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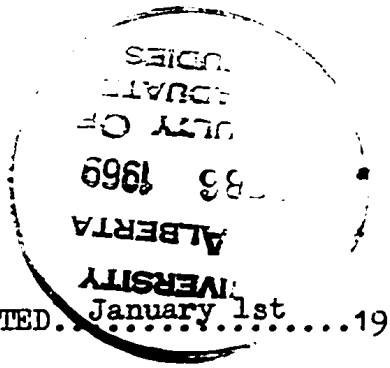

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STUDIES ON NUTRITIONAL CONDITIONAL LETHALS OF *DROSOPHILA MELANOGASTER*

by

© ERNEST ROBERT VYSE

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF GENETICS

EDMONTON, ALBERTA

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THE FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to The Faculty of Graduate Studies for acceptance a thesis entitled "Studies on Nutritional Conditional Lethals of Drosophila melanogaster" submitted by Ernest Robert Vyse in partial fulfilment of the requirements for the Doctor of Philosophy Degree.

David Nash
Supervisor

John Kuspira

P. J. Hastings

Wm. Paranchych

Date Dec. 6/68

Saul Zalik

ABSTRACT

Auxotrophic mutants are the basic tools of a large portion of genetic research. Despite extensive genetic knowledge in several animals no whole body auxotrophic mutants are known. Drosophila is a major genetic organism in which auxotrophic mutants should help to determine whether the biochemical pathways, genetic control mechanisms and genetic fine structure found in microorganisms apply to higher organisms.

The mutagen, ethyl methanesulphonate was used to produce sex linked recessive lethals on two different minimal media. Amino acid medium with RNA and casein medium without RNA were used as restrictive media. Yeast-agar medium and casein medium with RNA were used as complete media to check for possible nutritional supplementation of the recessive lethals. Three nutritional conditional lethals were found.

One mutant 1308 is viable in the presence of RNA or an RNA contaminant but is lethal in the absence of RNA. RNA precursors do not supplement 1308, but there is a suggestion that purine biosynthesis is involved in the nutritional deficiency. This mutant has been located within a small region of the X chromosome, with perhaps, a modifying factor also in the X chromosome.

The mutant 11523, which grows on yeast-agar medium, is lethal when grown on either amino acid or casein media. The nature of the nutritional deficiency has not been determined. Mutant 11523 maps within a small region of the X chromosome close to forked.

The third strain 1625 was isolated as an RNA requiring mutant but has since lost the requirement. An inversion from 3D5 - 6 to 20C2 of the X chromosome was found in this strain, this inversion includes the nucleolar organizer region but differs in two aspects from other known inversions which include the nucleolus organizer. The proximal break is closer to the nucleolus organizer than any other known inversion and the nucleolus is free from the chromocenter.

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INTRODUCTION

I. Auxotrophic Mutants

Auxotrophic mutants in microorganisms are useful tools of genetic research. Mutants which block enzymatic capabilities, many of them auxotrophic, have helped to elucidate biochemical pathways. Fine structure analysis of genes depends to a large extent on auxotrophic mutants. Studies with these mutants have helped to develop the current ideas on the mechanisms of gene action and gene control.

No auxotrophic mutants have been reported in animals despite extensive genetic studies on a number of species (e.g. Drosophila melanogaster, Mus musculus and Homo sapiens). In the crucifer, Arabidopsis thaliana in which a number of mutants is known, the only auxotrophic mutants involve thiamine biosynthesis. Auxotrophic mutants should help to determine whether biochemical pathways, genetic control mechanisms and genetic fine structure are similar to those in microorganisms. Little is known about the structure of chromosomes and auxotrophic mutants which block amino acid and nucleic acid metabolism should be useful in determining their structure. In addition, since the processes of differentiation and development are under genetic control, auxotrophic mutants should aid in their analysis.

There are many advantages to using Drosophila as a diploid organism for the development of auxotrophic mutants. It has a short life cycle with discrete developmental steps, progeny sizes are large, and the karyotype can be studied easily with routine cytological techniques. Moreover a vast store of accumulated genetic knowledge exists for this genera. The development of a

variety of defined media for culturing Drosophila (Shultz et al, 1946; Hinton et al, 1951 and Sang, 1956) should provide a basis for the selection of auxotrophic mutants.

The studies reported here have shown that nutritional conditional lethals can be selected for in Drosophila, using a standard mutagenic procedure on defined media. Nutritional conditional lethals are usually the first step in the isolation of auxotrophs as defined below. These are the first whole body nutritional conditional lethals reported in animals. The production of nutritional conditional lethals and possibly auxotrophs should stimulate investigation of many biochemical, developmental and genetic problems in Drosophila.

In the nutritional conditional lethals described here the specific metabolic block has not yet been determined. The term auxotroph is usually applied to mutants in which the metabolic block is known or implied and a specific defined compound supplements the nutritional deficiency; for that reason the term nutritional conditional lethals will be used in the discussion of mutants in this report. Nutritional conditional lethals are defined as mutants which do not survive through development to the imaginal (adult) stage under one nutritional regime but will do so under a second regime. Both nutritional conditions must, of course, support the unmutated strain.

A brief review of the literature pertaining to this study follows.

II. Auxotrophic Mutants in Diploids

Nutritional requirements have been found in mammalian tissue culture lines but not in whole organisms. Human diploid cell lines were reported by Eagle et al. (1966) to be unable to synthesize cystine from cystathione, while heteroploid cell lines were able to convert cystathione to cystine. Several inositol requiring strains have been isolated from Chinese hamster cells. A C3H mouse liver cell line required vitamin A (Price et al., 1966). A new selection technique for screening nutritionally deficient mutants from prototrophs has yielded several auxotrophic mutants in a Chinese hamster ovary cell line. Mutagen treated cells are plated on minimal medium in the presence of bromodeoxyuridine (BUDR), prototrophic cells incorporate the BUDR, rendering them sensitive to a killing dose of U.V. light. The remaining auxotrophic cells produce new colonies when they are covered with fresh complete medium. A glycine, an inositol and a combined thymidine, hypoxanthine and glycine auxotrophic strain have been isolated with this technique (Kao and Puck, 1968). Such apparent auxotrophic mutants in cell lines have limited value for genetic studies because cell lines are not amenable to genetic analysis through the sexual processes of recombination and segregation. These nutritionally deficient cell lines do, however, suggest the possibility of developing auxotrophic mutants in animals.

