EXPERIMENTAL ACUTE HEPATIC ENCEPHALOPATHY: COMPARISON OF THE ELECTROENCEPHALOGRAPHIC CHANGES IN THE LIVERLESS AND IN THE EVISCERATED RAT

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SUMMARY

1. The present work was carried out to compare the electroencephalographic changes in liverless rats and eviscerated rats and to determine whether substances released from the intestine, in particular ammonia, play a major part in the mechanism of hepatic encephalopathy.

2. The animals were prepared according to a three-stage procedure: ligation of inferior vena cava; 3 weeks later, end-to-side portacaval shunt; 2 days later, removal of the liver (liverless rats) or removal of the liver, spleen, stomach, intestine and pancreas (eviscerated rats).

3. In liverless rats, the electroencephalographic changes began 4–8 h after hepatectomy with a predominance of 'slow' sleep pattern followed by increasing changes, which consisted successively of (a) alteration of, then disappearance of, spindles of high-voltage waves; (b) predominance of slow waves; (c) depression in voltage and finally flat tracing. The mean duration of survival was 18.4 h. Mean plasma ammonia concentration 15 h after hepatectomy was 353 μmol/l.

4. In eviscerated rats, the electroencephalographic changes were similar. The mean duration of survival was 21.3 h, which is not statistically different from that of liverless rats. Mean plasma ammonia concentration 15 h after evisceration was 148 μmol/l, a value significantly lower than that of liverless rats.

5. These results suggest that ammonia, and substances released from the intestine in general, play no part or at most a minor role in the mechanism of hepatic encephalopathy of the liverless rat.

Key words: ammonia, electroencephalography, hepatectomy, hepatic coma.

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One of the consequences of hepatectomy in the rat (Meehan, 1954) as well as in other mammals (Lempinen, Soyer & Eiseman, 1973; Maddock & Svedberg, 1938; Mann, 1921; Mann, 1944; Svedberg, Maddock & Drury, 1938) is the development of neuropsychic disorders. These disorders are of two different types: in the first period after hepatectomy, they result from hypoglycaemia and are corrected by intravenous administration of glucose; in the second period, they are no longer corrected by glucose and correspond to hepatic encephalopathy. It is generally admitted that neurotoxic substances released from intestine, in particular ammonia, play a major part in the mechanism of hepatic encephalopathy (Breen & Schencker, 1972).

The present work was carried out (1) to establish the electroencephalographic (EEG) changes in liverless rats, and (2) to compare these changes with those observed in eviscerated rats. Evisceration consists in hepatectomy associated with removal of other splanchnic viscera, in particular the intestine; therefore, if hepatic encephalopathy develops in the eviscerated rat, it would be reasonable to conclude that its mechanism is independent of substances released from the intestine, such as ammonia.

**MATERIAL AND METHODS**

Male rats (Charles River, St Aubin-lès-Elbeuf, France) weighing 300–450 g, fed *ad libitum* with biscuits (Biscuits no. 113, Usine d’Alimentation Fonctionnelle, Villemoisson-sur-Orge, France), were used.

**Normal rats**

Behaviour, EEG and responses to stimuli were studied in twenty unrestrained normal rats. EEG was recorded from four silver electrodes implanted through the skull in contact with the brain and fixed with dentist’s cement. The implantation was performed 10–15 days before the recording, under pentobarbital anaesthesia. Two electrodes were placed symmetrically on the right and left parietal cortex, and two on the right and left frontal cortex. EEG was recorded by means of a polygraph (ECEM, Paris, France) with a time-constant of 0.3 s. A longitudinal, frontal–parietal, right or left indifferently, derivation and a transverse, parietal–parietal, derivation were recorded.

Motor and EEG responses to a tactile and an auditory stimulus were tested. The tactile stimulus involved stroking the rat the wrong way and the auditory stimulus was a brief, shrill sound. The motor or the EEG response was considered to be present when the stimulus was followed, respectively, by movements of the head, and/or limbs, and/or trunk, and/or tail, or by an active or a quiet wakefulness EEG pattern (see the Results section).

