ISOLATION AND PROPERTIES OF HYBRIDS BETWEEN SOMATIC MOUSE AND CHINESE HAMSTER CELLS[1]

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THE occurrence of hybridization between *in vitro* cultured cells of different species was first demonstrated in 1965 by EPHRUSSI and WEISS, who, by using the selective technique of DAVIDSON and EPHRUSSI (1965), succeeded in isolating viable, mononucleate hybrids between heteroploid mouse and diploid rat cells. Shortly thereafter, these investigators isolated a number of other rat × mouse hybrids (WEISS and EPHRUSSI 1966a).

Following these observations, crosses between cells of permanent lines of mouse and of chinese hamster origin have been undertaken. The aim of these experiments was to extend to another pair of species the observations referred to above. The choice of the species was motivated by the following considerations. The karyotype of the chosen pseudodiploid, drug resistant hamster line comprises a relatively small number of chromosomes, almost all of which can be identified by their characteristic morphology. Two lines of mouse cells were available which carry complementary selective markers and have karyotypes very different from that of the hamster cells. It was therefore expected that the hamster × mouse hybrids would be easily identified. Moreover, since many of the chromosomes of the hamster line can be distinguished from those of mouse lines, it was thought that hybrids between these two cell types would represent excellent material for the study of the evolution of their karyotypes.

As will be seen below, two types of hamster × mouse hybrids have been isolated and a certain number of observations on their properties and evolution have been made. It is the purpose of this paper to describe these observations.

MATERIALS AND METHODS

Cell lines used: Two permanent lines of mouse cells and one of Chinese hamster cells have been used in this work. Their designations and brief descriptions are as follows:

Line *2472–6–3* (mouse) is an 8-azaguanine resistant (3 μg/ml) subline isolated in this laboratory from the "high cancer" line NCTC 2472–6 (SANFORD, LIKELY and EARLE 1954; SCALETTA and EPHRUSSI 1965). The rate of reversion to drug sensitivity is 0.5–1.0 × 10^{-5}. Like the line of origin, it is heteroploid. Its very stable *1s* karyotype is characterized by 50 (49–52) chromosomes (the first number is the mode, the numbers in parentheses give the range), one or two of which are medium sized metacentrics, the remainder telocentrics (Table 1). Among the

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latter, is an extra long chromosome, which is present in every cell and therefore is an excellent "marker".

Line *A 9* (mouse), kindly given to us by DR. J. W. LITTLEFIELD, is a subline of L cells, resistant to 3μg/ml of 8-azaguanine. Reversions to drug sensitivity are extremely rare: only one has been detected in the course of many experiments performed in this laboratory during the past two years. A 9 cells are aneuploid with a total of 56(49–60) chromosomes. Of these, 20(18–25) are biarmed (metacentric or submetacentric) and 31–33(25–38) telocentric (Table 1). A metaphase and the karyotype of a modal A 9 are shown in Figure 1B.

Line $B_{14}I_{50}$ (Chinese hamster), kindly given to us by DR. T. C. HSU, is resistant to 50 μg/ml of 5-bromodeoxyuridine (BUDR). The rate of reversion to drug sensitivity is high: $2 - 3 \times 10^{-4}$. Cells of this line are pseudodiploid (HUMPHREY and HSU 1965). Karyological analysis of 32 metaphases revealed a total of 22(20–25) chromosomes which can be assigned to different classes as follows: 16(14–19) metacentrics [7(5–10) of small and 9(8–10) of large or medium size]; 5(3–6) sub-telocentrics; and 1(0–3) telocentric (Table 1). It may be added that the telocentrics are abnormal chromosomes (normal cells of the Chinese hamster contain no telocentric chromosomes); and that occasionally cells of line $B_{14}I_{50}$ contain a metacentric chromosome with an arm ratio not observed in the normal hamster karyotype. A metaphase and the karyotype of a near modal cell of $B_{14}I_{50}$ are shown in Figure 1A.

Culture technique and media: All cell lines were maintained in Dulbecco's modification of Eagle's medium supplemented with 10% calf serum. To prevent the accumulation of drug sensitive revertants, stock cultures of A 9 and 2472–6–3 were supplemented with 8-azaguanine (3 μg/ml), and those of $B_{14}I_{50}$ with 5-bromodeoxyuridine (50 μg/ml).

Selective medium (HAT): The medium used for the selection of hybrids is the same as above but contains in addition 4×10^{-7} M aminopterin, 1×10^{-4} M hypoxanthine and 1.6×10^{-5} M thymidine. The biochemical basis of this technique has been described by LITTLEFIELD (1964) and WEISS and EPHRUSSI (1966a). Suffice it to say that the drug resistant cells listed above do not grow in HAT because of correlated enzymatic deficiencies, while the hybrids resulting from the fusion of 8-azaguanine resistant cells with BUDR-resistant cells do.

Isolation and Karyological Identification of Hybrids

In the course of this work, hybrids were isolated from mixed cultures of lines $B_{14}I_{50}$ + A 9 and $B_{14}I_{50}$ + 2472–6–3. Although most of the investigations related in the present paper are concerned with a hybrid of the former type ($B_{14}I_{50}$/A 9), a brief description of the isolation and of the characteristics of the latter hybrid type ($B_{14}I_{50}$/2472–6–3) is given below.

