

SODIUM 4-HYDROXYBUTYRATE

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Summary—The author presents a general review of the pharmacology of sodium 4-hydroxybutyrate, the principal elements of which are:

- (a) very low toxicity, and metabolic application;
- (b) the hypnotic activity, which does not cause a decrease in the intensity of oxidative processes;
- (c) the potentiating action on anaesthetics and neuroplegics, and the antagonizing action against certain convulsants;
- (d) the absence of ventilatory depression;
- (e) the facilitating action on hypothermia.

The EEG study and the stereotaxic study of the cerebral evoked potentials, as well as the cardiovascular and antishock activity are described. The author insists on the strong potassium and cholesterol lowering effects of the drug, and on its protein sparing action. The mechanism of action is discussed. Numerous data permit to assume that this mechanism is essentially characterized by an activation of the pentose pathway. Finally, a brief summary is presented of its applications in anaesthesiology, obstetrics, psychiatry and in internal medicine.

INTRODUCTION

THE importance of butyric acid in cell metabolism as well as its possible role in the functions of certain organs such as the nervous system had induced our research group to study the effects of its i.v. injection in the animal (JOUANY, 1960). A definite hypnotic action was observed, but urine analysis showed that most of the drug injected undergoes β -oxidation.

In an attempt to modify this metabolic fate, an OH-group was introduced on carbon⁴ of butyric acid in the hope that its electro-negative properties would alter the electronic configuration of the molecule enough to interfere with β -oxidation. This led to the preparation of sodium 4-hydroxybutyrate starting from butyrolactone. At the same time, as γ -aminobutyric acid does not cross the brain-blood barrier, it was hoped that 4-hydroxybutyrate in the brain might function like a GABA precursor facilitating its synthesis in the brain. Recently BESSMAN and FISHBEIN (1963) have detected the presence of 4-hydroxybutyric acid in physiological amounts in the brain under normal conditions.

METHODS

Route of administration

The i.v. and i.p. routes were chosen for most experiments. The substance is also active orally, if the dose is doubled. In the clinic, until recently, the i.v. route had been used nearly exclusively (LABORIT *et al.*, 1960a and 1961a; BLUMENFELD *et al.*, 1962). Oral administration is being now actively investigated.

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Albert Lasker Award, 1957.

Quantitative determination of urinary excretion

M. REYNIER, in our group, developed a quantitative determination technique based on the conversion of 4-hydroxybutyrate into the lactone and then into succinic acid. 4-hydroxybutyrate urinary excretion can be determined on the basis of the succinic acid formed. (Technique described in JOUANY *et al.*, 1960).

TABLE 1a

Hours	Urine ml	Succinic acid mg	4-OH-Butyrate mg
1st	36.2	156	310
2nd	35	141	250
3rd	17.4	66	130
4th	3	traces	

TABLE 1b

Hours	Urine ml	Succinic acid mg	4-OH-Butyrate mg
1st	52	294	600
2nd	25	168	340
3rd	23	70	140
4th	15	12.4	25
5th		traces	

Urinary excretion of 4-hydroxybutyrate following conversion into succinic acid

Table 1a: 12 kg dog—6 gm of 4-OHB, i.v.

Table 1b: 24 kg dog—12 gm of 4-OHB, i.v.

Table 1a refers to a dog weighing 12 kg that had received 6 gm of sodium 4-hydroxybutyrate i.v. as a 20 per cent solution. Table 1b refers to a dog weighing 24 kg that had received 12 gm of sodium 4-hydroxybutyrate i.v. Urine was collected every hour after the injection until the animal returns to normal.

E.E.G. study

We will only give a brief description of our E.E.G. study in man. Our findings were confirmed by CAMPAN and ESPAGNO (1961). A more detailed study of these effects was carried out in the U.S.A. by DRAKONTIDES *et al.* (1962), and following our request, by SCHNEIDER *et al.* (1963). The derivations used in the works presented in Fig. 1 are mentioned.

Stereotaxic study of evoked potentials

Material: 40 rats and 10 cats

Animals are anaesthetized with ether for tracheotomy and craniotomy; they are then curarized and ventilated artificially. Temperature is maintained between 37° and 38°C. Cortical activity is recorded between a silver focal electrode terminated by a small sphere placed on the primary area of the cortex, and a larger silver electrode located on inactive tissues. Bipolar derivations with two concentric insulated electrodes, except at the tip,

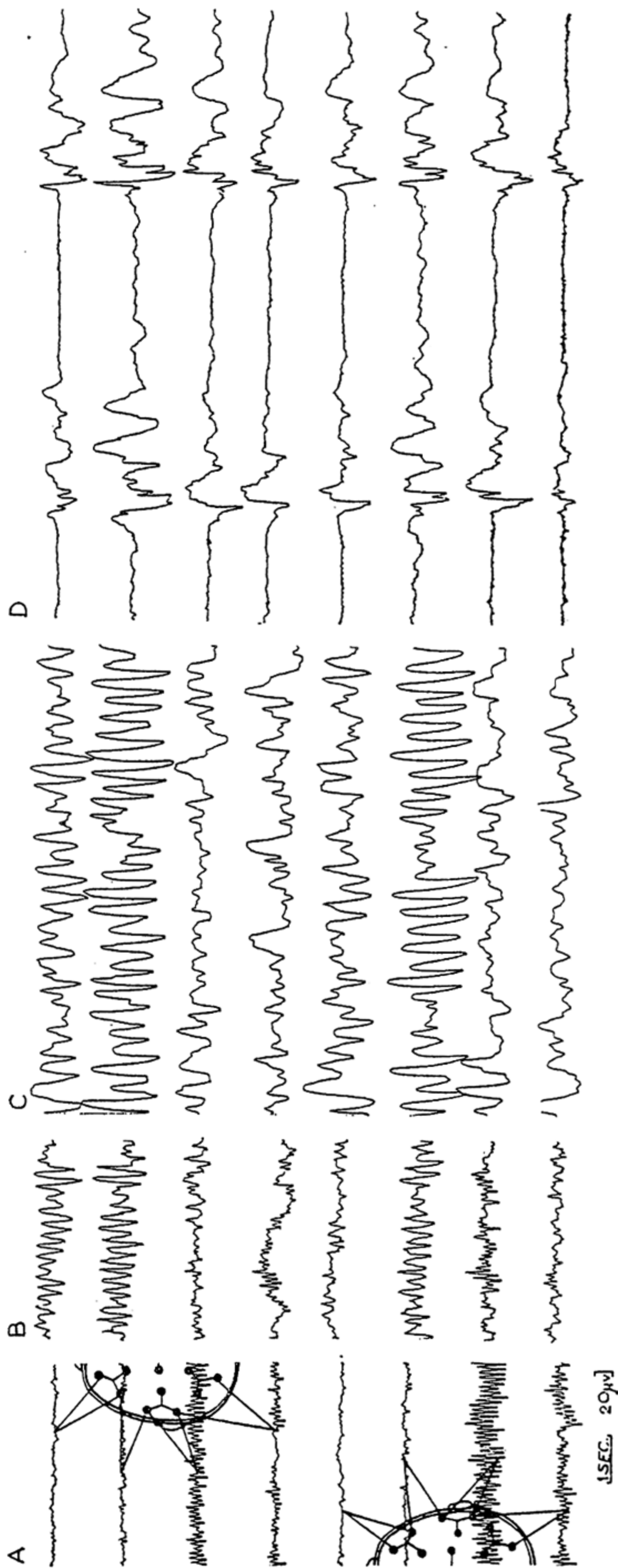


FIG. 1. The three EEG phases normally induced in man with 4-hydroxybutyrate (8 gm in 5 minutes, i.v.)

- A—tracing before injection.
 B—theta waves rhythmic phase (with progressive disappearance of the alpha activity).
 C—monomorphic beta wave phase.
 D—stage of electrical inactivity and of “K-complexes”.
 According to SCHNEIDER *et al.* (1963).

were used at the level of the thalamus and of the mesencephalic reticular formation. The distance between the external and focal electrodes is about 1 mm. Connections are such that a positive variation of the potential at the level of the focal electrode would produce a downward deflexion. Stimulus applied: 8 V electrical shock; duration: generally 0.5 sec. Peripherally, two needle electrodes are implanted at the extremity of each hind paw of the animal. Localization of thalamic and reticular structures according to the atlas of JASPER and AJMONE-MARSAN (1954). The animal is immobilized in a Horsley-Clarke type apparatus. Histological verification of placement of the intrathalamic and reticular electrodes was carried out following perfusion of the brain with 10 per cent formaldehyde and slicing of frozen tissue. Slices were stained by the Nissl method.

Monosynaptic reflexes

Material: 15 rabbits—average weight 2 kg

Decerebration under urethane anesthesia. Artificial ventilation. The activity of the monosynaptic medullary reflex is studied by recording the extension movements of the second segment of the hind leg in response to the excitation of the rotular tendon. The femur is fixed by two clamps at the level of the bone extremities. The tendon is stimulated every 10 sec with an electromagnetic reflex hammer. Simultaneous recording of the carotide pressure. The spinal cord of some animals was exposed by laminectomy of L₁ to L₇. The dura mater was incised. 4-hydroxybutyrate was administered i.v. at doses from 250 to 300 mg/kg. The action of a local application on the exposed cord was compared with that of GABA (20 per cent solution of each drug).

Action on oxygen consumption and on ventilation

O₂ consumption determination was made on small animals (rats) with a Bargeton apparatus; in the dog and in man the use of a Durupt apparatus permitted also the study of the ventilation.

Oxygen under pressure

Eighty mice are placed two by two in cylindrical boxes with glass tops so that the animals could be observed. Soda lime is spread at the bottom of the boxes. The boxes are flushed for 10 min before the beginning of the experiment. In each box, one mouse is injected i.p. with 4-hydroxybutyrate in varying doses from 200 to 500 mg/kg, the other mouse is used as a control. The experiment lasts 30 min. Pressure of pure oxygen = 3.5 atm.

