

Leucocyte Alkaline Phosphatase in Klinefelter's Syndrome

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A rise in the level of leucocyte alkaline phosphatase (LAP) has recently been reported in mongolism (Trubowitz, Kirman, and Masek, 1962; Lennox, White, and Campbell, 1962; King, Gillis, and Baikie, 1962; Alter, Lee, Pourfar, and Dobkin, 1963; O'Sullivan and Pryles, 1963), and it has been suggested that this might be the direct effect of a gene situated on the chromosome which is present in triplicate. Other abnormalities of the leucocytes in mongolism which have been described include an inhibition of the nuclear lobing (Turpin and Bernyer, 1947; Shapiro, 1949; Mittwoch, 1957, 1961), of drumstick formation (Mittwoch 1957, 1961, 1964), and an association with leukaemia (Krivit and Good, 1957; Stewart, Webb, and Hewitt, 1958; Wald, Borges, Li, Turner, and Harnois, 1961; Holland, Doll, and Carter, 1962).

Some abnormalities of the sex chromosomes have effects on leucocytes similar to those seen in association with mongolism. Leukaemia in association with Klinefelter's syndrome has been reported (Mamunes, Lapidus, Abbott, and Roath, 1961; Tough, Court Brown, Baikie, Buckton, Harnden, Jacobs, and Williams, 1962; Bousser and Tanzer, 1963). Inhibition of nuclear lobing and of drumstick formation in patients with additional X chromosomes, including Klinefelter's syndrome, has been described (Mittwoch, 1964). The question arises whether this list of parallels might also be extended to include an effect on LAP activity.

We have surveyed a group of hospital patients with Klinefelter's syndrome for LAP activity. In these studies we were concerned that LAP activity and lobe count alteration might result from related causes and thereby show an association.

Investigation of this possibility necessitated measurement of LAP and lobe count on the same cells. For this purpose the cytochemical technique for LAP by Kaplow (1955), modified to permit accurate lobe counting, was used.

The findings presented indicate that this group of patients with Klinefelter's syndrome have a raised LAP activity. They also suggest that an association between LAP activity and lobe count may be present.

Patients and Methods

Investigations were made on 36 adult subjects: 12 Klinefelter's and 24 mentally retarded controls (12 males and 12 females) matched closely in chronological and mental age. None of the patients had evidence of infection. Three of the Klinefelter patients had an XXXY chromosome constitution (Maclean, Mitchell, Harnden, Williams, Jacobs, Buckton, Baikie, Court Brown, McBride, Strong, Close, and Jones, 1962) and one was XYYY (Ellis, Miller, Penrose, and Scott, 1961). The remaining Klinefelters were assumed to be XXY on the basis of clinical and sex chromatin investigations.

Blood was taken from finger-pricks on to glass coverslips. Smears were dried quickly and fixed immediately in methanol/formol 90/10 at 0-5°C. for 30 seconds and washed for 10 seconds under cold running tap water. Staining for LAP was done by the method of Kaplow (1955) using sodium α -naphthyl phosphate and Brentamine Fast Garnet (Hayhoe and Quaglino, 1958) for 10 minutes at $20 \pm 0.5^\circ\text{C}$. The smears were quickly washed and transferred for counterstaining while still wet into a 1/4th dilution of aqueous Mayer's haematoxylin (Baker, 1950) at 4-8°C. for 30 seconds, washed quickly under cold running tap water, dried in air, and mounted in glycerol gelatin. LAP activity scored according to Kaplow's scale and lobe counts were recorded on approximately 100-200 cells from each patient. All observations were made without knowledge of the smear's origin.

Results

Results are summarized in Table I. Inspection suggested that there might be differences between all three groups. Accordingly, they were analysed

Received October 20, 1964.

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TABLE I

LEUCOCYTE ALKALINE PHOSPHATASE ACTIVITY AND LOBE COUNTS* IN CASES WITH KLINEFELTER'S SYNDROME AND CONTROLS

Case No.	Klinefelters			Controls			
	Karyotype	LAP	Lobes	Females		Males	
				LAP	Lobes	LAP	Lobes
1	XXY	143	204	26	289	55	245
2	XXY	109	171	104	270	34	247
3	XXY	71	253	23	250	164	191
4	XXY	89	245	134	218	101	244
5	XXY	106	203	96	249	44	240
6	XXY	82	248	94	259	17	274
7	XXXXY	96	206	38	281	74	236
8	XXY	65	252	94	235	105	234
9	XXY	107	209	75	230	39	259
10	XXXXY	121	188	100	194	18	255
11	XXXXY	68	225	77	220	37	261
12	XXXXY	29	231	155	225	31	264

*LAP activity and lobe counts are expressed for 100 cells.