In the crucifer Arabidopsis thaliana, vitamin requiring auxotrophic mutants have been reported by Langridge (1958, 1965) and Redei (1965). The thiamine requiring mutant reported by

Langridge (1958) could not use the pyrimidine or thiazole precursors of the thiamine molecules and hence was probably deficient at the coupling or subsequent steps. Five mutants, two in the pyrimidime precursor pathway and three in the thiazole precursor pathway were isolated by Redei (1965). Three temperature sensitive mutants of Arabidopsis, one requiring thiamine at 16°C but not at 29°C and two allelic mutants requiring biotin at 30°C but not at 25°C, were described by Langridge (1965). The metabolic step blocked in these mutants was not determined. A genetic block in the pyrimidine portion of the thiamine synthetic pathway has also been found in the tomato Lycopersicon esculentum (Langridge and Brock, 1961). These mutants have not been analysed in enough detail to determine whether the same synthetic steps for thiamine are used in diploid plants as those found in Neurospora crassa and Escherichia coli.

III. Biochemical Genetics of Drosophila

Although Drosophila has not enjoyed the explosive advances made in the areas of gene action and control in bacteria, the literature on its biochemical genetics is extensive. A brief review of the biochemical aspects of gene function and products will be attempted here. In the classical work on the eye color mutants in Drosophila, two genes, vermilion (v) and cinnabar (cn) were shown to control different steps in the same biochemical pathway (Beadle and Ephrussi, 1937; Tatum, 1939; Tatum and Beadle, 1940; Kikkawa, 1941). Not only did this work stimulate many subsequent developments in biochemical genetics but also led to the elucidation of the eye pigment synthetic system itself.

For example, the v mutant was shown to be deficient for tryptophan pyrrolase (Baglioni, 1960; Kaufman, 1962) and the cn mutant a deficiency for kynurenine hydroxylase (Ghosh and Forrest, 1967).

Several other enzyme systems have been shown to be more complex. Multigenic control of a functional protein has been demonstrated for xanthine dehydrogenase and phenol oxidase. Glassman (1965) and others have shown that three loci are involved in controlling xanthine dehydrogenase; rosy, (ry) maroon-like (mal) and low xanthine dehydrogenase (lxd). A large number of ry mutants have been demonstrated to be linearly arranged in one cistron (Chovnick et al., 1964). Extracts from flies carrying ry mutants show no xanthine dehydrogenase activity and some alleles show no crossreacting material (CRM) with xanthine dehydrogenase antibodies. The lxd alleles have low xanthine dehydrogenase activity, CRM with xanthine dehydrogenase antibodies and no pyridoxal oxidase activity, which is present in ry mutants. Alleles at the mal locus lack xanthine dehydrogenase activity, and pyridoxal oxidase activity, but display CRM with xanthine dehydrogenase antibodies. The relationship of these three loci in the control of xanthine dehydrogenase has not been determined, but several possible explanations were proposed by Glassman (1965). In addition to xanthine dehydrogenase, the purine catabolic pathway includes adenosine deaminase, inosine phosphorylase and guanine deaminase. A survey of the activities of these enzymes in extracts of ry, mal, lxd and other strains indicates that coordinate control of enzymes in

this pathway does not occur. The ry, mal and lxd loci do not appear to be regulatory loci affecting the synthesis of the enzymes in the purine catabolic pathway (Hodge and Glassman, 1967). Two forms of xanthine dehydrogenase, separable by electrophoresis, have been shown to be interconvertible by Drosophila extracts (Shinoda and Glassman, 1968). This conversion may be involved in an activation process in vivo.

A second complex system in Drosophila concerning phenol oxidase activity (variously called tyrosinase, phenol oxidase, dopa oxidase and phenolase) has been studied in some detail. Phenol oxidase is probably involved in cuticle formation, epinephrine synthesis and melanization, but the exact role in development has not been worked out (Mitchell, 1967). Horowitz and Fling (1954) demonstrated that the enzyme tyrosinase from Drosophila could be activated by a Drosophila extract. Subsequently it was shown by Mitchell and Weber (1965) that the phenol oxidase in Drosophila could be split into three active components and two components necessary for activation. The three active components were designated A_1, specific for tyrosine and A_2 and A_3, both specific for 3, 4 dihydroxyphenylalanine. Using strains differing in dihydroxyphenylalanine oxidase activity, one locus involved in the production of this enzyme complex was located at 52.4 map units on chromosome II (Lewis and Lewis, 1963). Extracts from lozenge glossy pupa contain no tyrosinase activity A_1 and less dihydroxyphenylalanine activity than wild-type pupa, indicating that the lozenge locus located at 27.0 on the X chromosome may be involved in the formation of the phenol oxidase complex (Peeples et al., 1967). Four of the

phenol oxidase components are found in the hemolymph, the fifth component (S) necessary for activation is secreted into the hemolymph by the salivary gland (Geiger and Mitchell, 1966). Seven peaks of phenol oxidase activity may be identified in sucrose gradients at various larval stages, the peaks being attributed to different states of aggregation of the protein components mentioned earlier. The size distribution of phenol oxidase varies with age during the larval period. The mutants black, ebony, Blond and yellow also alter the distribution of phenol oxidase in sucrose gradients. The exact role of the different states of aggregation has not been determined, but Mitchell et al. (1967) speculate that the phenol oxidase complex may have both structural and enzymatic functions.

In Drosophila, as in many organisms, an electrophoretic survey reveals many isozymes, of which the best documented will be discussed. The Canton-S and Samarkand wild-type strains of Drosophila melanogaster differ by a fast and slow electrophoretic variant for three different alcohol dehydrogenase isozymes. The F_1 hybrid has nine alcohol dehydrogenase bands in zymograms. One of the loci involved was mapped by Grell et al. (1965) at 50.1 on chromosome II. The alcohol dehydrogenase isozymes were assumed to be composed of two polypeptide subunits (Grell et al., 1965; Ursprung and Leone, 1965). Subsequently, three of the isozyme bands from hybrids were shown to have a greater specificity for long chain alcohols, particularly octanol, than for ethanol (Courtright et al., 1966). The octanol dehydrogenase was mapped at 49.2 on chromosome III. A more complex isozyme

series is alkaline phosphatase, which has seven different isozymes appearing at various stages, in different tissues during development. One of the adult isozyme bands is polymorphic and the locus responsible is on chromosome II (Schneiderman et al., 1966). A late third instar larval alkaline phosphatase band is controlled by a locus at approximately 47 on chromosome III. This locus has at least four alleles (Johnson, 1966 (a), (b); Wallis and Fox, 1968). Alkaline phosphatase is assumed to be a dimer (Wallis and Fox, 1968). One allele designated 1-Aph 0 is amorphic when homozygous but in F_1 hybrids with two other alleles, 1-Aph fast and 1-Aph slow, the presumed hybrid dimer is active. A pupal alkaline phosphatase band replaces the larval alkaline phosphatase band approximately 1.5 - 10 hours after pupation starts. This pupal isozyme has a slightly faster migration rate in electrophoretic gels than the late larval isozyme. Schneiderman (1967) has shown that the larval alkaline phosphatase can be converted to a protein with similar electrophoretic mobilities to that of the pupal isozyme by mild proteolytic digestion.

