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EMETINE MYOPATHY IN THE RAT

W.G. BRADLEY, J.D. FEWINGS,[1] J.B. HARRIS & MARGARET A. JOHNSON

Muscular Dystrophy Group Research Laboratories, Regional Neurological Centre, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne, NE4 6BE

1 (–)-Emetine (0.25–2.0 mg/kg i.p.) was administered to rats for up to 220 days.

2 At doses of 1.0 mg/kg or less, the animals continued to gain weight but more slowly than the untreated control animals. The physiological changes in the muscles from these animals were minimal; there was a small reduction in both the resting membrane potential and in the maximum rate of rise of the action potential. There was no atrophy or loss of muscle fibres although in the occasional muscle, hyaline fibres, necrotic fibres and split fibres were observed. There was a focal loss of myofibrillar adenosine triphosphatase (ATPase) and nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR) in Type II and Type III fibres, but no such loss in Type I fibres.

3 The animals receiving 2.0 mg/kg of (–)-emetine gained weight slowly for up to 20 days but then rapidly lost weight and by 30 days they were weak and emaciated. The muscles from these animals were severely atrophied and the total muscle wet weight was reduced by almost 20%.

4 The strength of the muscles from these animals was measured *in vitro* using direct stimulation. They were weaker than normal both in absolute terms and when expressed in terms of tension developed/unit wet weight.

5 There was no evidence of either functional or structural denervation but surgically denervated muscles from animals in this group were indistinguishable from denervated muscles from normal rats.

6 Severe structural damage was obvious in the fibres of both extensor digitorum longus and soleus. Necrotic, hyaline and splitting fibres were common and the focal loss of myofibrillar ATPase and NADH-TR activity was extensive and occurred in Type I fibres as well as in Type II and Type III fibres.

7 It is concluded that the muscular weakness induced by (–)-emetine is due to a direct effect on the muscle fibres and that this occurs at a subcellular level. There is no evidence that functional or structural denervation plays any role in the aetiology of emetine myopathy in the rat.

Introduction

The drug (–)-emetine, isolated from the root of *Cephaelis ipecacuanha*, has been used for more than 50 years in the clinical management of amoebiasis (Woolfe, 1963, 1965). The drug has also been used for the control of amoebiasis in experimental animals infested with *Entamoeba histolytica* (Williams, 1959).

Muscle weakness is a recognized side effect of the prolonged administration of the drug (Klatskin & Freidman, 1948; Fewings, Burns & Kakulas, 1973) and the symptoms have been graphically described by Young & Tudhope (1926). The precise cause of the weakness is not known, and it has been variously reported that emetine is capable of blocking transmission at ganglia and neuromuscular junctions (Ng, 1966; Salako, 1970) and that it is capable of directly damaging skeletal muscle fibres 'without recognizable morphological or physiological alteration in the neural components of the motor unit' (Duane & Engel, 1970). However, the role of the peripheral nervous system in the pathogenesis of (–)-emetine-induced muscle weakness is difficult to study because, under certain circumstances, the drug is capable of inhibiting protein synthesis (Grollman, 1966, 1968; Johnson, Donahue & Jondorf, 1971). Inhibitors of protein synthesis may delay the appearance of denervation-induced changes in mammalian skeletal muscle (Grampp, Harris & Thesleff, 1971; Llados & Zapata, 1974). Thus the absence of fibrillation potentials from the muscles of (–)-emetine-poisoned animals (Duane & Engel, 1970) does not necessarily imply that the peripheral nerve is intact and functioning normally. Moreover, in some instances, a morphologically normal motor nerve supply may be associated with a muscle exhibiting many of the usually accepted 'denervation-like' changes (Thesleff, 1960; Josefsson & Thesleff, 1961; Duchen & Stefani, 1971; Harris & Ward, 1974).

In this paper we describe our observations on the effect of (–)-emetine intoxication on the histology and physiology of mammalian skeletal muscle, and our

[1] Present address: Queen Elizabeth Hospital, Woodville, South Australia, 5011.

attempts to clarify the role of the nerve in the development of the syndrome.

Methods

Animals

Male Wistar rats were used which weighed 145–210 g at the start of the experiments. They were housed 6–8 per cage. They were allowed free movement and continual access to food and water. Each animal was weighed weekly and was subjected to regular clinical examination.

The animals were divided into 6 groups, each group comprising 12–24 animals. One group served as a normal control. The remaining groups of animals were given (–)-emetine by intraperitoneal injection for 5 successive days in each week, at a dose level of 0.25, 0.50, 0.75, 1.0 or 2.0 mg/kg respectively.

Animals receiving (–)-emetine, 0.25–1.0 mg/kg, were killed between 120 and 240 days after the start of the experiment; animals receiving 2.0 mg/kg were killed 21–30 days after the start of the experiment. The heart and pleural cavity were examined for obvious signs of cardiac or pulmonary distress. The muscles, usually extensor digitorum longus (EDL) and soleus (SOL), were then removed for physiological, histological or histochemical examination.

Physiology

Experiments *in vivo* were made on tibialis anterior muscles. The animals were anaesthetized with urethane (1.5–2.0 g/kg i.p.). The sciatic nerve was exposed in the thigh and all muscles other than the extensor muscles were denervated. The tibialis anterior muscle was exposed by removing the covering skin and connective tissue. The animals were placed in a bath containing Liley's (1956) bathing fluid (composition (mM): Na^+ 150; K^+ 5.0, Ca^{2+} 2.0, Mg^{2+} 1.0, Cl^- 148, $H_2PO_4^-$ 1.0, HCO_3^- 12.0, glucose 11.0), maintained at room temperature and equilibrated with 95% O_2 and 5% CO_2. The distal stump of the sciatic nerve was mounted on a pair of silver wire electrodes and stimulated with supramaximal rectangular stimuli of 0.05–0.10 ms duration. Action potentials, generated by the application of a single stimulus to the nerve, were recorded from single muscle fibres using an intracellular recording technique (q.v.). Action potentials generated by the application of repetitive stimuli (50 Hz for 200–250 ms) to the nerve were recorded from the muscle with a concentric needle electrode (Medelec, 0.5 mm diameter).

