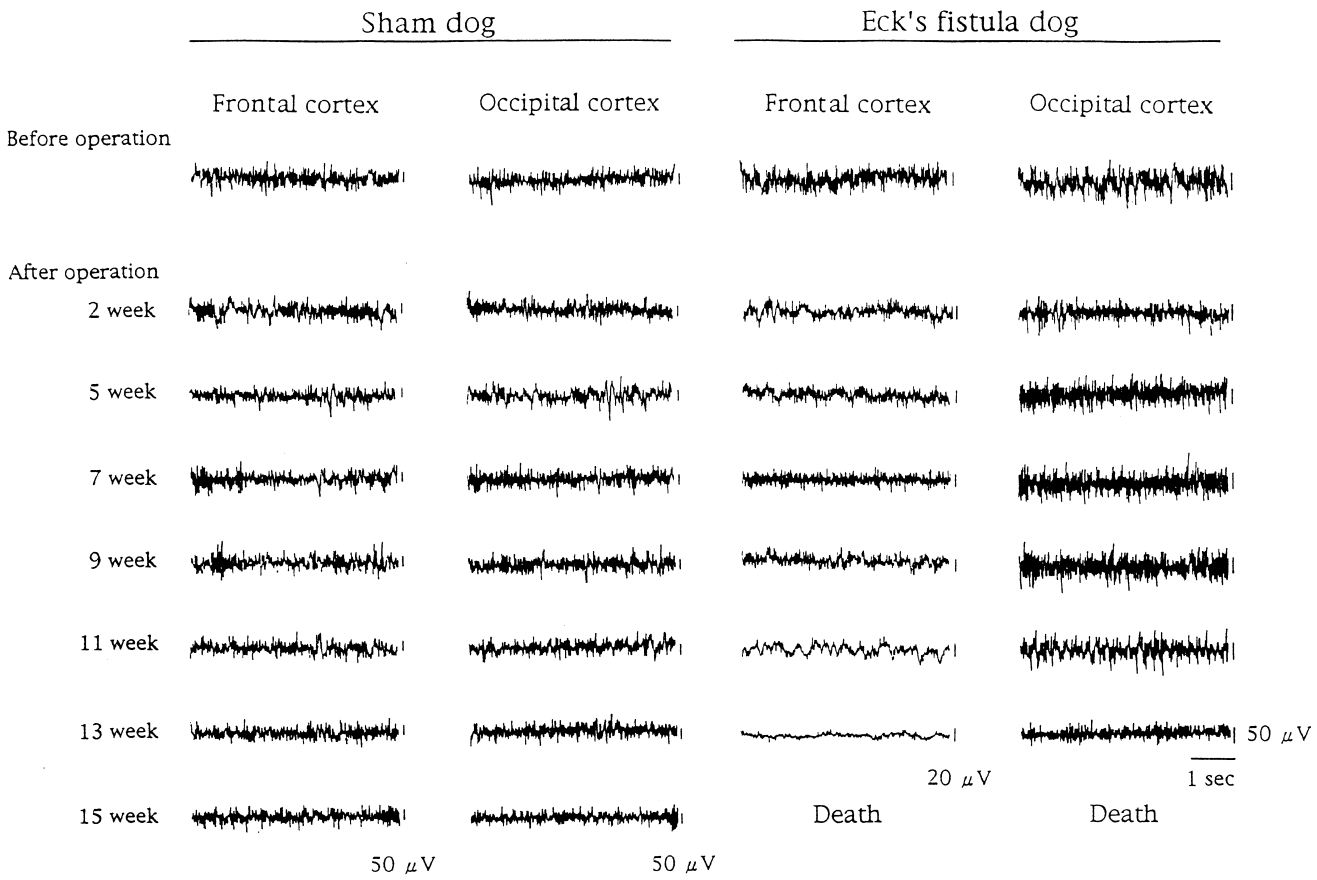

FIG. 3. Representative cortical EEG recordings before and after operation in Eck's fistula and sham operated dogs.

Implantation of EEG and VEP Recording Electrodes

For EEG and VEP recordings, electrodes were chronically implanted as described by Yamamoto and Kido (30). Animals were anesthetized with sodium pentobarbital (30 mg/kg, IV) and placed in a stereotaxic instrument (KN-397; Natsume, Tokyo, Japan). According to the brain atlas of Lim et al. (14), silver ball electrodes, 1 mm in diameter, were placed on the dura mater in the frontal cortex (R:30, T:12) and occipital cortex (R:0, T:12) for EEG recordings, and an indifferent electrode was placed on the cranial bone of the frontal sinus. For VEP recordings, an electrode similar to that used for cortical EEG was placed on the dura mater in the visual cortex (R:0, T:5). The lead wire from each electrode was soldered to a pin on a connector (HDA-15D; Hirose Electric, Tokyo, Japan), which was fixed to the cranial bone with dental cement (Yata Poliset; Yata Dental MFG, Osaka, Japan). Postoperative infection was prevented by an intramuscular injection of Doupen (0.1 ml/kg).

