TABLE I. Inhibition of Skeletal Muscle Cell Membrane Adenosinetriphosphatase by Quinidine. Figures are μmoles of inorganic phosphate formed in 15 minutes per mg of membrane protein. Figures in parentheses are percent inhibition.

Membrane preparation	Age*	Concentration of quinidine (M) None	1×10^{-5}	5×10^{-5}	1×10^{-4}	5×10^{-4}
1	24 hr	1.04	—	—	—	.52 (50)
2	Fresh	1.38	—	—	—	.79 (43)
"	24 hr	1.04	—	—	—	.58 (44)
3	Fresh	1.27	1.27 (0)	1.12 (11)	.98 (23)	.68 (46)
4	"	1.57	1.57 (0)	1.46 (7)	1.31 (17)	.90 (43)
"	7 days	.39	—	—	—	—

* Hours or days at 4°C. Fresh means experiment was performed within 4 hr after preparing membranes.

tal muscle and may reach a concentration above 1×10^{-4}M in myocardium (calculated from the data of Scherlis *et al*) (10).

Summary. The adenosinetriphosphatase activity of rat skeletal muscle membranes is inhibited about 45% by 5×10^{-4}M quinidine sulfate and slightly (about 9%) by 5×10^{-5} M quinidine sulfate. This enzymic activity requires sodium and magnesium. Higher concentrations of magnesium are inhibitory and the inhibitory effect of quinidine is decreased at high magnesium concentrations.

TABLE II. Quinidine Inhibition of Membrane Adenosinetriphosphatase at Various Magnesium Concentrations. Figures are as in Table I.

Mg^{++} added to reaction (M)	Adenosinetriphosphatase activity: No quinidine	5×10^{-4}M quinidine	Inhibition (%)
None	.13	.10	23
.0005	.66	.40	39
.0025	1.37	.72	47
.005	1.41	.81	43
.010	.97	.62	36
.015	.68	.49	28

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Influence of a Synthetic Analogue of Vasopressin on Survival After Hemorrhagic Shock in Rats.* (28903)

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The value of vasoexcitor-pressor drugs in treating shock is being vigorously questioned (1,2) primarily from experience with the amine pressors. The critical disadvantage of the latter is attributed principally to tissue ischemia resulting from their intense constrictor effects on the resistance arterioles. Little therapeutic distinction has been made between the unfavorable constrictor effects of

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the amine pressors on the resistance bed and their potentially favorable excitatory effects on the more distal vessels of the capillary bed. Evidence for the latter exists(3) but is difficult to demonstrate therapeutically in shock probably because of the unselective intense constriction produced by the amine pressors over a wide dose range on all muscular elements of the peripheral circulation as observed by direct microscopy(4).

Vasoactive polypeptides normally have an important role in regulation of local tissue blood blow(5); the critical hemodynamic factor in shock. These polypeptides and the catecholamines activate smooth muscle through different mechanisms(6) and may even be antagonistic at the cellular level. Because of these and other differences between the pressor amines and the vasotropic polypeptides and the recent availability of potent synthetic polypeptide pressor drugs, it was felt that the latter might be of therapeutic value in shock. The particular polypeptide examined in this study is an analogue of 8-lysine vasopressin in which phenylalanine replaces tyrosine in the 2 position; namely, 2-phenylalanine-8-lysine vasopressin or PLV-2.† Berde *et al*(7) have described the general pharmacological properties of PLV-2. The drug is prepared as a solution containing 5 International Units (pressor) per ml. One I.U. very closely approximates 20 μg of PLV-2.

Materials and methods. Wistar strain (CFN) female rats, 140-215 g body wt, after receiving tetracycline (10 mg/day) in their drinking water for 4-5 days, were anesthetized with pentobarbital sodium (3.0 mg/100 g body wt) and the femoral artery and vein of one limb were cannulated with plastic tubing. They were bled in graded fashion to a predetermined mean blood pressure level using an automatic bleed-out device incorporating a heparinized saline reservoir and conventional mercury manometer. Blood pressure levels were maintained at preselected levels for 2 hours with no reinfusion of blood or saline. Following this, 2.0 ml of normal saline containing the selected doses of PLV-2 were infused i.v. over a one-hour period. Controls received only saline and were run concurrently with treated animals. When the infusion was completed, the shed blood was reinfused in graded fashion (20-40 minutes total period), catheters removed, vessels ligated, incision closed and the animals observed for 48 hours for survival. Aseptic technic was used throughout. Gross autopsies were performed after death or sacrifice.

