CHANGES IN THE NUCLEIC ACID CONTENT IN THE BLOOD OF CATTLE AFFECTED WITH LEUKOSIS. I. DNA AND RNA LEVELS IN THE BLOOD OF HEALTHY CATTLE REPRESENTING DIFFERENT AGE GROUPS

The Veterinary Institute, Puławy
Department of Cellular Pathology
Head: Professor Dr. M. Grundboeck

MARIAN GRUNDBOECK, KRYSTYNA WILCZYŃSKA-CIEMIĘGA

It was established that in 2-12-year-old healthy cattle the nucleic acid content in the blood dropped with age whereby the DNA level difference between the youngest and the oldest age groups amounted to about 40%, that of RNA to 27%. The nucleic acid contents in one leukocyte showed no essential differeces depending on the age of animals. The DNA concentration in leukocytes was, on the average, 5 times higher than that of RNA. The determinations performed constitute a basis for the study of changes in the level of nucleic acids characteristic of leukosis.

A precise determination of the range of physiological changes in the level of nucleic acids in the blood of cattle affected with leukosis constitutes a basic condition for the estimation of the diagnostic value of this level. The determination of DNA and RNA contents in the blood cells or other cells of healthy cattle was the subject of numerous studies the results of which, together with the appropriate values with reference to humans, are presented in Tables 1 and 2.

The first part of this study aims at a precise determination of the dynamics of changes in the DNA and RNA levels in the blood depending on the age of the cattle. It also aims at the determination of the variance coefficient of DNA and RNA levels in the blood of individual animals and comparing that value with analogous ones concerning the dispersion of the number of leukocytes. It is generally known that a small dispersion of the parameter in considered to be a feature which increases the usefulness of this parameter in estimating deviations from the norm.

Material and Methods

One hundred and fifty three 2-12-year-old Friesian-Holstein cows were used in this study. They belonged to herds free from tuberculosis, brucellosis and leukosis, and they showed no manifestations of other diseases. The animals' blood was taken from the jugular vein and put into tubes containing heparin. From 20-ml samples leukocytes were separated by Weinhold's method (10). DNA and RNA were separated by Schmidt and Thannhauser's method (7) with the additional use of perchloric acid (Tsanev and Markov, 8). Also, following Glen (4), extraction of the material with fat solvents was abandoned and magnesium chloride was used to facilitate RNA precipitation.

In the obtained extracts DNA and RNA concentrations were determined by the use of Unicam SP 1700 spectrophotometer with SP 1805 programme controller. The DNA and RNA concentrations were determined in UV at 260 nm, path length 1 cm. The standard curves were determined by the use of pure DNA (L. Light,

Table 1

DNA or P-DNA contents in cells of blood and of some organs in cattle and man

Species (and kind of sample)	Tissue (organ)	Contents of the studied compound in 1 cell		Authors	Reference
		in units adopted by authors	$\times 10^{-9}$ DNA		
cattle:					
Fres.-Holst.	blood	0.584×10^{-6} µg P-DNA	5.84	Zielińska	12
Charolaise	„	0.611×10^{-6} µg P-DNA	6.11	„	12
Red Polish	„	0.615×10^{-6} µg P-DNA	6.15	„	12
cattle	„	6.97×10^{-9} mg DNA	6.97	Mandel et al.	5
cattle (calves)	thymus	7.15×10^{-9} mg DNA	7.15	Mirsky and Ris	6
cattle	lymph nodes	7.03×10^{-9} mg DNA	7.03	„ „	6
„	kidney	6.90×10^{-9} mg DNA	6.90	Vendrely	9
„	muscles	6.40×10^{-9} mg DNA	6.40	„	9
„	pancreas	6.90×10^{-9} mg DNA	6.90	„	9
„	somatic cells	5.9—6.9 pg DNA	5.9—6.9	Bielka	1
man	blood	0.73 pg P-DNA	7.3	Brückner	2
„	blood (lymphoc.)	7.37×10^{-9} mg DNA	7.37	Glen	4

Table 2

Contents of P-RNA and RNA in the blood cells in cattle and in man

Species and race	Kind of cell	P-RNA concentration in 1 cell	Recalculation to RNA $\times 10^{-9}$ mg	Author	Reference
cattle:					
Fries.-Holst.	leukocytes	0.173×10^{-6} µg	1.73	Zielińska	12
Charolaise	„	0.190×10^{-6} µg	1.90	„	12
Red Polish	„	0.134×10^{-6} µg	1.34	„	12
man	„	0.25 pg	2.5	Brückner	2
„	blood lymphoc.	0.19×10^{-6} µg	1.9	Glen	4
„	„ „	0.15×10^{-6} µg	1.5	Wojcierowski	11
„	„ „	0.24×10^{-6} µg	2.4	Davidson et al.	3

England) and yeast RNA (British Drug Houses Ltd). The DNA absorption curve (Fig. 1) was almost identical with that of RNA. On the basis of individual estimations, the mean values, standard deviations and variation coefficients were calculated for all age groups separately.

