menting with glucose slightly decreased sensitivity to fructose and had no effect on the response to glucose.

In order to ascertain whether or not the differences between Evans' results and ours could be attributed to the different techniques employed, we made a test with normal flies and with those raised on fructose that duplicated exactly his experimental technique and methods of comparison. For normal flies the values by which the stimulating effect of fructose exceeded that of glucose were 26 times (Evans), 25 times (our nonsterile technique), 23 times (our sterile technique), and 23 times (*7*). For flies raised on fructose the values were: less than 1 time (Evans), 20 times (our nonsterile technique), and 16 times (our sterile technique). Although the changes are all in the same direction when expressed this way, they are due to an increase in the sensitivity of these flies to fructose (Table 1).

The changes observed by us do not correlate with metabolic phenomena nor are they consistent with ideas concerning glucose repression of inductible enzyme synthesis. Additionally, analysis of mortality data collected over five generations of breeding on the respective fortified diets plus the fact that there were no differences in the slopes of the dosage-response curves argues against selection having occurred in this generation. The composition of the food consumed during development clearly affects the behavioral gustatory sensitivity of the adults, but the nature of the interaction is unresolved.

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Human Thymidine Kinase Gene Locus: Assignment to Chromosome 17 in a Hybrid of Man and Mouse Cells

Abstract. *The human chromosome retained in a hybrid clone derived from human cells and a mouse line deficient in thymidine kinase has the quinacrine-fluorescence pattern characteristic of chromosome 17.*

Weiss and Green described hybrids of human and mouse cells (man-mouse hybrids) containing a markedly reduced complement of human chromosomes (*1*). One human chromosome was consistently present when the hybrid cells were grown in medium containing hypoxanthine, aminopterin, and thymidine (HAT), which requires the presence of thymidine kinase for cell survival and growth; it was absent from hybrid cells grown in medium containing 5-bromodeoxyuridine, which selects for cells deficient in thymidine kinase. The gene specifying thymidine kinase was therefore presumed to reside on

Table 1. Characteristics of the karyotype of the hybrid cells.

Measurement	Migeon and Miller's data (*2*)	Our data
Number of cells analyzed	21	66
Range of number of chromosomes	48–54	48–55
Modal number of chromosomes	53	53
Modal number of large biarmed chromosomes	9, 10	9
Number of cells with no E-group chromosomes	4	10
Number of cells with one E-group chromosome	13	51*
Number of cells with two E-group chromosomes	3	5*
Number of cells with possible C-group chromosomes	1	1†

* Every one identified as chromosome 17 in the present study. † Not identified as a human chromosome.

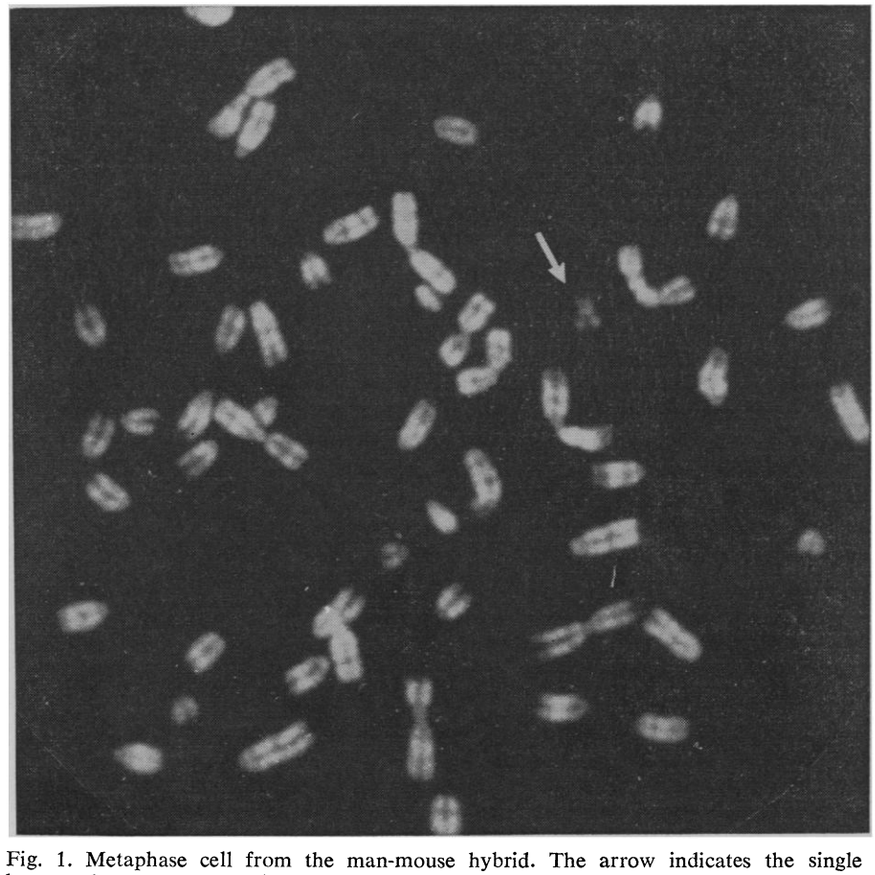

Fig. 1. Metaphase cell from the man-mouse hybrid. The arrow indicates the single human chromosome, number 17.

this chromosome, which was subsequently identified as a submetacentric member of the human E-group (chromosomes 17 and 18) by Migeon and Miller (*2*) and Matsuya *et al.* (*3*). The hypothesis that this chromosome carries the structural locus gained further support from electrophoretic studies which showed that the thymidine kinase present in the hybrid cells was of human rather than mouse type (*4*).

