to be better understood in terms of failure of intracellular retention rather than failure of delivery to the site of the detoxifying protein. We, therefore, believe that while these immune substances are an essential part of the defense mechanism, the host's ability to defend itself against endotoxin depends ultimately on the intracellular detoxifying principle.

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Renal Vascular Response to Hypothermia.* (29922)

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Past investigations(1-8,10) have been concerned with the response of the isolated or intact kidney to hypothermia. Results have varied, and interpretations are difficult due to complicating effects of increased blood viscosity and elevated extravascular pressure when blood temperature is decreased(7,10). Previous reports show that renal blood flow may be little affected(1), increase(4) or decrease(2-8,10) as a result of total body cooling. Levy(7) has attempted to separate viscosity influences from active renal vascular responses; however, his calculations were dependent on indirect procedures. The present study is devised to determine if renal vasodilation or vasoconstriction occur in response to hypothermia in the isolated denervated dog kidney. The complicating interfering components of increased blood viscosity and tissue pressure elicited by the hypothermic state have been avoided by the experimental design.

Methods. Experiments were carried out on a total of 22 dog-perfused kidneys. Five preparations served as controls in which body temperature was not altered. Dogs were intravenously anesthetized with sodium pentobarbital 30 mg/kg, and utilized as follows: One dog served as the experimental animal in each study and was subsequently immersed in the prone position in a large water bath

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TABLE I. Control Experiments: Dog Perfused Kidney.*

Time	RAP†	Blood temp (kidney)	Rectal temp (dog)	Heart rate	SAP‡
(min)	(mm Hg)	(°C)	(°C)	(beats/min)	(mm Hg)
0	95 ± 9	38.9 ± .3	36.7 ± .4	168 ± 13	149 ± 5
20	87 ± 7	39.2 ± .1	36.9 ± .4	166 ± 16	150 ± 7
40	83 ± 6	39.2 ± .1	37.3 ± .5	167 ± 16	149 ± 6
60	82 ± 7	39.2 ± .1	37.5 ± .4	170 ± 17	150 ± 6
80	85 ± 6	39.2 ± .2	37.9 ± .5	175 ± 20	149 ± 6
100	83 ± 5	39.2 ± .1	38.0 ± .5	178 ± 19	147 ± 7
120	86 ± 4	39.1 ± .1	38.0 ± .4	181 ± 16	144 ± 7
140	89 ± 6	39.1 ± .1	38.0 ± .4	176 ± 16	142 ± 8
160	92 ± 8	39.1 ± .1	38.0 ± .4	187 ± 15	140 ± 9
180	99 ± 9	39.1 ± .1	38.0 ± .3	181 ± 15	137 ± 8
200	109 ± 14	39.1 ± .1	38.0 ± .3	182 ± 16	137 ± 8
220	110 ± 15	39.1 ± .1	38.0 ± .3	182 ± 13	135 ± 10
240	112 ± 14	39.0 ± .1	38.0 ± .3	184 ± 12	134 ± 12

* Each entry is average value ± S.E. (5 experiments). (Renal blood flow constant; mean = 3 cc/min/g.)
† RAP = mean renal artery pressure.
‡ SAP = mean systemic artery pressure.

Time (min)	pH	Hematocrit
0	7.28 ± .03	38 ± 2
60	7.27 ± .03	40 ± 3
120	7.29 ± .02	43 ± 2
180	7.31 ± .02	41 ± 2
240	7.33 ± .02	43 ± 3

