No changes in the levels of non-esterified fatty acids and aminoacid nitrogen levels in blood serum were found. All CHE reactivators increase the secretion of corticosterone. This effect reaches statistical significance with methoxim and obidoxim.

After epinephrectomy, CHE reactivators no longer evoked an increase in glycogen concentration in heart muscle, but rather a trend to decrease was seen, but this change was not significant (Table).

Glycogen concentration in heart muscle from normal and epinephrectomized rats 2 h after administration of drugs (20 mg/kg s.c.)

Treatment	Normal rats			Epinephrectomized rats		
	n	mg/100 g	$s_{\bar{x}}$	n	mg/100 g	$s_{\bar{x}}$
Saline	43	279.9	13.1	4	235.2	36.4
Trimedoxim	11	334.0	29.8	5	201.4	38.2
Methoxim	21	354.7[a]	15.8	4	157.4	23.9
Obidoxim	14	419.9[a]	23.47	5	166.2	18.2

[a] Statistically significant difference from control values (saline); $p < 0.01$.

Discussion. Our previous paper (BENEŠOVÁ and HVIZDOŠOVÁ[1]) demonstrated clearly that CHE reactivators can induce an increase in glycogen concentration in liver, skeletal and heart muscle, and an increase in blood glucose level in rats. The first series of experiments confirmed again the rise of glycogen in muscle. If a rise in liver and muscle glycogen appears simultaneously with the rise of glycaemia in hungry animals, the most probable explanation is gluconeogenesis. The activation of adrenal cortex and increased release of corticosterone observed support this hypothesis. The lack of muscle glycogen rise in epinephrectomized animals may represent further evidence for this conclusion. Further findings – unchanged levels of non-esterified fatty acids and of amino acid nitrogen – need not be in contradiction, since the turnover of amino acids released from proteins and fatty acids from fats may be at such a speed that no change in these metabolites levels is detectable.

The estimation of catecholamines in adrenals, revealing a fall in concentration after the administration of CHE reactivators indicates also slight activation of the adrenal medulla. The lack of any change in the activity of liver tryptophane-pyrrolase and varying effects of CHE reactivators on the release of adrenal catecholamines (decrease of noradrenaline after methoxim and toxogonin, decrease of adrenaline after trimedoxim) is still difficult to explain.

Activation of Sustained Sympathetic Vasodilatation in Dog by Spinal Cord Stimulation[1]

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31 May 1976.

Summary. Electrical stimulation in lateral sites of the upper cervical spinal cord evoked vasodilatation after adrenergic blockade. Sympathetic fibres mediating sustained vasodilatation were shown to be separate from adrenergic sympathetic fibres since the adrenergic vasoconstrictor response in the paw evoked by vasomotor stimulation in the medulla was not reversed to vasodilatation after bretylium.

A vasodilator innervation which can be activated by sympathetic stimulation following adrenergic blockade exists in the cutaneous vasculature of the canine paw[3] and ear[4] and in the hind limb[5]. The vasodilatation brought about by stimulation of these sympathetic fibers is in part mediated by acetylcholine since the initial rapidly developing component of the response is blocked by atropine[5]. The majority of the response, however, which is of long duration is not attributable to cholinergic mediation or to release of other known transmitters[3,5] and has been termed sustained vasodilatation[5]. Because it is of importance to know whether central representation of this unique vasodilator system exists, stimulation of the lateral aspect of the upper cervical spinal cord was explored as a means of activating sustained vasodilatation. Experiments utilizing medullary vasomotor center stimulation were also conducted to try to separate physiologically the adrenergic and sustained vasodilator innervations.