X-ray irradiation

The mice are placed in a plexiglass block comprising 12 individual cells oriented identically around the center. The cells are surrounded by a 6 cm thick plexiglass partition, and the whole is placed on a 14 cm thick plexiglass stand so as to obtain a homogenous dose. The cells have a 5 mm thick plexiglass cover which the radiation has to penetrate. It is pierced by holes so that the animals can breathe. The ionization chamber (Massiot dosimetric chamber, current model) is placed either in a mouse cell or in the tunnel pierced into the plexiglass stand. The preliminary dose determination permits us to determine the ratio of the dose recorded by the ionization chamber in these two positions (the ratio is an average of 0.6). This permits to calculate later on the dose received at the level of the cells. The irradiation apparatus is an X-Ray apparatus for deep radiotherapy, a Securix model, functioning under 250 kV and 11 mA, and with an 0.5 mm copper + 0.5 mm aluminum

filter. Distance from the window of the apparatus to the irradiated object is of 63 cm. The irradiation field is of 22×22 cm with an overcoverage of 2 cm on each side of the cells. The output is of 25 to 28 r/min. Time of irradiation is of about 30 min.

Hemorrhagic shock and hepatic and renal blood output determination

Hemorrhagic shock in the rabbit is obtained with the conventional method of Wiggers. Hepatic and renal blood output determinations are made with the technique developed in our laboratory by LETERRIER and BARON (1963).

RESULTS

Toxicity—Dosage

In the animal. A 10 per cent solution of sodium 4-hydroxybutyrate was used. Sleep, as determined by the maintenance of the lateral decubitus, can be obtained in rat with 0.5 gm/kg by the i.p. route; in rabbit and dog, with 1 gm/kg by the i.v. route. In rat, the LD_{50} is 1.70 gm/kg; the LD_{100} , 2 gm/kg. The cause of death is respiratory depression, and under artificial respiration, rabbits can tolerate up to 7 gm/kg. The dog is less sensitive, and any noxious stimulus interferes with the onset of sleep. But even a very light pre-medication, in particular with phenothiazines, permits to lower the dose to 0.5 gm/kg.

In the rat, no significant differences are observed between controls and the group injected daily for 70 days with a 1/10th of the LD_{50} , particularly with respect to weight, bone marrow, liver and kidneys.

In man. 50 mg/kg by the i.v. route induce sleep. 4 gm must be injected as an initial dose. In surgery, depending on the duration of the intervention, 1 or 2 gm should be injected again when needed. The average dose is 4 to 6 gm. The practical dose for children is 100 mg/kg. No side-effects are produced with 6 to 8 gm daily for 8 to 10 days in sleep therapy. In all cases, both in the animal and in man, sleep sets in after a latent period of 5 to 10 min. Awakening is sudden with a return to consciousness and to motor activity within a few seconds.

Quantitative determination of urinary excretion

In a 12 kg dog, after the i.v. injection of 6 gm of 4-hydroxybutyrate, only 720 gm are excreted in 4 hours (Table 1a). In a 24 kg dog, after the i.v. injection of 12 gm, only 1.105 gm were found in 5 hours. Four to 5 hours after injection, no more of the drug can be detected (Table 1a) (JOUNAY *et al.*, 1960). It would, therefore, appear that most of the substance is utilized as a metabolic substrate.

Metabolism

Since, contrary to GABA, this substance does cross the brain-blood barrier, there was a possibility that it could be used as a precursor for the endogenous synthesis of γ -aminobutyric acid (GABA). Professor OSTEUX (unpublished) kindly made an electrophoretic study of cerebral aminoacids in the rat following administration of the drug, and did not observe any increase of γ -aminobutyric acid. More recently, however, M. WOLLEMANN (1963) has observed an increase in the GABA content of the brain after injection of 4-hydroxybutyrate, nevertheless, the action of 4-hydroxybutyrate does not appear to be related to an intense rate of GABA synthesis. We shall discuss later our interpretation of the possible mechanism involved.

Anticonvulsant activity

Table 2 compares the protective action of butyric acid and of sodium 4-hydroxybutyrate against convulsions induced by strychnine, cardiazol, izoniazide and ammonium chloride. The protective action is weak only against this latter compound (JOUANY *et al.*, 1960).

TABLE 2. PROTECTIVE ACTION OF BUTYRIC AND 4-HYDROXYBUTRIC ACIDS AGAINST CERTAIN CONVULSANTS

	Controls	Butyric acid 2 gm/kg	4-OH Butyric acid 0.5 gm/kg
Ammonium chloride 1st seizure	6 min 30 sec \pm 2 min 25 sec	7 min 5 sec \pm 4 min 20 sec	7 min 10 sec \pm 3 min 30 sec
1.20 gm/kg death	10 min \pm 5 min 10 sec	15 min 10 sec \pm 6 min 20 sec	14 min 25 sec \pm 5 min 40 sec
Strychnine 1st seizure	4 min 30 sec \pm 50 sec	10 min 30 sec \pm 4 min 10 sec	7 min 30 sec \pm 2 min 15 sec
4 mg/kg death	7 min 20 sec \pm 2 min 15 sec	61 min \pm 25 min 30 sec	2 mice at 9 min 2 mice at 16 min 2 mice at 70 min 2 mice at 100 min 3 survivals
Cardiazol 1st seizure	3 min 30 sec \pm 2 min 30 sec	6 mice out of 9 had seizures between 19 and 79 min	35 min \pm 3 min 30 sec
100 mg/kg death	from 12 to 45 min	death between 6 and 12 hours	95 min \pm 52 min
Isoniazide 1st seizure	25 min 40 sec \pm 1 min 50 sec	124 min \pm 26 min 10 sec	4 mice at 116 min 5 mice over 3 hours
750 mg/kg seizure			

Material—Male Wister rats, weighing from 180 to 200 gm.

The figures show the time interval between the i.p. injection of the toxic agent and the appearance of the disturbances (seizures, death) in controls and in the animals that had received simultaneously by the same route either butyric acid or 4-hydroxybutyrate (9 animals in each group except in that of strychnine where 11 animals were used).

Potentiation of anaesthetics and neuroplegics

In rat paradoxically, 0.25 gm/kg of 4-hydroxybutyrate delays the onset and shortens the duration of pentothal narcosis, in dogs however it produces pentathol as well as chloralose. 4-hydroxybutyrate action is also strongly potentiated by a number of phenothiazines (LABORIT *et al.*, 1960a and 1961a).

E.E.G. study (Fig. 1)

A progressive deterioration of the E.E.G. tracing is noted after 8 gm of 4-hydroxybutyrate. First the alpha waves are flattened, then appear theta waves which are followed by polymorphic delta waves on which rapid frequencies are superimposed. The delta waves do not mean that the sleep observed is of the anaesthetic type. The association with meperidine, however, can result in anaesthesia although the E.E.G. shows it as of superficial type.

E.E.G. awakening signs precede clinical awakening.

It is to be noted that aminoethylisothiuronium (AET) has a clear analeptic action against 4-hydroxybutyrate anaesthesia (CAMPAN and ESPAGNO, 1961).

Stereotaxic study of evoked potentials

This is a summary of the study carried out in our laboratory in Toulon, in collaboration with G. BERTHARION (1962). Following i.p. injection, 4-hydroxybutyrate was found to depress

the negative surface components in all types of primary cortical evoked potentials produced by the stimulation of a peripheral nerve of the contralateral paw (Fig. 2).

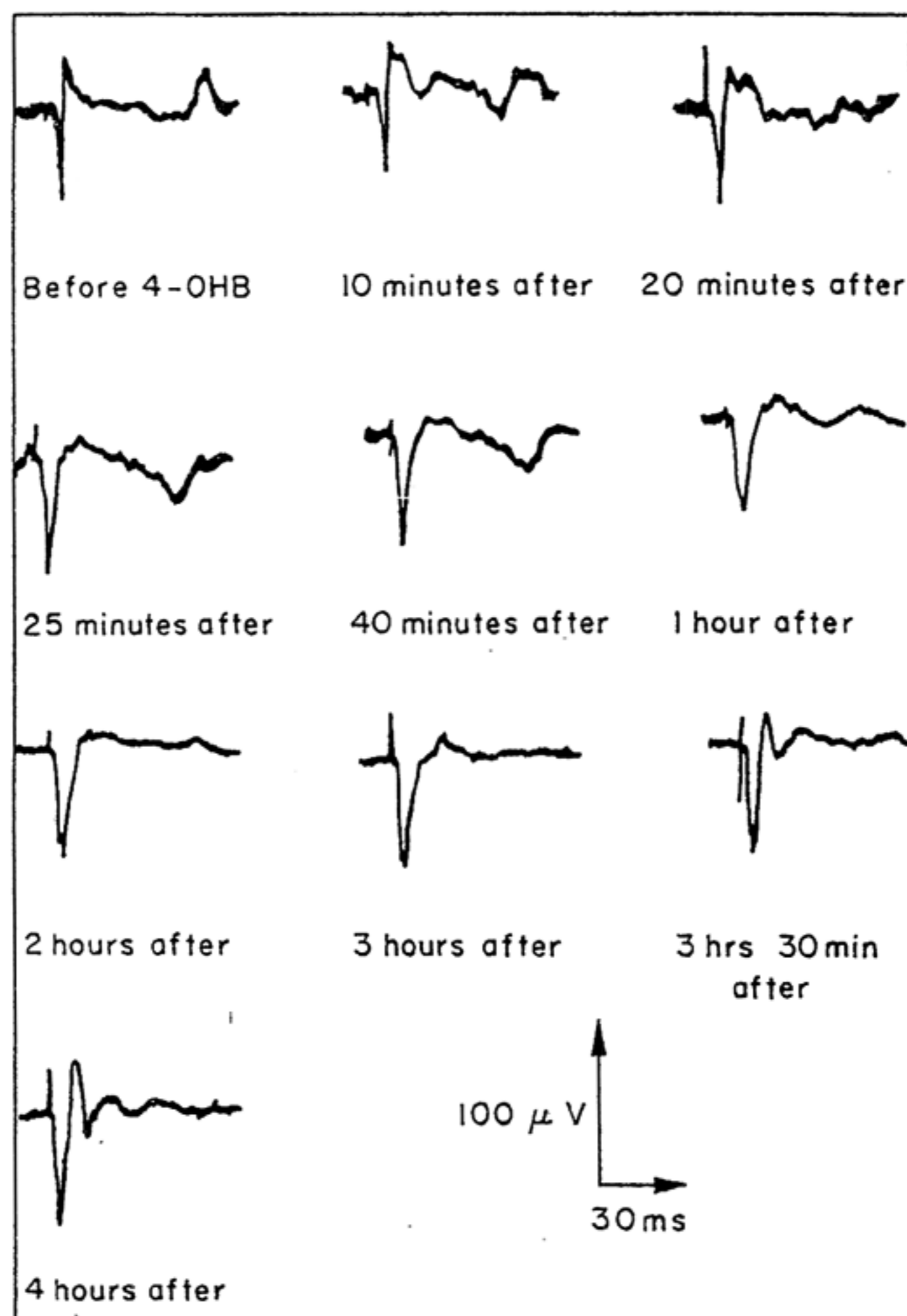


FIG. 2. Effect of 4-hydroxybutyrate on evoked potentials at cortical level.