in which the temperature was maintained at 1°C (± 0.2) during the cooling phase. Another dog provided blood for the perfusion circuit and a third animal was used in obtaining a kidney. The left kidney was removed as previously described(9) by cannulation of the aorta immediately distal to the renal artery and transferred to the perfusion circuit without interruption of the renal blood flow. The kidney was perfused at a constant arterial inflow by means of a Sigmamotor pump interposed between experimental dog and kidney(10). Blood from the severed renal veins was collected in a reservoir and returned to the femoral vein of the dog *via* a second Sigmamotor pump. Blood returned to the dog was first passed through tubing immersed in the large bath containing the dog. Inflow and outflow pumps were maintained in a balanced state so that there was no change of blood volume in the dog. Blood obtained from the femoral artery passed through coiled plastic tubing placed in a temperature controlled water bath maintained at 41°C. By this procedure, the kidney was perfused with normothermic blood while the rectal temperature of the experimental animal varied over a wide range (21°-38°C). Renal arterial blood temperature was continuously monitored with a temperature probe, and mean renal arterial pressure was recorded on a Sanborn recorder *via* a Statham pressure transducer. Following a control period, the dog was totally immersed, except for the head, in the water bath maintained at 1°C. When the rectal temperature reached 22-26°C, the water in the bath was quickly drained and replaced with heated water and rectal temperatures were increased toward control values. The responses of dog and kidney were followed during both the cooling and rewarming phases. Heart rate, pH and hematocrits were also recorded.

Results. Table I provides mean control data from 5 perfused kidney studies illustrating the relative stability of the preparation. There were no significant changes in renal artery pressure and other values were relatively constant.

Table II presents the average effects of hypothermia on 14 dog perfused kidneys in which the rectal temperature is progressively decreased from approximately 35° to 25°C. Results indicate no significant change in renal artery pressure (RAP) during the cooling period. Six kidneys, however, exhibited a mean decrease in RAP from 95 to 76 mm Hg in an average cooling time of 17 minutes.

TABLE II. Effects of Altering Body Temperature in Dog Perfused Kidney Preparation.*

Time	RAP		T_B		T_R		Heart rate		SAP	
(min)	M ± SE	N	M ± SE	N	M ± SE	N	M ± SE	N	M ± SE	N
				A. Decreasing Temperature						
−20	98 ± 9	12	33.6 ± .7	8	36.5 ± .3	8	131 ± 12	4	124 ± 4	12
0	94 ± 7	14	36.2 ± .4	9	35.1 ± .4	10	152 ± 10	13	124 ± 5	14
+20	101 ± 13	13	35.4 ± .5	12	30.8 ± .6	11	101 ± 19	7	113 ± 8	13
40	105 ± 13	7	35.1 ± 1.3	3	28.5 ± 1.4	5	72 ± 16	4	97 ± 14	7
60	136 ± 16	4	36.3 ± 1.9	3	24.4 ± 1.8	3	85 ± 19	3	72 ± 21	4
80	108 ± 12	2	34.9 ± 2.3	2	26.6 ± 3.6	2	69 ± 21	2	85 ± 13	2
100	102 ± 7	2	35.1 ± 2.7	2	25.1 ± 2.6	2	62 ± 19	2	90 ± 28	2
				B. Restoring Temperature						
0	82 ± 9	12	33.1 ± 1.2	9	25.5 ± .1	9	59 ± 9	8	84 ± 12	11
20	135 ± 24	10	35.1 ± .8	7	25.3 ± .1	7	69 ± 7	7	96 ± 12	9
40	123 ± 24	6	35.4 ± 1.1	4	30.1 ± .1	3	88 ± 3	4	92 ± 10	6
60	162 ± 43	4	35.0 ± 1.1	4	30.0 ± .7	4	120 ± 10	2	91 ± 13	4
80	161 ± 45	4	35.5 ± .6	4	32.1 ± .6	4	145 ± 10	3	112 ± 27	4
100	189 ± 93	3	35.8 ± 1.0	3	33.7 ± .9	3	163 ± 4	2	83 ± 25	3

* Renal blood flow constant; mean = 2.5 cc/min/g.
N = No. of observations.

