trifugation for 4 hours at 27,000 rev/min with the SW-27 rotor. Chloroplast ribosomes were prepared from phototrophically grown *E. gracilis*, strain Z, by a method similar to that reported by Rawson and Stutz (*1*). Various preparations of chloroplast ribosomes contained a large amount of free 50*S* subunits but few 30*S* subunits, probably due to the preferential retention of 30*S* subunits in the membrane fraction of chloroplasts. Subunits were prepared from *E. coli* and chloroplast ribosomes by incubation in 0.01*M* tris buffer (*p*H 7.6) containing 1 m*M* $MgCl_2$ and 0.1*M* KCl and then sedimenting through sucrose gradients containing the above buffer salts.

The ribosomes from chloroplasts of *E. gracilis* require 20 m*M* Mg^{2+} in the presence of 50 m*M* KCl to maintain ribosome integrity. At 10 m*M* Mg^{2+}, the chloroplast ribosomes dissociate into subunits, while the ribosomes from *E. coli* do not (Fig. 1). After incubation of chloroplast ribosomes with ^{14}C-labeled *E. coli* 50*S* subunits in the presence of 10 m*M* $MgCl_2$ and 50 m*M* KCl, analysis of the ribosome profile showed the formation of radioactive 70*S* ribosomes, which indicates the formation of hybrid monosomes resulting from the association of 30*S* chloroplast ribosome subunits and 50*S* *E. coli* ribosome subunits (Fig. 2). The formation of hybrid ribosomes was more extensive when a crude preparation of chloroplast ribosomes was used (Fig. 2A) than when purified chloroplast ribosomes were utilized (Fig. 2B) in the experimental protocol. When ^{14}C-labeled *E. coli* 70*S* monosomes were incubated with 50*S* chloroplast ribosome subunits and analyzed similarly, no radioactivity appeared in the 50*S* region.

The capacities of the heterologous combinations of subunits to carry out polyuridylic acid–directed incorporation of phenylalanine into products insoluble in hot 5 percent trichloroacetic acid (TCA) were examined in the presence of 10 m*M* $MgCl_2$. The purity of the subunits may be seen from the lack of phenylalanine incorporation when the subunits were tested alone (Table 1, lines 1 to 4). The mixture of 50*S* subunits of chloroplast ribosomes and 30*S* subunits of *E. coli* ribosomes failed to incorporate phenylalanine, but the mixture of 50*S* subunits of *E. coli* ribosomes and 30*S* subunits of chloroplast ribosomes incorporated an appreciable amount of phenylalanine. The inability of the homologous mixture of chloroplast ribosome subunits to incorporate phenylalanine is not surprising in view of the fact that these ribosomes dissociate into subunits in the presence of 10 m*M* $MgCl_2$.

Table 1. Polyuridylic acid–directed incorporation of phenylalanine into polyphenylalanine with hybrid ribosomes of *Escherichia coli* and *Euglena* chloroplast ribosomes. The reaction mixtures, in a volume of 0.5 ml, contained 1 m*M* adenosine triphosphate, 0.3 m*M* guanosine triphosphate, Mg^{2+} (as $MgCl_2$) as indicated, 50 m*M* KCl, 20 m*M* NH_4Cl, 20 m*M* tris buffer, 0.08 μc of [^{14}C]phenylalanine (175 count/min per picomole), 80 μg of polyuridylic acid, 50 μg of transfer RNA from dark-grown *Euglena*, 0.32 mg of *E. coli* enzyme mixture obtained in ammonium sulfate (between 22 and 50 percent) from postribosomal supernatant, 18 μg of protein from *E. coli* ribosome washing (the proteins that were released when ribosomes were allowed to dissociate; these proteins stayed at the top of the sucrose gradient when centrifuged, while ribosome subunits sedimented), and 90 or 180 μg, or both, of 30*S* and 50*S* subunits, respectively. The *p*H of the reaction mixture was 7.6. After 30 minutes of reaction at room temperature, TCA was added and the mixtures were heated to 90°C for 10 minutes. The precipitates were collected on Millipore filters, washed with four 3-ml portions of 5 percent TCA containing 0.1 percent cold phenylalanine. The filters were dried and radioactivity was counted with a gas flow Geiger-Müller counter.

