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J. H. LONGWELL, *Director*

Environmental Physiology and Shelter Engineering

With Special Reference to Domestic Animals

LIV. THE EFFECT OF LONG EXPOSURE OF ENVIRONMENTAL TEMPERATURES OF 50° AND 80° F ON GLUTATHIONE, BEI^{131}, AND GROWTH RATE OF DAIRY CALVES

TAYMOUR H. KAMAL, H. D. JOHNSON, AND A. C. RAGSDALE

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INTRODUCTION

Two important metabolic products of the body which influence growth are the thyroid hormone and the co-enzyme, glutathione. The importance of the thyroid hormone in the growth process has been demonstrated by the fact that the absence of this hormone in cattle through thyroidectomy resulted in retarded growth and a mature body weight reduction of 50 percent (11). Consistent acceleration of growth in other species was shown to be a consequence of mild hyperthyroidism (30). The fact that glutathione, a coenzyme in intermediary and cerebral carbohydrate metabolism (12, 42) and a mediating factor in many other biological (56) and hormone actions, is a direct participant in the formation of the structural machinery controlling cell division has been demonstrated by Mazia (39).

Growth, measured by weight gain, is also adversely affected by temperature (20, 43, 44). Much information concerning temperature induced changes in the biological functions of homeotherms has been reviewed (48). Such changes are regulated by a complex spatially and temporally interlocking series of hormones and enzymes (10), including the thyroid hormone and the coenzyme, glutathione. The effect of environmental temperature on the thyroid hormone level of dairy animals has been investigated (7, 17, 23, 41). However, neither the effect of temperature upon glutathione nor upon the relationship between glutathione and the thyroid hormone in dairy cattle has been studied.

This bulletin is a report on a specific phase of the investigation of growth of dairy cattle in the Missouri Climatic Laboratory at constant environmental temperatures of 50° F or 80° F.

*BEI^{131}: Blood Plasma Butanol Extract I^{131}.

The first purpose of this investigation was to determine the effect of continuous exposure to a relatively low (50° F) and to a relatively high (80° F) temperature upon dairy calves' blood glutathione, thyroid activity, and monthly gain in body weight. In most instances these data were obtained on the animals between the ages of 6 and 12 months.

The second objective of this study was suggested by the fact that age or length of exposure influences the magnitude of thermal stress.[1] A long exposure to cold or heat may allow compensation for some of the initial physiological displacements induced by short-term exposure at the beginning of an experimental study (1, 4, 14, 49, 62); the adjustment being an example of acclimation, as reported by Hart (19). The importance of age has been supported by the fact that older cattle have higher heat tolerance than do younger animals (2, 8, 29). Additional evidence by Kibler (25) demonstrated that the difference in pulse rate, ventilation rate, and heat production between beef calves reared at 50° F and those reared at 80° F decreased as age increased. The second goal of this study was, then, to observe any age trend differences between the heat and cold exposed groups.

MATERIALS AND METHODS

Two groups of calves, each consisting of three Brown Swiss, three Holsteins, and three Jerseys, were used in this study. The groups were maintained at constant temperatures and humidities in separate, independently controlled chambers of the Missouri Climatic Laboratory for one year. For more details consult Johnson and Ragsdale (24). The data for this study were obtained during the last six months of the growth experiment, when the calves were from 6 to 12 months of age. Three calves of each breed were placed in a pen in each chamber. One chamber was maintained at 50° and 62 percent relative humidity and the other at 62° F and 54 percent relative humidity. In each chamber illumination was provided by one 40-watt incandescent bulb which was on at all times and by six 200-watt incandescent bulbs which were on between 6 a.m. and 6 p.m. Air velocity was approximately 50 feet per minute.

Grain mix was restricted to 4.67 lbs. for Brown Swiss and Holstein and 3.33 lbs. for Jersey per calf per day, while alfalfa hay and water were available *ad libitum.* The amount of hay consumed was recorded and the volume of water consumed and frequency of drinks were recorded automatically. The calves of each chamber were weighed twice weekly at 6 a.m. with a scale platform stanchion balance located in the adjacent work room outside the test chamber.

Growth rate was measured as the absolute gain in the given magnitude per unit time and was plotted on semi-logarithmic paper and presented as body weight gain, Kg./month (9).

[1]Thermal stress in cattle is a physiological state manifested by various intensified or depressed biological reactions (heat production,, vaporization, respiration rate, pulse rate, thyroid activity, etc.). This state may occur in cattle at temperatures which are outside the "comfort zone." The comfort zone is defined in the Mo. Env. Physiol. Series (I-LV) (1948-1959).

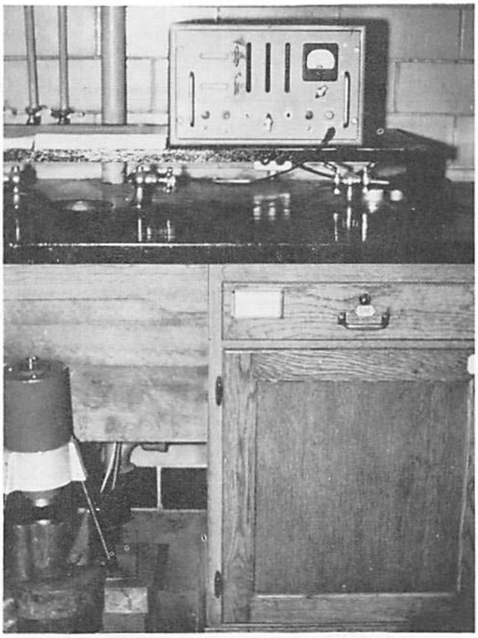

Figure 1. Radio-Isotope equipment for #BEI^{131} Determinations. In the lower left corner is the scintillation-well-counter which was used with the above Berkeley Decimal Scaler.

