Table I. Volumes of saliva (± SD) secreted by *Poecilometis punctiventris* within 10 min after stimulation with pilocarpine

	Saliva (µl)
Unoperated controls	3.4 ± 1.6
Accessory gland exposed but not cut	1.8 ± 0.6
Accessory gland cut on one side	0.011 ± 0.006
Accessory glands cut on both sides	0.008 ± 0.003

Table II. Volumes of saliva (± SD) secreted by *Amblypelta lutescens* within 10 min after stimulation with pilocarpine

	Saliva (µl)
Unoperated controls	0.45 ± 0.07
Accessory gland exposed but not cut	0.33 ± 0.04
Accessory gland cut on one side	0.13 ± 0.02
Accessory glands cut on both sides	0.03 ± 0.02

Attempts by us to cut these nerves in the live insects were unsuccessful in that they resulted in gross damage to the neural system.

In a further attempt to investigate the source of water in the saliva of Heteroptera, parts of the accessory glands were removed surgically. Fully fed 5th instar larvae of *P. punctiventris* were collected from beneath the bark of *Eucalyptus cladocalyx* (F. Muell.), chilled, and immersed in saline[7]. The accessory glands (and ducts) were pulled gently to their fullest extent through flaps cut in the pronotum. The glands either were not damaged further, or were cut on only the one side as near to the origin (at the hilus of the principal gland) as possible, or on both sides. On removal of the insect from the saline, the exposed parts of the salivary apparatus tended to retract themselves automatically. The integument was then carefully pushed back into place.

Treated insects lived for several days if disturbed no further. When an unoperated control was treated topically with 2 µl of 20% pilocarpine base in acetone, it usually began to salivate freely within 1 min. The volume was readily measurable to about 0.005 µl if collected into a calibrated capillary tube. Table I indicates the amounts of saliva produced by groups of 10 insects, 10 min after the completion of any operation, when stimulated with pilocarpine.

One of the insects from which the 'both sides' statistic was calculated produced the inconsistently large amount of 0.06 µl saliva. All operated insects were subsequently dissected to observe the actual state of the salivary glands and it was discovered that this particular insect (but no other) had not been operated on properly and had an appreciable amount of the sinuous proximal part of the accessory gland remaining on one side.

At this stage of the investigation it was the time of the year when the *P. punctiventris* larvae were beginning to become adults (the species is univoltine), and the insect could no longer be collected readily in numbers. A further experiment was therefore done on adults of *Amblypelta lutescens* (Dist.) (Coreidae) from a laboratory culture maintained on fresh broad beans. Table II indicates the results.

The secretion discharged from the rostrum in the experiments with either species was routinely tested on pH paper. The reaction was always >8 and was thus clearly distinguishable from that of the haemolymph or the contents of the gut or principal salivary gland, all of which were <7.

It is clear from these experiments that ablation of the accessory gland reduces the volume of saliva in a quantitative fashion and hence that the accessory gland contributes the bulk of the volume of the watery saliva secreted by these insects when stimulated to do so by the action of e.g. pilocarpine on the central neural system.

Relation of Efferent Impulse Activity in Splenic Nerve to Reflexly Induced Reactions of Resistance and Capacitance Vessels of Spleen

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Summary. Constrictory responses of splenic resistance vessels arising under pressor reflexes were abolished by hexonium (2 mg/kg) as well as the high amplitude (above 15 µV) impulses in sympathetic splenic nerves. Constrictory and dilatory responses of splenic capacitance vessels were preserved after administration of the same dose of hexonium and correlated as to the directivity with the changes of the low amplitude (15 µV and lower) impulsation in the splenic nerve.

It was previously shown[1-4] that under pressor cardiovascular reflexes on the background of constrictory response of resistance vessels, constriction or dilatation of capacitance vessels might take place or capacitance response was absent. Differently directed reactions of capacitance vessels under pressor reflexes were observed both in skeletal muscle and in splanchnic region. Analysis of mechanisms contributing to the differentiation of resistance and capacitance vessel responses showed[5,6] that the ganglionic blockade with hexonium (2 mg/kg) abolished reflexly-induced constrictory reactions of resistance vessels while the capacitance vessel responses were persistent. Additional dose of hexonium abolished reflexogenic reactions of capacitance vessels as well.

This study was intended to reveal the changes of postganglionic efferent activity in splenic sympathetic nerves after administration of 2 mg/kg of hexonium and the relation of the sympathetic efferent activity to the reactions of splenic resistance and capacitance vessels under pressor carotid sinus reflex.

Method. Experiments were performed on cats (14) anaesthetized with urethane (1 g/kg) and α-chloralose (20 mg/kg). Reflexogenic reactions of splenic resistance and capacitance vessels were studied with the aid of the

constant blood flow perfusion technique. Blood was withdrawned by a perfusion pump from the femoral artery and impelled into the splenic artery. To measure the venous outflow from a spleen it was directed into the U-shaped tube, from which the blood was returned to the femoral vein by means of the 2-d channel of the perfusion pump. Since the efficiency of both perfusion pump channels was constant and both channels operated in the same flow regime, the changes of perfusion pressure reflected the reactions of resistance vessels and the changes of venous outflow reflected the reactions of capacitance vessels.

