Results and discussion. Our results (figure) showed that acid phosphatase activity in the cuticle was significantly higher ($p = 0.05$) at 1% of the instar than at 10, 20, 30, or 40% of the instar. In the haemolymph, the acid phosphatase activity was significantly higher ($p = 0.05$) at 10% of the instar than at any other time period measured. In addition, the 1, 20, 30, and 40% measurements were all significantly higher than the 60% time period. There were no significant differences between 10% interval measurements in the midgut, fat body, testes, or salivary gland tissues.

These results indicate that acid phosphatase does exhibit variation during the fifth instar of the milkweed bug in the cuticle and haemolymph tissues. The role of the enzyme and the significance of the variation in activity is not known at this time.

Combined effect of veratridine and sodium aspartate on the rabbit retinas in vitro

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Summary. Combined effect of veratridine and sodium aspartate on rabbit retinas in vitro was studied. Observations suggested that mode of action of veratridine on the PII and PIII components of ERGs was modified by the presence of sodium aspartate in perfusate.

Veratridine (mol.wt 673.81) is an alkaloid extracted from veratrum subadilla, and is known to increase the negative after-potential, to decrease the threshold potential and to induce repetitive discharge in neurons and other tissue[1]. The mode of action has been considered such that veratridine increases the permeability of sodium and potassium across an excitable cell membrane[1]. In this study, the combined effect of veratridine with sodium aspartate on perfused rabbit retinas was investigated.

Methods. Albino rabbits weighing 3 kg each were used. The electroretinogram (ERG) was recorded from in vitro retinal preparations. Techniques for excision and perfusion of the retina were the same as those described in previous reports[2,3]. Ames' solution[4] was used as an incubating medium. No blood plasma was added. The temperature of the incubating medium was maintained at 36 °C. The osmotic pressure of the medium was 305 mOsm/l. The medium was equilibrated by perfusing it continuously with a mixture of 95% O_2 and 5% CO_2 prior to and during use. This retinal preparation was shown to produce ERGs of constant amplitude over a period of 3 h[5]. The stock solutions of veratridine and sodium aspartate were added to the incubating medium in a volume ratio of 1 (or less): 100. Concentrations of chemicals were shown as those after dilution in perfusate. Chemicals reached their active site by perfusion from the ganglion cell side of the retina.

Results and discussion. Effects of veratridine on the action potential of in vitro retinas appear in 2 ways[6,7]: no effect on ERG in appearance (weak veratrinization), or de-

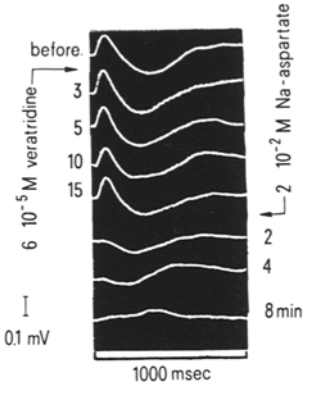

Fig. 1. Sodium aspartate of 2×10^{-2} M was administrated, 40 min after the administration of 6×10^{-5} M veratridine. The lapsing time on the right side was measured from the end of sodium aspartate administration. Responses were amplified by pre-amplifiers having a passband of 0.8–1000 Hz. Responses to 32 stimuli were summed on an averaging computer. The averaged responses were displayed on an oscilloscope and were photographed. The positivity of the active electrode, placed on the ganglion cell side of the retina, was recorded as an upward deflection. The reference electrode was placed on the receptor cell side. Stimuli were provided by a xenon flashtube (Grass PS-22) triggered by an electric stimulator. The light from the photo-stimulator was attenuated by polariod films. Radiometric measures, corrected for this attenuation, indicated that approximately 3.5×10^{-3} ergs/flash cm^2 of white light were falling at the position at which the perfused retinas were placed. The stimulus frequency was 1 flash/sec.

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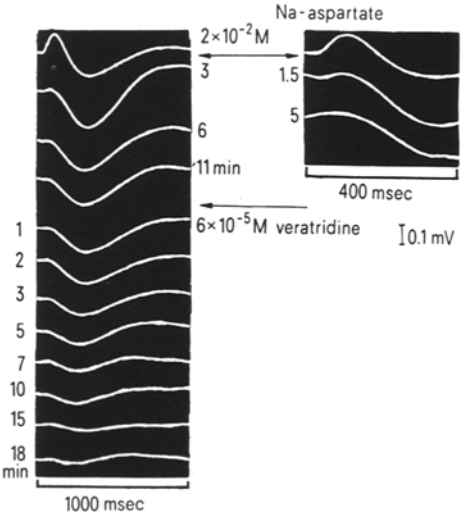

Fig. 2. To a fresh retina, sodium aspartate of 2×10^{-2} M was administrated, resulting in isolation of aspartate-insensitive PIIIs. 13 min after the administration, veratridine of 6×10^{-5} M was perfused. Analyzing times of the right and left columns were different, and were 400 and 1,000 msec, respectively. Lapsing time on the left side was measured from the end of veratridine administration.