Experiments *in vitro* were made on isolated EDL and SOL muscles. The muscles were mounted in a constant flow bath which was continuously perfused with gassed Liley's fluid maintained at 30–32°C.

Resting membrane potentials were recorded with intracellular microelectrodes. The electrodes were pulled from glass tubing (Corning Ltd, Type 7740) and were filled with 3M KCl. They had tip potentials less than 5 mV and d.c. resistances of 5–15 MΩ. The membrane potential was recorded using a unity gain input stage (input impedance, 10^{12} Ω) and an oscilloscope. Action potentials were generated and recorded by a double microelectrode technique. The current passing electrode, filled with 3M KCl, was inserted into the same fibre as the recording electrode at a separation of 50–100 μm. The muscle fibre membrane was locally hyperpolarized to between –90 and –95 mV before passing depolarizing current (cf. Redfern & Thesleff, 1971). Capacitance neutralization was achieved using feedback between the output and the input of the buffer amplifier. The time differential of the action potential was obtained by electronic differentiation. The frequency response of the differentiator was flat to frequencies in excess of 5 KHz.

Miniature end-plate potentials were recorded from putative end-plate regions by the described intracellular techniques. The noise level of the recording apparatus was approximately 150 μV peak to peak.

The ability of muscles to develop a contracture in response to the application of acetylcholine was assessed under isometric conditions. The muscles were mounted vertically in a 10 ml bath of gassed Liley's fluid at room temperature. A resting tension of 5.0 g was routinely used. Tension was measured with a strain gauge (Statham, G3). Extrajunctional sensitivity to acetylcholine was also examined by the technique of iontophoresis (Nastuk, 1953; Axelsson & Thesleff, 1959).

The ability of the (–)-emetine-poisoned muscles to develop tension in response to direct stimulation was measured *in vitro* under isometric conditions. The EDL muscles were mounted horizontally in a moist chamber on a multi-electrode assembly at a resting tension of 5.0 grams. The electrodes were platinum wires, 2.5 mm apart, assembled as alternating anode and cathode. Stimulation was with 90 V shocks of 1.0 ms duration at a frequency of 150–200 Hz and at room temperature. The responses were recorded with a strain gauge (Statham G1-8-350) and were displayed directly on a storage oscilloscope.

In some experiments we wished to study the effects of denervation on (–)-emetine-poisoned muscles. In these cases, EDL muscles were denervated by sectioning the common peroneal nerve at the knee. The muscles were removed 4 days later.

Histology and histochemistry

Blocks of tissue comprising the middle 4 mm of EDL and SOL muscles were sandwiched between thin slices of liver and orientated so that transverse sections could be obtained. The tissue blocks were then frozen in dichloro-difluoromethane, maintained at approx-

imately −150°C in liquid N_2. The transverse sections, 10 μm thick, were stained with haematoxylin and eosin and sections serial to these were used to demonstrate the activity of Ca^{2+}-activated myofibrillar adenosine triphosphatase (ATPase) (Hayashi & Freiman, 1966) and nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR, Pearse, 1972). Where appropriate the following histochemical methods were also applied: acid phosphatase (Burstone, 1958a, b), myophosphorylase (Godlewsky, 1963), periodic acid-Schiff (McManus, 1948), myofibrillar ATPase after acid-preincubation (Brooke & Kaiser, 1969).

The system of fibre type nomenclature adopted in this study is based on the properties of myofibrillar ATPase in rat skeletal muscle as defined by Stein & Padykula (1966). In rat EDL there is a relatively small number of fibres (approximately 2%) which have low myofibrillar ATPase and high levels of mitochondrial oxidative enzymes (Type I fibres). The remaining fibres consist of approximately equal numbers of small diameter fibres with high myofibrillar ATPase resistant to formalin fixation (Type III), and larger diameter fibres whose myofibrillar ATPase is more sensitive to the effects of formalin (Type II). Thus, the use of the technique for demonstrating myofibrillar ATPase as described by Hayashi & Freiman (1966) makes it possible to differentiate these fibre types on the basis of a single enzyme reaction.

Table 1 Correlation of enzyme characteristics and muscle fibre type in rat extensor digitorum longus muscles

Fibre type	*Myofibrillar ATPase activity (after formalin fixation)*	*Mitochondrial oxidative enzyme activity*
I	+	++
II	++	+
III	+++	++

The mitochondrial oxidative enzyme activity of the Type III fibres is approximately equal to that of the Type I fibres, whereas the Type II fibres have a much lower activity of oxidative enzymes. The correlation of the enzyme characteristics of the three fibre types is summarized in Table 1.

Drugs and chemicals

(−)-Emetine was used as the hydrochloride, and all doses refer to this salt. It was obtained in pure form from BDH Ltd and made up into solution in pyrogen-free distilled water. It was supplied by the Pharmacy Department, Newcastle General Hospital in the form of sterilized sealed 1.2 ml ampoules of 40 μg/ml.

Tetrodotoxin (Sankyo Chemical Co., Tokyo) was supplied by Koch-Light Laboratories Ltd. All other drugs and chemicals were obtained from the usual commercial sources.

Statistical treatment of results

Where necessary, the significance of the difference between two means was assessed with Students' t test; a level of significance of $P < 0.05$ was used.