TABLE I. Effect of PLV-2 on Survival of Rats After Various Degrees of Hemorrhagic Hypotension.

Group	mmHg B.P.	Controls	PLV-2
		%	%
1	50-55	13/16*(80)	14/14 (100)
2	40-45	12/16 (75)	10/10 (100)
3	35-40	10/15 (66)	11/12 (92)
4	30-35	14/30 (46)	13/16 (81)

B.P. maintained for 2 hr followed by infusion of 2.0 ml saline containing 5.0 I.U. of PLV-2 for period of 1 hr after which shed blood was reinfused.

* Numerator = survivors, denominator = total rats. Deaths determined at 48 hr.

In one series various blood pressure levels were utilized and the dose of PLV-2 kept constant at 5 I.U. (100 μg) per hour. In a second series blood pressure was maintained at 30-35 mmHg and various doses of PLV-2 given.

Results. In the first series (Table I) control survival rates were found to vary with blood pressure levels. Treated animals, at all blood pressure levels, showed a significantly‡ higher survival rate than controls irrespective of the blood pressure levels; most apparent at the lower-arterial pressures. PLV-2 administration uniformly resulted in a prompt and sustained rise in mean arterial pressure which fluctuated very little during the hour of drug infusion. In the more severely hypotensive animals the pressures during drug infusion were relatively close to pre-bleeding levels. In the less hypotensive treated rats the blood pressures reached (150-170 mmHg) were well above initial values (90-120 mmHg). But they never reached extreme hypertensive levels, even transiently, despite continuous administration and the large drug

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‡ Statistically significant at better than the 5% level by binomial confidence limits.

dose used. Tachyphylaxis during the hour of drug administration was not observed. Survivors, at autopsy, showed little or none of the visceral congestion typical of animals dying in shock.

In the second series at 30-35 mmHg, a significant‡ increase in survival was observed in treated rats at all doses of PLV-2. No correlation between survival and volume of shed blood (% body wt) was noted even though the latter varied considerably in both controls and treated rats (2.10-4.40 %). As in the first series, pressor responses were prompt, sustained, and stable both during and following drug infusion and the return of the shed blood. Blood pressures in treated animals were brought relatively close to initial levels with the higher drug doses and only moderately lower with the smaller doses. Treated animals, compared with controls, evidenced little or no respiratory difficulty during transfusion, regained consciousness and motor activity more rapidly and at autopsy showed little or no visceral congestion.

Discussion. The therapy of shock with any modality has as its essential purpose the maintenance of tissue blood flow. In principle, vasoexcitor drugs could exert a favorable influence on tissue flow by: 1. increasing the volume of and the pressure at which blood is delivered and 2. stimulating and/or potentiating the reactivity of the terminal arterioles, metarterioles and precapillaries distal to the major arteriolar resistance bed; the vessels which primarily condition tissue blood flow(5). The conclusion that the pressor amines cannot be readily administered so as to avoid intense constriction of the arterioles feeding the capillary bed does not *per se* invalidate the above principle.

These preliminary data seem to indicate that a polypeptide vasoexcitor can be readily administered to result in increased survival rates after severe hemorrhagic hypotension. It is reasonable to assume that this drug in some way favorably influences tissue blood flow, as the nature of the experiment (acutely depleted blood volume, no pre-treatment) makes it unlikely that the salutory effects of the drug are due to any "conditioning" effects on the tissues themselves. Despite the fact

TABLE II. Effect of Various Doses of PLV-2 on Survival After Hemorrhagic Hypotension (30-35 mmHg†).

Dose; PLV-2	Survival
1—Controls (2.0 ml saline)	31/54* (57)
2—5 I.U. (1.66 μg/min)	14/17 (82)
3—2.5 I.U. (.83 ")	10/12 (83)
4—1.25 I.U. (.42 ")	19/23 (84)
5— .63 I.U. (.21 ")	20/23 (87)
6— .32 I.U. (.11 ")	14/14 (100)
7— .16 I.U. (.06 ")	17/18 (98)

* Numerator = survivors, denominator = total rats. Deaths determined at 48 hr.

