the *fasted* rat) but not that mediated by the α-receptor (norepinephrine in the *fed* rat). The enhanced β-effect is apparently the result of a greater depletion of muscle glycogen. Hyperglycemic responses to epinephrine in *fasted* animals are enhanced in the lower dose range and depressed at higher doses. This is explained in terms of the effect of epinephrine on both α- and β-receptors. The importance of using both *fed* and *fasted* rats and dose-response curves rather than single doses in assessing the effects of adrenalectomy is demonstrated.

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Effects of a Synthetic Analogue of Vasopressin on Vascular Smooth Muscle.* (30152)

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PLV-2, (2-phenylalanine-8-lysine vasopressin) a synthetic analogue of vasopressin has been reported to increase survival of rats subjected to hemorrhage(1,2). Direct *in vivo* microscopy of the microcirculation in the mesoappendix of rats in hemorrhagic shock (3) indicated that PLV-2 maintained arteriolar and venular tone, sustained vasomotion of metarterioles and precapillaries and decreased the abnormally high reactivity to topically applied epinephrine characteristic of shock. The net influence of the above selective effects of PLV-2 was to improve true capillary inflow, distribution and outflow. In view of the foregoing observations in the shocked animal, the effects of PLV-2† on microvascular smooth muscle of normal intact rats and on isolated arterial muscle strip preparations were studied.

Materials and methods. 1. *In vivo.* Wistar strain female rats, 130-180 g, were anesthetized with pentobarbital sodium, 3.0 mg/100 g body wt i.m. The mesoappendix was exteriorized for direct microscopic visualization according to a modification of a previously described method(4). Microscopic observations were made at magnifications of 60× and 100×. The effects of various concentrations of PLV-2 (Tables I, II) on the microvessels were determined: A. By direct (topical) application. B. By i.v. injection followed by observation of the vascular responses to standard concentrations of epi-

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nephrine (0.5 μg/ml) and norepinephrine‡ (1.0 μg/ml) applied topically. All drugs were applied to the surface of the mesentery in volumes of 0.1 ml.

2. In vitro. To avoid systemic factors introduced *in vivo* by i.v. and topical administration of PLV-2 in assessing any direct effects of the drug on arterial smooth muscle, thoracic aortae were obtained from young male rabbits (1.5-2.3 kg in wt) immediately after sacrifice. Spiral strips were prepared according to Furchgott and Bhadrakom(5) and suspended vertically in an 8 ml organ bath containing Krebs-Ringer bicarbonate solution maintained at 37°C and aerated with 95% O_2, 5% CO_2. Each strip was equilibrated for 2 hours. Isometric contractions were measured with a Grass FT-03 force-displacement transducer. Isotonic contractions were obtained by means of a lever-type transducer designed by Invengineering, Inc., Belmar, N. J. All recordings were made on an Offner Type R Dynograph. Two grams of tension were applied to all of the strips. Graded dose-response relationships with norepinephrine (agonist) were determined at the start of each experiment prior to testing the strips for the contractile effects of PLV-2. A concentration of 0.8 μg of norepinephrine/8 ml of bath fluid, which produced approximately 60% of the maximal contractile response, was selected as the agonist test dose. Varying concentrations of PLV-2 were left in contact with the isolated strips for 5 minutes prior to adding the test dose of norepinephrine (Table III).

Results. 1. *In vivo.* Topical application of 0.05 I.U. of PLV-2 was the lowest concentration which regularly produced constriction of feeding arterioles, precapillaries, metarterioles and venules with restriction of overall true capillary inflow and outflow (Table I). Lower concentrations (0.02-0.03 I.U.) did not constrict arterioles but resulted in some metarteriolar, precapillary and venular constriction which restricted postcapillary outflow. A concentration of 0.01 I.U. affected only the venules.

