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J. H. LONGWELL, *Director*

Environmental Physiology and Shelter Engineering

With Special Reference to Domestic Animals

XXXIX. Environmental Temperature and Blood Volume

H. E. DALE, GLORIA J. BURGE, AND SAMUEL BRODY



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XXXIX. Environmental Temperature and Blood Volume

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INTRODUCTION

This is a continuation of Missouri Research Bulletins 433 and 488 on the effect of environmental temperature on blood composition but with special reference to serum volume, blood volume, and percent body water, changes of which affect the apparent blood composition. For instance, the initial increase in blood volume (of man) with increasing temperature is due to absorption of fluids (vasodilation) but without corresponding increases in hemoglobin, plasma protein, and other blood constituents. This has the effect of lowering the concentration of these blood constituents, that is, changing the apparent blood composition.

Increasing temperature above 80° F shifts heat dissipation in man from largely non-evaporative cooling by convection and radiation to evaporative methods. This tends to decrease the water and salt stores of the body. Lowering the environmental temperature tends to reverse the above processes: The rate of evaporative heat dissipation decreases with corresponding increase in salt and water stores and increase in urinary output to maintain the normal water equilibrium.

This general picture would change with many circumstances. For instance, it would vary with the degree of acclimatization or equilibrium of the subject with the environment and with the diurnal variability of the environment. A climate of constant environmental temperature of 80° F would be expected to have a very different effect from a climate having a diurnal temperature swing of 60° F, as in the California Imperial Valley where the diurnal temperature rhythm ranges from 60° to 120 F°. The low night temperature would keep the inner body temperature from rising during the hot day because of the stabilizing, or damping, effect of the large cattle body and therefore of the large heat capacity. By the time the rectal temperature begins to rise during the hot midday, the air begins to cool off, thus blocking the body temperature trend. The rectal temperature and associated physiological processes change, but the length of exposure to the deteriorating temperature is relatively short and is compensated in part by many homeothermic mechanisms.

The following are the most conspicuous homeostatic mechanisms controlling the blood volume and composition: The kidneys, the rate of activity of which is affected not only by the blood composition, but also by the neuroendocrine system. The posterior pituitary (and perhaps the neuro-hypophysis neurally connected with and under the control of the hypothalamus) produces an antidiuretic hormone which regulates facultative water resorption by the kidney tubules. The effect of temperature on the production of antidiuretic hormone is now under intensive investigation, particularly by Prof. W. V. MacFarlane in the Brisbane (Australia) Physiological Laboratory and in this country by Spealman *et al.* (1947).

Injury of the neuro-hypophysis, hypothalamus or connecting supraopticohypophyseal tracts greatly increases urine flow and therefore water consumption. While most dairy cows decrease their water consumption with increasing temperature above 80° F, one of our cows (Jersey 212) increased her urine output and water intake four-fold. This may have been caused by the effect of temperature on the above neurohormonal system. (Cow 212 should have been injected with posterior pituitary hormone to determine the effect on urinary output and water consumption.)

The adrenal cortex, also under the influence of the anterior pituitary, is greatly involved in water and salt balance. Removal of the adrenal cortical tissue reduces the blood sodium chloride by accelerating sodium chloride loss in the urine and decreasing its absorption from the gut. There is, on the other hand, an accumulation of potassium in the blood. The water content of the blood declines and the blood becomes concentrated as water passes to the tissue fluid and out through the kidneys. Temperature affecting the pituitary or adrenals would then affect blood volume and composition. The parathyroids maintain constant the calcium and phosphate ions by controlling their movements between bone and body fluids and favoring resorption of these ions by the kidney tubules. The importance of the hypothalamus and pituitary in water balance, blood volume and composition is indicated by the disorganization of these balances on removal of the hypothalamus and/or pituitary.

There is a voluminous literature on the physical dynamics, including influence of osmotic and other circulation factors on water balance and blood volume: Barcroft *et al.*, 1922; Bazett *et al.*, 1940; Forbes *et al.*, 1940; Maxfield *et al.*, 1941; Conley and Nicherson, 1945; Overman and Feldman, 1947; Reeve, 1948; Spealman *et al.*, 1948; Rodbard *et al.*, 1950; and D'Amato and Hegnauer, 1952. The effect of temperature on the distribution of body water, with particular reference to man, has most recently been reviewed by Bass and Henschel (1956).

These investigators report that in man increase in blood volume is associated with high temperature and dilation of the superficial capillaries, and decrease in blood volume is associated with low temperature and contraction of the capillaries. The increase in blood volume is associated with increase in extracellular fluid. All these changes are variable. Overman and Feldman (1947) reported extracellular fluid in monkeys as 182 ml/kg body weight in winter and 250 ml in summer. The increase of 37 percent of extracellular fluid in the monkey was associated with a 21 percent increase in blood volume. There is no doubt that there is a shift of heat dissipation to the evaporative methods above 80° F that alters the volume and distribution of blood and body water in profusely sweating man.

The water balance in slightly-sweating cattle may be different from that of the profusely sweating man at higher temperatures. This is because the rate of evaporative cooling in man rises at the fast pace of 8 to 13 percent per 1° F rise in temperature above 85° F, whereas in European-evolved cows the increase in evaporative cooling, under the given conditions, with increasing temperature above 80° F is very slight. Thus at 105° F, the ratio of evaporative cooling to heat production is 160 percent in man and only slightly above 100 percent in cattle. The purpose of the present research is to find out how changing temperature affects blood and water volumes, erythrocyte count, and distribution of body water between the various compartments. This is done on mature European cows kept under different diurnal rhythms (this bulletin) and on European and Indian heifers kept at constant temperatures of 50° and 80° F. (next bulletin).