At the median center level (Fig. 3), responses were greatly reduced. At the posterolateral ventricular nucleus level, the evoked potentials were not modified (Fig. 4). At the mesencephalic reticular level, responses were either little modified or in most cases increased (Fig. 5).

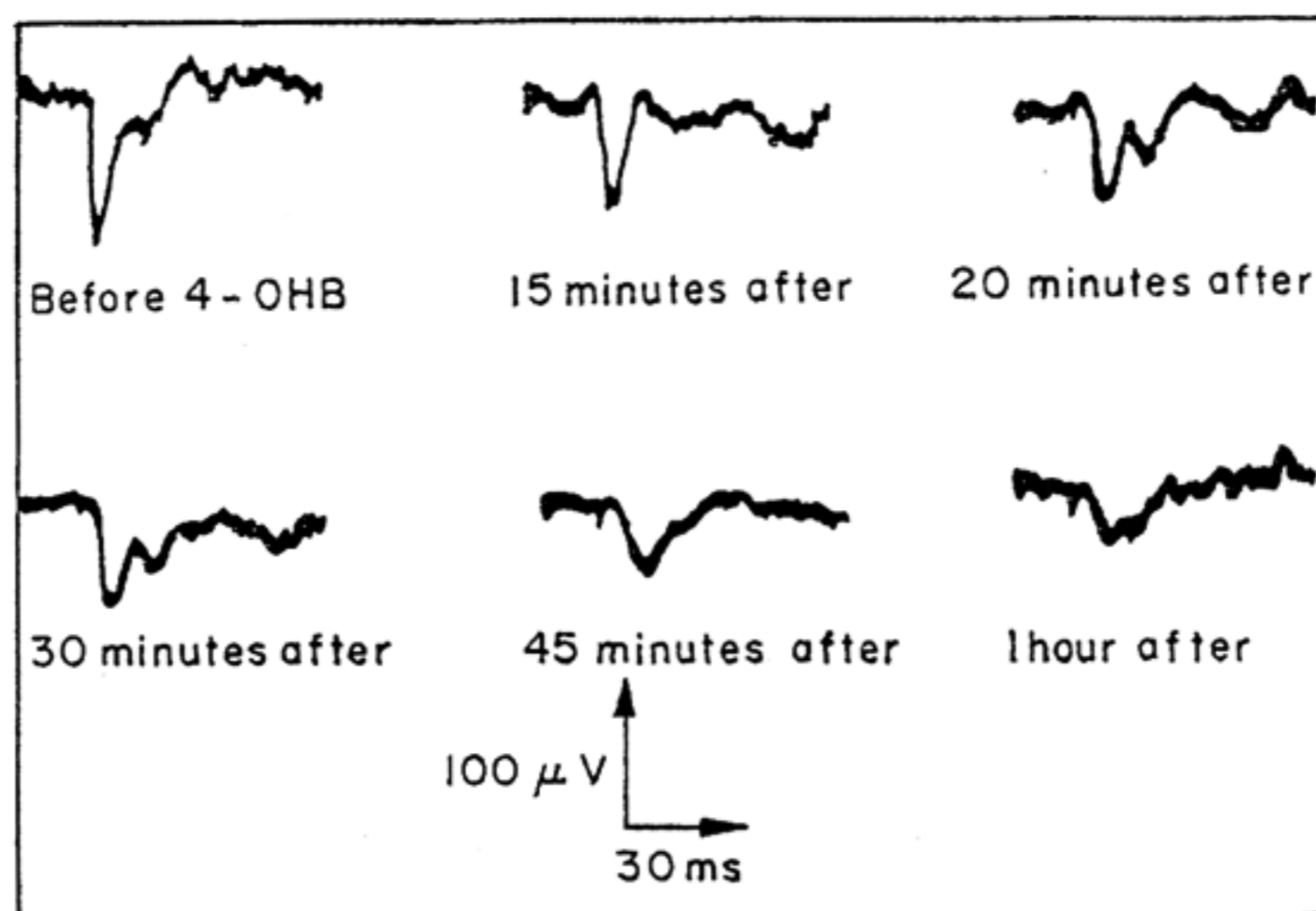


FIG. 3. Action of 4-hydroxybutyrate on evoked potentials at the level of the median center.

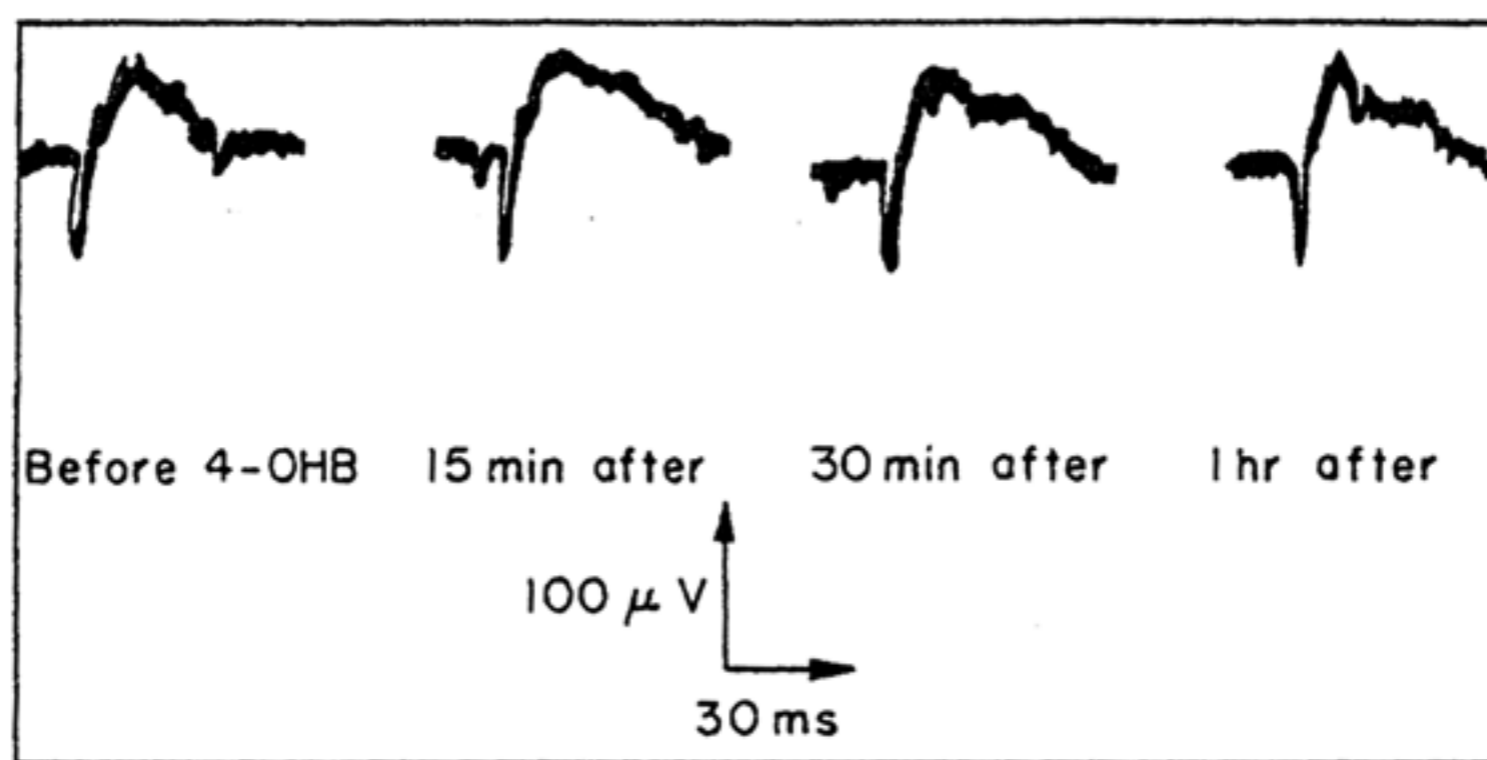


FIG. 4. Action of 4-hydroxybutyrate on evoked potential at the level of the posterolateral ventricular nucleus.