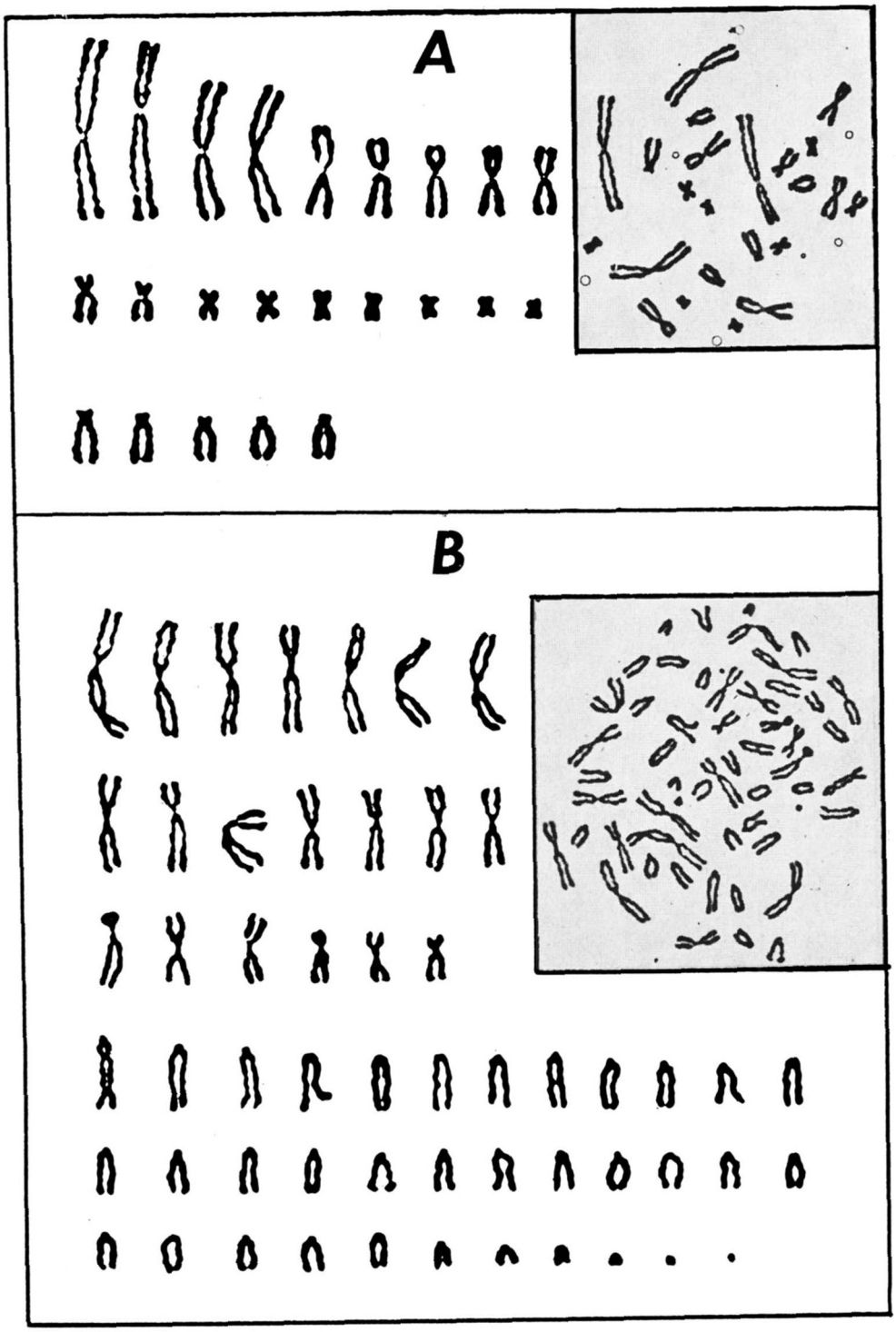
Hybrids $B_{14}I_{50}$/2472–6–3 (hereafter designated as H/M-5063): Because of the high reversion rate in both parental lines, the selective technique described above was modified as follows. Cultures in standard medium were initiated with 5×10^5 cells of a 1:1 mixture of 2472–6–3 and $B_{14}I_{50}$ and were incubated for three days at 29°C (*cf.* SCALETTA and EPHRUSSI 1965). Karyological analysis performed at the end of this period revealed 2% of hybrid metaphases identified by the presence of parental marker-chromosomes. The distribution of the karyotypes of these early hybrids is shown in Table 1. At this time the standard medium was replaced by HAT and the cultures shifted to a 37° incubator where they remained for 3 days. Thereafter, mitotic cells were suspended by shaking and seeded at low density in conditioned HAT in 6 cm petri dishes where they gave rise, within two weeks, to numerous colonies of parental revertant and fibroblast-like hybrid cells. Approximately 100 of these were pooled and subcultured for

TABLE 1

Karyotypic distribution of parental and hybrid lines

Parental lines	Cell lines: Total chromosome number Mode	Range	Metacentrics Total	Small	Medium to large	Subtelocentrics	Telocentrics	Minutes	No. of cells analyzed
(HAMSTER)									
$B_{14}I_{50}$	22	20–55	16(14–19)*	7(5–10)	9(8–11)	5(3–6)	1(0–3)	Rare	30
(MOUSE)									
A-9(L)	56	49–60	20(18–25)	1(0–2)	19(17–24)	0	31–33(25–38)	3(0–3)	61
NCTC-2472-6-3	50	49–52	2(1–2)	0	2(1–2)	0	48(47–51)†	0	25
Hybrid Lines									
A/H-1 (Observed)	75	71–76	33(32–35)	8(6–9)	25(24–28)	5(5–7)	35(31–36)	1(0–1)	25
A/H-1 (Expected)‡	78	69–85	36(32–44)	8(5–12)	28(25–35)	5(3–6)	32–34(25–41)	3(0–3)	. .
H/M 5063 (Observed)	72	65–74	19(14–22)	7(5–10)	12(8–13)	5(3–6)	48(44–51)§	0	19‖
H/M 5063 (Expected)‡	72	69–77	18(15–21)	7(5–10)	11(9–13)	5(3–6)	49(47–54)	0	. .

* First number—mode; in parentheses—range.
† Every cell analyzed had one extra long telocentric chromosome.
‡ Karyotypic distribution of hybrid lines expected from panmictic mating of the parental lines.
§ Seventeen of 19 cells analyzed had one extra long telocentric chromosome.
‖ Total analyzable hybrid cells found in mixed cultures from 3 to 13 days after initiation of the parental mixture.


A
B

three months. During this time the hybrid cells almost completely overgrew the parental (revertant) cells.