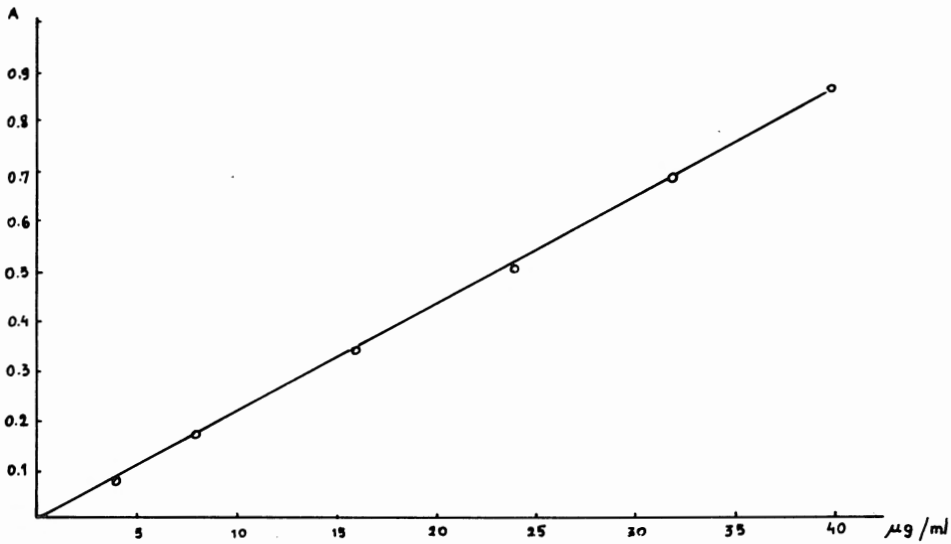

Fig. 1. Absorbance curve of DNA. λ = 260 nm, d = 1 cm

Results

The levels of DNA in the blood of cattle ranged from 2.30 to 9.50 mg/100 ml and, as seen in Table 3, decreased with age. The mean DNA level in animals above the age of 7 was about 40% lower than that of 2-3-year-old animals. The DNA content in 1 leukocyte showed a range of 3.70 — 9.96 $\times$ 10^{-9} mg. The mean values of this parameter, as calculated for the individual age groups, did not reveal any essential differences. The lowest values were found in 3-4-year-old animals.

The levels of RNA in the blood of the individual animals ranged from 0.39 to 1.87 mg/100 ml and, as in the case of DNA, tended to decrease in the older age groups. The mean RNA content of single leukocytes ranged from 0.71 to 2.25$\times$$10^{-9}$ mg and did not reveal any dependence on the animals' age. The DNA concentration in leukocytes was, on the average, 5 times higher than that of RNA.

As follows from Table 3, both the DNA and the RNA levels in the blood showed a somewhat higher coefficient of variance than the amount of leukocytes, which is indicative of a greater dispersion of the results.

Discussion

Comparing the mean DNA content in leukocytes established in the present study with appropriate values obtained by other authors and presented in Table 1, the values are found to be concurrent. The mean

RNA concentration in the white blood cells of the studied material is comparable to the results obtained by Zielińska (12), allowances having to be made only for our somewhat lower values than hers in Friesian--Holstein cattle and higher than those in Red Polish cattle. The RNA/DNA ratio calculated in our study was very similar to that found by Zielińska. The differences connected with the age of animals appeared to be very small in our experiment in comparison with those demonstrated by Zielińska.

Table 3

DNA and RNA contents in 100 ml of blood and in 1 leukocyte in cattle under normal conditions

Age of animals in years	2 — 3	3 — 4	4 — 5	5 — 6	6 — 7	7
Number of animals	22	24	29	21	28	29
DNA contents mg/100 ml of blood						
$\bar{x}$	7.15	5.61	4.84	4.95	4.62	4.40
S	2.23	1.39	1.12	1.07	1.65	1.52
W	31.6	26.4	25.2	22.9	38.2	36.6
DNA contents $\times 10^{-9}$ mg/leukocyte						
$\bar{x}$	7.55	6.33	6.80	6.93	7.15	7.07
S	1.21	0.91	1.02	0.94	0.99	0.93
W	16.5	15.6	16.3	14.1	14.2	13.9
RNA contents mg/100 ml of blood						
$\bar{x}$	1.25	1.18	1.00	1.03	0.98	0.91
S	0.22	0.19	0.19	0.17	0.29	0.22
W	33.2	29.6	21.5	30.6	32.1	25.2
RNA contents $\times 10^{-9}$ mg/leukocyte						
$\bar{x}$	1.40	1.38	1.44	1.46	1.53	1.46
S	0.35	0.33	0.33	0.27	0.29	0.23
W	26.2	26.4	25.0	20.1	18.8	17.0
RNA/DNA						
$\bar{x}$	0.19	0.22	0.22	0.21	0.21	0.21
S	0.05	0.06	0.06	0.04	0.04	0.03
W	27.3	30.9	29.6	19.6	21.1	17.1
Number of leukocytes in 1 μl of blood						
$\bar{x}$	9.340	8.900	7.120	7.300	6.500	6.330
S	2.000	1.910	1.270	1.200	1.280	1.540
W	22.8	23.0	19.1	17.5	20.7	25.6

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