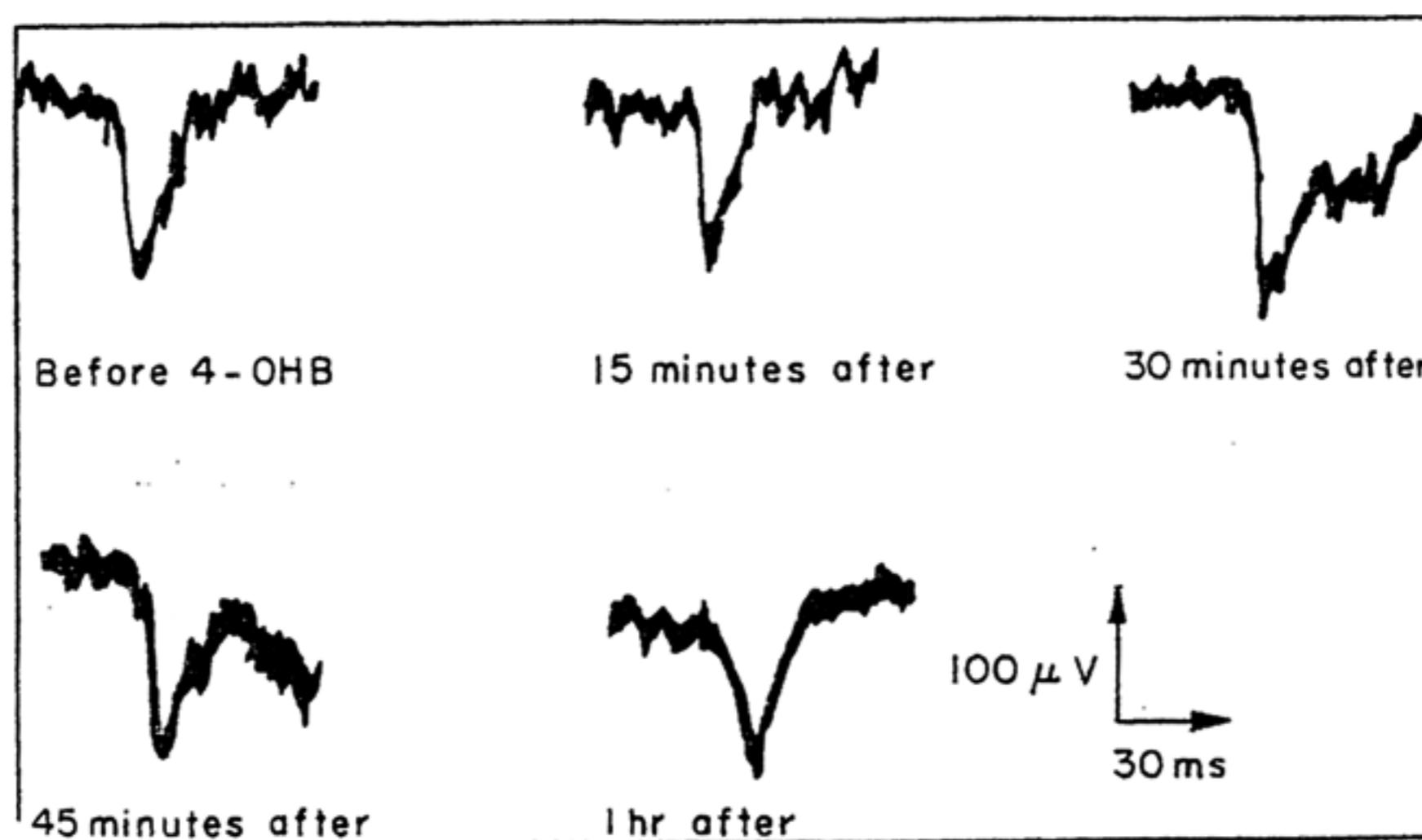


FIG. 5. Effect of 4-hydroxybutyrate on evoked potentials in the mesencephalic reticular formation.

These results would indicate that 4-hydroxybutyrate depresses the association pathways at the level of the cortex by hyperpolarization, a concept confirmed by the inhibition of the responses of the median center. This inhibition permits to explain the liberation of the reticular formation from cortical control.

The synergistic activity of 4-hydroxybutyrate with drugs that depress the midbrain reticular activity system, such as chlorpromazine in surgical anaesthesia, can also be well explained on this basis.

Action on monosynaptic reflexes

Decrease in amplitude was observed at various times after a 300 mg/kg i.v. injection. Doses as low as 250 mg/kg decrease the amplitude of the muscle response. Following local medullary application, the drug produces a clear inhibition of the monosynaptic reflex.

Pharmacological antagonists (JOUANY et al., 1961)

In the rabbit and the dog, the injection of 4-hydroxybutyrate does not affect the cardiovascular effects of epinephrine, acetylcholine, atropine, serotonin and pendiomide (Fig. 6). On the other hand, the *hypnotic effect* of the drug can be clearly antagonized; in this respect,

thyroxin and dinitrophenol are found to be inactive, epinephrine doubles the time interval between drug administration and onset of sleep (Table 3).

TABLE 3

	Delay for lateral decubitus
4-OHB 1 gm/kg 20% solution	11 min 12 sec \pm 2 min 20 sec
4-OHB 1 gm/kg + thyroxine 10 mg/kg 15 min before	11 min 25 sec \pm 1 min 58 sec
4-OHB 1 gm/kg + 2-4 dinitrophenol 10 mg/kg 15 min before	11 min 18 sec \pm 2 min 3 sec
4-OHB 1 gm/kg + monoiodoacetate 50 mg/kg 10 min before	8 min 10 sec \pm 2 min 50 sec
4-OHB 1 gm/kg + epinephrine 100 mg/kg 5 min after	21 min 36 sec \pm 6 min 50 sec
4-OHB 1 gm/kg + hydroquinone 25 mg/kg	12 min 30 sec \pm 3 min 10 sec
hydroquinone 50 „	13 min \pm 2 min 50 sec
4-OHB 1 gm/kg 10% solution	16 min 30 sec \pm 4 min 30 sec
4-OHB 1 gm/kg 10% + monoiodoacetate 20 mg/kg 10 min before	10 min 34 sec \pm 2 min

4-OHB = sodium 4-hydroxybutyrate

Material—Male Wister rats weighing from 180 to 200 gm

Series of 10 animals; i.p. injection.

We have demonstrated that doses as low as 80 mg/kg of hydroquinone cause a syndrome which is common to many chemical structures that possess a labile hydrogen susceptible to form free radicals. In view of its symptomatology, we have called this phenomenon the “excitation-hypotonia” syndrome with direct stimulation of all the central structures that we have been able to explore (LABORIT *et al.*, 1961b), (BROUSSOLLE and WERMUTH, 1961). 4-hydroxybutyrate antagonizes strongly this electro-encephalographic excitation produced by polyphenols at the level of the telencephalic centers in the “isolated brain”. But the “excitation-hypotonia” syndrome persists even after high sectioning of the cord due to the direct action of the drug on the cord. Under these conditions, 4-hydroxybutyrate will antagonize this syndrome.

Oxygen consumption

Both in the animal and in man, the sleep induced by 4-hydroxybutyrate *is not accompanied by a decrease in O₂ consumption*. In our opinion, this finding sets 4-hydroxybutyrate apart from any other known agent used in anaesthesia (LABORIT *et al.*, 1960b).

Action on ventilation

With hypnotic doses of 4-hydroxybutyrate, a decrease in ventilatory rate can be noted along with an increase in amplitude. The spirometric determination of ventilation per minute shows a maintenance of its initial value following anaesthetic doses both in the animal and in man. With high doses, and particularly in man after pre-medication, a Cheyne-Stockes rhythm appears, the mechanism of which will be discussed later. Even with high doses, however, the respiratory center remains always sensitive to an increase in pCO₂.

Action on temperature

In the rat, rabbit and dog, 0.50 gm/kg of 4-hydroxybutyrate causes a slight drop in body temperature. Since the O₂ consumption is not decreased, heat losses must be increased.

An essential observation is that when the animal is exposed to cooling, the temperature drop is not accompanied by shivering. Combination with neuroplegics leads to a very simple hypothermia both in the animal and in man. In the animal (rabbit and dog), if non-hypnotic doses of 4-hydroxybutyrate (1.5 gm/kg) are administered over a period of time (10-minute transfusion) in a cold environment (0° to 5°C), a hypothermia of about 20°C rectal temperature can be obtained with preservation of consciousness. An arousal reaction to noise has been observed on the E.E.G. in an animal at 20°C for 24 hours (LABORIT *et al.*, 1962). Finally, while the spontaneous contractions of the rabbit isolated atrium disappear generally at about 19°C, in certain cases the addition of 4-hydroxybutyrate to the bath permits to observe contractions at temperatures between 5° and 10°C. (Fig. 7) (LABORIT and BERTHOU, 1960).

Antagonistic action against oxygen under pressure and ionizing radiations

A hypnotic dose of 500 mg/kg protects all animals against convulsions (10 mice). With 250 mg/kg doses, a convulsion is noted in one mouse out of 10; this seizure is retarded and of short duration. In 20 mice, with 200 mg/kg, there were three slight and one typical convulsion.

We have noted that 1 gm/kg of 4-hydroxybutyrate injected 30 minutes to 1 hour before 750 to 1,050 R protects against irradiation (Fig. 8), (DANA, 1962). This action is not potentiated by aminoethylisothiuronium (AET), another radiation protecting agent.

This protective activity does not seem to be tied to the hydroxyl group, as demonstrated by a comparison of the effect of ethylalcohol and glycerol against irradiation with 850 R (Table 4). Moreover it does not seem to be tied to an anaesthetic effect (Table 5), or to a light hypothermia.

TABLE 4

Drug	Dose	Survivals total number	Average survival period
4-hydroxybutyrate	1 gm/kg	10/10	13 days
Ethylalcohol	360 mg/kg	4/9	13 days
Glycerol	250 mg/kg	1/9	19 days
NaCl, 9%	0.5 cc/mouse	4/10	14 days

Comparative protective action of 4-OHB and of compounds with an alcoholic group against ionizing irradiations.

TABLE 5

Drug	Dose	Survivals total number	Average survival period
4-hydroxybutyrate	1 gm/kg	5/8	8 days
Chlorpromazine	2 mg/kg	2/8	11 days
Thiopental Na	50 mg/kg	1/8	10 days
Controls (NaCl 9%)	0.5 cc	1/8	8.5 days

Comparative protective action of 4-OHB and of agents possessing an anaesthetic and hypothermic activity against ionizing irradiations.