Materials and methods. Experiments were carried out in 19 mongrel dogs anesthetized with 30 mg/kg of sodium pentobarbital and artificially ventilated after administration of 0.25 mg/kg of decamethonium bromide. Supplements of the anesthetic and neuromuscular blocker were given when necessary. With the animal in the prone position and its head placed in a holder, the medulla and uppermost portion of the cervical spinal cord were exposed through an incision over the cisterna magna. A bipolar concentric electrode was used to stimulate either the medulla (floor of the 4th ventricle) or the lateral spinal cord. Various sites 1–6 mm below the floor of the 4th ventricle and on the lateral aspect of the spinal cord just caudal to the medulla 1–2 mm beneath the surface were stimulated. The procedure of hindpaw perfusion which has been reported previously[4] entailed pumping blood from the femoral artery to the cranial tibial artery of the left hind paw with a Sigmamotor pump. The extracorporeal tubing encompassed 75 ml and included a 53 ml coil which delayed the passage into the paw of catecholamines released from the adrenal medulla during central elicitation of vasodilator responses. The animal was primed i.v. with 75 ml of 5% dextran to replace the blood

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taken up by the extracorporeal tubing. In order to vascularly isolate the paw during central stimulation, a ligature placed around the aorta exposed through a retroperitoneal incision was tightened prior to stimulation. This prevented passage of any vasoconstrictor substances in the systemic circulation from reaching the paw via collateral arteries. Systemic blood pressure and paw perfusion pressure were monitored from side-arms in the perfusion tubing proximal and distal to the pump, respectively. Pressures were measured with Statham transducers coupled to a Beckman dynagraph. Flow was initially adjusted to set perfusion pressure at approximately the level of systemic blood pressure and was maintained constant throughout the experiments. The left lumbar sympathetic trunk was exposed through a retroperitoneal incision so that it could also be stimulated. Parameters of stimulation were 10 V, 0.5–1 msec and 7–20 Hz for the sympathetic trunk, 5–10 V, 0.5 msec and 120 Hz for the spinal cord and 20 V, 0.5 msec and 240 Hz for the medulla. Vasodilator responses were expressed as a percentage of the control perfusion pressure (PP) which was considered as 100%. The paired *t*-test was employed to determine statistical differences.

Results. Prior to stimulating the left lateral spinal cord, bretylium, 10–15 mg/kg and atropine sulfate, 0.25 mg/kg were administered to abolish adrenergic and cholinergic responses to sympathetic nerve stimulation. The left lumbar sympathetic nerve was stimulated at 20 Hz for 60 sec intervals and these stimulations continued until a consistent sustained vasodilator response in the perfused left paw was obtained. Subsequently, responses to lateral cervical spinal cord stimulation (LCS) for 30 sec at 5 and 10 V, sympathetic stimulation for 2 and 15 sec at 20 Hz, histamine diphosphate and bradykinin administered i.a. were elicited (Figure 1). Bradykinin was employed as an internal vasodilator control. An experiment of this series is depicted in Figure 2. Responses to LCS were large in magnitude, ranging from 22 to 38% decreases in perfusion pressure, and often persisted for several min after termination of the stimulation (Figure 2). The sustained vasodilator responses evoked by LCS approximated those to sympathetic nerve stimulation (Figures 1 and 2) and were eliminated by lumbar sympathetic denervation. Increases in systemic blood pressure accompanied the vasodilatation in the paw evoked by LCS. The vasodilator response was also followed by delayed vasoconstriction in the paw in most cases. Because bretylium blocks adrenergic postganglionic nerves, but not the adrenal medulla, the pressor and vasoconstrictor responses are presumed to be due to centrally stimulated release of adrenal catecholamines or other vasoconstrictor substances. The volume of the perfusion tubing delays the delivery of circulating vasoconstrictor substances to the perfused paw by 3–5 min, thus allowing for observation of the vasodilator response to LCS. After the intraarterial administration of the antihistamines, chlorpheniramine (3–5.5 mg) and tripelenamine (3–5.5 mg), vasodilator responses to histamine were blocked or greatly reduced and those to LCS also reduced (Figure 1). The decrease in the response to LCS varied, since in some experiments there was little or no blockade (Figure 2). When all experiments were considered, however, there was a statistically significant decrease in the response at 10 V ($p < 0.05$). Responses to sympathetic stimulation and bradykinin were not significantly affected.

