

# CHANGES IN THE NUCLEIC ACID CONTENT IN THE BLOOD OF CATTLE AFFECTED WITH LEUKOSIS. II. DNA AND RNA LEVELS IN THE BLOOD OF COWS AFFECTED WITH SUBCLINICAL FORM OF THE DISEASE

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A statistically significant 2-3-fold increase of the nucleic acid content was found in the blood of cows with a subclinical form of leukosis. The nucleic acid level in 1 leukocyte was in the affected animals somewhat lower than in healthy ones, which most clearly manifested itself in the DNA level in older animals. The RNA/DNA ratio was almost double in over 6-year-old animals affected with leukosis. At the assumed top value of the normal range of  $\bar{x}+2S$ , it was found that in animals haematologically positive according to the Göttingen key, the DNA level showed unsuspected values in 40%, the RNA level in 5% of the cases.

The nucleic acid content in the blood of cattle affected with leukosis was subject to studies by a number of investigators who used either chemical (2, 3, 4) or cytochemical methods (6). In those studies as a rule an increase of the level of nucleic acids was found primarily as a consequence of the increased number of leukocytes. Most of the researchers, however, found in animals affected with leukosis a drop in the values of nucleic acids per 1 leukocyte. In the studies carried out so far no statistical data have been provided to indicate the significance of the differences between affected and healthy animals. Neither are any indications available as to how or in which cases such statistical data might be utilized for diagnostic purposes in leukosis. It is the aim of this study to fill the gap in this respect as well as to determine the diagnostic values of the DNA and RNA levels in the blood. As a result, it will be possible to decide whether in the diagnosis of leukosis it is advisable to replace or supplement morphological studies with nucleic acid determinations.

## Material and Methods

Forty Friesian-Holstein cows, aged 2—12 were used in the study. The cows originated from leukosis isolation units. In all the animals leukosis was diagnosed haematologically by the use of the Göttingen key, although no clinical symptoms were found in any of them. The number of leukocytes ranged from 11,100 to 58,800 per ml of the blood.

The methods of taking the blood samples, of isolating the leukocytes and determining the levels of nucleic acids were described in Part I of the study (1). The animals used for that part of the study constituted the control group to serve as comparative material.

## Results

As seen in Table 2, the mean content of nucleic acids in the blood of animals affected with a subclinical form of leukosis was 2—3 times higher than in the cattle of the control group of the same age. By the use of Student's *t* test it was found that the differences in the levels of the compounds in healthy and affected animals were statistically significant in all age groups. A difference of higher significance was found for RNA ( $p < 0.001$ ). It is worth noting that the number of leukocytes in those animals was 3—4 times higher than the normal level.

The mean DNA concentration per 1 leukocyte in the studied 2-4-year-old animals was close to that of comparable animals of the control group, while in the upper age groups it was a little below the normal level. The RNA concentration per cell in affected animals was, on the whole, slightly lower than in healthy ones. In the younger age groups the RNA/DNA ratio was similar to that of healthy animals, while in the two oldest age groups it almost doubled as compared to the control group.

## Discussion

The obtained results indicate that in subclinical leukosis in cattle a statistically significant increase of the DNA and RNA concentrations in the blood is found. The diagnostic value of this phenomenon can be judged precisely by confronting the increase of the leukocyte level with the number of normal standard deviations above the mean normal value. The Göttingen key for leukosis (Tolle, 8) rests on the principle that the sum of the mean normal value and the double standard deviation is considered to mark the lowest degree of suspicion of leukosis ( $\bar{x} + 2S$ ), while the lowest degree of the range of leukosis is constituted by the analogous value of  $\bar{x} + 4S$ . Using these criteria for the evaluation of the DNA levels established in this study, it was found that the investigated animals haematologically positive according to the Göttingen key showed unsuspected values in 16 cases (40%) and doubtful values in 3 cases (8%). The increase of the RNA level showed a greater concurrence with the Göttingen key, as only 2 cases (5%) remained within the normal range and 5 cases (13%) showed suspected values. In the rest of the cases the evaluation of the nucleic acid contents, similarly to the lymphocytosis judged according to the Göttingen key, led to the diagnosis of leukosis.