Mg^{2+} (conc.)	Ribosome subunits	Phenylalanine incorporated (count/min)
10 m*M*	*E. coli* 30*S*	23
10 m*M*	*E. coli* 50*S*	42
10 m*M*	Chloroplast 30*S*	21
10 m*M*	Chloroplast 50*S*	12
10 m*M*	*E. coli* 30*S* + chloroplast 50*S*	12
10 m*M*	Chloroplast 30*S* + *E. coli* 50*S*	910
10 m*M*	*E. coli* 30*S* + *E. coli* 50*S*	4030
10 m*M*	Chloroplast 30*S* + chloroplast 50*S*	16
10 m*M*	None	22
20 m*M*	Chloroplast 30*S* + chloroplast 50*S*	230

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15 March 1971

Blowflies: Alteration of Adult Taste Responses by Chemicals Present during Development

Abstract. *The addition of certain sugars to the food consumed during larval development increases the taste sensitivity of adult blowflies to some sugars, decreases it to others, and is without effect on the sensitivity to still others. No correlation with metabolic phenomena is apparent. The hypothesis that repression of inductible enzyme synthesis by glucose is a relevant model is not supported.*

The chemical nature of food ingested by insects during larval life apparently can influence various behavioral responses of the adults (*1*). Of particular interest is the report of Evans (*2*) describing experiments in which the addition of specific sugars to a basal larval diet lacking all sugars except lactose reduced the gustatory sensitivity of adult blowflies (*Phormia regina* Meigen) to the particular supplementary sugar. For example, in flies reared in the presence of fructose the mean behavioral response to tarsal stimulation was 46 times higher than that of control flies reared in the absence of fructose. The sucrose threshold of these flies also was elevated. Evans suggested that the effective sugars acted by depressing sensitivity specifically to themselves, and to other sugars that act at the same site, because of a decrease in the number or affinity of their combining sites on the sugar-sensitive neuron. A possible parallel was drawn between this action and the depressive effect of regulator genes in *Escherichia coli* (*3*).

Certain critical controls were missing from Evans' experiments; consequently alternative explanations of his results are equally plausible. Because of the potential significance of the phenomenon described we have repeated and extended his experiments to supply the missing controls. One major flaw was that the larval medium employed was not sterile. It supported a luxuriant growth of bacteria and fungi. There was no assurance that the diet actually contained the intact supplementary sugar during the course of larval ingestion and development. Another cause of concern was the high mortality rate encountered; therefore, the possibility that differentially sensitive strains of flies were being selected from a heterogeneous population was not eliminated.

The method of testing also introduced potential sources of error. The ascending technique (*4*) is not the most rigorous because each fly is exposed to more than one test solution, and sensory adaptation becomes a confounding factor. Finally, the use of a relative measure of sensitivity is less desirable than an absolute measure. Evans analyzed his results in terms of the relative sensitivity (n) of two sugars expressed as a function of ascending steps of doubled concentrations according to the formula: fructose threshold $\times 2^n =$ glucose threshold. This procedure was adopted to compensate for the variability of absolute threshold occurring with different degrees of feeding and starvation. By instituting a rigid control of age, nutritional state, and state of water balance, and by using very large numbers of flies (up to 900), we were able to substitute the kind of test typically used for measuring response as a function of dosage.

The flies tested in our experiments were of the same stock as those used by Evans. They had been maintained in continuous culture since 1947; since 1955 they had been raised on a diet consisting of yeast, powdered whole milk, agar, and Tegosept. Eggs were sterilized within 24 hours after oviposition by being soaked in 1 percent NaOH for 10 minutes and rinsed twice in 70 percent ethanol (*5*). They were then placed in a sterile medium. The medium consisted of 200 g of powdered yeast, 200 g of powdered whole milk, 8.5 g of Tegosept, 40 g of agar, and 3 liters of water, all of which had been poured while hot into sterilized flasks, plugged with cotton, and autoclaved 7 minutes at 121°C. Media supplemented with sugars were prepared the same way except that sugar was added. Each supplemented medium had per liter 0.1 mole of one of the following: arabinose, fructose, galactose, glucose, mannose, or sorbose. The integrity of the sugar in the medium after sterilization was indicated by the creamy color of the mixture (brown coloration indicates carmelization and breakdown plus the formation of protein complexes) and by other tests. One test, the enzymatic-colorimetric determination of glucose (Sigma Chemical Co., kit No. 510-DA) in a sample of glucose-fortified medium upon which larvae had been feeding for 6 days, showed that the larvae were actually exposed to glucose and not to some product of degradation.

Table 1. The tarsal sensitivity to sugars of adult blowflies raised on media supplemented with sugars. The sensitivity is expressed as median acceptance threshold. Values that are significantly different at $P = .01$ are shown with an asterisk. The direction of the difference is indicated by + or −.

