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Environmental Physiology and Shelter Engineering

With Special Reference to Domestic Animals

XXX. Thermal Stress and Acid-Base Balance in Dairy Cattle

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Missouri Agricultural Experiment Station and the United States Department of Agriculture Cooperating

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INTRODUCTION

The CO_2-combining capacity, or alkali reserve, of the blood plasma of the cows in the Climatic Chambers remained roughly constant[1]* (about 60 Vol. %) between temperatures 40° and 85°F, and decreased sharply with further increase in temperature[1]* (to about 30 Vol. %).

The decline in the alkali reserve with rising temperature could reflect a *metabolic acidosis:*[2] The feed consumption was sharply reduced with increasing temperature with resulting dietary carbohydrate reduction and increased body-fat oxidation, thus possibly leading to increased production of acetoacetic, B-hydroxybutric and related acids, which would constitute metabolic acidosis.

The decline in alkali reserve with rising temperature could, however, also reflect a *respiratory alkalosis:*[3] The hyperventilation associated with panting blows off the CO_2 and reduces the H_2CO_3 to $BHCO_3$ ratio, followed by a compensatory increase in $BHCO_3$ excretion, and therefore is followed by a lowered CO_2-combining capacity. The blood pH could decrease or increase, depending on the relative decline in the H_2CO_3 and $BHCO_3$ levels.

Which of these two interpretations represents the situation? Are we dealing with a metabolic acidosis or a respiratory alkalosis? This bulletin reports an attempt to clarify the situation by a study of changes in the metabolic pattern of the major cations and anions, along with changes in the blood pH and CO_2-combining power, with changing environmental temperature.

DEFINITIONS AND THEORY

Acid-Base Balance vs. Blood Reaction. Acid-base balance properly refers to the balance between all anions and cations. The blood reaction

*See Literature Cited, page 27.

refers to its pH. The relation between the pH and acid-base balance is conventionally expressed by the Henderson-Hasselbalch equation.[4]

$$pH = pK + \log \frac{BHCO_3}{H_2CO_3}.$$

The simultaneous determination of any two of these three variables in the blood is said to fix the third and so defines its state of the acid-base balance.

Acidosis and Alkalosis. Acidosis was first used in 1906 by Naunyn as a synonym for ketosis; in 1917 it was used to designate a depressed blood bicarbonate level.[5] Currently, acidosis has come to mean a decreased blood pH. Uncontrolled diabetic acidosis is characterized by all these features: ketosis, depressed bicarbonate, and low pH. Conditions are, however, encountered in which pH, bicarbonate and ketone bodies do not vary together. Thus, at high temperatures in our cows, the bicarbonates were depressed, the pH elevated, and ketone bodies remained unaltered. Hence, the designations of *respiratory alkalosis,* as occurred in our cows, and *metabolic acidosis,* as occurs in uncontrolled diabetes or during starvation when the pH declines along with the decline in $BHCO_3$. Peters and Van Slyke[2] also characterized the acid-base balance of the blood by "CO_2 excess or deficit" or by "alkali excess or deficit" and by qualifying prefixes "compensated" or "uncompensated."

Some measurements were taken to supplement the Henderson-Hasselbalch variables, including total ketone bodies in blood and urine, the major anions and cations, the CO_2 *content* of arterialized and venous plasma and the CO_2-*combining power* of venous plasma.

MATERIALS AND METHODS

The plan of this experiment was complicated by the need of synchronizing and adapting it to several other experiments done by others at the same time on the same cows. This, in part, accounts for the non-committal nature of the title—The Thermal Stress—including changes not only in air and wall temperature, but also in radiation intensity. Some of the exposures were of seven days duration and constant light (day and night); some were "acute" exposures of 14 hours duration; some of the exposures were to "variable light" simulating the outdoor diurnal rhythm.

Climatic Chambers. The Climatic Chambers,[6] the lamp installations for controlling thermal stress by radiation,[7] and the animals[7] have already been described. For convenience of reference, Table 1 summarizes some of the pertinent data about the cows and temperature and radiation schedules.

Starvation Experiment. Since decline in feed consumption—as occurred with rising heat stress—may lead to acidosis independently of temperature, one Guernsey and three Holstein cows were subjected during the

1952 summer to a five-day fast at the comfortable temperature of 60°F (16° C) to determine the effect of fasting, i.e., shift to body-fat catabolism, independently of temperature, on acidosis.

Radiation. During the initial phase of 1953, an attempt was made to determine the maximal temperatures that the cows could tolerate. A predominantly white Holstein cow (357) was therefore subjected for 10 hours to a radiation intensity (from lamps) of about 500 kg-cal/m^2/hr while the air temperature was gradually increased from 85° to 105°F. Four predominantly black Holstein cows were similarly subjected for 14 hours to an environmental temperature of 95°F while gradually increasing the radiation intensity from dim light to about 500 kg-cal/m^2/hr. It appeared that the maximal thermal stress the Holsteins could bear at this radiation intensity was an environmental air temperature of 85°F.

Collection of Blood Samples. Venous blood, collected from the jugular vein in heparinized flasks, was used for all analyses and determinations, except when otherwise specified. Blood was collected on the fourth day of the seven-day exposure periods, and during the last hour of the shorter exposure periods. The serial samples, collected at intervals of several hours during the periods of "variable light" and during the "acute" experiments, were obtained from indwelling polyethylene catheters in the jugular vein.

Urine Collections. Urine was collected for approximately 24 hours on the second and third days of the seven-day exposure periods. The volume of a 24-hour sample was estimated from the volume collected during 20 to 28 hours.

Two collection methods were used. During the starvation experiment, a body harness* was used to hold a rubber bag* over the vulva (Fig. 1). A wire screen allowed urine—but not feces—to pass into the bag (Fig. 2). The rubber bags were examined at 30-minute intervals during the period of collection and the urination time was recorded. The urine was stored in large glass jars.

During the light experiments, the urine collection method of Hobbs, Hansard, and Barrick[8] was used. Their apparatus was modified so that the rubber collecting tube was detachable from the straps cemented to the cow (Fig. 3). Gasoline cans (Fig. 3), coated on the inside with a rubberized paint, served as receptacles for the collected urine. The rubber tubes were kept taut between the cow and the can by a lead weight attached to the lower end of the tube inside the can. The shield over the spout of the can was designed to prevent the entrance of feces. While these collectors were in use, the cows were observed only long enough to determine the time of the beginning and end of the urinations.

*Obtained from the Upjohn Richland Farms, Richland, Mich. Fig. 1 shows the Upjohn equipment.

TABLE 1 -- TEMPERATURE AND RADIATION CALENDAR

Weekly Periods 3 P.M. to 3 P.M.		Holsteins			Brahmans & Jerseys		
		Air Temp. °F	Radiation+ Btu/ft²/hr	Relative Humidity, %	Air Temp. °F	Radiation+ Btu/ft²/hr	Relative Humidity, %
1953							
Jan. 9	Jan. 16	46	variable*	66			
Jan. 16	Jan. 22	45	5	65			
Jan. 22	Jan. 29	46	42	60			
Jan. 29	Feb. 5	48	86	62	45	variable*	65
Feb. 5	Feb. 12	46	131	59	45	5	62
Feb. 12	Feb. 19	46	179	54	46	39	53
Feb. 19	Feb. 26	69	variable*	63	46	94	49
Feb. 26	Mar. 5	70	7	63	45	136	51
Mar. 5	Mar. 12	70	44	64	45	190	52
Mar. 12	Mar. 19	70	98	56	69	variable*	62
Mar. 19	Mar. 26	70	135	60	70	5	67
Mar. 26	Apr. 2	71	180	55	70	40	63
Apr. 2	Apr. 9	47-93	irregular**	61	48-67	irregular**	61
Apr. 9	Apr. 16	83	variable*	64	70	82	55
Apr. 16	Apr. 23	80	7	68	70	129	55
Apr. 23	Apr. 30	80	40	69	70	175	51
Apr. 30	May 7	80	95	64	80	variable*	62
May 7	May 14	80	138	58	80	12	69
May 14	May 21	80	161	57	80	40	63
May 21	May 28				80	90	61
May 28	June 4				80	130	56
June 4	June 11				80	156	54

+ Radiation values (net amounts of energy that would be absorbed from overhead by a flat horizontal black surface) were taken 52 inches from the floor along each stall divider. These readings were representative of the average radiation at the stall platform center 36 inches from floor. For convenience, the radiation levels are sometimes referred to as follows:

Level	Btu/ft^2/hr	Kg-cal/m^2/hr
Zero or "visible"	5	14
1/4	40	108
1/2	90	244
3/4	130	352
full	180	488

*Variable period in which the radiation was changed during the day:

5-7 a.m.	5 Btu/ft^2/hr	2-3 p.m.	130 Btu/ft^2/hr
7-8 a.m.	40 Btu/ft^2/hr	3-4 p.m.	90 Btu/ft^2/hr
8-9 a.m.	90 Btu/ft^2/hr	4-5 p.m.	40 Btu/ft^2/hr
9-10 a.m.	130 Btu/ft^2/hr	5-6 p.m.	5 Btu/ft^2/hr
10-2 p.m.	180 Btu/ft^2/hr	6 p.m.-5 a.m.	dark

**Irregular period in which the temperature as well as the radiation were varied to determine the highest temperature at full radiation intensity the Holstein cows would be able to endure for a week without serious consequences.

