Am J Hum Genet 31:586–600, 1979

Regional Localization of Human Gene Loci on Chromosome 9: Studies of Somatic Cell Hybrids Containing Human Translocations

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SUMMARY

Somatic cell hybrids were derived from the fusion of (1) Chinese hamster cells deficient in hypoxanthine guanine phosphoribosyltransferase (HPRT) and human cells carrying an X/9 translocation and (2) Chinese hamster cells deficient in thymidine kinase (TK) and human cells carrying a 17/9 translocation. Several independent primary hybrid clones from these two series of cell hybrids were analyzed cytogenetically for human chromosome content and electrophoretically for the expression of human markers known to be on human chromosome 9. The results allow the assignment of the loci for the enzymes galactose-1-phosphate uridyltransferase (GALT), soluble aconitase ($ACON_s$), and adenylate kinase-3 (AK_3) to the short arm of chromosome 9 (p11→pter) and the locus for the enzyme adenylate kinase-1 (AK_1) to the distal end of the long arm of human chromosome 9 (band q34). Earlier family studies have shown that the locus for AK_1 is closely linked to the *ABO* blood group locus and to the locus of the nail-patella (*Np*) syndrome. Thus the regional localization of the AK_1 locus permits the localization of the AK_1-*Np*-*ABO* linkage group.

Received November 28, 1978; revised February 2, 1979.

This work was supported in part by grants (HD-05615) and (HD-04612) from the National Institute of Child Health and Human Development.

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INTRODUCTION

Rodent-human somatic cell hybrids have been used extensively for human gene mapping [1]. The formulation of a comprehensive map of the genome requires subchromosomal localization of the human gene loci known to be on specific chromosomes. The best systematic approach to assign gene loci to human chromosome regions is genetic analysis of cell hybrids derived from the fusion of rodent cells with human cells obtained from individuals carrying known, defined chromosome arrangements. Further facilitation of regional localization can be achieved by use of translocations in which one of the chromosomes involved has a marker which can be selectively retained or lost from the hybrid cells. We have utilized this approach to regionally localize gene loci known to be on human chromosome 9.

The two translocations used in the experiments described here involved human chromosome 9 with breaks at different points; the second chromosome involved was the X chromosome, in one case, and chromosome 17, in the other. The human X chromosome carries the gene for HPRT (E.C.2.4.2.8) on its long arm [2]. Hence, fusion of the cells carrying the X/9 translocation with rodent cells deficient in HPRT and selection of hybrid cells in growth medium containing hypoxanthine, aminopterin, and thymidine (HAT) [3] retain the rearranged chromosome with the human *HPRT* locus. Counter-selection of these cells, in medium containing 8-azaguanine which selects for cells lacking HPRT, generates hybrid cells without the rearranged chromosome which carries the human *HPRT* locus. Thus, it is possible to obtain hybrids with and without a rearranged chromosome and analysis of these permits regional localization of loci known to be on the chromosomes involved.

Translocations involving chromosome 17 can be similarly utilized because the human gene for soluble TK (E.C.2.7.1.75) is on the long arm of chromosome 17 [4]. Fusion of human cells having a 17/9 translocation with TK deficient rodent cells and growth in HAT medium selects for the presence of the human *TK* locus, which can be eliminated from the hybrid cells by growth in medium containing 5-bromodeoxyuridine (BrdU).

The human gene loci for AK_1 (E.C.2.7.4.3), AK_3 (E.C.2.7.4.10), and $ACON_s$ (E.C.4.2.1.3) have been previously assigned and confirmed on chromosome 9 [5–8]. The gene locus for GALT (E.C.2.7.7.12), the deficient enzyme in classical galactosemia, has recently been assigned to human chromosome 9 by three different laboratories [9–11]. However, the assignment of *GALT* to human chromosome 9 contradicts earlier assignments of this gene locus to chromosome 2 [12] and 3 [13], also using the somatic cell hybrid approach.

Results presented here, based on our studies using Chinese hamster-human somatic cell hybrids which segregated rearranged human chromosome 9, provide additional evidence for the assignment of the *GALT* locus to chromosome 9. Further, these results show that *GALT*, $ACON_s$, and AK_3 are on the short arm of human chromosome 9 and AK_1 is on the long arm in band q34.

