α-blockade has almost no further effect on metabolic acidosis. Conversely, the increased blood loss effected by additional β-blockade does not aggravate the metabolic status ($p > 0.05$). These findings are amplified by the changes of lactate/pyruvate metabolism depicted in Figure 3. Without treatment and with pure β-blockade, tissue perfusion is impaired following shock, as witnessed by an average arterial L/P ratio of 75. At all levels of α-blockade, the ratio remains low ($p < 0.01$). Again, the blood loss increment produced by additional β-blockade causes no metabolic deterioration.

Three hours after reinfusion, metabolic recovery as judged by arterial pH and base excess was better following combined blockade than after α-blockade only, as seen from the Table. A significant interaction ($p < 0.05$) between α- and β-blocker was, however, present. At all levels of α-blockade (A_1, A_2, A_3), metabolic acidosis decreased with a small dose of β-blocker (B_1), whereas it increased again with higher levels of β-blockade (B_2 and B_3).

Combined adrenergic blockade at the lowest dosage level employed in this study (1.0 mg/kg α-blocker + 0.1 mg/kg β-blocker) thus increases tolerance to an acute blood loss as compared with pure α-blockade, without sacrificing the metabolic protection provided by that measure. It therefore represents an optimum in the sense that it combines a minimum decrease of tolerated blood loss with a maximum inhibition of its metabolic sequels. The results obtained with this regimen in shocked dogs will be reported separately[7].

Generic and trade names of drugs: Dibenzyline® = Phenoxybenzamine; Trasicor® = 1-Isopropylamino-3-(*o*-allyloxyphenoxy)-2-propanol-hydrochloride; Nembutal® = Pentobarbital.

pH and base excess 3 h after reinfusion $\bar{x}$ and 95% confidence intervals. No overlapping = $p < 0.05$

	Combined blockade mg/kg $1.0\alpha + 0.1\beta$	α-Blockade mg/kg 1.0	2.5	4.0
pH	7.447 (7.402–7.492)	7.323 (7.315–7.331)	7.345 (7.301–7.389)	7.255 (7.215–7.295)
BE	−2.1 (−1.1 to 3.1)	−8.9 (−8.3 to −9.5)	−8.5 (−7.8 to −9.2)	−11.5 (−10.5 to −12.5)

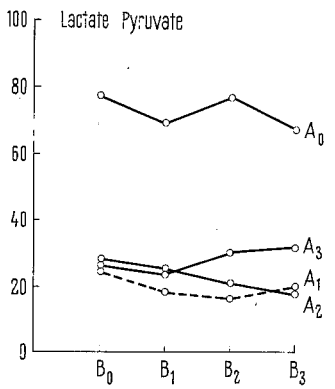

Fig. 3. Means of arterial lactate/pyruvate ratios in arterial blood at the end of the 30 min hypotensive period, cf. Figure 1.

Zusammenfassung. Die Kombination von 1,0 mg/kg α-Blocker und 0,1 mg/kg β-Blocker ergibt im experimentellen hämorrhagischen Shock die geringste Reduktion des tolerierten Blutverlustes zusammen mit einer maximalen Hemmung seiner metabolischen Konsequenzen und erscheint daher als optimal.

G. Zierott, H. Riedwyl
and P. Lundsgaard-Hansen

University Department of Experimental Surgery, Inselspital, and Institute of Mathematical Statistics, University of Bern, 3008 Bern (Switzerland), 1 April 1969.

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Preparation of 'New'-Vasoconstrictine (SVPx), a Vasoconstrictor Hormone of Plasma

A previously undescribed vasoactive hormone[1–4] has been separated from plasma by paparchromatography and counter-current distribution[5–7], and can be characterized by the following properties: (1) the material causes the isolated aorta of the rabbit to contract; (2) its contracting action is not antagonized by serotonin antagonists[6]; (3) unlike catecholamines, it leads to a contraction of intestinal muscle of the rabbit; and (4) it can be distinguished from histamine and from a number of commonly known vasoactive polypeptides[8].

Battelli (1905)[6] coined the term 'vasoconstrictine' for the constrictor material present in serum, for the most part the result of serotonin released from blood platelets during the clotting of the shed blood. We shall refer to the serum principle(s) as 'old'-vasoconstrictine. In contrast, when arterial blood is freshly collected in the presence of heparin, there is no evidence for newly formed constrictor material such as serotonin[9]. Since the plasma of heparinized blood incubated at 37 °C for as long as 1 h showed no additional constrictor activity, it appears reasonable to assume that the vasoconstrictor potency

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is essentially the same as that in the plasma circulating in vivo.

In a recent paper[6] we showed that the total contractile activity of plasma was a composite of known and an additional unknown principle, termed in 1962 SVPx[1], and we are suggesting for it presently the term 'new'-vasoconstrictine. We showed that SVPx can be separated by paperchromatography[6], and that counter-current distribution can be used as a preparative procedure. The details of this latter method for beef plasma are reported below.