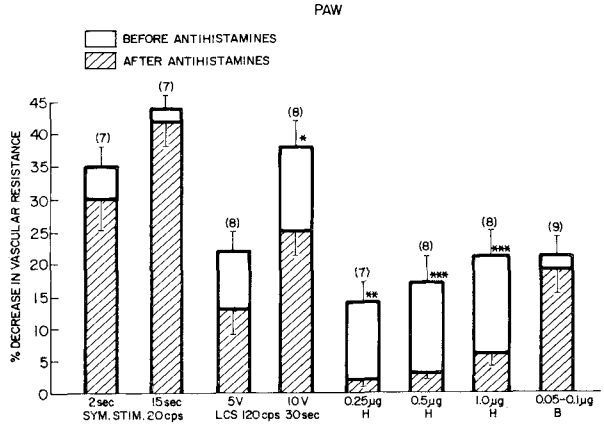

Fig. 1. Vasodilator responses evoked by sympathetic stimulation, LCS, histamine, and bradykinin before and after combined intraarterial infusions of antihistamines, chlorpheniramine and tripelennamine (3–5.5 mg of each) in 9 experiments. Control responses are depicted as the entire bar and responses after antihistamines as striped portion of bar. Number of experiments are within parentheses. CPS = Hz. $*p^*_{\Delta} < 0.05$; $**p < 0.01$; $***p < 0.001$.

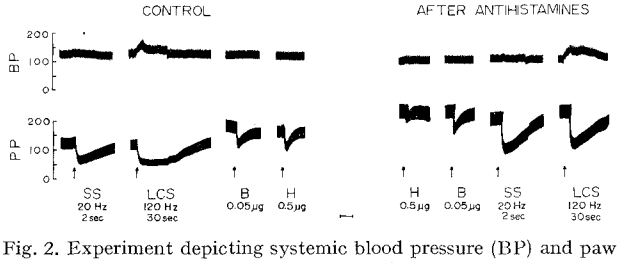

Fig. 2. Experiment depicting systemic blood pressure (BP) and paw perfusion pressure (PP) during sympathetic stimulation, LCS, and intraarterial administration of histamine and bradykinin before and after tripelennamine and chlorpheniramine (3 mg of each). Paw blood flow was 17.5 ml/min. A 1 min time calibration is indicated.

Medullary stimulation. These experiments were undertaken to demonstrate that sustained vasodilatation is mediated by sympathetic nerve fibres separate from the sympathetic adrenergic innervation. Stimulation of pressor sites in the medulla and the lumbar sympathetic trunk produced vasoconstrictor responses in the paw (Figure 3). The frequency of lumbar nerve stimulation was adjusted in each experiment so that the vasoconstriction evoked in the paw was comparable in magnitude to that caused by medullary stimulation. Following bretylium the usual marked degree vasodilatation in the paw was obtained during sympathetic stimulation, but the vasoconstrictor response elicited by medullary stimulation was blocked, and little or no vasodilatation occurred (Figure 3).

Discussion. Previous investigations of the sustained sympathetic vasodilator system have been limited to its peripheral activation, i.e. by direct stimulation of the sympathetic innervation of the paw[3,4,6] ear[4] or hind limb[5,7]. A brief report of non-cholinergic vasodilatation in the dog hind limb evoked by stimulation of centers in the hypothalamus[8] has also been presented. The results of the current study demonstrate conclusively that a central representation of the sustained vasodilator system exists. Electrical stimulation of the lateral spinal cord after adrenergic blockade elicits vasodilatation in the cutaneous vasculature of the paw which resembles closely the vasodilator response produced by lumbar sympathetic stimulation. The vasodilatation is noncholinergically mediated and is long in duration. Localiza-

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tion of the centers in the brain or spinal cord from which this non-cholinergic innervation of the cutaneous vessels is derived was not sought in the present study.

Inasmuch as the neurotransmitter mediating sustained vasodilatation has not been delineated it remained to be determined whether the adrenergic nerves even after adrenergic neuronal blockade may have mediated the vasodilator effect. Results have been presented indicating that release of 'pseudo transmitter' in the form of the blocking agent itself did not mediate this response[7]. In the present work it is strongly suggested that sympathetic vasodilator fibres separate from the adrenergic nerves mediate sustained vasodilatation. Medullary vasomotor center stimulation and sympathetic nerve stimulation evoked comparable vasoconstrictor responses in the paw prior to adrenergic blockade, presumably by activation of a similar adrenergic nerve discharge. However, after adrenergic blockade medullary stimulation did not cause vasodilatation, whereas stimulation of the sympathetic trunk did. Stimulation of these sites in the medulla caused selective adrenergic stimulation which was shown to be separate from the sustained vasodilator innervation.

