THE EFFECT OF LOW ENVIRONMENTAL TEMPERATURE ON PLASMA CORTICOSTEROID AND GLUCOSE CONCENTRATIONS IN THE NEWBORN CALF

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SUMMARY

Exposure of newborn Holstein and Jersey calves to −4 °C did not significantly increase the plasma corticosteroids (cortisol and corticosterone) concentrations compared with calves kept at 16 °C. Two Holstein calves exposed to −12 °C showed a slight decrease of plasma corticosteroid concentrations and one Holstein calf at −18 °C responded with a marked increase in both hormones during cold exposure. In the animals at 16 and −4 °C the plasma cortisol and corticosterone concentrations fell steadily during the sampling period. There was also a marked, and almost linear, decrease in the packed cell volume during the sampling period; this occurred in all groups. That this was not due entirely to the withdrawal of blood was shown by a similar decrease in two calves from which only small quantities of blood had been taken. Thus, the decrease in plasma corticosteroids may have resulted to some extent from haemodilution.

An increase in glucose concentration was observed in both the control and cold-exposed calves. There was no correlation between the changes in plasma glucose and plasma corticosteroids.

INTRODUCTION

In 1942, Tyslowitz & Astwood reported that hypophysectomized or adrenalectomized rats were unable to maintain normal body temperature when exposed to an environmental temperature of 0 °C. Subsequently, Levin (1945) showed that the adrenal cortical cholesterol level of cold-exposed rats decreased after 16–22 h but that, after 72 h of cold exposure, the concentration had returned to normal. Long (1947) observed that the depleted adrenal cortical ascorbic acid in rats subjected to 0–4 °C for 1 h returned to normal after 6 h of treatment. In 1955, Heroux found normal cold resistance in adrenalectomized rats previously acclimated to low temperature. The role of the adrenal medulla in cold adaptation was demonstrated by Cottle & Carlson (1956). Zegaya & Chatonnet (1966), in dogs, have shown that both medulla

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and cortex are important in thermogenesis on exposure to cold. Heroux (1969) has suggested that increased adrenal cortical activity is required only during the initial phase of cold adaptation and that noradrenaline is the principal mediator of non-shivering thermogenesis.

The reactivity of the pituitary–adrenal cortical system in the newborn is controversial and appears to be species-dependent (Milkovic & Milkovic, 1969). In calves, Balfour (1953) could not stimulate corticosteroidogenesis by corticotrophin (ACTH) until the 8th or 10th day after birth. This author found that cortisol was the major secretory product in the newborn calf and that corticosterone did not appear until about 10 days of age. Chester Jones, Jarrett, Vinson & Potter (1964) detected both cortisol and corticosterone in the adrenal venous plasma of a 1-day-old lamb though the concentration of corticosterone was low.

The experiments reported here were designed to obtain information on adrenal cortical function and plasma glucose concentration in newborn calves after exposure to low temperature.

**MATERIALS AND METHODS**

*Experimental design.* The 21 animals used were obtained from the dairy herd of Washington State University. All were procured as soon after birth as possible and birth weights were recorded. Upon the arrival of a calf at the experimental quarters, the neck region was clipped, disinfected with ethanol and a 14-gauge, 2-in thin-walled needle inserted centrally into the jugular vein. A 6-in polyethylene catheter (Clay Adams, Intramedic tubing, PE 190) was then directed into the vein through the needle and the needle withdrawn. No anaesthetic was employed. After the withdrawal of the first blood sample, the catheter was filled with heparin solution and plugged until the next blood sampling. Blood samples were collected with heparin as the anticoagulant and the catheter was filled with heparin solution between samples. The absence of colostral ingestion before catheterization was confirmed by the absence of γ-globulin in the initial sample. At catheterization, the calves were given 950 ml of colostrum/27 kg body weight and this was repeated at 8-h intervals up to 48 h. From 48 to 72 h, they were given vitamin-supplemented skim milk.

The animals were placed in the following four treatment groups: (1) seven controls at 16 °C, (2) nine at −4 °C, (3) two at −12 °C and (4) one calf at −18 °C. In addition, two calves, kept at 16 °C, were employed in an experiment in which only small quantities of blood were withdrawn for determination of the packed cell volume.

In all cases, the first sample was obtained upon catheterization. The second sample was withdrawn 4 h and subsequent samples every 8 h until the third day. In three cases, all at −4 °C, the second sample was obtained after 2 h of cold exposure. The time from catheterization to cold exposure varied from ½ to 4 h. In the cold-exposed calves, a final sample was obtained 4 h after transfer from the cold room to 16 °C.