The n-butanol I^{131} extract (BEI^{131}) determinations were made following injection of 200μc of sodium I^{131} into the jugular vein of each animal. Blood samples were collecred from the jugular vein one week after the injection. The method used in the BEI^{131} determination was similiar to that described by Blincoe and Brody (7). Correction for decay was made by preparing a srandard solution of 1/2000 of the dose concentration and the radioactivity of equal volume to the dose was determined along with rhe samples. A well scintillation detector was used for that purpose and the radioactivity (counts per minute) was recorded on a Berkeley Decimal Scaler (Figure 1).

Blood glutathione (GSH) determinarions were made on bi-monthly individual blood samples collected from rhe jugular vein into heparinized plastic tubes. Blood samples for glutathione were always kept in ice baths before and during the analysis which was carried out at 5° C immediately after sampling. The nitroprusside method modification by Grunert and Philips (18) was used in this study. Data were analyzed for variance as well as differences between groups and breeds according to the method described by Snedecor (53).

RESULTS

Influence of Breed and Temperature on Blood GSH, BEI^{131} and Monthly Gain in Body Weight.

Table I shows that in both 50° and 80° F reared calves the Brown Swiss maintained the highest average values of body weight gain, and that they were followed by the Holstein and Jersey calves respectively. The differences in the weight gain of the Brown Swiss and Jersey and of the Holstein and Jersey were

TABLE 1--EFFECT OF TEMPERATURE ON BLOOD GSH, BEI^{131} AND GAIN IN BODY WEIGHT

Breed	GSH mg./100 ml. 50°F	GSH mg./100 ml. 80°F	BEI^{131} x 10^{-3}% of Dose/100 ml. plasma 50°F	BEI^{131} x 10^{-3}% of Dose/100 ml. plasma 80°F	Body Weight Gain, kg./month 50°F	Body Weight Gain, kg./month 80°F
Brown Swiss	21.04 ± 2.56****	34.72*** ± 3.90	23.70 ± 2.48	13.66*** ± 1.66	28.65 ± 1.24	27.21* ± 1.08
Holstein	27.39 ± 3.11	42.59*** ± 2.03	18.66 ± 3.43	9.10*** ± 1.32	28.25 ± 1.88	25.79** ± 1.58
Jersey	31.88 ± 2.78	52.24*** ± 1.94	33.54 ± 1.98	19.50*** ± 2.25	16.71 ± 0.99	17.71 ± 2.34
All Breeds	27.23 ± 1.80	43.22*** ± 3.33	25.66 ± 1.55	14.29*** ± 1.27	24.53 ± 1.23	23.06* ± 1.29

*** Statistically significant higher or lower than 50°F group at P 0.01

** Statistically significant higher or lower than 50°F group at P 0.05

* Statistically significant higher or lower than 50°F group at P 0.10

**** Standard error values.

significant, but those of the Brown Swiss and Holstein were not significant (Table 2). The Jersey calves, however, had the highest BEI^{131} values and were followed by the Brown Swiss and Holstein calves. Blood gutathione was highest in the Jerseys, intermediate in Holstein, and lowest in the Brown Swiss and Holstein calves. Blood glutathione was highest in the Jerseys, intermediate in Holstein, and lowest in the Brown Swiss. The breed differences for GSH and BEI^{131} were statistically significant (Table 2). The breed does not modify the

TABLE 2--BREED AVERAGES OF BLOOD GSH, BEI^{131} AND GAIN IN BODY WEIGHT

Breeds / Test Significance	GSH mg./100 ml.	BEI^{131} x 10^{-3}% of Dose/100 ml. plasma	Body Weight Gain, kg./month
Brown Swiss	28.13 ± 2.80	19.76 ± 1.87	27.92 ± 0.81
Holstein	35.42 ± 3.82	16.63 ± 2.35	27.04 ± 1.24
Jersey	42.04 ± 3.87	23.79 ± 1.07	16.42 ± 1.26
All Breeds	35.34 ± 2.12	20.06 ± 1.21	23.79 ± 0.89
Br. S. - Holst.	***	***	-
Br. S. - Jersey	***	***	***
Holst. - Jersey	***	***	***

*** Statistically significant breed difference at P 0.01.

temperature effect on GSH, BEI^{131} and gain in body weight as shown by the insignificant interaction between temperature and breed (Table 3). Table 1 shows that the three breeds reared at 50° F have lower values of blood glutathione but higher values of n-butanol I^{131} extract and monthly gain in body weight than the 80° F group. The influence of temperature on GSH and BEI^{131} was significant for all breeds, while the temperature effect on weight gain was significant for only Brown Swiss and Holsteins. The fact that body weight gain apparently was not significantly influenced by temperature in the analysis of variance, was probably caused by irregularity or "acclimation" of Jersey animals during growth.

Influence of Age on Blood GSH, BEI^{131} and Monthly Gain in Body Weight.

Figure 2 and tables 4 and 6 showed that blood GSH and gain in body weight generally declined with the advance in age in both 50° and 80° F groups of all three breeds. This effect of age upon blood GSH and body weight gain was statistically significant (Table 3). However, the BEI^{131} shows a generally constant or relatively slight decline with age in the 50° F group (Figure 2 and table 5). The heat exposed group which had markedly depressed BEI^{131} values at the beginning of the experiment showed a gradual increase in BEI^{131} with advancing age. The difference in trends of the two groups contributed an insignificant effect of age on BEI^{131} in the analysis of variance (Table 3). The difference between the two groups at earlier age was greater than that at the 12th month of age in most measurements. There were no statistically significant interactions between age and temperature for gain in body weight or between age and breed for either GSH or gain in body weight, although there were statistically significant interactions between temperature and age for GSH and BEI^{131} (Table 3).