**Sham-operated, liverless and eviscerated rats**

Sham-operated, liverless and eviscerated rats were prepared according to a three-stage procedure. The first and second stages, identical in the three groups of rats, consisted of ligation of the inferior vena cava between the renal and hepatic veins, followed, 3 weeks later, by end-to-side portacaval shunt (Bismuth, Benhamou & Lataste, 1963; Lee & Fisher, 1961). The third stage was performed 2 days later and consisted of either removal of the liver with the retrohepatic segment of the inferior vena cava (liverless rats), or removal of the liver with
the retrohepatic segment of the inferior vena cava and of spleen, stomach, intestine and pancreas (eviscerated rats), or laparotomy lasting 20 min (sham-operated rats). All the surgical procedures were performed under ethyl ether anaesthesia. Cephalothin (Keflin; Eli Lilly, Paris, France), 10 mg/100 g body wt., and gentamycin (Gentaline, Unilabo, Levallois, France), 0·2 mg/100 g body wt., were administered intraperitoneally every day after the second stage. The rats were fasting for the 24 h preceding the third stage.

After the third stage, the animals were immobilized in restraining cages placed in a room where temperature was regulated so that rectal temperature was maintained between 26° and 28°C. In the liverless and the eviscerated rats, a glucose solution was infused into an iliac vein in order to supply 1·1 mmol h⁻¹ kg body wt.⁻¹ for the first 8 h, and 2·22 mmol h⁻¹ kg body wt.⁻¹ for the following hours: this supply was achieved by infusion of 0·5 ml/h, and then 1 ml/h, of a solution with a glucose concentration ranging from 0·56 to 0·83 mol/l. In the eviscerated rats, in addition to glucose, insulin (Insulin Novo), 0·40 unit h⁻¹ kg body wt.⁻¹, was injected intravenously. In the sham-operated rats, glucose was replaced by a solution of sodium chloride (0·15 mol/l); 0·5 ml/h was infused for the first 8 h, and 1 ml/h for the next hours.

Sham-operated rats were subdivided into two subgroups. The animals of one subgroup were not refrigerated: their rectal temperature remained between 34° and 36°C. The animals of the other subgroup were refrigerated by means of an extracorporeal circulation between the iliac artery and vein, blood being passed through tubes in water at 4°C; extracorporeal circulation flow was set so that rectal temperature was maintained between 26° and 28°C.

In all animals, a catheter was inserted in the iliac artery for measuring blood pressure and taking blood samples.

Behaviour, EEG and responses to stimuli were studied in the same way as in normal animals. Implantation of the electrodes was performed 10–15 days before the recording, between the second and the third surgical stages. The EEG was recorded continuously after the third surgical stage. Responses to stimuli were looked for every 30 min after the third surgical stage.

Arterial plasma ammonia concentration was measured by the technique of Miller & Rice (1962) based on the use of a cation-exchange resin (Hyland, Costa Mesa, California, U.S.A.) and arterial blood glucose by the glucose oxidase technique (Biotrol, Paris, France). Measurements were performed 1, 5 and 15 h after the third surgical stage.

The groups were compared using the Wilcoxon two-sample rank test (Snedecor & Cochran, 1967).

RESULTS

Normal rats

In unrestrained normal rats, four states of wakefulness and sleep were recognized on the basis of behaviour, the EEG obtained from the two derivations employed (Fig. 1), and motor and EEG responses to stimuli. (1) The state of active wakefulness was characterized by alertness and explorative behaviour; the EEG consisted, on the transverse derivation, of low-voltage, fast activity (20–30 Hz, 30–50 μV) and, on the longitudinal derivation, of intermediate voltage, almost sinusoidal, regular waves (6–8 Hz, 200–250 μV) mixed with a low-voltage, fast activity similar to that recorded from the transverse derivation; the shape and frequency of these waves are those of theta rhythm. (2) The state of quiet wakefulness was characterized by absence of movements; the EEG consisted, on both derivations, of a low-voltage, fast
activity (20–30 Hz, 30–50 μV); motor and EEG responses to stimuli were present and immediate. (3) The state of ‘slow’ sleep was characterized by absence of movements; the EEG consisted, on both derivations, of consecutive segments of intermediate voltage, slow waves (1–3 Hz, 100–400 μV) and spindles of high-voltage waves (11–18 Hz, 300–600 μV); motor and EEG responses to stimuli were present and immediate. (4) The state of paradoxical sleep was characterized by muscular hypotonicity and absence of movements except for sudden motor episodes; the EEG was similar to that of the state of active wakefulness; motor and EEG responses to stimuli were absent or obtained only with intense stimulation.