Larval medium	Median acceptance threshold (mole/liter) to					
	Arabinose	Fructose	Galactose	Glucose	Mannose	Sorbose
Control	0.105	0.114	0.506	0.263	>1.0	0.479
Arabinose	.531	.0108	.586	.247	>1.0	.374−*
Fructose	.175	.011−*	.760+*	.179−*	>1.0	.535
Galactose	.090	.007−*	.418	.177−*	>1.0	.242−*
Glucose	.129	.054+*	.622+*	.330	>1.0	.859+*
Mannose	.131	.005−*	.513	.244	>1.0	.460
Sorbose	.132	.009	.690	.323	>1.0	.360

For tests of behavioral sensitivity 3- to 4-day-old flies that had received only water since eclosion were anesthetized with CO_2 and mounted by the wings on Tackiwax holders. They were allowed to rest undisturbed for at least 1.5 hours to ensure complete recovery from narcosis. All testing was done in the early afternoon to minimize possible effects of variation in the degree of activity. Just before testing, each fly was given free access to water. The random technique of testing was employed (*4*). A series of sugar solutions of doubling steps of concentration from 0.0019 to 1 mole/liter was prepared. The flies were divided into four to six groups of at least 20 each. The approximate effective range of concentrations for each sugar was known from previous work (*6*). The first group of flies was tested at one of these concentrations; then, depending upon the percentage responding, successive groups were tested, each at a different concentration greater or less than that of the first. A test consisted of lowering the legs of each fly first into water until the proboscis did not extend and then placing the legs into the test solution for 10 seconds. The percentages of each group which extended the proboscis fully within this period at each of six concentrations were converted to probability units and plotted against the logarithms of the respective concentrations, yielding typical dosage-response curves. The median acceptance threshold was then calculated according to the method of Bliss (*7*). Chi-square tests were made to ascertain whether or not the slopes of these regression curves and their medians were significantly different at $P = .01$. From 300 to 900 flies were tested under each experimental condition (Table 1).

There were significant differences in the median acceptance thresholds in some cases; there were no differences in the slopes of the dosage-response curves in any case. This means that when changes in sensitivity did occur in a population, they were not changes in the distribution of sensitivities within the population but changes that affected all members.

The changes in threshold that did occur are not in agreement with Evans' data. For one thing, they are very small. Whereas he found differences between control and experimental groups as great as 46 times, the greatest difference that we recorded was only 2.5 times. The rest were smaller. Furthermore, the directions of the differences were not in complete agreement. Supplementing the diet with a sugar did not always induce a decrease in sensitivity of the adults to that added sugar. Specifically, supplementing the diet with fructose slightly increased adult sensitivity to that sugar. Supple-

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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8. Supported by NSF grant GB 1472. We thank Prof. G. S. Watson for advice and assistance in statistical matters.

12 February 1971

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.

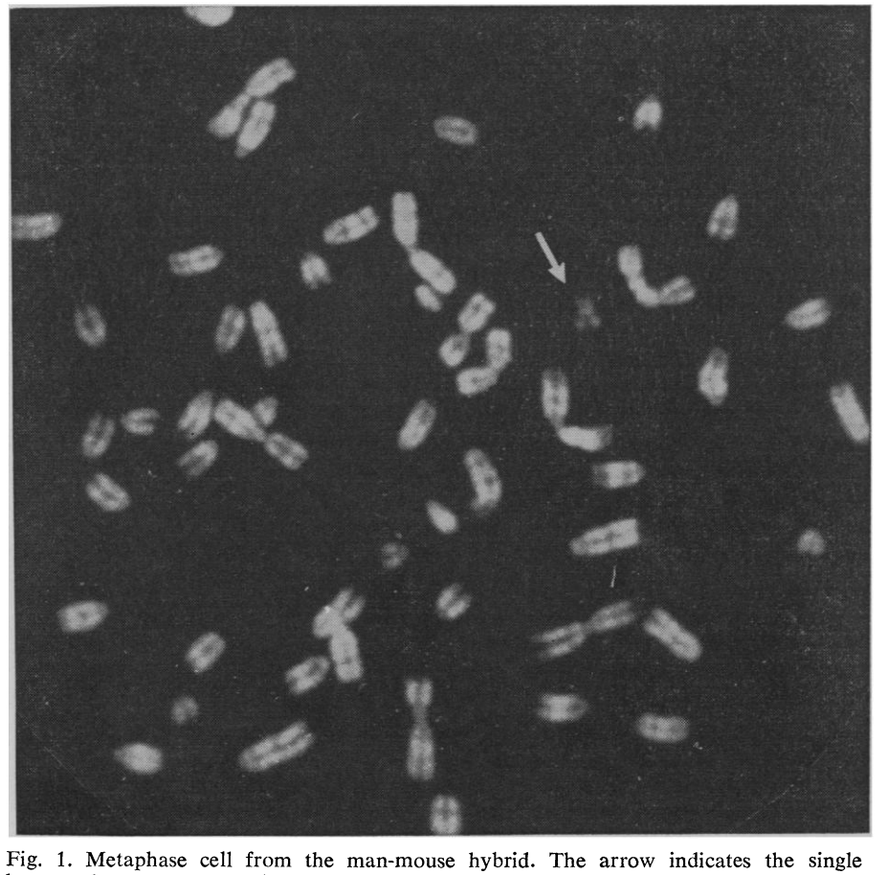

Fig. 1. Metaphase cell from the man-mouse hybrid. The arrow indicates the single human chromosome, number 17.