6-hydroxydopamine, i.e. it was of small magnitude and outlasted the period of stimulation, arterial pressure fell slightly or did not change, intestinal motility was unaltered and pretreatment with intravenous atropine 0.5 mg/kg failed to affect it (Figure 1). The maximum reduction in mesenteric vascular resistance (mean ± SE, $n = 6$) was 17 ± 4% of the pre-stimulation value. No mesenteric flow or resistance changes could be induced by nerve stimulation in three of the nine cats even after increasing the total dose of bretylium to 60 mg.

Discussion. These experiments have shown that stimulation of the nerves surrounding the superior mesenteric artery after adrenergic neurone blockade produces vasodilatation within the area of distribution of this vessel. Since the pancreatico-duodenal branch was tied and the branches to the large intestine were divided, it seems likely that the dilatation occurred in the small intestine although it cannot be excluded that dialtation also occurred in the vessels supplying the fat and lymph nodes of the mesentery. The response was not secondary to changes in intestinal tone or motility since no significant alteration in these variables occurred. Cholinergic mechanisms were probably not involved since the response was unaltered by atropine. Moreover, mesenteric vasodilatation does not occur when the abdominal vagi are stimulated[1].

The present study provides additional evidence indicating the possible existence of intestinal neurogenic vasodilator mechanisms which are neither adrenergic or cholinergic. This possibility was suggested by the previous observation that pelvic nerve stimulation in cat produced an atropine-resistant dilatation of the mucosal vessels of the colon[2]. Moreover mechanical stimulation of the small intestinal mucosa in cats induces a non-cholinergic vasodilatation possibly mediated by an intramural nerve reflex[3].

There are several possible explanations of the vasodilatation observed in my experiments:

1. The mesenteric nerves may contain specific efferent fibres which release a vasodilator transmitter. Recent studies suggest that the vasodilator agent adenosine triphosphate may be released by non-adrenergic inhibirotory nerves innervating the gut of several species[4,5]. However, inhibition of gut motility was not observed in the present study. The slow onset of the dilator response and its persistence after the stimulus is withdrawn is also rather uncharacteristic of a neurotransmitter.

2. A vasodilator substance may be released from the pre-terminal portions of adrenergic nerves during stimulation but can only produce vasodilatation when the release of the vasoconstrictor transmitter, noradrenaline, is prevented. One possible vasodilator which might be involved is prostaglandin E 1 which occurs in sympathetic nerves[6] is a mesenteric dilator[7] and is released when sympathetic nerves are stimulated[8].

3. The dilatation may be associated with secretory changes induced in the gut by stimulation of non-adrenergic nerve fibers in the periarterial nerve plexus. No evidence bearing on this possibility is available[9].

Resume. La stimulation électrique du plexus nerveux périartérial mésentérique provoque une vasodilatation résistante à l'atropine de la couche vasculaire mésentérique chez le chat anesthésié après blocage des neurones adrénergiques.

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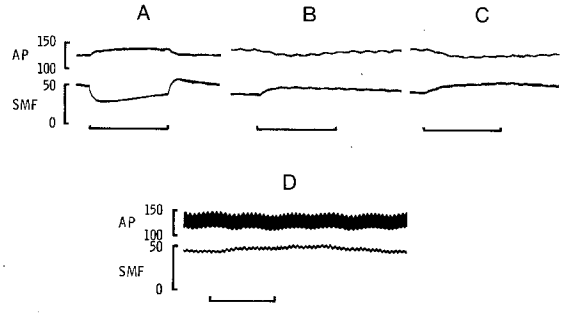

Effects of mesenteric periarterial nerve stimulation (10 v, 2 ms, 10/s) on arterial pressure (AP, mm Hg) and superior mesenteric arterial flow (SMF, ml/min), A) no drugs; B) after bretylium tosylate 30 mg infused into the superior mesenteric artery; C) same as B) but i.v. atropine 0.5 mg/kg also given; D) after chronic pre-treatment with 6-hydroxydopamine. The bars under panels A), B), C) indicate a period of 3 min stimulation and the bar under panel D) indicates 2.5 min stimulation.

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Histamine Sensitizing Activity of Lentinan, an Antitumour Polysaccharide

Lentinan, a β-(1 → 3) glucan obtained from *Lentinus edodes*, an edible mushroom, exhibits an excellent antitumour activity against Sarcoma 180 of mice[1,2]. As one of new biological properties of this substance, we found that lentinan was able to increase the susceptibility of the mice to histamine. The finding caused us to investigate the histamine sensitizing (HS) activity of lentinan.