TABLE I. Effects of Topical Application of PLV-2 on Micro-Vessels in Rat Mesentery.*

Dose (I.U.)†	Arteriole	Metarteriole	Precapillary	Venule
<.01	0	0	0	0
.01	0	0	0	+ ‡
.02	0	0	+	++
.03	0	+	+	+++
.05	+	++	++	++++

* PLV-2 applied to surface of mesentery in volumes of 0.1 ml.

† One I.U. (pressor unit) = 20 μg of active polypeptide.

‡ Symbols (+) represent degree of microvascular constriction and numbers of vessel types responding.

The i.v. injection of PLV-2 (0.01-0.1 I.U. in 0.2 ml of saline) produced a transient increase in capillary circulation within 1-2 minutes and a definite narrowing of the arteriolar vessels. Blood pressure was not affected with these doses of PLV-2. These non-pressor i.v. doses of PLV-2 did, however, potentiate the constrictor responses to the topically applied threshold doses of epinephrine (0.05 μg) and norepinephrine (0.10 μg) in relation both to the vessel types which responded and to the magnitude of their responses (Table II). In the untreated control the above threshold doses of the catecholamines resulted in complete closure of precapillary sphincters and a slight narrowing of the metarterioles for a duration of 20-45 seconds. Within 1-2 minutes following i.v. injection of the lowest dose of PLV-2 (0.01

TABLE II. Effects of I.V. Administration of PLV-2 on Vascular Reactivity.*

	Response to topical catecholamines†			
Dose (I.U.)	Arteriole	Metarteriole	Precapillary	Venule
Saline, .2 ml	0	+‡ 0	+	0
PLV-2, .01	+	++	++	0
" .05	+	++	+++	+
" .10	Generalized vasoconstriction of microbed			

* PLV-2 injected via tail vein in 0.2 ml of saline.

† Responses to topical epinephrine (0.05 μg) and norepinephrine (0.10 μg) determined 1-60 min after PLV-2 injection.

‡ Symbols (+) indicate degree of positive response.

‡ Norepinephrine used in this study was Levophed® bitartrate; 0.2% sol. = 0.1% base. All doses were calculated as the base. Epinephrine used was Adrenalin® Chloride; 1:1000 = 1 mg/ml salt. All epinephrine doses were calculated as the salt.

TABLE III. Effect of PLV-2 on Rabbit Aortic Strip Contraction.

Agonist = 0.8 μg norepinephrine	No. of strips	PLV-2* (Dose: I.U./8 ml bath)	% Inhibition (mean)	Range
Isometric	3	1.0	31	27 to 36
	3	2.0	40	37 to 43
	3	4.0	70	67 to 74
Isotonic	3	1.0	54	47 to 60

* All strips pre-exposed to PLV-2 for 5 min prior to addition of agonist.

I.U.), potentiated responses to the topically applied catecholamines were apparent in that many arterioles and metarterioles, in addition to the precapillaries, were markedly constricted for a period of 1-3 minutes. The intermediate dose of PLV-2 (0.05 I.U.) additionally produced some venular narrowing. The highest dose (0.10 I.U.), with topical application of threshold concentrations of both catecholamines, caused a generalized vasoconstriction of the entire microbed. PLV-2 potentiation of the topical effects of epinephrine and norepinephrine was apparent for up to one hour after a single i.v. administration; long after the initial microcirculatory vasomotor effects of the polypeptide, itself, had disappeared.

2. *In vitro.* PLV-2 in concentrations up to 4.0 I.U. per 8 ml of bath fluid failed to contract any of the isolated aortic strip preparations. When tested for potentiating action, PLV-2 inhibited norepinephrine-induced isometric and isotonic contractions. The degree of inhibition expressed as % decrease in contractile response compared with the uninhibited muscle tension (isometric) or contractile height (isotonic) is presented in Table III.

