cells neither go through to metaphase nor remain blocked in prophase but regress to interphase. This effect is similar to that observed when treatment is carried out with other protein synthesis inhibitors under the same experimental conditions. In the presence of 6-DMAP no prophasic accumulation is observed, the telophase ratio, however, is markedly different between 6-DMAP and the control.

In this study 6-DMAP has been shown to have similar effects on cytokinesis but to differ from puromycin in that it does not affect protein synthesis and subsequently mitosis. Similarly, puromycin has been shown to inhibit cleavage in marine eggs[10] and Rebhun et al.[11] have suggested that puromycin, 6-DMAP and other substituted 6-purines affect the cleavage of marine eggs via the same process. To our knowledge neither puromycin nor 6-DMAP has previously been reported as blocking cell plate formation in plant cells. It was concluded that puromycin and 6-DMAP both interfere with cytokinesis in plant cells through a similar mechanism. In addition, the present results indicate that the physiological action of puromycin may involve the inhibition of protein synthesis and also other effects related to the purine moiety of puromycin.

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Male mouse submaxillary gland secretes highly toxic proteins

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Summary. Submaxillary gland saliva induced by phenylephrine from male mice was highly toxic to guinea-pigs, rats and hamsters, whereas the toxicity was relatively low to mice. One of the toxic components in the saliva was isolated as a kallikrein-like enzyme.

It is well known that there are marked sexual differences in the morphology and biochemistry of the submaxillary gland (SMG) of the mouse; the SMG of male is more abundant in serous-like granules in cells of the convoluted tubules[1,2] and in proteins such as esteroprotease[3], kallikrein[4], renin[5], nerve growth and epidermal growth factors[6,7] than that of female. Little is known, however, about the physiological significance of such sexual differences. SMG is generally considered to be an exocrine organ and recent studies have demonstrated that nerve growth and epidermal growth factors are also secreted into saliva[8,9]. We report here that SMG saliva elicited by phenylephrine from male mice is highly toxic to animals and that one of the toxic components in the saliva is a kallikrein-like enzyme.

Materials and methods. ICR-strain male mice, 14–16 weeks old, were anesthetized with pentobarbital and salivation was induced by injection of an α-adrenergic agent, phenylephrine (1 mg/kg, i.v.). The saliva elicited was collected by washing the oral cavity with 1 ml of distilled water using a pipet 10 min after phenylephrine injection. The protein content of the saliva was determined by the method of Lowry et al.[10]; an average of 5.2 mg protein can be collected per mouse in this way. The salivas collected from 1,600 mice were pooled, filtered through a 0.45 µm Millipore membrane and concentrated by lyophilization. SMGs were excised from 250 male mice aged 14 weeks. They were pooled, homogenized with 5 vol. of saline and the supernatant was obtained by centrifugation at 5000 × g for 30 min. The saliva and SMG extract were injected i.p. into test animals and LD_{50} was determined by the method of Litchfield and Wilcoxon[11] on the basis of the mortality rate within 24 h. Isoelectric focusing was carried out using a column with 110-ml capacity (pH range; 3 ~ 10)[12]. Kallikrein activity was measured by the 2 methods, a) hydrolysis of α-N-benzoyl-L-arginine ethyl ester (BAEE)[13], b) kinin-releasing activity from kininogen using rat isolated uterus[14]. Polyacrylamide gel electrophoresis was carried out in 7% gels at pH 9.5[15].

Results. Phenylephrine-induced saliva was highly toxic to guinea-pigs, rats and hamsters whereas the toxicity was relatively low to mice (table). The lethal effect appears within 24 h with weakness and prostration of test animals. Phenylephrine-induced saliva of SMG-ectomized male mice was nontoxic, indicating that the origin of toxic component(s) is

Toxic activity of phenylephrine-induced saliva and SMG extract of ICR-strain male mice

Species	Sex	LD_{50} (µg protein/g b.wt) Phenylephrine-induced saliva	SMG extract
Guinea-pigs	♂	6.6 (4.4–9.9)	24.1 (18.3–31.8)
Rats	♂	15.1 (10.4–21.7)	41.2 (29.9–56.9)
	♀	13.3 (9.8–18.1)	39.6 (30.7–51.1)
Hamsters	♂	26.3 (19.1–36.3)	80.9 (61.3–106.8)
Mice (ICR)	♂	127.6 (98.9–164.6)	380.0 (279.4–516.8)
	♀	119.4 (87.8–162.4)	321.6 (255.2–405.2)
Mice (C3H)	♂	95.3 (64.4–141.0)	286.2 (216.8–377.8)
	♀	84.7 (61.4–116.9)	241.8 (187.4–311.9)

Values in parenthesis shows 95% confidence limits. Hartley guinea-pigs, 4 weeks old, Wistar rats, 4 weeks old, Golden hamsters, 5 weeks old, and ICR and C3H mice, 4 weeks old, were used as test animals.