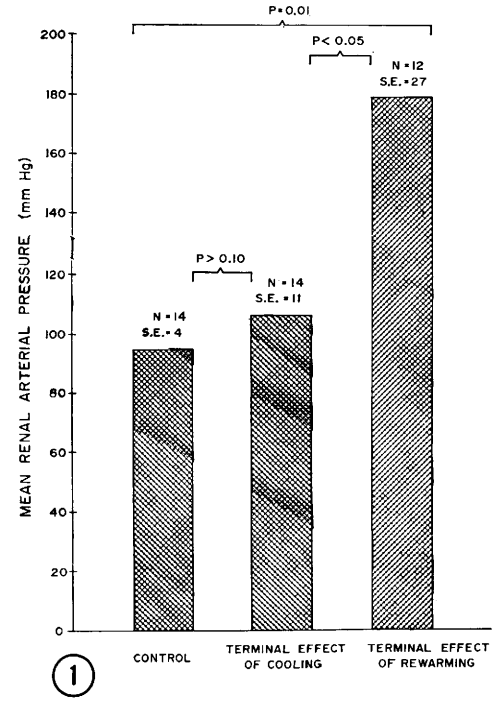
Three kidneys showed a marked increase in RAP from a mean of 76 to 181 mm Hg at the end of 43 minutes cooling time. Heart rate and systemic arterial pressure (SAP) progressively fell as a function of decreased rectal temperature. The hypothermic period was concluded at varying times in each preparation. Two animals died during the cooling period. The lower portion of Table II shows data obtained during the rewarming period, as rectal temperature was progressively increased from 25°-33°C. Marked increases in renal arterial pressure were noted. Since renal arterial blood flow rate was constant, a change in renal artery pressure indicated a like change in total renal vascular resistance. Inasmuch as the temperature of blood perfusing the kidney was maintained relatively constant, changes in renal resistance were due to altered vessel radius resulting from alterations in concentrations of vasoactive agents derived from the dog. Mean SAP did not show a recovery over the 100-minute period. Control mean pH was 7.24 ± 0.03; at termination of cooling was 7.17 ± 0.02, and at end of rewarming period, 7.12 ± 0.02. Hematocrit was 44 ± 3 for the control period; 47 ± 6 at termination of cooling, and 44 ± 5 at end of rewarming period.

Fig. 1 presents the mean changes in RAP and renal vascular resistance in 14 experiments. Values from each kidney were recorded at the end of both cooling (average, 45 minutes) and rewarming (average, 43 minutes) periods. It is seen that a decrease in body temperature elicits an insignificant change in renal resistance, but that there is a profound renal vasoconstriction while rewarming the animal. RAP increased from a mean control of 94 mm Hg to 177 mm Hg which indicates a like change in renal resistance since blood flow rate is constant.

Fig. 2 provides evidence in additional kidney experiments for the role of adrenergic agents in promoting the increase in renal resistance as a result of cooling and/or recovery from cooling. The marked rise in RAP, occurring during the recovery (rewarming) period, was obliterated by injection of 2.5 to 5.0 mg of phentolamine into the renal arterial inflow tubing. Injection of phentolamine in control experiments had no effect on renal resistance.

Discussion. These experiments were primarily useful in distinguishing the active and passive effects of hypothermia on the kidney. If blood viscosity is maintained relatively constant and extravascular pressure is not permitted to increase during the cooling period(10) it is seen that active renal vasoconstriction occurs during the rewarming period. This increase in resistance is apparently due to circulating adrenergic agents released as a result of the stress of cooling and/or rewarming. The delayed release of constrictor agents is not explained. Individual variations

in renal resistance observed during the cooling period agree with published data in which both renal vasodilation and constriction have been reported(2-8). Previous publications (13-16) have evaluated the role of catecholamines in hypothermia. Although increases have been reported in most instances(13,15, 16), depressions of catecholamine levels have been shown to occur(14). Data from the present study suggest that rewarming following a severe bout of acute hypothermia provides a strong stimulus for catecholamine release. Since systemic arterial pressure remains at borderline shock levels and pH is depressed, the stress of rewarming may be exceedingly severe. This possibility is substantiated by the data of others(17,18). Changes in heart rate and mean systemic arterial pressure during cooling appear to be related directly to rectal temperature and are presumably expressions of diminished metabolic activity(11,12).