BLOOD VOLUME OF CATTLE

Investigator	Method	Animals	Plasma cc/Kg Body Weight	Blood cc/Kg Body Weight
Turner & Herman (1931)	Vital Red	54 growing dairy cows	35.0	58.1
		24 non-lactating cows	37.8	63.8
		41 lactating cows	49.2	81.1
Miller (1932)	Vital Red	19 animals, 400-1300 lbs.; 81 detns.		59.7
Hansard et al. (1953)	RBCs Tagged With P ³²	2 2-6 weeks		120.0
		1 3 weeks		85.0
		3 2-3 months		62.0
		5 6-8 months		58.0
		3 14-15 months		57.0
		4 8-12 years		57.0
Reynolds (1953)	T-1824	1 heifer; 11 detns.	36.9	52.1
		10 non-preg., non-lact.; 10 detns.	38.8	57.4
Reynolds (1953a)	T-1824	20 pregnant Guernseys	38.5	59.2
		7 lactating Guernseys	44.1	64.1

METHODS

Data were obtained in the temperature-controlled psychroenergetic laboratory under four diurnally-variable temperature rhythms: 10° to 40° F rhythm ("Midwest Cold"); 40° to 70° F rhythm ("Midwest Normal"); 60° to 110° F rhythm ("Imperial Valley"); and 70° to 100° F rhythm ("Midwest Hot").

The temperatures were controlled so that the temperature troughs occurred at 5 to 7 a.m. and peaks at 3 to 4 p.m. The diurnal temperature and humidity patterns are shown in Figures 2 and 3, Missouri Research Bulletin 578.

Serum volume was measured with the blue dye, T-1824, as originally suggested by Dawson *et al.* (1920). This method, refined for clinical use by Gregersen *et al.* (1935, 1937, 1938, and 1943), has been used to measure serum volume in man (Reeve, 1948, and Gregersen, 1951), in a variety of animals (Barcroft *et al.*, 1939; Courtice, 1943; Bonnycastle, 1947; Reeve, 1948; Aikawa, 1950) and in cows (Reynolds, 1953 and 1953a).

The animals were deprived of feed for 12 hours before the serum volume determination and were not allowed water while the determination was in progress. During the injection of the dye and the withdrawal of the blood samples (both through a polyethylene catheter in the jugular vein) the animals were restrained with a rope halter; at all other times the animals were standing free in the stanchion. The dye solution T-1824 was injected at the rate of 20 mg/100 kg body weight for dry cows and 25 mg/100 kg body weight for the lactating cows. Blood samples were withdrawn at approximately 10-minute intervals for 1 hour; the samples were allowed to clot, the serum was obtained by centrifugation, and the density of the dye was read at 420 m μ on a Coleman Junior spectrophotometer. The hematocrit of a heparinized blood sample was read after 30 minutes centrifugation at 1500 rpm (radius 19 cm); the white cells were included with the plasma fraction. No correction was made for trapped plasma (estimated at 6 percent by Reynolds, 1953) or for the difference between the venous and the general body hematocrit (Stead and Ebert, 1941, and Gibson *et al.*, 1946).

Antipyrine for the determination of total body water was administered at the rate of 2.5 Gm/100 Kg body weight. The injection and sampling procedures were in general similar to the blood volume technique; however, blood for the antipyrine determinations was collected at approximately 1-hour intervals for 6 hours after the injection. The concentration of antipyrine in the blood serum was determined by the method of Brodie *et al.* (1949) using a Beckman DU spectrophotometer. The percent of serum water was determined by drying to a constant weight.

RESULTS AND DISCUSSION

The overall results for serum volume determination and blood volume calculation on lactating Jersey and Holstein cows, and on dry cows are given in Table 1 and Figures 1 and 2.

