CHANGES IN THE ACID-SOLUBLE NUCLEOTIDE COMPOSITION OF RED CELLS OF CATTLE AT VARIOUS AGES

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SUMMARY: The free nucleotide content of red blood cells from heifers was determined by column chromatography on the day of birth and at 1, 3, 6, and 12 months. Uric acid riboside, which was relatively low on the day of birth, was the major ultraviolet-absorbing compound in the nucleotide pool at 3 months and at the later sampling times. The concentration of UDPglucose, UDP-N-acetylglucosamine, ATP, GTP, and UTP decreased between the day of birth and 1 month and between 1 month and 3 months. The concentrations of these nucleotides were the same at 6 and 12 months as they were at 3 months. The concentrations of NAD, AMP, UMP, and IMP also decreased with time; the concentration of NADP was practically constant.

Although UAR[1] was reported to be the major ultraviolet-absorbing compound in the erythrocytes of cattle (1, 2), UAR was not present in the blood of cattle fetuses (3). The red blood cells of fetuses of cattle also contained higher levels of ATP, UTP, GTP, UDPglucose, and UDP-N-acetylglucosamine than red cells from the mature animals (3). The present study was conducted to ascertain the nucleotide composition of red blood cells of newborn calves and to determine when the changes in levels of UAR and other nucleotides occur.

METHODS: Three female calves that were produced artificially with semen from Simmental bulls and out of crossbred Charolais x British cows were used in this experiment. The calves were born between May 24 and June 3, 1975. On the day of birth and when the calves were 1, 3, 6, and 12 months old, 20 to 40 ml of blood was collected from the jugular vein in tubes containing EDTA. The blood was cooled in an ice bath and in less than 30 min it was taken to the laboratory. There it was immediately centrifuged in a 40 ml centrifuge tube at 2300 g for 10 min in a model RC-3 Sorvall centrifuge with a no. HG-4L swinging bucket head. The red cells were washed twice with 0.9% sodium chloride and the supernatant solution removed by aspiration; on the last wash, the buffy coat of white cells was removed. Washed red cells (5 to 15 ml) were added to 10 volumes of ice-cold

[1]Abbreviation ; UAR, uric acid riboside

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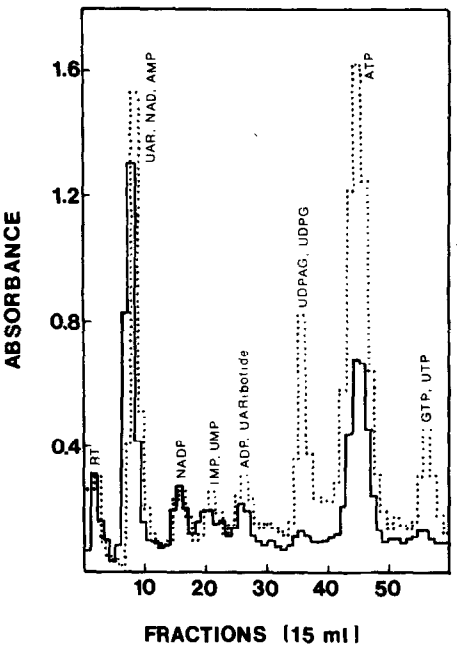

FIG. 1. Column chromatography of the cold TCA-soluble fraction from 15 ml of red blood cells from a newborn calf (·····) and from a 6 month old heifer (———). The runthrough (RT) is the material not absorbed by the column. The absorbance was measured at 260 nm.

6% trichloroacetic acid and after 10 min, the extract was centrifuged. The supernatant solution was extracted 5 times with equal volumes of cold diethyl ether saturated with water to remove the trichloroacetic acid. The ether remaining in the samples was removed by aeration, the pH adjusted to 7 to 9 with 1 N NH_4OH, and the samples lyophilized. Once dried, the samples were dissolved in 10 to 20 ml of water, diluted, and the spectra determined with a Beckman model 25 recording spectrophotometer. The remainder of the sample was applied to a Dowex-1 column from which the nucleotides were quantitated and identified (2, 3). Typical elution patterns of the nucleotides from red blood cells of a heifer on the day of birth and at 6 months are shown in Fig. 1. Treatment means were compared using Student's t-test.

RESULTS AND DISCUSSION: The ultraviolet absorbance spectrum of the trichloroacetic acid-soluble portion of the red blood cells of newborn calves had a maximum at 259 nm with a shoulder at approximately 298 nm (Fig. 2). When the heifers were 1 month old, there were absorbance maxima at 259 and 298 nm. At 3, 6, and 12 months, the absorbance maxima at 298 nm had increased and the absorbance maxima at 259 nm had decreased.