TABLE II

LEUCOCYTE PHOSPHATASE ACTIVITY AND LOBE COUNTS IN DIFFERENT GROUPS OF PATIENTS

	No.	LAP/100 Cells*		Lobes/100 Cells	
		Mean LAP	Mean Log LAP ± S.D.	Mean	S.D.
Klinefelters	12	85	1.93 ± 0.18	220	27
Females	12	72	1.86 ± 0.27	243	28
Males	12	48	1.68 ± 0.30	246	21

*Alter *et al.* (1963) found that logarithmic transformation of LAP values fitted them to a normal distribution. Comparisons of mean LAP activities in this Table have been made using this criterion. Significance (t) tests on log LAP activity, for Klinefelters vs. males, $t_{22} = 2.47$ ($0.02 > p > 0.01$), Klinefelters vs. females, $t_{22} = 0.75$ ($p > 0.4$) and females vs. males $t_{22} = 1.55$ ($0.4 > p > 0.1$); on lobe count, for Klinefelters vs. males, $t_{22} = 2.62$ ($0.02 > p > 0.01$), Klinefelters vs. females, $t_{22} = 2.05$ ($0.1 > p > 0.05$) and females vs. males, $t_{22} = 0.3$ ($p > 0.4$).

TABLE III

MEAN LEUCOCYTE ALKALINE PHOSPHATASE ACTIVITY IN CELLS CLASSIFIED ACCORDING TO LOBE COUNTS

	Case No.	Number of Lobes							
		I		II		III		IV	
		No. of Cells	LAP	No. of Cells	LAP	No. of Cells	LAP	No. of Cells	LAP
Klinefelters	1	54	74	76	121	45	59	8	8
	2	89	82	72	89	29	37	2	2
	3	11	6	71	51	92	72	8	8
	4	15	12	79	68	68	63	13	13
	5	19	20	85	87	24	29	0	0
	6	9	8	41	35	43	35	7	4
	7	23	20	49	52	27	23	1	1
	8	11	4	35	28	45	27	9	6
	9	19	23	55	56	24	25	2	3
	10	24	27	64	83	12	11	0	0
	11	19	13	41	24	36	28	4	3
	12	8	2	56	15	33	12	3	3
Females	1	14	2	14	3	31	11	32	8
	2	17	20	42	39	89	95	24	26
	3	28	5	56	16	88	18	17	5
	4	29	36	104	141	53	73	5	6
	5	15	12	67	69	69	64	13	12
	6	9	6	33	30	48	48	10	10
	7	4	3	24	7	59	26	13	2
	8	6	10	56	54	35	25	3	5
	9	8	9	56	44	34	24	2	2
	10	29	25	50	53	19	20	2	2
	11	16	12	50	45	32	19	2	1
	12	8	11	60	92	31	51	1	1
Males	1	21	2	46	24	60	46	12	5
	2	12	2	34	13	41	14	8	4
	3	39	64	52	86	23	37	3	5
	4	17	16	82	82	77	75	11	16
	5	6	3	43	19	40	17	4	2
	6	6	0	26	5	56	10	12	2
	7	8	5	51	33	38	33	3	3
	8	8	8	51	59	40	38	1	0
	9	4	3	39	14	51	20	6	2
	10	11	0	35	6	42	12	12	0
	11	5	3	34	14	56	18	5	2
	12	7	2	33	8	49	16	11	5
Totals		628	548	1,862	1,655	1,639	1,231	269	166
Mean LAP/100 cells			87		89		75		62

TABLE IV

MEAN LEUCOCYTE ALKALINE PHOSPHATASE ACTIVITY IN CELLS CLASSIFIED ACCORDING TO LOBE COUNTS IN DIFFERENT GROUPS OF PATIENTS

	No. of Cells	I		II		III		IV				
		LAP	Mean LAP*	No. of Cells	LAP	Mean LAP*	No. of Cells	LAP	Mean LAP*			
Klinefelters	301	291	97	724	709	98	478	421	88	57	42	74
Females	183	149	81	611	593	97	588	474	81	124	78	63
Males	144	108	75	526	363	69	573	336	59	88	46	52

* For 100 cells.

separately (Table II). The data are also presented in another form to show the distributions of LAP activity (Tables II and IV) and lobe count (Table V) in cells classified into 4 groups according to lobe count.