Discussion. These observations indicate that this synthetic polypeptide exhibits selective microvascular effects which are apparently paradoxical in that its actions are out of the normal profile of reactivity of the microvessels to the usual physiological stimuli. The gradient of reactivity for PLV-2 progresses from the venular toward the arteriolar side in contrast to the arteriolar to venular gradient for epinephrine and norepinephrine. There is increasing evidence that such selective activity of vasoactive substances, some of which are normal tissue components, is not unusual particularly in abnormal circumstances. Serotonin(6) can influence microvessels from the venous to arteriolar loops. In acute endotoxemia the venules show abnormal hyperreactivity to locally applied catecholamines relative to other types of microvessels(7). The dose-graded microvascular constriction induced by locally applied vasopressin closely resembles the selective effects noted here with PLV-2 (6,8).

The observations noted here and previously reported by our group(3) indicate that PLV-2 can induce either epinephrine potentiation or epinephrine inhibition on vascular smooth muscle. Comparable microvascular effects can be produced by vasopressin which in contrast to PLV-2 are dose dependent; low doses of which are epinephrine inhibitory while high doses are epinephrine potentiating(4). Other vasoactive substances, such as serotonin, have been shown to be either dilators or constrictors depending upon the vessel tone present at any given time(9).

Although PLV-2 failed to elicit contractions of isolated aortic strips, it did however markedly attenuate contractions induced by norepinephrine. Similar effects on isolated large vessel musculature have been noted with pitressin by other workers(10).

Definitive identification of the mechanisms participating in the microcirculatory effects of vasopressin-like polypeptides must be deferred pending further study. Interest in such agents seems warranted in view of the therapeutic potential of drugs with selective vasomotor effects on the exchange vessels of the microbed.

Summary. PLV-2, a synthetic analogue of vasopressin, applied topically to the microvessels in the rat mesoappendix produces a graded dose response reduction in true capillary blood flow. The various vessel types contract from the venular to the arteriolar loops;

the opposite of their normal profile of reactivity. This polypeptide, when injected i.v. in a single dose potentiates constrictor responses to topically applied catecholamines. In contrast to these microcirculatory effects, PLV-2 inhibits norepinephrine-induced contractions in the isolated rabbit aortic strip.

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Blood Lactate and Pyruvate and Evidence for Hypocapnic Lacticacidosis in the Chicken.* (30153)

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In mammals there is an inverse relationship between plasma P_{CO_2} and level of blood lactate and pyruvate(1-3). A similar relationship for an avian species has not been established.

Materials and methods. Adult male white Leghorn chickens (2.5 kg to 3.1 kg) kept in outdoor pens were used in these experiments. Experiments were conducted between February and May so the animals were adapted to cold. The tracheas, left carotid arteries and right brachial veins were cannulated under local anesthesia (ethyl chloride). Incisions were closed and 20 mg/animal of heparin (sodium) was administered intravenously. After waiting approximately 15 minutes, a control sample of blood was drawn. Succinylcholine was injected i.v., initially in a dose of 40 mg/animal and before each additional ventilating regimen in a dose of 20 mg/animal. The animals were overventilated artificially by connecting the tracheal cannula of the animal to a respiratory pump which ran at a stroke volume of 38 ml and a rate of 50 breaths per minute (1.9 l/min). Gas mixtures of 21% O_2, and 0, 5 or 10% CO_2 with the remainder as N_2 were delivered to the animals on the over ventilation regimen. Presentation of gas mixtures was randomized to eliminate the possibility of sequence of presentation influencing the results. In some experiments only 0 and 5% CO_2 were used.

At the end of 30 minutes on a given gas mixture a 4 ml sample of arterial blood was drawn after first drawing ½ ml to clear the cannula. The portion of blood sample to be analyzed for lactate and pyruvate and glucose was drawn into a 2 ml syringe, transferred directly into a centrifuge tube containing 2 ml of chilled 10% trichloroacetic acid and mixed. Volume of blood was determined on the basis of weight increase assuming a specific density of 1.06 g/ml for chicken blood. The remainder of the sample was drawn into a heparinized syringe and stored in an ice bath until appropriate determinations could be made.

Lactate concentrations were determined by the method of Friedland and Dietrich(4).

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