Fig. 1—Urine collection by the Upjohn method, side and rear views.

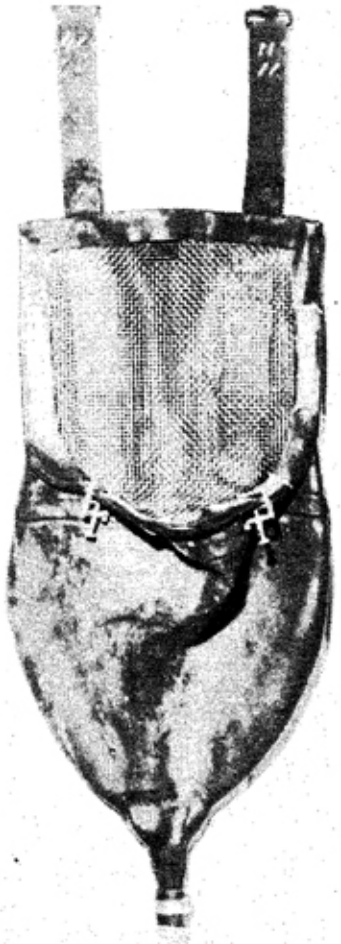

Fig. 2—The Upjohn rubber bag used for urine collection.

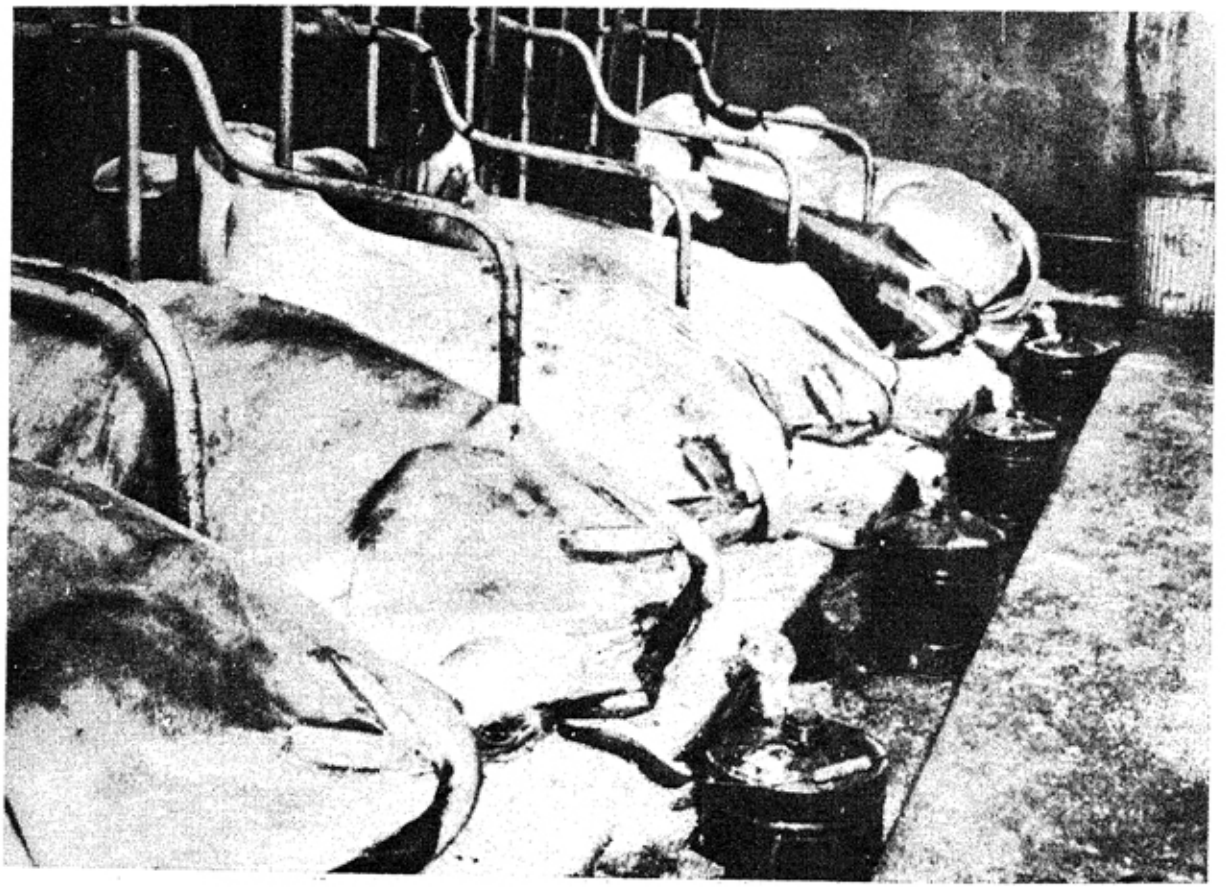

Fig. 3—Urine collection by the Hansard method, modified by attachment of collecting tube to the body strap. Gasoline cans were used as receptacles.

At temperatures above 60°F chloroform was added to the urine as a preservative. At the end of the collection period the weight, temperature, and specific gravity of the mixed urine were recorded, and the urine was sampled for chemical analysis.

Blood pH Determinations. The blood pH was measured according to the procedure of Davenport,[9] employing a Beckman "Model G" pH meter and a Beckman "Blood Type" electrode. This apparatus was checked with buffers of pH 7.4 and pH 4.0 before and after each period of use.

The electrode system was thermostated in a kerosene bath at a temperature of 38.5°C (Fig. 4). Before each determination the electrode assembly of 1 ml. capacity was rinsed and filled with physiological saline solution. Injecting 10 ml. of blood into the electrode assembly sufficed to displace this saline and to flush the electrode jacket.

Blood, obtained with minimum stasis, was aspirated into a 10 ml. heparinized syringe with the least possible negative pressure; exposure to air was negligible throughout the entire procedure. Thirty seconds after the blood was drawn into the syringe it was injected into the electrode assembly, and the pH reading was made 90 seconds later. These time intervals, measured with a stop watch, were designed to avoid the acid shift in shed blood described by Havard and Kerridge.[10]