Surgical Procedure

An end-to-side PCS was performed according to a modification of Kimoto's procedure (13). In dogs fasted overnight and anesthetized with sodium pentobarbital (30 mg/kg, IV), an abdominal midline incision was made. The portal trunk from the hepatoportal region to the bifurcation to the gastroduodenal vein was separated. The inferior vena cava was separated from the retroperitoneum, and the anastomosed site (between the hepatic vein and the right renal vein) was clamped with a Mera Satinsky vascular clamp (Senko Medical Instruments, Kanazawa, Japan) to ensure venous patency during anastomosis. Then an incision as long as half the circumference of the portal vein was made inside the vascular clamp. The portal trunk was clamped with a Mera Hepner vascular clamp (Senko Medical Instruments) immediately above the gastroduodenal vein. The portal vein was ligated with two sutures of 1-0 suture stitch (Nescosuture Silk Braided, Nippon Shoji, Osaka, Japan) and severed at a point central to the clamp (less distant from the liver). The portal stump on the intestinal side was anastomosed to the incision in the inferior vena cava from the posterior to the anterior wall with a continuous suture of 6-0 vascular stitch with a needle (Nicho Kogyo, Tokyo, Japan). Following suture, the two vascular clamps were removed and hemostasis was achieved by applying pressure with sterile gauze. After confirming hemostasis and resolution of venous congestion of the intestine, 10 mg of nafamostat mesilate dissolved in 10 ml of 5% glucose solution was dispersed over the pancreas to prevent pancreatitis. The intestine was allowed to fall back into the peritoneal cavity. Infection was prevented by dispersing 1 g of Viccillin-S in 10 ml of saline (Otsuka Pharmaceutical) in the peritoneal cavity. The abdominal muscle layer and the skin were closed with sutures, and the same dose of the Viccillin-S preparation was subcutaneously injected around the sutures.

Behavioral Observations

Each animal was observed for 1 h during EEG recordings to assess its general condition and behavior. Based on behav-

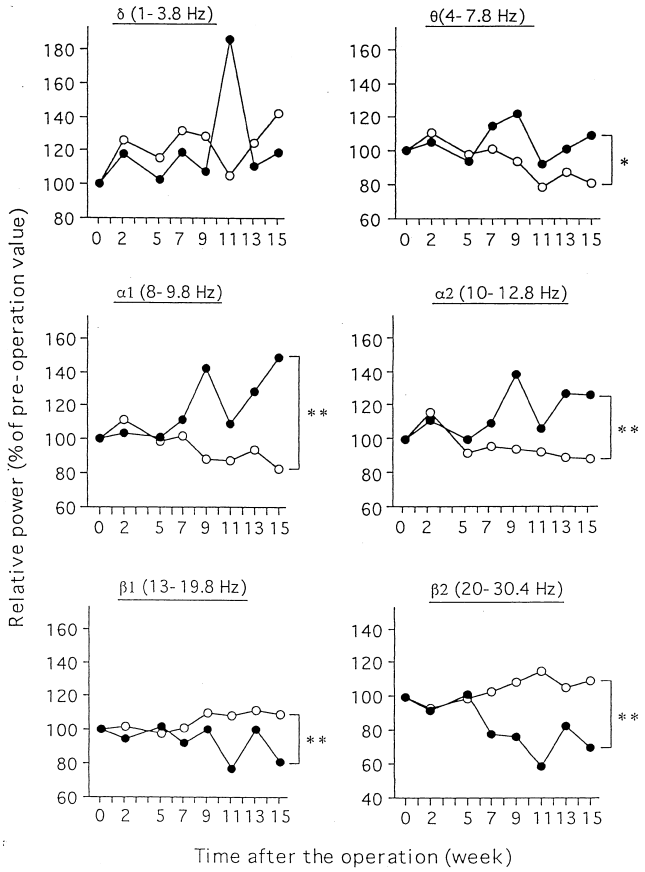

FIG. 4. Relative power of each waveband of the EEGs in the frontal cortex of Eck's fistula and sham operated dogs. The effects of group and time on the power spectra from the 2nd to the 15th week after the operation were compared by two-way ANOVA, followed by the analysis of the effect of Eck's fistula operation by LSD test. -○-, sham operated dogs; -●-, Eck's fistula dogs. Data represent the mean of 7 dogs. *$p < 0.05$, **$p < 0.01$ vs sham.