Non-refrigerated, sham-operated rats

Eight sham-operated rats, immobilized for 24 h in restraining cages, were studied. None of the animals died. Systolic blood pressure ranged from 140 to 180 mmHg. After the third surgical stage, the animals were continuously in the state of active wakefulness or in the state of quiet wakefulness; occasionally, slow sleep EEG patterns of 4–7 s duration or spindles of high-voltage waves occurred. Motor and EEG responses to stimuli were immediate and intense. Blood glucose, measured 1, 5 and 15 h after the third surgical stage, ranged from 4.45 to 7.79 mmol/l. The mean values for plasma ammonia are given in Table 1.

Refrigerated, sham-operated rats

Eleven refrigerated sham-operated rats, immobilized for 24 h in restraining cages, were studied. None of the animals died. Systolic blood pressure ranged from 140 to 160 mmHg. The behaviour, EEG, and motor and EEG responses were similar to those of non-refrigerated, sham-operated rats, except for a reduced voltage of the EEG. Blood glucose, measured 1, 5 and 15 h after the third surgical stage, ranged from 4.45 to 11.11 mmol/l. The mean values for plasma ammonia are given in Table 1.

Liverless rats

Twenty-four rats were hepatectomized; three of them died less than 11 h after hepatectomy
Experimental acute hepatic encephalopathy 603

because of intraperitoneal haemorrhage and were not included in this study. The mean duration of survival of the twenty-one other rats was 18.4 h (SD 4.9). Systolic blood pressure ranged from 140 to 160 mmHg during the period 8-18 h after hepatectomy, and then gradually decreased until death; the onset of the fall in blood pressure occurred within period 3 (which is defined below).

The course of brain disorders after hepatectomy was divided into three periods on the basis of the changes in behaviour, EEG and response to stimuli.

Period 1 lasted from 1 to 2.5 h. The animals were motionless. The EEG consisted of intermediate voltage, slow waves (1–3 Hz, 200–300 μV). Motor and EEG responses to stimuli were absent. Period 1 ended suddenly with the arousal of the animals.

Period 2 lasted from 2 to 11 h. This period was characterized by a relative, transient normalization in behaviour, EEG and responses to stimuli. In early period 2, the animals were restless, and then became less agitated, and eventually were motionless. In early period 2, the EEG consisted of alternation of active and quiet wakefulness patterns; then, 3–5 h after hepatectomy, slow sleep patterns occurred, increased gradually in duration, and finally represented the whole of the tracings; these slow sleep patterns were never followed by paradoxical sleep patterns. The motor response to stimuli was present; the EEG response was present and consisted in active wakefulness patterns lasting 20 s or more.

Period 3 began 4–13 h after hepatectomy and lasted from 5 to 25 h. This period was characterized by increasing alterations in EEG and responses to stimuli. This period was subdivided into periods 3A, 3B and 3C. Period 3A lasted from 5 to 12 h; the animals were motionless; the EEG tracings were roughly similar to those of normal slow sleep, except that the spindles, albeit distinguishable, deteriorated as indicated, in particular, by a widening of the high-voltage waves (Fig. 2); the motor response to stimuli was present; the EEG response to stimuli was present and consisted in active wakefulness patterns lasting from 15 to 20 s. Period 3B was observed only in sixteen out of the twenty-one liverless rats, five animals having died during period 3A; period 3B lasted from 2 to 11 h; the animals were motionless; the EEG consisted almost exclusively of slow waves (2–4 Hz, 150–250 μV) (Fig. 2); a few spindles of high-voltage

Table 1. Plasma ammonia in rats 1, 5 and 15 h after sham-operation, hepatectomy or evisceration

<table>
<thead>
<tr>
<th></th>
<th>1 h</th>
<th>5 h</th>
<th>15 h</th>
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<tbody>
<tr>
<td>Sham-operated, non-refrigerated rats (n = 8)</td>
<td>95 ± 21</td>
<td>97 ± 29</td>
<td>151 ± 68</td>
</tr>
<tr>
<td>Sham-operated, refrigerated rats (n = 11)</td>
<td>91 ± 21</td>
<td>109 ± 18</td>
<td>98 ± 18</td>
</tr>
<tr>
<td>Liverless rats (n = 21)</td>
<td>151 ± 22</td>
<td>272 ± 47</td>
<td>353 ± 69</td>
</tr>
<tr>
<td>Eviscerated rats (n = 7)</td>
<td>173 ± 23</td>
<td>151 ± 16</td>
<td>148 ± 18</td>
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waves were distinguishable in early period 3B, and then gradually intermingled with the rest of the tracings; the motor response to stimuli fluctuated, being present at one time, absent at another in the same animal; the EEG response consisted during early and in late period 3B respectively of active wakefulness patterns, abnormal in that the theta rhythm was replaced