Simultaneously with the vasomotor responses, the efferent impulse activity was recorded in small branches of the splenic nerve. Action potentials were taken off the central part of severed splenic nerve branches by means of bipolar silver electrodes, and after the amplification they were recorded on the cathode oscillograph. The pressor carotid sinus reflex was brought about by the clamping of carotid arteries for the period of 30–40 sec. Ganglioblocking agent hexonium was administered i.v. in the dose of 2 mg/kg. Heparin was used to prevent blood coagulation. Experimental data were processed in digital computer 'Minsk-32' correlation and regression analysis was used.

Results. After the clamping of carotid arteries, the arterial perfusion pressure in spleen increased in all the experiments by 6.0 $\pm$ 1.5% above control. Simultaneously with the constriction of the resistance vessels, the constriction of capacitance vessels was observed in 52% (venous outflow was increased by 6.5 $\pm$ 1.4% above the control flow through the organ); in 44% of cases the dilatation of capacitance vessels took place (venous outflow decreased by 6.5 $\pm$ 1.0%) and in 4% the capacitance response was absent.

After clamping off carotids, the rate of impulsation in post-ganglionic sympathetic efferents of the spleen was increased in all cases. The increase of sympathetic outflow reached its maximal value during the first seconds after the stimulation and preceeded the reflexogenic increased of the perfusion pressure. The sympathetic activity was increased mostly at the expense of the high amplitude (above 15 μV) impulses. The rate of impulses increased on the average by 34.4 $\pm$ 4.8% as compared with controls. The initial increase of the sympathetic impulsation was followed by its decrease on the background of continued carotid clamping. In spite of this, the perfusion pressure was above the control or even rizing for some time. It leads us to consider the high amplitude sympathetic impulsation as a trigger mechanism for the constriction of spleen resistance vessels.

After hexonium (i.v. 2 mg/kg), sympathetic activity in the splenic nerve was significantly changed. It was expressed in the sharp decrease of the high amplitude impulse rate or in complete abolishing of the activity. Meanwhile the low amplitude impulsation (15 μV and lower) was preserved after the hexanium. In most experiments, the correlation of impulse volleys with the arterial pulse and respiratory cycles after ganglionic blockade became significantly less distinct.

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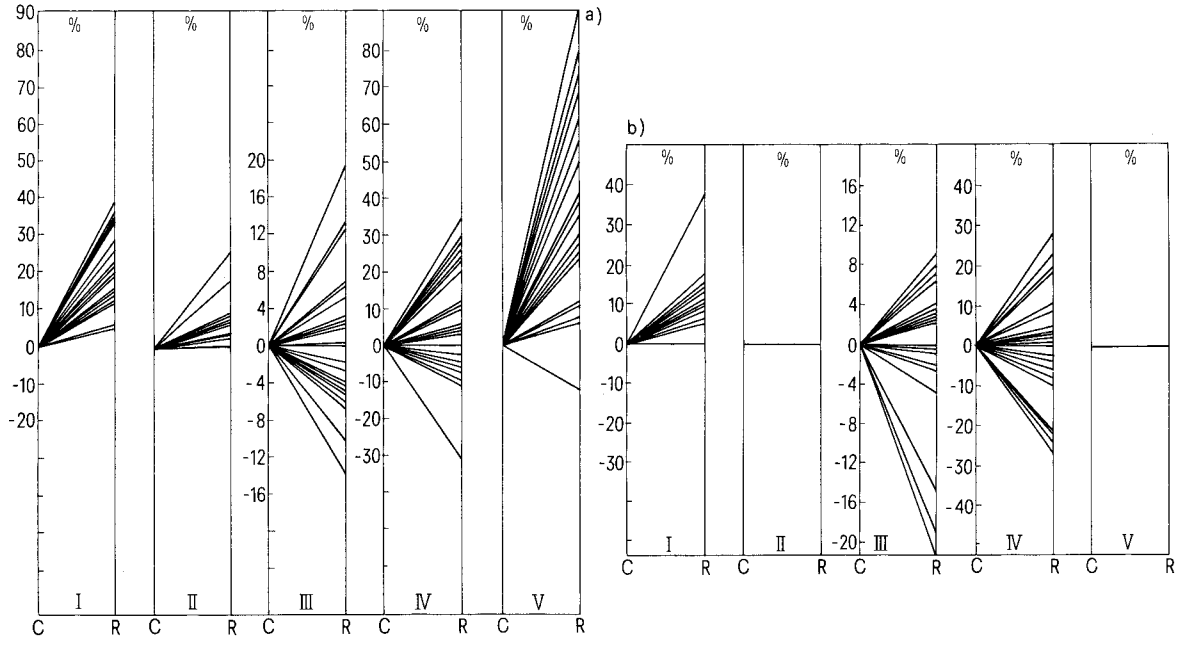

Fig. 1. Magnitude and directivity of resistance and capacitance vessel reactions, arterial pressure and changes of sympathetic activity in splenic nerve under pressor carotid sinus reflex.
From left to right: changes of arterial (I) and perfusion (II) pressures in % to control, venous outflow (III) in % to control flow, mean rate of impulses through the period of stimulation (IV) in % to mean rate of impulsation in prestimulation period, rate of impulses for 3 sec in the peak of reactions (V) in % to control rate. C, control; R, reaction; a) before and b) after hexonium (2 mg/kg).