When comparing the results of the present study with those of Soviet authors (2, 3, 4), considerable differences in the contents of nucleic acids in the blood and in individual cells, particularly in the control groups,

Table 1  
DNA and RNA contents in the blood of cattle affected with leukosis and in control animals

State of animal	Leuko- cytes in 1 $\mu$ l of blood	Contents in blood		Contents in 1 leukocyte *		Authors	Refe- rence
		DNA mg%	RNA mg%	DNA $\times 10^{-9}$ mg	RNA $\times 10^{-9}$ mg		
Lymphatic leukosis	54,500	33.4	37.0	6.13	6.79	Klimov, Koromyslov	2
Haemocytoblastosis	72,000	47.0	46.4	6.53	6.44	"	
Lymphosarcoma	30,800	26.5	32.5	8.60	10.55	"	"
Reticulosarcoma	20,500	15.8	20.5	7.71	15.32	"	
Healthy animals	7,100	10.5	21.3	14.79	30.00	"	"
Lymphatic leukosis stage I	41,700	31.2	29.8	7.48	7.15	Kudriavceva et al.	
Lymphatic leukosis stage II	96,900	46.9	46.5	4.84	4.80	"	
Haemocytoblastosis stage I	41,700	29.9	29.5	7.17	7.07	"	"
stage II	195,500	125.3	83.3	6.41	4.26	"	
Lymphosarcoma stage I	32,300	27.7	33.2	8.58	10.28	"	"
Healthy animals	9,200	14.7	28.4	15.98	30.87	"	
Lymphatic leukosis	63,500	38.7	57.8	6.09	9.10	Koromyslov	3
Haemocytoblastosis	70,000	44.9	49.3	6.41	7.04	"	
Lymphosarcoma	29,500	25.1	31.9	8.51	10.81	"	"
Reticulosarcoma	27,300	20.7	33.1	7.58	12.12	"	
Healthy animals	6,500	14.4	32.9	22.20	50.62	"	"

\* — The DNA and RNA values per 1 leukocyte were not given in the quoted items of literature, but were established by the authors of this study.

Table 2  
Nucleic acid contents in the blood and in 1 leukocyte in cows affected with leukosis and comparison of these values with analogous ones in control cattle (L : C)

Age of animals in years	2—3	3—4	4—5	5—6	6—7	> 7
Number of animals	3	6	10	8	7	6
DNA contents mg/100 ml of blood L : C	19.0 ± 7.1 2.7	16.5 ± 8.1 2.9	14.7 ± 3.2 3.1	15.6 ± 9.8 3.2	11.0 ± 6.4 2.4	10.1 ± 5.7 2.3
DNA contents × 10 <sup>-9</sup> mg/leukocyte L : C	7.7 ± 0.9 1.0	6.4 ± 2.5 1.0	5.4 ± 2.6 0.8	6.1 ± 3.8 0.9	5.9 ± 3.2 0.8	5.0 ± 2.6 0.7
RNA contents mg/100 ml of blood L : C	3.0 ± 0.4 2.4	2.6 ± 0.6 2.2	3.0 ± 0.1 3.0	2.6 ± 0.9 2.5	2.6 ± 0.8 2.6	2.7 ± 0.7 3.0
RNA contents × 10 <sup>-9</sup> mg/leukocyte L : C	1.3 ± 0.3 0.9	1.1 ± 0.2 0.8	1.3 ± 0.5 0.9	1.1 ± 0.3 0.7	1.6 ± 0.7 1.1	1.3 ± 0.2 0.9
RNA : DNA L : C	0.19 ± 0.05 1.0	0.23 ± 0.16 1.1	0.33 ± 0.18 1.5	0.28 ± 0.19 1.3	0.38 ± 0.25 1.8	0.38 ± 0.20 1.9
Number of leukocytes in 1 µl of blood × 10 <sup>-3</sup> L : C	26.5 ± 6.1 2.8	27.3 ± 4.1 3.1	27.9 ± 10.00 3.9	30.2 ± 15.3 4.1	21.2 ± 9.6 3.1	22.4 ± 4.5 3.5

became apparent. Yet, similarly as the Soviet studies, the present report indicated a tendency for the DNA and RNA concentrations in 1 leukocyte to decrease in affected animals. A comparable phenomenon was observed also with reference to leukosis in humans (Seitz, 5). Stöber (16), using a semiquantitative method in cytochemical studies, demonstrated an increase of the RNA content in the lymphocytes of animals affected with leukosis. Yet, because of the considerable dispersion, a part of the RNA value as established by the author for affected animals remained below the normal range of variance.

The leukocytes of affected animals are more delicate and decompose more easily (7), nevertheless the lower level of nucleic acids in the cells of affected animals should first of all be ascribed to metabolic changes and not to the loss of the compounds during the process of isolation.

The obtained results indicate that estimates of the nucleic acid contents in the blood may have some importance for the diagnosis of leukosis, although — since it is very laborious the method cannot be widely used in regular practice. The established changes in the nucleic acid contents in individual leukocytes are indicative of metabolic disturbances in the cell which are associated with leukosis. The findings should be enlarged by studies embracing clinical forms of leukosis.

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