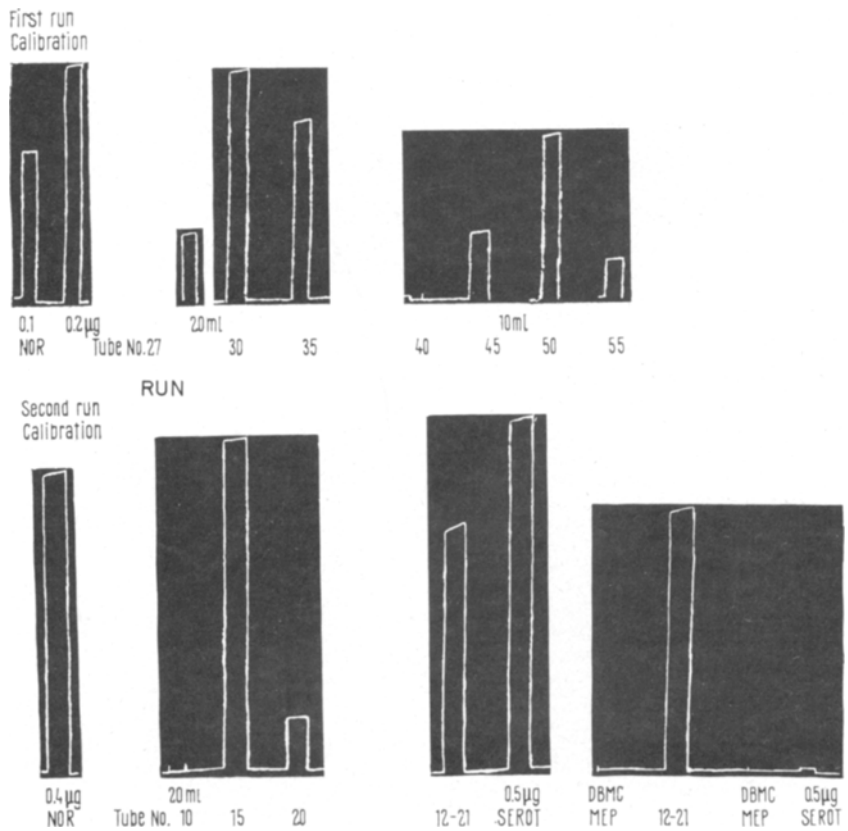
Methods and results. (1) *Bioassay of 'new'-vasoconstrictine* was performed on the spirally cut rabbit aortic strip suspended in oxygenated Ringer's solution, as described before[6]. The antagonists used were an antiserotonin agent, DBMC (N-β-dimethyl-aminoethyl-N-benzyl-m-methoxy-cinnamamide, Dombro and Woolley 1964) 20 μg/ml, and an antihistaminic agent, mepyramine, 10 μg/ml[6]. Samples taken from individual tubes of the counter-current distribution (CCD) apparatus were dried in a rotary evaporator, and the residue dissolved in Ringer's solution for application on the isolated organ. The resulting contraction height was referred to a calibration curve previously obtained with norepinephrine (NOR), and the potency expressed in equivalents of NOR.

(2) *Preparation of 'new'-vasoconstrictine from beef plasma* (Figure). Blood flowing from the severed blood vessels of the neck was collected in a plastic bucket which contained sufficient heparin to yield a final concentration of approximately 10 U/ml. The blood was centrifuged at 10,000 *g* in refrigerated centrifuge ajusted to 4 °C, and the plasma decanted by suction. *Deproteinization by dialysis.* Visking cellulose dialyzer tubing was filled with plasma and placed into an Erlenmeyer which contained the same volume of glass-distilled water. After shaking for 3 h in the cold, the contents of the bags were discarded, and only the protein-free diffusate was further processed.

Preliminary purification by counter-current distribution technique (CCD). Concentration of the plasma diffusate in this stage, whether by rotary evaporation at room temperature or by freeze-drying, usually resulted in excessive loss of potency. Therefore, the 1:1 plasma diffusate was used unconcentrated. The solvent mixture consisted of equal volumes of 0.1 *N* acetic acid and *n*-butanol equilibrated at room temperature. Accordingly 1 l of the plasma diffusate was also acidified and equilibrated with the same volume of *n*-butanol.

The CCD apparatus, manufactured by H.O. Post Scientific Instrument Co., contained 60 tubes, each holding 40 ml of the aqueous, and 40 ml of the organic phase. The first 22 tubes were loaded with 40 ml of each phase of the starting material. Then the organic phase of the solvent mixture was introduced during 200 transfers, which take about 2 days. The settling time between 2 transfers was 15 min because of emulsions formed in several tubes upon equilibration of the solvents. Residual vasoconstrictor activity not antagonized by the serotonin antagonist, DBMC, was detected by bioassay in the tubes from 27–38. Their contents were stored frozen. The vasoconstrictor material detected around tube 50 was discarded because it was antagonized by DBMC, it had the approximate partition ratio and therefore, likely represented serotonin.