Results

Clinical observations

All animals receiving (−)-emetine at dose levels of 0.25–1.0 mg/kg gained weight, although not quite as rapidly as normal. The animals receiving 2.0 mg/kg ceased to grow as soon as drug administration began, and by 14–20 days began to lose weight. Details of mean body weight of all groups of animals are given in Table 2. In Figure 1 the mean weight of selected groups of animals are plotted, with all weights expressed relative to the initial body weight (which has been termed 100%). It can be seen that animals receiving a low dose of (−)-emetine gain weight normally for about 6 weeks, and only then begin to fall

Table 2 The mean body weight (± s.e.) of the various groups of animals obtained at different times after the start of the experiments; animals were assigned to either a control group or to a group receiving emetine, 5 days in every 7, at the stated dose

Time	*Control*	*0.25 mg/kg*	*0.50 mg/kg*	*0.75 mg/kg*	*1.0 mg/kg*	*2.0 mg/kg*
0	155 ± 9.5	157 ± 2.3	160 ± 2.8	182 ± 4.1	181 ± 1.9	201 ± 4.7
2	213 ± 13.0	224 ± 13.9	227 ± 5.1	235 ± 4.2	233 ± 4.1	202 ± 4.3
4	276 ± 14.5	273 ± 4.9	277 ± 5.2	291 ± 7.2	277 ± 5.5	175 ± 9.4
6	308 ± 15.0	314 ± 6.0	309 ± 6.0	306 ± 7.3	284 ± 5.3	—
12	370 ± 16.3	370 ± 6.3	359 ± 7.0	340 ± 9.9	298 ± 5.2	—
18	434 ± 16.7	414 ± 8.8	395 ± 7.0	358 ± 9.6	298 ± 8.8	—
28	474 ± 19.0	444 ± 10.0	432 ± 14.0	379 ± 11.2	312 ± 6.5	—

Each group contained 12–24 animals

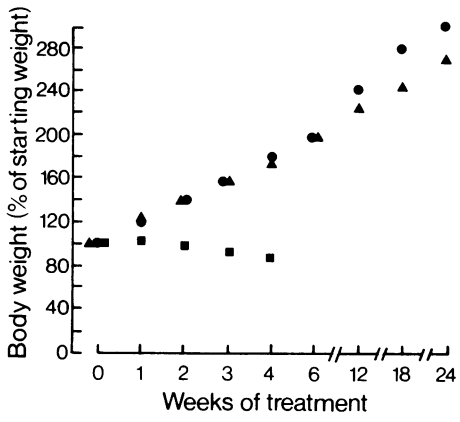


Figure 1 Growth curves of normal rats (●), rats treated with emetine 0.5 mg/kg 5 days per week (▲) and rats treated with emetine, 2.0 mg/kg 5 days per week (■). For each group of animals, the weight at time zero has been designated 100% and its weight thereafter referred to that standard.

behind normal animals. We have never observed the acute weakness following the injection of (–)-emetine that was reported by Salako (1970).

Physiology

The resting membrane potential and the characteristics of the directly elicited action potential were recorded from isolated EDL and SOL muscle fibres. The muscles were removed from control animals and animals that had received (–)-emetine at dose levels of 0.50 mg/kg and 2.0 mg/kg respectively.

(–)-Emetine administration caused a significant fall in the mean resting membrane potential of muscle fibres of both EDL and SOL muscles. The fall was larger in the muscles removed from animals that had received (–)-emetine at a dose level of 2.0 mg/kg rather than 0.5 mg/kg, even though the higher dose was given for a much shorter time period.

Action potential generation was also affected by (–)-emetine administration. Thus in EDL muscle fibres, the mean maximum rate of rise of the action potential, and the amplitude of the overshoot were reduced in muscles from animals treated at dose levels of (–)-emetine of either 0.5 mg/kg or 2.0 mg/kg, the muscles from animals receiving the higher dose being the more severely affected. In SOL muscle fibres, the maximum rate of rise of the action potential was reduced significantly only in those muscles removed from animals treated with (–)-emetine, 2.0 mg/kg. Treatment with (–)-emetine had no effect on the amplitude of the overshoot of the action potential in SOL muscle fibres. The threshold for action potential generation was increased in EDL muscle fibres after treatment with (–)-emetine at either dose level; in SOL muscle fibres, the change in threshold was significant only in those muscles removed from animals treated with the higher dose of (–)-emetine. These results are summarized in Table 3, and some representative records of action potentials are shown in Figure 2.

Miniature end-plate potentials in the (–)-emetine-treated muscles were recorded from all fibres penetrated in the end-plate region (a total of 60 fibres in EDL muscles and 40 in SOL muscles). The

Table 3 Some properties of muscle fibres in isolated extensor digitorum longus (EDL) and soleus (SOL) muscles from normal rats and rats treated with emetine, 0.5 mg/kg and 2.0 mg/kg respectively

			Action potential		
		Membrane potential (mV)	*Max. rate of rise (V/s)*	*Overshoot (mV)*	*Threshold (mV)*
Normal rats	EDL	−77.6 ± 0.4 (30)	856 ± 17 (30)	43.0 ± 0.7 (30)	51.9 ± 0.4 (30)
	SOL	−71.7 ± 0.7 (32)	660 ± 14 (32)	32.1 ± 0.9 (32)	50.6 ± 0.5 (32)
Rats treated with emetine (0.5 mg/kg)	EDL	−71.4 ± 1.2* (27)	792 ± 25* (27)	35.9 ± 1.4* (27)	49.5 ± 0.4* (27)
	SOL	−68.5 ± 0.7* (20)	629 ± 21 (20)	30.9 ± 1.3 (20)	50.9 ± 0.7 (20)
Rats treated with emetine (2.0 mg/kg)	EDL	−65.6 ± 0.8* (54)	419 ± 19* (55)	33.5 ± 0.6* (54)	41.5 ± 0.8* (42)
	SOL	−64.5 ± 0.8* (30)	430 ± 14* (30)	33.2 ± 0.9 (30)	43.1 ± 0.6* (30)

Figures quoted are means ± s.e. mean. The number of fibres sampled is given in parentheses.
*Value differs significantly ($P < 0.05$ or better) from normal.

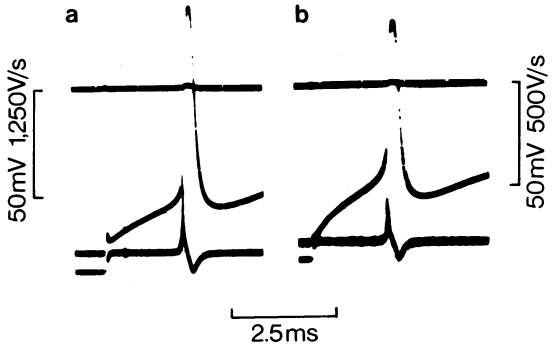


Figure 2 Muscle fibre action potentials generated and recorded by a double-microelectrode technique in isolated extensor digitorum longus muscles from (a) a normal rat and (b) an emetine (2.0 mg/kg)-treated rat. The top trace (horizontal) represents zero potential, the middle trace the first derivative of the voltage recording and the bottom trace the muscle fibre membrane potential. In each fibre, the membrane potential was artificially set to −90 mV before the passage of depolarizing current. Note the different vertical calibration bars in this figure.

