CYTOGENETIC INVESTIGATIONS IN SOME STATES OF PSEUDOHERMAPHRODITISM

M. T. Tzoneva-Maneva, E. Bozadjieva, B. Petrov

The progress achieved in the study of chromosomes in man and their numerical and structural aberrations threw new light upon conceptions on the nature of certain diseases in humans.

In this respect, the results of the studies on the aberrations of sex chromosomes constitute the basis for a new classification of the various gonada, dysgeneses and intersexual states (15). Among the latter, the testicular dysgenesia or Kleinfelter syndrome, encountered in 2,06‰ of newborn boys (15)- occupies an important place.

This syndrome was originally described in 1942 by Kleinfelter and assoc., and is characterized by gynekomastia, small testes, lack of spermatogenesis despite intact Leydig's cells and hyalinization of the seminiferous tubules, elevated urinary pituitary gonadotropin and insufficient development of secondary sexual signs (8). However, Reifenstein (1964) underlines the heredofamilial incidence of the affection (8). Subsequently, in some of the patients, presenting the clinical picture just described, chromatin positive nuclei have been found. The latter finding justifies the subdivision of the syndrome in "true" and "false" form with inclusion of the former in female pseudohermaphroditism. Plunkett and Barr, on the other hand, assumed that in "true" Kleinfelter's syndrome aberrations are concerned of the type-XXY sexual chromosomes (8). This supposition was supported for the first time by the investigations of Jacobs and Strong (1959) and Ford and assoc. (1959). Thus, modern cytogenetic investigations on the "true" Kleinfelter justified considering the latter as dysgenesia of the testes (15).

In the first part of the work, we follow up the incidence of this aberration in patients with primary hypogonadism, exhibiting in part of them, a clinical picture similar ot that of the Kleinfelter syndrome.

The investigations are carried out on a series, comprising 13 male patients, hospitalized at the Higher Medical Institute — Varna during 1964. Twelve of them are recruits, postponed from military service because of negative (subnormal) indices of physical fitness and one (K. A. S., № 2, table I) — a labour conscript. In addition to the routine clinical examinations, a spermogram was performed in all patients and accordingly, 17KS and 17-KGS traced. The urinary pituitary gonadotropin was not investigated nor was biopsy of testes made on account of refusal of the patients.

For the chromosome analysis of the patients metaphase plates were utilized from the cultivated in vitro lymphocytes of the peripheral blood, according to a personal modification of the method, described by Moorhead and associates.

Table 1

No.	Name	Weight in kg.	Height in cm	Length of arm extension	Length of testes mm	Consistency of testicles	Sex Hair	Gynecomastia	Osteal age	Intelect	Blood cholesterol	P in blood	17 KS mg/24 h.
1	D.M.B.	48,5	158	166	17	dense	Well pronounced	—	normal	oligophrenia	136	—	22,44
2	K.A.S.	53	168	171	22	"	Feminine type, absent in the face and axillae	+	"	poor	136	4,34	2,20
3	M.Y.A.		169	169	35	"	Masculine type, absent in the face	—	—	mediocre	160	4,65	2,70
4	M.A.A.	48	160	164	25	"	Feminine type	+	—	"	109	5,89	2,86
5	N.N.Sh.		148	149	18	"	Weakly manifested, absent in the face and axillae	—	—	fair	152	5,27	11,0
6	Sn.K.S.		147	158	normal	"	"	+	—	mediocre		4,65	20,0
7	S.M.T.	59	173	175	50	"	"	—	—	fair	144	4,96	16,0
8	L.H.H.	38	139	139	25	"	"	—	delayed milk teeth	mediocre	144	6,20	8,9
9	T.S.D.	44	165	168	10	"	"	—	delayed	good	128	8,20	2,97
10	T.S.T.	45	156	158	35	"	"	—	"	fair	158	—	7,58
11	K.K.T.	52	163	179	15	"	"	—	"	excellent	79	—	—
12	S.R.P.	51	145	—	38	"	"	+	"	fair	200	4,65	—
13	B.S.M.	53	167	173	20	"	"	+	—	"	147	4,34	3,84

Table 1 illustrates the results of investigating the basic clinical indices in the individuals of the series reviewed with hypogonadism, manifested in various degrees.