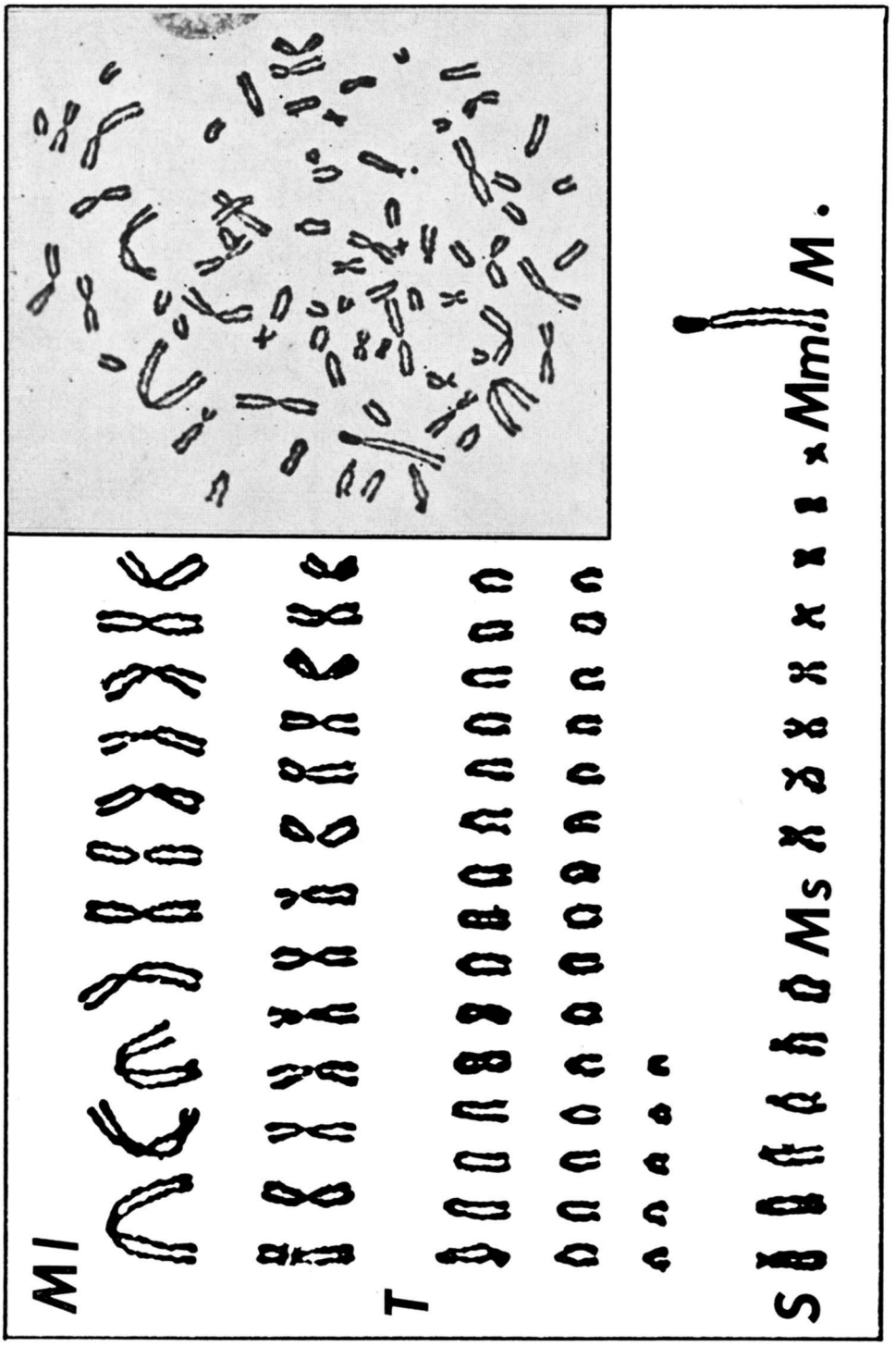
Hybrid $B_{14}I_{50}$/A 9 (hereafter designated A/H-1): Culture bottles were inoculated with 1×10^6 cells of a 1:1 mixture of $B_{14}I_{50}$ and A 9 cells and incubated 48 hours at 37°C; at this time the standard medium was replaced by HAT. Massive degeneration of the parental cells began after a few days. Within a week, many colonies began to appear. All but one of these were composed of granular, epithelial cells like those of the hamster parent, and undoubtedly represented drug sensitive "revertant" hamster cells. The exceptional colony of fibroblast-like cells, detected two weeks after the cultures had been placed in HAT, was isolated and gave rise to a culture which was examined karyologically two weeks thereafter. This analysis proved the cells to be hybrids. The observed modal numbers of metacentrics (33) and telocentrics (35) were, respectively, slightly below and slightly above the number expected from fusion of *modal* cells of the two parental lines (Table 1). A metaphase and the karyotype of a modal cell of A/H-1 are shown in Figure 2 (notice in particular the simultaneous presence of six subtelocentrics, characteristic of the hamster cells, and of the many telocentrics contributed by the mouse parent).

Phenotypic Characteristics

Growth: Hybrid line A/H-1 has now been in continuous culture for nine months, and has undergone approximately 300 cell generations, while hybrids H/M-5063 (mixed population and one of its clones) have undergone approximately 100 generations during three months of culture. Like the rat × mouse hybrids described by WEISS and EPHRUSSI (1966a), both hamster × mouse hybrids therefore appear to be capable of continuous propagation. This statement is based on the observation that the cultures showed at no time the reduction of the growth rate, characteristic of "senescence." On the contrary, their generation times seem to have decreased.

Phenotypic variation: In describing the isolation of the two types of hybrid cells, it was mentioned that these were, to begin with, of the fibroblast type, similar to their mouse parents and very different from the cells of the epithelial-like hamster parent. However, several weeks after the isolation of hybrid A/H-1, a marked morphological change was observed in cultures of this line. As can be seen in Figure 3A, epithelial islands, a single cell thick, developed, which were surrounded by areas of multilayered fibroblast-like cells of the original morphological type. The epithelial cells remained in the mass hybrid population for about three months, before they finally disappeared. While they were still present in large numbers, (11.5 weeks after isolation of the original hybrid colony), a

FIGURE 1.—A. Metaphase and karyotype of a nearly modal cell of Chinese hamster line $B_{14}I_{50}$. Note the presence of nine large and medium and nine small metacentric chromosomes and five subtelocentric chromosomes. B. Metaphase and karyotype of a cell of mouse line A 9. This nearly modal cell has 55 chromosomes, of which 20 are biarmed, 33 are telocentrics and two are minutes.


MI
T
Ms
Mm
M.

culture of A/H-1 was cloned; 30% of the colonies which developed were of the epithelial type, the remainder of the fibroblast type (Figure 3B, C). The epithelial clone shown in Figure 3B was maintained in culture approximately 2½ months with no alteration in its morphology.

Karyological analysis performed on the mass population at approximately the time of cloning, revealed a bimodal distribution of karyotypes (Figure 4A). This suggested the possibility that epithelial and fibroblastic cells were grossly different in chromosome number. Indeed, examination of the karyotypes of a few clones isolated in the described cloning experiment provided support for this view. Surprisingly though, these analyses showed that cells of the epithelial clones have a higher chromosome number than those of the fibroblast clones, i.e., a chromosome number which deviates less from the original one than that of the fibroblast hybrids (Figure 4B). The correlation between fibroblast *vs.* epithelial morphology and chromosome number is further supported by the results of karyological analysis of the mass hybrid culture *after* the disappearance of the epithelial cells. The results of this analysis, given in Figure 4C, show the disappearance of the karyotypes previously grouped around the higher mode. It is however clear from the above observation on the karyotypes of individual clones that epithelial *vs.* fibroblastic morphology must be correlated with some unidentified chromosomal change(s) rather than with the chromosome number *per se*.