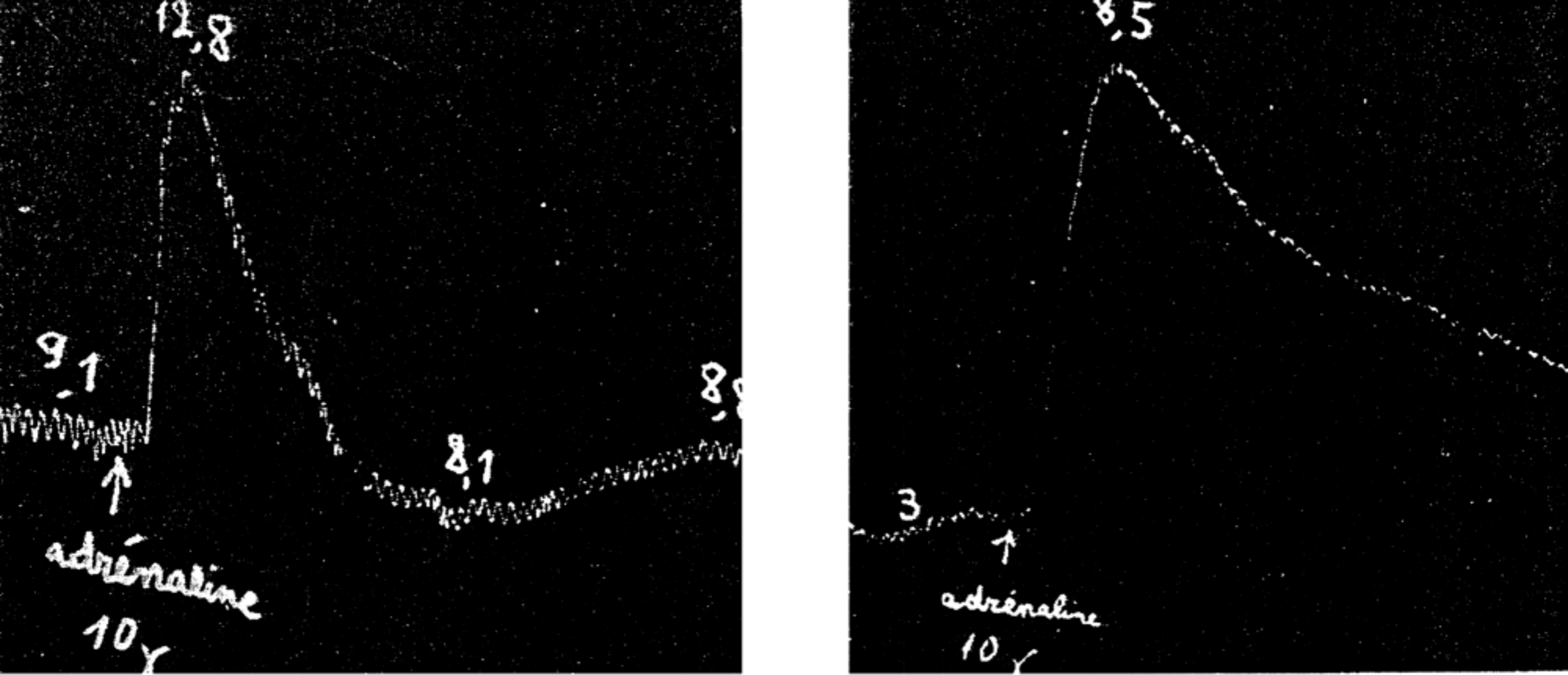


FIG. 6

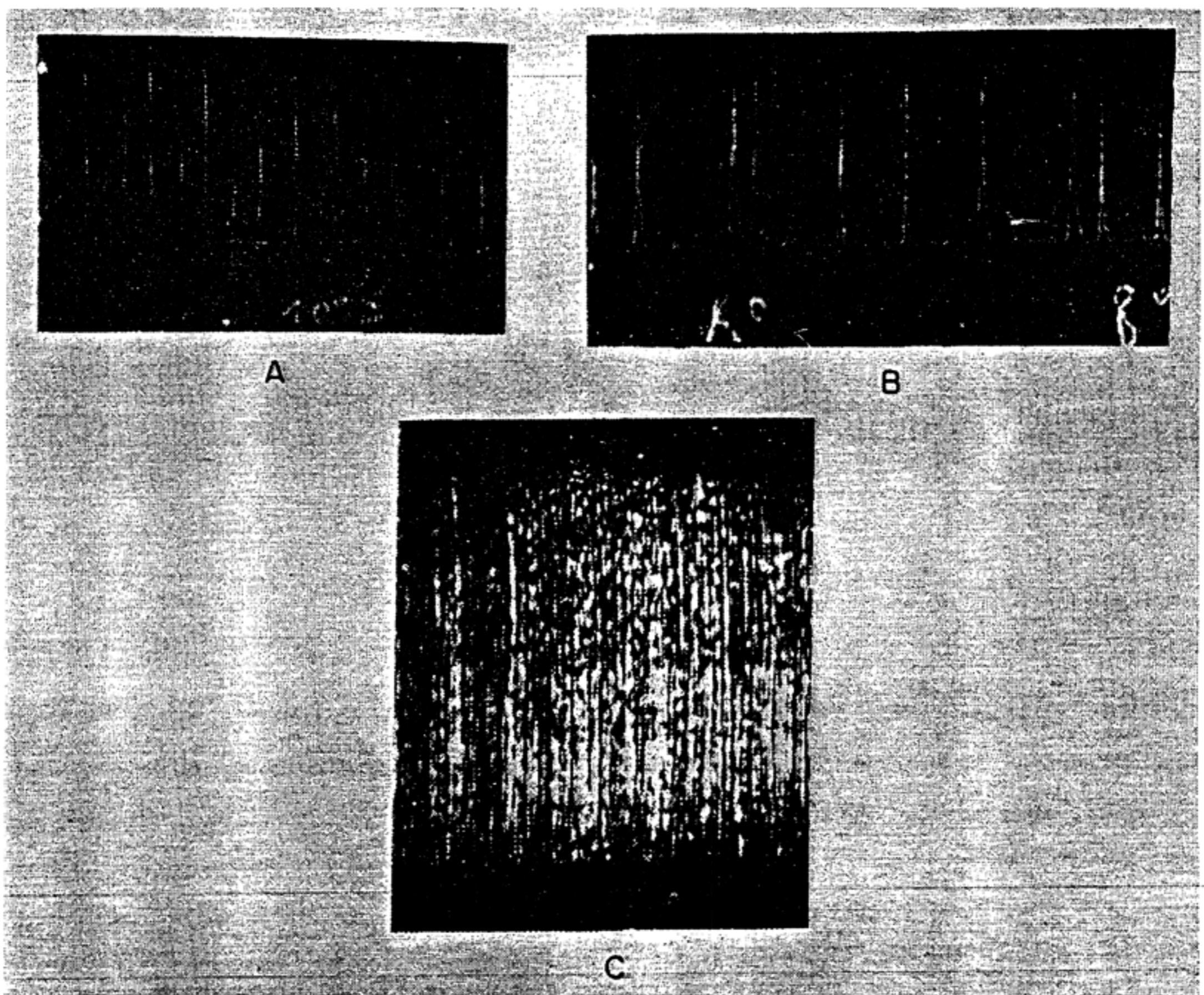


FIG. 7. Recording of rabbit isolated atrium under hypothermia (Locke solution, in which one-third of the NaCl has been replaced by sodium 4-hydroxybutyrate).

A—bath temperature = 10.5°C.

B—bath temperature = 8°C.

C—After warming, bath temperature = 25°C.

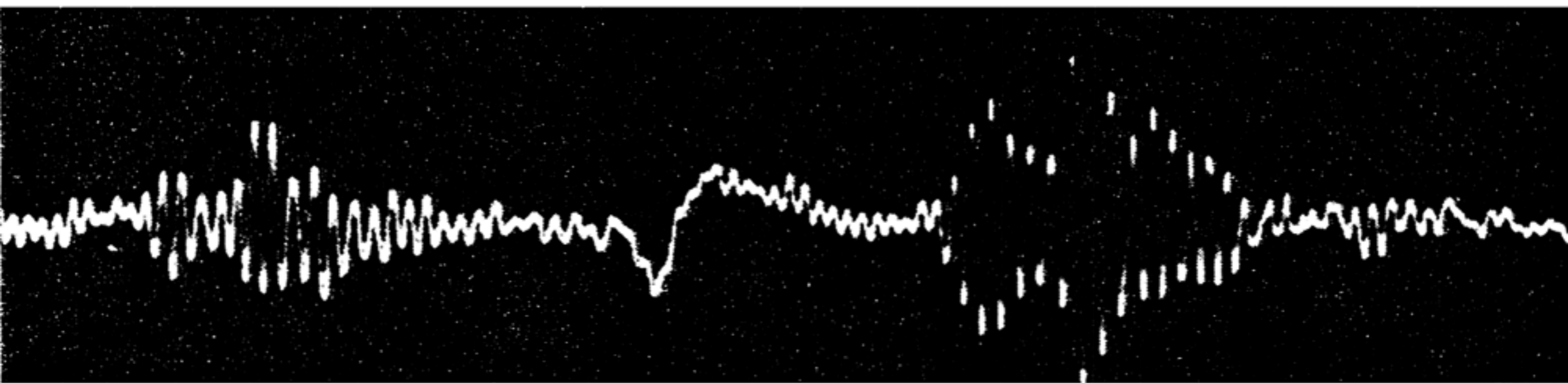
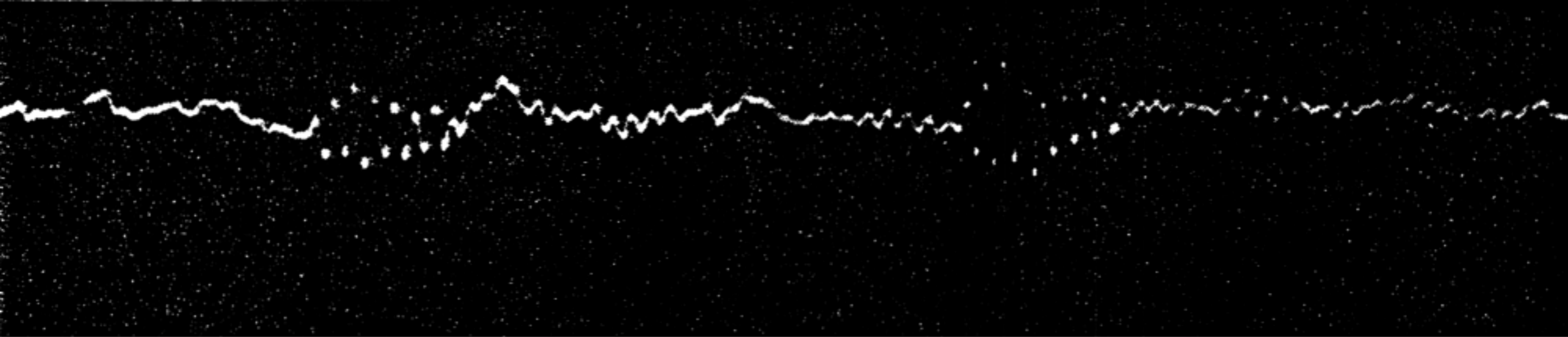


FIG. 9. Corticogram of an animal anesthetized with 4-hydroxybutyrate.

Above: 1/2 hour after injection.

Below: 1 hour after injection.