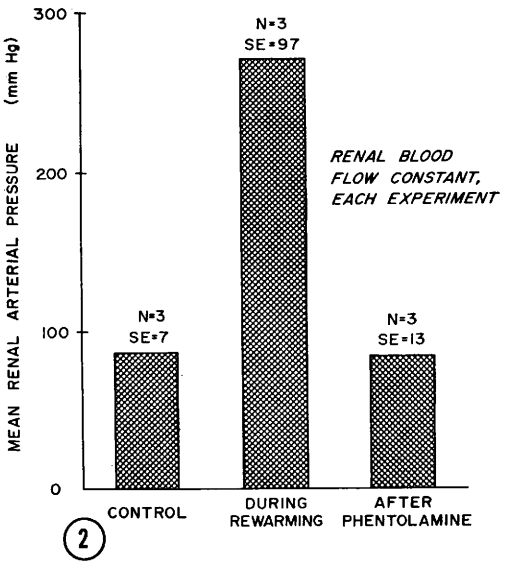


FIG. 1. Terminal effects of cooling and rewarming anesthetized dog on renal artery pressure and vascular resistance (renal blood flow maintained constant).

FIG. 2. Effect of phentolamine on renal vasoconstriction due to circulating humoral agents derived from hypothermic animal. Phentolamine injected during recovery (rewarming) period.

Summary. The vascular response of the perfused isolated dog kidney to acute periods of hypothermia has been carried out in specially designed experiments to remove interfering vascular effects of altered blood viscosity and extravascular pressure. Results show an active renal vasoconstriction occurring during the rewarming period, resulting from the release of adrenergic agents by the stressed animal. It is concluded that both active and passive components of renal resistance are involved during certain phases of hypothermia.

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Properties of Rous Sarcoma Hyaluronic Acid.* (29923)

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Morphological and biochemical studies(1, 2) of connective tissue in cancer have led to the view that the invasive growth of malignant tumor may be due to destruction of the ground substances of connective tissues adjacent to the tumor or to proliferation of undifferentiated connective tissues. Little is known concerning the biochemical characteristics of cancerous connective tissue components; however several workers(3,5) have indicated that the Rous sarcoma hyaluronic acid or its protein complex has a lower particle size than that of normal tissue as determined by viscosity and sedimentation constant.

In the work described here, the hyaluronic acid of Rous sarcoma was found to behave differently from that of normal tissues in the differential precipitation procedure with a cation detergent(6,7) and in ECTEOLA cellulose anion exchange column chromatography(8). The hyaluronic acid preparation from the sarcoma was also found to increase the rate of spreading of trypan blue in skin (9) and to increase capillary permeability (10).

Experimental. Sources of hyaluronic acid. Rous sarcoma was grown subcutaneously under the wings of white Leghorn chickens. Forty or fifty days after inoculation of Rous virus, the rapidly growing tumors were collected as a source for preparation of mucopolysaccharide. The necrotic brei, if any, in the central part of the tumor was discarded. Necrotic areas, when present, could easily be distinguished from viable tissue by the darker color and comparative softness. After the necrotic material was removed together with adjacent intact tumor tissue, the remaining tissue was washed with tap water before proceeding with preparation of mucopolysaccharides. Bovine vitreous humor and human umbilical cord were used as a source of normal hyaluronic acid. All tissues were defatted with acetone, dried and then ground in a Wiley laboratory mill.

Preparation of crude mucopolysaccharide. The tissue powder was digested with pepsin and then with trypsin according to the method of Jeanloz *et al*(11) and Schiller *et al* (12). Perchloric acid was added to precipitate the residual protein; the supernatant was dialyzed against distilled water and then lyophilized. The uronic acid content of isolated crude mucopolysaccharide preparation, estimated with the carbazole reaction of Dische(13), was as follows: Rous sarcoma, 26.5%; vitreous humor, 38.8%; umbilical cord, 35.9%.

Preparation of alkali-degraded hyaluronic acid. The mucopolysaccharide preparation of vitreous humor and of umbilical cord was dissolved in 1 M NaOH and incubated at 37° for 48 hours. After neutralizing, the solution was dialyzed against distilled water for 48 hours.

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