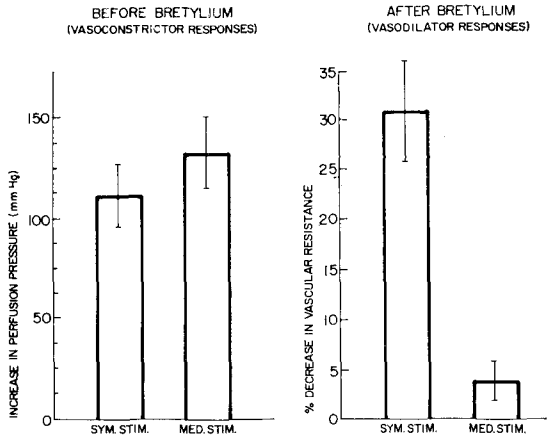

Fig. 3. Vasoconstrictor responses evoked by sympathetic stimulation (7–20 Hz) and medullary stimulation (240 Hz) before bretylium 10 mg/kg and vasodilator responses evoked after bretylium. Vasoconstrictor responses are indicated by increases in PP, in mm Hg and vasodilator responses as % decrease in vascular resistance. Values are mean of 10 experiments.

In previous studies it was reported that antihistamines were partially effective in blocking sustained vasodilatation, whereas in other reports no effect of antihistamines was obtained[3–6]. Certain antihistamines such as tripelenamine potentiate adrenergic responses[9]. If an adrenergic contribution to sympathetic stimulation remains after adrenergic neuronal blockade, the antihistamine may partially antagonize sustained vasodilatation by potentiating this residual adrenergic component rather than by a specific antihistaminic effect[6]. It is conceivable that this action of the antihistamine combination utilized in the present study accounts for the decrease in vasodilatation induced by LCS. We cannot rule out the possibility, however, that histamine release evoked by LCS may partially contribute to the vasodilator response and that the antihistamines may be acting specifically. The fact that a comparable vasodilator response elicited by sympathetic stimulation was not antagonized by the antihistamine would suggest involvement of histamine at least in some of the experiments involving LCS.

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Early Investigations on the Effect of Methyl Mercuric Chloride upon DMN-acute Hepatotoxicity[1]

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Summary. Acute toxicity induced by DMN was partially prevented by previously administering methyl mercuric chloride (MMC), a chemical inhibitor of the drug metabolizing enzyme system (DMES). We have studied the early changes occurring during the course of DMN-intoxication, namely disaggregation of polysomal profiles and necrosis, evaluated morphologically and by the release of S-GPT.

Mercury is an important environmental pollutant, largely used in industry and agriculture, and is very well known for its toxic effects on the peripheral and central nervous system[2]. Lucier et al.[3] and Alvares et al.[4] showed that low doses of mercury administered to rats inhibited the activity of the hepatic drug metabolizing enzyme system (DMES). Mercury does in fact reach high concentration in the microsomal fraction of the hepatocyte[5].

Pathophysiological alterations of the DMES may alter the course of toxicity induced by those drugs which act after their biochemical transformation occurring in the DMES. Carlson[6] has in fact found a protection by methyl mercury against CCl_4-poisoning. This haloalkane is a very well-known hepatotoxin which exerts its poisoning effects through a metabolite, the free radical $\cdot CCl_3$ formed in the DMES[7,8].

Dimethylnitrosamine (DMN), as CCl_4, is also metabolized within the DMES into derivatives which induce acute hepatic damage and carcinogenic effects as well[9].

The course of DMN toxicity is dependent on the rate of its metabolism. The inhibition of the DMES, obtained by feeding the rats with a protein-free diet, decreases DMN-

[1] This work was supported by 'Consiglio Nazionale delle Ricerche', Roma, Italy.

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