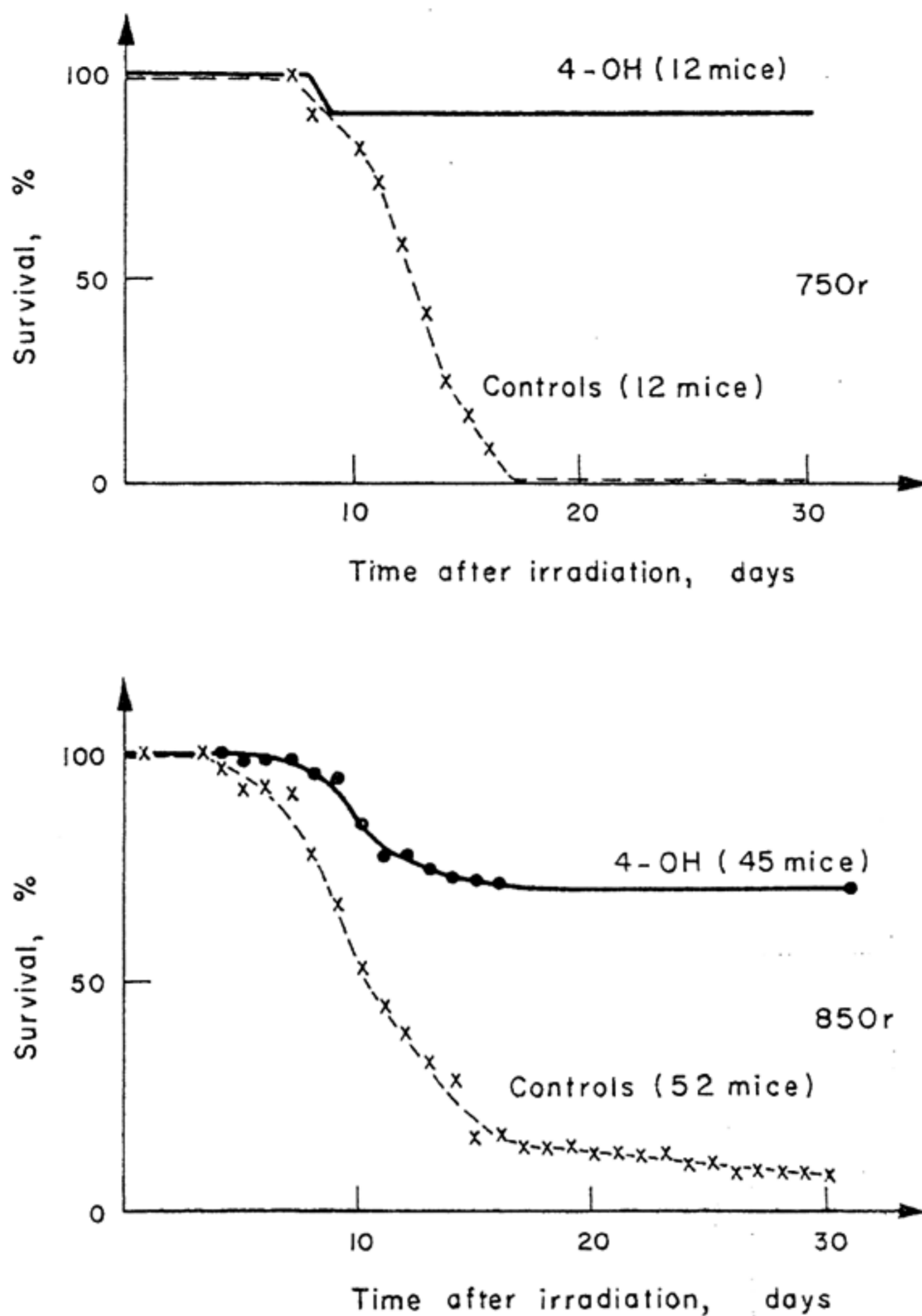


FIG. 8

Action on the cardiovascular system

No obvious action was observed on the isolated heart of rabbit, isolated atrium, ileum or aortic strip.

In the *rabbit*, 4-hydroxybutyrate causes a constant but short drop in blood pressure, which persists after section of the two vagi (BRUE *et al.*, 1962a and b). In the *dog*, it has either no effect or causes a slight and progressive increase in blood pressure, which appears even under controlled ventilation and is not tied to an increase of the $p\text{CO}_2$.

In *man*, an injection of 2 to 4 gm has no effect on blood pressure. In surgery, however, in the case of insufficient inhibition of the reticular formation by a concomitant neuroplegia, a progressive hypertensive episode may be seen occasionally, but this is never observed following adequate neuroplegic premedication.

In all animal species, a constant and often important *bradycardia* is observed. Cardiac output does not seem changed in the dog. Actually, pulse pressure increases substantially. The drug antagonizes effectively phenothiazine induced tachycardia and hypotension.

Antishock action

In the animal, we have observed a strong hepatic and renal vasodilating action, which is

particularly marked during hemorrhagic shock (LETERRIER, 1963). This property explains in part its antishock activity, clearly observed in the animal and frequently seen in man.

Electrocardiogram

Fifty patients were used in this study, most of whom were atherosclerotic and some of them had a history of infarct sequellae. No unfavorable effect was observed under 4-hydroxybutyrate anaesthesia (G. LABORIT *et al.*, 1963). However, a frequent decrease in the amplitude of the T-wave was noted, even to a point of *inversion*, especially if the drug was combined with glucose, insulin and neuroplegia, a combination frequently used by us. This inversion accompanies the hypokaliemia constantly noted with the drug. These electrocardiographic changes, which testify of an increase in the $\frac{[K_i]}{[K_o]}$ ratio consecutive to intracellular potassium loading, disappear with the injection of potassium, either as the chloride or even better still, as the aspartate, and in some cases considerable quantities are required. Moreover, HEROLD *et al.* (1961) have demonstrated that in the mouse under anoxia following curarization, 500 mg/kg of 4-hydroxybutyrate prolong cardiac activity from 438 (± 44.3) to 869 seconds (± 58.9).

Blood pressure effect and carotid reflexes (BRUE *et al.*, 1962a, b and c)

In the rabbit and the dog, the constant hypertension caused by bilateral novocain infiltration of the carotid sinuses is abolished by 4-hydroxybutyrate injection. On the other hand, the hypertension caused by trachea clamping in the animal, and by induced hypercapnia in man during anesthesia, remains unchanged. It appears, therefore, that 4-hydroxybutyrate elevates the sensitivity threshold of the pressure receptors without having any obvious action on the chemoreceptors.

Effect on blood constituents

The only major effect of sodium 4-hydroxybutyrate on blood constituents in the dog is a decrease in serum potassium (JOUANY *et al.*, 1960). The systematic investigation of various blood constituents in 100 patients under 4-hydroxybutyrate made at our request by the Institute of Hematology in Warsaw demonstrated the constant hypocholesterolemic action of the drug (ROSENGARTEN *et al.*, 1963).

Metabolic activity

We demonstrated in our laboratory (GERARD and WEBER, 1961), (LABORIT *et al.*, 1960b), (WEBER, 1961) that in the rat:

- (a) Fasting potentiates the hypnotic activity of 4-hydroxybutyrate. A threshold dose (1.33 gm/kg by gastric intubation) causes, in the fed animal (animals weighing an average of 350 gm), only a temporary somnolence, while in the animal fasting for 36 hours, it induces deep sleep.
- (b) In the fed animal, the same threshold dose can induce sleep if one unit of insulin, free of any hypnotic action by itself, is injected simultaneously by the subcutaneous route.
- (c) Following a 36-hour fasting, a simple administration of 3 gm/kg of glucose by gastric intubation causes labile but deep sleep which lasts from one hour to one hour and a half.

- (d) Following a 36-hour fasting, 4-hydroxybutyrate, which never causes any ketonuria in the normally fed animal, causes a urinary excretion of ketone bodies if it is administered at a 1 gm/kg dose.
- (e) Finally, HEROLD *et al.* (1962) have noted that the drug causes an increase in the total lipids of the liver.

DISCUSSION

(a) *Effects on various functions*

On account of its chemical relationship with GABA, it would appear that the decrease in muscle tone due to 4-hydroxybutyrate can be attributed, at least in part, to an action on stretch receptors. Moreover, this action might extend to all sensitive pressure receptors. Such a hypothesis would account for the inhibition of the baroreceptors, and would also furnish an interpretation for the occasional hypertension, although reticular stimulation, a secondary result of cortical inhibition, may also participate in this hypertension.

The elevation of the excitability threshold of the pulmonary stretch receptors would furnish an explanation for the decreased ventilatory rate with increase of amplitude, as well as for the Cheyne–Stokes rhythms, since there is no decrease of sensitivity of the respiratory centers to CO_2 . Similarly, the depression of the Bainbridge reflex would furnish a mechanism for the bradycardia.

The elective cortical action of the drug explains the antagonism by epinephrine, a reticular stimulant, as well as by nociceptive stimuli, against the hypnotic action of the drug. The reticular liberation, that results from cortical inhibition, as shown by the investigation of evoked potentials at this level, may possibly be an additional factor in the elevation of the sensitivity threshold of the pressure receptors, causing the occasional hypertensions observed. It can then be understood why hypertensive manifestations are brought about by surgical procedures, and controlled by neuroplegics, which are reticular formation inhibitors. Under such conditions, it is evident that the hypnotic action of the drug will be potentiated by neuroplegics, which then permit a true surgical anaesthesia in man.

If the activity of the drug can thus be rather well explained at the level of the interrelation mechanisms between various systems, we believe however, that the fundamental mechanism of its action must be sought at the metabolic level.

(b) *Metabolic action*

The synthesis and metabolic study of 4-hydroxybutyrate fall within the framework of our concept of the "orientation of metabolic pathways" (LABORIT, 1961).