MATERIALS AND METHODS

Cell Lines

An HPRT deficient, pseudodiploid Chinese hamster line, CHW 1102, [14] and a TK deficient Chinese hamster line, RJK, (obtained from Dr. Frank Ruddle's laboratory) were used as the rodent parental cells in the fusion experiments. CHW 1102 is resistant to 10 μg/ml of 8-azaguanine, and RJK is resistant to 30 μg/ml of BrdU in the media. White blood cells were used as human parental cells and these were obtained from: (1) a male subject carrying an inherited, apparently balanced translocation between chromosomes 9 and 17 [46,XY,t(9;17)(p11;p11)] [15] (fig. 1); and (2) a female carrying an apparently balanced translocation between chromosomes X and 9 [46,X,t(X;9)(q13;q34)] (fig. 2). The normal X chromosome is late replicating in this individual [16].

Cell Fusion and Isolation of Hybrid Cells

Cell fusion was done in suspension with the aid of inactivated Sendai virus (Connaught Laboratories, Toronto, Ontario), according to the procedure of Giles and Ruddle [17]. Human cells with the 9/17 translocation were fused with RJK, and the cells carrying the X/9 translocation were fused with CHW 1102. Following the cell fusion protocol, the cell mixture was divided and plated into a number of 60 mm culture dishes (Falcon Plastics, Oxnard, Calif.) containing the selection medium HAT supplemented with glycine [17]. Hybrid cell colonies were visible in 2–3 weeks, and one colony was isolated from a culture dish using a cloning cylinder to obtain a series of independently derived primary hybrid lines. Back selection of lines, to eliminate the chromosomes carrying the human *HPRT* or *TK* gene, was achieved by growing the cells in full growth medium for a short period followed by selection in media containing 10 μg/ml 8-azaguanine or 30 μg/ml BrdU, respectively.

Enzyme Analysis

Biochemical and cytogenetic analyses were done on cells at the same passage level. Harvested tissue culture cells were washed in buffered saline. Lysates were prepared by adding a volume of 2% Triton X-100 solution (containing NADP and EDTA) equal to that of the cell pellet, followed by freeze thawing at least twice. Previously described techniques were used for starch gel electrophoretic evaluation of the enzymes GALT [18], $ACON_s$ [19], AK_1 [20], AK_3 [21], and the X chromosome markers glucose-6-phosphate dehydrogenase (G6PD) [22] and phosphoglycerate kinase [23].

Cytogenetic Analysis

Standard air dried chromosome preparations made from the hybrid cell cultures were Q-banded [24], and well-spread banded metaphases were photographed. Cytogenetic analysis

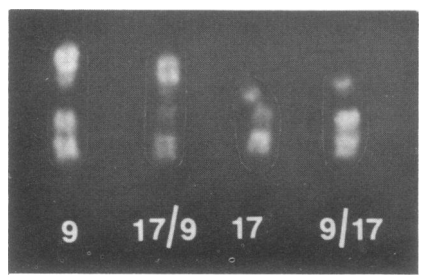

FIG. 1.—Q-banded partial karyotype from cell of individual carrying 17/9 translocation: 46,XY,t(17;9)-(p11;p11). Short arm of chromosome 9 is translocated to long arm of chromosome 17 (17/9); short arm of 17, in turn, is translocated to long arm of 9 (9/17).

was done on photographs, and an average of 20–30 metaphases were analyzed per hybrid line. The percentage of analyzed cells containing a particular human chromosome was then determined.

RESULTS

Electrophoretic Studies

Examples of starch gel electrophoretic patterns of GALT, AK_1, and $ACON_s$ are presented in figures 3, 4, and 5, respectively. In hybrids expressing human GALT, the electrophoretic pattern consistently showed an intermediate band, which is presumably a hamster-human heteropolymer; some hybrids showed human band(s) in addition to the intermediate band. The 17/9 translocation carrier was heterozygous (type 2-1) for AK_1; as a result, hybrids with human AK_1, type 1, and human AK_1, type 2, were seen depending on the chromosome 9 material retained. The results of the biochemical evaluations are summarized in tables 1, 2, and 3, along with cytogenetic results.