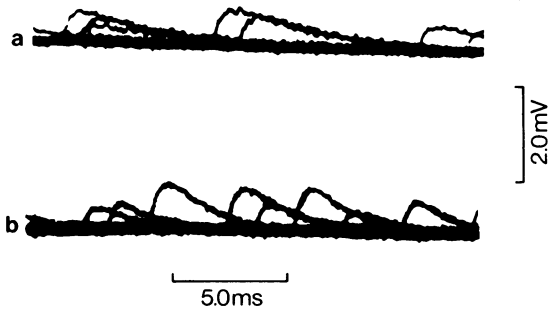


Figure 3 Miniature end-plate potentials recorded from muscle fibres in extensor digitorum longus muscles from (a) a normal rat and (b) an emetine (2.0 mg/kg)-treated rat. The recordings were made onto a storage oscilloscope, and several traces have been superimposed.

miniature end-plate potentials were generally of normal amplitude and frequency (Figure 3) although in some fibres of SOL and EDL muscles from animals treated with (–)-emetine 2.0 mg/kg, the miniature end-plate potentials exceeded 1 mV in amplitude. This is rather larger than normal (0.2–0.6 mV: these laboratories, unpublished) and probably reflects the atrophy that was noted in many of these muscles. The relationship between fibre diameter and miniature end-plate potential amplitude was demonstrated unequivocally by Katz & Thesleff (1957) to be the

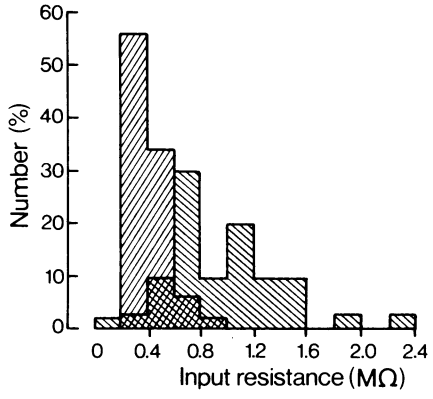


Figure 4 The distribution of 'input resistance' measurements of muscle fibres in extensor digitorum longus muscles from normal rats (cross hatch right to left) and from emetine (2.0 mg/kg)-treated rats (cross hatch left to right).

consequence of the relationship between the 'input resistance' of a muscle fibre and its diameter. It was of interest therefore, to note that the input resistance of EDL muscle fibres from animals treated with (–)-emetine 2.0 mg/kg was increased from a normal value of 0.426 ± 0.020 MΩ to 1.259 ± 0.10 MΩ ($n = 50, 30$, respectively). The distribution of these results is shown in Figure 4.

It has been suggested (Ng, 1966) that the cause of muscular weakness seen during (–)-emetine therapy is an action of (–)-emetine 'at or near the neuromuscular junction' and Salako (1970) has suggested that the drug has a 'tubocurarine-like action' on the neuromuscular junction. If an action at either a pre-synaptic or a post-synaptic site were primarily responsible for muscle weakness, the implication would be that either individual muscle fibres or entire motor-units were paralysed. Moreover this paralysis would have to be of considerable duration in order to account for the clinical features of the weakness. Such affected muscle fibres would then resemble the 'functionally denervated' muscle fibres seen, for example, in botulinum poisoning (Thesleff, 1960), inherited motor and end-plate disease (Duchen, 1970) and α-bungarotoxin poisoning (Chuang & Huang, 1975). In all of these conditions, the muscle fibres become sensitive to acetylcholine at extrajunctional regions, and generate action potentials in the presence of tetrodotoxin (Thesleff, 1960; Duchen & Stefani, 1971; Harris & Ward, 1974; Chang *et al.*, 1975). It may be of significance therefore, that we could find no 'tetrodotoxin-resistant' action potentials in the muscles of animals receiving 0.5 mg/kg (–)-emetine (20 fibres tested in each of EDL and SOL), and of a total of 87 fibres tested in muscles of rats treated with 2.0 mg/kg (–)-emetine, we found only 2 fibres (both in EDL

muscles) which would generate such an action potential. The potentials were very small, and would probably not have been propagated along the muscle fibre outside the hyperpolarized region of membrane (see methods section) due to inactivation.

Moreover, extrajunctional sensitivity to acetylcholine was found to be absent when the muscles were examined by the technique of iontophoresis, and no contracture could be obtained in the muscles of (−)-emetine-treated animals even in response to acetylcholine, 0.1 mM.

These results indicated that the administration of (−)-emetine at doses large enough to cause emaciation in the animal and marked changes in the properties of the muscle fibres did not induce 'denervation-like' changes in the muscle. Further evidence that the peripheral nerve was not severely damaged was obtained by recording the electrical activity of tibialis anterior muscles *in vivo* in response to indirect stimulation in anaesthetized animals that had received (−)-emetine 2.0 mg/kg. In the first series of experiments, intracellular action potentials were recorded. All muscle fibres tested responded to indirect stimulation by generating an action potential, and the latency between stimulation and excitation of the muscle was normal. As in the experiments *in vitro*, the resting membrane potential and the maximum rate of rise of the action potentials were reduced. The amplitude of the overshoot of the action potentials was not significantly reduced. The results are summarized in Table 4.

In the second series of experiments, the electrical response of the muscle was recorded using standard EMG techniques in response to stimulation of the nerve at a frequency of 50 Hz (see methods section). The EMG revealed no 'myasthenia-like' response (see Simpson, 1966) indicating that under normal circumstances, there was little likelihood that muscular weakness would be caused by a low safety-factor of neuromuscular transmission. Some typical records are shown in Figure 5.