Table 2

№	Name	Sex	Age	Number of chromosomes					Total number cells	Number Karyograms	Karyotype
				less 45	45	46	47	more 47			
1	D.M.B.	m	22	3	5	9	82	1	100	7	44+XY(44+XXY)44+XXXY
2	K.A.S.	m	20	1	2	6	88	3	100	8	44+XY(44+XXY) 44+XXXY
3	M.Y.A.	m	19	2	—	19	1	—	22	5	44+XY,44+XXY — translocation of the lower arm of X over chromosome 3
4	M.A.A.	m	19	1	2	40	—	—	43	7	44+XY
5	M.N.Sh.	m	18	—	6	43	—	—	49	7	44+XY, 44+XY with chromosome breaks and presence of fragments
6	S.K.S.	m	19	—	—	6	—	—	6	1	44+XY
7	S.M.T.	m	19	—	—	6	—	—	6	2	44+XY
8	L.H.H.	m	19	—	—	6	—	—	6	2	44+XY
9	T.S.D.	m	18	6	—	94	—	—	100	9	44+XY, chromosomic breaks
10	T.S.T.	m	17	—	—	20	—	—	20	4	44+XY
11	K.K.T.	m	23	1	—	20	—	—	21	3	44+XY
12	S.R.P.	m		—	—	22	—	—	22	2	44+XY
13	B.S.M.	m	19	—	3	42	—	—	45	6	44+XY
14	K.L.M.	f	9	—	4	55	9	2	70	11	44+XO(44+XX)(44+XXX) 44+XXXX
15	M.P.S.	f	40	—	—	16	28	with 48—17 more 48—13	74	4	44+XXX(44+XX)44+ +XXXX

Table 2 presents an account of the results of the cytogenetic investigations carried out on 13 cases with varying degree manifestation of hypogonadism in males and two ases with congenital adrenal cortical hyperplasia.

The lymphocytic mitotic activity (mitosis) was stimulated with the preparation phaseosaxin (phytohemagglutinin form P obtained from a Bulgarian sort Ph vulgaris).

The cells were stained according to Romanovski-Gimsa. The chromosome number was made on photocopies or by means of drawing under 1000 X magnification. The structure of the chromosomes was analysed in the constructed karyograms from the photocopies.

The results of the clinical investigations are summarised in table 1, and the results of cytogenetic studies — in table 2.

The clinical analysis of the results of our investigations shows that the stature of the patients is average or low-height. In some of them eunuchoid proportions are noted. Secondary morpholcgic criteria of sex are weakly pronounced, of varying degree. The voice of the patients is different — ranging from childish to normal male voice. The male organ size is most varying. The size of testes likewise, displays a wide range of variety. Absence of ejaculation is observed in two instances (L. H. H. № 8 — table 1 and K. K. T. № 11 — table 1), whereas azoospermia — in all the remainder. The facial skin is usually wrinkled and on the extremities — marmoreal. The osseous age in three patients is normal and in the remainder — retarded. The urinary 17-ketosteroids in the majority of the patients are