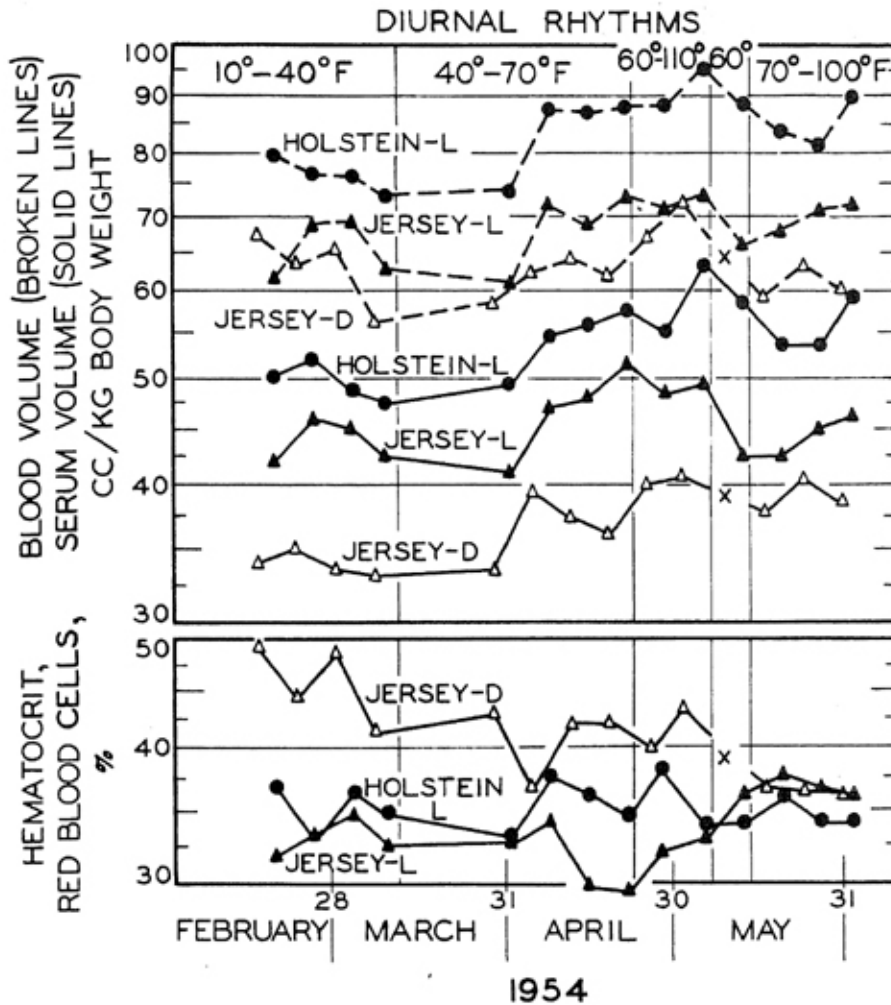


Fig. 1—Blood volume (broken line, upper three curves), serum volume (solid line) in cubic centimeters per kilogram body weight, and hematocrit (red-blood cell %, bottom segment) plotted against time (involving four diurnal temperature rhythms, upper axis) on arithlog paper (on which equal percentages are represented by equal slopes). Note that lactating (L) cows have higher blood and serum volumes but lower red blood percentages than dry (D) cows; that Holsteins have higher blood and serum volumes than Jerseys; and that the blood and serum volumes tend to increase with increasing average temperature of the diurnal rhythm.

TABLE 1 -- BLOOD AND SERUM VOLUME
(Mean values with standard deviation*)

Temperature Condition	No. of Cows	No. of Obs.	Serum Volume		Blood Volume		Hematocrit	
			cc/Kg Body Weight	Standard Deviation	cc/Kg Body Weight	Standard Deviation	% RBC	Standard Deviation
<u>Dry Jerseys</u>								
"Midwest Cold" (10° to 40°F)	3	12	34.03	2.80	63.20	2.25	45.88	3.65
"Midwest Normal" (40° to 70°F)	3	12	36.64	3.67	62.01	5.05	40.88	3.41
"Imperial Valley" (60° to 110°F)	3	9	40.16	3.95	68.19	11.87	40.72	3.59
"Midwest Hot" (70° to 100°F)	3	9	39.00	4.50	61.19	5.05	36.32	4.34
<u>Lactating Jerseys</u>								
"Midwest Cold" (10° to 40°F)	2	7	44.23	0.60	66.41	3.80	33.20	2.49
"Midwest Normal" (40° to 70°F)	2	8	47.06	4.67	68.84	7.00	31.56	2.65
"Imperial Valley" (60° to 110°F)	3	5	49.20	7.44	72.82	9.09	32.56	3.33
"Midwest Hot" (70° to 100°F)	3	7	44.49	8.60	70.19	10.91	36.90	2.62
<u>Lactating Holsteins</u>								
"Midwest Cold" (10° to 40°F)	3	9	49.17	2.32	75.69	3.34	35.18	1.66
"Midwest Normal" (40° to 70°F)	3	12	54.49	5.65	84.38	9.76	35.30	2.00
"Imperial Valley" (60° to 110°F)	3	5	58.40	6.64	91.56	7.28	36.32	3.54
"Midwest Hot" (70° to 100°F)	2	6	55.50	3.55	85.22	4.38	34.65	1.38