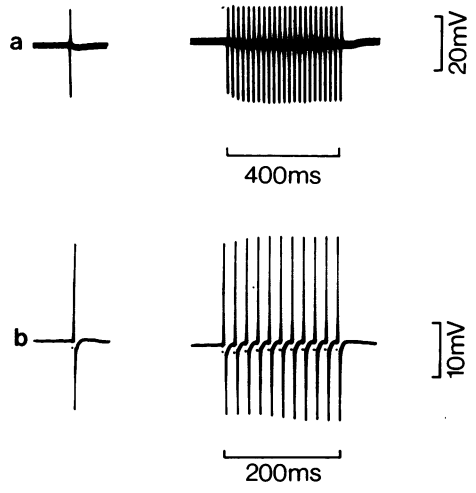


Figure 5 EMG records from the tibialis anterior muscles of two emetine (2.0 mg/kg)-treated rats (a, b) *in vivo*. A standard concentric needle electrode technique was used. The responses of the muscle to single shocks (left) and repetitive stimulation (50 Hz) are shown. The small increase in total EMG amplitude during stimulation is normal. The animals were anaesthetized with urethane.

We next considered the possibility that either an acute or sub-acute denervating process might not give rise to physiological and pharmacological symptoms of denervation as a result of the protein-synthesis-inhibiting action of (−)-emetine. Accordingly, the

Table 4 Some properties of tibialis anterior muscle fibres *in vivo* in normal rats and in rats treated with emetine, 2.0 mg/kg.

	Muscles in normal rats	*Muscles in rats treated with emetine, 2.0 mg/kg*
Membrane potential (mV)	−78.9 ± 0.6 (24)	−71.9 ± 1.5* (22)
Action potential max. rate of rise (*V/s*)	545 ± 19 (24)	433 ± 29* (22)
Action potential overshoot (mV)	34.0 ± 2.2 (24)	28.5 ± 3.1 (22)
Action potential latency (ms)	3.10 ± 0.14 (24)	3.24 ± 0.14 (22)

Animals were anaesthetized with methane. Figures quoted are means ± s.e. mean. The number of fibres sample is given in parentheses.
* Value differs significantly ($P < 0.05$ or better) from normal.

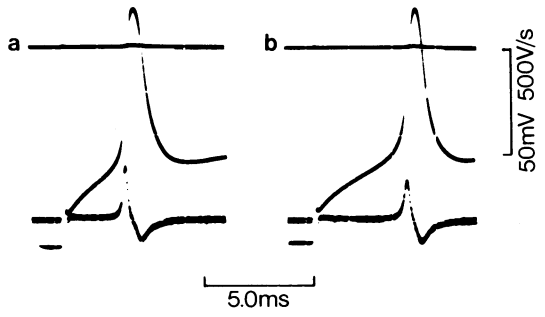


Figure 6 Typical denervated muscle fibre action potentials recorded from isolated extensor digitorum longus muscles *in vitro* in the presence of tetrodotoxin, 1 μM. (a) Record from a denervated muscle of a normal rat; (b) record from a denervated muscle of an emetine (2.0 mg/kg)-treated rat.

extensor muscles of one hind limb of six animals were denervated by sectioning the common peroneal nerve at the knee. Two animals were untreated controls, two animals had received (−)-emetine 0.5 mg/kg and two had received (−)-emetine 2.0 mg/kg. Four days after the operation the denervated muscles were removed for examination. The results (Table 5) made it clear that treatment with (−)-emetine is not able to inhibit the onset of denervation-induced changes in the muscle. Some typical tetrodotoxin-resistant action potentials are shown in Figure 6.

Since the muscles of the most severely affected animals were atrophied we wished to determine whether or not muscles were physically 'weaker' than normal when 'strength' was expressed as tension developed/unit wet weight of muscle. Tetanic contractions were elicited in isolated directly stimulated EDL muscles from normal rats and rats treated with (−)-emetine 2.0 mg/kg. The results (Table 6) show that the muscles from (−)-emetine-treated animals were not only weak, but were unable to generate the same tension as normal when expressed on the basis of their individual wet weights.

Table 6 Tetanic tension generated by isolated directly stimulated extensor digitorum longus muscles from normal rats and rats treated with emetine, 2.0 mg/kg, expressed in terms of muscle wet weight

	Muscles from normal rats	*Muscles from rats treated with emetine, 2.0 mg/kg*
Tetanic tension (g/mg wet wt.)	0.93 ± 0.03	0.60 ± 0.05*
n	7	4

Figures quoted are means ± s.e. mean. *n* = number of muscles studied.
* Value differs significantly ($P < 0.05$ or better) from normal.

Table 5 Some properties of muscle fibres in isolated denervated extensor digitorum longus muscles from normal rats and from rats treated with emetine 0.5 mg/kg and emetine 2.0 mg/kg. The muscles were removed 220 days and 29 days respectively after treatment started. The muscles were denervated 4 days before the experiment.

	Muscles from normal rats	Muscles from rats treated with emetine	
		0.5 mg/kg	*2.0 mg/kg*
Membrane potential (mV)	−62.0 ± 0.5 (11)	−62.4 ± 0.6 (10)	−60.0 ± 1.3 (21)
Action potential			
Max. rate of rise (*V/s*)	582 ± 36 (11)	530 ± 42 (10)	376 ± 20 (21)
Overshoot (mV)	30.6 ± 2.0 (11)	33.0 ± 1.4 (10)	26.0 ± 0.3 (21)
Action potential in tetrodotoxin† occurence*	14/14	14/14	16/16
Max. rate of rise (*V/s*)	212 ± 8 (14)	235 ± 25 (14)	203 ± 18 (16)
Overshoot (mV)	21.5 ± 1.9 (14)	18.7 ± 2.8 (14)	14.1 ± 1.9 (16)

Figures quoted are means ± s.e. mean. The number of fibres sampled is given in parentheses. * No. of fibres responding/No. of fibres tested. † A concentration of 1 μM was used in this experiment.