The experimental quarters consisted of two adjacent rooms; the cold chamber was 91 \( \times 121 \times 81 \) ft and could be controlled from −35 ± 2 °C to 45 ± 2 °C, the control room, of approximately the same dimensions, was kept at 16 ± 2 °C. Humidity was 60% for the controls, 25% for the −4 °C group and less than 10% at −12 and −18 °C.

*Chemical determinations.* Fifty ml blood were taken at each sampling. In the two
animals in which only the packed cell volume was determined, less than 25 ml blood was withdrawn daily. A sample of blood was taken for immediate determination of glucose and packed cell volume. The remainder was centrifuged to obtain plasma which was frozen and stored at -20 °C; no systematic study of the long-term stability of plasma corticosteroids in the frozen state was made but such stability has been reported (Weichselbaum & Margraf, 1955; Péron, 1962).

Corticosteroids were determined in duplicate in 226 plasma samples, using an extended fluorimetric procedure similar to that reported by Frankel, Cook, Graber & Nalbandov (1967). Single determinations were made on a further 21 samples (insufficient plasma). Before extraction of each plasma sample, approximately 10,000 c.p.m. of [4-14C]cortisol (sp.act. 53.8 mCi/mmol) and a like amount of [4-14C]corticosterone (sp.act. 52.8 mCi/mmol) were added. An equal aliquot of each radioactive steroid was used to determine the radioactivity added. Plasma samples were washed with petroleum ether (b.p. 30–60 °C) (Moncloa, Péron & Dorfman, 1959), 0.1 N-sodium hydroxide (Silber & Busch, 1956), extracted twice with methylene chloride (Peterson, 1957) and washed twice with distilled water. This extract was dried under nitrogen, redissolved in 10% ethanol: methylene chloride and chromatographed on paper using a benzene: methanol: water system (Bush, 1952). Cortisol and corticosterone standards were carried through the entire procedure simultaneously. Chromatograms were scanned for radioactivity using a 4-pi windowless counter and examined in ultraviolet light to detect the cortisol and corticosterone peaks to be eluted. The $R_F$ values ranged from 0.23 to 0.26 for cortisol and 0.70 to 0.73 for corticosterone while those reported by Bush (1952) in this system were 0.32 and 0.85, respectively. The difference in $R_F$ values was not surprising since many factors cause variable mobility of these compounds (Dominguez, 1967). The cortisol and corticosterone peaks were cut out, eluted with ethanol, dried and redissolved in ethanol. An aliquot was taken for the determination of radioactivity and the remainder dried and redissolved in 65% ethanolic sulphuric acid for fluorimetry. Radioactivity was measured by liquid scintillation spectrometry (Packard Tri-Carb Model 314 DC). Steroid recovery was estimated as the ratio of radioactivity in the eluate to that originally added. After 45 min development in the 65% ethanolic sulphuric acid, cortisol or corticosterone fluorescence was read in a Model 110 Turner fluorimeter (primary filters 48 + 48 + 3; secondary filters 2-A 15) and steroid concentration determined from a standard curve. The concentration of each sample was adjusted for recovery of its radioactive cortisol and corticosterone. For the entire series of samples ($n = 247$), the recovery of added radioactive cortisol and corticosterone was 69.9 ± 3.4 (s.d.) and 77.7 ± 15.1%, respectively. In two experiments, in duplicate, various amounts of cortisol and corticosterone were added to calf plasma and the corticosteroid concentrations were determined. Recovery was linear and the concentration of endogenous cortisol averaged 5.7 μg/100 ml.

Plasma glucose was determined by a glucose oxidase method (Mager & Farese, 1965). Packed cell volume (PCV) was determined by the capillary tube technique of Guest & Siler (1934).
RESULTS

Effect of cold exposure on corticosteroid concentrations

Since there were no significant or consistent differences in corticosteroid concentration between Jersey and Holstein calves the results from the two breeds were pooled. Examination of the results from the control (16 °C) group showed that both the cortisol and corticosterone values were lower in the morning than in the afternoon or evening but the differences were insignificant.

Table 1. Effect of low temperature on plasma corticosteroids, blood glucose and packed cell volume in newborn calves (means ± S.E.)