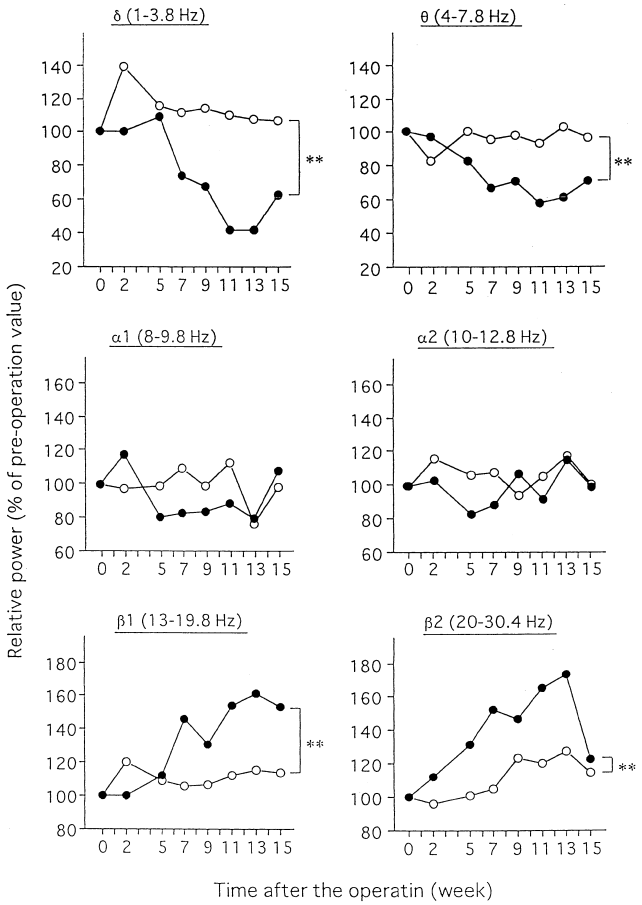

FIG. 5. Relative power of each waveband of the EEGs in the occipital cortex of Eck's fistula and sham operated dogs. For further details, see Fig. 4. Data represent the mean of 7 dogs. **$p < 0.01$ vs sham.

ior, the level of consciousness was graded according to Condon's criteria (3) (Table 1). Grades 0, I, II, III and IV were scored as 0, 1, 2, 3 and 4. Death was assigned to a score of 4.

Electrophysiological Studies

EEG recordings. EEGs were recorded for 1 h in conscious and freely moving dogs which were placed in individual cages (700 [W]× 700 [D] × 900 mm [H]) in a sound-proofed, electrically shielded room. EEG recordings were made with an electroencephalograph (EEG4400, Nihon Kohden, Tokyo, Japan), and EEG data were stored on magnetic tapes using a data recorder (RD-200T; TEAC, Musashino, Japan). Signals obtained during a 1 min period were analyzed by fast Fourier transformation (sampling time, 5 s; summation, 12) using an optional program (Power Array No. 200-A, Nihon Denki Sanei, Tokyo, Japan) run on a signal processor (7T18A, Nihon Denki Sanei). From the power spectra obtained, the power of each of the following 6 frequency bands was determined and expressed as a percentage relative to the total power (relative power): δ (1.0–3.8 Hz), θ (4–7.8 Hz), α1 (8–9.8 Hz), α2 (10–12.8 Hz), β1 (13–19.8 Hz) and β2 (20–34.8 Hz).

VEP recordings. Following an EEG recording, VEPs were recorded in dogs placed prone in a specific restraining chamber (250 [W] × 700 [D] × 250 mm [H]) in a sound-proofed, electrically shielded room. A flash unit of a photic stimulator (SMP-3100; Nihon Kohden) was held 30 cm from the eye. The chamber room was darkened, and mydriasis was induced by applying two drops of Mydrin-P to the eye. Following 30 min of dark adaptation, light stimuli with an intensity of 0.6 J were delivered at a frequency of 0.5 Hz to the eye, and monopolar recording of the VEPs from the visual cortex was made. The signals were passed through an amplifier (ACB-11; Nihon Kohden) and recorded using a data recorder (RD-200T; TEAC), while the waveforms were observed on an oscilloscope (VC-11; Nihon Kohden). The responses to 100 flashes were averaged using Mac Lab Data Recording System (Scope Ver. 3.3.5; Mac Lab AD Instruments, Castle Hill, N.S.W., Australia). In the mean VEP waveform, the latencies to peak P30 (a positive peak with a latency of about 30 ms) and peak N45 (a negative peak with a latency of about 45 ms) were determined together with the amplitude between the two peaks.

Statistical Analysis

EEG and VEP data were analyzed by two-way ANOVA using group and time as factors. When significant group effects were detected, differences between groups were subsequently tested for significance using the least significant difference

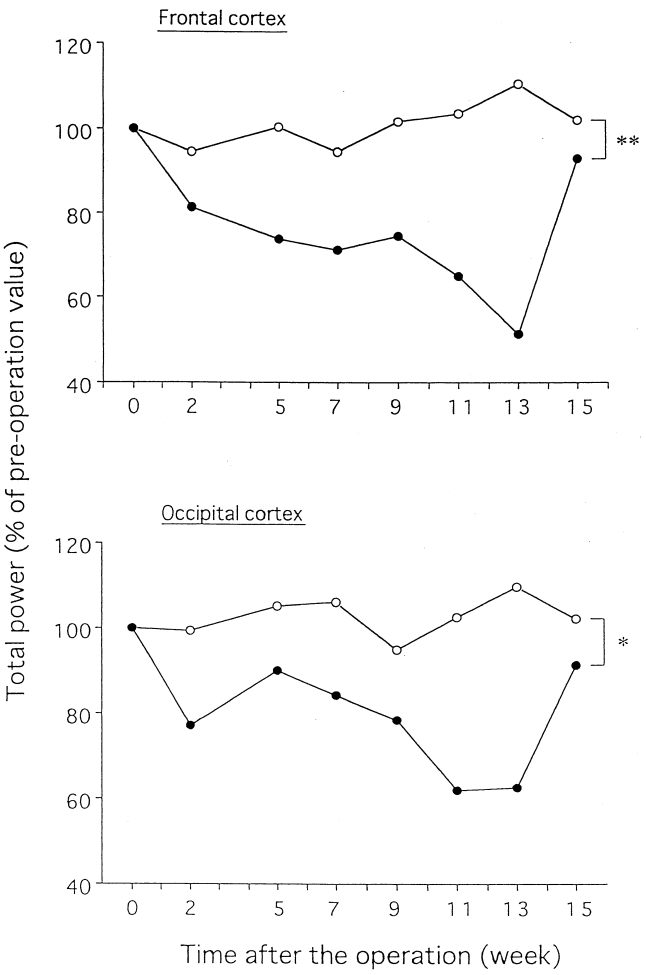

FIG. 6. Total power of the cortical EEGs in Eck's fistula and sham operated dogs. For further details, see Fig. 4. Data represent the mean of 7 dogs. *$p < 0.05$, **$p < 0.01$ vs sham.