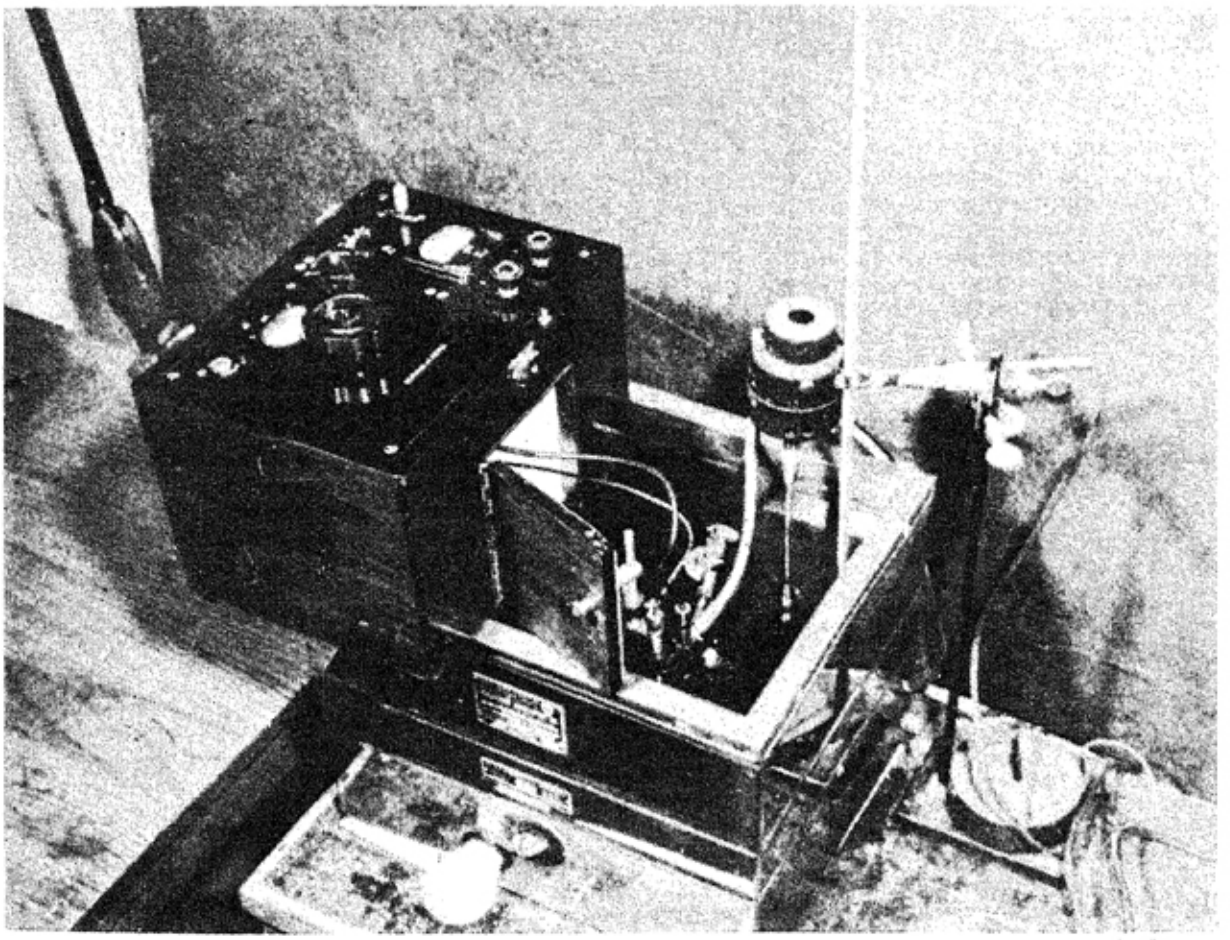

Fig. 4—pH meter, electrode assembly, and kerosene bath.

CO_2 Determinations. Several categories of CO_2 determinations were made: CO_2-*combining capacity* of the blood plasma, measured by the manometric method of Van Slyke and Neil as outlined by Hawk, Oser, and Summerson;[11] CO_2 *content* of venous plasma; CO_2 content of "true" or "arterialized" plasma[11] by the method of Peters and Van Slyke.[11]

The CO_2 content refers to the amount of CO_2 that can be extracted from the plasma as it is collected. Blood for the CO_2 content determinations was collected in oxalated tubes under oil and handled with minimum agitation and air exposure. Venous plasma was obtained from blood centrifuged in the collecting tube. "Arterialized" plasma was obtained from whole blood saturated with normal human alveolar air at 38.5°C, following which it was centrifuged under oil and the plasma CO_2 content determined.

Ketone Body Determinations. The method of Greenberg and Lester[12] was used for the determination of total ketone bodies in blood and urine. As suggested by Sargent,[13] this method was modified by replacing the elaborate refluxing tube with a small, rubber capped bottle (Fig. 5). The protein-free filtrate and acid dichromate solution were pipetted into this bottle, which was then tightly stoppered and placed in a boiling water bath for 10 minutes. The bottles were then cooled, 10 percent dichromate solution injected through the rubber stoppers with a 26 gauge needle, and the bottles again boiled for 10 minutes. After cooling, 3 ml. of the fluid in each

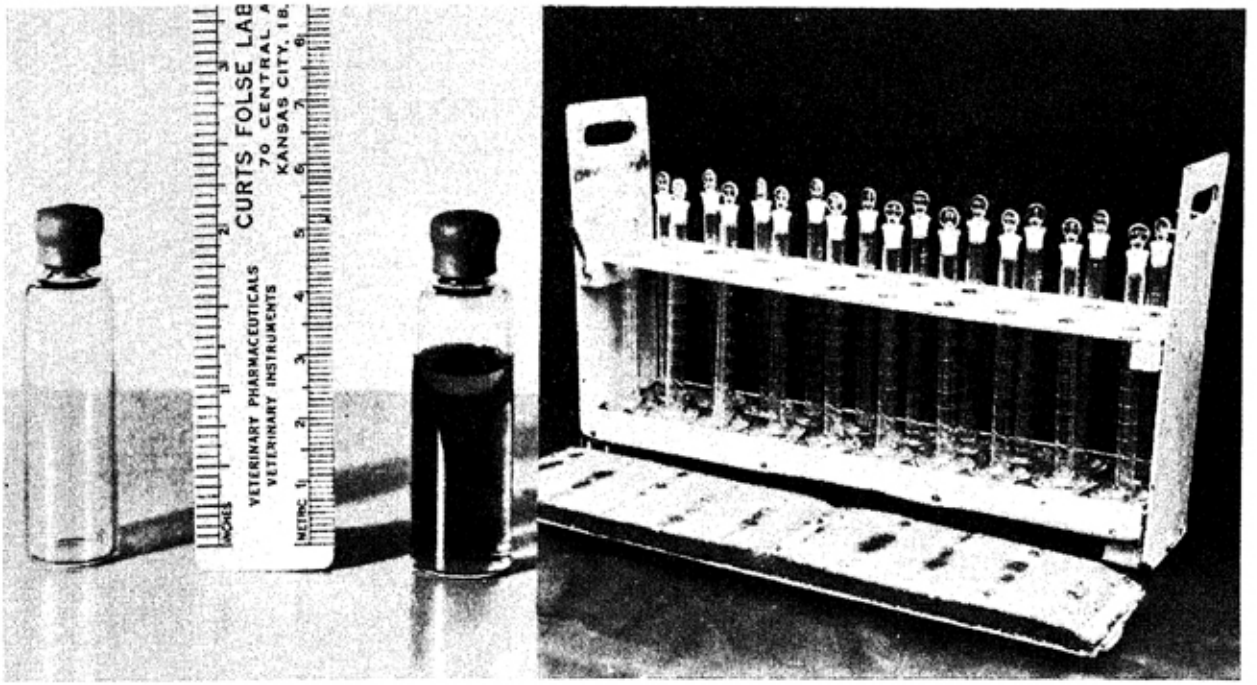

Fig. 5—Bottle used in place of refluxing tube for ketone body estimation, and rack with cylinders.

bottle was removed with a 5 ml. syringe for processing.[12] For uniformity, the stoppered cylinders were shaken with the rack shown in Fig. 5.

Major Anion and Cation Analysis. Samples of the blood plasma and urine were analyzed for sodium, potassium, calcium, magnesium, chloride, phosphate, and sulfate and also, in the urine only, for carbonate. These data will be presented at a later date.

RESULTS ON 7-DAY EXPOSURE TO CONSTANT LIGHT OF DIFFERENT INTENSITIES

CO_2-Combining Capacity. Fig. 6 shows that in dim light (absence of thermal stress from the light), increasing air temperature depressed the CO_2-capacity to a greater extent in the large Holsteins than in the smaller Jerseys and Brahmans. At maximal radiation (heat stress), however, increasing air temperature to 80°F induced the maximal decline in CO_2 capacity in all breeds, so that breed differences in their reaction disappeared.

CO_2 Content. Fig. 7 and Table 2 show considerable variation between the CO_2 content of "arterialized" and venous plasma, depending on the outdoor temperature (Table 2). In the absence of thermal stress (at 45°F under dim light), the blood plasma *lost* about 3.1 volumes percent CO_2 during arterialization. This is approximately the normal difference between arterial and venous blood and shows that the CO_2 tension in this venous blood was higher than the CO_2 tension in normal human alveolar air. But when the cows were exposed to 80°F and full light intensity, the blood plasma *gained,* on the average, 3 volumes percent CO_2 during the process of arterialization, showing that the CO_2 tension of this venous blood was lower than that of normal human alveolar air. This reversal of the normal