Three weeks after the cloning experiment, 10^6 hybrid cells of the uncloned population were exposed to 3 μg/ml of 8-azaguanine to which the mouse parent is resistant. (It will be recalled that the hybrid cells are presumably sensitive to both 8-azaguanine and BUDR since they grow in HAT; *cf.* LITTEFIELD 1964). Forty colonies of *both* fibroblastic and epithelial types were detected and a few of these were successfully propagated in the presence of the drug for a prolonged period of time.

On the other hand, 10^6 cells from the same population were exposed to 50 μg/ml of 5-bromodeoxyuridine with essentially similar results; epithelial and fibroblast-like hybrids resistant to BUDR were detected and a resistant population of hybrid cells was isolated and propagated in the presence of BUDR.

In a parallel experiment, 3×10^6 hybrid cells were exposed simultaneously to both drugs. No doubly resistant clones were detected.

Drug resistant hybrids from the two former experiments have been exposed to HAT and were found to degenerate in this medium. Thus there is no doubt that the isolated colonies are stable (drug resistant) variants which have arisen either by chromosome loss resulting in the "uncovering" of the parental recessive "resistance genes" or by mutation(s) analogous to those which permitted the

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FIGURE 2.—Metaphase and karyotype of a modal cell of hybrid line A/H-1. The 75 chromosomes include 24 large and medium sized metacentrics (Ml), 35 telocentrics (T), 6 subtelocentrics (S), 8 small metacentrics (Ms) and one each of the characteristic submetacentric (Mm) and minute (M) chromosomes. All chromosomes in class S and the majority in Ms are derived from the Chinese hamster line, while most of those in class T are of mouse origin.

isolation of the drug resistant parents. The data at hand do not permit one to distinguish between these two possible mechanisms.

It should be mentioned at this point that evidence for the occurrence of "rever-

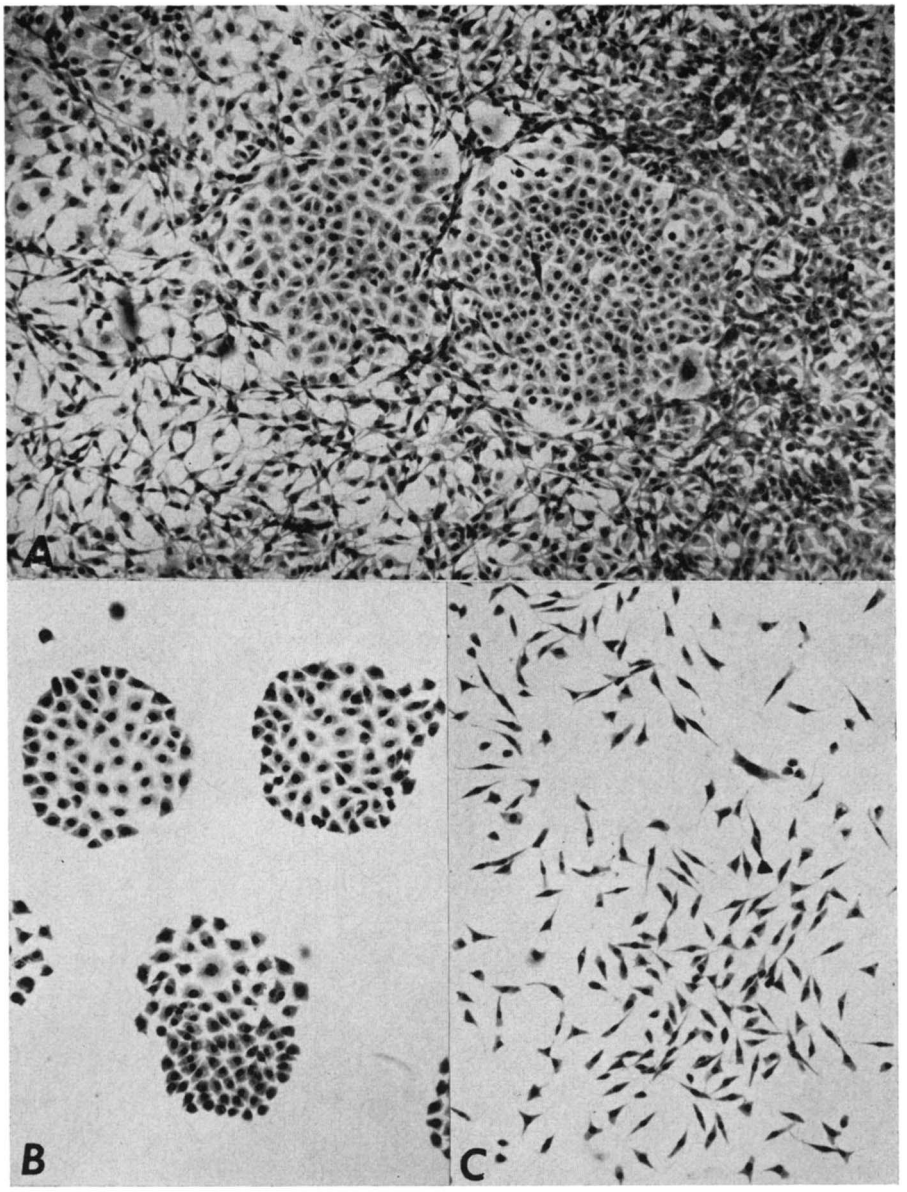

FIGURE 3.—A. Culture of cells of line A/H-l, fixed and stained at a time when epithelial cells were present in large numbers in the predominantly fibroblastic population. Note the epithelial islands, a single cell layer thick, surrounded by multilayered fibroblast growth. B. Fixed and stained colonies of epithelial cells isolated from hybrid line A/H-l. C. Fixed and stained colony of fibroblast-like cells isolated from the same hybrid line.