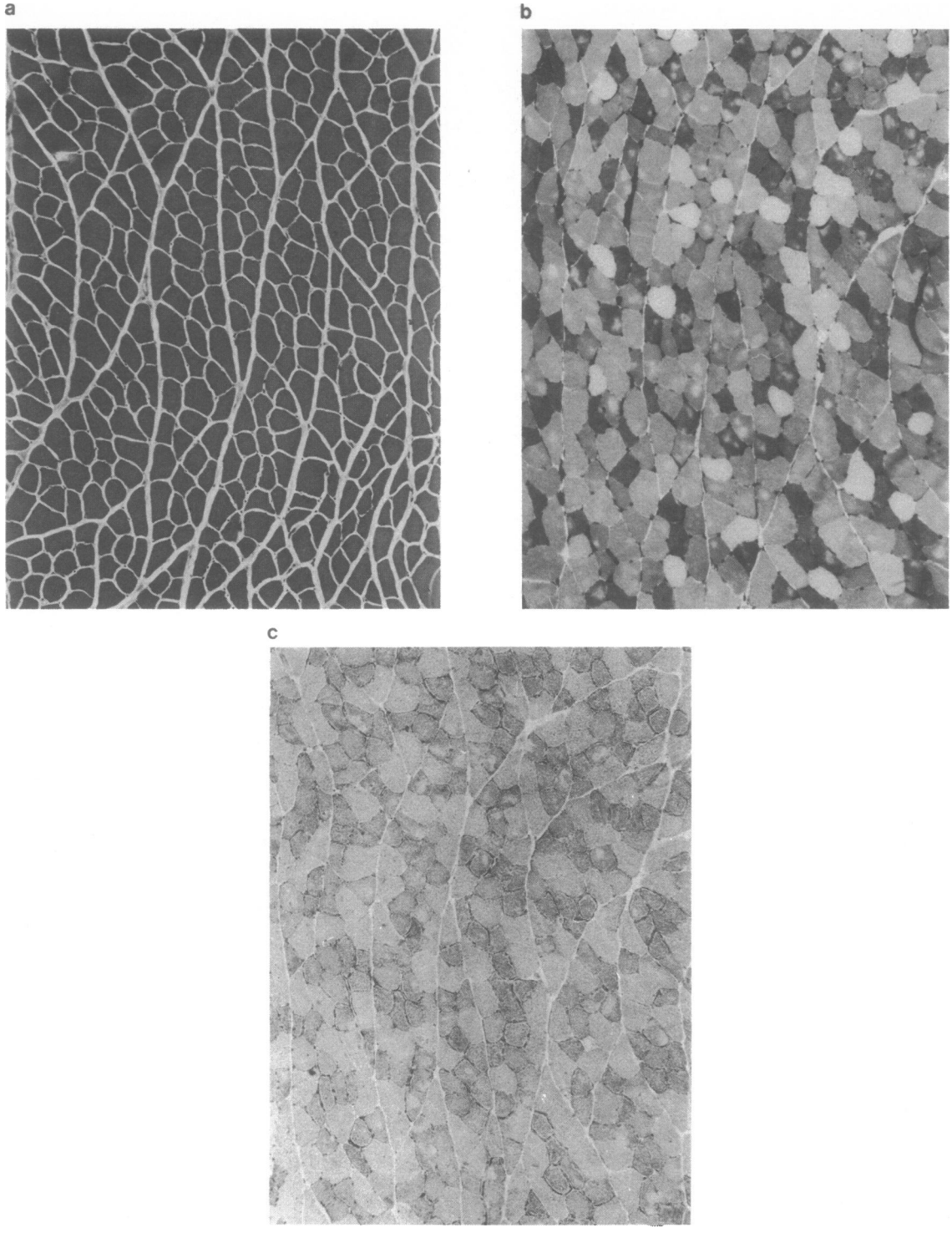


Figure 7 (a) Transverse section ×150 of part of an extensor digitorum longus muscle from an emetine (0.5 mg/kg)-treated rat stained with haematoxylin and eosin. Note the normal histological appearance of this muscle. (b) Transverse section ×150 serial to (a), stained with myofibrillar ATPase. Numerous areas of myofibrillar ATPase loss (pale areas in fibres) are seen, even though from haematoxylin and eosin staining in (a) the fibres are histologically normal. (c) Transverse section × 150 serial to (a) and (b) stained with NADH-TR. Foci of enzyme loss are seen in this preparation (pale areas in fibres) although the areas of loss are less well defined than in the myofibrillar ATPase preparation (b).

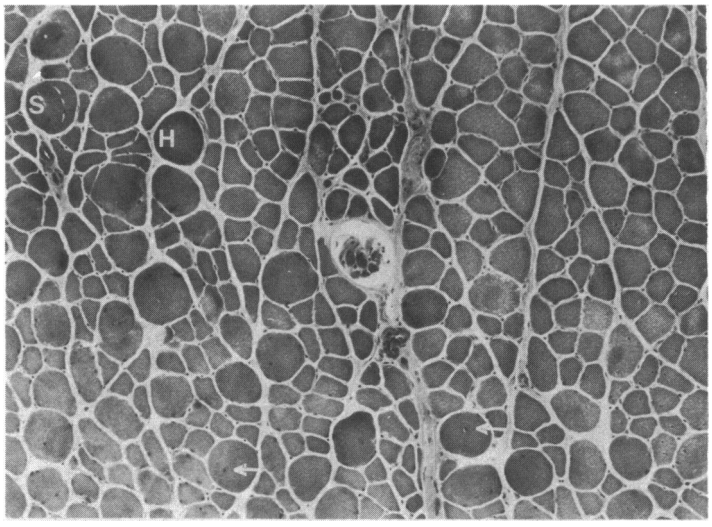


Figure 8 Transverse section x 150 of part of an extensor digitorum longus muscle from an emetine (2.0 mg/kg)-treated rat, stained with haematoxylin and eosin. Hyaline fibres (H) centrally nucleated fibres (arrows) and splitting fibres (S) can be seen.