$$*\text{Standard deviation} = \sqrt{\frac{\sum x^2}{n-1}}$$

$$\sum x^2 = \sum X^2 - (\sum X)^2/n$$

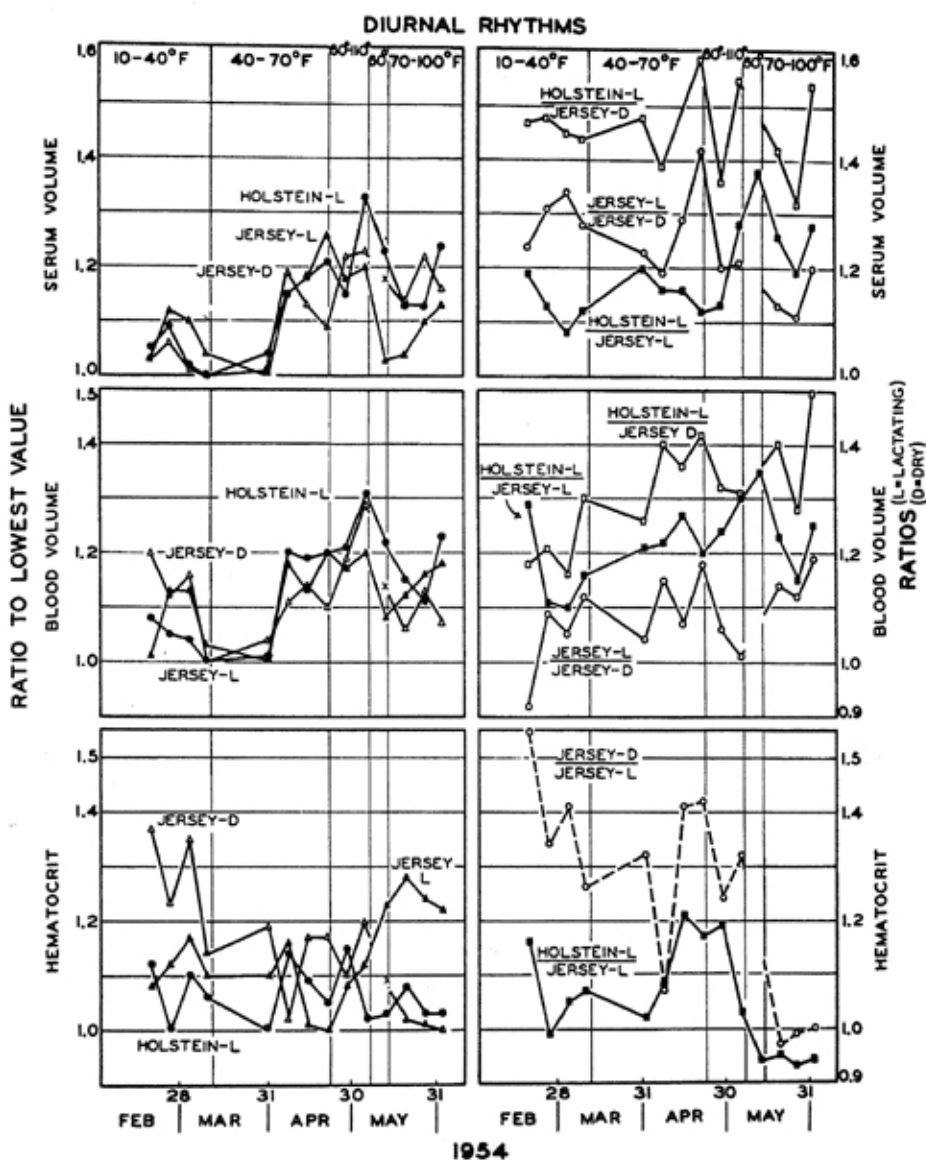


Fig. 2—The same data as in Fig. 1 also plotted on an arithlog grid, but in terms of ratios of each value to the lowest-level value (left side). These curves show that the blood and serum volumes were 1.3 times as great at the 60°-110° F than at the 10°-40° F and 40°-70° F level. It also illustrates the presence of a lag: the values during March-April (40°-70° F cycle) seem to reflect the reactions during the (preceding) 10°-40° F cycle in February. The right side of Fig. 2 represents the ratios of the Holstein to the Jersey and lactating (L) to dry (D) values. The Holsteins have higher water and serum volumes than the Jerseys.

As outlined in the introduction, based on the recent literature on man, volume and distribution of blood and body water are regulated by many homeostatic mechanisms, kidneys, posterior pituitary, adrenal cortex, hypothalamus, and hydrostatic and osmotic factors which translocate water across the capillary membranes.

Figures 1 and 2 and Tables 1 to 5 (Tables 2 to 5 in Appendix) reflect the effects particularly of the following three factors.

Effect of Temperature: Rising environmental temperature was associated with increased plasma and blood volumes in all the three cattle categories. As suggested by Bazett *et al.* (1940), this increased blood volume is probably related to the vascularity of the skin. The closing of arterio-venous anastomoses, with resultant greater capillary circulation, increases the total capacity of the vascular system; blood pressure falls and fluid is moved from the tissue spaces to the blood. Yet the pulse rate of cows usually declines until the environmental temperature surpasses 100° F and a slow pulse is usually associated with a higher than normal blood pressure. Other factors may be involved; yet, our measurements of percent serum water (see antipyrine section) indicate no appreciable change in total serum solids and therefore, presumably, no appreciable change in the protein osmotic pressure of the blood plasma. The other factor which would move fluid from the tissue spaces to the blood, the hydrostatic pressure of the tissue fluid, was not studied.

The hematocrit for lactating Jerseys and especially for lactating Holsteins remained relatively constant at all environmental conditions; apparently, the RBCs increased almost in proportion with serum volume. Our dry Jerseys, however, showed a decreasing hematocrit with increasing environmental temperature; the total volume of RBCs remained nearly constant. The stimulus to hematopoiesis is anoxia; perhaps the higher oxygen consumption of lactating cows is responsible for the difference in hematocrits between dry and lactating animals as the environmental temperature increases.

The increase in both serum volume and blood volume with rising temperature was greater for lactating Holsteins than for lactating Jerseys. This may be related to the greater difficulty of heat dissipation because of the lower body surface per unit body weight in Holsteins than Jerseys.

Effect of Lactation: The mammary gland is more vascular than other tissues of lower metabolic activity; yet it seems improbable that this alone could account for the serum volume difference between our dry and lactating Jerseys, a difference of 5 liters in a 1000-pound cow. It is more probable that the increased feed consumption and increased metabolism associated with lactation increases the vascularity of all of the visceral organs. In this respect the working (lactating) cow differs from working man. At high environmental temperatures working man does not volun-

rarily drink enough water to replace his losses, the resultant dehydration reduces plasma water about 2.5 as much as other compartments of body water and leads ultimately to the circulatory failure of heat exhaustion (Adolph, 1947). Apparently cows become hydrated instead of dehydrated when allowed free access to drinking water at high temperatures.

Effect of Breed: Lactating Holsteins had larger serum and blood volumes in proportion to body weight than lactating Jerseys. This breed difference was particularly striking during the 60° to 110° F diurnal temperature rhythm where it amounted to almost 20 cc of blood per kilogram of body weight, or more than 2 gallons in a 1000-pound cow. This mechanism of the breed difference in exchange of water between tissues and capillaries needs investigation.

TOTAL BODY WATER

Total body water, determined by the dilution volume of antipyrine, ranged between 45 and 75 percent of body weight. Repeated at weekly intervals, our results on any one animal were so inconsistent as to be worthless (Fig. 3). There was no correlation between the antipyrine space and the environmental temperature.