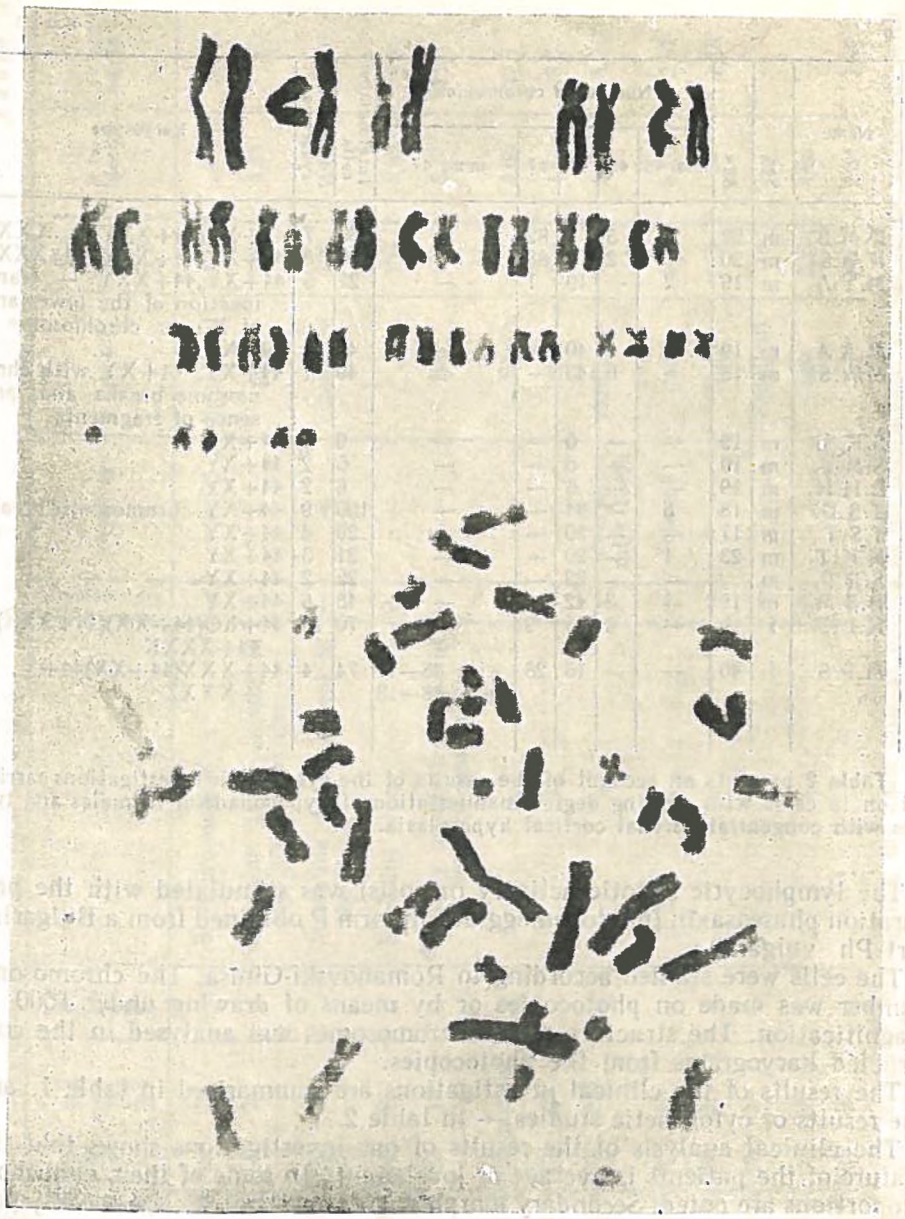

Fig. 1. Cell in metraphase from K. A. S. with karyogram 2n=44+XXY

low or at the lowermost limit of the normal value. The basal metabolic rate in most of the patients is depressed. Nevertheless, in all the patients of the series, the blood cholesterol is lowered or at the lower limit of the normal value,

Fig. 2. Cell in metaphase with karyogram 2n=74+XY

The serum phosphorus, on the contrary, is at the top limit of the normal value or exceeds it. A great number of authors state (1, 9) that the Leydig's cells in the Kleinfelter syndrome, although undergoing hyperplasia, show histologic and histiochemical signs of reduced functional activity and

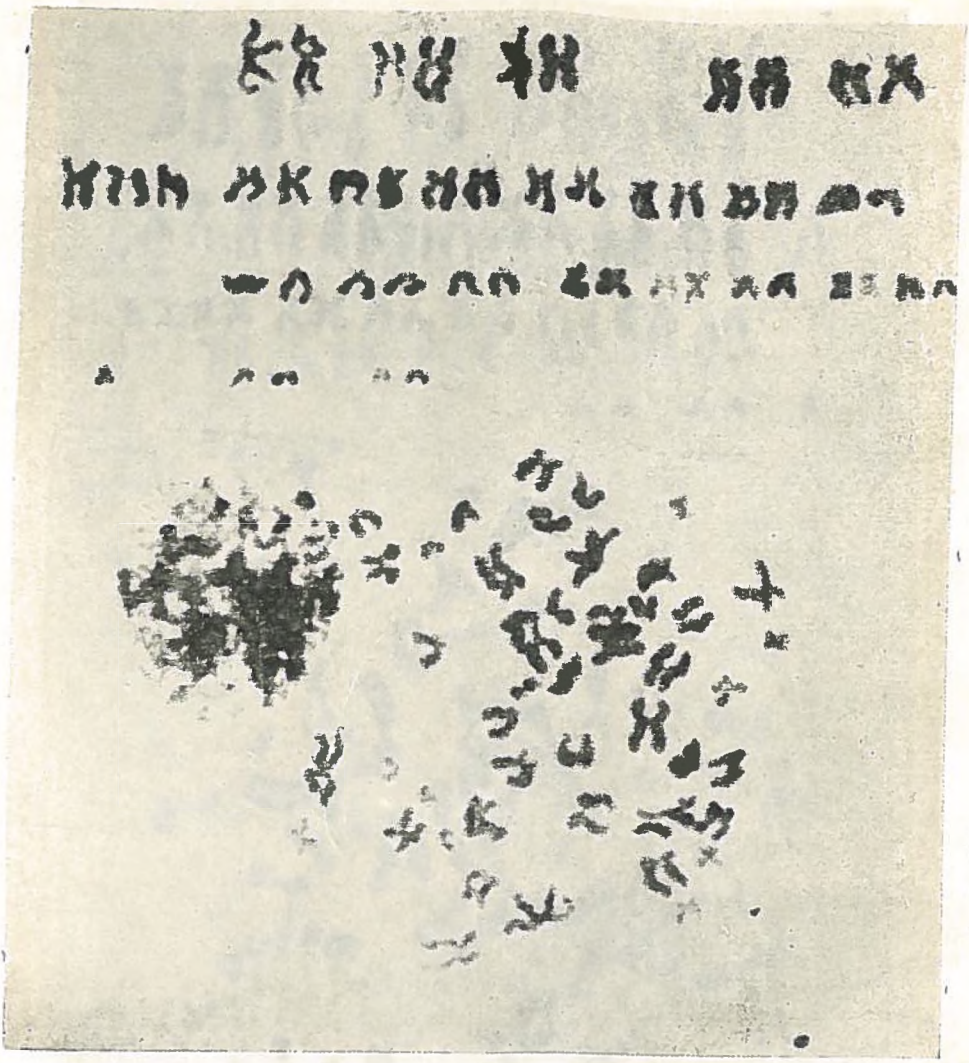

Fig. 3. Cell in metaphase from D. M. B. with karyogram 2n=44+XXXY

more particularly reduced lipid and cholesterol content. Whether such a reduction of lipids and cholesterol, in particular, is related to the total cholesterol reduction in blood and to the lowered urinary 17-ketostero ds, is rather difficult to be stated definitely for the time being; it is beyond doubt, any way, that in the latter case a disorder is concerned of the cholesterol metabolism.