![Graph showing EEG recordings from FP and PP derivations in periods 3A, 3B, and 3C.](image)

**FIG. 2.** Liverless rat. EEG recorded from a longitudinal, frontal-parietal derivation (FP) and a transverse, parietal-parietal derivation (PP) in periods 3A, 3B and 3C (see the Results section for the definition of these periods).

by intermediate voltage, sinusoidal slow waves (2–4 Hz, 200–250 μV), and quiet wakefulness patterns; immediately after stimulation and preceding the wakefulness EEG patterns, bursts of high-voltage waves (5–6 Hz, 200–600 μV), lasting 1–2 s, took place (Fig. 3); the duration of the EEG responses decreased from 15 to 1 s from the onset to the end of period 3B. Period 3C was observed only in nine out of the twenty-one liverless rats, the others having died during

![Graph showing EEG recordings from FP and PP derivations in period 3B.](image)

**FIG. 3.** Liverless rat. EEG recorded from a longitudinal, frontal-parietal derivation (FP) and a transverse, parietal-parietal derivation (PP) in period 3B. The auditory stimulus is followed by a burst of high-voltage waves.
Experimental acute hepatic encephalopathy

period 3A or 3B; period 3C lasted from 1 to 9 h; the EEG voltage decreased (Fig. 2) and finally the tracings were flat; the motor response to stimuli was absent; the EEG response consisted only in a burst of waves whose voltage became progressively lower.

The course of the EEG changes in the twenty-one liverless rats under study is shown schematically in Fig. 4: it appears that the EEG alterations were gradually increasing and that remissions were observed in none of the animals.

Blood glucose, measured 1, 5 and 15 h after hepatectomy, ranged from 7.23 to 16.11 mmol/l. The mean values for plasma ammonia 1, 5 and 15 h after hepatectomy are given in Table 1.
Eviscerated rats

Eight animals were eviscerated; one of them died 11 h after evisceration because of intra-abdominal haemorrhage and was excluded. The mean duration of survival of the seven other animals was 21.3 h (SD 2.5). Changes in systolic blood pressure, behaviour, EEG and responses to stimuli were not notably different from those observed in the liverless rats. The course of the EEG alterations is shown schematically in Fig. 5.

Blood glucose, measured 1, 5 and 15 h after evisceration, ranged from 7.23 to 15.56 mmol/l. The mean values for plasma ammonia 1, 5 and 15 h after evisceration were significantly lower than those of the liverless rats (Table 1).

DISCUSSION

The techniques of hepatectomy and evisceration employed in this work have been designed in preliminary experiments not reported in this paper. The first surgical stage, ligation of inferior vena cava, is intended to promote the development of anastomoses between inferior and superior caval territories; in the rat, a segment of inferior vena cava is included in the liver, and the removal of this segment is required to achieve total hepatectomy; the caval-caval anastomoses enable the rat to tolerate well the interruption of blood flow through inferior vena cava when the liver is removed. The second surgical stage, end-to-side portacaval shunt, is an indispensable prerequisite to hepatectomy; if not performed before hepatectomy, acute ligation of the portal vein would result in severe circulatory collapse and death 3–4 h after hepatectomy. Portacaval shunt and hepatectomy have been performed in two distinct stages separated by an interval of 2 days: preliminary experiments have shown that operative mortality amounts to 20% when portacaval shunt and hepatectomy are performed in a single surgical stage, and that operative mortality is less than 5% when they are performed in two successive distinct stages. Portacaval shunt is not an indispensable prerequisite to evisceration; however, it has been performed before evisceration in order that a valid comparison between the eviscerated and the liverless animals can be made. The levels of glucose infusion have been chosen to prevent post-hepatectomy hypoglycaemia. Rectal temperature has been maintained at 26°C: in preliminary experiments, it had been observed that liverless rats with rectal temperature of 28°C or more were subject to episodes of seizures occurring repeatedly, with ensuing perturbations in the course of the EEG changes, and that the duration of survival was less than 15 h; this inverse relation between body temperature and duration of survival has already been observed in liverless animals (Ingle & Nezamis, 1950; Bollman & Hook, 1968).