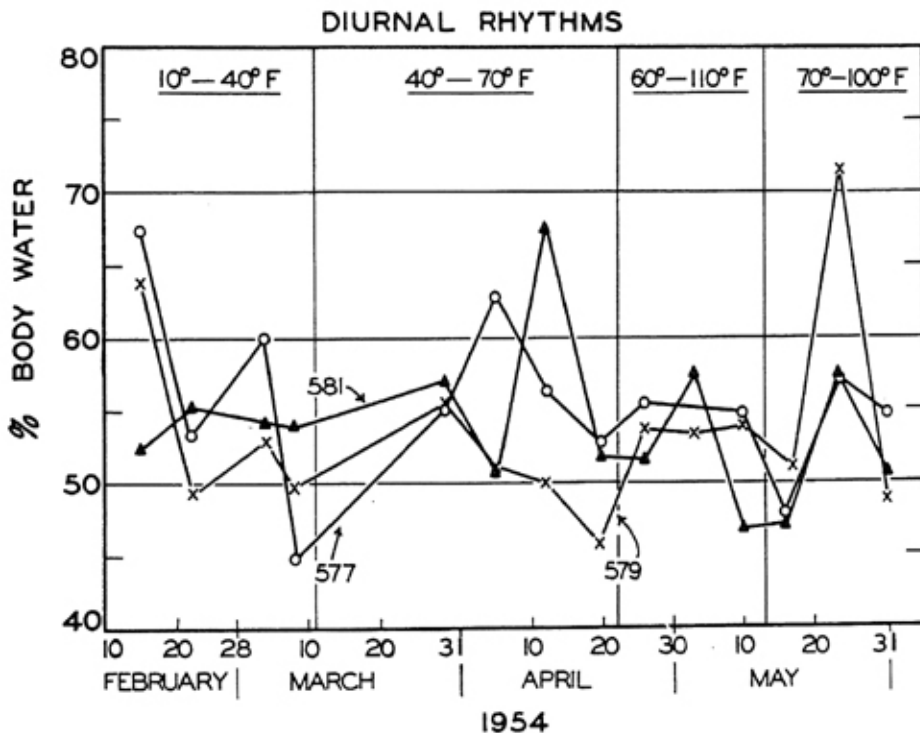


Fig. 3—Antipyrine space ("total body water") as percentage of body weight.

In all three cows, the rate at which antipyrine disappeared from the blood stream was quite variable (Fig. 4). In two of the three cows, the rate of disappearance varied with the environmental temperature and/or with the repeated administration of antipyrine (Fig. 5). In cow 577 a pronounced decrease in the rate of disappearance of antipyrine was associated

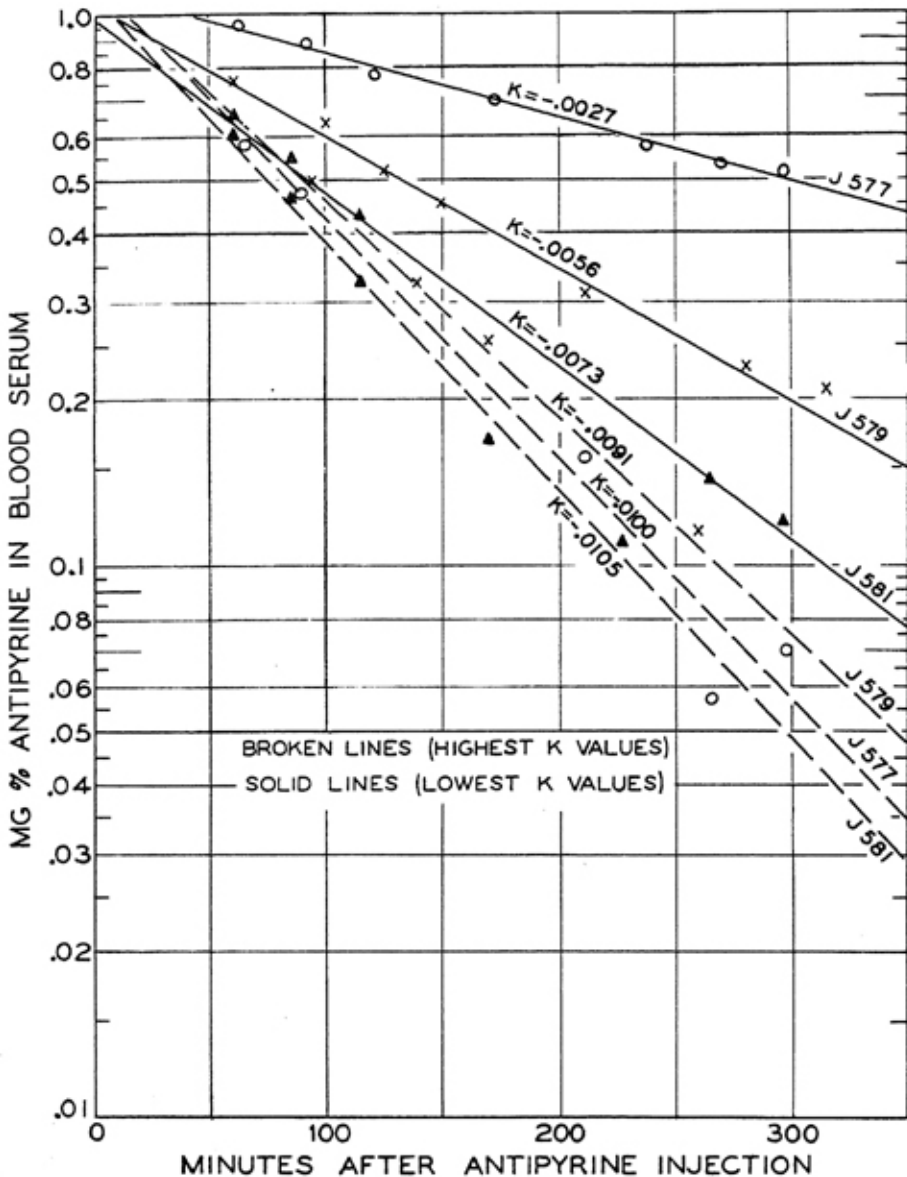


Fig. 4—Decline in blood antipyrine concentration with time after injection in different cows and in several experiments plotted on an arithlog grid. 100 k is the percentage decline. Thus the declines in blood antipyrine in Jersey cow 579 ranged from .56 to .91 percent per minute at various times.