The familial nature of the disease is noted in T. S. D. (table 1) whereas in patient B. C. D. it is only suspected; one of his elder brothers is unmarried, whereas the others are childless. Anosmia is not established in any of the patients of the series reviewed. Changes in the eye ground are not found.

Macroscopically gynecomastia is observed in five cases, mostly pronounced in K. A. S. (table 1). In two cases (M. Y. A. and S. M. T.) digital pterygium is noted. In other two patients (N. A. A. and S. M. T. — table 1) linea albea is pigmented — a finding which could be interpreted as a sign of certain degree adrenal insufficiency.

The histological changes in the testes and the increased urinary gonadotropins are still considered the most reliable criteria for the diagnosis of the Kleinfelter's syndrome. In the opinion of most of the authors, gynecomastia is not an invariably found symptom, whereas according to others (9), it is present (marked or microscopic), in all cases, its etiology being rather obscure. According to Heller and Nelson (cited by 9), the habitus in the Kleinfelter syndrome could be either eunuchoid, moderately eunuchoid or normal. It is stressed by Stewart that the osseous age likewise might be dependent on the androgenic production within the organism and even in eunuchoid tyre of the Kleinfelter, the epiphyseal lines might disappear in due time (13).

As stated by the vast majority of authors, the azoospermia, small testes as well as elevated urinary gonadotropin might be lacking, depending on the degree and speed of evolution of hyalinization processes within the testes. Similar deviations are observed in 5 per cent of the patients with Kleinfelter's syndrome.

Unlike eunuchoidism, in which the urinary 17-ketosteroid is elevated, in the Kleinfelter's syndrome the urinary 17-ketosteroid, which is known to be of adrenal origin, is low or at the lowermost limit of the normal value (9).

As already stressed, determination of the urinary gonadotropins and biopsy of testes in our series have not been carried out. Yet, the presence of tiny compact testes in the majority of individuals with low or average stature, azoospermia, low level of urinary 17-ketosteroids provided sufficient ground (in some of the patients) to accept and in others — merely to suspect the Kleinfelter's syndrome (9). Probably, only in L. H. H. №8 — table 2) a primary lesion of hypophysis is concerned with involvement of the growth hormone and secondary hypogonadism (7).

The results of the cytogenetic investigation (table 2) revealed the characteristic for the Kleinfelter syndrome karyotyre $2n = 44+XXY$ in two subjects — № 1 (82% of the cells) and № 2 (88% of the cells) see table 2, fig. 1. With them, however, cellular clones are observed, although in relativey small percentage, with $2n = 44+XY$ (fig. 2) and $2n = XX+XXXY$ chromosomes (fig. 3). The latter phenomenon is indicative for the nondisjunction susceptibility during cell division in these individuals. Mosaicism is noted in № 3 also, but with modal number $2n = 44+XY$ chromosomes and single cells with $44+XXY$ chromosomes. In subject № 3 (table 2) we established abnormally large lower arms of one of the chromosomes № 3, with shortened lower arms of one X chromosome. In all likelihood a translocation of the X/3 fragment is concerned (fig. 4). Van den Berghe described a case of hypogonadism with abnormal karyotype-translocation of the chromosome 2 fragment over the chromosome Y (16). Chromosome breaks and chromatid fragments are established in individuals № № 5 and 9. The limited number of cells investigated from the subjects 6, 7 and 8 does not warrant the discarding of mosaicism XY/XXY in them. The results obtained by the study are based on karyotype examination wi-

Fig. 4. Cell in metaphase from M. Y. A. with karyogram. The large lower arms of one of the chromosomes № 3 and shortened lower arms of the X chromosome are seen. Probably, translocation of the X/3 chromosome