Lentinan was administered i.p. in the total amounts of 100 to 500 μg fractionated for several days to the female 5-week-old mice (ddY strain). Three or 4 days after the last administration of lentinan, the mice were treated with the i.p. injection of 6 mg histamine (as histamine dihydrochloride, Sigma Co.) and 2 h later the death of the mice was observed. As shown in Table I, a number of dead mice were observed in the lentinan-histamine treatment group, but not in both control groups treated only with lentinan or histamine. The mortality of mice due to lentinan-histamine treatment was rather irregular. The

Table I. Histamine sensitizing activity of lentinan

Materials	Dose per mouse/day (μg)	Frequency of dose Times	Days	Total dose (μg)	Interval of treatments[a] (days)	Mortality
Experiments						
Lentinan	50	5	7[b]	250	4	10/10[c]
	50	5	6	250	3	4/10
	50	10	11	500	4	2/10
	50	5	7	250	3	3/10
	25	10	11	250	4	4/5
	10	10	11	100	3	2/10
	10	10	11	100	4	2/10
HSF	0.1	1	1	0.1	3	10/10
Controls					histamine	
Lentinan	50	5	7	250	—	0/10
	25	10	11	250	—	0/10
Histamine[d]	—	—		—	+	0/10

[a] Interval between the last administration of lentinan and histamine challenge. [b] Total days spent for the administration of materials. [c] Numerator presents the number of dead mice, denominator the total number of mice used. [d] No administration of materials except for histamine.

major cause of the irregularity may relate to the following finding. Recently we found that the manifestation of HS activity of lentinan in mice was largely dependent on the temperature of the animal-holding room immediately after histamine administration. The study on the effect of temperature is in progress.

The fact that lentinan has the HS activity in mice furthermore compelled us to investigate the relation between lentinan and the well-known histamine sensitizing factor (HSF) of *Bordetella pertussis*. The HSF preparation was partially purified from the filtrate of shaking culture of *B. pertussis* phase I (Tohama strain)[3,4]. The HS activity was assayed by i.p. injection of histamine (6 mg/mouse) in mice 3 days after the i.v. administration of HSF preparation (fraction ZpS_2)[4]. The effect of pretreatment with lentinan on the HS activity of HSF was studied. Contrary to our expectation, the pretreatment could not enhance the HS activity of HSF but rather suppressed it (Table II).

In addition to HSF there is, furthermore, a lymphocytosis promoting factor (LPF)[5] as one of interesting products from *B. pertussis*. The effect of pretreatment with lentinan on the lymphocytosis promoting (LP) activity of LPF was then studied in mice. As shown in Table III, the pretreatment with lentinan did not give any effect on the LP activity of LPF but also no effect on lymphocyte counts by itself. The LP activity was assayed according to the method described[6].

Lentinan and HSF showed the similar HS activity in mice, though their mechanisms are obscure at present. It was suggested that some differences may exist between them, because the pretreatment with lentinan induced neither the additive nor multiplying effect on the HS activity of HSF but showed rather a suppressing effect. As far as the HS activity of these substances is concerned, a few differences could be demonstrated between them; HSF is known to be relatively heat-stable, losing its activity by heating at 80 °C for 10 min[4], but lentinan was much more heat-stable. Besides, the histamine sensitizing dose of lentinan was much larger than that of HSF. It

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Table II. The effect of lentinan on HS activity of HSF

Amount of HSF (μg/mouse)	Mortality of mice treated with		Mortality of mice treated with	
	Lentinan + HSF[a]	HSF[a]	Lentinan + HSF[a]	HSF[a]
0.17			6/10[b]	9/10[b]
0.09	2/10[b]	7/10[b]	5/10	5/10
0.05	2/10	7/10	1/10	4/10
0.03	3/10	5/10		

[a] Lentinan was administered in total amount of 250 μg fractionated (50 μg/mouse/day) for 7 days. [b] Numerator presents the number of dead mice and denominator the total number of mice used.

Table III. The effect of lentinan on lymphocytosis by LPF from *B. pertussis*

Lentinan (μg)	LPF[a] (μg)	Number of lymphocytes (counts/mm³)
250[b]	0	1.68×10^4
0	0	1.71×10^4
250	22	7.09×10^4
0	22	7.34×10^4
250	87	1.77×10^5
0	87	1.76×10^5

[a] LPF was administered at 24 h after the last administration of lentinan. [b] Total amount of lentinan administration fractionated for 10 days.

seems that these facts may rather support the existence of different mechanisms between them. We were much interested in linking the HS activity with the regressive effect of lentinan against transplantable tumour.