Histology and histochemistry

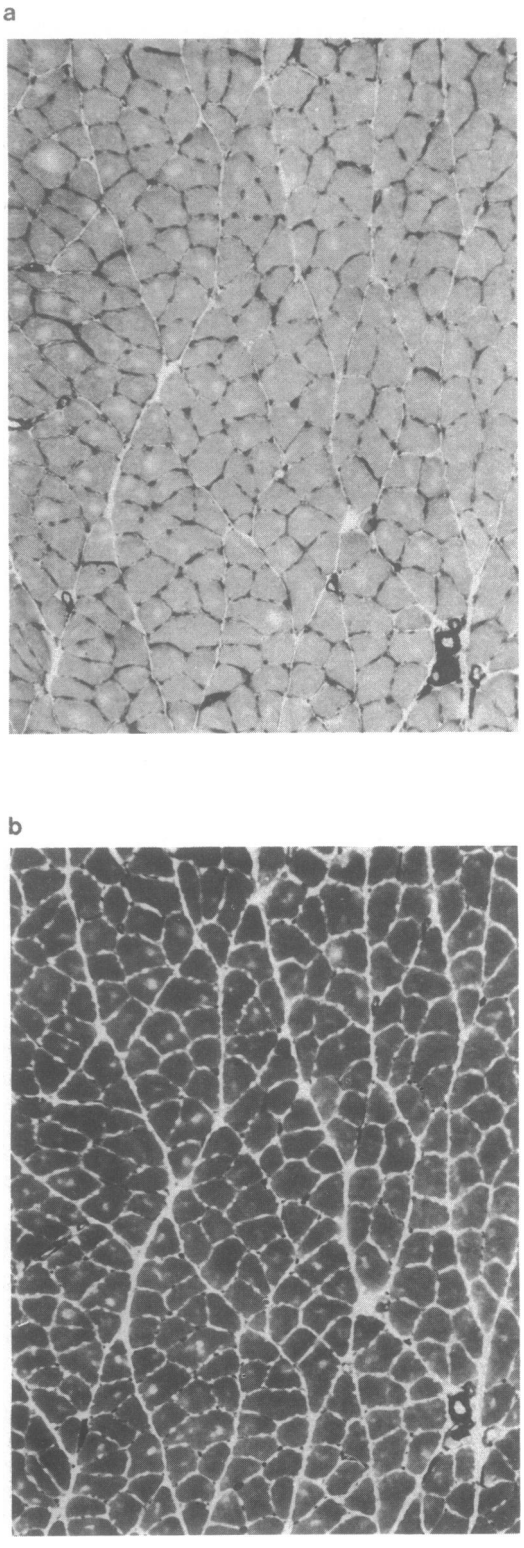
The chronic administration of low doses of (–)-emetine (0.5 mg/kg 5 days weekly for 180–220 days) had little effect on the histology or either EDL or SOL. There was no atrophy or loss of muscle fibres although an occasional necrotic fibre was seen in a few of the muscles studied. In less than 10% of the muscles studied, hyaline fibres (Bethlem, 1970) were seen but even in these muscles, the incidence of hyaline fibres was very low (< 1% of all fibres). There was also some evidence of fibre splitting and central nucleation.

Muscle fibres in EDL muscles which appeared normal when stained with haematoxylin and eosin often revealed a local loss of myofibrillar ATPase (Figure 8). This was a variable phenomenon. Thus of 12 EDL muscles studied, 4 were unaffected, 3 were severely affected and in the remaining 5, the changes were moderate. In the severely affected muscles up to 25% of the fibres were affected; in the least affected less than 5% of the fibres were affected. The reduction in ATPase activity was seen in both Type II and Type III fibres; since Type I fibres have low myofibrillar ATPase activity any further reduction in activity caused by (–)-emetine would be difficult to detect by the standard histochemical techniques. However, the acid-preincubation method of Brooke & Kaiser (1969) results in a 'reversal' of the ATPase staining characteristics of the main muscle fibre types. Thus, Type I fibres appear darkly stained in comparison to Type II and III, and the technique can therefore be used to detect any focal loss of ATPase activity in such fibres. However, no such focal loss of ATPase was seen in the Type I fibres of SOL, and only rarely was the phenomenon observed in the Type I fibres of EDL.

Type II and Type III fibres appeared to be equally susceptible to the focal loss of myofibrillar ATPase. In many fibres only one such area of reduced myofibrillar ATPase activity was seen, but as many as 3 such areas in a single fibre were not uncommon. The edges of these areas were not well defined, and suggested less severe disruption of myofibrillar material at the periphery (Figure 8). It is of interest to note that serial sections demonstrating the activity of NADH-TR revealed corresponding areas where the activity of this mitochondrial enzyme was also reduced (Figure 7). The loss in activity of NADH-TR appeared to be less severe than that of myofibrillar ATPase.

The demonstration of total myophosphorylase activity showed that this was virtually normal, except in the areas of total myofibrillar ATPase loss, and there was no evidence that the activity of either acid phosphatase or any other lysosomal hydrolytic enzyme was changed in the areas of myofibrillar ATPase loss.

Almost all of the animals receiving (–)-emetine over long periods of time showed loss of Type III fibres from SOL. This loss was not due to a selective atrophy of Type III fibres, but appeared in conjunction with an increase in the proportion of



'intermediate' fibres, suggesting that over the course of the experiment there was a transition of Type III fibres to Type I. However, control experiments (Johnson, unpublished) revealed that transition of Type III fibres to Type I fibres in (−)-emetine-treated SOL muscles was an index of maturation, rather than being drug-induced.

The systematic administration of larger doses of (−)-emetine (2.0 mg/kg) gave rise to similar but more severe changes as those previously described. In both SOL and EDL, hyaline fibres and splitting fibres were commonly seen (Figure 8). Similarly, the muscles with apparently normal histology as depicted by haematoxylin and eosin showed loss of myofibrillar ATPase activity and local loss of NADH-TR activity. However, unlike muscles from animals treated with low doses of (−)-emetine there was also a loss of myofibrillar ATPase in Type I fibres (demonstrated by the ATPase reversal technique; Figure 9) in the muscles of animals receiving these high doses of (−)-emetine.

Muscles from these animals were atrophied. The average wet weight of the EDL muscles for example fell from a normal value of 120 ± 3.5 mg ($n = 14$) to 97 ± 7.6 mg ($n = 6$).

Discussion

An attempt has been made to determine some of the features of (−)-emetine-induced myopathy in the rat. The study is not simply of academic interest since (−)-emetine is still occasionally used in the treatment of severe amoebiasis even though metronidazole is probably the drug of choice (Manson-Bahr & Ormerod, 1971). The highest dose we used in this study (2.0 mg/kg daily for 5 days in every 7) is not excessive by clinical standards; the dose of (−)-emetine used clinically is 60 mg/adult by injection daily for 10 days or more, often followed by chloroquine (which may also be myotoxic; Aguayo & Hudgson, 1970). This recommended dose, in the average adult, approximates to 1.0 mg/kg, and it is given by intramuscular or subcutaneous injection.