Cytogenetic Studies

17/9 translocation. Seventeen independent primary hybrid lines were analyzed biochemically and could be grouped into six phenotypic classes (table 1). Nine of these lines, belonging to five classes, were analyzed cytogenetically. The human chromo-

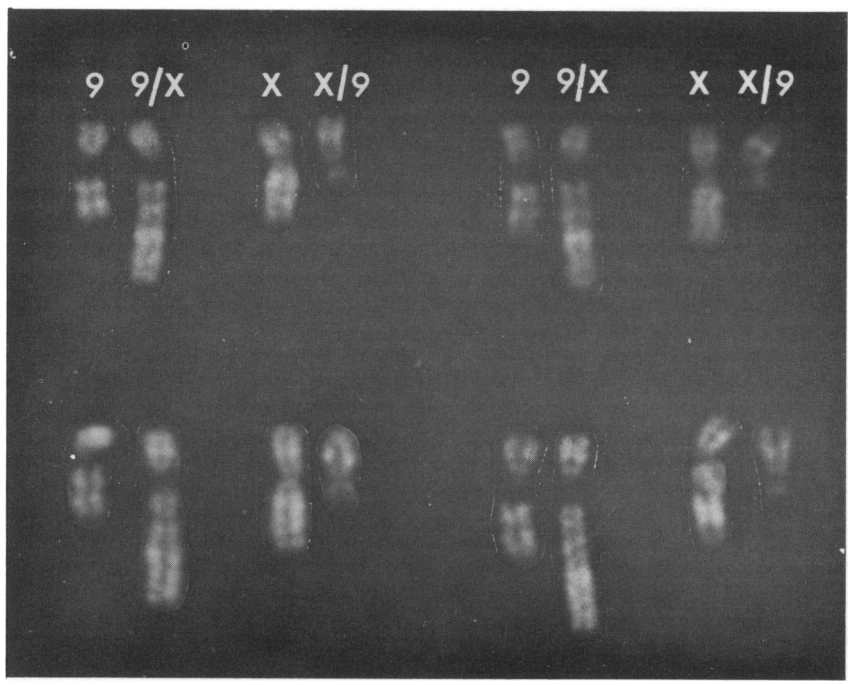

FIG. 2.—Q-banded partial karyotypes from four different cells of individual carrying X/9 translocation: 46,X,t(X;9)(q13;q34). Long arm of the X chromosome distal to band q13 is translocated to extreme end of long arm of chromosome 9 at band q34.

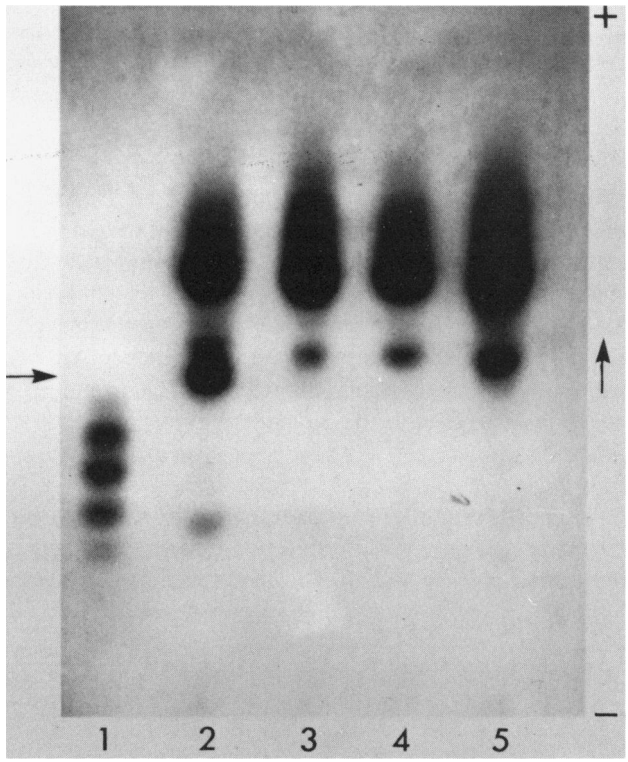

FIG. 3.—Starch gel electrophoretic patterns for GALT. *Vertical arrow* indicates direction of migration. Channels contain the following samples: *1*, human fibroblasts; *2–4*, Chinese hamster-human hybrids; *5*, Chinese hamster cells. *Horizontal arrow* indicates hybrid enzyme band. Hybrid in channel *2* is positive for expression of human GALT as indicated by presence of an intermediate, presumably hybrid, enzyme band and a band in area of human enzyme. Hybrids in channels *3* and *4* are negative for expression of human GALT.