Two sex linked loci controlling enzymes have been extensively studied in Drosophila. Two alleles (A and B) are known for both glucose-6-phosphate dehydrogenase (Zw) at position 63 and 6-phosphogluconate dehydrogenase (Pgd) at position 0.9 (Young, 1966; Kazazian et al., 1965). In females heterozygous for 6-phosphogluconate dehydrogenase, both A and B forms of the enzyme and a hybrid form (AB) are found. In glucose-6-phosphate dehydrogenase heterozygous females, only the hybrid band of intermediate mobility is found. A similar hybrid form can be made in vitro

by dissociating and recombining A and B isozymes. Kazazian et al., (1965) interpret this result as indication that both X-chromosomes must function for the synthesis of glucose-6-phosphate dehydrogenase. A sex influence on the electrophoretic mobility and kinetics of G6PD has been demonstrated (Komma, 1966, 1968 (a), (b).

Other enzyme polymorphisms in Drosophila include amino acid peptidases (Beckman and Johnson, 1964) esterases (Wright, 1963) and amylase (Kikkawa, 1960) and many other examples. (See Mitchell (1967) and Shaw (1964) for references.)

A special example of the utility of Drosophila as a genetic tool for molecular problems was the establishment of the nucleolus organizer as the site of ribosomal RNA synthesis (Ritossa and Spiegleman, 1965). Drosophila stocks with one, three and four nucleolar organizer regions were demonstrated to have one-half, one and one-half, and twice the amount of DNA hybridizable with ribosomal RNA, when compared to DNA extracted from a normal stock with two nucleolar organizers. Since Drosophila stocks carrying bobbed alleles have reduced amounts of DNA hybridizable with ribosomal RNA, Ritossa et al. (1966) hypothesize that the site of ribosomal RNA synthesis and the bobbed locus are the same. The bobbed alleles are presumed to be deletions of some part of the tandem repeats of DNA complementary to ribosomal RNA, the "ribosomal RNA cistrons", which Ritossa and Spiegelman (1965) suggest as composing the nucleolar organizer.

IV. A Brief Description of the Biochemical Composition of Drosophila

Genetic lesions which block metabolic steps, are expected to change the metabolic levels of compounds in affected and related

pathways. The biochemical composition of Drosophila should form a basis for the analysis of nutritionally deficient mutants.

A comprehensive study of the lipid, protein, amino acid and nucleic acid composition of Drosophila melanogaster throughout the life cycle has been made by Church and Robertson (1966). The amounts of RNA, DNA and protein increase throughout the larval period but the rate of synthesis varies. At 25°C the rate of DNA synthesis is initially high, decreasing until approximately 48 hours, then increasing to approximately 72 hours and again decreasing until pupation at 96 hours. The rate of RNA synthesis follows a similar pattern except for an early rise until 24 hours. The rate of protein synthesis increases until approximately 36 hours then declines until pupation. The newly laid eggs have large quantities of ribosomal RNA. High molecular weight RNA is the major RNA component synthesized until about 65 hours when ribosomal RNA becomes the largest RNA component synthesized. The lipid content increases during the larval stages and decreases during the pupal stage. Keith (1966) has shown that the lipid content of adults varies with the diet, the greatest difference depending upon the concentration of glycerides. Drosophila hemolymph has been shown by Hadorn and Mitchell (1951) and others to contain many free amino acids and peptides (see review by Chen, 1966). Church and Robertson found that the free amino acid level decreases markedly from 40 to 60 hours of larval life and then remains relatively constant until an occurrence of sharp increase at pupation, probably due to degradation of larval proteins, during histolysis. Boyd and Mitchell (1966) have shown that there is a turnover of hemolymph proteins

during larval development. The free amino acid content of larva varies with the diet used and is presumably a function of the amino acids supplied in the medium (Burnet, 1968).

V. Drosophila Nutrition and Conditions for the Selection of Auxotrophs

The selection of auxotrophic mutants in Drosophila requires a defined medium, in which the nutritional requirements for growth are at least adequate, if not optimal. The nutritional requirements of Drosophila are greater than those of microorganisms but a number of amino acids and nucleic acid precursors are normally synthesized.

Drosophila melanogaster will grow in the absence of RNA but development time is decreased by approximately five days with the addition of RNA to the medium (Sang, 1956). Nucleotides or nucleosides in combination can replace RNA in the diet (Villee and Bissell, 1948; Hinton, 1956; Sang, 1957). The ribonucleic acid requirement of Drosophila varies widely with the wild-type strain used. Geer (1963) has shown that the Canton-S strain has a marked requirement for RNA but two other wild-type strains, Oakland and Oregon-R, show very little improvement in growth with increasing RNA supplementation. An adenine requiring strain of Drosophila, carrying an inversion in the left arm of chromosome II was reported by Hinton et al. (1951). This strain In(2LR)40d would grow with adenine, adenosine or adenylic acid in the medium but not in the presence of other RNA precursors. Attempts to separate the adenine requirement from the inversion failed (Hinton, 1955). Deoxyribonucleic acid is not equivalent to ribonucleic acid in Drosophila media, but inhibits larval growth (Schultz et al., 1946; Burnet and Sang, 1963).

Drosophila does not require any of the fat soluble vitamins but does require vitamins of the B group (Sang, 1956). The early workers in the development of defined medium for Drosophila could demonstrate a requirement for thiamine (B_1), riboflavin (B_2), nicotinic acid and pyridoxine (B_6) (Van Hoog, 1935; Tatum, 1939). The work was not definitive because undefined extracts of yeast were required for growth. When a completely defined medium was developed by Schultz et al. (1946) and Hinton et al. (1951) the growth response in relation to vitamin concentration could be determined. Biotin was first shown to be a requirement by Schultz et al. (1946). Hinton et al. (1951) found that some pupae formed but no imagos emerged in the absence of folic acid.

Drosophila requires cholesterol and choline or cholesterol and lecithin. Lecithin is a source of choline but when choline is added in optimum concentration, lecithin still increases development rate (Sang, 1956). Lecithin is not an essential requirement in the presence of cholesterol and choline, but does stimulate growth. Ergosterol was added to the medium used by Begg and Robertson (1950) but it does not appear to be an essential requirement (Sang, 1956).

Casein supplied all of the amino acids required, but some may not be in optimal concentration (Sang, 1956). Casein hydrolyzate supplemented with tryptophan has also been used (Tatum, 1939). Replacement of whole casein with L-amino acids was first accomplished by Schultz et al. (1946) and confirmed by Hinton et al. (1951). Casein added to L-amino acid medium does improve growth, which may result from polypeptides in casein (Schultz et al., 1946; Sang, 1956).