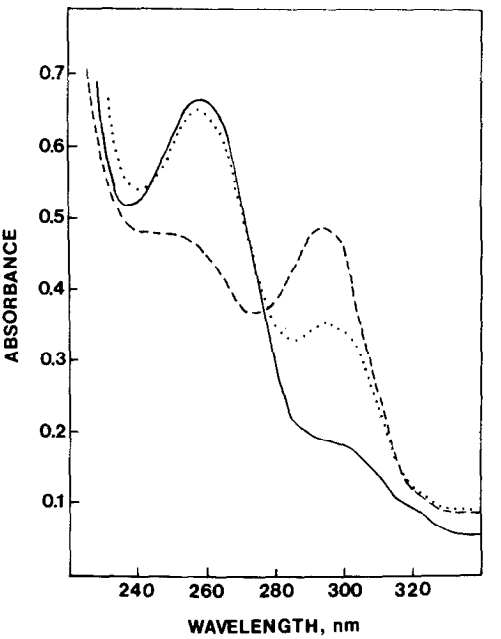

FIG. 2. Ultraviolet absorption spectra of the cold TCA-soluble fraction of blood from a newborn calf (———); at 1 month (·····); and 6 months (---).

Only the spectrum at 6 months is given; the spectra at 3 and 12 months were similar.

The changes in the absorbance spectra of the extracts of the red cells were reflected in the changes that occurred in the nucleotide composition of the cells (Table 1). The quantity of UAR was low in the samples from the newborn calves and increased from day 1 to 1 month and from 1 month to 3 months after which the content of UAR was unchanged. Because of the large amounts of UAR present in the fractions that contained NAD and AMP, it was difficult to obtain good quantitative data on NAD and AMP unless they were also chromatographed on paper. However, the concentration of both of these compounds decreased as the age of the animals increased.

The concentration of NADP in the red cells was the same at all sampling times. The concentration of IMP and UMP was the same the first 4 sampling times, but neither was detected at 12 months. Uric acid ribotide was not

TABLE 1

ACID-SOLUBLE NUCLEOTIDE COMPOSITION OF BLOOD OF HEIFERS

(μMOLES PER 100 ML RED BLOOD CELLS)*

NUCLEOTIDE	AGE				
	1 DAY	1 MONTH	3 MONTHS	6 MONTHS	12 MONTHS
UAR	7.96±2.10[a]**	28.17±2.77[b]	55.60±10.14[c]	51.73±3.16[c]	56.56±4.51[c]
NAD	10.36±0.77[a]	11.48±1.37[a]	8.90±1.92[a]	5.54±0.05[b]	6.04±0.47[b]
AMP	3.80±0.29	N.Q.	N.Q.	N.Q.	N.Q.
NADP	1.83±0.17[a]	1.99±0.20[a]	1.89±0.76[a]	3.02±0.55[a]	2.85±0.45[a]
IMP+UMP	4.69±0.44[a]	3.72±0.48[a]	3.50±1.79[a]	4.64±1.37[a]	N.D.
URIC ACID RIBOTIDE	N.D.	trace	trace	trace	trace
ADP	5.06±1.91[a]	5.40±1.55[a]	2.89±0.17[a]	2.28±0.54[a]	2.50±0.31[a]
UDPG+UDPAG	17.97±3.20[a]	11.71±1.87[b]	0.96±0.11[c]	0.65±0.35[c]	0.89±0.45[c]
ATP	40.28±2.33[a]	29.52±1.11[b]	16.38±2.10[c]	18.21±0.40[c]	17.04±1.25[c]
GTP+UTP	9.42±1.66[a]	3.34±0.78[b]	1.10±0.31[c]	1.03±0.39[c]	0.79±0.15[c]

*Values represent means ± standard errors.
**Mean in each row with different superscript letters are significantly different, $p<.05$. N.D., not detected, N.Q., not quantitated.

detected on day 1, but traces of it were found at the other sampling times. ADP appeared to decrease as the animals aged, although as reported previously, the ADP values were more variable than most of the nucleotides and the means were not significantly different.

The concentration of UDPglucose and UDP-N-acetylglucosamine decreased about 35% between day 1 and 1 month. By 3 months the level of UDPglucose and UDP-N-acetylglucosamine was about 5% of the value at day 1. Mandel (4) has described the nucleotides of muscle and red blood cells as the energetic type where adenine nucleotides predominate and the nucleotides of liver, brain, and spleen as the metabolic type with an abundance of nucleoside diphosphate anhydrides. The red blood cells of the newborn calves had high levels of UDPglucose and UDP-N-acetylglucosamine and resemble the metabolic type of cells. In the red cells of older cattle, with the exception of UAR, the adenine nucleotides predominate and resemble the energetic type of tissue.

ATP decreased about 25% from day 1 to 1 month, and then decreased to about 50% of the value at day 1. The level of ATP has been reported to be higher in younger human erythrocytes compared to older erythrocytes (4, 5).

UTP and GTP decreased about 65% from day 1 to 1 month and at 3 months had decreased to about 10% of the level of day 1. Brown et al. (6) reported that higher order species of animals had low levels of GTP and no UTP; lower order species had high levels of GTP and detectable amounts of UTP. The concentration of both GTP and UTP were high in both newborn calves and in calves at 1 month of age, but reached much lower levels by 3 months of age.

The nucleotide content of blood from cattle fetuses is markedly different from that of blood of mature cattle (3). The present study has shown that the nucleotide composition of the red cells of newborn calves is different from that of older animals. Most of the changes in

amounts of these nucleotides had taken place by the time the cattle were 3 months old. The significance of the changes in levels of the nucleotides is unknown. Further studies are necessary to determine whether these changes in nucleotide content are related in any way to maintenance of the shape of the cell or of the binding of oxygen to hemoglobin (5).

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