some content in relation to chromosomes 17 and 9 are also presented in table 1. The detailed cytogenetic results are presented in table 2. As can be seen from table 1, in the presence of the 17/9 chromosome (9pter→9p11::17p11→17qter) (Class VI), hybrids express human GALT, $ACON_s$, and AK_3 but not AK_1, thus showing that *GALT*, $ACON_s$, and AK_3 are on the short arm of human chromosome 9, and AK_1 is on the long arm. In the presence of the 9/17 chromosome (17pter→17p11::9p11→9qter) (Class II), human AK_1, type 2, is expressed, but not human GALT, $ACON_s$, or AK_3, which shows that (1) AK_1 is indeed on the long arm and *GALT*, $ACON_s$, and AK_3 are on the short arm of human chromosome 9, and (2) the rearranged chromosome 9 carries the AK_1, type 2, allele, whereas the normal chromosome 9 carries the AK_1, type 1, allele. Thus in class IV hybrids, which express GALT, $ACON_s$, AK_3, and AK_1, type 1, one can assume the presence of a normal chromosome 9, although cytogenetic analyses were not done on any of these lines. In the presence of 17/9 and 9/17 chromosomes (class V), all markers except AK_1, type 1, are expressed, as expected.

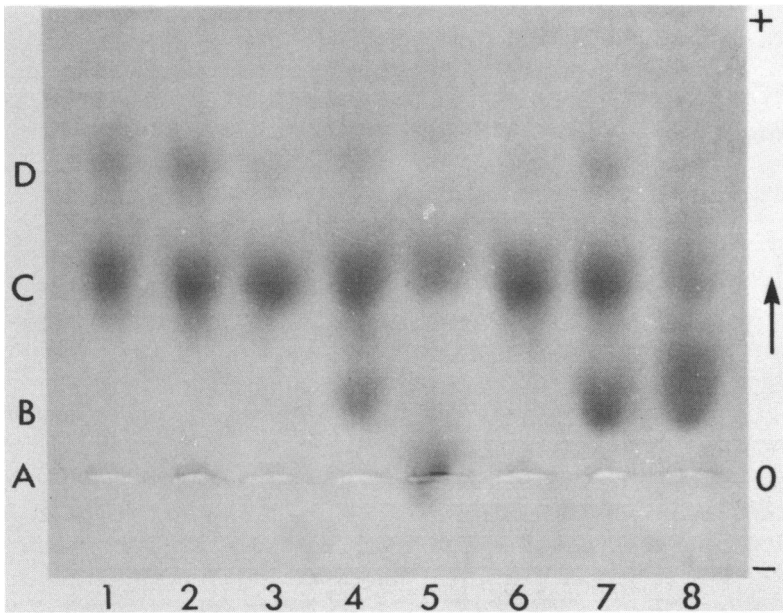

FIG. 4.—Starch gel electrophoretic patterns for AK_1 in 17/9 translocation experiment. *Vertical arrow* indicates direction of migration. Channels contain the following samples: *1*, Chinese hamster cells; *2–7*, Chinese hamster-human hybrids; *8*, human fibroblasts. *B* indicates AK_1 type 1 band; *A*, AK_1, type 2, band. Hybrids in channels *4* and *7* are positive for human AK_1, type 1. Hybrid in channel *5* is positive for human AK_1, type 2. Human control fibroblasts used in gel were not derived from individual carrying 17/9 translocation and show human AK_1, type 1. *C* and *D* indicate Chinese hamster AK bands in channels 1–7.

The detailed cytogenetic results of the nine independent primary lines and two back selected lines (in BrdU) from two of the above nine lines are presented in table 2, along with the biochemical results on these lines. The expression of GALT is concordant only with the short arm of human chromosome 9 and not with any other human chromosome, thus confirming the assignment of *GALT* on human chromosome 9. Two independent primary lines (CF17-7 and CF17-12) retaining the 17/9 chromosome were back selected in BrdU, and the cytogenetic findings (table 2) show that the 17/9 chromosome was absent in back selected clones with concomitant loss of expression of human GALT, $ACON_s$, and AK_3, further confirming the assignment of *GALT*, $ACON_s$, and AK_3 to the short arm of human chromosome 9. Note that line CF17-12 upon back selection lost only the 17/9 chromosome and not any of the other human chromosomes.