Drosophila requires the same ten essential L-amino acids as required by mammals: tryptophan, phenylalanine, histidine, isoleucine, leucine, valine, lysine, arginine, methionine, and threonine (Rudkin and Schultz, 1947). Other amino acids (glycine, cystine and glutamic acid) definitely increase survival (Hinton et al., 1951). Alanine, aspartic acid, proline, hydroxyproline and tyrosine when added to the thirteen amino acids above, also increase the development rate (Hinton et al., 1951). In the medium developed by Geer (1965), tyrosine is the only amino acid added above the basic thirteen used by Hinton et al., (1951). Racemic mixtures of isoleucine, methionine, phenylalanine and valine were used by Geer (1965); all other amino acids were the L-form. Hinton et al. (1951) found D-Serine to be toxic and L-Serine slightly toxic. Drosophila requires isoleucine, but the optimal concentration is critically defined and above this concentration isoleucine becomes toxic. The L-form is only slightly better for growth compared with the racemic mixture (Hinton et al., 1951).

Hassett (1948) has tested extensively the utilization of sugars by Drosophila, the most suitable sugars for adult flies in order of decreasing usefulness being fructose > maltose > sucrose > glucose > galactose > xylose > lactose. The sugar which increases the rate of larval development the most is fructose, followed by sucrose. Larvae appear to be incapable of using maltose.

The salt mixture, (K_2HPO_4, KH_2PO_4, $MgSO_4$, NaCl, $MnSO_4$, $FeSO_4$ and $CaCl_2$) of Tatum (1939) has been used in media devised by Begg and Robertson (1950) and Hinton et al. (1951) without checking the necessity of these ions. Sang (1956) found that Drosophila could develop with a mixture of Mg, K and Na salts, and suggested that commercial casein

and agar preparations contain large quantities of salts with very few additional salts being necessary. In an amino acid medium (Hinton et al., 1951 and Geer, 1965), additional salts may be required. Other possible supplements included were inositol and para-amino benzoic acid (Schultz et al., 1946) but these were shown by Hinton et al. (1951) to be superfluous. Cyanocobalamin (vitamin B_{12}) was claimed by Hinton et al. (1951) to increase development rate, but this observation was not supported by Sang (1956).

From the previous description of nutrition, a list may be compiled of compounds which Drosophila probably synthesizes and for which nutritional conditional lethals may be selected. Nine amino acids, alanine, aspartic acid, glutamic acid, glycine, cystine, hydroxyproline, tyrosine and serine are listed by Hinton (1959) as possible compounds for mutant supplementation in Drosophila. Hydroxyproline is probably produced from proline within the polypeptide chain, and hence is not a likely nutritional supplement. Glycine, serine, alanine, aspartic acid and glutamic acid are fundamental to the synthesis of many metabolites from the citric acid cycle. Although mutants have been found requiring these amino acids in microorganisms, all of the mutants have been shown to be only partially supplemented by one metabolite. This result is expected when the centralized position and the high turnover rates are considered (Wagner and Mitchell, 1964). The remaining amino acids, cystine, proline and tyrosine are the only amino acids in which a single supplement could be expected to replace an enzyme deficiency in a synthetic pathway. The ribonucleic acid and deoxyribonucleic acid precursors are the

most likely candidates for mutants deficient in a synthetic pathway. Hinton (1959) suggests inositol, para-amino benzoic acid and cyanocobalamine as potential supplements.

MATERIALS AND METHODS

I. Drosophila Stocks

The stocks used in the experimentation were obtained from universities and Drosophila stock centers. The following list gives the description and the institution supplying the stock. A detailed description may be found in Lindsley and Grell (1968).

Stock Symbol	Description	Location	Source
+	Oregon wild-type		Univ. of Wisconsin
Basc or M-5	B^a - Bar eye w^a - apricot eye sc - scute bristles Associated with complex inversion. In(1) sc^{S1L} sc^{8R+S}, sc^{S1L} sc^{8} w^a B.		Amherst College
y cv v f	y - yellow body cv - crossveinless v - vermilion eye f - forked bristles	0.0 13.7 33.0 56.7	Calif. Inst. of Tech.
w m f	w - white eye m - miniature wing	1.5 36.1	Calif. Inst. of Tech.
Df(1)bb, y v car bb^1/In(1) AM	Df(1)bb - Deficiency (1) bobbed car - carnation bb^1 - bobbed lethal In(1)AM - Inversion (1)AM. Homozygous female sterile		Calif. Inst. of Tech.
In(1)dl-49, ty-1, bb/y v f car	In(1)-49 - Inversion (1) deletion-49. ty-1 - tinylike bristles y v f car - females with y v f car attached X chromosomes		Calif. Inst. of Tech.

Stock Symbol	Description	Location	Source
w^e bb^1/C(1) DX, y f/B^sY	w^e - white eosin bb^1 - bobbed lethal C(1)DX - Compound (1) Double X, y f B^sY - Y chromosome with portion of X chromosome attached including B^s		Oak Ridge National Laboratory
Y-bb/w sn bb/ y v f	Y-bb - Y chromosome deficient for bobbed sn - singed bristles y v f - females with y v f attached X chromosomes		Oak Ridge National Laboratory

II. Culture Media

The medium used for routine stock maintenance early in the research program was a standard cornmeal medium. The yeast agar medium of Nash and Bell (1968) without chloramphenicol was used for the production of large numbers of flies and was later used for stock cultures. The composition of these media is described below.

Cornmeal Medium:

Sodium potassium tartrate	24	gm.
Calcium chloride	1.5	gm.
Agar	18.5	gm.
Dried brewers yeast	88	gm.
Cornmeal	210	gm.
Dextrose	173	gm.
Sucrose	87	gm.
Distilled water	3500	ml.
Propionic acid	35	ml.

Yeast-Agar-Medium: Modified from Nash and Bell (1968)

Brewer's yeast	100 gm.
Sucrose	100 gm.
Agar	10 gm.
Propionic acid	10 ml.
Distilled water	1000 ml.

Approximately 25 ml. of the medium was poured into one quarter pint bottles and stopped with cotton plugs. The experimental work described required Drosophila cultures free from microorganism contamination. A complete bacteriological medium was used in routine checking for contamination in experimental cultures. The composition is shown below.

Bacteriological Medium

Bacto Minimal Agar Davis	26.6 gm.
Casein Hydrolysate	5 gm.
Yeast Extract	5 gm.
Distilled water to	1000 ml.

In selecting nutritionally deficient mutants, a defined minimal medium was required. The amino acid medium of Geer (1965) has the smallest number of constituents.

Geer's Amino Acid Medium

L-Arginine HCL	0.08 gm.	Cholesterol	0.03 gm.
L-Cystine	0.03 gm.	Nicotinic Acid	0.02 mg.
L-Glutamic Acid	0.840 gm.	Riboflavin-5-Phosphate	1.0 mg.
Glycine	0.04 gm.	Ca pantothenate	1.6 mg.