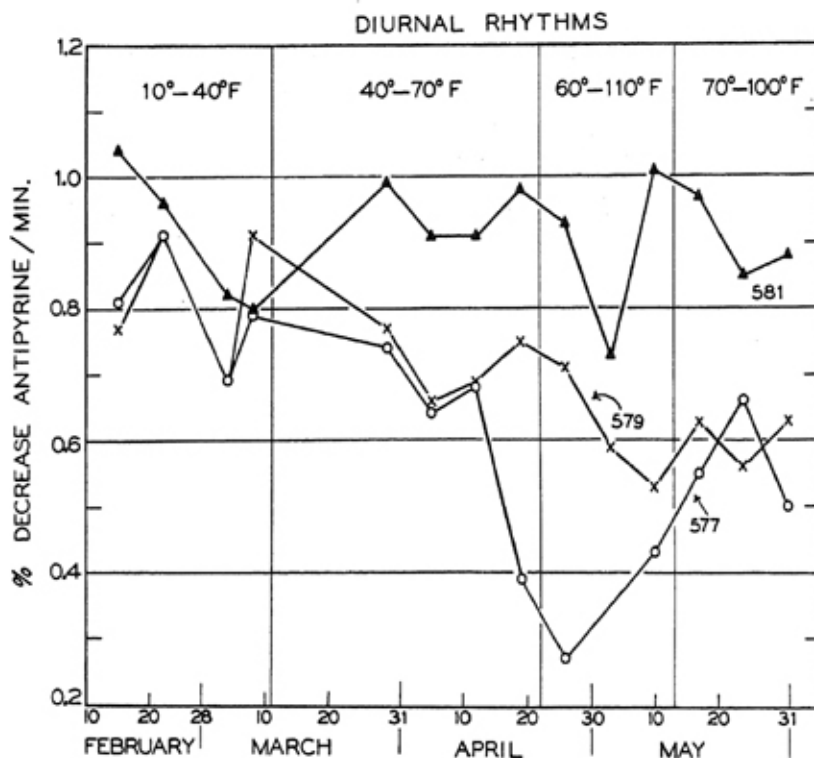


Fig. 5—Percentage decline of antipyrine during each of the four diurnal cycles. The percentage decline was obtained by plotting the antipyrine against time on semilog paper (Fig. 4) and computing the slope, k , in equation $y = ae^{kt}$ in which y is concentration antipyrine, t is time in minutes, and $100k$ is the percentage decrease antipyrine per minute.

with a blank value of antipyrine high enough to justify stopping the determination for two weeks. We cannot as yet interpret this change in the rate of disappearance of antipyrine from the blood. The metabolism of antipyrine is slow in man but rapid in dogs and rabbits. About 5 percent of the administered antipyrine is excreted unchanged in the urine; 30 to 40 percent is oxidized to 4-hydroxyantipyrine then conjugated with glucuronic and perhaps sulfuric acids and excreted in the urine; the fate of the remaining 60 percent is unknown (Goodman and Gilman, 1955). It is possible that the pyrazolon ring of the remaining 60 percent is broken up during its metabolism; it is possible that the change in the rate of disappearance of antipyrine is associated with an impairment of liver or kidney function. We found no correlation between the rate of disappearance of antipyrine and the volume of distribution of antipyrine.

Because of the relationship to the exchange of fluid through the capillary wall, the percent serum water used in calculating total body