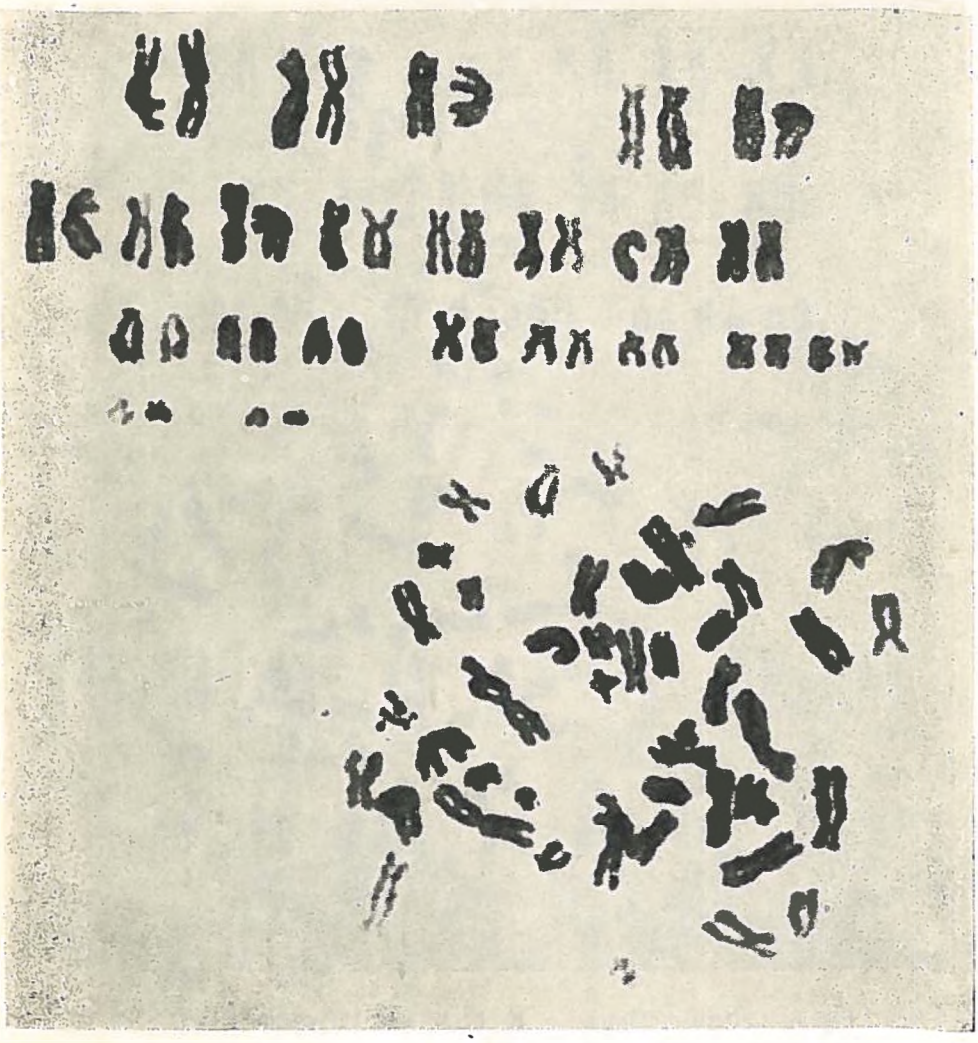

Fig. 5. Cell in metaphase from K. L. M. and karyogram 2N=44+XX

ıhin the cells of a single tissue and aberrations are not excluded in the karyotype in other tissues of the individuals with the karyotype, established by the authors — 44+XY.

Owing to the fact that clinical and cytogenetic investigations of the Kleinfelter syndrome in Bulgarian medical literature are rather limited (1, 3, 2), the authors of the present paper assume the task to present a more detailed past history and clinical data concerning the three patients with proved aberrations.

Case report I — D. M. B., 21-year-old several times postponed from military service enrollment on account of low weight. Since childhood, he is small-sized and rather thin; he has difficulties in school. No relations