DISCUSSION

Effect of Temperature, Breed, and Age on BEI^{131}.

The 80° F Brown Swiss, Holstein, and Jersey calves used in this experiment had lower thyroid activity than the 50° F calves. This result agrees with those of similar studies on dairy cows (7, 23). However, using the extrapolation technique in calves, Lewis *et al.* (35) were not able to demonstrate seasonal differences in thyroxine secretion rate in different breeds of calves. On the other hand, Premachandra *et al.,* (41) using a technique similar to the latter showed that thyroxine secretion rate was depressed in summer to about 30 percent of the winter value.

The mechanism involved in the depression of thyroid gland activity at high temperature is not clearly understood. Heat effects may possibly be transmitted by the Ruffini's endings in the skin to the regulatory centers of the hypothalamus through the afferent nerves. The hypothalamus in some manner stimulates the pituitary to secrete adrenocorticotropin which, through the adrenal

TABLE 3--ANALYSIS OF VARIANCE OF GSH, BEI^{131}, AND WEIGHT GAIN

Source of Variation	(Symbol)	GSH			BEI^{131}			Weight Gain		
		Mean Square	DF	F	Mean Square	DF	F	Mean Square	DF	F
Total		321.81	70	2.66***	100.60	68	3.02***	56.76	71	2.79***
Temperature	T	4530.00	1	374.78***	2213.00	1	66.48***	39.00	1	1.92
Age	A	2644.00	3	218.75***	43.60	3	1.30	190.67	3	9.38***
Breed	B	1135.00	2	93.90***	301.50	2	9.06***	990.00	2	48.70***
T X A	TA	377.67	3	3.12**	356.67	3	107.14***	32.33	3	1.59
T X B	TB	3.50	2	0.03	77.50	2	2.33	5.00	2	0.25
A X B	AB	41.50	6	0.34	161.83	6	48.61***	39.00	6	1.92
Error		120.87	53		33.29	51		20.33	54	

*** Significant at the 0.01 level ($P<0.01$)

** Significant at the 0.05 level ($P<0.05$)

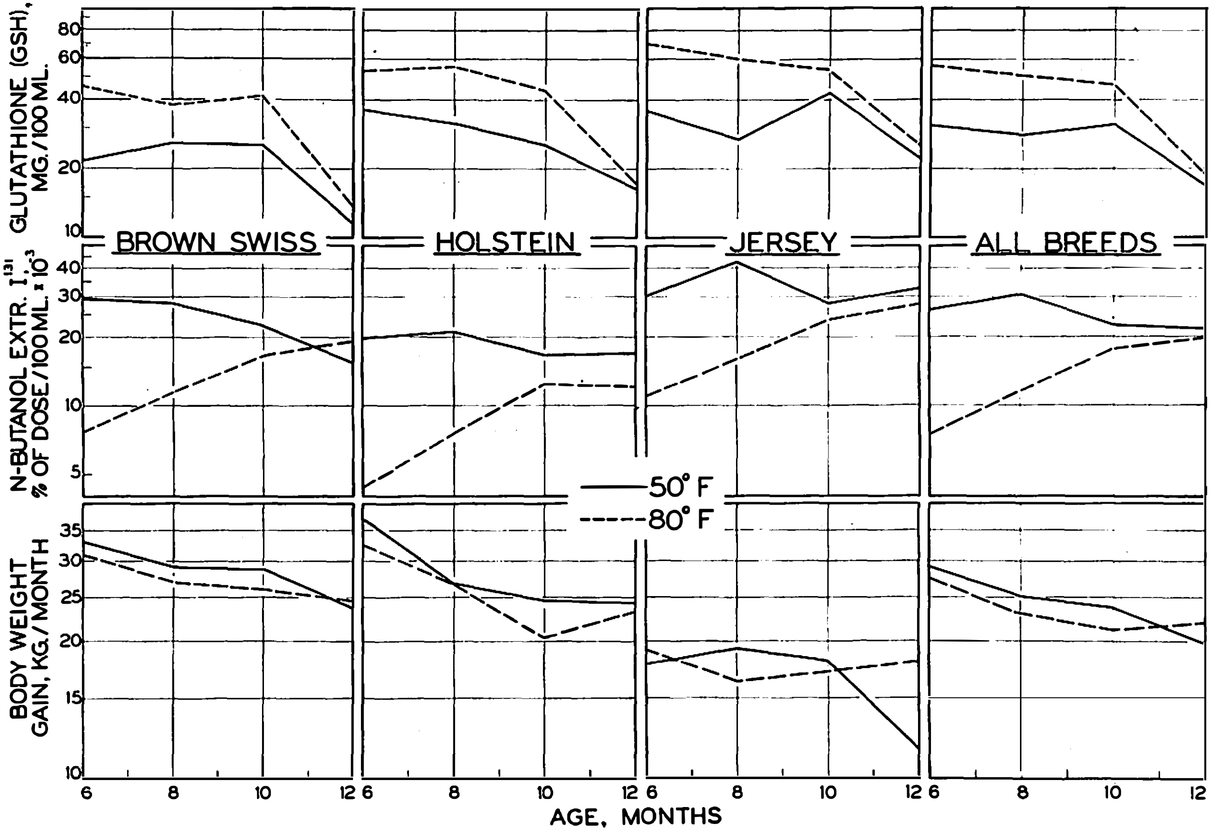

Figure 2. Effect of advancing age on the blood glutathione, n-butanol extract—I^{131} and body weight gain of calves raised at 50° and 80° F.

