CHANGES IN THE MACROMOLECULAR STRUCTURE OF CHROMATIN IN HEMATOPOIETIC TISSUE OF BALB /c MICE IN THE PERIOD OF INDUCTION OF RAUSCHER'S LEUKEMIA

N. A. Grigorovich

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In the early stages after infection of BALB/c mice with Rauscher's virus, changes occur in the normal quaternary structure of the chromatin isolated from the hematopoietic tissue of the animals. Later the tertiary structure of the chromatin is disturbed.

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The writer has previously shown that the macromolecular structure of chromatin is disturbed in the hematopoietic tissue of mice exposed to the action of radiation or steroid hormones in doses giving the greatest leukemogenic effect [3, 4]. It appeared interesting, therefore, to examine the state of the macromolecular structure of the chromatin during the development of leukemia induced by a leukemogenic virus, because there is no information of this type in the literature [5].

The object of this investigation was to study the macromolecular structure of the chromatin of hematopoietic tissue of BALB/c mice infected with Rauscher's virus, which induces leukemia 2-4 weeks after inoculation [13].

EXPERIMENTAL METHOD

Experiments were carried out on 140 BALB/c mice of both sexes, weighing 20-22 g. The course of the virus was a cell-free extract of the spleen of mice with Rauscher's leukemia, prepared by Rauscher's method [10, 13]. The animals were inoculated by intraperitoneal injection of 0.2 ml of the extract per mouse.

The state of the macromolecular structure of the chromatin was judged from the results of a study of its rheological properties, by Spitkovskii's method. Observations were made on the formation and relaxation of regularly arranged chromatin structures [2, 7]. The technique of isolation of chromatin from the hematopoietic tissue (the spleen) of the mice and the conditions under which its rheological properties were studied remained the same as in previous investigations [4]. The mice were sacrificed 4 h and 1, 2, 4, 7, and 14 days after inoculation with the leukemogenic virus. For each test material was taken from 5 animals. The results of the observations were subjected to statistical analysis by methods for small samples.

EXPERIMENTAL RESULTS

The results illustrated in Fig. 1 show that by the 2nd day after inoculation of the BALB/c mice with Rauscher's virus the weight of their spleen had increased by 36%, and on the 4th day by 53%. Seven days after inoculation of the mice the weight of their spleen was more than 2.5 times greater, and 14 days after almost 5.5 times greater, than the weight of this organ in healthy animals.

The results given in Fig. 2 show the initial length of the regularly arranged structures formed from the same volume of chromatin solution of equal concentration. It follows from these results that the regularly arranged structures formed from chromatin of the spleen of mice taken into the experiment at

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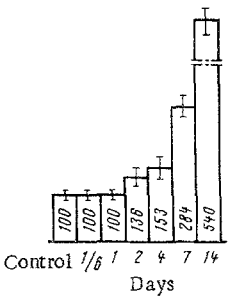

Fig. 1. Changes in weight of spleen of BALB/c mice (in % of initial weight) after inoculation with Rauscher's virus.

different times after inoculation with Rauscher's virus had the same initial length as the corresponding structures from the splenic chromatin of healthy mice. Only the regularly arranged structures from the splenic chromatin of mice sacrificed 4 h after inoculation were slightly shorter than the original length ($P < 0.05$).

Once it had begun, the process of high-elastic deformation of the regularly arranged structures formed from splenic chromatin of mice followed a different course depending on the time after inoculation with the virus that the animals were sacrificed. It follows from the curves given in Fig. 3 that the process of relaxation of the regularly arranged structures formed from the splenic chromatin of mice used in the tests 4 h and 2 days after inoculation with virus-containing material followed the same course as in the corresponding structures formed from splenic chromatin of healthy animals. Relaxation took place most intensively in the first 6 min. During this time the length of the regularly arranged structures was reduced by 35%. From the 6th to the 10th minute the threads shortened by a further 2-3%, while their length at the 10th minute of observation was 39% less than initially (Fig. 3, curves 1, 2, 4).

High-elastic deformation of the regularly arranged structures formed from splenic chromatin of mice tested 1 and 4 days after inoculation with Rauscher's virus was less marked. The dynamics of their relaxation was identical in character. As will be seen from curves 3 and 5, shown in Fig. 3, relaxation of the regularly arranged structures took place most intensively in the first 5 min. During this time their length was reduced by 28-29%. From the 6th to the 10th minute these structures were shortened by a further 4%, so that by the end of the observation their length was 33% less than initially. The regularly arranged structures formed from splenic chromatin of healthy mice were shortened by 39% during this period. These differences are statistically significant.