(LSD) test. Differences with $p < 0.05$ were considered significant.

RESULTS

Behavioral and Electrophysiological Changes Following PCS

Gross behavior. No abnormal behaviors were induced in the sham-operated group. Changes in the body weight and the grade for consciousness disturbance with time following PCS are shown in Fig. 1 and Fig. 2, respectively. Body weight of dogs with PCS was gradually decreased after the operation due to anorexia. Following PCS, all of the 7 Eck's fistula dogs became listless (grade I) at week 2. At week 5, four animals became sluggish, developing altered gait and other symptoms of cloudiness of consciousness (snapping and transient catatonia-like symptoms (grade II). Three out of these 4 animals became apparently blind (grade III) at week 7 and comatose (grade IV) at weeks 9–11 and died at weeks 13–15. Comatose animals showed no response to the examiner or any other external stimuli. The remaining 3 animals developed grade II disturbance at 7–13 weeks, and one of these became apparently blind (grade III) at week 13.

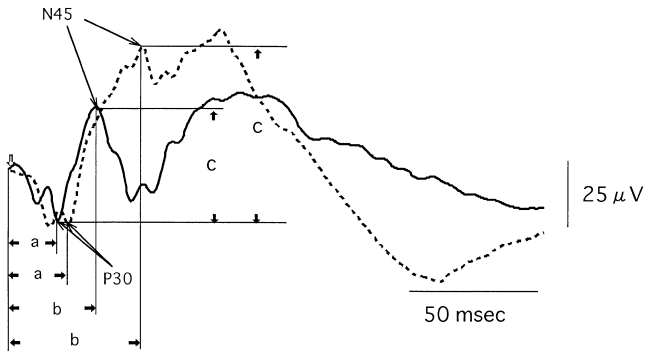

FIG. 7. Schematic diagrams of a visually evoked potential (VEP) from a normal dog (solid line), the same dog with encephalopathy caused by PCS (dashed line). The arrow shows the time of stimulation a) latency of p30; b) latency of N45; c) amplitude of P30-N45.

EEG. Figure 3 shows representative EEG recordings from a sham-operated and an Eck's fistula dog. In the sham-operated group, no obvious change occurred in the EEG pattern after surgery. In the Eck's fistula group, the EEG from the frontal and occipital cortices showed a slight decrease in the amplitude at week 2, when the animal seemed to be listless. At week 5, there were an increase in the slow-wave components and a decrease in the amplitude in the EEG from the frontal cortex, and an increase in the amplitude of fast-wave components in the EEG from the occipital cortex. At weeks 7–9, when the dog showed snapping and catatonia-like symptoms, EEGs with an increase in the low voltage slow waves in the frontal cortex and an increase in the high voltage fast waves in the occipital cortex were manifested. At week 11, when the dog became comatose, more prominent EEG changes were observed in the frontal and occipital cortices. At week 13, EEG flattening was observed in the frontal cortex and a marked decrease in the amplitude was noted in the EEG from the occipital cortex.

In the frequency analysis, the percentages of the relative power for individual frequency bands relative to the corresponding preoperative values were calculated. They were plotted against time after surgery (Figs. 4 and 5). Figure 6 shows the time-course of the percentage of total power relative to its preoperative level after surgery. Post-hoc comparison revealed that in the Eck's fistula group compared to the sham-operated group, there were a significant increase in the relative power of bands θ, $\alpha 1$ and $\alpha 2$ and a significant decrease in the relative power of bands $\beta 1$ and $\beta 2$ and the total power (i.e., an increase in low voltage slow waves) in the EEGs from the frontal cortex, and a significant decrease in the relative power of bands δ and θ and the total power and a significant increase in the relative power of bands $\beta 1$ and $\beta 2$ (i.e., an increase in low voltage fast waves) in the EEGs from the occipital cortex.

VEP. A schematic representation of the individual components of VEP is shown in Fig. 7. Before PCS, photic stimulation evoked a P30 peak with a latency of about 30 ms and an N45 peak with a latency of about 45 ms from the visual cortex. The percent changes in the latencies for P30 and N45 and the amplitude between the two peaks relative to the corresponding preoperative values were calculated and plotted against time in Fig. 8. In the sham-operated group, no significant changes occurred in the VEP pattern after surgery. Compared to those in the sham-operated group, the VEPs in the Eck's