TABLE 4--EFFECT OF TEMPERATURE AND AGE ON BLOOD GLUTATHIONE (GSH)

Month of Age	Breed	GSH mg./100 ml. 50°F	GSH mg./100 ml. 80°F	Standard Error 50°F	Standard Error 80°F
6	Brown Swiss	21.50	45.50	12.49	1.73
	Holstein	36.00	53.67	7.68	2.00
	Jersey	35.67	69.67	4.24	6.92
	All Breeds	31.06	56.28	4.58	4.12
8	Brown Swiss	25.83	37.83	4.12	2.24
	Holstein	31.67	55.50	2.24	5.57
	Jersey	26.83	60.13	2.83	11.79
	All Breeds	28.11	51.15	3.23	5.10
10	Brown Swiss	25.17	41.67	1.73	5.10
	Holstein	25.50	44.00	1.00	3.87
	Jersey	43.00	54.00	3.74	5.66
	All Breeds	31.22	46.56	3.00	3.16
12	Brown Swiss	11.67	13.87	2.65	0.57
	Holstein	16.37	17.17	3.46	2.58
	Jersey	22.00	25.17	2.24	3.65
	All Breeds	16.68	18.74	2.00	1.73

TABLE 5--EFFECT OF TEMPERATURE AND AGE ON BUTANOL EXTRACT I^{131} (BEI^{131})

Month of Age	Breed	$BEI^{131} \times 10^{-3}$ % of Dose/100 ml. plasma 50°F	$BEI^{131} \times 10^{-3}$ % of Dose/100 ml. plasma 80°F	Standard Error 50°F	Standard Error 80°F
6	Brown Swiss	28.88	7.63	2.00	0.27
	Holstein	19.80	4.35	3.16	2.23
	Jersey	30.64	10.47	2.00	2.32
	All Breeds	26.44	7.48	2.00	1.41
8	Brown Swiss	28.00	11.36	6.08	0.26
	Holstein	21.05	7.45	1.41	1.41
	Jersey	42.91	15.98	1.41	2.45
	All Breeds	30.65	11.60	3.74	1.41
10	Brown Swiss	22.48	16.60	0.92	2.83
	Holstein	16.71	12.52	1.73	2.24
	Jersey	28.08	23.64	4.47	2.65
	All Breeds	22.42	17.59	2.65	2.24
12	Brown Swiss	15.46	19.07	2.83	2.83
	Holstein	17.07	12.10	0.84	2.00
	Jersey	32.56	27.92	4.58	0.87
	All Breeds	21.70	19.70	3.16	2.45

TABLE 6--EFFECT OF TEMPERATURE AND AGE ON MONTHLY GAIN IN BODY WEIGHT

Month of Age	Breed	Body Weight Gain, kg./month 50°F	80°F	Standard Error 50°F	80°F
6	Brown Swiss	33.00	30.97	1.73	3.05
	Holstein	37.17	32.66	0.45	2.03
	Jersey	17.84	19.16	1.00	1.09
	All Breeds	29.34	27.60	2.80	2.45
8	Brown Swiss	29.09	27.02	1.41	1.87
	Holstein	26.92	26.76	1.73	1.99
	Jersey	19.33	16.42	1.00	1.99
	All Breeds	25.11	23.40	1.41	1.41
10	Brown Swiss	28.92	26.09	1.00	0.89
	Holstein	24.59	20.50	2.03	3.09
	Jersey	18.09	17.17	1.73	1.31
	All Breeds	23.87	21.25	1.41	1.41
12	Brown Swiss	23.59	24.75	1.64	1.56
	Holstein	24.33	23.25	3.98	2.04
	Jersey	11.59	18.09	0.89	1.11
	All Breeds	19.84	22.03	1.73	0.95

hormones, may depress the thyroid activity. Also heat, as a systemic stressor, evokes a generalized adaptive pattern in which the whole body and, particularly, the endocrine system participate (51). One of the most prominent systemic responses is the enlargement of the adrenal cortex and an increase in the release of cortical steroids (52). Most of the work indicates that adrenocortical hormones have depressing effects upon protein-bound iodine (22, 47.) The low BEI^{131} value of 80° F reared calves may therefore be attributed to higher adrenocortical activity stimulated by thermal stress. It is interesting to observe that the BEI^{131} of the Jersey and Brown Swiss calves raised at 80° F was only 41.56 percent and 42.36 percent respectively lower than the corresponding values of 50° F group, while the Holstein calves showed the greatest depression, 51.23 percent. This relatively greater depression would logically follow if the thyroid glands of the apparently more heat tolerant Jerseys and Brown Swiss (61) are less affected by thermal stress than those of the Holstein calves.

Quite high, statistically significant breed differences have been obtained in the BEI^{131} (tables 2, 3). The Jersey and Brown Swiss maintained a higher thyroid hormone concentration in their plasma than did the Holstein calves (tables 1, 2). However, the total amount of the thyroid hormone circulated in the blood plasma of the former animals is possibly less than that in the latter since as assumed there is less plasma volume in Jersey and Brown Swiss than in Holstein (13, 54). In agreement with these results, PBI (36, 37) and thyroid release rate studies (23), showed similar breed differences. Perhaps the relatively high concentration of BEI^{131} in the Jerseys and Brown Swiss may be of significance with respect to heat tolerance. Whether the high BEI^{131} concentration is due to low thyroid hormone utilization, excretion or to a reduced effect of the metabolites and derivatives of the circulated thyroid hormone on thyrotropin in the heat tolerant animals, is a problem that warrants investigation.