The characterization of the four states of sleep and wakefulness used in this work is based on incomplete criteria, since electric activity of subcortical formations, electromyograms and eye movements have not been recorded; however, these incomplete criteria appeared to be sufficient to distinguish the well-recognized states of wakefulness and sleep, at least in the normal rat. The choice of derivations employed for the EEG resulted from the following considerations: in the states of active wakefulness and of paradoxical sleep, hippocampal theta rhythm and neocortical fast activity have been demonstrated in different species of mammals (Green & Arduini, 1954; Jouvet, 1967), in particular in the rat (Brugge, 1965); the hippocampal theta rhythm is well collected by surface electrodes on the parietal cortex (Calvet, Holingue, Guillard & Scherrer, 1960); the hippocampal theta rhythm, being synchronous and symmetrical, is
cancelled on the tracings recorded from the transverse parietal–parietal derivation. By contrast, a longitudinal frontal–parietal derivation collects both hippocampal theta rhythm and neocortical fast activity. Thus a longitudinal and a transverse derivation are sufficient to characterize the four states of wakefulness and sleep as they have been defined in the rat (Brugge, 1965).

In the liverless rat, the EEG alterations of period 1 are likely to be the unspecific consequence of anaesthesia and surgery: these alterations clear up spontaneously in 1–2 h. In period 2, EEG is essentially normal; in early period 2, EEG mainly consists of patterns of active wakefulness or quiet wakefulness, a finding which parallels the normal behaviour of the liverless dog in the first hours after hepatectomy when blood glucose is maintained at normal concentrations (Mann, 1944). In the late period 2, patterns of slow sleep appear and increase progressively in number and duration; this slow sleep, although its EEG pattern is normal in shape, is abnormal in that (1) it is not observed in the restrained sham-operated rats and (2) it is not followed by paradoxical sleep patterns. This slow sleep might result from a depression of the waking centres or from a block in the transition from slow to paradoxical sleep, as produced by certain drugs (Passouant, Cadilhac & Ribstein, 1972). In the liverless rat, the EEG response to stimulation decreases steadily; in particular, the duration of active wakefulness patterns after stimulation becomes gradually shorter. By contrast, the motor response fluctuates more than the EEG response, since this motor response may be absent at one moment and present a moment later in the same liverless rat. Thus, in the liverless rat, the course of the alterations of the EEG and the EEG response to stimuli is characterized by a gradual deterioration, without any improvement.

The EEG alterations after hepatectomy are not related to hypoglycaemia, since blood glucose was maintained above 6.1 mmol/l in all the animals under study. Nor are these EEG alterations related to hypothermia, since they were not observed in refrigerated sham-operated rats. Nor are they the consequence of low blood pressure, since they developed in animals with normal systolic arterial pressure; in addition, they are not present in all the animals with low blood pressure.

In the eviscerated rat, the EEG alterations are similar to those observed in the liverless rat: this resemblance does not prove, but suggests, that encephalopathy induced by hepatectomy and encephalopathy induced by evisceration are of the same nature. The initial values for plasma ammonia have been found to be abnormally high in the liverless rats as well as in the eviscerated and the sham-operated rats; this initial hyperammonaemia is the consequence of portacaval shunt performed previously to the third surgical stage in the three groups of animals. However, the later values for plasma ammonia are significantly different in the liverless and in the eviscerated rat, increasing in the former and remaining unchanged in the latter; this difference is probably related to the presence or the absence of the intestine, a major source of ammonia. Although the 5 h and 15 h values for plasma ammonia are different in the liverless and in the eviscerated rat, behaviour, EEG changes and responses to stimuli are similar in these two groups of animals; this suggests that the increase in plasma ammonia has no part or plays only a minor role in the pathogenesis of the encephalopathy induced by hepatectomy. This speculation could be extended to all the hypothetical neurotoxic substances released from intestine which have been suspected as being responsible for hepatic encephalopathy.

The stereotyped, progressive, remissionless course of the EEG alterations after hepatectomy renders this model of experimental hepatic encephalopathy particularly suitable to the assess-
ment of the procedures proposed for the treatment of this condition: any transient alleviation induced by the procedure tested would certainly indicate therapeutic efficiency, since improvement never occurs spontaneously.

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