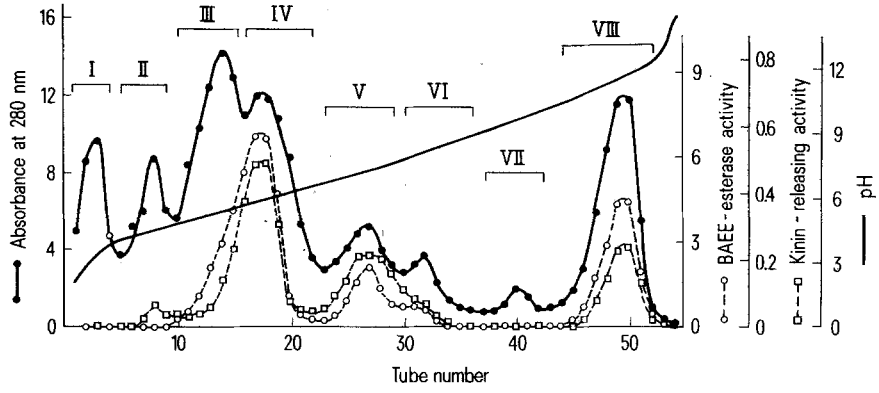

Fig. 1. Isoelectric focusing of phenylephrine-induced saliva. The saliva, containing 400 mg protein, was isoelectrofocused at 700 V for 48 h at 0–2 °C and then 2-ml fractions were collected. Enzyme units were expressed as mmoles of hydrolyzed BAEE and/or mg of bradykinin equivalent per min per ml fraction. Each of the protein peaks was concentrated, dialyzed against water for 24 h and injected i.p. into guinea-pigs (4-week-old males). Mortality rates of 15 µg protein/g b.wt were as follows. Peak I, II, III, VI and VII; 0/10, peak IV; 10/10, peak V; 2/10, peak VIII; 3/10.

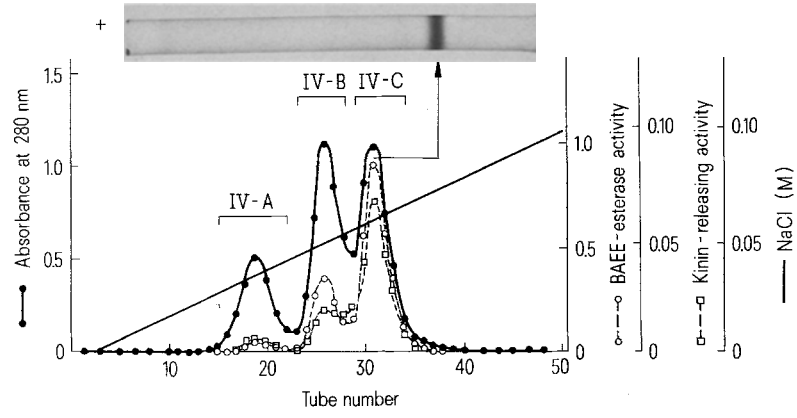

Fig. 2. Chromatography of fraction IV with DEAE-Sephadex A-50. The sample containing 80 mg protein was applied to the column (1.6×23 cm) equilibrated with 10 mM Tris-HCl buffer (pH 7.0). Elution was carried out with a linear gradient of 0–0.1 M NaCl in 10 mM Tris-HCl buffer (pH 7.0). Flow rate was 10 ml/h and 5-ml fractions were collected. Enzyme units were expressed as described in figure 1.

SMG. Indeed, SMG extract of male mice is also highly toxic (table). Most of the toxic activity of the saliva and SMG extract was destroyed by heating for 30 min at 65 °C. Dialysis against water did not affect the toxic activity.

In an attempt to purify the toxic component(s), phenylephrine-induced saliva was fractionated by isoelectric focusing (figure 1). Among the protein peaks (I–VIII), toxic activity was found mainly in the peaks IV, V, and VIII (see legend to figure 1). These 3 peaks contain kallikrein-like enzymes because they hydrolize BAEE and liberate kinin from kininogen. The pool of the peak IV, which is the highest toxic fraction, was further fractionated with DEAE-Sephadex A-50 (figure 2). Among the protein peaks (IV-A, -B, and -C), toxic activity was found only in the peak IV-C, which appeared as a single protein band with polyacrylamide gel electrophoresis. The mol. wt estimated by Sephadex G-100 gel filtration was about 31,000. This protein has a kallikrein-like activity. It is highly toxic when injected i.p. into guinea-pigs [LD_{50}; 4.9 (3.5–6.9) µg protein/g b.wt].