With advancing age the 50° F Brown Swiss and Holstein group showed a slight decline in the BEI^{131}. This indicates a decrease in the relative thyroid secretory activity (per unit volume of blood) with age. This conclusion is consistent with those of other experimenters (36, 37) who have investigated dairy calves and cows. The 80° F group which had a marked depression at early age showed a tendency to restore its normal level until it approximately approached or even exceeded the 50° F group at the 12th month of age as in the case of the Brown Swiss calves. These data correspond to those reported by Blincoe (6) on the rate constant of thyroid hormone release (K4) in beef calves. These different effects of age at the two temperature levels are expressed by an insignificant effect of age on BEI^{131} and a high, statistically significant interaction between age and temperature effects on BEI^{131} (Table 3). There is a possibility that a restoration of blood concentration from a hemodilution to a normal state has taken place, and that it has contributed to the elevation of BEI^{131} concentration with longer exposure to heat. This possibility, however, cannot account for the same results obtained in previous study (6) using thyroid hormone release technique. Young calves seemed to be more affected by heat than older animals (29). Egyptian cattle and buffaloes (2) and Afrikaner or European beef breed (8, 25) have been affected in like manner by age. During long exposure, the calves either succeeded in resistance or failed, and subsequently succumbed. It appears that the calves used in this study exhibited an adaptive mechanism which facilitated the restoration of physiological functions and the withstanding of heat. The possibility that the thyroid gland may be involved in long-term adaptation to thermal stress in dairy cattle has been suggested (6, 23). The adrenal cortex may play a large role in this mechanism as well as in the initial phase of resistance (51). Evans (16) showed that adrenocorticotrophic preparations and hydrocortisone produced thyroid stimulation and restored the function and morphological characteristics in hypophysectomized rats. It may be true that although the adrenocorticoids have depressive effects on the thyroid gland function (15, 58, 60) they may have a restoring effect on the thyroid activity when its secretion is at a minimum.

Effect of Temperature and Age on Blood Glutathione (GSH).

The 80° F group (Table 1) had statistically significant higher GSH values than the 50° F group in contrast to their significantly lower thyroid activity. Evidence concerning the GSH and BEI^{131} relationship is replete with conflicts and inconsistencies. On the one hand, an increased level of blood GSH occurred after thyroidectomy or administration of thiourea, methyl thiouracil, and propythiouracil, while on the other hand, diminished blood GSH levels in most patients with hypothyroidism has been reported (34). The adrenocorticotrophic hormone and adrenal cortocoids, moreover, which previously had been reported to depress thyroid activity have been shown more recently to increase the GSH in the other (5, 63, 21, 32, 33). These contradictions in the reviewed results may, however, be attributed to the different levels of dosage, size and duration of the dose, the time of sampling, and the purity of the preparation used. However, as indicated by the above studies, the possibility that the increase in blood GSH in 80° F group may be induced by low thyroid activity or factors depressing its activity such as high secretion of adrenocorticotrophin or adrenal corticoids, cannot be excluded. It has also been observed that short term temperature studies on aged ewes (6 years or older) showed no influence of temperature changes on blood GSH (27). The change in blood GSH with temperature may also be explained by the recent work of Phillips and Langdon (40), Ball and Cooper (3), and others who showed that thyroxine has stimulated TPN-cytochrome c reductase and glucose-6-phosphate dehydrogenase enzymes. This suggested a stimulation of the hexose monophosphate shunt by thyroxine and consequently would show a lowered production of the enzyme, glyceraldehyde-3-phosphate dehydrogenase, as well as its prosthetic group GSH (42) at low than at high temperature. This may be responsible for the low blood GSH which is associated with the high thyroid activity in 50° F group and vice-versa in the 80° F group.

Another explanation for the increase of the blood GSH in the 80° F group is derived from many investigations demonstrating that GSH is definitely essential in growth (38, 39, 45, 55). Its role in growth is thought to be either through its direct participation in the formation of the structural machinery that carries out the process of cell division, on which growth is dependent (39), or through its action as a coenzyme in the enzyme glyceraldehyde-3-phosphate dehydrogenase (42). The latter enzyme provides the first energy production step in the glycolytic cycle which is generally required for protein synthesis and consequent growth. GSH also participates in the transpeptidation reaction by transferring the glutamyl radical to amino acids and peptide acceptors (59). It has also been suggested that it may be an intermediate between free amino acids and protein transferring amino acids or a regulating mechanism.

Since GSH is definitely essential in growth it is expected to be more readily utilized from the blood by rapid growth tissues and consequently to decrease in the blood. It is not surprising therefore to find out that the rapidly growing group (50° F reared calves) has lower blood GSH than the thermally stressed group. It may be that a relatively lower adrenal corticoid secretion, possibly in the 50° F calves, may enhance GSH utilization and thus result in a lower blood GSH level. Lazarow (33) has shown that adrenalectomy in dogs was followed by increased muscle GSH and decreased blood GSH.

Figure 2 shows that the advance in age or as assumed increase in body weight has a depressing effect on blood glutathione in both groups. This is also demonstrated by a significant effect of age on GSH (Table 3). Reid *et al.* (46) showed that the blood of new-born calves, either before or after colostrum ingestion, contained significantly greater quantities of both reduced and total glutathione than that of dams or any of the animals studied. They, however, reported that these levels appeared to decline sometime between birth and 18 months of age. Although Kidwell *et al.* (26) demonstrated a significant negative correlation between blood GSH and the rate of gain during the first 40 days of the feeding experiment with two years of fattening, they found that low blood GSH was associated significantly with the most economically or rapidly gaining animals. Their results, however, were similar to their previous observation in mature beef cattle (28) or later studies on sheep (27).

