standable that L-Dopa enhanced the effects of apomorphine. Amantadine, on the contrary, inhibited the gnawing compulsion induced by a dose of 10 mg/kg of apomorphine. A similar dose-dependent inhibition by amantadine was observed when a dose of apomorphine (4 mg/kg) inducing half the maximal effect was used (Table II). Amantadine behaved in this respect as the dopaminergic receptor blocking agents, metoclopramide and chlorpromazine.

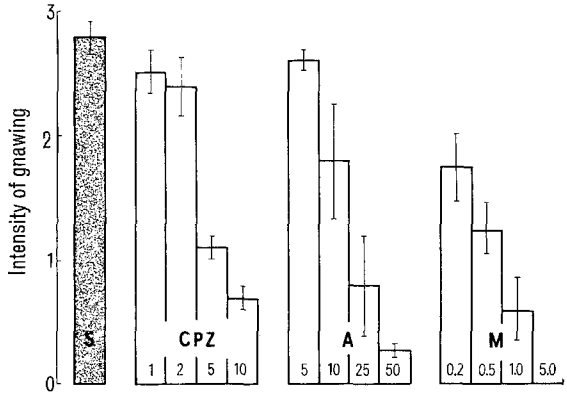

Fig. 2. Effect of chlorpromazine (CPZ), amantadine (A), metoclopramide (M) or saline (S) on the gnawing compulsion induced by apomorphine. CPZ, A, M or S were given in the doses indicated (mg/kg i.p.) 30 min before the injection of apomorphine (10 mg/kg) and the gnawing compulsion was noted after a further 30 min using an arbitrary scale from 0 to 3. The means ± S.E. of 5–10 rats are given.

The inhibition of apomorphine gnawing by amantadine cannot be explained only by the release of dopamine, which has been suggested as a mechanism for the action of amantadine[7]. We propose that amantadine, in addition to its amine releasing properties, has the ability partially to occupy the dopaminergic receptors without causing a marked agonistic action of its own, and thus competes with apomorphine at the receptor sites.

Zusammenfassung. Amantadin bewirkte bei Ratten eine dosisabhängige Hemmung der sogenannten Apomorphin-«Gnawing Compulsion» und verhielt sich somit ähnlich wie ein Dopaminrezeptorblocker.

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Catecholamine Depleting Effect of Black Widow Spider Venom on Fibres Innervating Different Guinea-Pig Tissues

The venom of the black widow spider *Latrodectus mactans tredecimguttatus* (Rossi) has been shown to cause acetylcholine (ACh) depletion of rat superior cervical ganglia[1], of brain cortex slices[2] and of *Torpedo* electric tissue slices[3], when added in vitro to the tissue incubation medium. These findings are in good agreement with electrophysiological experiments where the venom was assayed in vitro on cholinergic systems such as frog[4] and cat[5] muscle end-plates and rat superior cervical ganglia[1,6], indicating a presynaptic site of action of the venom, affecting the mechanism of transmitter release. *Latrodectus* venom has been demonstrated to affect also other types of nerve endings besides the cholinergic ones, namely, lobster excitatory and inhibitory neuromuscular junctions[7], locust excitatory neuromuscular junctions[8] and rat iris adrenergic nerve-terminals[9]. The last result was obtained by subjecting irises incubated in vitro to fluorescence histochemistry for the detection of catecholamines; addition of the venom was followed by the disappearance of the specific yellow-green fluorescence of the adrenergic nerve fibres. This catecholamine-depleting effect was not secondary to ACh release, as demonstrated by the normal appearance of controls where irises were incubated in the presence of ACh and eserine or of carbachol.

Extending the observations on rat iris, we have now assayed the venom on thin innervated tissues of guinea-pig, equally suited for stretch preparations. In addition to irises, fragments of mesentery, spleen capsule, inferior vena cava, gut longitudinal muscular layer (including