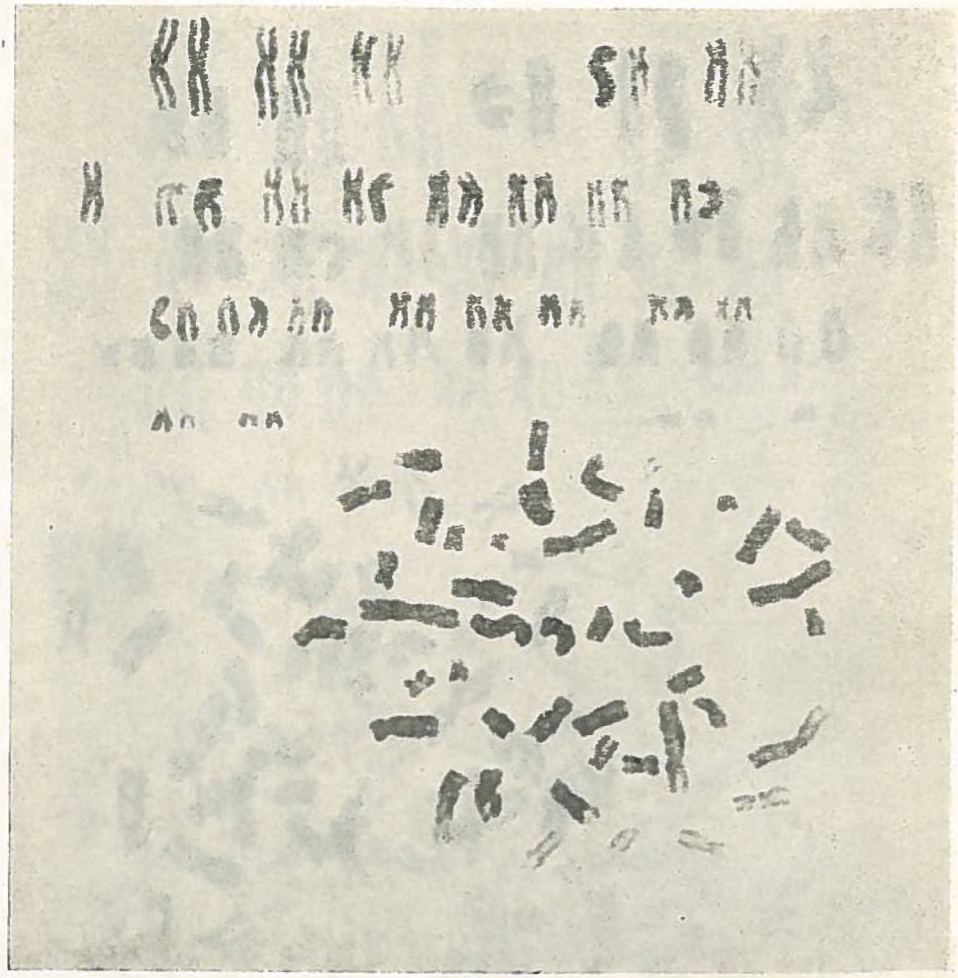

Fig. 6. Cell in metaphase of K. L. M. and karyogram 2n=44+XO

with women and sexual contact. Since two years he shaves his beard but very seldom — monthly. Hairing of sex organs occurs 5—6 years ago. Familial history — unburdened.

Habitus — asthenic. Sex hair — male type, well pronounced;on, the chest — absent. Facial hair — weakly pronounced on the chin and upper lip. Sex organs and scrotum — well developed. The testes measure the size of beans with dense consistency. Macroscopically, no gynecomastia is established. No pathological deviations discovered in terms of internal organs. Syncs'osis of all bones is completed. The neurologic investigation reveals debilitas. Blood sugar 106 mg %, total serum lipids 870 mg %, blood cholesterol 136 mg %, beta-lipoproteins 28 U, hepatic functional tests — within normal limits. Sodium in the blood serum — 325 mg %, potassium — 23,4 mg %, chlorides — 585 mg %. Insulin, glucose and adrenalin