Following this concept, it had appeared to us that the orientation of glucose-6-phosphate, the common initial substrate, toward the hexose-monophosphate shunt, or pentose pathway, at the expense of its use in the Embden–Meyerhof could be responsible for physiological inhibition phenomena and sleep. To cause this orientation, NADPH_2 must be oxidized, and we believed that this could be achieved by stimulating lipogenesis. To this end, butyryl–CoA, the initial building block of the long chain fatty acids by further addition of C_2 fragments, is needed, and the reaction involved requires the concomitant oxidation of NADPH_2 . Only indirect proofs of the existence of such a mechanism in mammals are available. But even if none of the observations we have made can bring a proof individually, their accumulation appears strongly suggestive that inhibition and sleep are associated with an orientation of glucose-6-phosphate toward the pentose pathway. Here is a list of such indirect proofs:

- (1) The orientation of glucose-6-phosphate in the Embden–Meyerhof pathway under the action of epinephrine antagonizes the hypnotic activity of 4-hydroxybutyrate, while, on the other hand, the latter is potentiated by insulin which orients glucose-6-phosphate toward the pentose pathway.
- (2) Glucose has a hypnotic action in the rat after 36 hours of fasting (GERARD and WEBER, 1961). Similarly TEPPERMANN (1961) has demonstrated recently with labelled glucose that after an identical fasting period, it is the C_1 that is most actively metabolized.
- (3) The neurohormonal origin of sleep slow waves that appear in the cat under the same experimental conditions has been demonstrated by SUDAKOV (1963), since these waves are maintained even following anterior medulla oblongata and vagi sections.
- (4) 4-hydroxybutyrate after fasting causes ketonuria, this does not appear in the well fed animal probably because under fasting conditions, there does not exist any longer a sufficient supply of $NADPH_2$ to ensure lipogenesis, and of glucose to permit the utilization of C_2 fragments by the tricarboxylic acid cycle (GERARD and WEBER, 1961).
- (5) The original mechanism of action of the drug is demonstrated in anaesthesia by the absence of any decrease in O_2 consumption. It does not probably effect the energy liberating pathway of Embden–Meyerhof, Krebs, in contrast with conventional anesthetics, although it may be admitted that its alcoholic OH-group could reduce NAD, limiting thereby the entry of glucose-6-phosphate into the Embden–Meyerhof pathway (HEROLD *et al.*, 1962).
- (6) 4-hydroxybutyrate stimulates hepatic lipogenesis and decreases cholesterolemia (ROSENGARTEN *et al.*, 1963). The latter action may be explained by the utilization of the C_2 fragments for lipid synthesis; this would then take them away from cholesterol synthesis.
- (7) Since 4-hydroxybutyrate enters into lipid metabolism, it may also secondarily furnish β -oxidation processes with a substrate capable to supply the carrier chain with H_2 molecules directly and through the C_2 fragments consumed in the Krebs cycle; this cycle actually operating then at a low level. Such an interpretation would explain the need for a minimal supply of glucose to avoid ketonuria. It would also explain the protein sparing action (LABORIT *et al.*, 1960b), (WEBER, 1961), (LABORIT *et al.*, 1961c).
- (8) This type of pathway parallel to that of the Embden–Meyerhof, Krebs would finally explain its favorable action in hypothermia. Besides, the elevation of the excitability threshold of the stretch-receptors could participate in the inhibition of shivering.
- (9) Experimental observations led us to consider that the pentose pathway is particularly developed in the neuroglia. BARONDES *et al.* (1961) have shown recently that under the action of epinephrine, cortex slices use preferentially the C_1 of labelled glucose. Since 9/10th of the cortex are formed by glial cells, we believe that the physiological role of the neuroglia and of the pentose pathway in neurophysiology can be very considerable. The preferentially cortical action of 4-hydroxybutyrate would then be understandable (Fig. 9). The central effect of 4-hydroxybutyrate on neurone activity would be indirect and mediated essentially through the neuroglia. Indeed, neurone activity is conditioned by oxidative phosphorylation that 4-hydroxybutyrate does not seem to be able to affect directly.

- (10) Once again, we stress that all substances facilitating the functioning of the pentose pathway lower the potassium level in serum (LABORIT, 1961). Insulin and glucose are excellent examples, but to our knowledge, 4-hydroxybutyrate is the most potent agent. We believe that this action is due to the decrease in the intracellular redox potential as well as to the early liberation of CO_2 which, in the presence of carbonic-anhydrase, increase the $\frac{[\text{CO}_3\text{H}_i]}{[\text{CO}_3\text{H}_0]}$ ratio and are responsible for repolarization. Potassium loss by red blood cells of preserved blood is delayed by 4-hydroxybutyrate (REYNIER *et al.*, 1962).
- (11) The protective action against ionizing radiations and oxygen under pressure could result from the decrease in lipoperoxides arising from the action of the free radicals produced by the radiations, or from oxygen itself, a double free radical. For quite a few years now, we have believed that the lesions caused by these two agents, as well as those that characterize aging, are lesions of membrane lipids that alter cell nucleus and mitochondria permeability by changing the physical structure of their membranes. We also believe that NADPH_2 is essential to reduce these peroxides. Any agent that can stimulate the functioning of the pentose pathway, i.e. reduce NADPH_2 and the redox potential, can in our view protect against radiations and oxygen under pressure.

As mentioned, we realize that none of these experimental findings are conclusive individually, however, their accumulation add support to our hypothesis. The very low toxicity of 4-hydroxybutyrate could result from its ability to act like a substrate that orients metabolic pathways, and not like an enzyme inhibitor. The recent observation of HARDMAN and STADMAN (1963) showing that gamma-amino- and gamma-hydroxybutyrates are metabolized by *Clostridium butyricum* to form butyryl-CoA suggests the possible existence of a similar mechanism in mammals and in man, as mentioned earlier in our hypothesis.

Finally, we believe that 4-hydroxybutyrate sheds light on an essential aspect of the mechanism of sleep. In view of our past and present studies on this drug and on other chemical structures, we believe that the process of physiological inhibition must be considered as an active metabolic phenomenon linked with a preferential orientation of glucose-6-phosphate toward the pentose pathway. We have described why we believe that the relative functional importance of this pathway over that of the Krebs cycle can vary, and that it can be either dominant, similar or negligible, according to the cell or organ considered (LABORIT, 1961). If this is accepted, it can then be understood how identical drugs can favor either stimulation or inhibition depending on the enzymatic structure characteristic of the cell or organ upon which they act.

Clinical applications

We shall touch on this subject only very briefly since quite a number of experiments have already been published, and their importance can be gathered from the general study just outlined.

In anaesthesia (G. LABORIT et al., 1962)

G. LABORIT has already collected over 6,000 report-cases on the use of 4-hydroxybutyrate in general anaesthesia. Its main advantages are:

- (a) low toxicity;

- (b) the absence of respiratory depression and the maintenance of response of the respiratory center to CO_2 ;
- (c) the muscle relaxing action which permits to avoid the use of curares or decreases substantially the dose required. *Intubation* can thereby be performed on spontaneously breathing patients;
- (d) non-hypotensive bradycardiac action;
- (e) the absence of a venous irritation. Pleasant induction, and perhaps above all, the ease with which mechanically controlled respiration can be initiated since the patient has no defense reaction against the imposed apparatus, a fact which, in our opinion, confirms the inhibiting action on the alveolar stretch-receptors;
- (f) the ease with which hypothermia can be induced and maintained;
- (g) finally, its antishock activity.

Limitations

Although this drug is more hypnotic than hydroxydione sodium succinate (Viadryl) and methyl-4- β -chloroethyl-5-thiazol ethane disulfonate (Hemineurine-SCTZ), it does not produce surgical anaesthesia when used alone, except in children. Premedication must be chosen among those drugs that depress reticular activity, such as chlorpromazine or promethazine.

The strong lowering effect of potassium on serum levels caused by the drug might be considered as a problem. In our opinion it is not a real one, however, since we classify this action as a beneficial effect similar to that of glucose and insulin. Indeed, the serum potassium lowering is associated with cellular repolarization, and some electrocardiographic lesions have been improved by 4-hydroxybutyrate. However, the association of certain drugs (insulin + glucose + neuroplegics) remains a potential risk, because a drop in serum potassium caused by these drugs *per se* may summate with that caused by 4-hydroxybutyrate, consequently the total effect may go beyond the result sought, if—as in the case of an important bradycardia—the precaution is not taken then to perfuse a potassium salt capable of re-establishing the $\frac{[K_i]}{[K_o]}$ ratio (LABORIT *et al.*, 1960b, G. LABORIT *et al.*, 1953).

In any event, since the organic reaction to changes of homeostasis favors always the escape of intracellular potassium and a negative potassium balance, we have always found logical in our routine therapy to limit, or even reverse this phenomenon by potassium administration.

This being so, we believe that 4-hydroxybutyrate, both practically and theoretically, offers a new orientation to surgical anaesthesia, which—and we would like to stress this again—for the first time seems to be produced by a substrate that orients rather than disturb cellular metabolism.

In the post-operative phase

For reasons outlined, among which its protein sparing action, the drug finds a place in parenteral feeding, and 8 to 12 gm doses per 24 hours are then required. In many instances, we have found its use very beneficial for the placement of an artificial kidney (LABORIT, 1960b).

In obstetrics

Recent observations would indicate that this drug can be very useful in delivery. Independently of the decrease in anxiety and consciousness obtained, it showed an absolutely

spectacular action on the dilation of the cervix. This finding reminds us of its action on stretch-receptors in general. It is probable that it depresses the contraction reflex which originates from the pressure on the cervix. In the course of anaesthesia in obstetrical surgery, the absence of respiratory depression in the infant, and the protection it offers against cardiac anoxia are also beneficial properties (BARRIER, 1962).

In psychiatry

Since the injectable form is the only one that has been available until recently, the study of the drug in psychiatry has been limited (LANGLOIS *et al.*, 1961). Nevertheless, it appears to be a major medication of anxiety, as shown by the preliminary study we set up with DANON-BOILEAU *et al.* (1962) in schizophrenics. So far, the effect observed has been temporary.

In a few cases, this drug or butyrolactone has been used *per os*, both appeared to be pleasant hypnotics without any side-effects. The apparently elective cortical site of action should lead to more precise therapeutic indications. Preliminary unpublished studies show that this agent has a very favorable action in alcohol and narcotic withdrawal symptoms.

Action on lipid metabolism

Some of our studies indicate that the most interesting effect of this drug could be in the treatment and/or prevention of arteromatosis. Although the mechanism of action of this drug on lipid metabolism is not yet well understood, it would seem to be particularly physiological since based not on an enzymatic inhibition with more or less foreseeable secondary effects, but on a new and more physiological orientation of the C₂ fragments produced by lypolysis toward lipogenesis (ROSENGARTEN *et al.*, 1963).