Generally, we found morphological and physiological evidence of muscle damage without any sign of involvement of the peripheral nervous system. Thus, even in animals receiving the highest doses of

Figure 9 (a) Transverse section ×150 of part of a soleus muscle from an emetine (2.0 mg/kg)-treated rat stained for myofibrillar ATPase. Foci of enzyme loss (pale areas) are seen in many of the Type I muscle fibres in this muscle. (b) Transverse section ×150 serial to (a) stained for myofibrillar ATPase after pre-incubation with formalin at pH 4.5. Type I fibres appear dark in this preparation, and the foci of enzyme loss are more easily distinguished.

the drug, neuromuscular function, as judged by the presence of miniature end-plate potentials and normal EMG records, was not impaired. Moreover, the muscles themselves exhibited none of the features associated with 'denervation' (see also Duane & Engel, 1970) and this lack of 'denervation-like changes' was not an indirect effect reflecting the ability of (−)-emetine to inhibit protein synthesis. The physiological changes that were demonstrated (i.e. fall in resting membrane potential, reduction in maximum rate of rise of the action potential) were more severe in the animals receiving high doses of (−)-emetine for 21–30 days than in those receiving lower doses for much longer periods. However, the precise significance of these changes is not clear since they occur in so many pathological conditions, involving directly or indirectly either peripheral nerve, skeletal muscle or both (see for example Harris, 1971; Albuquerque, Warnick, Tasse & Sansone, 1972; Harris & Ward, 1974) that they must be considered non-specific. The large increase in threshold for action potential generation seen in the muscles of the more severely affected animals may be of some significance. The threshold was shifted by 10 mV, from −50 mV in muscles from normal animals to approximately −40 mV in mucles from animals treated with 2.0 mg/kg (−)-emetine and this could have the effect of reducing the safety factor for neuromuscular transmission.

However, such a postulated reduction in safety factor cannot possibly account for the degree of weakness observed in these muscles since the muscles are weaker than normal when stimulated directly, and when their strength is expressed in terms of their individual wet weights. This latter observation would imply a direct involvement of the contractile material and/or its associated enzymes.

Our physiological and pharmacological observations of the properties of muscles removed from (−)-emetine-treated rats suggest to us that the demonstrable muscle weakness is primarily mediated by drug-induced damage to the muscle fibre rather than to an effect involving neuromuscular transmission. This conclusion is in direct contrast to that reached by Ng (1966) and Salako (1970). It is difficult to reconcile our differing points of view, particularly when it is considered that both Ng (1966) and Salako (1970) mainly described the acute effects of (−)-emetine administration to isolated or *in situ* preparations.

The observation that is most difficult to reconcile with our own is that of Salako (1970), who reported that following the injection of 1 mg/kg (−)-emetine subcutaneously the rat showed 'no spontaneous muscular activity' 'felt limp' and 'did not right itself when placed on its back'. We have never observed such a response, and have no explanation for this behaviour.

Salako (1970) also reported that rat sciatic nerve-gastrocnemius preparations, made *in vivo* in rats that had received a series of injections of emetine, had smaller contractions than normal and fatigued more rapidly than normal. This observation is not inconsistent with our findings, however, since Salako's results could be a demonstration of the inability of the muscle to generate tension, rather than a manifestation of partial neuromuscular failure induced by the prolonged administration of (−)-emetine.

Similarly, direct evidence of muscle damage was the observation of a dose-dependent focal loss of myosin ATPase suggesting a localized disruption of myosin filaments in (−)-emetine-damaged muscles. Preliminary observations by our colleagues (Bindoff & Cullen, personal communication) on the ultrastructural changes induced by emetine reveal such a disruption at least in Type II fibres. Similarly, the reduction of NADH-TR activity demonstrated by histochemistry is suggestive of mitochondrial damage, and autolytic changes involving the mitochondria have been seen by Bindoff & Cullen in both Type I and Type II fibres. In this context it is of interest that in the liver, (−)-emetine has been found to be associated with the mitochondria (Miller & Jondorf, 1970). Further, in the cardiac muscle of (−)-emetine-treated dogs, Murphy, Bullock & Pearce (1971) have correlated reduced mitochondrial oxidative phosphorylation with structural damage to the mitochondria.

Clearly a major disruption of mitochondrial metabolism would lead to the rapid loss of the appropriate cell; thus it might be expected that the mitochondria-rich muscle fibres (i.e. Type I) would be more susceptible to the drug than the relatively less mitochondria-rich Type II and Type III fibres. Similarly, muscles such as SOL that contain almost exclusively mitochondria-rich fibres, might be expected to be more severely affected than muscles such as EDL which contain a mixture of mitochondria-rich and mitochondria-poor fibres. It should be noted though, that the precise degree of damage caused by the drug in the different types of muscle can only be determined by quantitative electron-microscopic techniques. Until this determination is made, the role of mitochondrial damage in (−)-emetine-induced muscle damage remains unclear.

In summary, our observations, physiological, pharmacological and histological, all lead to the conclusion that the primary cause of muscular weakness, seen during the prolonged use of (−)-emetine is a direct effect of (−)emetine on the skeletal muscle fibres. We cannot deny that the acute administration of (−)-emetine either to the whole animal, or to isolated mammalian tissues and organs may reveal other direct effects of the drug on, for example, ganglionic and neuromuscular transmission. Neither can we deny the possibility that there may be a slight, barely measurable effect of (−)-emetine on the safety factor of neuromuscular transmission in treated animals. However, it seems unlikely that the drug

seriously impairs neuromuscular transmission either by inducing a neuropathy (Young & Tudhope, 1926) or by affecting the neuromuscular junction directly (Ng, 1966; Salako, 1970). It would appear, therefore, that (−)-emetine myopathy is a condition caused by the direct effects of the drug on skeletal muscle fibres.

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