creasing amplitude of the b-wave (strong veratrinization) isolating the slow-decaying negative potential which might be referred to as the PIII component. Difference between effective doses of weak and strong veratrinization in the retina is small[6,7], as in other tissue[1]. In figure 1, almost no effect was observed in appearance by veratridine administration (weak veratrinization). However, by administration of 2×10^{-2} M sodium aspartate (which is usually enough to abolish the PII component and to isolate the PIII component[8]), PIII as well as PII disappeared. In figure 2, the PIII component was isolated at first by administration of 2×10^{-2} M sodium aspartate. Sodium aspartate by itself is a very safe chemical as sodium salt, and PIII, isolated by sodium aspartate of this level of concentration, is always very stable[8]. To this retina, veratridine of 6×10^{-5} M was administrated. Then, PIII was markedly reduced in amplitude and disappeared in 10–15 min. On the recording of 18 min, a moderate positive wave of 600–700 msec peak latency was observed. An important finding in these experiments was that generation of PIII component as well as the PII component of ERGs was severely damaged by simultaneous presence of veratridine and sodium aspartate in perfusate. Hanitzsch[8] studied effects of sodium aspartate upon the rabbit retina and analyzed PIII into 3 subcomponents, a) an aspartate-insensitive distal PIII, b) an aspartate-sensitive PIII, and c) an aspartate-insensitive slow PIII. PIII isolated in this study might be referred to a) or c), or a mixture of both subcomponents of Hanitzsch[8]. Further analysis is impossible in this study employing superficial electrodes, but it might be said that veratridine effects (weak and strong veratrinization) on the PII and aspartate-insensitive PIII components of perfused rabbit retinas were modified by presence of sodium aspartate, or in other words, that effects of sodium aspartate were modified by the presence of veratridine.

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Sex factors and plasma levels of oxytetracycline (OTC) in rats[1]

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Summary. Sex related differences in plasma OTC concentrations were found in rats 4–24 h after a single i.p. application of 100 and 50 mg/kg OTC.

Reports on bone retention of tetracyclines in rats indicate that age and sex significantly influence the deposition of these antibiotics in the skeleton[4–6]. The differences were either not specially commented upon[5] or the authors tried to interpret them as an indicator of different bone growth[4]. It was also assumed that they could be due to other changes in the metabolism of antibiotics in the body caused by age or sex[6]. In the present paper the influence of sex factors on plasma levels of OTC was investigated as a possible explanation of different OTC retention in the bone, and as a factor which might be relevant for assessing the general efficiency of the OTC treatment.

Methods and results. The experimental animals, 12-month-old male and female albino rats (body weights 425 ± 18 g and 254 ± 13 g, respectively) and 12-month-old ovariectomized animals (body weight 280 ± 8 g; ovaries removed at the age of 4 months) were injected i.p. with 100 mg/kg OTC (Geomycin, Pliva, Zagreb). 2 lower doses of OTC, 10 and 50 mg/kg were injected in the same way to male and female rats of similar age (12–13 months old) and body weights (434 ± 7, 256 ± 5 g). Blood samples were taken 1, 4, 8, 24 and 48 h after OTC application from the orbital venous plexus and plasma was separated by centrifugation at 5000 rev/min (3000 × g) for 10 min. The concentration of the antibiotic was assayed on agar plates against Sarcina Lutea. It can be seen on table 1 that 1 h after administration of 100 mg/kg OTC plasma levels of OTC were practically the same in all groups of animals. After 4 h, the values for males and ovariectomized females

Table 1. OTC concentrations in plasma (μg/ml) after a single i.p. injection of 100 mg/kg*

		Time after dose (h) 1	4	8	24
Female rats					
A) controls		48.9±1.0	46.0±1.4	20.0±2.0	2.7±0.1
B) ovariectomized		49.2±0.6	81.1±4.7	33.5±5.0	3.7±0.3
Male rats					
C) controls		47.1±3.3	76.0±5.4	48.5±3.9	4.0±0.6
	A:C	$p>0.1$	$p<0.001$	$p<0.001$	$p<0.05$
	A:B	$p>0.1$	$p<0.001$	$p<0.02$	$p<0.001$
	B:C	$p>0.1$	$p>0.1$	$p<0.02$	$p>0.1$

*Each figure represents the mean of 8 animals ± SE.

Table 2. OTC concentrations in plasma (μg/ml) after a single i.p. injection*

Dose mg/kg	Sex		Time after dose (h) 1	4	8
50	A) female		22.0±1.8	10.5±1.5	0.5±0.01
	B) male		19.0±1.2	8.9±1.3	3.5±0.3
10	C) female		4.1±0.2	1.3±0.1	
	D) male		3.8±0.4	1.4±0.2	
		A:B	$p>0.1$	$p>0.1$	$p<0.001$
		C:D	$p>0.1$	$p>0.1$	

*Each figure represents the mean of 8–12 animals ± SE.