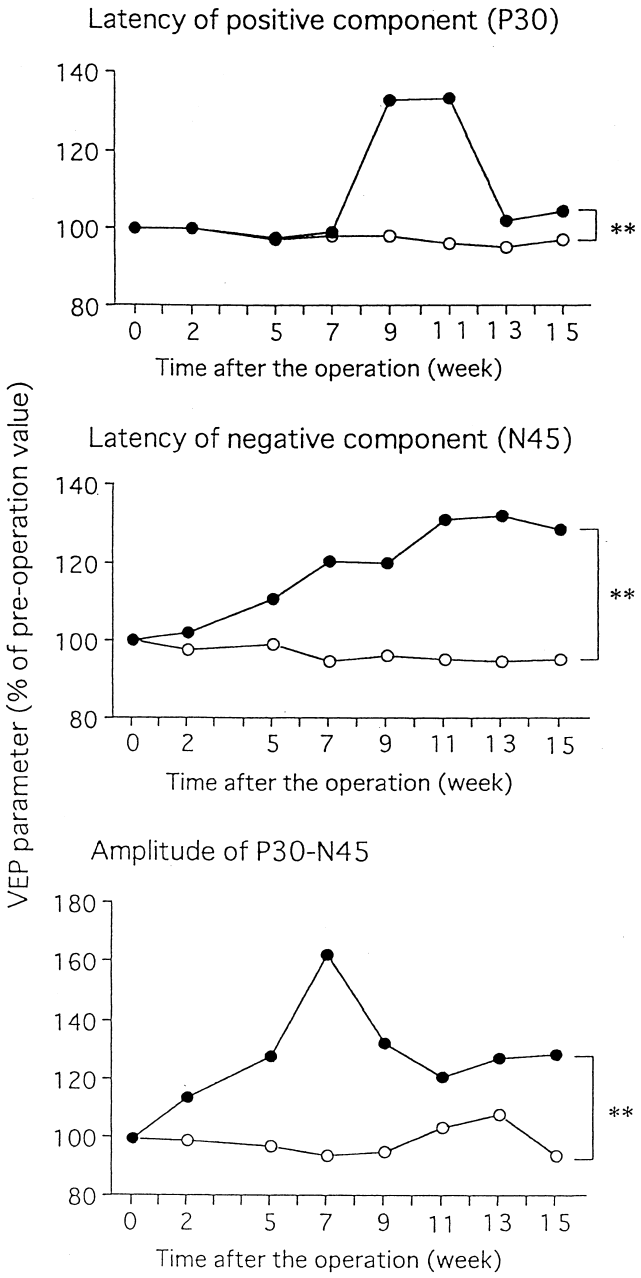

FIG. 8. VEPs in Eck's fistula and sham operated dog. VEPs were recorded before and at various times after the operation. The latency of P30 and N45 and the amplitude of P30-P45 were determined for each VEP. The effect of group and time on VEP parameters from 2nd to 15th week after the operation were compared by two-way ANOVA, followed by an analysis of the effect of Eck's fistula operation by LSD test. -○-, sham operated dogs; -●-, Eck's fistula dogs. Data represent the mean of 7 dogs. $^{**}p < 0.01$ vs sham.

fistula group showed a significant increase in the latencies for P30 and N45 and in the amplitude between the two peaks.

Effects of Carbamazepine, Flumazenil and TRH on Consciousness Disturbance

Effect of carbamazepine on grade II disturbance. In 2 out of the 3 dogs treated with carbamazepine, the snapping behavior and catatonia-like symptoms (positive mental symptoms) were all ameliorated from 30 to 120 min after drug administration, whereas no signs of alleviation were observed in the remaining animal whose symptoms were so severe as to become apparently blind. In the 2 animals with behavioral improvement, the EEG amplitude increased in the frontal cortex and decreased in the occipital cortex from 20 to 80 min after drug administration (Fig. 9).

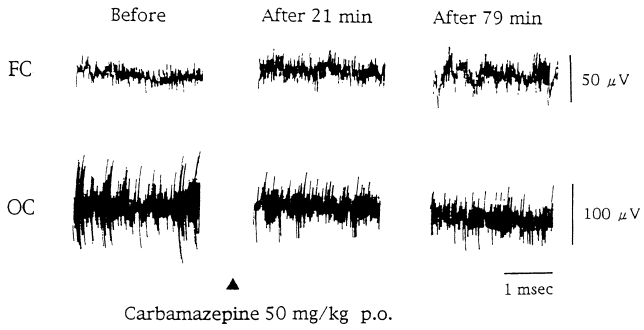

FIG. 9. Effects of carbamazepine on EEGs in Eck's fistula dog.

Effects of flumazenil and TRH on grade IV disturbance. The animal treated with flumazenil recovered from coma immediately after drug administration; it returned to a standing position and slightly regained walking ability. This effect lasted for 2 h, but the EEG pattern did not change (Fig. 10). The animal treated with TRH also recovered from coma immediately after drug administration. It continuously moved in the cage, indicating marked behavioral improvement. The EEG amplitude increase slightly in the frontal cortex for up to 30 min after the drug administration (Fig. 11).