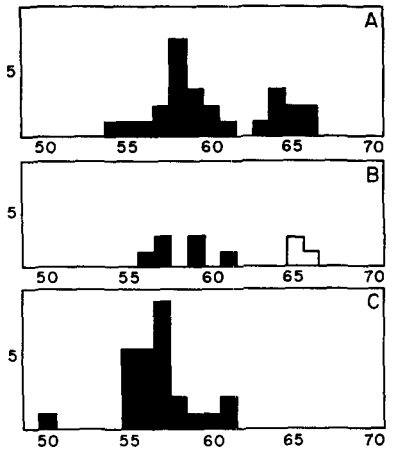

FIGURE 4.—A. Chromosome numbers of cells of line A/H-1 at the time when numerous epithelial cells were present in the predominantly fibroblastic population. Notice the bimodal distribution. B. Mean chromosome numbers of nine clones isolated from hybrid line A/H-1. Each black square represents a fibroblast-like clone, each white one, a clone of epithelial morphology. C. Chromosome numbers of cells of the hybrid population A/H-1 after disappearance of the epithelial cells.

sions" to drug resistance in (intraspecific) somatic hybrids has previously been described by LITTLEFIELD (1964, 1966). Our data confirm the occurrence of this process and, moreover, show the independence of these variations from those in the morphological characteristics of the hybrids.

β-glucuronidase of hybrid A/H-1: Different lines of mice are known to carry different forms of the enzyme β-glucuronidase, recognized by their different thermostabilities (PAIGEN 1961). GANSCHOW (1966) has studied the β-glucuronidase of somatic hybrids resulting from the fusion of *in vitro* cultured cells of two different mouse lines carrying different forms of the enzyme. His results clearly showed that the genes of both parents specifying the structure of β-glucuronidase are active in the hybrid cells: the kinetics of heat inactivation of the enzyme of hybrid cells was, roughly, intermediate between those of the parental enzymes.

WEISS and EPHRUSSI (1966b) performed similar experiments on rat × mouse hybrids and similarly concluded that the genomes of both species are active in the hybrids. However, a closer comparison of the heat inactivation curves of the enzymes from the parental cell lines (containing β-glucuronidases of very different heat sensitivities) with heat inactivation curves of the enymes from the hybrids led these authors to conclude that the latter probably contain a "hybrid (polymeric) enzyme" rather than a mixture of enzymes of the two parental types.

In view of the "biological novelty" of the hamster × mouse hybrid obtained in the present work, it was of interest to ascertain whether in these hybrids also both the mouse and hamster genes specifying the structure of β-glucuronidase are active. The following experiment was therefore performed on hybrid A/H-1.

Trypsinized suspensions of parental and hybrid cells were homogenized and partially purified by the method of PAIGEN (1961), as modified by WEISS and EPHRUSSI (1966b). Heat inactivation was carried out at 63°C using enzymes from 2–4 × 10^6 cells for each sample. After heating (0 to 63 minutes), the samples were assayed for β-glucuronidase by the spectrophotometric method of GANSCHOW (1966) utilizing para-nitrophenol as substrate (NIMMO-SMITH 1961). As can be seen in Figure 5, at 63°C the heat stability of the enzyme(s) of the hybrid

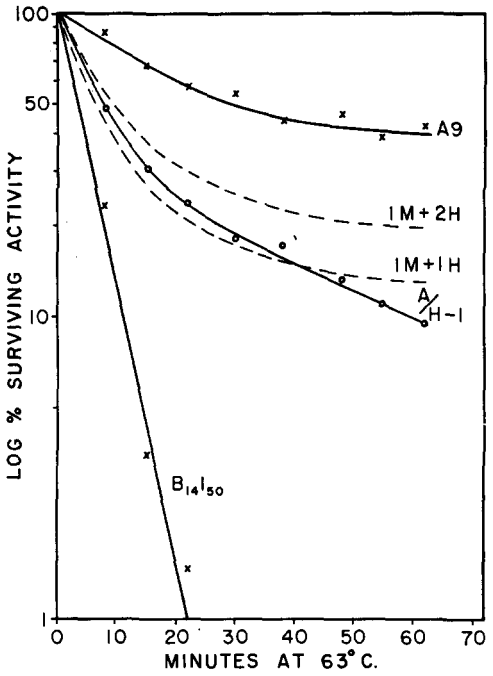

FIGURE 5.—Heat inactivation curves of β-glucuronidase from parental (A 9 and $B_{14}I_{50}$) and hybrid lines are represented as solid lines. The broken lines show the expected inactivation curves of "calculated 1:1 and 2:1 mixtures" of hamster and mouse activities.

is, roughly, intermediate between those of the mouse and hamster enzymes. The enzyme activities of the three cell lines (solid lines), after various intervals at 63°C, are given as the log percent surviving activity per unit time. The broken lines are theoretical curves calculated for 1:1 and 2:1 mixtures of hamster and mouse enzymes.

While the "intermediacy" of the curve of the enzyme from hybrid A/H-1 leaves no doubt that both mouse and hamster enzyme units are formed in hybrid cells, it will be noticed that the slope of the curve of the surviving enzyme activity from the hybrid does not conform to any of the theoretical inactivation curves calculated for mixtures of hamster and mouse enzyme in different proportions. This is a result similar to that consistently observed by WEISS and EPHRUSSI (1966b) on all rat × mouse hybrids tested. It can therefore be concluded that in the hamster × mouse hybrids, as in the rat × mouse hybrids, there is some association between the enzyme subunits of parental types.

Evolution of the Karyotype of Hybrid A/H-1

As stated at the outset, one of the main reasons hybridization of mouse cells with cells of the Chinese hamster was undertaken was that many of the chromosomes of the hamster line are identifiable and different from those of the mouse lines. Thus these hybrids offer a unique opportunity for the study of chromosome "segregation." Such a study was undertaken on hybrid A/H-1 and its results are recorded and discussed below.

