The Nuclear Configuration of Leucocytes in Males in Prepuberty

By

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Davidson and Smith [3] have shown that the nuclear configuration of the polymorphonuclear neutrophil leucocytes constitutes a valuable tool for the determination of true genetic sex. A person in whose blood picture at least six out of 500 segmented leucocytes contain the sex chromatin types A and B has to be considered a female. The determination is more reliable if not only the number of forms A and B but also that of C is taken into account because the latter type is 3 to 4 times more frequent in males than in females. Kosenow established the following formula for the determination of the female sex:

$$\frac{A+B}{C} \geqq 0.3$$

The factors influencing, and the phenomena associated with the frequency of the three chromatin forms have repeatedly been investigated. There appears to exist a connection between lobe count and the number of these forms: hypersegmentation means a higher, hyposegmentation a lower frequency of all of the three types [3, 7, 8, 9, 14]. It follows that the lobe count must not be disregarded when making comparative examinations. It has been demonstrated by Harnack and Strietzel [4] that the number of sex chromatins diminishes both absolutely and with reference to 500 mature cells if the number of immature leucocytes exceeds 25 per cent.

The lobe count is so low in Down's syndrome [15] and Pelger's nuclear anomaly [10] that the blood smear may not allow the determination of sex chromatins. Lüers and Struck [9] showed that constitutional hypersegmentation was associated with a higher frequency of sex chromatins in goats and sheep, and likewise a constitutional low lobe count with a lower frequency of the nuclear appendages in swine.

Harnack and Strietzel [4] were the first to show that, irrespective of sex, all of the three forms were more frequent in childhood than at any later age of life. Kosenow [8] found that the number of both the sex chromatins and the C forms decreased after the first postnatal week, and that the number of the former was no

longer related to age in later life, while that of the latter became subsequently higher than in childhood.

MÉHES et al. [12, 13] found in the rat that the number of *A* and *B* forms remained unchanged, whereas that of type *C* gradually increased from birth to sexual maturity. The rate of increase could be accelerated by means of androgens and decelerated by the administration of progesterone. The number of type *C* chromatin dropped to the newborn level in castrated male animals.

The number of forms *A* and *B* is not influenced either by hormonal factors or changes in the leucocyte count [1, 4, 6, 8, 13, 15].

The purpose of the present study was to ascertain whether changes in the number of type *C* chromatins was hormonally regulated in humans; since, however, literature contains contradictory data regarding the number of these structures and also because the available reports offer no information as to the age at which their number begins to increase, it seemed necessary to elucidate these questions.

Material and Method

Fifty-two boys and 34 girls between 1 and 14 years of age, all clinically healthy, and 20 adult males and 20 adult females (healthy volunteers of the hospital staff and blood donors) and, in addition, 21 young male patients admitted on account of endocrine disorders (9 with hypogonadotrophic hypogonadism and 12 with prepuberty basophilism) were examined.

Results

It can be seen from the results listed in Table I that in the boys both the mean and the extreme values of the type *C* structures gradually increased with advancing age and reached the adult level between 10 and 14 years, whereas no such increase was observed in the girls.

The figures in Table I show a wide scattering, but with Student's *t*-test the differences between the increases in the male age groups 4 to 10 and 10 to 14 years were significant statistically, $t = 2.64$, even at the 1 per cent level of confidence; the corresponding value for the male age group of 4 to 10 years and the male adult group was 1.93, significant statistically at the 5 per cent level.

Table II shows a comparison of KOSENOW's [6, 7, 8], BURGOLD and SPREER's [2] and our own data. Except those in respect of the female age group 4 to 10 years, KOSENOW's data are essentially different from ours. Our data regarding adults are more in harmony with those of BURGOLD and SPREER, but it should be noted that these authors did not regard "small-club" structures as belonging to type *C*.

Our data obtained in humans are in good agreement with those of MÉHES et al. [12, 13], established in rats. The incidence of *C* chromatins was 12 per cent in sexually mature male rats against 12.4 per cent in adult men, and 3 to 4 per cent in female rats against 2.54 per cent in adult human females.