DISCUSSION

In the present study, the dogs gradually became listless and sluggish, developing altered gait and other symptoms of cloudiness of consciousness (snapping and transient catatonia-like symptoms), and became apparently blind following PCS. These symptoms progressed to coma in some animals, although the timing of their occurrence varied among individuals. Previously, Thompson et al. (29) reported that dogs with Eck's fistula which were fed standard dog chow manifested anorexia, weight loss and neurologic signs; however, these symptoms were prevented by adequate nutrition maintained with a palatable diet. In accordance with their study, our Eck's fistula dogs fed with standard dry dog chow, of which compositions were similar to those used by Thompson et al. (29), manifested the similar degree of the encephalopathic syndrome. These behavioral changes mimicked those related to neuropsychiatric disorders associated with hepatic encephalopathy in humans. Since a rapid increase in the blood and CSF levels of ammonia following PCS was reported in Eck's fistula dogs (32), ammonia seems to play a major role in the development of encephalopathy. In the present study, snapping and catatonia-like symptoms developed at 5–13 weeks after PCS. A previous electroencephalographic study using cats suggested that these symptoms were primarily caused by disorders in the limbic system and the posterior part of the hypothalamus (31). Since Eck's fistula dogs have an increased low voltage slow component in the EEG's from the frontal cortex, the above-mentioned symptoms may be due to the cancellation of the cortical suppression of the limbic system

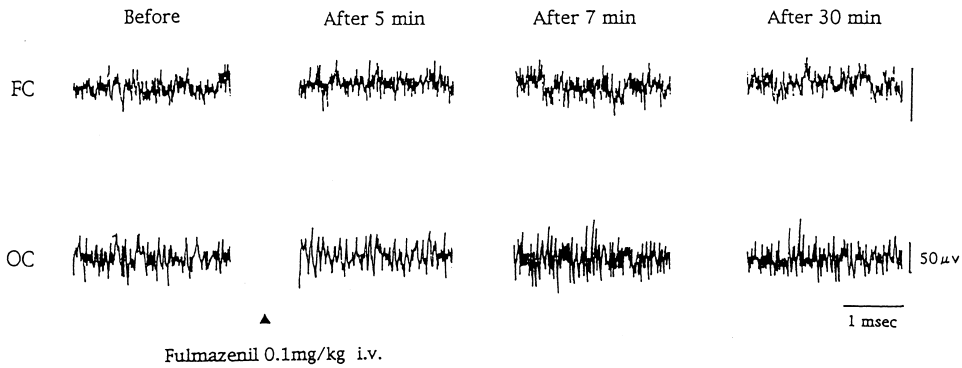

FIG. 10. Effects of flumazenil on EEGs in Eck's fistula dog.

(hippocampus and amygdaloid nucleus) as a result of a decrease in cortical activity. To confirm the involvement of the limbic system in these symptoms, the effect of carbamazepine, which is known to improve the positive symptoms of manic-depressive illness and schizophrenia, on these symptoms was assessed in the present study. Carbamazepine exerted a suppressive effect on aggression, anxiety and epilepsy (26), and has been shown to suppress the activity of the olfactory bulb-limbic pathway, the limbic system, and the hypothalamus-limbic pathway (1,10). The reversal of the positive symptoms by the drug in 2 of 3 dogs suggests the association of limbic abnormalities with grade II consciousness disturbance. At 9 weeks or more after PCS, some dogs showed no response to the examiner or any other external stimuli. The loss of the righting reflex in PCS rats has been considered to be attributable to a suppression of the ascending reticular activating system due to ammonia-induced impairment in energy metabolism in the brainstem (25). To determine if such a suppression occurred in this dog model, a comatose Eck's fistula dog was treated with TRH, which is through to ameliorate consciousness disturbance by stimulating the ascending reticular activating system (20,21). Immediately after the administration, the dog recovered from the coma and continuously moved around, indicating a marked behavioral improvement. This finding suggests that coma in Eck's fistula dogs might be due to a decrease in the activity of the ascending reticular activating system. Recent studies using a benzodiazepine receptor antagonist (5,16) demonstrated the activation of the GABA/benzodiazepine receptor complex in an animal model of hepatic encephalopathy. In the present study, flumazenil, a benzodiazepine antagonist (16), promoted recovery from coma in an Eck's fistula dog. Our findings suggest that GABA/benzodiazepine receptors are activated in comatose (grade IV) Eck's fistula dogs.

In Eck's fistula dogs, an increase in the low voltage slow-wave components in the frontal cortical EEG was observed 5 weeks after PCS, and EEG flattening was observed at weeks 13–15. Since an increase in the slow-wave components is thought to indicate a decrease in cerebral function (11,18), hypofunction of the frontal cortex may occur in Eck's fistula dogs. In contrast, there was an increase in the low voltage fast-wave components in the occipital cortical EEG at 5 weeks after PCS. At weeks 7–9, the high voltage fast-wave components increased, and this increase coincided with a peak severity of positive symptoms (snapping and catatonia-like symptoms). The development of such positive symptoms may be related to disturbed activity of limbic system associated with the increase of high voltage fast-wave components in the occipital cortex.