<table>
<thead>
<tr>
<th>Treatment (°C.)</th>
<th>Value after cannulation</th>
<th>Hours of treatment</th>
<th>Value after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1-12</td>
<td>13-36</td>
</tr>
<tr>
<td>Cortisol (mg/100 ml)</td>
<td>9.20 ± 0.65 (18)*</td>
<td>7.43 ± 1.15 (9)</td>
<td>6.85 ± 1.7 (6)</td>
</tr>
<tr>
<td>Corticosterone (mg/100 ml)</td>
<td>1.29 ± 0.16 (18)</td>
<td>1.68 ± 0.38 (8)</td>
<td>1.15 ± 0.33 (6)</td>
</tr>
<tr>
<td>Glucose (mg/100 ml)</td>
<td>70.4 ± 4.7 (19)</td>
<td>70.5 ± 6.4 (9)</td>
<td>98.6 ± 7.8 (7)</td>
</tr>
<tr>
<td>Packed cell volume (%)</td>
<td>43.0 ± 1.7 (12)</td>
<td>35.2 ± 4.7 (5)</td>
<td>30.2 ± 5.1 (4)</td>
</tr>
</tbody>
</table>

* The temperature before and after exposure to cold was 16 °C. Number of animals in parentheses.

Because of the large individual and intragroup variation, the effect of cold exposure was assessed by coding the data into time intervals of low-temperature treatment. Five intervals were used: cannulation; 0–12 h in the cold; 13–36 h in the cold; 37–72 h in the cold and values after return to 16 °C. These results are shown in Table 1; the cannulation measurements and the results after return to 16 °C are shown as overall means since all animals, regardless of treatment, were kept at 16 °C at these times. A nearly linear decline in both cortisol and corticosterone concentration was observed in both control and the −4 °C groups. There appeared to be a slight increase in cortisol concentration during the first 36 h of exposure to −4 °C, but no significant difference was found between the two groups when individual time intervals were examined. The decreasing corticosteroid concentration and the similarity between the control and the calves kept at −4 °C is particularly evident when the data are coded into 8 h after treatment intervals. This is shown in Fig. 1 in which the first bar represents the mean cannulation value for the group and no values obtained after exposure to cold are given.

In both the control and −4 °C animals, the cortisol concentration in samples obtained at cannulation was significantly higher (P < 0.01) than that in samples
obtained between 36 and 72 h or in samples obtained after 72 h. There was a similar decrease in corticosterone but the difference between the concentration at cannulation and that of the 36–72 h time interval was significant only in the − 4 °C group.

Fig. 1. Concentrations of cortisol and corticosterone in the plasma and blood glucose concentrations in newborn calves exposed to different environmental temperature.

Individually, 11 out of the 15 calves in these two groups had increased cortisol levels at cannulation as compared with their overall mean cortisol concentration. In six instances, recannulation was necessary; this procedure was accompanied with increased cortisol values in three out of the six cases. Of ten samples collected during...
attacks of moderate diarrhoea, only two showed an increased cortisol concentration. However, a possible association of sickness with corticosteroid concentration was observed in one calf exposed to \(-4^\circ C\); this animal was listless and had to be given colostrum by stomach tube for the first 48 h after cold exposure. Blood samples taken during this period showed markedly increased cortisol levels. In the two calves exposed to \(-12^\circ C\), cortisol concentration decreased from cannulation to 36 h and the values were lower than those observed in the control or \(-4^\circ C\) groups. From 36 to 72 h the cortisol level increased until the concentration in the three groups was similar. No statistical evaluation was attempted because of the small number of animals. As in the other groups, corticosterone concentration approximately paralleled that of cortisol.

The concentration of both corticosteroids was high after exposure of one calf to \(-18^\circ C\). In this animal, the high cannulation value (Fig. 1) may have been due to an unusually long delay between birth and colostrum administration (12 h). This degree of cold exposure was judged to be too severe (frozen ears, weakness, distress, diarrhoea) and was not repeated.

**Effect of cold exposure on glucose concentration**

Hyperglycaemia was observed in all four treatment groups during the sampling period and occurred in 16 out of the 19 calves studied. There was a significant \((P < 0.01)\) increase when either cannulation or 1–12 h means were compared with 13–26 h samples in the 16 or \(-4^\circ C\) groups or when cannulation means were compared with 37–72 h values in the 16 \(^\circ C\) calves. Hyperglycaemia also occurred in the animals exposed to \(-12^\circ C\) and \(-18^\circ C\); in these three animals, blood glucose concentrations reached higher levels than in the control or \(-4^\circ C\) groups. Hyperglycaemia was not maintained as long in the \(-4^\circ C\) group and in the calf exposed to \(-18^\circ C\) as in the controls or the two calves kept at \(12^\circ C\). As with the corticosteroids, there was no difference between Jersey and Holstein calves.