Data presented in this investigation indicated that during the last two months of the experiment the 80° F group (Jersey and Holstein) showed a general increase in body weight gain, whereas the corresponding blood GSH level was markedly depressed (Figure 2). This observation is in agreement with that of Kidwell *et al.* (26) who suggested that the low blood GSH levels in the most rapidly and economically gaining animals are indicative of a depletive state, and such animals might gain even faster or more economically if given additional GSH or that means of synthesizing it.

It seems that the advance in age reduces the depressive effect of heat on GSH utilization. This conclusion is indicated by a more rapid depletion of blood GSH coinciding with faster growth in the 80° F group than that in the 50° F group during the last two months (Figure 2), and by a statistically significant interaction between age and temperature effects on blood GSH (Table 3).

A statistically significant breed difference in blood GSH was also obtained (Table 3). Again the Jerseys had the highest concentration of blood GSH (Table 1). Could this be due to its far lower body weight in comparison to the other breeds or to a heat tolerance characteristic? Kunkel, *et al.* (31) have shown that the heat tolerant breed, Brahman, had significantly higher blood GSH than the Hereford and from heritability estimation, they concluded that blood GSH was a highly heritable characteristic in young beef cattle.

Effect of Temperature and Age on the Gain in Body Weight.

The 50° F Brown Swiss and Holstein calves gained more in body weight than did the 80° F animals, the differences being significant at the 0.05 and 0.10 levels of probability respectively (Table 1). There was no statistically significant difference between the 50° and 80° F Jerseys which exhibited considerable variability in their individual data. These data, nevertheless, do indicate that 80° F heat has a depressive effect upon growth. The results of this study are consistent with those of dairy heifers, dairy cattle (44), and beef calves (43). Whatever the mechanism responsible for the relationship between growth and heat, the glutathione and the thyroid hormone appear to be involved. This conclusion seems justified since a significant decrease in thyroid activity and a significantly higher level of blood GSH in the slow growing calves, the 80° F group, has been demonstrated.

However, with advancing age the two groups progressively gained less weight per month (Figure 2). This was demonstrated by a highly significant effect of age on the gain in body weight (Table 3). Similar occurrences were observed on beef calves (43), (24).

It seems, therefore, that an adaptive pattern had taken place in the 80° F group of calves due to their long exposure to heat. Their levels of BEI^{131}, blood GSH, and body weight gain tended to approach the corresponding levels of the 50° F groups with advancing age. They attempted to correct for the displacements that had occurred during the short term thermal stress at the beginning of the experiment. The mechanism responsible for this type of acclimation is not yet clarified.

The question remains whether the 80° F reared group would hold this heat acclimation it had acquired when both groups were exposed later on to extremely low followed by high temperature levels. An investigation in this report will be presented in a separate bulletin.

SUMMARY

Blood glutathione (GSH), plasma butanol extract I^{131} (BEI^{131}) and monthly gain in body weight were measured on three breeds of dairy calves during growth (from 6-12 months of age) at 50° and 80° F. All breeds of the 50° F reared group showed statistically significant lower values of GSH and higher levels of BEI^{131} than the 80° F reared group. The 50° F Brown Swiss and Holstein made significantly greater gains in body weight than the corresponding 80° F groups. The all breed values of the GSH, BEI^{131} and monthly weight gain for the 50° F group were respectively 27.23 mg. percent, 25.66 X 10^{-3} percent of dose/100 ml. plasma and 24.53 kg., while for the 80° F group they were 43.22 mg. percent, 14.29 X 10^{-3} percent of dose/100 ml. plasma and 23.06 kg. There were statistically significant breed differences in all measurements except that of weight gain between Brown Swiss and Holstein. At an early age the high temperature had more pronounced effect on the GSH, BEI^{131} and gain in body weight than at an advanced age. The differences between the two groups in GSH and BEI^{131} at early age (sixth month) were 25.22 mg. percent and 18.96 X 10^{-3} percent of dose/100 ml. plasma respectively, while they were only 2.06 mg. percent and 2.00 X 10^{-3} percent of dose/100 ml. plasma respectively at the termination of the experiment (12th month).

REFERENCES

1. Adolph, E. F., and Lawrow, J. W. Acclimatization to Cold Air, Hypothermia and Heat Production in the Golden Hamster. *Am. J. Physiol.,* 166:62. 1951.
2. Asker, A. A., Ragab, M. T., and Ghany, M. A. Some Factors Affecting Heat Tolerance of Cattle and Buffaloes. *Nature,* 171:1074. 1953.
3. Ball, E. G., and Cooper, O. The Oxidation of Reduced Triphosphopyridine Nucleotide as Mediated by Transhydrogenase Reaction and its Inhibition by Thyroxine. *Proc. Nat. Acad. Sci.* 43:357. 1957.