More precise identification of the solitary submetacentric chromosome in these hybrids has not been possible on the basis of comparative morphology or replication pattern since both require the presence of chromosomes 17 and 18 together in the cell. However, individual human chromosomes can be identified by their distinctive pattern of fluorescence with quinacrine mustard (*5*) or quinacrine hydrochloride (*6*). These patterns appear to be an inherent characteristic of each chromosome under the culture conditions studied. Consequently, identification may be independent of the presence of other human chromosomes in the cell.

We, therefore, undertook to identify the submetacentric human chromosome present in one of the man-mouse hybrid cell lines, clone 6, isolated by Migeon and Miller (*2*) and maintained in HAT medium to retain the human thymidine kinase in the hybrid genome. The cells of clone 6 used for these experiments had been frozen as a sixth subculture at the time when the karyotype of most of its cells included the pertinent E-group chromosome (*7*). After thawing, the cells were maintained in HAT medium for 6 days before karyotyping. Metaphase cells were then removed from the Falcon plastic flasks by shaking and transferred to previously warmed (37°C) centrifuge tubes containing Colcemid. After 35 minutes, the medium was replaced with warmed trypsin (0.25 percent weight per volume), which was replaced after 8 minutes with 1 percent sodium citrate. Cells were fixed with acetic acid and alcohol (1 : 3) 20 minutes later. Cells were air-dried on good quality microscope slides (Clay-Adams). The slides were immersed in 1 percent quinacrine hydrochloride (Atebrin, G. Gurr) for 5 to 6 minutes, rinsed for 3 minutes in tap water, rinsed and then mounted in tris-maleate buffer at *p*H 5.6.

Cells were examined with a Leitz Ortholux microscope by transmitted

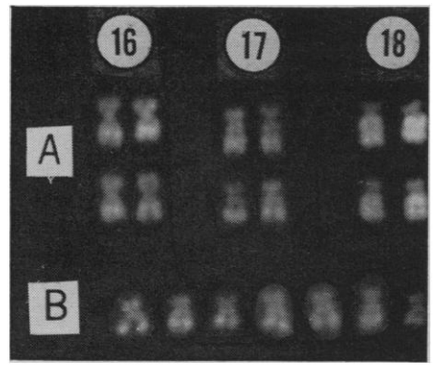

Fig. 2. (A) Chromosomes 16, 17, and 18 from two normal human cells. (B) The small submetacentric chromosome (human number 17) from seven man-mouse hybrid cells.

ultraviolet light from an HBO 200 watt mercury vapor lamp with a 1.5-mm BG-12 exciter filter, a dark-field condenser (D1.20), and a K530 barrier filter. All observations were made with an apochromatic oil immersion objective (×90). Suitable metaphase figures were photographed on Kodak Panatomic-X film, at exposure times of 20 to 40 seconds. Photographic prints were made on Kodabromide or Fotorite paper of contrast grades 3 or 4.

Sixty-six cells were examined (Table 1). Fifty-one of these contained a single, small submetacentric chromosome of the size and morphology of a member of the human E-group (Figs. 1 and 2), and five cells contained two such chromosomes. The fluorescence patterns of chromosomes 16, 17, and 18 in human metaphase cells are illustrated in Fig. 2. Chromosome 16 has a brightly fluorescent band in the middle of its long arm and a nearly even degree of intensity of fluorescence throughout the rest of the chromosome. Chromosome 17 has a generally low level of fluorescence and a banded appearance; only the distal half of the long arm is relatively bright. Chromosome 18 has a bright band in both the proximal and distal portions of the long arm.

The fluorescence pattern of the presumptive human E-group chromosome in the hybrid cell was typically that of a human chromosome 17, and quite different from that of either chromosome 18 or 16. No other human chromosome could be identified in any of the 66 cells examined. In one cell a submetacentric chromosome was present whose size and shape were compatible with its being a member of the human C-group. However, its pattern of fluorescence was unlike that of any human chromosome, but it could have been derived from centric fusion of two acrocentric mouse chromosomes. Cells of the parental LM(TK$^-$) clone 1D line (*8*), examined by quinacrine fluorescence, contained no small submetacentric chromosome, but did have the other biarmed chromosomes seen in the hybrid.

Our results indicate that the great majority of the man-mouse hybrid cells in this clone have retained a human chromosome 17, and that a minority of cells have two chromosomes 17. Since the viability of these hybrid cells in HAT medium is dependent upon the presence of this human E-group chromosome (*2, 3*) and human thymidine kinase (*4*), we conclude that the thymidine kinase locus is on human chromosome 17.

The use of characteristic quinacrine fluorescence patterns to identify human chromosomes in man-mouse hybrid cells should greatly enhance the value of these hybrids for linkage studies.

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