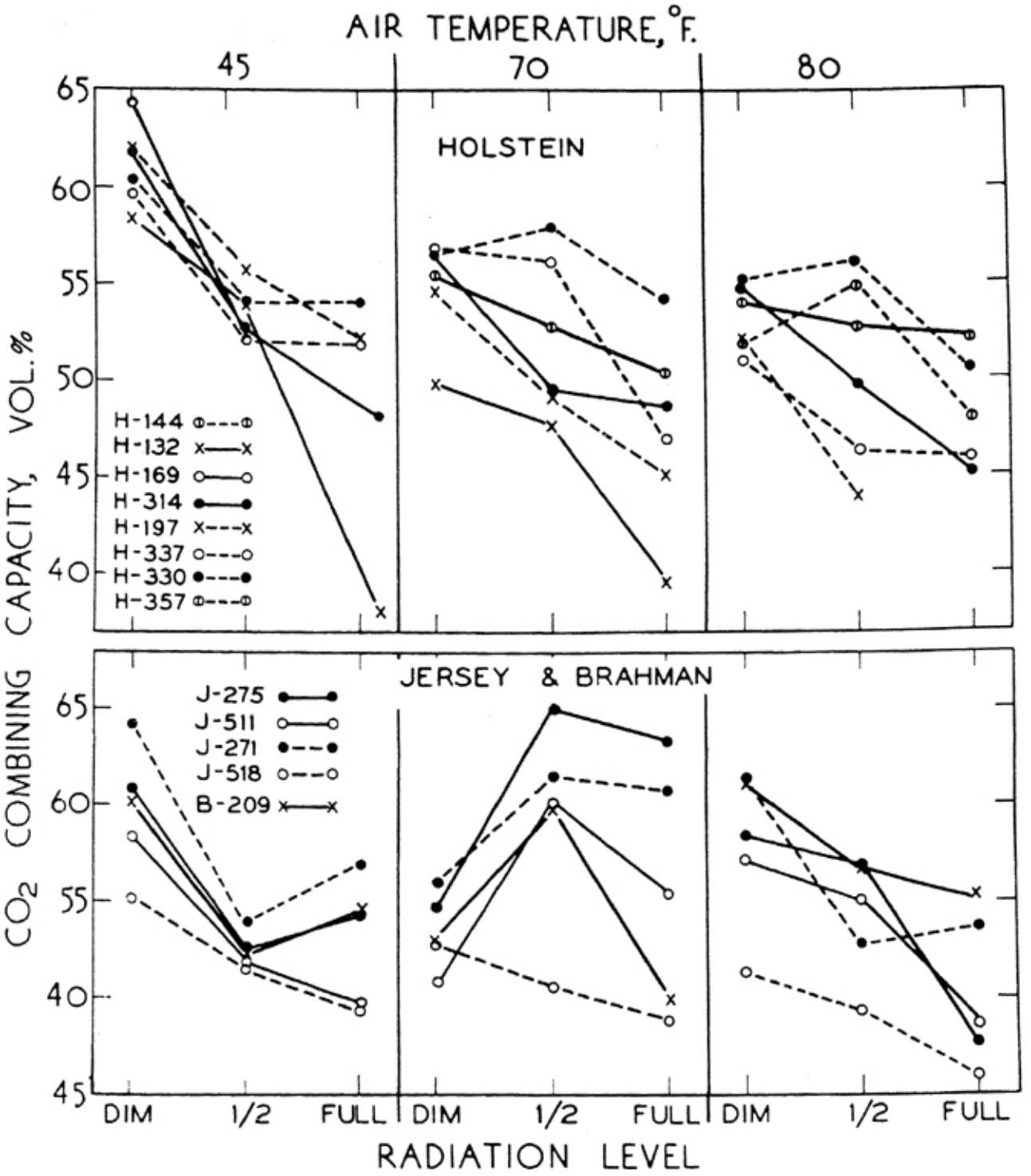

Fig. 6—CO_2 combining capacity as function of radiation level (500 kg-cal/m²/hr. is "full" radiation) at 45°, 70° and 80°F air temperature levels.

relationship between the partial pressure of CO_2 in venous blood and the partial pressure of CO_2 in alveolar air is attributed to the "washing out" effect of the thermal hyperpnea (panting), affecting first the alveolar air and arterial blood and ultimately the tissues and venous blood. This reversal of the normal relationship also indicates that the 50 percent decrease

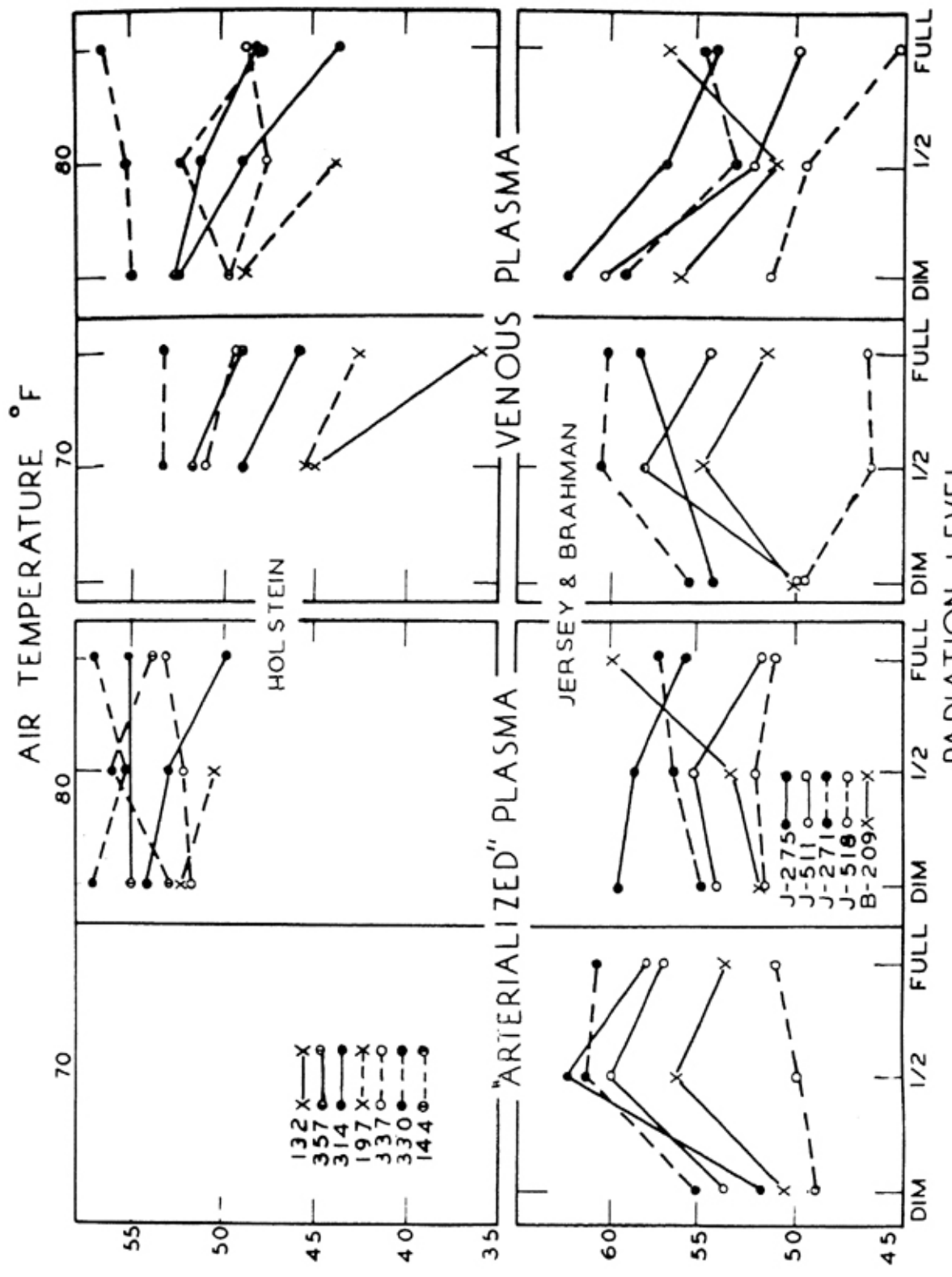

Fig. 7—CO_2 content of "arterialized" plasma and venous plasma as function of radiation intensity level at four air temperature levels.