L-Histidine HCL	0.1 gm.	Pyridoxine HCL	0.25 mg.
DL-Isoleucine	0.30 gm.	Biotin	0.03 mg.
L-Leucine	0.20 gm.	Folic Acid	1.0 mg.
L-Lysine HCL	0.19 gm.	Choline Chloride	8.0 mg.
DL-Methionine	0.08 gm.	$FeSO_4$	1.0 mg.
DL-Phenylalanine	0.13 gm.	$CaCl_2$	1.29 mg.
DL-Threonine	0.20 gm.	$MgSO_4$ $7H_2O$	24.6 mg.
L-Tryptophan	0.05 gm.	$MnSO_4$ H_2O	1.29 mg.
L-Tyrosine	0.08 gm.	$NaHCO_3$	0.1 gm.
DL-Valine	0.28 gm.	KH_2PO_4	0.183 gm.
Agar	1.5 gm.	Na_2HPO_4	0.189 gm.
Sucrose	1.0 gm.	Water to	100 ml.
RNA	0.1 gm.		

The amino acids, agar, sucrose and RNA were weighed from powders with each preparation. The pH of the medium was adjusted to pH 7.0 with NaOH after the addition of the amino acids. Cholesterol, vitamins and salts were added from stock solutions stored in the refrigerator. The medium was autoclaved for fifteen minutes, and about 5 ml. was dispensed into sterile 25 ml. vials capped with 25 mm bacti-capalls.

This medium contains 14 amino acids, not all of which are required (Hinton et al., 1951; Schultz, 1946). Preliminary tests were run to check the amino acid requirements on this medium (See table).

Amino Acid Absent	No. of Eggs	No. Adults	% Survival
None	100	60	60
Glycine	50	38	76
Glutamic Acid	60	17	28
Tyrosine	30	15	50
Cystine	50	41	92

Absence of tryptophan (60), leucine (50), valine (40), isoleucine (50), arginine (50), histidine (50), methionine (50), lysine (50), threonine (50) and phenylalanine (50) resulted in complete lethality (the number of individuals tested is given in parenthesis). The average time to pupation was twelve days and all flies had emerged by the nineteenth day. The variability in suvivorship could be due in part to the handling of eggs during transfer. Glycine and glutamic acid, being essential to metabolic conversions, are not likely to result in a single requirement, hence only tyrosine and cystine were omitted from the minimal medium. RNA precursors are potential sources of metabolic blocks in Drosophila, so the minimal medium was tried with and without RNA. The results are shown below.

	No. of Eggs	No. Adults	% Survival
With RNA	175	95	54.8
Without RNA	200	4	2.0

RNA is a very important component of this minimal medium.

The casein medium of Burnet and Sang (1963) was used for the selection of RNA requiring mutants because RNA is not a requirement on this medium. The composition of Burnet and Sang's medium is shown below.

Burnet and Sang's Casein Medium

Agar	3.00 gm.	Thiamine HCL	0.2 mg.
Casein	5.5 gm.	Riboflavin	0.1 mg.
Sucrose	0.74 gm.	Niacin	1.2 mg.
Cholesterol	0.83 gm.	Ca pantothenate	1.6 mg.
Lecithin	0.40 gm.	Pyridoxine HCL	0.25 mg.

Biotin	0.016 mg.	KH_2PO_4	0.183 gm.
Folic acid	0.3 mg.	Na_2HPO_4	0.189 gm.
$NaHCO_3$	0.140 gm.	Water to make	100 ml.

RNA (when added 0.40 gm.)

The agar, casein, sucrose and RNA, when added, were weighed from powders with each preparation. The addition of other components, autoclaving, and dispensing into tubes was handled as in the minimal medium. Casein, amino acids and vitamins were purchased from Nutritional Biochemical Corporation, except where otherwise designated. The agar (Difco-Bacto) and salts were purchased from Fisher Scientific.

III. Egg Collection, Sterilization and Larval Transfer

Drosophila normally feeds on microorganisms, usually yeast, and a flora is usually found in the gut tract. To develop an axenic culture of Drosophila, the eggs must be freed of the chorions and the clinging microorganisms, and then placed on sterile medium. Intracellular or intestinal flora may exist in adult flies and larvae grown on defined media, but smears of tissue from adults and third instar larvae on bacteriological plates have not revealed any contamination. Sterilization of eggs by the elaborate procedures of Yoon and Fox (1965) and Sang (1956) met with varied success. A modification of the procedure described by Geer (1963) proved simpler and more effective.

Eggs were normally collected on petri dishes containing 1.5% agar gels. A petri dish was taped over the mouth of an empty 1/4 pint milk bottle, containing 100-200 males and females for 10-12 hours. Eggs were then brushed into sterile 15 ml. centrifuge tubes containing 10 ml. of 3% freshly filtered calcium hypochlorite. After 20 minutes the eggs were spun down at 100 G for 1 minute in a Phillips Drucker

clinical centrifuge. The supernatant was then decanted and the eggs were rinsed three times with sterile insect Ringer's solution (Ephrussi and Beadle, 1936). The eggs were centrifuged and the supernatant decanted with each rinse. After rinsing, 1 ml. of Ringer's was added and eggs were pipetted onto complete bacteriological plates or into yeast—agar bottles. For lethal supplementation test, 25 or 40 larvae 12-24 hours old, were transferred from the complete plates to the test medium using a flamed scalpel and sterile technique in a U.V. sterilized isolation chamber. Forty-eight hours after larval transfer the complete plates were checked for contamination with microorganisms. Cultures set up from plates showing contamination were routinely discarded. All cultures which showed visual signs of contamination on the synthetic media were discarded.

IV. Mutant Selection

Screening for nutritional mutants was confined to the X-chromosome because there is no a priori reason why nutritional lethals should not be scattered throughout the genome, and sex linked lethal tests require one less generation of testing than autosomal lethals.

The routine Muller-5 or Basc technique described by Spencer and Stern (1948) was used for the production of sex linked recessive lethals. This technique is diagrammed below.

P_1 Basc/Basc ♀ x +/Y ♂ (Mutagen Treated or Control)