TABLE I

C chromatin count in children (1 to 14 years) and adults of both sexes, in 500 segmented leucocytes

	Age	No	Average $\bar{x}$	Scattering s	Mean deviation $s_{\bar{x}}$	Extreme values
♂	1—4 years	12	21.33	7.75	2.23	12—42
	4—10 „	20	41.75	26.26	5.77	10—99
	10—14 „	20	66.72	31.13	6.96	16—118
	Adults	20	62.20	37.86	8.44	19—128
♀	1—4 years	12	9.16	6.08	1.75	0—22
	4—10 „	10	15.00	8.06	2.55	5—26
	10—14 „	12	14.58	10.44	3.00	0—28
	Adults	20	12.7	8.94	2.00	0—28

TABLE II

Mean and extreme values of C chromatin counts according to age

	Age	Kosenow		Own data		Burgold	
		Average $\bar{x}$	Extreme values	Average $\bar{x}$	Extreme values	Average $\bar{x}$	Extreme values
♂	1 week	79.9	14—142				
	2 weeks—1 year ...	43.5	8—110				
	1—4 year			21.33	12—42		
	4—1 „	27.4	6—61	41.7	10—99		
	10—14 „			66.7	16—118		
	Adults	42.0	7—111	62.2	19—128	70.6	16—137
♀	1 week	40.8	19—65				
	2 weeks—1 year	23.6	4—64				
	1—4 year			9.16	0—22		
	4—10 „	11.9	0—24	15.0	5—26		
	10—14 „			14.5	0—28		
	Adults	37.3	10—57	12.7	0—28	9.3	0—48

It has been claimed by MÉHES et al. that the changes in the *C* chromatin number are regulated by sex hormones in the male rat. They have, however, failed in demonstrating a regulative influence of the pituitary and the adrenal in their experiments.

We determined the number of type *C* chromatins in 21 patients with endocrine disorders. Results are illustrated in Fig. 1, from which it is evident that the number of *C* chromatins was near or less than the lowest control value in cases of hypogonadism, while it was near or above the highest control value in case of basophilism. There was only one case of hypogonadism in which the observed value surpassed the normal mean value of the patient's age group, while it was near the lowest limit in 6, and still lower in 2, cases. As regards the group of basophilic children, in 9 cases the number of *C* chromatins was more than the extreme value corresponding to the given age groups, in 3 cases it was above and in one case below, the normal mean. The results, as

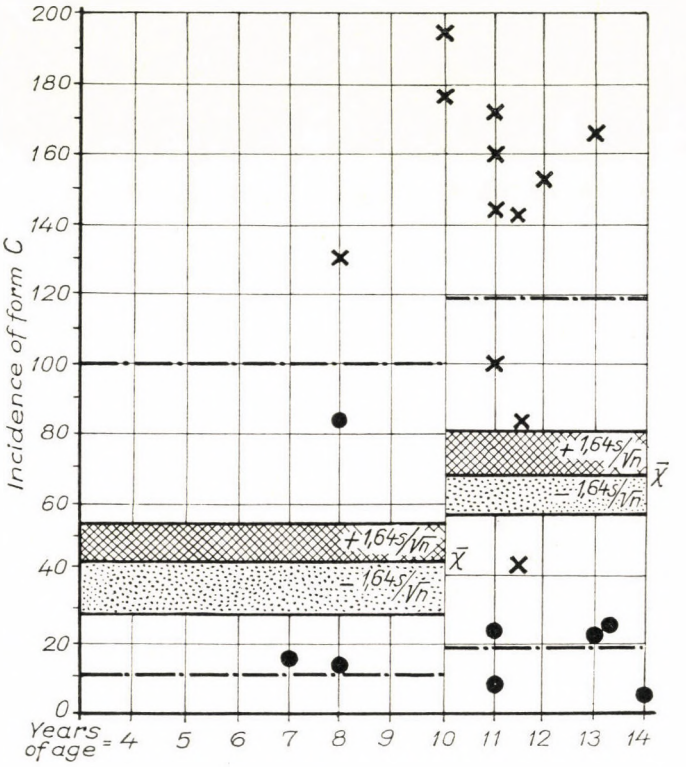

FIG. 1. Number of C forms in pituitary hypofunction (●) and prepuberty basophilism (x)

shown in Fig. 1, allow the conclusion that the nuclear configuration is influenced by the pituitary especially by the behaviour of the pituitary basophils. Hypofunction is accompanied by a decrease, hyperfunction by an increase, in the number of *C* chromatins.

A comparison of the results obtained in the controls with those observed in the diseased children admits of the inference that an increase in the *C*-count in prepuberty is due to a hypophysial change, presumably a change in the activity of the hypothalamo-hypophysial apparatus.

Although the examination of nuclear configurations promises to represent a valuable aid in sex determination, its reliability requires a further elucidation of hormonal mechanisms.

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Summary

The possible correlation of age with the number of type *C* chromatin in polymorphonuclear leucocytes has been studied. The amount of *C* forms was found gradually to increase in male children from the age of one year and to reach the adult level in prepuberty. No similar change has been observed in female children. Examination of patients with endocrine disorders revealed that the number of *C* forms was below normal in hypogonadotrophic hypogonadism, and above normal in cases of pituitary basophilism.

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