TABLE 2 -- COMPARISON OF CO_2 CONTENT OF VENOUS PLASMA WITH CO_2 CAPACITY OF VENOUS PLASMA AND CO_2 CONTENT OF ARTERIALIZED PLASMA
(Mean Values)

Climatic Laboratory °F Light	Mean Outdoor Dairy Temp.*	CO_2 Content Venous Plasma (vol. %)	CO_2 Content Arterialized Plasma**	CO_2 Capacity** Venous Plasma
		Jersey and Brahman		
45 dim	49	55.0	-3.1	+1.8
70 dim	50, 39, 39	52.0	+0.1	+1.5
70 ½	44	56.9	+1.1	+2.5
70 full	51, 70	54.2	+2.0	+0.5
80 dim	62	58.0	-3.5	-0.1
80 ½	82	52.5	+2.7	+1.7
80 full	84	52.2	+3.0	-1.9
		Holstein		
45 dim	49	53.2	-0.3	+1.8
70 ½	46, 54, 53	48.4		+2.4
70 full	54	46.0	+2.8	+1.5
80 dim	42, 56	51.5	+2.5	+1.7
80 ½	52, 62	49.8	+3.6	+0.9
80 full	64, 71	48.9	+3.9	-0.5

*U. S. Weather Bureau.
**Difference in volumes percent from CO_2 content of venous plasma.

in CO_2-capacity previously observed at high temperatures is associated with a *primary CO_2 deficit* or *respiratory alkalosis.*

The differences between the CO_2 *content* and the CO_2 *capacity* of venous blood are apparently partly dependent on the outdoor daily temperature (as recorded by the U. S. Weather Bureau at the Columbia Airport, two miles north of the laboratory) and, therefore, on the temperature of the analytical laboratory. For example, when the mean daily Weather Bureau temperature was 44°F, the plasma gained, on the average, 2.5 volumes percent CO_2 in the process of saturation; when the mean daily temperature was 84°F the plasma lost, on the average, 1.9 volumes percent CO_2 in the process of saturation. The consistency of this relationship suggested that changes in outside temperature led to changes in the temperature of the analytical laboratory, and that changes in the temperature of the analytical laboratory altered the plasma CO_2 capacity.

The general schedule of operations in the Climatic Laboratory has been to have low temperatures in the winter and high temperatures in the summer. The winter-summer differences in the temperature of the analytical laboratory would, on the basis of the above evidence, account for a 5 volumes percent change in the CO_2-combining capacity of the venous blood plasma. If, however, whole blood were allowed to stand at room temperature for an appreciable length of time before centrifugation, this same factor would cause a decrease in the CO_2 capacity several times the magnitude of

that actually demonstrated above; since, in whole blood, a decrease in H_2CO_3 entails a simultaneous decrease in $BHCO_3$.

Blood pH. Table 3 shows an increase in blood pH with increase in thermal stress, apparently caused by the pulmonary over-ventilation (panting) associated with the stress. The increase in blood pH along with the decrease in blood bicarbonate confirms the condition as *respiratory alkalosis.*

The magnitude of pH changes reported herein is small, perhaps reflecting acclimatization or a compensation of the blood pH after continued exposure to thermal stress. For example, the pH data reported under "Acute Experiments" (next section) show a larger change after four hours of exposure to 95°F and full light intensity than after four days of exposure to 80°F and full light intensity. Although four hours at maximal light intensity at 95°F may be more of a strain than four days of 80°F, a comparison of changes in the blood bicarbonate indicates the reverse to be true. The chronic stress imposed in the experiment shown in Table 3 either does not produce abrupt changes in blood pH or else time is allowed for the abrupt changes to be followed by a gradual return to values more nearly normal.

Although the pH changes reported herein are small, they have added significance because the measurements were made on venous blood. Arterial blood is saturated with CO_2 at the partial pressure of CO_2 in alveolar air; the pH of arterial blood changes simultaneously with changes in alveolar CO_2. The pH of venous blood, however, is slower to change. After passing through tissue capillaries, the pH of the blood is determined by the CO_2 tension of the interstitial fluid which, in turn, is affected by the CO_2 tension within the cells.

Blood accounts for about 7 percent of body weight, interstitial fluid for about 15 percent and intracellular fluid for about 50 percent of body weight. For alveolar CO_2 tension to change the pH of venous blood, a volume of fluid at least two times and possibly nine times that of blood volume must first undergo the same pH change.

Ketone Bodies in Blood and Urine. A voluminous literature indicates that when carbohydrate oxidation is greatly deficient, as during prolonged starvation in normal individuals, or in severe uncontrolled diabetics, there is an acceleration of fat catabolism which leaves some of the fatty acid in incompletely oxidized state, particularly aceto-acetic acid, B-hydroxybutyric acid, and acetone. These bodies are readily excreted (ketonuria). If the ketone bodies are produced more rapidly than excreted, they accumulate in the blood and use up the blood buffers, thus lowering the alkali reserve. While the feed consumption of our cows was greatly depressed on increasing the environmental temperature above 80°F, they did not develop ketosis or even ketonuria. The concentration of ketone bodies in the blood plasma (Table 4) and the excretion of ketone bodies in the urine (Table 5) remained within the normal range, showing no tendency to increase with in-

TABLE 3 -- EFFECT OF RADIATION INTENSITY AT 45°, 70°, AND 80°F ON BLOOD pH, 1953

Temperature, °F	Radiation	H132	H169	H357	H314	H197	H337	H330	J275	J511	J271	J518	B209	Average Holstein	Average Jersey
45	dim	7.32	7.35		7.30	7.36	7.36	7.36	7.34	7.32	7.33	7.33	7.30	7.34	7.33
	½	7.39	7.38		7.29	7.37	7.39		7.23	7.35	7.32	7.34	7.34	7.36	7.31
	full	7.30			7.38	7.38	7.40	7.40	7.38	7.39	7.40	7.38	7.39	7.37	7.39
70	dim	7.37		7.37	7.35	7.34	7.34	7.35	7.34	7.34	7.35	7.34	7.32	7.35	7.34
	½	7.34		7.33	7.32	7.32	7.32	7.31	7.34	7.37	7.35	7.34	7.35	7.32	7.35
	full	7.40		7.38	7.39	7.39	7.39	7.39	7.37	7.35	7.38	7.37	7.34	7.39	7.37
80	dim	7.38		7.37	7.32	7.35	7.35	7.36	7.35	7.37	7.38	7.37	7.34	7.36	7.37
	½	7.35		7.38	7.37	7.37	7.38	7.38	7.37	7.39	7.40	7.40	7.38	7.37	7.39
	full	7.37		7.38	7.37	7.39	7.35	7.38	7.37	7.40	7.39	7.41	7.38	7.37	7.39

TABLE 4 -- EFFECT OF RADIATION INTENSITY AT 45°, 70°, AND 80°F ON TOTAL KETONE BODIES IN BLOOD, 1953
(as mg acetone per 100 ml plasma)

Temperature, °F	Radiation	H144	H357	H314	H197	H337	H330	J275	J511	J271	J518	B209	Average Holstein	Average Jersey
45	dim			1.7	2.2	2.8	2.2	3.1	1.9	2.3	2.8	0.9	2.2	2.5
	½			3.0	4.4	4.1	4.0	2.7	2.8	5.8	4.9	2.6	3.9	4.1
	full			6.6	6.1	6.7	7.3	1.1	2.0	0.5	2.7	2.2	6.7	1.6
70	dim		4.2	2.7	2.8	3.0	3.1	3.2	2.4	2.5	5.1	1.4	3.2	3.3
	½		1.5	1.9	2.7	3.3	2.8	1.8	2.6	3.5	5.7	1.8	2.1	3.4
	full		2.1	2.1	2.1	1.3	1.6	1.8	1.6	2.2	3.6	0.9	2.0	2.3
80	dim	0.5	1.2	0.9	2.8	2.1	1.7	1.4	1.4	1.8	2.7	1.5	1.6	1.8
	½	1.0	0.2	1.4	0.9	0.8	1.6	3.6	2.0	2.8	3.9	1.5	1.0	3.1
	full	2.4	4.6	2.8		2.5	3.2	3.8	2.9	4.2	4.1	2.6	3.1	3.8

creasing thermal stress. The data in Tables 4 and 5 indicate that heat, which is very stressful to dairy cattle, apparently does not, by itself, increase the blood or urinary ketone bodies.

RESULTS ON SHORT, ACUTE EXPERIMENTS

Determination of Maximal Temperature with Full Light Intensity. A predominantly white Holstein, No. 357, was first studied to determine the maximal temperature which could be tolerated under a full light (500 Cal/m^2/hr.) intensity. On the basis of her reactions (Table 6), 95°F was tentatively selected as the maximal temperature.

The blood pH increased and the CO_2 capacity decreased with increasing respiration rate. This *respiratory alkalosis* appeared within *five* hours after exposure to the thermal stress.

After *six* hours exposure, the respiration rate, rectal temperature and blood pH declined; but there was a simultaneous decrease in CO_2 capacity. Acclimatization seems to have been affected at the expense of change in blood composition.