↓

F_1 +/Basc ♀ x Basc/Y ♂

↓

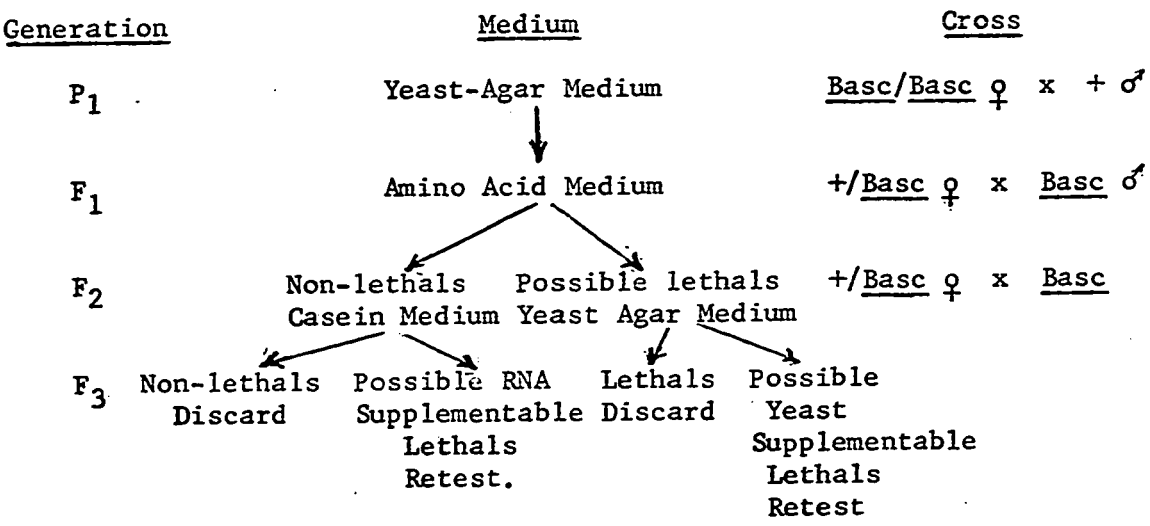
F_2 Basc/Basc ♀ +/Basc ♀ +/Y ♂ Basc/Y ♂

F_2 cultures are scored for the presence or absence of wild-type males. If the mutagenic treatment in the P_1 generation has induced a lethal on the chromosome tested no wild-type males are expected in the F_2. Cultures containing one or more wild-type males are assumed to be nonlethal. Cultures containing eight or more Basc males, Basc/Basc females and +/Basc females but no wild-type males are assumed to be lethals. Cultures with less than eight Basc males but no wild-type males are retested. Using the criterion of eight or more Basc males and no wild-type males before a culture is classified as a lethal giving less than 1% chance of misclassifying a culture.

The mutagen ethyl methanesulphonate (EMS) ($C_2H_5OSO_2CH_3$) supplied by K and K Laboratories Incorporated was used for the mutation experiment. For the mutagenic treatment, males (0-48 hours old) were placed on Kleenex saturated with 0.025 M solution of EMS in 1% sucrose for 24 hours, according to the method of Lewis and Backer (1968). Control males were fed on the sucrose solution without the mutagen.

Nutritional conditional lethal mutants are selected by using synthetic media in the critical crosses. The P_1 generation cross of a single male by 3 or 4 Basc females was done on yeast-agar medium. The F_1 crosses (pair matings of +/Basc females and Basc males) were performed on the restrictive amino acid medium. Cultures were scored as either non-lethals having one or more wild-type males and possible lethals, having only Basc males. Cultures with wild-type males in the F_1 were transferred to the casein medium without RNA to test for a possible RNA requirement.

The cultures producing wild-type males on the amino acid medium but no wild-type males on the casein medium were considered to be nutritional mutants supplementable by RNA. Cultures with only Basc males in the F_2 were retested on the restricted amino acid medium and the yeast-agar medium. Those cultures which produced 8 or more Basc males and no wild-type males on the amino acid medium but produced wild-type males on the yeast-agar medium were assumed to be nutritional lethals supplementable by a yeast constituent. A diagram summarizing the different media used in each generation is shown below.



In the mutation tests an attempt was made to maintain germ free cultures and germ free conditions throughout. Wild-type males and virgin Basc females for the mutation experiments were collected from yeast-agar germ free cultures. The medium from stock bottles was routinely checked for contamination by plating once a week. Flies were etherized in autoclaved glass etherizers and handled on sterile pieces of white cardboard with sterile brushes in the isolation chamber described above.

The screening process for RNA requiring mutants take approximately six weeks in which cultures must be kept free of microorganisms. Sources of contamination include contaminating many flies from one culture in bulk handling of males and virgin females, and contamination during fly handling between generations.

V. Linkage Tests

Oregon wild-type males or the mutant to be tested were mated to y cv v f or w m f females on yeast-agar medium. Heterozygous females were mated to the multimarked males on casein or yeast-agar medium for the recombination test. All the progeny to emergy for a period of one week to ten days were scored.

Using the recombinant data a map position of the lethal may be estimated from the following considerations.

1. The recombinant males which do not receive the lethal will be found in the progeny in proportion to the map distance between the markers used and the lethal to be mapped.
2. All recombinant males receiving the lethal die under restrictive conditions.
3. The control cross using the strain of flies from which the mutant is derived, should give the best estimate of the number of recombinants expected in any recombinant class.
4. The multimarked male class, common to both the experimental and control cultures, may be used as a reference to calculate the expected number of males in the experimental class.
5. The proportion of recombinant males which receive the lethal will be equal to one minus the observed number of non-lethal males recombinants divided by the expected number of recombinants.
6. [illegible]

6. The location of the lethal between two adjacent markers is equal to the proportion of lethal recombinants in one marker class, divided by the total number of lethal recombinants in the adjacent marker classes, multiplied by the distance between these two markers.

7. The map position of the lethal is equal to the map position of the lethal plus the map position of the marker used above.

A hypothetical example using algebraic expression should help to illustrate the calculations used.

Experimental

a + b c

+ l + +

Recombination between a and b will result in 4 classes of recombinants

a l + + lethal

+ + b c viable

a + + + viable

+ l b c lethal

Control

a b c

+ + +

Recombination between a and b results in two classes of recombinant chromosomes.

a + + viable

+ b c viable

The expected number of males in the experimental recombinant classes a + and + b may be calculated with the following expressions.

$$E_1 = \frac{(C_1)\ M}{P}$$

where:

E_1 = No. of recombinant males expected in the a + class.

C_1 = No. of recombinant males observed in the control a + class.

M = No. of males observed in the experimental multimarked class abc

P = No. of males observed in control multimarked class abc.

Similarly,

$$E_2 = \frac{(C_2)\ M}{P}$$

where:

E_2 = No. of recombinant males expected in the + b class

C_2 = No. of recombinant males observed in the control + b class.

M = same as above.

P = same as above.

The proportion of recombinant males lost as a result of lethality in the marker classes a + and + b may be calculated with the following expressions.

$$X = 1 - \frac{O_1}{E_1}$$

where

X = Frequency of recombinant males lost in the marker class a +

O_1 = No. of recombinant males observed in the marker class a +

E_1 = Same as above.

Similarly,

$$Y = 1 - \frac{O_2}{E_2}$$

where

Y = Frequency of recombinant males lost in the marker class + b

O_2 = No. of recombinant males observed in the marker class + b

E_2 = Same as above.

The estimated location of the lethal between the two adjacent markers can be calculated as shown below:

$$L = \frac{X\,(D)}{X + Y}$$ where

L = Location of the lethal between the markers.

D = Map distance between the two adjacent markers.

The location of the lethal will therefore be estimated as L + map position of the marker a.

The proportion of the recombinant males lost in the reciprocal classes adjacent to the lethal should total one. This total will exceed one if more than the expected number of recombinant males die. The calculation of map distance by this method is thought to be the best estimate of the map position of the lethal, but many factors may influence the results.