Discussion

The cytochemical findings indicate that the overlap between LAP distributions is extensive and that the distributions of LAP in the cases with Klinefelter's syndrome and female groups are very similar.

Both groups have appreciably greater mean LAP activity than males (Table II). Tests on the differences between the means of cases with Klinefelter's syndrome and controls were significant for males ($t_{22} = 2.47, 0.02 < p > 0.01$) and not significant for females ($t_{22} = 0.75, p > 0.4$). It should be emphasized that all subjects surveyed in this study were adults. Since Alter *et al.* (1963) noted that LAP increases attributable to chromosomal aberration were present in young mongols but were absent in an older group, it may be that a more pronounced rise in LAP activity would be found if young cases of Klinefelter's syndrome were investigated.

The data on mean lobe counts (Table II) show that X chromosome polysomy in males is associated with inhibition of nuclear lobulation, confirming an earlier finding by one of us (Mittwoch, 1964). They also show that the mean LAP increases as the mean lobe count decreases. The LAP scores in cells classified according to the number of lobes are given in Table III. The total LAP values for all patients suggest that there may be an increase in enzyme activity as the number of lobes increases from one to two followed by a decrease in LAP in cells with higher lobe numbers. At present it is not possible to observe this trend in individual patients, since the number of cells observed for any given lobe number is too small. However, a falling off of LAP in cells with increasing lobe numbers is also observed when the total scores for Klinefelters, females and males, are taken separately (Table IV). It can be seen that for cells of given lobe numbers, LAP is highest for Klinefelters, intermediate for females, and lowest for males. A somewhat comparable situation was reported earlier (Mittwoch, 1964) for drumsticks: though the incidence of drumsticks is correlated with the number of lobes, mongols and Klinefelters have a lower incidence of drumsticks for cells of given lobe numbers compared with normal females. Finally these figures show that the cells with the fewest lobes and the highest LAP activity make up an appreciably greater proportion of the total in Klinefelters than in either control group, as is apparent in Table V.

TABLE V

FRACTION OF CELLS CLASSIFIED ACCORDING TO LOBE COUNT IN DIFFERENT GROUPS OF PATIENTS

	Total No. of Cells	Fraction of Cells With Stated Number of Lobes				Mean Lobe Count/100 Cells
		I	II	III	IV	
Klinefelters	1,560	0.19	0.46	0.31	0.04	220
Females	1,507	0.12	0.41	0.39	0.08	243
Males	1,331	0.11	0.39	0.43	0.07	246

The present suggestion that the LAP activity in cases with Klinefelter's syndrome and controls is diminished in cells with more than two lobes has not been reported before. Though Klinefelters and mongols have increased mean LAP activity, both also have an increased proportion of low-lobed cells. The latter might provide an explanation for the observed increase in this enzyme activity in these conditions. The increased LAP activity would thus be correlated in both conditions with a disturbance in the maturation of the polymorphonuclear leucocytes, and the existence of a specific gene locus need not be involved.

Summary

The leucocyte alkaline phosphatase activity (LAP) was investigated cytochemically in 12 patients with Klinefelter's syndrome and 12 male and 12 female mentally retarded control patients. The number of nuclear lobes was counted in the same cells.

The mean LAP values of cases with Klinefelter's syndrome were higher than those of the controls. The increase over male controls was statistically significant, and that over female controls not significant. The mean lobe counts of Klinefelters were lower than those of controls (significant for male, not significant for female controls).

It is suggested that in cells with increasing lobe count LAP activity may fall after an initial rise.

We gratefully acknowledge the co-operation of Dr G. M. Tucker and his staff at Darenth Park Hospital, and of Dr A. Shapiro and his staff at Harperbury Hospital; and of the late Dr D. H. H. Thomas and his staff at Cell Barnes Hospital.

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