The anions and cations in the blood plasma are quite variable, possibly as a result of changes in the fluid compartments of the animal body. Chlorides, however, increased progressively more or less in parallel with decrease in CO_2 capacity. It should be remembered that migration of chloride between red cells and plasma in response to changes in O_2 and CO_2 tension transfers the buffering action of the cell proteins to the relatively poorly buffered plasma. This may account for the decrease in CO_2 capacity without equivalent loss of fixed base. The data in Table 6, however, must be interpreted with caution. Blood samples for chloride analyses were not collected under paraffin oil, and an *in vitro* "chloride shift" may have occurred; nevertheless, the time between collection and centrifugation of samples was almost the same, and the error was probably of similar magnitude in each case.

Although the reactions of Holstein 357 indicated 95°F as the maximal temperature, it was felt that animals not predominantly white, with lower reflectance of radiations, might be more susceptible to the heating effect of light. For this reason, four predominantly black Holsteins were exposed to 95°F with and without full light intensity. The reactions of these animals (Table 7) necessitated lowering the temperature first to 90°F then to 85°F, and eventually 80°F was selected for the maximal temperature. The powerful heating effect of the lights is indicated by the increased rectal temperatures and respiration rates after the lights had been turned on for four hours at 95°F.

Blood pH was highest at the time of the highest respiration rate. The mean increase in blood pH was greater during this relatively short exposure than during the light experiments of longer duration previously reported; apparently compensation of blood pH occurs after continued exposure to high temperatures.

TABLE 5 -- EFFECT OF RADIATION INTENSITY AT 45°, 70°, AND 80°F ON TOTAL KETONE BODIES IN URINE, 1953
(expressed as grams of acetone excreted per 24 hours)

Temperature, °F	Radiation	H144	H314	H197	H337	H330	J275	J511	J271	J518	B209	Average Holstein	Average Jersey
45	dim						0.6	0.7	3.7	1.0	0.6		1.5
	½		2.1	2.1	2.0	1.3						1.9	
	full		3.6	3.6	2.7	2.9	0.7	0.9	0.8	1.4	0.4	3.2	0.9
70	dim		2.4	2.0	1.9	1.8	1.3	1.2	0.7	1.4	1.4	2.0	1.2
	½												
	full		1.9	1.0	1.9	0.6	0.8	1.1	1.1	0.4	0.9	1.3	0.8
80	dim	1.4	1.7	1.4	1.5	1.3	2.9	1.0	2.1	0.9	0.9	1.5	1.7
	½												
	full	2.8	2.9	1.1	1.8	1.3	1.5	1.2	1.2	0.5	1.0	2.0	1.1

TABLE 6 -- REACTIONS OF HOLSTEIN 357 TO INCREASING TEMPERATURE WITH FULL RADIATION (180 Btu/ft^2/hr) INTENSITY

Hours	Air Temp. °F	Respirations per min.	Rectal Temp. °F	Blood pH	Ketone Bodies* in blood	mEq/liter of plasma CO_2 Capacity	Na	K	Ca	Mg	SO_4	Cl	PO_4	Total Anions	Total Cations
Control**			101.0	7.47	1.78	25.2	141.3	4.9	5.9	2.3	5.0	103	5.7	138.9	154.4
½	87	52	102.6	7.53											
2½	90	72	102.0	7.55	2.51	27.4	143.4	3.9	4.8	2.4	6.2	106	6.0	145.6	154.5
3½		98	102.0	7.59											
5	93	80	102.0	7.61	1.48	27.3	147.8	3.7	4.9	2.3	5.2	106	5.7	144.2	158.7
6¼	92	72	101.5	7.50	2.10	24.1	137.4	4.1	5.6	1.9	4.8	106	5.7	140.6	149.0
7½	102	84	102.0	7.51											
8½	104	114	102.4	7.60	2.97	23.7	143.2	3.5	5.1	2.5	5.4	109	4.7	142.8	154.3

*Total ketone bodies expressed as mg acetone/100 ml blood plasma.
**Measured in dairy barn one hour before cow was placed in Climatic Laboratory.

TABLE 7 -- REACTIONS OF LACTATING HOLSTEIN COWS EXPOSED SUCCESSIVELY TO 70°, 90°, AND 95°F UNDER DIM, ½ FULL AND FULL RADIATION INTENSITIES

Cow No.	Air Temperature and Light Intensity	Respirations per Minute	Rectal Temperature °F	Blood pH	CO_2 Content, Vol. % "true" Plasma	CO_2 Content, Vol. % Venous Plasma	Ketone Bodies In Blood
357	70°F	36	101.6	7.32	54.3	52.8	1.93
314	dim light	36	101.3	7.31	55.0	55.9	3.26
337	48 hrs.	46	101.2	7.30	52.6	53.0	1.70
330		40	101.3	7.36	57.4	59.5	2.13
mean		40	101.4	7.32	54.8	55.3	2.26
357	95°F	52	102.0	7.32	53.2	53.2	1.13
314	dim light	50	101.8	7.29	49.1	48.1	3.30
337	1 hr.	74	101.6	7.31	51.9	51.0	2.13
330		54	101.4	7.30	52.3	50.7	1.91
mean		58	101.7	7.31	51.6	50.8	2.12
357	95°F	44	102.0	7.36	54.6	52.4	2.50
314	dim light	40	101.7	7.35	50.7	50.7	1.81
337	14 hrs.	54	102.4	7.30	47.4	44.5	2.65
330		44	101.9	7.37	55.3	55.8	.60
mean		46	102.0	7.35	52.0	50.9	1.89
357	95°F	104	103.5	7.40	52.4	49.8	2.44
314	1/2 light: 1 hr.	124	104.8	7.38	52.0	49.4	2.13
337	3/4 light: 1 hr.	144	105.4	7.38	48.6	45.5	.76
330	full light: 2 hrs.	100	103.3	7.38	52.3	46.0	3.31
mean		118	104.3	7.39	51.3	47.7	2.16
357	95°F	80	102.8	7.37	50.4	47.2	
314	full light: 1 hr.	112	103.9	7.38	50.6	48.6	
337	90°F	118	105.4	7.39	52.8	50.8	
330	full light: 4 hrs.	78	102.9	7.38	51.9	49.8	
mean		97	103.8	7.38	51.4	49.1	

*Total ketone bodies expressed as mg acetone/100 ml blood plasma.

The CO_2 content of both "arterialized" and venous plasma decreased with increasing radiation intensity on the cows; however, as previously indicated, even more significant was the change in the relationship of these quantities to each other. Blood obtained at 70°F and dim light *lost* CO_2 during saturation, while blood obtained at 95°F and full light intensity *gained* CO_2 during saturation. This changed relationship also identifies the condition of *respiratory alkalosis.*

Starvation Experiment. The effects of high temperature on the cows were complicated by reduction of feed intake with increasing temperature above 80°F (about 25°C). Changes in the acid-base balance may then be due to high temperature directly affecting the tissues and fluids or indirectly, as previously explained, by decline in carbohydrate oxidation along with rise in fat catabolism and increase in incompletely oxidized fatty acid using up the buffers. There could, of course, be a summation of effect of starvation and heat stress. To differentiate between these possibilities, four dairy cows were fasted for five days at the comfortable temperature of about 65°F with results shown in Figs. 8 to 10 and Table 8.

The CO_2 capacity (lower segment, upper left Fig. 9) and blood pH* (upper right, Fig. 10) were relatively constant during the five days of food deprivation. This differs from changes in the acid-base balance of the blood plasma associated with high temperature and indicates that these changes are not due primarily to feed deprivation but to other factors associated with heat stress, perhaps primarily to increased respiration rate (panting) with starvation as a reinforcing factor.

In contrast to the high temperature experiments, five days of starvation at thermoneutrality increased the concentration of ketone bodies in the blood plasma and also increased the excretion of ketone bodies in the urine (left side, Fig. 10). This confirms two recently published reports.[16] The manner in which ketone bodies were handled by the kidney was apparently unique among the metabolites studied in this experiment. Despite a large increase in the excretion of ketone bodies, there was a parallel increase in the concentration of ketone bodies in the blood plasma. This relationship between plasma concentration and renal excretion suggests that the ketone bodies are *low threshold substances*[17] which pass from the glomerular filtrate back to the blood only by diffusion.

The relationship between plasma concentration and renal excretion of phosphate and sulfate (also low threshold substances), differs from that of the ketone bodies. Small changes in the plasma concentration of phosphate or sulfate resulted in large changes in the renal excretion of these substances (Fig. 9).

The plasma concentrations and the renal excretions of the principal

*pH values approximately 0.15 pH units high because of improper standardization.