CONCLUSIONS

At a very early stage of our study, the unique metabolic action of 4-hydroxybutyrate led us to the conclusion that it is the most important compound in the list of those we have discovered since the days of chlorpromazine (LABORIT, 1952).

Whatever may be the value of the hypothesis on the pharmacological orientation of the metabolic pathways that led us to the synthesis and study of 4-hydroxybutyrate, this substance, and its unquestionable original action, demonstrate a new aspect of the metabolic as well as systemic mechanism of sleep and of its induction. By overlooking temporarily the concept of chemical mediators in nerve influx, of brain nuclei, of cell groups and of other specific brain areas, we were able to pay more attention to the concept of the variability of the metabolic make-up of these central structures. In our opinion, this is an important step toward a non entirely empirical approach to neuropsychopharmacology, based not on inhibition, but on selective orientation of metabolism.

REFERENCES

- BARONDES, S. H., JOHNSON, P. and FIELD, J. B. (1961). Stimulation of anterior pituitary and cerebral cortical glucose oxidation by neurohumoral agents. *Endocrinology* **69**: 809-818.
- BARRIER, G. (1963). Première utilisation du 4-hydroxybutyrate de sodium en obstétrique. *Ann. Anesth. Française* **1**: 11-14.
- BERTHARION, C. and LABORIT, H. (1962). Etude stéréotaxique des potentiels évoqués sous gamma-hydroxybutyrate de Na. *Agressologie* **3**: 489-496.
- BESSMAN, S. P. and FISHBEIN, W. N. (1963). Gamma-hydroxybutyrate—A new metabolite in brain. *Fed. Proc.* **22**: 334.

- BLUMENFELD, M. *et al.* (1962). Sodium gamma-hydroxybutyric acid: A new anesthetic adjuvant. *Anesthesia and Analgesia* 41: 721-726.
- BROUSSOLLE, B. and WERMUTH, C. G. (1961). Structure chimique et activité corticale. *Agressologie* 2: 513-520.
- BRUE, F., LABORIT, H. and BARON, C. (1962a). Etude des effets tensionnels immédiats du 4-Hydroxybutyrate chez le lapin et de leur modification par l'injection d'Hydroquinone. *Agressologie* 2: 627-630.
- BRUE, F., LABORIT, H. and BARON, C. (1962b). Etude des effets tensionnels du 4-Hydroxybutyrate de Na et de l'Hydroquinone chez le lapin, après section des deux pneumogastriques et après infiltration bilatérale à la novocaïne des régions sinu-carotidiennes. *Agressologie* 2: 631-4.
- BRUE, F., LABORIT, H. and BARON, C. (1962c). A propos de l'action du 4-hydroxybutyrate de Na sur la pression artérielle du lapin. Hypothèse d'une inhibition des baro-recepteurs vasculaires. *Agressologie* 2: 635-640.
- CAMPAN, L. and ESPAGNO, M. T. (1961). Essai du 4-Hydroxybutyrate en narcoanalgésie. *Agressologie* 2: 283-293.
- DANA, M., BARON, C. and LABORIT, H. (1962). Action radioprotectrice du 4-Hydroxybutyrate de Na. *Agressologie* 3: 497-508.
- DANON-BOILEAU, H., LAVITRY, S., LAB, P., LEVY, F., RUFFIOT, S. and LABORIT, H. (1962). Utilisation en psychiatrie du gamma-Hydroxybutyrate de Na. *La Presse Médicale*, 70: 2205-2207.
- DRAKONTIDES, A. B., SCHNEIDER, J. A., FUNDERBURCK, W. H. (1962). Some effects of sodium gamma-hydroxybutyrate on the central nervous system. *Pharmacol. Exper. Ther.* 135: 275-284.
- DROUET, J., and LABORIT, H. (1962). Reflète monosynaptique et 4-Hydroxybutyrate de Na. *Agressologie* 3: 481-488.
- DROUET, J. and LABORIT, H. (1963). Action de l'acide gamma-amino-butérique et du gamma-hydroxybutyrate de Na sur le reflète monosynaptique. *Agressologie* 4: 153-156.
- GÉRARD, J. and WEBER, B. (1961). Action du glucose sur le sommeil et la cétonurie provoquée par le 4-Hydroxybutyrate de Na chez le rat à jeun. *Agressologie* 2: 611-614.
- HARDMAN, J. K. and STADMAN, T. C. (1963). Metabolism of gamma-aminoacids III and IV, *J. Biol. Chem.* 238: 2081-2037 and 2088-2093.
- HEROLD, M., KABACOFF, O. and CAHN, J. (1961). Recherches sur la prolongation du temps de fonction du coeur par le 4-Hydroxybutyrate de Na chez la souris curarisée. *Agressologie* 2: 551-554.
- HEROLD, M., KABACOFF, O., GAUTIER, E., MATHIAS, G., BARRE, N. and CAHN, J. (1962). Lipides, 4-Hydroxybutyrate de Na et fonction cardiaque. *Agressologie* 2: 837-842.
- JOUANY, J. M., GÉRARD, J., BROUSSOLLE, B., REYNIER, M., ORSETTI, A., WERMUTH, C. and BARON, C. (1960). Pharmacologie comparée des sels de l'acide butyrique et 4-Hydroxybutyrique. *Agressologie* 1: 417-430.
- JOUANY, J. M., GÉRARD, J., WEBER, B. and LABORIT, H. (1961). Interférence de certains agents pharmacologiques sur l'établissement et les caractères du sommeil provoqué par le 4-Hydroxybutyrate de Na. *Agressologie* 2: 45-50.
- LABORIT, G. and KIND, A. (1961). Une utilisation particulière du 4-Hydroxybutyrate de Na. *Agressologie* 2: 543-550.
- LABORIT, G., LARCAN, A. and KIND, A. (1963). Etude électrocardiographique du 4-Hydroxybutyrate de Na. *Agressologie* 4: 77-88.
- LABORIT, G., KIND, A. and HOUDART, R. (1962). Le gamma-Hydroxybutyrate en anesthésie neuro-chirurgicale. *Neuro-chirurgie* 8: 104-107.
- LABORIT, H. and BERTHOU, J. (1960). Etude sur l'oreillette isolée du lapin de l'essai des variations de différents facteurs physico-chimiques, aux différentes températures. *Agressologie* 1: 407-416.
- LABORIT, H., JOUANY, J. M., GÉRARD, J. and FABIANI, P. (1960a). Généralités concernant l'étude expérimentale et l'emploi clinique du 4-Hydroxybutyrate de Na. *Agressologie* 1: 397-406.
- LABORIT, H., BUCHARD, E., LABORIT, G., KIND, A. and WEBER, B. (1960b). Emploi du 4-Hydroxybutyrate de Na en anesthésie et en réanimation. *Agressologie* 1: 549-560.
- LABORIT, H., BARTHÉLÉMY, L. and PERRIMOND-TROUCHET. (1960c). Etude de l'action du 4-Hydroxybutyrate de Na sur les convulsions hyperoxiques de la souris. *Agressologie* 1: 601-603.
- LABORIT, H. (1961). Mécanisme d'orientation des voies métaboliques en fonction de l'environnement et des agents pharmacologiques. *Agressologie* 2: 432-460.
- LABORIT, H., JOUANY, J. M., GÉRARD, J. and FABIANI, P. (1961a). Première note sur l'étude expérimentale et l'emploi clinique du 4-Hydroxybutyrate de Na en anesthésiologie et en neuropsychiatrie. *Neuropsychopharmacology* 2: 490-497.
- LABORIT, H., JOUANY, J. M., GÉRARD, J. and DROUET, J. (1961b). Sur un syndrome expérimental d'"excitation-hypotonie" *Arch. int. pharmacodyn.* 131: 151-163.
- LABORIT, H., WEBER, B. and GÉRARD, J. (1961c). Action de l'Hydroquinone sur la cétonurie provoquée par le jeûne et le 4-Hydroxybutyrate de Na. *Agressologie* 2: 615-618.
- LANGLOIS, M., LACROTTE, J. and FERRIER, F. (1960). Action du gamma-Hydroxybutyrate de Na sur les syndromes névrotiques et psychopathiques avec agitation psychomotrice. *Agressologie* 1: 431-434.

- LETERRIER, F., BARON, C., BRUE, F., DELCAMBRE, J. P. and LABORIT, H. (1963). Etude de nouvelles thérapeutiques des états de choc basée sur l'exploration physique et biologique des circulations hépatiques et rénales. *Agressologie* 4: 343-360.
- REYNIER, M., BROUSSOLLE, B., DROUET, J., DANOY, B., and LABORIT, H. (1962). Effets comparés des perfusions de glucose seul et de glucose associé au 4-Hydroxybutyrate de Na avec ou sans carnitine sur la survie du lapin soumis au jeûne. *Agressologie* 3: 711-722.
- REYNIER, M., ARDISSON, H., DANOY, B. and LABORIT, H. (1962). Variations du taux de potassium plasmatique du sang conservé en présence de 4-Hydroxybutyrate de Na. *Agressologie* 2: 843-848.
- ROSENGARTEN, H., LABORIT, H. and WEBER, B. (1963). Diminution du taux de cholestérol sanguin après administration intraveineuse du 4-Hydroxybutyrate de Na. *Agressologie* 4: 285-290.
- SCHNEIDER, J., THOMALSKA, G., TRAUTMANN, P., SMOLARZ, R. and SABBAGH, R. (1963). Le comportement E.E.G. de l'Homme et de l'animal soumis à l'action progressive du 4-Hydroxybutyrate de Na. *Agressologie* 4: 55-70.
- SUDAKOV, K. V. (1963). A neuro-humoral mechanism of activation of the frontal cortex during physiological starvation. *Bul. of Exper. Biol. and Med.* 54: 817-820.
- TEPPERMAN, J. and TEPPERMAN, H. M. (1961). Metabolism of glucose 1 C¹⁴ and glucose 6 C¹⁴ by liver slices of refed rats. *Amer. J. Physiol.* 260: 1065-1075.
- WEBER, B. (1961). Effet d'épargne protidique du 4-Hydroxybutyrate de Na. *Agressologie* 2: 533-542.
- WOLLEMAN, M. (1963). The metabolism of 4-OH butyric acid. *Agressologie* 4-6: 593-597.