Riassunto. E dimostrato che lentinan, polisaccaride antineoplastic, è capace di aumentare la sensibitità del topo contro istamina. La presomministrazione di lentinan invece non aumenta l'attività del fattore istamina sensibilizzante della *Bordetella pertussis*. Lentinan non induce linfocitose nel topo.

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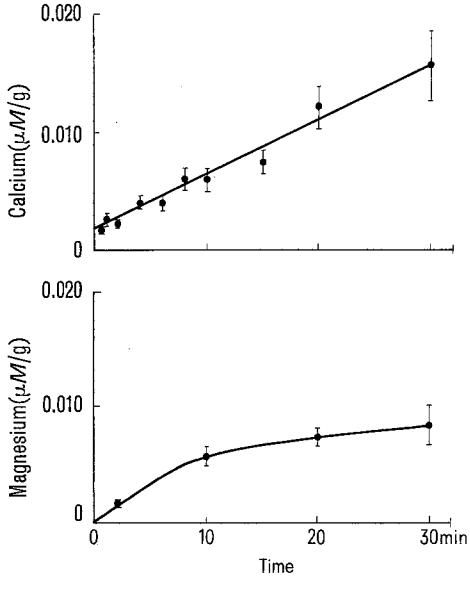
Fluxes of Magnesium and Calcium During Induced Activity of Frog Sartorius Muscle

Fluxes of calcium have been extensively studied in relation to excitation-contraction coupling in muscle, in particular the increases in both influx and efflux which can be elicited in frog skeletal muscle either by caffeine or by electrical stimulation[1–3]. Data on magnesium transport and permeability is by comparison sparse[4–6]. GILBERT[4] found that although frog sartorius was permeable to Mg the extent of exchangeable Mg was only about one-quarter of the muscle total, and that exchange occurred in 3 phases in whole muscle, the slowest phase being interpreted as truly intracellular Mg. Elevated Mg abolishes frog muscle response to indirect stimulation without influencing resting or surface potential[7], and stimulation in vivo has been reported to significantly increase the exchange of rat gastrocnemius Mg with that of plasma[8]. The present experiments investigated the fluxes of both Ca and Mg during electrical stimulation and caffeine contracture in excised sartorius muscles of frog.

Materials and methods. All experiments used whole sartorius muscles of male *R. temporaria*; muscles (from both winter and summer frogs) ranged in fresh weight from 30–90 g. Mg in freshly-dissected muscles, determined by atomic absorption on nitric acid digests, was 10.08 ± 0.24 (mean, s.e., $n = 16$) μM/g wet weight, uncorrected for muscle compartments. This value did not vary with season. For influx measurements the excised muscles were secured with one end fixed and the other attached to a strain gauge, transducer and pen recorder which monitored tension. Resting influx was measured by bathing the muscles, mounted at slightly more than skeletal length, in frog Ringer[9] containing ^{45}Ca or ^{28}Mg at 20°C for periods from 0.5 to 30 min. At the conclusion of incubation the radioactive Ringer was quickly withdrawn from the bath and replaced by nonradioactive medium of identical composition, to allow efflux of isotope from surface and extracellular components of Ca or Mg[1–4]. The efflux was continued for 90 min, and the muscles were then removed from the bath, cut free of tendon, blotted and weighed. ^{28}Mg was counted on neat extracts of muscles in 1 ml conc. HNO_3; ^{45}Ca was counted by liquid scintillation on aliquots of an aqueous solution of HNO_3 extract which had been evaporated to dryness. Counts were converted to concentrations by use of standards prepared from the radioactive solutions and fluxes were calculated as μM/g wet weight.

Caffeine was incorporated in Ringer at 5 mM and invariably elicited contracture for a 10-min period of incubation at this concentration. Electrical stimulation was submaximal and direct, by square-wave pulses of 50 msec duration at frequency 0.5/sec for the last 6 min of a 10-min incubation. Both contracture and electrical stimulation experiments were limited to 10 min duration in order to reduce fatigue or damage to fibres, and the effects of these treatments were assessed by comparing the influx with that in companion muscles incubated at rest under the same conditions. The influxes were again measured after 90 min efflux had been allowed.

Results and discussion. Influxes of Ca and Mg into resting muscles are shown in the Figure. Ca influx was linear with time up to 30 min, after the bulk of the



Time-course of influx of Ca and Mg into resting frog sartorius at 20 °C. Concentrations in Ringer were Ca 1.8 mM, Mg 2 mM. Each point is the mean of 6 muscles, 3 frogs. Vertical bars are standard errors.

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