As the level of consciousness lowers in patients with hepatic encephalopathy, the high frequency component decreases and abnormal triphasic waves appear in the EEG from the frontal cortex. Furthermore, flattening of EEGs is occasionally recorded in comatose patients (17). EEGs from schizophrenic patients are characterized by an increase in δ waves in the frontal cortex and an increase in β waves in the occipital cortex

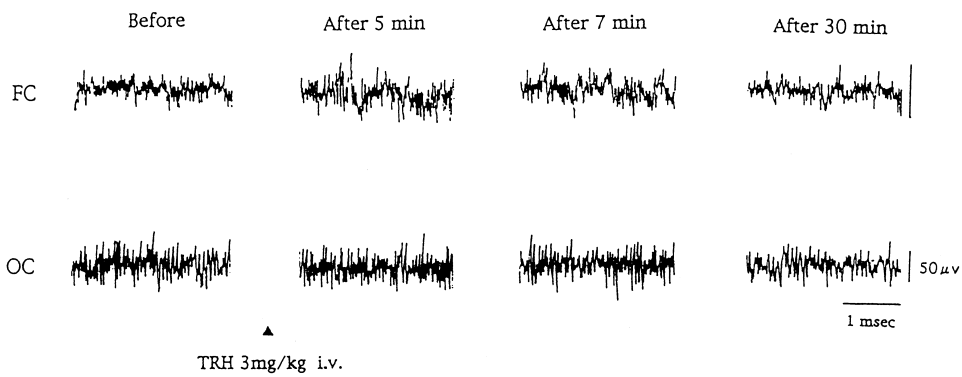

FIG. 11. Effects of TRH on EEGs in Eck's fistula dog.

(28). Development of epileptic discharges in association with positive symptoms has also been reported (4). EEG findings in manic-depressive patients also include an increase in β waves and a decrease in the voltage in the occipital cortex (6,22). In the Eck's fistula dogs, flattening of the EEGs was recorded from the frontal cortex as observed in patients with hepatic encephalopathy. These findings indicate that severe CNS disorders develop in this dog model.

The VEP changes following PCS in the Eck's fistula dogs consisted of an increase in the latencies for P30 and N45 and the amplitude between the two peaks. Visual stimuli produced an excitation in the visual cortex via the retina and the lateral geniculate body. The VEP changes observed in the Eck's fistula dogs may be related to changes occurring in the lateral geniculate body.

Previously we examined the effect of lactitol, which lowers the blood ammonia levels by preventing the absorption of ammonia from the cecum and colon, in the Eck's fistula dogs (33). Chronic treatments with lactitol suppressed the behavioral and EEG changes caused by PCS. Combined with the clinical evidence that lactitol improves the hepatic encephalopathy (14), the results with lactitol support the suggestion that the present Eck's fistula dog is an appropriate animal model for the human hepatic encephalopathy.

In summary, Eck's fistula dogs showed consciousness disturbance and behaviorally abnormal symptoms, including snapping, catatonia-like symptoms, blindness and coma. This dog model also showed pronounced EEG changes, i.e., a gradual decrease in the voltage and frequency in the frontal cortex after PCS and the appearance of high voltage fast waves in association with the positive mental symptoms at weeks 7–9 and a subsequent decrease in the voltage in the occipital cortex. Both the abnormal behavioral symptoms and EEG patterns observed in Eck's fistula dogs were ameliorated by either carbamazepine, flumazenil or TRH treatment. Based on these behavioral and EEG findings, it can be concluded that Eck's fistula dogs provide a useful model of hepatic encephalopathy.