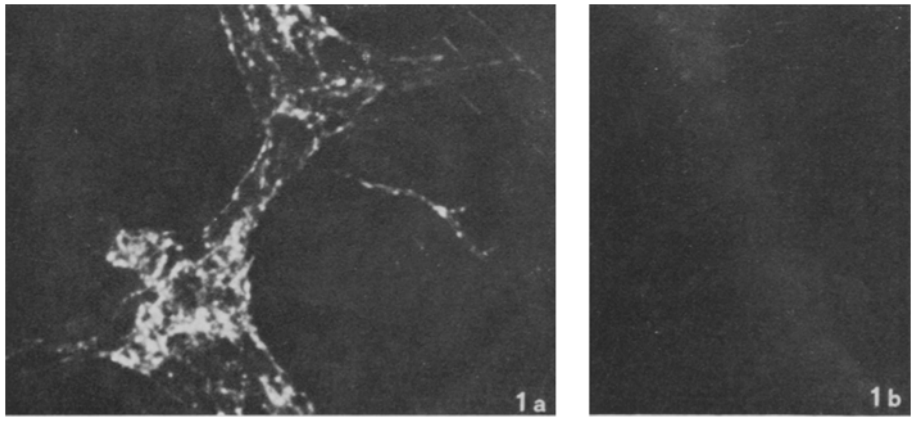
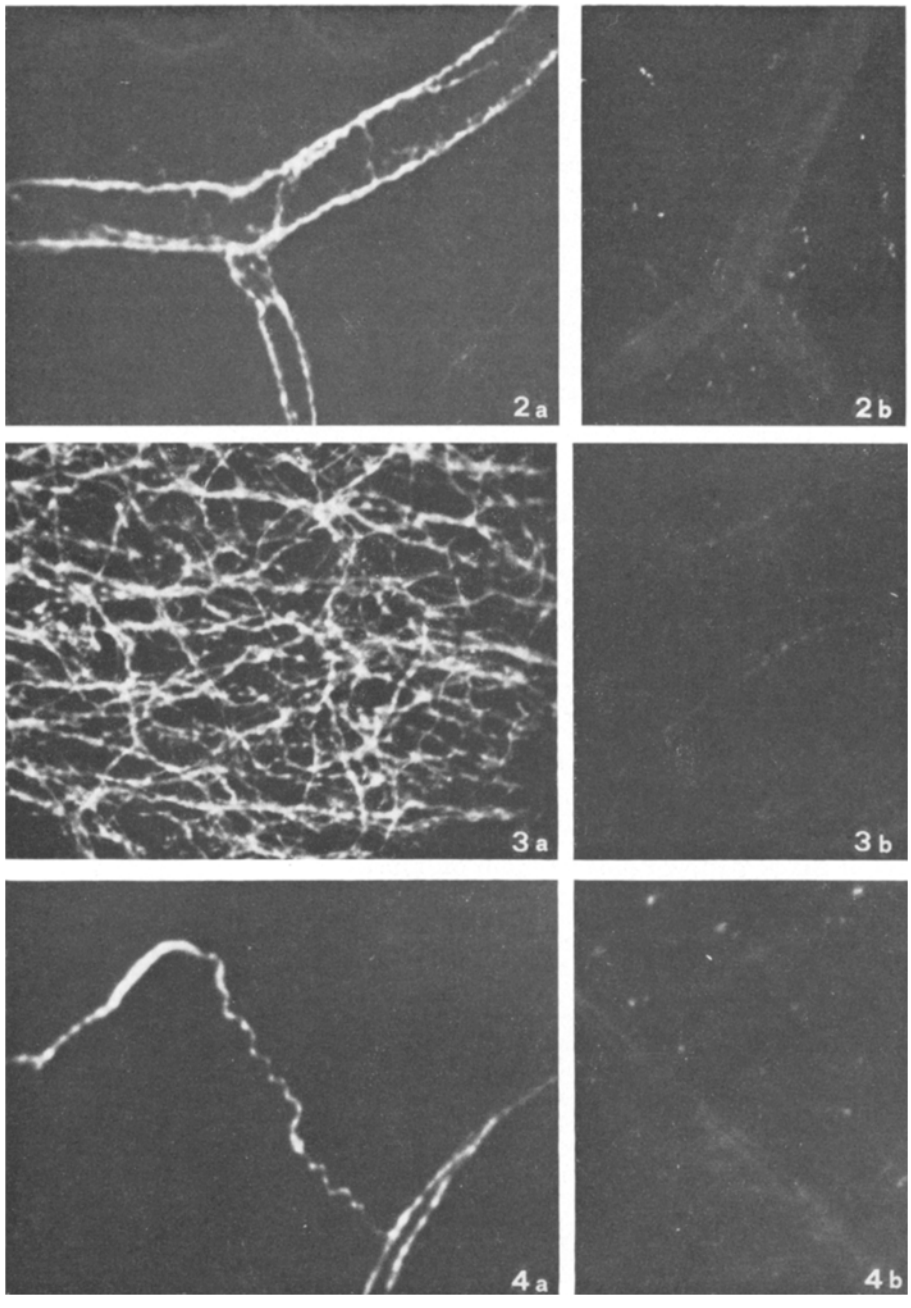


Fig. 1. Longitudina muscular layer of guinea-pig gut incubated in the absence (a) and in the presence (b) of the venom (0.5 pairs of glands/ml of medium). The yellow-green fluorescence of the adrenergic nerve fibres forming the Auerbach plexus disappears as a consequence of venom addition.



Figs. 2, 3, 4. Guinea-pig mesentery (2), iris (3) and spleen capsule (4) incubated in the absence (a) and in the presence (b) of the venom (0.01 pairs of glands/ml of medium). At this venom concentration, the disappearance of the fluorescence is complete or almost complete.

the Auerbach plexus) were dissected out from young adult male and female guinea-pigs and incubated in an oscillating water bath at 37 °C in O_2 gassed vessels in a medium with the following composition (mM): NaCl, 119.5; KCl, 4.2; $CaCl_2$, 1.3; $MgSO_4$, 1.2; Na_2HPO_4-HCl buffer (pH 7.4), 16.3; glucose, 10; ascorbic acid, 1.14. Details regarding the preparation of the venom and the determination of its protein content and toxicity have been reported in a preceeding paper[2]. Up to 10 µl of the venom solution, appropriately diluted in 0.9% NaCl, were added to the experimental vessels, and equal amounts of 0.9% NaCl were added to the controls. After 1 h incubation, the tissues were washed in several changes of fresh medium, stretched on microscope slides, dried in a desiccator, treated with formaldehyde gas (70% humidity) and observed with a fluorescence microscope[10]. The yellow-green fluorescent adrenergic fibres had the same appearance in control tissues, incubated without the venom, as in non-incubated tis-

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sues; the incubation conditions employed had therefore apparently not altered the structures and the noradrenaline stores.