Second purification by counter-current distribution. The active material obtained in 3 or 4 preliminary runs, i.e. prepared from 3–4 l of plasma diffusate, was concentrated



Preparation of 'new'-vasoconstrictine by counter current distribution. Bioassay on rabbit aortic strip (*n*-butanol: 0.1 *N* acetic acid).

in a rotary evaporator to approximately 100 ml without ever carrying the evaporation to dryness as a precaution against loss of activity. The first 2 tubes of the apparatus were now loaded with the starting material, and again run for 200 transfers. The settling time remained 15 min.

Samples taken from the second run left barely noticeable residues upon evaporation. Contracting activity was usually contained in the range of tubes extending from 11–20, with a clear cut peak at 15. This corresponds to a distribution coefficient of 0.08. The purification of 3-4 l of 1:1 plasma diffusate processed in the first run provided in the second run vasoconstrictor material approximately equivalent in potency to 100–200 µg NOR in 800–900 ml solvent mixture, which was then stored in the frozen state.

(3) *'New'-vasoconstrictine causes the isolated rabbit intestine to contract.* Since on rabbit aorta NOR is not antagonized by a mixture of DBMC and mepyramine, it appeared important to differentiate it from 'new'-vasoconstrictine with such conclusive biological evidence.

Discussion. Comments on the method of preparation. It was found important to use heparinized blood in order to prevent release of serotonin from platelets. Otherwise separation of 'new'-vasoconstrictine might be handicapped by an overwhelmingly large concentration of serotonin.

Meaningful figures on yields cannot be given until a standard preparation of 'new'-vasoconstrictine becomes available. By expressing the amount of 'new'-vasoconstrictine in equivalents of NOR in a bioassay it is tacitly assumed that from one preparation to the other there is no change in the relative sensitivity of the tissue to NOR and to 'new'-vasoconstrictine. This possibility may however not be rigorously correct.

Differentiation from other biologically active substances. As reported in the results, 'new'-vasoconstrictine could be distinguished from serotonin, histamine, or catecholamines. Oxytocin, vasopressin, and bradykinin were also excluded[8], because these substances did not affect the aortic strip at all. Substance P is relatively inactive on the rabbit artery (unpublished). Angiotensin was eliminated earlier on account of biological evidence[8]; and more recently by a preliminary estimate of the molecular weight of 'new'-vasoconstrictine which is half of that of angiotensin. None of the plasma proteins has any contracting activity[10].

Our observations indicate that the bulk of the vasoconstrictor potency of plasma which causes the rabbit artery to contract well beyond 50% of its maximal response, can be attributed to a mixture of serotonin and 'new'-vasoconstrictine[11].

Résumé. La «nouvelle»-vasoconstrictine (SVPx 1962), une hormone vasoconstrictrice du plasma, a été préparée par distribution à contre-courant en quantité suffisante pour l'étude de sa structure chimique et de ses propriétés biologiques.

M. Wurzel, L. C. Craig and B. W. Zweifach

Department of Physiology, University of Ottawa (Ontario, Canada), The Rockefeller University (New York, USA), and Department of Aerospace and mechanical Engineering Science, University of California San Diego, La Jolla (California, USA), 8 April 1969.

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After-Potentials Due to an Electrogenic Pump in Molluscan Giant Neurons

A previously undescribed hyperpolarizing wave following the after-potential of every somatic spike was consistently found in 'H' neurons, i.e. neurons showing cholinergic inhibition[1], of the abdominal mass ganglia of the Argentinian land snail *Cryptomphallus aspersa*. Experiments were carried out to determine the underlying mechanism of this wave and the results to be reported suggest that it may be due to an electrogenic pump.

Materials and methods. Isolated ganglia were immersed in a saline solution for mollusc[2] containing: NaCl, 127.8 mM; $MgSO_4$, 3.5 mM; $NaHCO_3$, 3 mM; CaCl, 6 mM. Neuronal somata were impaled with simple or double glass microelectrodes. The methods for recording and stimulation in this preparation have been previously described[3,4].

Results and discussion. The hyperpolarizing wave, hereafter called Ap_2 or second after potential invariably follows each somatic spike and begins about 10 msec after the typical after-potential, hereafter called Ap_1. The hyperpolarization reaches its maximum amplitude (about 4 mV), 75 msec after Ap_1 and the total duration is about 800 msec (Figure 1A).

Ap_1 has the well-known properties of the common after-potential[3,5]; i.e. (a) When the membrane potential is changed by passing hyperpolarizing or depolarizing currents through the cell, the size of Ap_1 varies (see Figure 1). It becomes smaller as the cell is hyperpolarized and beyond a point of reversal potential, it becomes a depolarizing wave. (b) The reversal potential of Ap_1 is related to the external potassium concentration. When this is increased from the normal 4.9 mM to 15 mM, the equilibrium potential is displaced from -60 to -45 mV. On the other hand, at zero potassium concentration it is about -68 mV. (c) Variations of chloride in the solution produce no changes, either in the size or in the reversal potential of Ap_1. These data are in accordance with the potassium permeability theory for the after-potential[3,5] and the reversal potential of Ap_1 may be considered equivalent to the equilibrium potential for potassium (E_K).

Ap_2 behaves very differently from Ap_1. (a) Its size is independent of the membrane potential (see Figure 1)

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