Five karyological analyses of this hybrid were performed after 1, 3, 4, 5½ and 6½ months

of its *in vitro* propagation. These times will be designated as t_1, t_2, t_3, t_4 and t_5 respectively. At all these times, the karyotype was examined for changes[4] in the total number of chromosome arms (Ca), in the total number of chromosomes (Cn) and in the several morphologically distinct classes of chromosomes, namely: large and medium metacentrics (Ml), small metacentrics (Ms), subtelocentrics (S), telocentrics (T) and a minute chromosome (M) present in 88% of the cells at time t_1. Although it was difficult to identify individual chromosomes within the major classes Ml, Ms, S and T, each chromosome could be assigned to the appropriate class on the basis of length and general morphology. A single exception is the long biarmed (submetacentric) chromosome which falls within the size range of class Ml, but can be distinguished from any member of this class. This chromosome (Mm) has an arm ratio of 3.4:1; its long arm appears to be slightly longer than the long arm of chromosome No. 1 of the Chinese hamster; the deep staining of the short arm suggests that it is composed predominantly of heterochromatin. Chromosome Mm is probably a translocated chromosome of hamster origin, but it has never been observed in either the hamster or the mouse parental lines. The estimated proportion of mouse : hamster chromosome initially present in the major morphological chromosome classes of the early hybrid (assuming that the hybrid arose from the mating of modal cells) is as follows: Ml = 2:1, Ms = 1:7, S = 0:6, T = 34:1. Numerical changes in classes Ms and S can therefore be ascribed primarily to changes in the hamster genome, while changes in class T and, to a lesser extent, in class Ml, can be attributed to changes in the mouse genome. The karyotype of an early modal hybrid from t_1 is shown in Figure 2, where the different chromosome classes have been indicated.

The changes observed in the karyotype of hybrid A/H-1 during the time period t_1–t_5 are given in Table 2. Included in the table are the means, standard deviations, modes and ranges, and the percentage changes for Ca, Cn and each of the six classes of chromosomes. These changes at times t_2, t_3, t_4 and t_5 were computed with reference to the data of t_1. In calculating the total number of chromosome arms, Ca, chromosomes in classes Ml, Ms and Mm were each counted as two. Subtelocentric chromosomes (S), because of the shortness of one arm, were assigned unit values as were chromosomes in classes T and M. Although it was impossible to perform the first analysis until one month after the mixed cultures were initiated, i.e., until two weeks after the hybrid colony was first detected, the clear modes, narrow ranges and small standard deviations of chromosome numbers seen in all chromosome classes in t_1 suggest that only minor karyotypic changes occurred prior to the first analysis. The modal chromosome numbers recorded in t_1 were therefore assumed to represent the original hybrid karyotype.

As can be seen in Table 2, the following major changes in the karyotype of hybrid line A/H-1 occurred during the 5½ month period t_1–t_5:

1. *Decrease of Ca:* There occurred a decrease of chromosome arms (12.8%). This decrease is within the range of that observed in intraspecific (mouse × mouse) hybrids after an equivalent time (EPHRUSSI *et al.* 1964). Its rate clearly decreased after t_3 and possibly fell to 0 between t_4 and t_5, indicating that a "stabilization" of the karyotype had occurred.

[4] In previous puplications, changes in the numbers of chromosomes were referred to as "losses" or "gains." As will be seen below, the present work, as well as that of WEISS and EPHRUSSI (1966a), indicates the possibility of frequent occurrence, in interspecific hybrids, of chromosome rearrangements such as centric fusion. Such changes result in the diminution of the number of chromosomes without loss of genetic material. In the present paper we prefer, therefore, not to speak of "losses" and "gains," but rather of "decreases" and "increases,"—purely descriptive terms which imply no particular mechanism.

TABLE 2

Evolution of the karyotype of hybrid line A/H-1

	Karyological analysis	Total No. of chromosome arms Ca	Total No. of chromosomes Cn	Chromosome numbers for specific chromosome classes						Numbers of cells analyzed	Months since first analysis
				Ml	Ms	S	T	Mm	M		
t_1	Mean	107.4	74.1	24.4	8.0	5.7	34.2	0.92	0.88		
	standard deviation	1.3	2.0	0.9	0.6	0.5	1.2	0.28	0.33	25	0
	mode (range)	108(104–110)	75(71–76)	24(24–28)	8(6–9)	6(5–7)	35(31–36)	1(0–1)	1(0–1)		
t_2	Mean	98.7	64.9	25.5	7.4	5.0	25.4	0.81	0.73		
	standard deviation	4.5	4.1	2.2	0.7	0.8	4.6	0.40	0.61	26	2
	mode (range)	97(86–100)	66(56–72)	24(22–30)	7(6–9)	5(3–6)	23(17–32)	1(0–1)	1(0–2)		
	percent change	—8.1	—12.4	+4.5	—7.5	—12.3	—25.7	—12.0	—17.0		
t_3	Mean	95.2	60.0	27.1	7.2	4.8	19.6	0.76	0.52		
	standard deviation	3.8	3.5	2.3	0.8	0.8	4.5	0.43	0.51	25	3
	mode (range)	97(86–101)	58(54–66)	27(21–31)	7(6–9)	5(4–7)	17(13–28)	1(0–1)	1(0–1)		
	percent change	—11.4	—19.0	+11.1	—10.0	—16.8	—42.7	—17.4	—41.0		
t_4	Mean	93.1	57.2	28.0	7.1	4.7	16.0	0.80	0.63		
	standard deviation	2.2	2.1	2.0	0.9	0.7	3.3	0.41	0.49	35	4.5
	mode (range)	94(89–99)	57(53–63)	29(23–33)	7(6–9)	5(3–6)	15(8–23)	1(0–1)	1(0–2)		
	percent change	—13.3	—22.8	+14.8	—11.2	—17.5	—53.2	—13.0	—28.4		
t_5	Mean	93.6	56.7	29.1	7.0	4.2	15.1	0.76	0.60		
	standard deviation	3.1	2.2	1.6	0.7	0.6	2.4	0.43	0.50	25	5.5
	mode (range)	94(82–100)	57(50–61)	29(24–31)	7(5–8)	4(3–6)	15(11–20)	1(0–1)	1(0–1)		
	percent change	—12.8	—23.5	+19.2	—12.5	—26.3	—55.8	—17.4	—31.8		

2. *Decrease of Cn:* The decrease of chromosome number exceeded the decrease of Ca in all periods. The decrease of Cn was greater than those observed in any of the intraspecific (mouse × mouse) (EPHRUSSI *et al* 1964) or interspecific (rat × mouse) (WEISS and EPHRUSSI 1966a) somatic hybrid lines thus far studied. The reduction of Cn, like that of Ca, appears to have slowed down or to have stopped altogether by t_4, again suggesting a stabilization of the karyotype. Such stabilization of the karyotype after the first few months of growth was also observed in a rat × mouse hybrid (WEISS and EPHRUSSI 1966a).

3. *Decrease of Ms and S:* An analysis of variance performed on the number of chromosomes in classes Ms and S (both predominantly of hamster origin) revealed that significant ($P < .005$) decreases had occurred in both classes during the time period t_1–t_5. An analysis of variance performed on the ratios Ms/S indicated significant ($P < .005$) differences in the relative losses of chromosomes in classes Ms and S during the same period. However, the same statistical examination indicated that the relative losses of chromosomes in these two classes were not significantly ($P > .10$) different for the period t_1 to t_4.