REFERENCES

1. Albright, P. S.; Burnham, W. M. Development of a new pharmacological seizure model: effects of anticonvulsants on cortical- and amygdala-kindled seizures in the rat. Epilepsia 21:681–689; 1980.
2. Bassett, M. L.; Mullen, K.; Skolnick, P.; Jones, E. A. Amelioration of hepatic encephalopathy by pharmacologic antagonism for the $GABA_A$ benzodiazepine receptor complex in a rabbit model of fulminant hepatic failure. Gastroenterology 93:1069–1077; 1987.
3. Condon, R. E. Effect of dietary protein on symptoms and survival in dogs with an Eck fistula. Am. J. Surg. 121:107–114; 1971.
4. Flor-Henry, P. Psychosis and temporal lobe epilepsy: a controlled investigation. Epilepsia 10:363–395; 1969.
5. Gammal, S.; Basile, A. S.; Geller, D.; Skolnick, P.; Jones, E. A. Reversal of the behavioral and electrophysiological abnormalities of an animal model of hepatic encephalopathy by benzodiazepine receptor ligands. Hepatology 11:371–378; 1990.
6. Harding, G.; Jeavons, P. M.; Jennet, F. A.; Drummond, P.; Sheridan, M.; Howells, G. W. The electroencephalogram in three cases of periodic psychosis. Electroenceph. Clin. Neurophysiol. 21:59–66; 1966.
7. Hindfelt, B.; Plum. F.; Duffy, T. E. Effect of acute ammonia intoxication on cerebral metabolism in rats with portacaval shunts. J. Clin. Invest. 59:386–396; 1977.
8. Inui, K.; Matsuda, A.; Chaki, H.; Makita, I.; Kokuba, Y.; Kataoka, M.; Sato, H. Effect of MSH-G, an amino acids granule, on hepatic encephalopathy in portacaval anastomosis rats. Folia Pharmacol. Jpn. 102:379–388; 1993.
9. James, J. H.; Hodgman, J. M.; Funovics, J. M.; Fishcer, J. E. Alterations in brain octopamine and brain tyrosine following portacaval anastomosis in rats. J. Neurochem. 27:223–227; 1976.
10. Jurna, I. Electrophysiological effects of antiepileptic drugs. Handb. Exp. Pharm. 74:611–658; 1985.
11. Karson, C. N.; Coppola, R.; Morihisa, J. N.; Weinberger, D. R. Computed electroencephalographic activity mapping in schizophrenia. Arch. Gen. Psychiatry 44:514–517; 1987.
12. Kido, Y.; Sugiyama, K.; Nakao, S.; Kashiyama, E.; Suda, T.; Miyamoto, G.; Shimazu, T.; Shintani, S.; Kohri, H. Study of the ameliorating effects of an enteral nutrient for liver failure on hepatic encephalopathy: Effect of SF-1008C on plasma and brain free amino acids, intracerebral amine concentrations and electroencephalogram in portacaval shunted rats with ammonia loading. Folia Pharmacol. Jpn. 88:47–56; 1986.
13. Kimoto, S. Handbook of experimental surgery for medicine and biology. Tokyo: Igaku-Shoin; 1967:271–290.
14. Lanthier, P. L.; Morgan, M. Y. Lactitol in the treatment of chronic hepatic encephalopathy: an open comparison with lactulose. Gut 26:415–420; 1985.
15. Lim, R. K. S.; Chan-Nao, Liu; Moffitt, R. L. A stereotaxic atlas of the dog's brain. Springfield: C. C. Thomas Publisher, 1960.
16. Luo, Z.; Watanabe, H.; Ohta, H.; Matsumoto, K. Antagonistic effects of a benzodiazepine antagonist, flumazenil, on hypnotic sedative midazolam. Jpn. Pharmacol. Ther. 177:2533–2543; 1990.
17. Maier, K. P. Diagnose der hepatischen Enzephalopathie. Dtsch. med. Wschr. 113:1882–1885; 1988.
18. Miyauchi, T.; Tanaka, K.; Hagimoto, H.; Miura, T.; Kishimoto, H.; Matsushita, M. Computerized EEG in schizophrenic patients. Biol. Psychiatry 28:488–494; 1990.
19. Mousseau, D. D.; Butterworth, R. F. Current theories on pathogenesis of hepatic encephalopathy. Proc. Soc. Exp. Biol. Med. 206: 329–344; 1994.
20. Ogawa, N.; Mizuno, S.; Nukina, I.; Tsukamoto, S.; Mori, A. Chronic thyrotropin releasing hormone (TRH) administration on TRH receptors and muscarinic cholinergic receptors in CNS. Brain Res. 263:348–350; 1983.
21. Ogawa, N.; Yamawaki, Y.; Kuroda, H.; Ofuji, T.; Itoga, E.; Kito, S. Discrete regional distribution of thyrotropin releasing hormone (TRH) receptor binding in monkey central nervous system. Brain Res. 205:169–174; 1981.
22. Perris, P. EEG techniques in the measurement of the severity of depressive syndromes. Neuropsychobiology 1:16–25, 1975.
23. Record, C. O. Neurochemistry of hepatic encephalopathy. Gut 32:1261–1263; 1991.
24. Schenker, S.; Breen, K. J.; Hoyumpa, A. M. Hepatic encephalopathy: current status. Gastroenterology 66:121–151; 1974.
25. Schener, S.; Mendelson, J. H. Cerebral adenosine triphosphate in rats with ammonia-induced coma. Am. J. Physiol. 206:1173–1176; 1964.
26. Schumtz, M. Carbamazepine. Handb. Exp. Pharma. 74:479–506; 1985.
27. Szerb, J. C.; Butterworth, R. F. Effect of ammonium ions on synaptic transmission in the mammalian central nervous system. Prog. Neurobiol. 39:135–153; 1992.
28. Takizawa, Y.; Wada, Y.; Horita, M.; Kitazawa, S.; Futamata, H.; Hashimoto, T. Quantitative analysis of EEG background activity in drug native schizophrenic patient. Jpn. J. Clin. Pathol. 42:759–763; 1994.
29. Thompson, J. S.; Schafer, D. F.; Haun, H.; Schafer, G. J. Adequate diet prevents hepatic coma in dogs with Eck fistulas. Surg. Gyn. & Obs. 162:126–130; 1986.
30. Yamamoto, K.; Kido, R. Comparative studies on the effects of tranquilizers, barbiturates and morphine with implanted electrodes in cats and dogs. Brain and Nerve 14:591–608; 1962.

31. Yamamoto, K.; Sawada, T.; Naito, Y.; Kido, R. Analysis of central inhibitory mechanisms of nitrazepam. Brain and Nerve 21:488–502; 1969.
32. Watanabe, M.; Ozaki, T.; Hirata, Y.; Yoshikuni, Y.; Kimura, K.; Suzuki, K.; Satoh, S. The effect of lactitol (NS-4) on the concentrations of ammonia and amino acids in blood and cerebrospinal fluid in Eck fistula (portacaval-shunted) dogs. Folia Pharmacol. Jpn. 105:389–402; 1995.
33. Watanabe, M.; Ozaki, T.; Mushiroi, T.; Ukai, Y.; Ueda, F.; Kimura, K.; Katoh, M.; Matsumoto, A.; Kotani, E.; Itoh, S.; Yamaguchi, K.; Kyuki, K.; Suzuki, K.; Sato, S. Ameliorating effect of lactitol on experiemtnal hepatic encephalopathy in Eck fistula dogs. Folia Pharmacol. Jpn. 105:403–413; 1995.