4. Bianca, W. The Effect of Repeated Short Exposures to Heat on the Volume and Hydration of the Blood of the Calf. *Brit. Vet. J.,* 113: 227. 1957.
5. Binet, L. and Poutonnet, M. Maladie D'addison et Glutathionemie. *Ann Endocrinol. Paris,* 4:111. 1943.
6. Blincoe, C. The Influence of Constant Ambient Temperature on the Thyroid Activity and Iodine Metabolism of Shorthorn, Santa Gertrudis, and Brahman Calves During Growth. *Mo. Agr. Exp. Sta. Res. Bul.* 649. 1958.
7. Blincoe, C., and Brody, S. The Influence of Ambient Temperature, Air Velocity, Radiation Intensity and Starvation on Thyroid Activity and Iodide Metabolism in Cattle. *Mo. Agr. Exp. Sta. Res. Bul.,* 576. 1955.
8. Bonsma, J. C. Breeding Cattle for Increased Adaptability to Tropical and Subtropical Environments. *J. Agr. Sci.,* 39:204. 1949.
9. Brody, S. Bioenergetic and Growth. Reinhold Pub. Corp., New York, 1945.
10. Brody, S. Influence of Ambient Temperature, 50 to 100° F, on the Blood Composition of Jersey and Holstein Cows. *Mo. Agr. Exp. Sta. Res. Bul.* 433. 1949.
11. Brody, S., and Frankenbach, R. F. Growth and Development. LIV. Age Changes in Size, Energy Metabolism and Cardio-respiratory Activities of Thyroidectomized Cattle. *Mo. Agr. Exp. Sta. Res. Bul.* 349. 1942.
12. Crook, E. M. Biochemistry of Glutathione. *Nature,* 181:887. 1958.
13. Dale, H. E., Brody, S., and Burge, G. J. Effect of Environmental Temperature Rhythms on Blood and Serum Volumes and Body Water in Dairy Cattle. *Fed. Proc.* 15:43. 1956.
14. Depocas, F., Hart, J. S., and Heroux, O. Energy Metabolism of The White Rat After Acclimation to Warm and Cold Environments. *J. App. Physiol.,* 10:393. 1957.
15. Epstein, D., Cantarow, A., Friedler, G., and Paschkins, K. E. Inhibition of Thyroid Function by Cortisone and ACTH in Hypophysectomized Rats. *Proc. Soc. Exp. Biol. Med.,* 82:50. 1953.
16. Evans, E. S., Contopoulos, A. N. and Simpson, M. E. Hormonal Factors Influencing Calorigenesis. *Endocrinol.,* 60:403. 1957.
17. Flamboe, E. E. and Reineke, E. P. Thyroid Secretion Rates of Lactating and Nonlactating Dairy Goats. *J. Animal Sci.,* 16:1061. 1957.
18. Grunert, P. R. and Philips, P. H. A Modification of the Nitroprusside Method of Analyses for Glutathione. *Arch. Biochem.,* 30:217. 1951.
19. Hart, J. S. Climatic and Temperature Induced Changes in the Energetics of Homeotherms. *Revue Canadienne de Biologie,* 16:133. 1957.
20. Heitman, H., Jr., Kelly, C. F., and Bond, T. E. Ambient Air Temperature and Weight Gain in Swine. *J. Animal Sci.,* 17:62. 1958.
21. Hess, W. C., Kyle, L. H., and Doolan, P. D. Effect of Administration of ACTH and Cortisone Upon Blood Glutathione Levels. *Proc. Soc. Exp. Biol. Med.,* 76:418. 1951.
22. Hill, S. R., Jr., Reiss, R. S., Forsham, P. H. and Thorn, G. W. The Effect of Adrenocorticotrophin and Cortisone on Thyroid Function: Thyroid—Adrenal—Cortical Interrelationships. *J. Clin. Endocrinol.,* 10:1375. 1950.
23. Johnson, H. D. and Ragsdale, A. C., with technical assistance of Delano Robertson. Temperature Effects on Thyroid I^{131} Release Rate of Dairy Calves. Part I. Changes in Thyroid I^{131} Release Rate of During Growth in Holstein, Brown Swiss, and Jersey Calves at Constant Environmental Temperatures (50° and 80° F). Part II. The Effect of Rising Environmental Temperatures (35°-95° F) on Thyroid I^{131} Release Rate of Holstein, Brown Swiss, and Jersey Heifers. *Mo. Agr. Exp. Sta. Res. Bul.,* LIII, 1959. (In press).
24. Johnson, H. D., and Ragsdale, A. C. Effects of Constant Environmental Temperatures of 50° and 80° F on the Growth Responses of Holstein, Brown Swiss, and