4. *Decrease of T:* Class T showed a more extensive decrease than any other class through t_4, at which time the average reduction per cell exceeded 50% of the initial (t_1) number of these chromosomes (mostly of mouse origin). An analysis of variance test showed significant ($P < .001$) loss in telocentric chromosomes over the entire period t_1 to t_5. As observed for the other classes (except S), stabilization appeared to occur between t_4 and t_5. An analysis of variance of the ratio T/(Ml + Ms + S + Mm + M) showed a significant ($P < .001$) decrease of T relative to other chromosomes throughout the period t_1 to t_5. The same analysis of the ratio T/(Ms + S + Mm + M) gave similar results, showing a greater decrease of T than of chromosomes in classes Ms, S, Mm and M.

5. *Increase of Ml:* Unlike the three classes of chromosomes discussed above, the large and medium sized metacentrics (Ml) steadily increased in number in each successive analysis. An average increase of almost five chromosomes per cell (19.2%) was seen during the 5½ months of observation. An increase in metacentric chromosomes has also been reported for other interspecific hybrids (WEISS and EPHRUSSI 1966a). An analysis of variance test revealed that the increases in number of Ml chromosomes were statistically significant ($P < .005$) over all periods from t_1 through t_5. Similar tests of the ratios Ml/(T+Ms+S+Mm+M) and Ml/(Ms + S + Mm + M) showed significant ($P < .001$) increases of Ml relative to the other chromosome classes throughout the entire period investigated.

6. *Changes of M and Mm:* A chi-square test showed significant decreases ($P < .05$) in the number of M during the period t_1–t_3. The number of Mm did not change significantly ($P > .10$) during this period.

The previous analyses were all concerned with changes in either the mean chromosome numbers in various classes or in ratios between chromosome numbers in two classes. In the hope of finding significant associations between some of these changes, which could be enlightening as to the underlying mechanisms, correlation coefficients were determined between pairs of chromosome classes using the numbers of Ml, T, S and Ms in each of the five karyological analyses.

Such coefficients, presented in Table 3, give measures of association between chromosome numbers in the various classes. They show whether cells having a larger than average number of chromosomes of one class, tend to have a smaller (negative coefficient) or larger (positive coefficient) than the average number of chromosomes of another class. *A priori*, high and statistically significant correlation coefficients may be suggestive of mechanisms producing related changes in the pairs of chromosome classes examined.

A statistical test (RUSHFORTH 1964) indicated that there were significant ($P < .05$) differences among the sets of correlation coefficients obtained for each of the five karyological analyses. It is evident from Table 3 that Ml and T are consistently and significantly ($P < .01$) negatively correlated in t_2 t_3, and t_4. The increasing negative coefficients from analyses t_1–t_3 (but decreasing between t_4 and t_5) imply that cells having the higher numbers of large and medium sized metacentric chromosomes increasingly tended (up to t_4) to have smaller than average numbers of telocentric chromosomes (and *vice versa*). No consistent association was detected between the pairwise combinations of other chromosome classes.

Summarizing the results of the above statistical analyses, it may then be stated that the following relationships have been revealed between the changes of the various classes of chromosomes: (1) Except during the last time period t_4–t_5. decreases of Ms and S were not significantly different. (2) A significant preferential decrease of chromosomes in class T was observed throughout the period of study. (3) In contrast, chromosomes in class Ml were found to increase significantly relative to other chromosome types throughout the entire period of observation. (4) Correlation coefficients between Ml and T revealed an increasing negative association over the periods t_1 and t_4, significant at the 1% level, between t_2 and t_4. There was, however, a decrease of this negative association between t_4 and t_5. No consistent association was detected between other combinations of classes.

The most notable feature of the karyotypic changes recorded for A/H-1 appears to be the increase of large and medium sized metacentric chromosomes clearly negatively correlated with the decrease of telocentric chromosomes. In considering possible reasons for this negative correlation one naturally raises the

TABLE 3

Correlation coefficients of classes M_l, M_s, S and T

Karyological analysis	Number of cells analyzed	M_l, T	M_l, S	M_l, M_s	T, S	T, M_s	M_s, S
I	25	—.33	—.46*	—.67**	—.12	.12	.27
II	26	—.60**	.11	—.15	.09	.18	.04
III	25	—.72**	—.23	.05	.11	.02	—.07
IV	35	—.72**	.02	—.45*	—.11	.09	—.08
V	25	—.12	—.30	—.22	—.48*	—.17	.00

* Significant at 5% level. ** Significant at 1% level.

question as to the existence of a causal relationship between these two processes. Metacentric chromosomes are known to arise often by misdivisions of the centromeres resulting in the production of metacentric isochromosomes, and by centric fusion of telocentric chromosomes. Either of these mechanisms could account for the observed correlated increase of metacentrics and decrease of telocentrics. Were it not for the selection which takes place in cell populations, a distinction between the two mechanisms could be made by the type of analysis described in the Appendix. However, intense selection does operate in rapidly growing cultures, making inferences from this type of analysis hazardous. Direct evidence of selection in A/H-1 was provided by the appearance of epithelial-like cells several weeks after t_1. The proportion of these cells gradually increased and they maintained themselves for about three months before gradually disappearing from the culture. The data at hand do not enable us to evaluate the role of these cells in the recorded changes of the correlation coefficients with time. Selection might favor cells with increased numbers of metacentrics and decreased numbers of telocentrics resulting from centric fusion or isochromosome formation. The observed correlation between increase of metacentrics and decrease of telocentrics could also be due to a selective advantage of the rare cells in which chance loss of certain telocentric chromosomes happened to coincide with (and was compensated by) the gain of certain metacentrics (for example by nondisjunction). A choice between these possibilities cannot be made on the basis of the available data. It must be stressed, however, that the relative changes seen in the chromosome numbers in the various classes up to t_4 do not hold for time period t_4–t_5. This period is characterized by (a) an increasing reduction in subtelocentrics, (b) a reduction in the negative association between telocentrics and metacentrics and (c) a general reduction in the variability of chromosome numbers in the various classes. Clearly, during the period t_4–t_5 a different karyotype was being selected.

It is worth recalling the striking similarities between the observations of this study and those of WEISS and EPHRUSSI (1966a). This similarity points to the possibility that the intracellular environment of interspecific hybrids may be conducive to some types of chromosomal rearrangements, such as centric fusion and isochromosome formation.