**Packed cell volume**

The PCV decreased from the first to the last sample in all animals. That this was due only in part to the removal of blood was indicated by the results obtained in two calves subjected to the same experimental procedure as the controls except that small blood samples only were withdrawn, for assessment of the PCV. In these two animals the PCV decreased from 41.5 to 29.5% after 72 h as compared with a decrease from 43.0 to 23.8% in the experimental calves.

**DISCUSSION**

The corticosteroid concentrations found in this study cannot be compared with published values since no report has been found in which peripheral plasma from newborn calves has been analysed for corticosteroids. The concentrations reported by us are lower than those found by Venkataseshu & Estergreen (1970) in lactating, non-pregnant dairy cows but our cortisol concentrations are higher than those reported by Lindner (1964) for adult cattle. Balfour (1953) reported that corticosterone did not appear in the adrenal venous blood during the first 10 days after birth. In our
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experience, there were only a few instances in which corticosterone was below the limit of detection of the method used. This may be due to the use of a fluorimetric procedure which is four times more sensitive for corticosterone than for cortisol on a \( \mu g \) basis. On the other hand, corticosterone concentration in peripheral plasma should be considerably less than that in adrenal effluent.

The higher concentration of cortisol at the time of cannulation may be attributed to increased maternal levels at parturition, the stress of cannulation, handling, transport and delay in food intake, or to haemodilution during the sampling period.

In cattle, the maternal corticosteroid concentration during the last days of pregnancy has been reported as decreased (Paterson, 1957; Shaw, Dutta & Nichols, 1960) or only slightly increased (Brush, 1958). The report of Chester Jones et al. (1964) indicates that the syndesmochorial placenta may not be permeable to corticosteroids.

In our experiments, recannulation did not consistently elevate the cortisol concentration and there was no significant correlation \( r = 0.09 \) between initial concentration and the number of hours from birth to first feeding. However, it is well known that environmental changes result in a rapid adrenal cortical response in sheep (Reid & Mills, 1962). In this species, cold stress (2–3 °C) did not detectably increase the concentration of corticosteroids and the adrenal response to cold combined with wind and rain was delayed as compared with that elicited by movement to an unfamiliar environment (Reid, 1962).

The decreased PCV found during the first 72 h of life, even in two calves from which very small quantities of blood were withdrawn, suggests an increase in plasma volume during this period. Thus, the consistently decreasing blood cortisol and corticosterone values might result from dilution alone. This would imply that the rate of adrenal cortical secretion did not increase and that the pituitary–adrenal system of the newborn calf was relatively unresponsive to peripheral corticosteroid concentration.

The marked adrenal cortical response in the one calf subjected to severe cold stress (−18°C) as compared to the lack of response in the calves kept at −4 °C and the apparently negative response in the two calves exposed to −12 °C is similar to the findings of Nelson, Egdaahl & Hume (1956) and Egdaahl & Richards (1956) in the dog. The former authors reported no consistent or significant increased secretion of 17-hydroxycorticosteroids during exposure to −10 °C and the latter authors found marked adrenocortical stimulation upon exposure to very low temperatures (−46° to −50 ° and −75 ° to −79 °). The results in this calf furnish evidence, however limited, that the adrenal cortex of the newborn calf can respond to stress.

Increases of corticosteroid concentration were observed in some samples in the absence of a known cause. Such non-specific increases have been reported by others (Nelson et al. 1956). Whatever the cause, these ‘bursts’ were responsible for the considerable variations encountered. It is possible that steroid catabolism and excretion, as well as production and secretion, are not stabilized in the newborn animal. In the newborn rabbit, Sereni, Castegnaro, Perletti & Sala (1965) have observed a deficiency of the liver enzyme reducing the A ring and the 20-oxo group of cortisol.

The rise in blood glucose concentration during cold exposures or after cannulation may be a result of adrenaline released from the medulla in response to non-specific stress or a specific response to cold. In the 16 and −4 °C group the similarity in blood glucose concentrations indicate that hyperglycaemia resulted from non-specific
stress. However, in the two calves exposed to \(-12^\circ\text{C}\), hyperglycaemia occurred earlier and the blood glucose increased to a higher level; it is probable that in these two animals cold stress was a factor in the hyperglycaemic response. The results of Leduc (1961) suggest that increased adrenaline secretion upon cold exposure occurs only after the maximum secretion rate of noradrenaline is attained and that either a greater intensity or longer duration of cold stress will evoke the adrenaline response. Hypoglycaemia was found before cold exposure (Fig. 1) in the calf subjected to \(-18^\circ\text{C}\); this was probably due to the 12 h delay between birth and feeding. Table 1 and Fig. 1 show that there was no correlation between blood glucose and plasma cortisol or corticosterone.

REFERENCES


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