Jersey Calves. *Mo. Agr. Exp. Sta. Res. Bul.* (In press).
25. Kibler, H. H. Energy Metabolism and Cardiorespiratory Activities in Shorthorn, Santa Gertrudis, and Brahman Heifers During Growth at 50 and 80° F Temperatures. *Mo. Agr. Exp. Sta. Res. Bul.,* 643. 1957.
26. Kidwell, J. F., Bohman, V. R., Wade, M. A. and Hunter, J. E. The Effect of Nutritional Environment on Blood Glutathione Level in Beef Cattle. *Growth,* 21:1. 1957.
27. Kidwell, J. F., Bohman, J. R., Wade, M. A., and Hunter, J. E. An Investigation of Blood Glutathione Levels in Sheep. *Growth,* 22:63. 1958.
28. Kidwell, J. F., Wade, M. A., and Hunter, J. E. The Relation of Blood Glutathione Level to Growth and Body Size in Beef Cattle. *Growth,* 19:177. 1955.
29. Klemm, G. H., and Robinson, K. W. The Heat Tolerance of Two Breeds of Calves From 1 to 12 Months of Age. *Aust. J. Agr. Res.,* 6:350. 1955.
30. Koger, M. and Turner, C. W. The Effects of Milk Hyperthyroidism on Growing Animals of Four Species. *Mo. Agr. Exp. Sta. Res. Bul.* 377. 1943.
31. Kunkel, H. O., Stutts, E. C., and Shrode, R. R. The Individuality of the Level of Blood Glutathione in Young Beef Cattle. *J. Animal Sci.,* 13:852. 1954.
32. Lazarow, A. Glutathione Potentiation of Cortisone-Induced Glycosuria in the Rat. *Proc. Soc. Exp. Biol. Med.,* 74:702. 1950.
33. Lazarow, A. The Relation of Glutathione to the Diabetogenic Effect of Adrenal Steroids. *Diabetes,* 1:171. 1952.
34. Lazarow, A. Relation of Glutathione to Hormone Action and Diabetes. Glutathione, Academic Press Inc. Pub. N. Y. 1954.
35. Lewis, R. C., Lodge, J. R. and Reineke, E. P. The Thyroxine Secretion Rate of Dairy Calves Estimated by the Extrapolation Technique. *J. Animal Sci.,* 16:1063. 1957.
36. Lewis, R. C. and Ralston, N. P. Protein-Bound Iodine Levels in Dairy Cattle Plasma. *J. Dairy Sci.,* 36:33. 1953.
37. Long, J. F., Gilmore, L. O., Curtis, G. M., and Rife, D. C. Bovine Protein-Bound Iodine and its Relation to Age and Breed. *J. Dairy Sci.,* 35:603. 1952.
38. Mapson, L. W. and Moustafa, E. M. Ascorbic Acid and Glutathione as Respiratory Carriers on the Respiration of Pea Seedlings. *Biochem. J.* (London), 62:248. 1956.
39. Mazia, D. SH and Growth. Glutathione, Academic Press. Inc. Pub., N. Y. 1954.
40. Philips, A. H., and Langdon, R. G. The Influence of Thyroxine and Other Hormones on Heptic TPN-cytochrome Reductase Activity. Biochim. Biophys. ACTA 19:380. 1956.
41. Premachandra, B. N., Pipes, G. W. and Turner, C. W. A Study of Thyroxine Secretion in Cattle. *J. Animal Sci.,* 16:1063. 1957.
42. Racker, E. Glutathione as a Coenzyme in Intermediary Metabolism. Glutathione, Acad. Press, Inc. Pub. N. Y. 1954.
43. Ragsdale, A. C., Cheng, C. S., and Johnson, H. D. Effects of Constant Environmental Temperatures of 50° F and 80° F on the Growth Responses of Brahman, Santa Gertrudis, and Shorthorn Calves. *Mo. Agr. Exp. Sta. Res. Bul.* 642. 1957.
44. Ragsdale, A. C., Thompson, H. J., Worstell, D. M., and Brody, S. Influence of Increasing Temperature 40° to 105° F on Milk Production in Brown Swiss and Brahman Cows and Heifers. *Mo. Agr. Exp. Sta. Res. Bul.* 471. 1951.
45. Rapkine, L. Sur Les Processus Chimiques au Cows de la Division Cellulaire. *Ann. Physiol. Physicochim. Biol.,* 7:382. 1931.
46. Reid, J. T., Ward, G. M. and Salsbury, R. L. Blood Glutathione in The Bovine. *Am. J. Physiol.,* 152:633. 1948.
47. Robertson, W. S., Lennon, H. D., Jr., Bailey, W. W., and Mixner, J. P. Interrelationships among Plasma 17—Hydroxy Corticosteroid Levels, Plasma Protein-

Bound Iodine Levels and Ketosis in Dairy Cattle. *J. Dairy Sci.,* 40:732. 1957.

48. Robinson, S. Physiological Effects of Heat and Cold. *Ann. Rev. Physiol.,* 14:73. 1952.
49. Robinson, S., Turrel, E. S., Belding, H. S., and Horvath, S. M. Rapid Acclimatization to Work in Hot Climates. *Am. J. Physiol.,* 140:168. 1943.
50. Riek, R. F., and Lee, D. H. K. Reactions to Hot Atmosphere of Jersey Cows in Milk. *J. Dairy Res.,* 15:219. 1948.
51. Selye, H. The Physiology and Pathology of Exposure to Stress. ACTA, Inc. Med. Pub., Montreal. 1950.
52. Selye, H., and Heuser, G. Fifth Annual Report on Stress. Med. Pub. Inc., New York, N. Y. 1956.
53. Snedecor, G. W. Statistical Methods. 4th Ed. Iowa State College Press, Ames, Iowa. 1946.
54. Stahl, P. R. and Dale, H. E. Effect of Constant Environmental Temperature and Growth on Blood Volume of Calves. Unpublished data.
55. Stern, H. Sulfhydryl Group, and Cell Division. *Science,* 124:1292. 1956.
56. Szent—Gyorgi, A. Bioenergetics. Academic Press Inc. N. Y. 1957.
57. Turner, C. W., Yammamoto, H., and Ruppert, H. L., Jr. Endocrine Factors Influencing the Intensity of Milk Secretion.-A. Estrogen, Thyroxine, and Growth Hormone, *J. Dairy Sci.,* 40:37. 1957.
58. Verzar, F. and Vidovic, V. The Action of Thyrotrophic Hormone and Cortisone on the Uptake of I^{131} By The Thyroid Gland. *J. Endocrinol.,* 8:321. 1952.
59. Waelsch, H. Glutathione Metabolism in Mammals. *Advances in Enzymology,* 13: 273. 1952.
60. Woodbury, D. M., Ghosh, B. N., and Sayers, G. Modification of TSH Action by ACTH and Cortisone in Hypophysectomized Rats. *J. Clin. Endocrinol.,* 11:761. 1951.
61. Worstell, D. M. and Brody, S. Comparative Physiological Reactions of European and Indian Cattle to Changing Temperature. *Mo. Agr. Exp. Sta. Res. Bul.* 515. 1953.
62. Wyndham, C. H. and Jacobs, G. E. Loss of Acclimatization After Six Days of Work In Cool Conditions On The Surface of a Mine. *J. App. Physiol.,* 11:197. 1957.
63. Zunz, E., Et Vesselovsky, O. Recherches Sur L'Influence de Diverses Hormones Sur la Teneur du Sang en Glutathion I. *Adrenaline et Cortine Enzymologia,* 3:281. 1937.