Experiments showed that, when high amplitude impulsation had been abolished by hexonium, the carotid clamping was not followed by the constriction of spleen resistance vessels, while a capacitance vessel response (constriction or dilatation) was persistent in most cases. Venous outflow increased by 4.2 ± 0.8% above control in 50% of experiments, decreased by 6.8 ± 2.9% in 33%, and was unchanged in 17% of experiments.

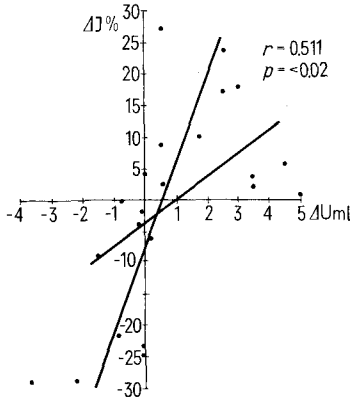

Fig. 2. Direct correlation between changes of venous outflow from spleen vessels and the rate of low amplitude impulses in splenic nerve under pressor carotid sinus reflex after hexonium (2 mg/kg). Abscissa, magnitude of outflow changes in ml (ΔU, ml); ordinate, impulse rate in % to control (ΔI, %).

Changes of the low amplitude impulse rate in the splenic nerve were of the same direction as those of the venous outflow in 79%. The increase of impulse rate (by 8.5 ± 2.4% on the average) in 15% of cases was accompanied by a rise of venous outflow, and in 33.3% of cases decrease of the impulse rate (by 14.3 ± 4.7%) was accompanied by an outflow decrease. These data are summarized in Figure 1.

Correlation and regression analysis showed that a direct correlation existed between the changes of low amplitude impulse rate and the changes of venous outflow ($r = 0.511$, $p < 0.02$): the more the increase of low amplitude impulse rate, the more the magnitude of venous outflow increase; and the more the decrease of the impulse rate, the lesser was the outflow (Figure 2).

Thus, the study of the vasomotor reactions of the spleen with simultaneous recording of the efferent sympathetic activity permits us to suggest that high amplitude impulsation is related to the responses of resistance vessels, and low amplitude impulses, to the responses of capacitance vessels.

'Binding' of Glutamate and Aspartate to Synaptosomal Fractions of Six Regions of the Feline Brain[1]

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Summary. The order of potency of 'binding' of both glutamate and aspartate to synaptosomal fractions of brain regions was: cerebellar cortex ⩾ caudate nucleus ≧ cerebral cortex > medulla ≅ pons > corona radiata. Glutamate was bound to a greater extent than aspartate to particles of all regions studied, except for cerebral cortex.

L-Glutamate and L-asparate, leading candidates for roles as excitatory neurotransmitters, are the most potent and quickest-acting excitatory agents present in the mammalian CNS[2-4]. Since 'high'- and 'low-affinity' uptake processes for these amino acids in various CNS regions[5-7] and their subcellular distributions[8] are very similar, we have devised a simple method by which regional differences in their 'binding' can be demonstrated, as has been shown for GABA, glycine and taurine[9-13].

Materials and methods. Adult male cats (2.8–3.3 kg) were anesthetized with pentobarbitone-Na+ (30 mg/kg) and then killed by air embolism. Regions of the brain were dissected out on a chilled, moist surface, weighed, and homogenized in 10 volumes of ice-cold isosmotic (0.32 *M*) sucrose solution. All further operations were conducted at 0 °C. The subcellular fractionation procedure employed was essentially that of GRAY and WHITTAKER[14], as modified[10], except that the 'binding' of L-glutamate and L-aspartate to synaptosome-enriched fractions was examined in a balanced, bicarbonate-buffered medium, rather than in sucrose solutions. The osmolarities of the ions present in this glucose free medium were; Na^+, 147.3; K^+, 3.5; Ca^{++}, 1.3; Mg^{++}, 1.2; Cl^-, 128.5; HCO_3^-, 24.55; PO_4^{---}, 0.45 and SO_4^{--}, 1.2 mOsmoles/liter; total osmolarity ≅ 308 mOs*M*; pH was 7.4 after equilibration with 95% O_2/5% CO_2.

Portions (4.0 ml) of homogenates representing 0.364 g tissue, were centrifuged at 1000 × *g*, 10 min to prepare first supernatant (S_1) fractions. Centrifugation of 3.0 ml portions of S_1 fractions at 17,000 × *g*, 30 min provided synaptosomal mitochondrial (P_2) fractions, which were re-suspended in 3.0 ml of 0.32 *M* sucrose solution and re-centrifuged at 17,000 × *g*, 30 min. Washed P_2 fractions were re-suspended in 3.0 ml of physiological medium,

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