X/9 translocation. Results of cytogenetic and biochemical analyses of six independently derived primary hybrid lines and two lines back selected from two of the six lines are presented in table 3. The rearranged chromosome 9/X (9pter→9q34::Xp13→Xpter) is present in each of the six lines selected in HAT medium. This is expected because the normal X chromosome is inactivated in the human parent, and the *HPRT* gene on the X portion of 9/X chromosome is required for the survival of the hybrid cells. The data presented in table 3 show that in the presence of the 9/X

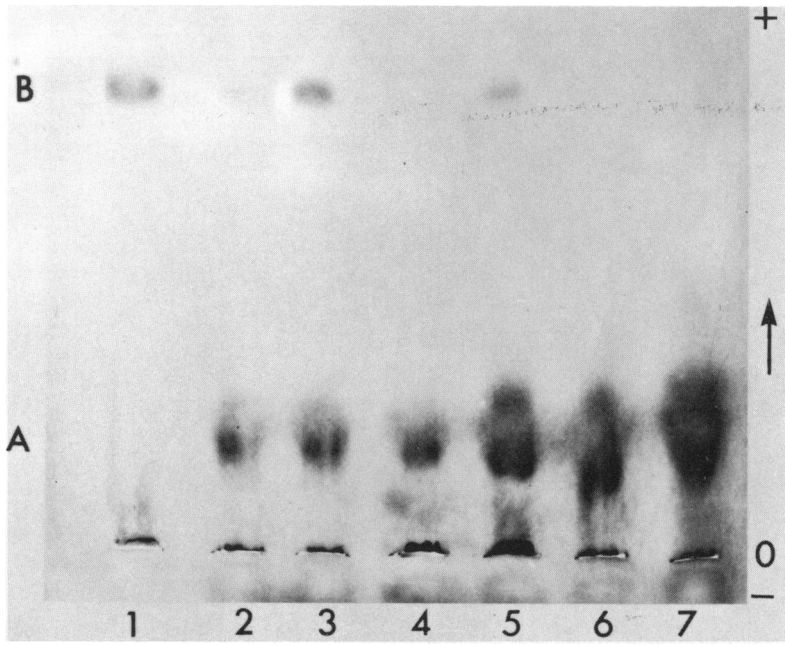

FIG. 5.—Starch gel electrophoretic patterns for $ACON_s$. *Vertical arrow* indicates direction of migration. Channels contain the following samples: *1*, human fibroblasts; *2–6*, Chinese hamster-human hybrids; *7*, Chinese hamster cells. *A* indicates Chinese hamster $ACON_s$; *B*, human $ACON_s$ bands. Hybrids in channels *2*, *3*, and *5* express human $ACON_s$.

chromosome (CF11-1; CF12-2; CF11-4), human chromosome 9 markers GALT, $ACON_s$, and AK_3 are expressed, but not AK_1. This shows that *AK_1* is in the q34 band of chromosome 9, although a more proximal location for *ABO* locus cannot be ruled retaining the human 9/X chromosome also expressed the human X markers G6PD and PGK, thus assigning these two loci to the q13→qter region of the X chromosome, which is consistent with data published to date on regional localization of these two human loci [25].

One hybrid line (CF11-14) had a deleted 9/X chromosome, resulting in the loss of 9p13→9pter material, and did not express the human markers GALT, $ACON_s$, and AK_3, an observation consistent with the results from the 17/9 translocation experiment assigning these loci to the short arm of human chromosome 9. However, any further conclusions with regard to localization of *GALT*, *$ACON_s$*, and *AK_3* on 9p (i.e., 9p13→9pter) based on just one hybrid line with a de novo deletion of a human chromosome in the interspecific hybrid is tenuous.