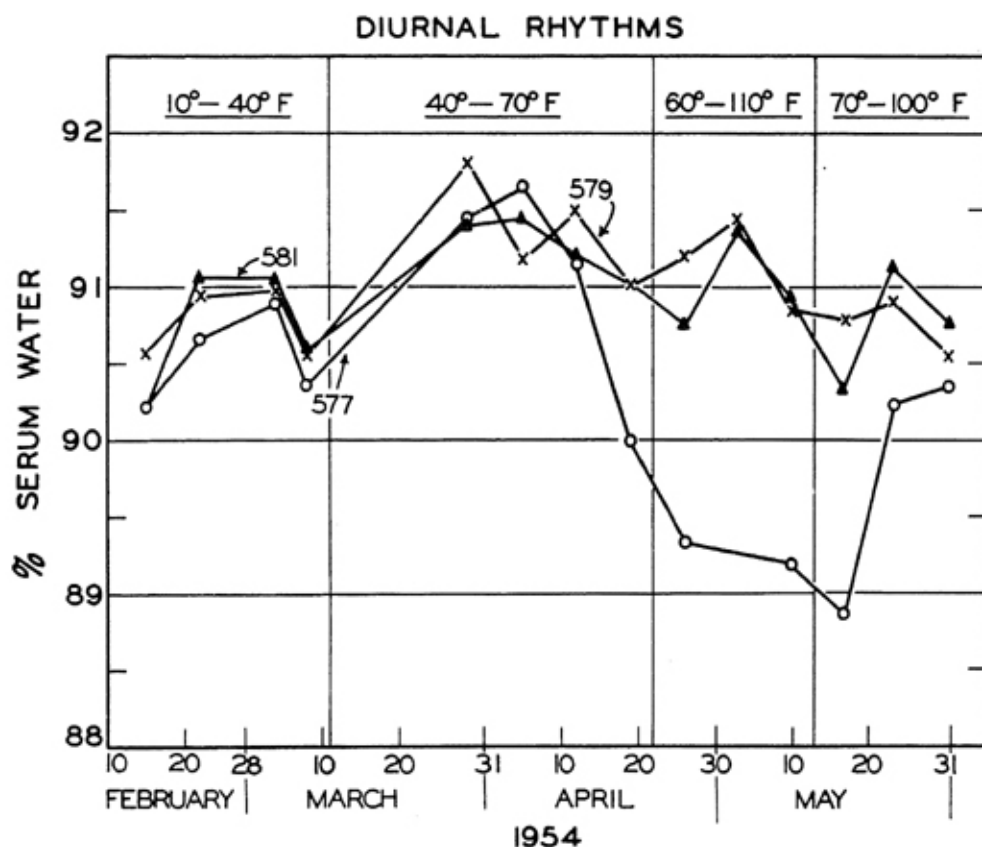


Fig. 6—Percentage serum water determined by drying to constant weight. Total solid (100 - % serum water) and presumably protein osmotic pressure remained relatively constant except in cow 577.

water is plotted in Figure 6. There was no consistent change with the different environmental conditions, although in cow 577 the decrease in percent serum water was remarkably similar to the decrease in the rate of metabolism of antipyrine (Fig. 5).

SUMMARY

Serum and blood volumes were estimated with the T-1824 hematocrit method in three dry and three lactating Jersey cows and in three lactating Holstein cows. Total body water was estimated in the dry Jerseys with the antipyrine dilution technique. All cows were exposed to each of four diurnally-variable temperature rhythms: 10° to 40° F; 40° to 70° F; 60° to 110° F; and 70° to 100° F.

All the cows had increased serum and blood volumes at the higher temperature ranges; this was most noticeable in the lactating Holstein

cows with a difference of 9 ml of plasma and 16 ml of blood per Kg of body weight between the 10° to 40° F rhythm and the 60° to 110° F rhythm. In lactating Jerseys cows, the serum volumes were 5 to 10 and the blood volumes 3 to 9 ml per Kg body weight greater than were the serum and blood volumes of dry Jerseys cows under the same conditions. In lactating Holstein cows the serum volumes were 5 to 11 and the blood volumes 9 to 19 ml per Kg of body weight greater than were the serum and blood volumes of lactating Jersey cows under the same conditions. The hematocrit remained relatively constant except in the dry Jersey cows where it decreased from 45.8 percent RBC at the 10° to 40° F rhythm to 36.2 percent RBC at the 70° to 100° F rhythm.

The antipyrine space varied between 45 and 75 percent of body weight; consecutive determinations on any one cow were very inconsistent. In two of our animals the rate of metabolism of antipyrine was found to decrease at the higher temperature rhythms (or possibly with increasing repetition of administration of antipyrine). Serum water ranged between 91.68 and 88.85 percent; there was no consistent change with the different temperature rhythms.

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TABLE 2 -- BLOOD AND SERUM VOLUME

Temperature Condition	Serum Volume	Blood Volume	Hematocrit	Serum Volume	Blood Volume	Hematocrit	Serum Volume	Blood Volume	Hematocrit
	cc/Kg Body Wt.	cc/Kg Body Wt.	% RBC	cc/Kg Body Wt.	cc/Kg Body Wt.	% RBC	cc/Kg Body Wt.	cc/Kg Body Wt.	% RBC
	<u>Holstein 178 (Lac.)</u>			<u>Holstein 144 (Lac.)</u>			<u>Holstein 184 (Lac.)</u>		
"Midwest Cold" (10° to 40°F)	50.3	79.6	36.8	45.9	71.7	36.0	53.9	79.0	33.0
	50.0	74.7	33.1	48.4	72.9	33.6	51.5	78.1	35.5
	48.9	78.6	37.6				49.4	75.8	34.8
	45.2	70.8	36.2						
"Midwest Normal" (40° to 70°F)	50.8	76.7	33.8	55.2	81.9	32.6	42.5	63.3	32.8
	48.6	77.7	37.4	50.9	80.4	36.7	65.1	105.5	38.4
	53.0	83.0	36.9	56.4	86.5	34.8	58.8	92.6	36.5
	55.6	87.7	36.6	58.8	88.8	33.8	58.8	88.2	33.3
"Imperial Valley" (60° to 110°F)	48.9	81.4	39.9	54.4	91.0	40.2	61.6	93.8	34.0
	58.4	90.0	35.1	68.6	101.6	32.4			
"Midwest Hot" (70° to 100°F)	54.9	84.4	34.9	52.7	83.4	36.8			
	50.9	78.7	35.3	56.8	84.5	32.8			
	60.1	90.6	33.7	58.8	89.7	34.4			
	<u>Jersey 274 (Lac.)</u>			<u>Jersey 564 (Lac.)</u>			<u>Jersey 310 (Lac.)</u>		
"Midwest Cold" (10° to 40°F)	45.0	68.6	34.3	42.2	61.8	31.8			
	43.9	70.3	37.6	46.9	69.3	32.2			
	43.1	66.1	34.7	46.3	68.3	31.5			
"Midwest Normal" (40° to 70°F)				42.2	60.0	30.3			
	42.5	64.7	34.3	39.7	57.6	31.0			
	49.5	77.0	35.7	44.9	66.9	32.9			
	49.7	70.5	29.4	47.1	67.4	30.2			
"Imperial Valley" (60° to 110°F)	54.7	79.8	31.4	48.4	66.8	27.6			
	54.6	80.9	32.5	42.6	62.2	31.5	39.8	64.4	38.1
	55.9	81.6	31.5	53.1	75.0	29.2			
"Midwest Hot" (70° to 100°F)	49.6	75.6	34.4	43.3	70.6	38.7	35.3	58.8	39.9
	52.8	82.2	35.7				37.5	60.1	37.6
	56.6	84.3	32.9				36.3	59.7	39.1
	<u>Jersey 577 (Dry)</u>			<u>Jersey 581 (Dry)</u>			<u>Jersey 579 (Dry)</u>		
"Midwest Cold" (10° to 40°F)	33.2	67.0	50.5	37.4	73.9	49.3	31.8	61.3	48.1
	35.7	65.7	45.6	36.2	66.0	45.2	33.5	58.2	42.4
	36.5	73.1	50.1	34.2	65.9	48.1	30.2	58.0	47.9
	34.6	59.6	42.0	36.8	62.0	40.6	28.3	47.7	40.8
"Midwest Normal" (40° to 70°F)	35.0	58.4	40.0	34.2	60.5	43.4	31.3	57.2	45.3
	38.6	59.9	35.5	43.4	71.3	39.1	36.6	56.7	35.3
	39.8	65.8	39.6	39.7	68.1	41.6	32.9	59.7	44.8
	38.3	62.4	38.6	38.7	67.8	43.8	31.8	56.3	43.5
"Imperial Valley" (60° to 110°F)	43.1	71.2	39.5	42.0	72.9	42.4	36.1	57.7	37.5
	46.0	88.0	47.8	40.0	71.6	44.1	36.6	59.1	38.0
	42.7	67.9	37.1	41.2	70.6	41.7	33.7	54.7	38.4
"Midwest Hot" (70° to 100°F)	42.5	63.5	33.0	37.4	63.2	40.9	33.5	52.4	36.3
	47.1	68.0	30.8	39.7	66.1	40.0	35.1	56.7	38.0
	42.9	60.5	29.1	38.2	63.6	40.0	34.7	56.7	38.8