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SUMMARY

Two types of mononucleate interspecific somatic hybrids were isolated from the mating of cells of each of two biochemically marked mouse lines with cells of a marked Chinese hamster line, and cultured over prolonged periods of time. The hybrid nature of these cells, was confirmed by karyological analysis and by the demonstration in one of them (A/H-1) of the synthesis of β-glucuronidase(s) characteristic of both parental species.—Two types of phenotypic variants were isolated from hybrid line A/H-1: one characterized by morphological change

(from fibroblastic to epithelial morphology), the other by changes from drug sensitivity to drug resistance. Changes to resistance to 8-azaguanine and 5-bromodeoxyuridine were shown to be independent of the morphological changes and of each other.—The evolution of the karyotype of hybrid line A/H-1 was followed over a period of 5½ months. During this time a decrease in chromosome number occurred at a rate which exceeded that observed in other somatic hybrids. A preferential decrease of telocentric chromosomes, mostly of mouse origin, was observed which was apparently correlated with an increase of metacentric chromosomes.

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APPENDIX

It was noted in the preceding paper that several mechanisms could lead to an increase of metacentric chromosomes negatively correlated with a decrease in telocentric chromosomes. The following analyses were performed to determine if mechanisms of centric fusion and isochromosome formation, assuming the absence of selection, were compatible with the observed karyotypic changes.

The expected numbers of chromosomes and chromosome arms per cell were derived for a given time, t', in terms of those recorded at the time of the previous karyological analysis t, as follows:

Let Ml denote the number of chromosomes in class Ml and T the number of those in class T at time t. Suppose that between t and t' proportions α and β of the T telocentric chromosomes give rise to metacentric chromosomes by centric fusion and isochromosome formation respectively. It is postulated that the probability of random loss of chromosomes of any type is $1-p$, where p is the probability of chromosome retention between t and t'. If it is assumed that no selection occurs for chromosomes of any class, then the number of chromosomes and chromosome arms at t and those expected at t' are given by:

Time		Metacentrics	Telocentrics	Metacentrics and telocentrics
t	Chromosome number	M_L	T	M_L+T
	Chromosome arms	$2M_L$	T	$2M_L+T$
t'	(a) Random loss ($\alpha=0,\beta=0$)			
	Chromosome number	pM_L	pT	$p(M_L+T)$
	Chromosome arms	$p2M_L$	pT	$p(2M_L+T)$
	(b) Random loss and centric fusion ($\beta=0$)			
	Chromosome number	$p(M_L+\alpha T/2)$	$p(1-\alpha)T$	$p(M_L+T-\alpha T/2)$
	Chromosome arms	$p(2M_L+\alpha T)$	$p(1-\alpha)T$	$p(2M_L+T)$
	(c) Random loss and isochromosome formation ($\alpha=0$)			
	Chromosome number	$p(M_L+\beta T)$	$p(1-\beta)T$	$p(M_L+T)$
	Chromosome arms	$p(2M_L+2\beta T)$	$p(1-\beta)T$	$p(2M_L+T+\beta T)$
	(d) Random loss, centric fusion and isochromosome formation			
	Chromosome number	$p(M_L+\beta T+\alpha T/2)$	$p(1-\alpha-\beta)T$	$p(M_L+T-\alpha T/2)$
	Chromosome arms	$p(2M_L+2\beta T+\alpha T)$	$p(1-\alpha-\beta)T$	$p(2M_L+T+\beta T)$

From the above relationships the expected ratios of metacentric and telocentric chromosomes, and chromosome arms at t' to those at t are given by:

	Chromosomes	Chromosome arms
(a) Random loss	$\dfrac{p(M_L+T)}{(M_L+T)}=p$	$\dfrac{p(2M_L+T)}{(2M_L+T)}=p$
(b) Random loss and centric fusion	$\dfrac{p(M_L+T-\alpha T/2)}{(M_L+T)}<p$	$\dfrac{p(2M_L+T)}{(2M_L+T)}=p$
(c) Random loss and isochromosome formation	$\dfrac{p(M_L+T)}{(M_L+T)}=p$	$\dfrac{p(2M_L+T+\beta T)}{(2M_L+T)}>p$
(d) Random loss, centric fusion and isochromosome formation	$\dfrac{p(M_L+T-\alpha T/2)}{(M_L+T)}<p$	$\dfrac{p(2M_L+T+\beta T)}{(2M_L+T)}>p$

It is seen that, if centric fusion occurred, there would be fewer chromosomes in the combined

TABLE 4

Observed ratios of numbers of metacentric plus telocentric chromosomes and chromosome arms and the estimated probabilities of chromosome retention

Time period	Estimated probability of chromosome retention	Observed ratio of chromosome number	Observed ratio of chromosome arms
$(t'-t)$	$p = \frac{(M_s + S + Mm + M)'}{(M_s + S + Mm + M)}$	$\frac{(M_L + T)'}{(M_L + T)}$	$\frac{(2M_L + T)'}{(2M_L + T)}$
t_2-t_1	.90	.87	.92
t_3-t_2	.95	.92	.97
t_4-t_3	.99	.94	.98
t_5-t_4	.95	1.00	1.02

class (metacentrics + telocentrics) at t' than expected with random chromosome loss. Fusion, however, would not affect the expected number of chromosome arms in this class. In contrast, if isochromosomes were formed, the number of chromosomes would not be affected but there would be more chromosome arms than expected with random loss alone. The ratios of the observed numbers of metacentric and telocentric chromosomes at t' to those at t, and similar ratios for chromosome arms were determined for the four time periods. These ratios are compared in Table 4 with the estimated probabilities of chromosome retention (p), based on changes in the number of chromosomes in the combined class $M_s + S + Mm + M$. The combined class contains all chromosomes other than telocentrics and metacentrics. Such chromosomes are assumed to be subject to random loss alone (and the latter is assumed to be independent of chromosome length).

From Table 4 it is seen that the observed ratios of chromosome numbers are less than the estimated value of p for all periods except $t_5 - t_4$. In addition the observed ratios in numbers of chromosome arms are greater than the estimated value of p in all periods except $t_4 - t_3$. Such changes except in the two periods noted above are consistent with mechanisms of centric fusion and isochromosome formation both occurring. However, since none of the changes are statistically significant ($P > .10$) the results of this analysis are not conclusive. Nevertheless the analysis has been presented here since its principle may be applied in cases where selection is negligible or absent.