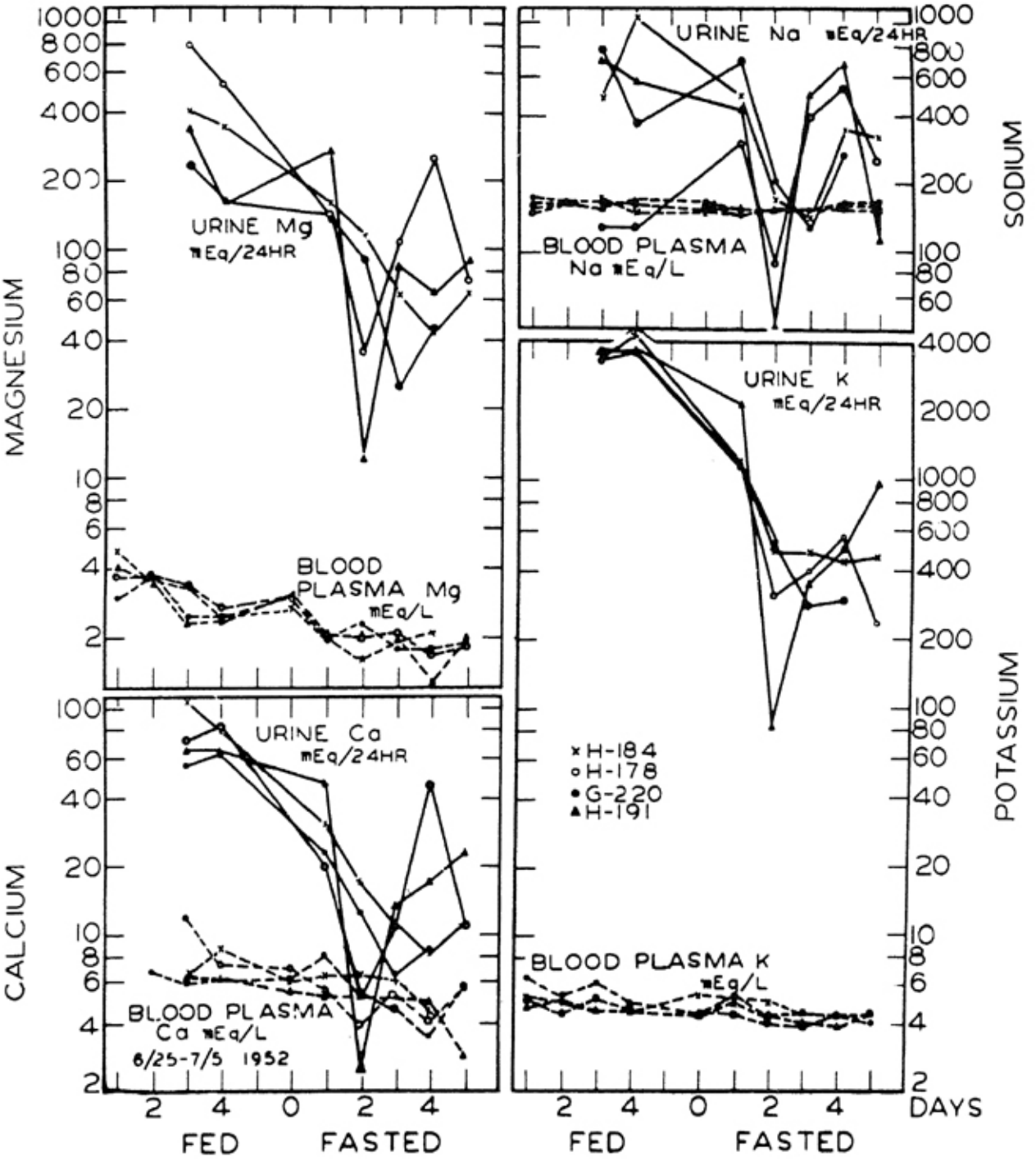

Fig. 8—Effect of five-day fast on some cation levels in plasma and excretion in urine.

cations and anions (Fig. 8 and 9, and Table 8) dramatize the homeostatic function of the kidney. Very small changes in plasma concentration are associated with huge changes in urinary excretion.

The change from a base-rich herbivorous metabolic mix, under normal feed intake, to a base-poor metabolic mix, under conditions of fast when the animal catabolized its own tissues, resulted in the decreased excretion of cations observed in Fig. 8.

TABLE 8 -- EFFECT OF STARVATION ON PLASMA CONCENTRATION AND URINE EXCRETION OF PRINCIPAL ANIONS AND CATIONS, 1952
(Mean Values)

	Plasma Concentration				Urine Excretion			
	Control Period		Last Day of Starvation		Control Period		Last Day of Starvation	
Constituents	mEq/liter	% Total	mEq/liter	% Total	mEq/liter	% Total	mEq/liter	% Total
Cation								
Sodium	162	91.1	157	93.3	529	11.4	239	29.6
Potassium	5.1	2.9	4.4	2.6	3658	78.9	485	60.2
Calcium	7.5	4.2	4.8	2.9	73.8	1.6	13.6	1.7
Magnesium	3.2	1.8	2.0	1.2	374.2	8.1	68.3	8.5
Total	177.8		168.2		4635.0		805.9	
Anion								
Sulfate	14.3		12.1*		759	13.8	272	16.1
Phosphate	7.4		7.6		4.2	0.8	824	48.7
Carbonate	25.4		23.5		4500	81.6	540	31.9
Chloride					250	4.5	55**	3.3
Total					5513.2		1691	

*From third day of starvation.
**Value of 678 mEq/liter for H-197 not included in calculation of mean.

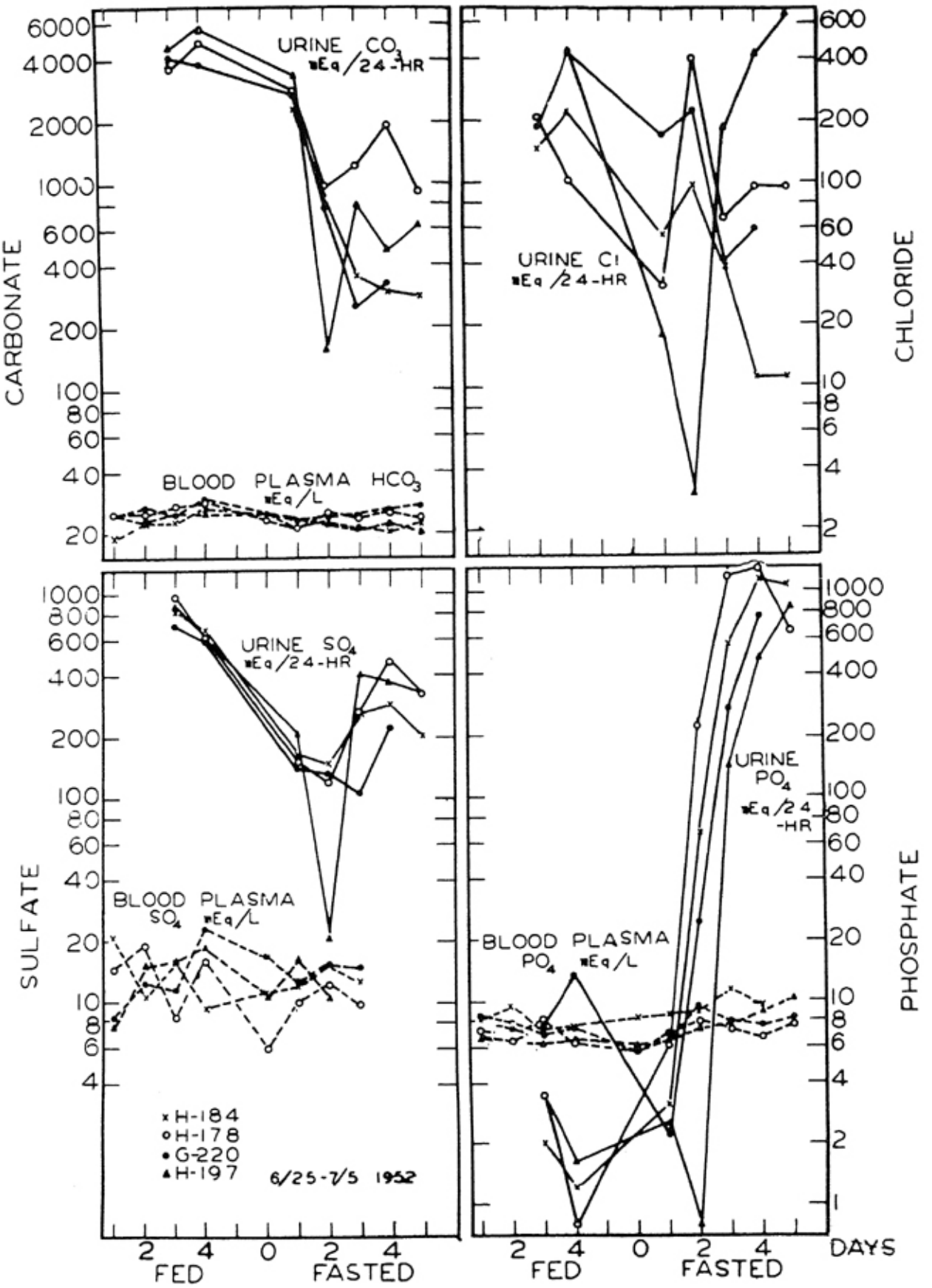

Fig. 9—Effect of five-day fast on some anion levels in plasma and excretion in urine.