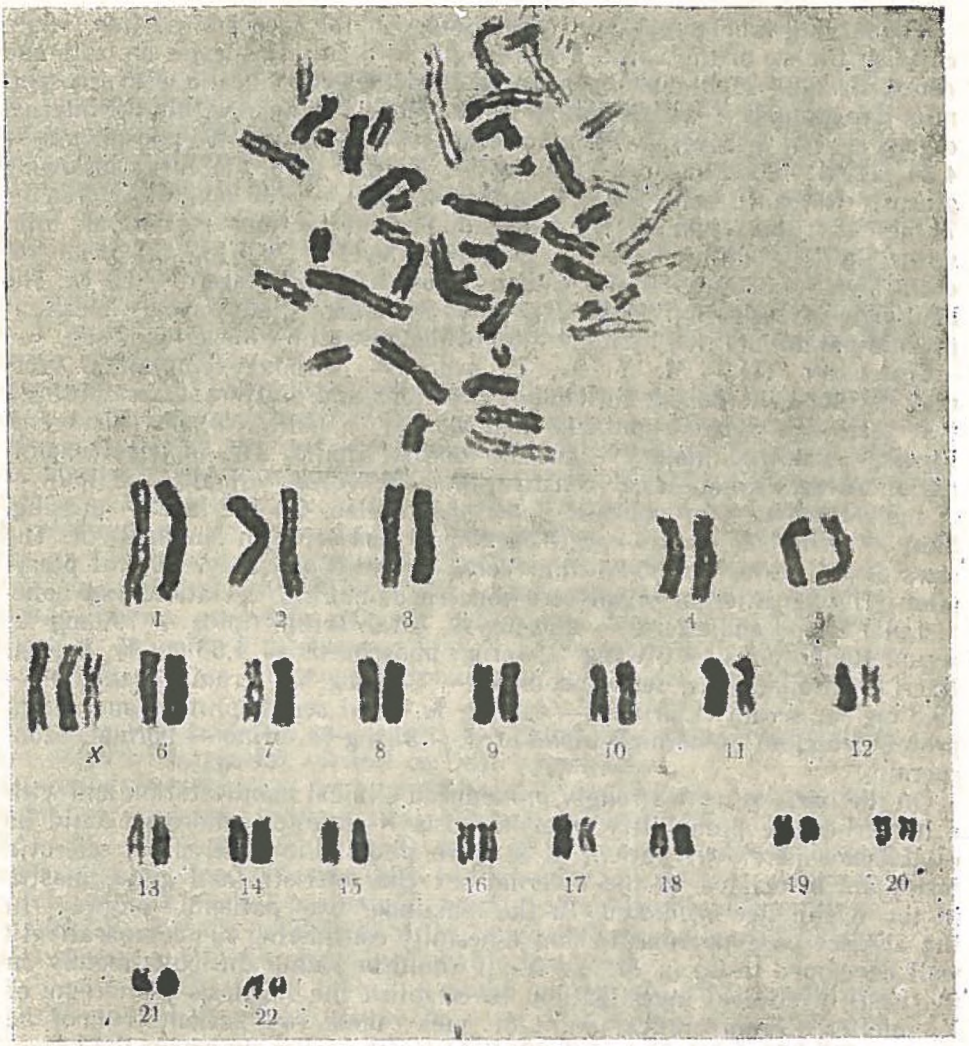

Fig. 7. Cell in metaphase from K. L. M. and karyogram 2n=44+XXX

(epinephrine) tolerance tests show a normal course. Eye grounds — normal. X-ray of pituitary fossa does not reveal deviations of the norm. Azoospermia.

Case report II — K. A. S., aged 20. The first erections received at the age of 12—13 years. After the 17th year of life, his right mammary gland shows gradual growth. Graduated the 7th class, after repeating two classes. At the time of birth, his mother was 54 and his father 64. His brothers and sisters are living and in good health, all of them married with children. The objective examination (physical examination) reveals eunuchoid proportions of the body with broad, feminine pelvis.

The sex hair is of female type. It is missing on the face and axillae. High, childish timbre of the voice. Penis length — 5 cm. The testes are soft and dense, sized 2—24/10 mm. Pronounced rightside gynecomastia with azoospermia. Eye grounds — within normal limits. Morbid changes within the internal organs are not discovered. Serum calcium level — 11 mg %, phosphorus — 4,34 mg %, blood sugar — 110 mg %. Normal glycemic curve following glucose tolerance test. Total blood cholesterol — 136 mg %, esteren — 71 mg %. Total serum protein and serum protein fractions — normal, total serum lipids — 800 mg %, serum potassium level — 20,4 mg %, serum sodium level — 336 mg %, Weltmann 8 test tubes, Mclagen — 50 U. The histologic investigation of the extirpated mammary gland reveals: clear-cut predomination and rich hyalinization of the grown up connective tissue.

Case report III — M. Y. A., 19 years — without complaints. Normal development during childhood. Erections and masturbations date back two years ago. Sexual contacts with women — none. No past history of illnesses. Familial history — within normal limits. The physical examination reveals symmetrical constitution. The voice — male. Sex hair — of male type, well pronounced under the axillae. On the face — missing. Skin — wrinkled, penis — well developed measuring in length 9 cm. The sizes of the testes are 35/25 mm; dense-elastic consistency. Digital pterygium. Insofar internal organs are concerned, morbid deviations are nonexistant. Blood cholesterol — 160 mg %, total serum lipids — 790 mg %, serum lipoproteins — 616 mg %, serum phosphorus — 4,65 mg %, normal liver functional tests, serum sodium — 340 mg %, serum potassium — 19,8 mg %, serum chlorides — 644 mg %, total serum protein and serum protein fractions — normal, blood urea — 31 mg %, urine — normal, azoospermia.

On the basis of more strongly pronounced clinical manifestation and with a higher degree probability, the diagnosis Kleinfelter syndrome could be established merely in patient K A. S. in whom, the small sized, sclerotic testes are associated to the external sex characteristic and gynecomastia in the Kleinfelter syndrome. In the remainder two patients, anyway, in the absence of gynecomastia and especially considering the comparatively well developed testes in M. Y. A., it would be rather difficult, merely on the basis of clinical investigation, to establish the diagnosis „syndrome of Kleinfelter". From clinical point of view, these two patients cannot be distinguished from the rest of the patients in the series, in whom the karyotype is within normal limits. Thereby, it is assumed by the authors (without being able to prove it by means of biopsy of testes) that, in all likelihood, a false form of the Kleinfelter's syndrome (Pseudkleinfelter) is concerned in the majority of cases. The difficulties in the classification of male hypogonadism are emphasized by a great number of authors (4). According to De La Chapelle, the study of urinary gonadotropins and the spermorgam have a limited significance insofar establishing of diagnosis is concerned (4). The same author finds chromosome aberrations of the Kleinfelter type in 12 out of a total of 43 patients with manifest or suspected hypogonadism. The ratio established in our series is analogous. Chromosome aberrations were established by the same author not merely in the Kleinfelter syndrome, but also in cryptorchism and infantilism; on the other hand, the presence

of a normal karyotype was noted in cases with clinical signs of Kleinfelter's syndrome.