Our preliminary experiments showed that salivas collected from phenylephrine-stimulated female mice and unstimulated normal male mice were also toxic but the toxicity was extremely low (about 11% in the former and 9% in the latter as compared to phenylephrine-induced saliva of male mice). Phenylephrine-induced salivas of rats, hamsters and guinea-pigs were nontoxic.

Discussion. Liuzzi and Angeletti[16] and Huang et al.[17] have reported that crude extract of male mouse SMG shows a lethal effect when injected into mice or some other animals. But they did not examine in detail the nature of the toxic components in the SMG. Our present study has demonstrated that toxic components in male mouse SMG are secreted into saliva and that the toxic property of saliva may be due to kallikrein-like enzymes.

Mouse SMG is one of the target organs of androgen[18,19]. Typical androgen-dependent proteins such as esteroprotease, nerve growth and epidermal growth factors are secreted into saliva and their secretions are regulated through α-adrenergic receptors[8,20,21]. These proteins are known to be localized in serous-like granules in cells of the convoluted tubules[22–24]. Our preliminary experiments showed that the secretion of toxic components in the mouse SMG was also stimulated by phenylephrine or norepinephrine and the stimulative effects were completely inhibited by pretreatment with α-blockers such as phentolamine or phenoxybenzamine. This suggests that toxic components in the mouse SMG are also localized in the serous-like granules.

It is generally accepted that, in the mouse, the male is more aggressive than the female. Our results presented here suggest that the male mouse SMG is a toxic organ, which secretes toxic proteins (kallikrein-like enzymes) into saliva, as an effective weapon.

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Frequency distribution of *Trypanosoma cruzi* in macrophages from resistant and susceptible strains of mice

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Summary. The frequency distribution of *Trypanosoma cruzi* inside macrophages from normal or chronically infected resistant and susceptible mice obeys a negative binomial type of distribution. This implies that an "aggregating mechanism" operates in *T. cruzi:* macrophage interaction.

Chagas' disease, a systemic disease, is caused by infection with *Trypanosoma cruzi*, an intracellular protozoan flagellate. It has been shown[3,4] that 2 different strains of mice, A/J (H-2^a) and C57 B1/10 (B10) (H-2^b) are susceptible and resistant to experimental *T. cruzi* infection. Moreover, these strains present different levels of intramacrophage parasitism after in vitro incubation of the parasites with resident peritoneal macrophages[4]. When peritoneal washout macrophages from normal A/J and B10 mice are incubated with trypomastigotes the time course curves for the percentage of infected macrophages are exactly the same. However, the accumulated means are higher in A/J than in B10 animals. When the washout macrophages are derived from chronically infected mice (mice at 60 days post-infection) both the percentage of infected macrophages and the accumulated means are significantly higher in the A/J than in the B10 mice (figure 1). Here we show that irrespective of the genetic profile or the stage of infection, the frequency distribution pattern of intracellular parasitism obeys a contagious distribution, mainly the negative binomial one. Resident peritoneal macrophages (Mø) from normal and chronic A/J and B10 mice were incubated in vitro with blood-form trypomastigotes of the Y strain. The number of intracellular parasites per cell, up to a total of 250 Mø, were counted at 1, 2, 8, 14 and 24 h of incubation. The frequency distribution curves were fitted to a negative binomial type of distribution[5]. The goodness-of-fit was based on the usual χ^2 statistic. The distribution curves of a typical experiment are shown in figure 2. In all the experimental situations, the distribution pattern fits a negative binomial type, except for the 24 h points where a best fit is found with a negative binomial truncated at the point $K=0$.

The contagious type of frequency distribution is very commonly found describing frequency distribution of living organisms in their natural habitat[6]. Natural populations tend to obey aggregated patterns of spatial distribution unless very improbable contingencies are taking place. True randomness would be very seldom found. A number of mathematical models comprising the compound Poisson series has been described as explanatory for those observed distributions[7], the negative binomial being one of them. The basic assumption is that a randomly distributed variable would have its Poisson lambda parameter varying over the entire field of observation.

Sample variances and means of the frequency distribution of *Trypanosoma cruzi* inside macrophages in normal and chronically infected A/J and B10 mice after different times of incubation

Time (h)	A/J				B10			
	Normal		Chronic		Normal		Chronic	
	s^2	$\bar{x}$	s^2	$\bar{x}$	s^2	$\bar{x}$	s^2	$\bar{x}$
1	0.8505	0.3360	0.3662	0.2080	0.4110	0.2160	0.1770	0.0880
2	1.8960	0.4600	1.7315	0.6160	0.4110	0.2160	0.2215	0.1240
8	7.7593	1.0880	14.7578	2.9280	2.0535	0.6440	4.3470	0.8800
14	4.9468	1.0320	10.5560	2.5160	0.8470	0.4200	5.7871	1.0640
24	3.8587	0.8560	9.3369	1.9800	1.6859	0.6040	0.4494	0.1280