TABLE 3 -- RESULTS OF ANALYSIS OF VARIANCE
(These values were computed on the basis of a two criteria
and disproportionate frequency analysis.)

Analysis of Variance Criteria	Source of Variation	"F" Values and Significance		
		Serum Volume	Blood Volume	Hematocrit % Cells
Breed and Climatic Condition	Among Breeds	11.22 sig at 1%	13.94 sig at 1%	6.4337 sig at 5%
	Among Climatic Conditions	9.867 sig at 1%	15.84 sig at 1%	2.4492 not sig
	Interaction	1.3115 not sig	1.1529 not sig	41.98 sig at 1%
Lactation Status and Climatic Condition	Among Lactation Status	6.221 sig at 5%	6.077 sig at 5%	3.1304 not sig
	Among Climatic Conditions	10.67 sig at 1%	3.431 sig at 5%	8.0439 sig at 1%
	Interaction	2.318 not sig	2.118 not sig	3.5225 not sig

Note: Analysis of variance method taken from, "Calculation and Interpretation of Analysis of Variance and Covariance", Snedecor, G. W., Collegiate Press Inc., Ames, Iowa, 1934, 96 pp.

TABLE 4 -- MEANS USED IN COMPUTING THE SIGNIFICANCE OF THE
DIFFERENCE BETWEEN CLIMATIC CONDITIONS BY THE "t" TEST

Analysis of Variance Criteria	Temperature Condition	Means		
		Serum Volume	Blood Volume	Hematocrit % Cells
Breed and Climatic Condition	"Midwest Cold" (10° to 40°F)	47.0063	71.6313	34.3125
	"Midwest Normal" (40° to 70°F)	51.5200	78.1600	33.8050
	"Imperial Valley" (60° to 110°F)	53.7900	82.1900	34.4400
	"Midwest Hot" (70° to 100°F)	49.6615	77.1231	35.8615
Lactation Status and Climatic Condition	"Midwest Cold" (10° to 40°F)	37.7894	64.3842	41.2105
	"Midwest Normal" (40° to 70°F)	40.8100	64.3898	37.1500
	"Imperial Valley" (60° to 110°F)	43.3857	69.8429	37.8071
	"Midwest Hot" (70° to 100°F)	41.3563	65.2500	36.5750

TABLE 5 -- DIFFERENCE BETWEEN MEANS TESTED BY "t" TEST FOR SIGNIFICANCE OF DIFFERENCE

Analysis of Variance Criteria	Temperature Condition	Mean Difference		
		Serum Volume cc/Kg Body Wt.	Blood Volume cc/Kg Body Wt.	Hematocrit % Cells
Breed and Climatic Condition	(10-40°F) - (40-70°F)	4.5137*	6.5287*	.5065
	(10-40°F) - (60-100°F)	6.7837*	10.5587*	.1275
Lactation Status and Climatic	(10-40°F) - (70-100°F)	2.6553	5.4918	1.5490
	(40-70°F) - (60-110°F)	2.2700	4.0800	.6350
	(40-70°F) - (70-100°F)	1.8585	1.0369	2.0565*
	(60-110°F) - (70-100°F)	4.1285	5.0669	1.4215
	(10-40°F) - (40-70°F)	3.0206	.3608	4.0605
	(10-40°F) - (60-110°F)	5.5963*	5.4586*	3.4034
Lactation Status and Climatic	(10-40°F) - (70-100°F)	3.5668	.7408	4.6355*
	(40-70°F) - (60-110°F)	2.5757	5.0978	.6571
	(40-70°F) - (70-100°F)	.5462	.3800	.5750
	(60-110°F) - (70-100°F)	2.0295	4.7178	1.2321

*Significant at 5% level.

Note: Method of testing the differences was adapted from "Calculation and Interpretation of Analysis of Variance and Covariance", Snedecor, G. W., Collegiate Press Inc., Ames, Iowa, 1934, 96 pp.