Hybrid line CF11-7 had a normal chromosome 9 and 9/X chromosome and expressed all the human 9 markers evaluated as well as the X-linked markers G6PD and PGK. Line CF11-4 was unique in that it retained only the 9/X chromosome (fig. 7), and upon back selection in 8-azaguanine, the 9/X was lost from these hybrids along with the loss of expression of all human markers evaluated. Line CF11-11 expressed all human chromosome markers, including AK_1, and had the 9/X chromosome and the

TABLE 1

CYTOGENETIC AND BIOCHEMICAL ANALYSES OF CELL HYBRIDS DERIVED FROM FUSION OF CHINESE HAMSTER CELLS AND HUMAN CELLS CARRYING 9/17 TRANSLOCATION

		BIOCHEMICAL ANALYSIS					CYTOGENETIC ANALYSIS				
		HUMAN MARKERS									
PHENOTYPIC CLASS	NO. PRIMARY HYBRID CLONES	GALT	$ACON_s$	AK_1		AK_3	NO. CLONES ANALYZED	HUMAN CHROMOSOMES			
				Type 1	Type 2			9	17	17/9*	9/17†
I	6	−	−	−	−	−	2	−	+	−	−
II	1	−	−	−	+	−	1	−	+	−	+
III	2	+	+	+	+	+	1	+	−	+	+
IV	2	+	+	+	−	+	NA‡				
V	1	+	+	−	+	+	1	−	−	+	+
VI	5	+	+	−	−	+	4	−	−	+	−

NOTE. — Absence of human marker or human chromosome is indicated by −; presence of human marker or human chromosome is indicated by +.

* 17/9: translocation chromosome, 9pter→9p11::17p11→17qter.

† translocation chromosome, 17pter→17p11::9p11→9qter.

‡ Not analyzed.

TABLE 2

HUMAN CHROMOSOME CONTENTS AND BIOCHEMICAL PHENOTYPES OF ELEVEN HYBRID LINES DERIVED FROM FUSION OF CHINESE HAMSTER CELLS AND HUMAN CELLS CARRYING 9/17 TRANSLOCATION

	HYBRID LINES										
	CF17-3	CF17-13	CF17-14	CF17-8	CF17-22	CF17-1	CF17-24	CF17-17	CF17-17R*	CF17-12	CF17-12R†
	Cytogenetic Analysis										
No. metaphases analyzed	30	27	26	28	26	21	25	32	32	24	27
Human Chromosomes:											
1	57‡	19	★	4	...	...	...	25	...	...	...
2	...	...	...	...	46	71	...	...	...	...	...
3	...	...	★	...	...	...	...	...	...	96	11
4	...	96	...	96	85	...	...	...	...	88	7
5	...	30	96	...	85	...	...	...	...	...	...
6	77	100	...	82	...	71	...	...	...	88	96
7	...	30	...	...	...	29	...	...	...	...	...
8	90	96	...	...	100	...	32	91	69	...	...
9	...	...	...	...	65	...	...	...	...	...	...
10	...	...	...	79	88	...	...	...	...	...	...
11	93	85	96	36	88	...	...	...	...	100	81
12	...	96	...	...	81	71	...	...	...	...	...
13	...	93	...	...	92	★	...	...	...	100	89
14	97	96	100	...	96	81	...	...	...	100	21
15	...	...	...	86	73	81	4	81	84	...	...
16	...	81	96	...	...	...	...	...	...	100	67
17	87	93	96	...	...	...	...	...	...	...	...
18	...	89	...	...	...	...	...	...	...	100	19
19	27	85	...	...	85	...	...	...	...	83	70
20	70	...	...	...	88	62	...	...	...	100	22
21	...	100	100	...	54	...	40	...	...	96	96
22	...	96	100	...	77	...	52	94	97	100	93
X	...	93	...	...	...	...	...	...	...	...	...
Y	...	...	96	...	73	95	...	...	...	100	81
17/9§	...	...	...	89	96	100	100	100	...	100	...
9/17‖	...	...	96	93	88	...	...	...	...	...	...

TABLE 2 (*continued*)

No. metaphases analyzed	30	27	26	28	26	21	25	32	32	24	27
					Biochemical Analysis						
Enzymes:											
GALT	−	−	−	+	+	+	+	+	−	+	−
$ACON_s$	−	−	−	+	+	+	+	+	−	+	−
AK_1:											
Type 1	−	−	−	−	+	−	−	−	−	−	−
Type 2	−	−	+	+	+	−	−	−	−	−	−
AK_3	−	−	−	+	+	+	+	+	−	+	−

NOTE:—★ indicates presence of chromosome or part thereof in rearranged form; −, absence of human enzyme; +, presence of human enzyme.

* Back selected in BudR from CF17-17.

† Back selected in BudR from CF17-12.

‡ No. refers to percentage of analyzed cells with at least one copy.