Due to the fact that the Kleinfelter syndrome is rather more frequently met in psychically retarded and mentally ill individuals (15) (similar observations are reported also in the Bulgarian literature), Nowakowski and Lenz (7) make an attempt to assess comparatively the psychic development and the changes in the karyotype in the „true" Kleinfelter's syndrome. They find out that intellectual level is lowered more frequently and impaired more severely in patients with mosaicism and deviations, in positive and negative direction, of the XXY conjunction than in carriers of XXY aberrations merely. The results of the present investigations, although carried out on a limited number, are in compliance with the conclusions reached by the above cited authors.

The second part of our work is dedicated to the adrenogenital syndrome in two cases of congenital adrenal cortical hyperplasia — pathological condition also included in the group of pseudohermaphroditism. As it is well known, the congenital adrenocortical hyperplasia is brought about by enzymic defects in the cortisone biosynthesis, usually absence of the C-21-β and C-11-β hydroxylase. In the opinion of the great majority of authors, the affection is connected to a recessive autosome gene and is recorded approximately in a ratio of 1 : 50000 births. In accordance with modern genetics, congenital diseases, caused by enzymic defects and developing at the level of molecules, are related to abnormalities in single genes; it is furthermore pointed out that in the latter case chromosomic aberrations are not established.

In the literature reviewed, no reports are found concerning chromosome abnormalities in congenital adrenal cortical hyperplasia.

Cytogenetic investigations were carried out in two female patients with adrenogenital syndrome in congenital bilateral adrenal cortical hyperplasia: K. L. M. — 9 years old and M. P. S. — 40 years old, treated at Higher Medical Institute — Varna during 1964. In the former, it concerns a compensatory form of C-21 block in postnatal congenital adrenal cortical hyperplasia.

In patient M. P. S., a natal form of the congenital adrenal cortical hyperplasia is observed, brought about by C-21 block as well.

In patient K. L. M. (№ 14, table 2) a modal number of cells is established (80%) with normal female karyotype 2n = 44 + XX. We established also cell clones with karyotype 2 n = XX + XO and 2 n = 44 XXX and 2 n = 44 XXXX (Figs. 5, 6, 7). Such a mosaicism, involving 20% of the cells, is presumably the consequence of impaired hormonal balance or occasional treatment.

In the second case (№ 15 — table 2), strongly pronounced mosaicism is established within the karyotype of cells from 2n = 44 XXXX—trisomy X (38%), 2n = 44 + XXXX — tetrasomy X (23%), 2n = 44 + XX normal female karyotype (22%) and polysomy X (17%).

The modal number cells are with trisomy X — a finding warranting the assumption that initially the karyotype was 2 n = 44 + XXX and the clones described were subsequently developed.

The cytogenetic investigations of various authors in individuals wiht manifested congenital adrenal cortical hyperplasia do not demonstrate the presence of chromosome aberrations (5, 6, 10, 14). In the two patients just

reported, variously manifested cellular mosaicism was established with karyotype aberrations, involving the sex chromosomes both to the left and right in the first case (№ 14) which is still a child (9 years), whereas, in the second (№ 15) which is 45-year-old, the deviation is exclusively right-sided, with marked proneness towards polysomy X. It is rather difficult to accept a relationship existing between the karyotype aberrations and the development of the affection in the individuals analyzed. It is possible that the aberrations in question are the consequence of the disease; the latter depends on the disturbances of the hormonal balance, which, on its part, might lead to secondary karyotype aberration. The data established in the present study are of particular interest and therefore, worth of publication.

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ЦИТОГЕНЕТИЧЕСКИЕ ИЗУЧЕНИЯ ПРИ НЕКОТОРЫХ СОСТОЯНИЯХ ПСЕВДОГЕРМАФРОДИТИЗМА

М. Т. Цонева-Манева, З. Бозаджиева, Б. Петров

РЕЗЮМЕ

Проведены цитогенетические изучения 13 мужчин с выраженным и подозреваевым гипогонадизмом и двух лиц женского пола с адреногенитальным синдромом. У двух больных с гипогонадизмом установлено наличие кариотипа, характерного для синдрома Клеинфельтера — 47 (ХУ,

48) XXXУ хромосом. У одного из исследованных лиц установлена абнормная хромосома № 3 и укорочение верхних плеч Х-хромосомы — вероятно, транслокация фрагмента Х-хромосомы № 3. Разрывы хромосом и хроматидных фрагментов установлены у трех больных, а у остальных лиц не были обнаружены отклонения от нормального кариотипа.

У обоих лиц с адреногенитальным синдромом установлены: у одного — модальное число клеток с нормальным кариотипом 46/XX и клеточными ветвями с XO (XXX) XXXX хромосомами, а у второго — модальное число клеток 47/XXX и клеточными ветвями XX, XXXX и полисомия Х-хромосом.