All the tissues assayed reacted to the addition of the venom with a striking reduction of the specific yellow green fluorescence; however, the concentration necessary to obtain this effect was not the same for all tissues. The addition of an extract corresponding to 0.1 pairs of venom glands/ml of incubation medium (13 µg protein/ml) was followed by complete disappearance of the specific fluorescene in all the tissues examined, with the exception of the longitudinal muscular layer of the gut. This tissue showed an almost total depletion only when incubated at a 5 times higher concentration (Figure 1). With the addition of 0.01 pairs/ml the iris, the mesentery, the inferior vena cava and the spleen capsule still showed a total or subtotal disappearance of the specific fluorescence (Figures 2, 3, 4), while at a 10 times lower concentration the depleting effect was still clearly visible in the mesentery, but only very slightly in the other tissues. These quantitative differences in the response of various tissues could be due to differences either in penetration of the venom, or in sensitivity of the nerve terminals towards it. Guinea-pig irises were sensitive to the same concentrations of venom as rat irises, although the whole organisms differ in sensitivity. The amounts of venom/ml of incubation medium which proved effective in vitro may be compared with the LD_{50}/g of animal body weight in vivo, which was established for mice: 0.01 gland couples corresponded to 2 LD_{50}'s/h.

The neurotransmitter depleting action of *Latrodectus* venom, which has already been shown not to be restricted to cholinergic nerve terminals, extends therefore to adrenergic nerve fibres and terminals of different mammalian organs, although with different degrees of susceptibility. These findings may help to explain the symptoms of *Latrodectus* poisoning.

Riassunto. Diversi tessuti di cavia, adatti per l'allestimento di preparati in toto per la dimostrazione delle catecolamine (per mezzo della fluorescenza specifica che si sviluppa in seguito al trattamento con formaldeide gassosa) vengono incubati in vitro in presenza o in assenza di un estratto di ghiandole velenifere del ragno *Latrodectus mactans tredecimguttatus* (Rossi). I tessuti incubati in presenza del veleno (in concentrazioni che per certi tessuti sono di 1,3 µg di proteina/ml) mostrano una scomparsa della fluorescenza, evidentemente dovuta a uno svuotamento delle riserve di noradrenalina dalle terminazioni adrenergiche.

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Development of Tolerance to Δ^9-THC in the Frog

Although 1-Δ^9-tetrahydrocannabinol (Δ^9-THC) is now generally recognized as the principle active component of marihuana (*Cannabis sativa*)[1], it is still a matter of dispute whether the biological activity resides in Δ^9-THC, its hydroxylated metabolites, particularly 11-hydroxy-Δ^9-THC, or both[2]. The latter metabolite has been reported to be markedly more potent in the mouse than the parent compound following intracerebral administration[2]. However, it has also been noted that while Δ^9-THC is extensively metabolized to 11-hydroxy-Δ^9-THC by the liver, the amount of this metabolite that reenters the blood is rather negligible, the 11-hydroxy metabolite apparently being excreted rather than recirculated[3]. This suggests that the activity of the 11-hydroxy metabolite may be negligible if the parent compound is being examined. This might explain Sofia and Barry's[4] finding that SKF-525, which inhibits the microsomal enzyme systems responsible for much of the metabolism of Δ^9-THC, did not attenuate the depressant effect of Δ^9-THC. However, SKF-525 A has recently been shown to increase the latency of the onset of activity of Δ^9-THC[5], which supports the contention that a metabolite may in fact be the active component in Δ^9-THC. However, 2 aspects of the latter experiment make this result somewhat tenuous. First, SKF-525 A was shown to have a slight excitatory effect on the behavioral response being measured, (operant bar pressing), and second, the same animals were used to assess the onset of the effects of Δ^9-THC alone and in the presence of SKF-525 A, thus introducing the possibility that tolerance to the effects of Δ^9-THC may have been responsible for the increased latency of onset[6].

To investigate the problem somewhat differently, we examined the effects of Δ^9-THC in the frog. The reason for using this animal is that the mechanism responsible for hydroxylation of drugs by the liver is not present in these animals[7]. Nor is it possible to induce these drug metabolizing enzymes in the frog by treatment with phenobarbital, although this has been shown for a variety of other species[8]. Consequently, unless other tissues are actively metabolizing the drug, any behavioral effects observed following administration of Δ^9-THC ought to be due to the parent compound and not its hydroxylated

Duration of loss of righting reflex in the frog following chronic administration of Δ^9-THC

	N	Median time (sec)		
		Day 1	Day 3	Day 5
Drug (60 mg/kg)	7	2435	76	1
Control	6	1	1	1

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