VI. Preparation of Salivary Gland Slides

Slides of salivary gland chromosomes were prepared following the procedure of Plaut (1963). Microscope slides were coated with 0.5% gelatin to achieve better tissue adherence. Clean new slides were dipped in a hot 0.5% gelatin solution and allowed to dry in an upright position. Salivary glands were disected from sexed third instar larvae in Ringer's solution and then transplanted to a drop of 45% acetic acid on a labelled microscope slide. The tissue was squashed under number 2 cover slips. The cover slips were removed with a razor blade after freezing the tissue in liquid nitrogen. The slide was immediately post-fixed in ethanol acetic acid 3:1 for 3 minutes and then transferred to 98% ethanol. Slides were passed through an alcohol series to water, into 45% acetic, stained for 5 minutes in aceto-orecin (2% aceto-orecin in 60% acetic acid), and then the above series was reversed to

98% ethanol. A drop of Euparal was added to the tissue and then a number one coverslip was applied. A Zeiss photomicroscope was used for slide observation and photography.

The pyronin/methyl green double stain has been shown by Brachet (1953) to be specific for RNA and DNA. Other cellular components will not bind these dyes if fixatives containing alcohol are used. Alcohol containing fixatives remove lipid components which might interfere with the basic stain's binding to nucleic acids. Methyl green binds to DNA in a stoichiometric manner. Pyronin does not have the same affinity for RNA and must be used only as an approximation of the amount of RNA present. The purpose of this double stain was to show the nucleolus because the orcein slides do not allow good photography of the nucleolus. The procedure of Brachet (1953), as modified by Jordan and Baker (1955), was used. The 0.5% aqueous solution of methyl green was extracted with chloroform until the chloroform was colorless; seven or eight extractions were usually required. Then the stain was prepared as shown below.

Pyronin	0.5%	37 ml.
Methyl green	0.5%	13 ml.
Acetate buffer at pH.	4.8	50 ml.

The acetate buffer was prepared by mixing 81 ml. of M/5 acetic acid and 119 ml. of M/5 sodium acetate.

The slides were handled as above for the orcein stain except after the hydration, when they were placed directly into the methyl green pyronin stain for 30 minutes. From the stain the slides were passed through two changes of acetone, acetone:xylene (1:1), xylene and then mounted in D.P.X. or Euparal.

RESULTS

I. Mutagenesis

In the ethyl methanesulphonate (EMS) treated male cultures, 604 cultures (17.72%) of the 3388 original cultures were carried through the entire mutagenesis screening procedure. Two hundred and nineteen of the 604 screened cultures were scored as nonsupplementable lethals, and thirteen were scored as possible supplementable lethals. In the 171 control cultures no lethals or supplementable lethals were found. In retesting the thirteen possible supplementable lethals, five were non-supplementable lethals in which one male had "escaped", five were non-mutant, and three were supplementable lethals. The frequency of induction of recessive lethals with EMS under these conditions was 219 + 5 + 3/604 or 37.58%. The frequency of nutritional supplementable lethals was 3/604 or approximately 0.5%. The percentage of recessive lethals which were supplementable was 3/227 or 1.32%.

A large percentage of each generation was lost as the result of contamination: 4.66%, 18.77% and 19.61% in experimental cultures, and 13.87%, 11.87% and 10.52% in control cultures. An additional 57.94%, 31.31% and 22.98% were lost in the experimental cultures, and 38.92%, 14.57% and 26.90% were lost in the control cultures as a result of infertility. The difference in fertility between experimental and control cultures could be the result of the EMS treatment, inducing male sterility in the first generation or leading to dominant female sterility in the second generation. Pair matings and cultures set up with small numbers of flies quite frequently fail to produce progeny in any experiment, and this may also account for infertility.

Table 1

F_1 RESULTS - YEAST-AGAR MEDIUM

	NUMBER OF MALES	INFERTILE CULTURES		CONTAMINATED CULTURES		PAIR MATINGS ON AMINO ACID MEDIUM	
		NO.	%	NO.	%	NO.	%
TREATED	3388	1962	57.94	158	4.66	1268	37.51
CONTROL	519	202	38.92	71	13.87	246	47.39

F_2 RESULTS - AMINO ACID MEDIUM

	INFERTILE CULTURES		CONTAMINATED CULTURES		NONLETHALS		POSSIBLE LETHALS >8 Basc ♂		≤8 Basc ♂	
	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%
TREATED	397	31.31	243	18.77	383	30.20	195	15.38	50	3.87
CONTROL	46	14.57	29	11.78	171	69.51	0	0	0	0

F_3 RESULTS - CASEIN MEDIUM WITHOUT RNA

	INFERTILE CULTURES		CONTAMINATED CULTURES		NONLETHALS		POSSIBLE RNA SUPPLEMENTABLE LETHALS	
	NO.	%	NO.	%	NO.	%	NO.	%
TREATED	88	22.98	75	19.61	210	54.84	10	2.65
CONTROL	46	26.90	18	10.52	107	62.51	0	0

F_3 RESULTS - YEAST MEDIUM

	INFERTILE CULTURES		LETHALS		POSSIBLE SUPPLEMENTABLE LETHALS	
	NO.	%	NO.	%	NO.	%
TREATED	23	9.34	219	89.38	3	1.21

Table 1 continued

Total recessive lethals	$\frac{219 + 5 + 3}{604} = 37.58\%$
Putative nutritional conditional lethals	$\frac{13}{604} = 2.15\%$
Nutritional conditional lethals	$\frac{3}{604} = 0.5\%$ (0.4966)
Recessive lethals which are nutritional, conditional	$\frac{3}{227} = 1.32\%$

In the retest of the thirteen possible supplementable lethals, two RNA supplementable lethals and one yeast supplementable lethal were found. The RNA supplementable lethals were in cultures numbered 1308 and 1625. The yeast supplementable lethal was in culture number 11523 (table 2). The nutritional conditional mutants were designated by the number of the culture in which they were found, hence 1308, 1625 and 11523.

II. Strain 1308

Genetic Mapping

The nutritional conditional lethal 1308 was isolated as an RNA requiring mutant. By the method outlined in Materials and Methods, 1308 has been located within seven map units on the X chromosome.

The results of the w m f/1308 and control backcrosses, under restrictive and permissive conditions, are given in table 3. In the wild-type controls, the reciprocal recombinant classes are almost equal with a small excess of + + + over w m f chromosomes and females over males.

A deficiency of males was found in the + + + and + + f classes in the 1308/w m f backcross on the restrictive medium. The reduction of males in the w + + and + m f reciprocal classes in the 1308 data indicates that 1308 lies between w and m. The excess of w + males over + m indicates that 1308 lies closer to the white locus than to the miniature locus. When using the method previously described, 1308 is found to map at position 15.7. RNA added to the casein medium increases the survival of all male classes in which 1308 may be expected to be found.