§ 17/9: translocation chromosome, 9pter→9p11::17p11→17qter.

|| 9/17: translocation chromosome, 17pter→17p11::9p11→9qter.

TABLE 3

HUMAN CHROMOSOME CONTENTS AND BIOCHEMICAL PHENOTYPES OF EIGHT HYBRID LINES DERIVED FROM FUSION OF CHINESE HAMSTER CELLS AND HUMAN CELLS CARRYING X/9 TRANSLOCATION

	HYBRID LINES							
	CF11-14	CF11-1	CF12-2	CF11-7	CF11-4	CF11-4R*	CF11-11	CF11-11R†
Cytogenetic Analysis								
No. metaphases analyzed	21	22	30	17	16	30	29	21
Human chromosomes:								
1	...	...	...	...	...	...	...	...
2	...	...	...	...	...	...	...	...
3	90‡	...	...	82	...	...	...	...
4	...	5	100	...	...	...	97	95
5	...	...	...	59	...	...	...	...
6	95	...	...	100	...	...	...	...
7	...	...	...	82	...	...	...	...
8	81	...	...	100	...	...	...	...
9	...	...	...	65	...	...	...	5
10	...	...	100	...	...	...	86	...
11	...	91	83	...	...	...	...	...
12	...	...	...	...	...	...	...	...
13	...	...	...	94	...	...	97	67
14	95	91	...	71	...	...	...	...
15	90	100	...	...	...	...	...	...
16	86	73	...	29	...	...	...	...
17	...	55	...	...	...	...	...	...
18	...	...	...	...	...	...	...	...
19	...	...	...	...	...	...	...	...
20	81	...	...	...	...	...	...	...
21	...	100	100	47	...	...	76	67
22	14	...	...	94	...	...	10	38
X	...	...	...	...	...	...	...	...
9/X§	p-,95‖	100	97	100	100	...	86	
X/9#	...	...	...	76	...	...	66	48
Biochemical Analysis								
Enzymes:								
GALT	−	+	+	+	+	−	+	+
$ACON_s$	−	+	+	+	+	−	+	+
AK_1	−	−	−	+	−	−	+	+
AK_3	−	+	NA**	+	+	−	+	+
G6PD	+	+	+	+	+	−	+	−
PGK	+	+	+	+	+	−	+	−

NOTE.—Absence of human enzyme indicated by −; presence of human enzyme by +.
* Back selected in 8-azaguanine from CF11-4.
† Back selected in 8-azaguanine from CF11-11.
‡ No. refers to percentage of analyzed cells with at least one copy.
§ 9/X: translocation chromosome, 9pter→9q34::Xq13→Xqter.
‖ 9/X p-: 9/X chromosome with 9p13→9pter deleted.
X/9: translocation chromosome, Xpter→9q13:: ?9q34→9qter.
** Not analyzed.

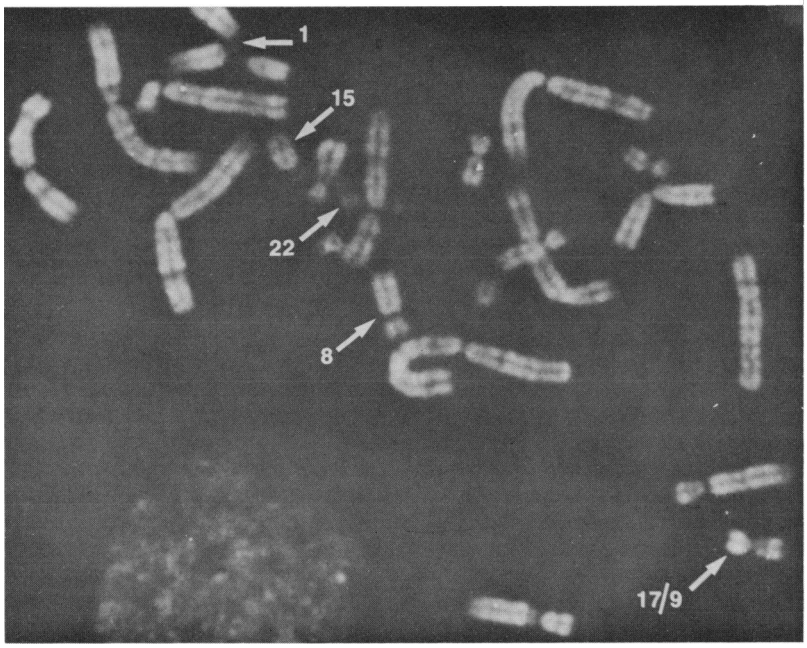

FIG. 6.—Q-banded metaphase from Chinese hamster-human hybrid line from 17/9 translocation experiment. *Arrows* indicate the human chromosome present. Notice 17/9 translocation chromosome retained.