Seven and 14 days after inoculation of the mice with leukemogenic virus the rheologic properties of the chromatin isolated from splenic tissue likewise were disturbed (Fig. 3, curves 6 and 7). However, these disturbances differed in character from those in the earlier periods, and were manifested as an increase in the high-elastic deformation of the regularly arranged structures.

The macromolecular structures formed from splenic chromatin of mice tested 7 and 14 days after inoculation with Rauscher's virus were more intensively relaxed than those formed from the splenic chromatin of healthy animals. During the first 6 min their length was reduced by 40%. The structures formed from splenic chromatin of healthy mice shortened by 35% during this time (Fig. 3, curves 1, 6, and 7). By the 10th minute the length of the macromolecular structures formed from the splenic chromatin of the experimental animals was reduced by 43-44%. The regularly arranged structures formed from chromatin of the splenic tissue of healthy animals, as a result of relaxation during this period, shortened its length by 39%. The differences described above in the kinetics of high-elastic deformation of the macromolecular structures formed from chromatin of the hematopoietic tissue of experimental and control animals are statistically significant.

These investigations thus showed that in BALB/c mice the development of leukemia induced by Rauscher's RNA-containing virus is preceded, and subsequently is accompanied, by changes in the rheologic properties of the chromatin of the hematopoietic tissue. Induction of leukemia by Rauscher's virus in the period immediately after infection of the animals is accompanied by changes in the quaternary structure of the chromatin which are probably due to the appearance of additional cross-linkages between the DNP molecules in the chromatin. In the later periods no changes in rheologic properties of the chromatin indicative of changes in its quaternary structure can be detected.

Changes in the rheologic properties of the chromatin of the hematopoietic tissue of the mice during this period was most probably due to changes in its tertiary structure, reflecting a disturbance of the bond between DNA and protein in the DNP molecules and a change in the conformation of the DNA in the nucleoprotein. The decrease in the degree of high-elastic deformation of the macromolecular structures formed from the splenic chromatin of mice sacrificed on the 1st and 4th days after inoculation with the virus is most probably due to a decrease in the number of bonds between the DNA strands and protein in the DNP

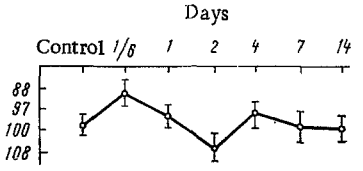

Fig. 2

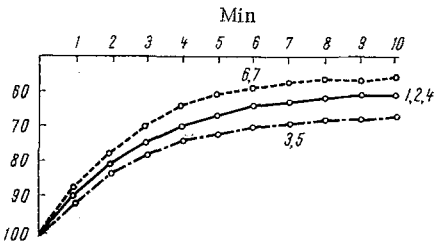

Fig. 3

Fig. 2. Initial length of regularly arranged structures formed from splenic chromatin of BALB/c mice tested at various times after inoculation with Rauscher's virus. Abscissa, time of observation; here and in Fig. 3, ordinate: length of regularly arranged structures (in % of initial length K).

Fig. 3. Dynamics of high-elastic deformation of regularly arranged structures formed from splenic chromatin of BALB/c mice tested at various times after inoculation with Rauscher's virus: 1, 2, 4) curves of relaxation of regularly arranged structures from splenic chromatin of healthy animals and of animals tested 4 h and 2 days after inoculation; 3, 5) the same 1 and 4 days after; 5, 7) the same 7 and 14 days after.

molecule, and to "restoration of the native type" of double-helical structure of the DNA in the DNP molecule. The increase in the degree of high-elastic deformation of the macromolecular structures formed from splenic chromatin of the mice tested 7 and 14 days after inoculation with the virus, in the period of increased proliferation of the hematopoietic tissue, must be due to increased flexibility of the DNA and DNP, probably in connection with an increase in the degree of denaturation of the DNA in the chromatin and to an increase in the number of bonds between the DNA strands and protein in the DNP molecule [1, 2, 6, 8].

It has recently been shown that the RNA of Rauscher's virus possesses loci which are homologous to those of the DNA of mouse hematopoietic tissue [11]. It can therefore be postulated that the changes described above in the macromolecular structure of chromatin of the hematopoietic tissue of mice after inoculation of the animals with Rauscher's virus reflect different stages of interaction between viral and cell genomes. The conformation changes in the macromolecular structure of chromatin of mouse hematopoietic tissue in the early period of induction of leukemia by Rauscher's virus may be genetically important [9, 12], and they may be directly related to the transformation of normal cells of hematopoietic tissue into leukemic cells.

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