† B.P. maintained for 2 hr followed by infusion of 2.0 ml saline containing various doses of PLV-2 for period of 1 hr after which shed blood was reinfused.

that PLV-2 is a potent vasopressor and a potent arteriolar constrictor(8), unlike the amine pressors it seems to develop a "ceiling" on the degree of such constriction as evidenced by the plateau-like blood pressure response even when administered continuously over a wide dose range. A submaximal constriction of the arterioles of the rat mesoappendix during hemorrhagic hypotension has been observed when angiotensin (a polypeptide pressor) was administered.

In relation to shock therapy with drugs the value of increased systemic blood pressure resulting from vasoconstriction has also been questioned. The important factor in this regard is the tacit assumption that the increase in delivery pressure from the pressor drugs is insufficient to drive the blood through the almost obliterated arterioles into the capillary bed. This assumption does not negate the obvious value of an increased systemic pressure on capillary flow in circumstances where arteriolar constriction would be less intense and did not close down arteriolar outflow mechanisms as completely. Referable data, by direct microscopy, on the effects of PLV-2 on the terminal vascular bed in normal and shocked animals is not yet available. More precise substantiation of the mechanisms involved in the favorable effects of this drug on survival must, therefore, be deferred. Nevertheless, these data add support to the logical thesis, now apparently being abandoned, that vasoexcitor principles may have a valid place in shock therapy.

Summary. Rats subjected to hemorrhagic

shock, when given a vasoexcitatory synthetic polypeptide, PLV-2, showed increased survival rates. The mechanism(s) involved in the beneficial action of this drug cannot yet be precisely identified but may be related to a limited vasoconstrictor action as evidenced by a plateau-like pattern of the blood pressure response to its administration.

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Phosphorylation in a Partially Restored Bacterial System. (28904)

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Studies on the electron transport system of *Bacillus stearothermophilus* indicated that the light sensitive naphthoquinone component in the succinate oxidase complex did not participate in the direct line of electron flux but apparently functioned in a collateral manner(4). Such data are in conformity with the sequence of components in the succinoxidase chain as formulated for mammalian systems (6). However, the mechanism of the quinone mediated oxidation of succinate and NAD linked substrates in the microbial system appears to differ in various species(7,8). Recent studies on the electron transport sequence in the thermophile indicated a commensal relationship between soluble and particulate oxidative components was possible.

Methods and materials. The microorganism, electron transport particles (ETP) and soluble fraction were prepared as described previously(4). Soluble NADH—cytochrome *c* reductase was obtained from a supernatant (100,000 × *g*; 90 min) in the manner of Segel *et al*(11). The reduced nicotinamide adenine dinucleotide (NADH) and beef heart cytochrome *c* were obtained from Sigma Chemical Co., St. Louis; antimycin A, Kyowa Fermentations, LTD., Tokyo, Japan; 2,3-dimercapto propanol (BAL), gramicidin and ubiquinone 30, Mann Research Laboratories, N. Y., and amytal sodium, Eli Lilly & Co., Indianapolis.

Results and discussion. A cytochrome-bearing, electron transport particle (ETP) and a non-cytochrome, soluble fraction, isolated from *B. stearothermophilus*(4), both catalyzed the oxidation of reduced nicotinamide adenine dinucleotide (NADH). The oxidative rate in the soluble fraction was aided considerably by addition of cytochrome *c* and to a lesser extent by a naphthoquinone (NQ_b) isolated from the thermophile (Table I). No oxidation of added NADPH was observed.

The oxidation of NADH in the ETP was aided slightly by addition of cytochrome *c* or NQ_b. Examination of the catalysis in a mixture of the 2 systems indicated the rates were additive. These results suggested that the soluble NADH oxidase is limited by the availability of acceptor whereas the ETP maintains its initial degree of respiratory control and is limited by some other factor.

Flavin analyses, in the manner of Burch *et al*(2) disclosed a ratio of 3 to 1 between

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