reciprocal X/9 chromosome with no evidence of a normal 9 in the 29 cells analyzed. When it was back selected, it continued to express all the chromosome 9 markers, but not the X markers. One out of 21 cells analyzed in the back selected line was found to have a normal human chromosome 9. The expression of all human chromosome 9 markers (i.e., GALT, $ACON_s$, AK_3, and AK_1) in this line could be due to the presence of the normal 9 in a small proportion of cells. It was not possible to determine the phenotype of the hybrid line in the absence of a normal 9 and the presence of the translocation chromosomes X/9 and 9/X.

DISCUSSION

The data clearly show that the gene locus for human GALT is on chromosome 9 and further that *GALT*, $ACON_s$, and AK_3 are on the short arm of chromosome 9 (p11→pter). The localization of $ACON_s$ is in agreement with the results obtained by Westerveld et al. [26] who assigned this locus to the 9pter→9q13 region. The assignment of $ACON_s$ and AK_3 is also consistent with the results of Carritt and Povey [27] localizing these loci to the 9pter→9p13 region.

Our results also show that AK_1 is in the q34 band of human chromosome 9. Ferguson-Smith et al. [28] have assigned AK_1 to this region using the gene dosage method; a patient trisomic for the terminal band of chromosome 9 was found to have an elevated level (43% more) of red cell AK_1, whereas patients trisomic for all other parts of 9 were found to have normal enzyme activity. The data presented here confirm the

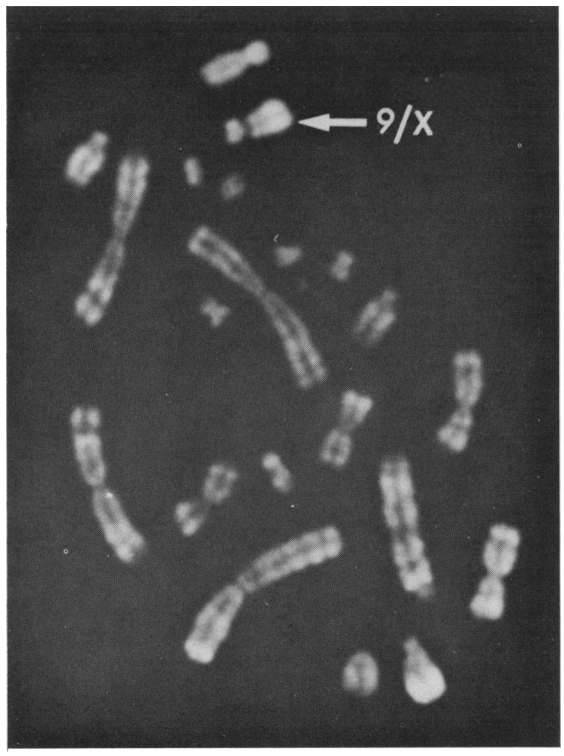

FIG. 7.—Q-banded metaphase from hybrid line CF11-4. Only human chromosome retained was 9/X (*arrow*).

regional localization of AK_1 to band q34 of chromosome 9. These results are in agreement with our own preliminary findings [29] and the data obtained by Carritt and Povey [27]. Pedigree analyses have shown that the locus for the Np syndrome is extremely closely linked to AK_1 and the locus for ABO blood group is linked to AK_1 with estimated recombination fractions of 8% in the male and 24% in the female [30–34]. In addition, studies by Cook et al. [35] of families with rearranged chromosome 9 show that AK_1 and *ABO* loci are near the end of this chromosome, which is in agreement with the assignment of AK_1 to the terminal band on the long arm of chromosome 9 (i.e., 9q34). It has not been possible to definitely establish the order of the three loci AK_1-*Np*-*ABO* from the family linkage studies. The assignment of AK_1 to 9q34 thus permits the assignment of *Np* and, probably, the *ABO* locus to this region of chromosome 9, although a more proximal location for *ABO* locus cannot be ruled out at this time.

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