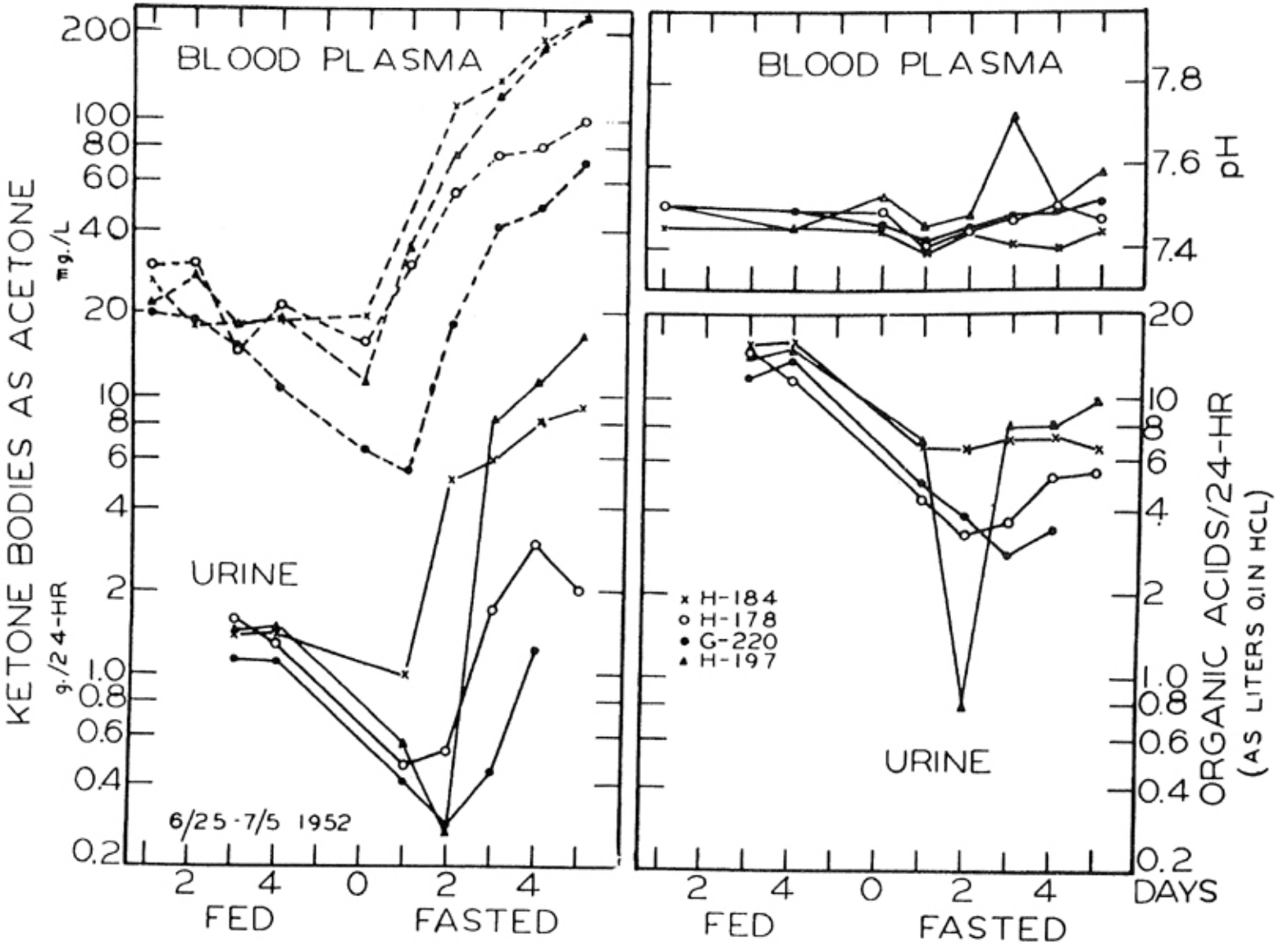

Fig. 10— Ketone body levels in plasma and ketone excretion in urine during a five-day fast, also plasma pH.

The steep rise in the excretion of inorganic phosphate presumably reflects tissue destruction associated with decline of exogenous energy supply.

Sulfate excretion (lower left, Fig. 9) which decreased to a low value on the second day of starvation and then returned to an intermediate value, reflects transition from exogenous to endogenous metabolic mixtures. Apparently the sulfur-containing amino acids are more abundant in the feed consumed by a herbivorous animal than they are in the tissues of that animal.

The decreased excretion of carbonate during starvation (upper left, Fig. 9) indicates the excretion of proportionally less cations than anions during starvation. This is confirmed by the values summarized in Table 8, showing an 83 percent decrease in the excretion of cations but only a 69 percent decrease in the excretion of anions.

The ratio of sodium to potassium in the blood plasma increased from 31.8 during the control period to 35.7 on the last day of starvation; during the same time the ratio of sodium and potassium excreted in the urine increased from 0.145 to 0.493. This decreased ratio of sodium to potassium in the blood and increased ratio of sodium to potassium excreted in the urine may reflect deficiency of 11-desoxy compounds from the adrenal cortex. The effect of starvation is paradoxical in that the ratio is increased in both blood and urine. A plausible explanation is that the 11-oxy compounds, some with both mineral and gluconeogenic activities, are secreted in increased amounts during starvation.

The excretion of organic acids in the urine (lower right, Fig. 10) is decreased by starvation. Since the ketone bodies were included in the organic acids, there was apparently some organic acid fraction that underwent more of a decrease than is indicated in Fig. 10. This decrease presumably reflects decreased production of organic acid in the rumen during starvation.

SUMMARY AND CONCLUSIONS

1. The CO_2 combining capacity of the blood plasma of dairy cattle decreased on increasing thermal stress (high air temperature and varying radiation intensity of short and long duration). The decrease was greater in large, lactating animals than in small, non-lactating animals.

2. The decline in CO_2 combining capacity was associated with rise in blood pH and with change in the relationship between the CO_2 content of arterialized plasma and the CO_2 content of venous plasma as explained below. This decline in the CO_2 combining capacity with rise in pH is termed *respiratory alkalosis.* Cattle may, therefore, be said to develop a respiratory alkalosis on exposure to environmental temperature exceeding 85°F.

3. The CO_2 *content* of "arterialized" and venous plasma was more variable than the CO_2 *capacity* of the same plasma. This difference was correlated with the climatic conditions to which the animals were exposed. Collected under conditions of thermoneutrality, the plasma lost CO_2 during "arterialization;" collected under conditions of thermal stress, the plasma gained CO_2 during "arterialization." This altered relationship between the CO_2 tensions of venous blood and alveolar air occurred after both long and short exposure to thermal stress.

4. The difference between the CO_2 *content* and CO_2 *capacity* of venous plasma was correlated with the mean outside daily temperature and consequently with the temperature of the analytical laboratory. When the mean outside temperture was low, the plasma gained CO_2 during the saturation for capacity measurement; when the outside temperature was high, the plasma lost CO_2 during the saturation.

5. While the blood pH increased initially with increasing thermal stress, there often developed compensation of blood pH, as indicated by greater changes after short than after long exposures to thermal stress.

6. The concentration of total ketone bodies in the blood plasma and the excretion of total ketone bodies in the urine did not increase with exposure of the cows to thermal stress.

7. Five days of starvation at about 65°F did not affect the blood pH and bicarbonate content. The concentration of ketone bodies in the blood plasma and the excretion of ketone bodies in the urine were, however, increased. The excretion of organic acids in the urine was decreased during starvation.

8. Five days of starvation produced only slight changes in the concentration of the principal inorganic anions and cations in the blood plasma but resulted in dramatic changes in their urinary excretion, particularly decreased excretion of cations and carbonate and increased excretion of phosphate.

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