Table 2

RETESTS FOR CONDITIONAL NUTRITIONAL MUTANTS

MUTANT CULTURE NUMBER	YEAST MEDIUM		CASEIN MEDIUM WITHOUT RNA		CASEIN MEDIUM WITH RNA		RESULT
	Basc	Wild-type[1]	Basc	Wild-type	Basc	Wild-type	
	♂	♂	♂	♂	♂	♂	
1308	42	44	42	0	42	9	requires RNA
1625	21	18	16	0	7	8	requires RNA
11210	34	0	0	0	0	0	lethal
11137	+*	0	+	0	0	0	lethal
11223	68	0	0	0	0	0	lethal
13045	47	0	0	0	0	0	lethal
13059	40	5	59	50	0	0	non-lethal
13218	+	+	63	14	0	0	non-lethal
13330	+	0	+	0	0	0	lethal
13361	+	+	11	0	0	0	lost, probably mutant
11523	8	18	8	0	35	0	requires yeast
1811	+	+	+	+	0	0	non-lethal[2]
1645	13	1	0	0	0	0	lost, probably non-mutant[2]

* + Indicates estimated 25 or more flies, not counted.

1 Wild-type with respect to B, w^a, sc but carrying the suspected lethal being tested.

2 Wild-type males on amino acid medium.

Applying similar arguments to the 1308/y cv v f data, 1308 can be shown to lie between cv and v. The excess of cv + over + v chromosomes indicates that 1308 is closer to cv than to v. The map position calculations with table 4 place 1308 at map position 22.3. In recombination tests when many markers and various media are used, differential viability resulting from the different responses of alternative alleles to changes in culture conditions and linkage relationships may be expected. Although the results presented here are relatively clear, at least one factor appears to influence the results. A factor near f contributes to the lethality of the 1308 chromosomes because + + + f males are more viable than the + + + + males on the restrictive medium. The males in the + + + + class and y + + + class could either be lethal free multiple recombinants or escapees from lethality, possible resulting from larval cannibalism of dead larvae and flies in the crowded cultures in which these flies were grown.

The calculations from the w m f and y cv v f backcross data place 1308 at 15.7 and 22.3 respectively. The estimated position of 15.6 for 1308 may be too low because of the small number of recombinants found between w and m, approximately one-third the expected 35% in wild-type cultures. 1308 is therefore estimated to lie between 15.7 and 22.3.

Growth Requirement of 1308

In the 1308 supplementation tests the percentage of larvae to emerge as adults was calculated as an index of the effectiveness of the medium used to support growth. The results were compiled from culture tubes set up under the same conditions and incubated in the same incubator at 24-25°C, with a relative humidity of 80°C.

Table 3

1308/w m f LINKAGE TEST

The percentage of phenotypes within each sex on different media is presented

Progeny classes	1308				Wild-type			
	Without RNA[1]		With RNA (4 mg/ml)		Without RNA		With RNA (4 mg/ml)	
	♀	♂	♀	♂	♀	♂	♀	♂
w m f	39.9	81.5	34.9	57.2	32.6	34.5	27.1	33.8
+ + +[2]	37.9	0.3	38.2	15.2	40.8	39.8	48.2	41.4
w + +	5.0	4.4	5.7	6.7	5.1	4.1	5.0	4.2
+ m f	4.8	3.0	5.4	4.7	5.1	5.7	4.2	3.9
w m +	8.0	10.2	5.9	10.2	8.7	7.1	6.9	7.1
+ + f	7.9	.5	9.0	5.3	7.6	7.6	8.2	9.2
Multiple Recombinants	0.8	0.1	0.9	0.6	0.8	1.0	0.2	0.5
Total no.	1651	773	1643	1037	1342	1202	402	382

1 Casein medium with or without RNA
2 + indicates wild-type alleles for markers

Using calculations described in the materials and methods the map position of 1308 may be estimated as follows:

$$E_1 = \frac{(C_1)\ (M)}{P} \qquad E_2 = \frac{(C_2)\ (M)}{P}$$

$$X = 1 - \frac{O_1}{E_1} \qquad Y = 1 - \frac{O_2}{E_2}$$

$$= 1 - \frac{34}{\frac{49}{415}\ (630)} \qquad = 1 - \frac{23}{\frac{69}{415}\ (630)}$$

$$= 1 - 0.46 = 0.54 \qquad = 1 - 0.22 = 0.78$$

$$L = \frac{(X)\ (D)}{X + Y}$$

$$= \frac{0.54\ (34.6)}{1.32} = 14.2$$

1308 estimated map position is 1.5 + 14.2 = 15.7

Table 4

1308/y cv v f LINKAGE TEST

The percentage of phenotypes within each sex on different media is presented

Progeny classes	Casein Medium without RNA		Yeast Medium		Casein Medium without RNA		Yeast Medium	
	♀	♂	♀	♂	♀	♂	♀	♂
y cv v f	26.2	66.4	23.4	30.4	18.4	18.0	23.7	20.4
+ + + +[1]	32.5	2.2	34.2	24.3	32.6	34.0	34.3	39.0
y + + +	2.4	0.2	3.4	3.7	4.8	4.8	3.3	3.0
+ cv v f	2.3	5.3	2.5	4.4	2.5	2.6	1.3	1.5
y cv + +	7.7	6.6	7.3	11.1	8.4	6.2	5.3	5.6
+ + v f	6.8	4.5	9.1	7.7	13.0	11.0	9.8	9.3
y cv v +	8.9	10.5	7.4	9.6	4.5	8.8	7.3	7.1
+ + + f	9.1	1.4	9.3	7.0	11.0	9.9	12.9	11.3
Multiple Recombinants	4.0	2.9	3.4	1.7	4.6	4.5	2.2	2.2
Total no.	3156	1188	1018	700	1036	726	604	602

1 + indicates wild-type alleles for markers.

Using calculations described in the materials and methods the map position of 1308 may be estimated as follows:

$$E_1 = \frac{(C_1)\ (M)}{P} \qquad E_2 = \frac{(C_2)\ (M)}{P}$$

$$X = 1 - \frac{O_1}{E_1} \qquad Y = 1 - \frac{O_2}{E_2}$$

$$= 1 - \frac{78}{\frac{45}{131}\ (789)} \qquad = 1 - \frac{53}{\frac{80}{131}\ (789)}$$

$$= 1 - 0.29 = 0.71 \qquad = 1 - 0.11 = 0.89$$

$$L = \frac{(X)\ (D)}{Y + X} = \frac{0.71\ (19.3)}{0.89 + 0.71} = 8.6$$

1308 estimated map position is 13.7 + 8.6 = 22.3

On the yeast-agar medium, 1308 larvae survive almost as well as the wild-type, as shown in table 5. The large loss of wild-type probably results from egg sterilization or larval handling during transfer. On casein medium, in the absence of RNA, about 40% of the wild-type and none of the 1308 larvae survive (table 6). RNA from several sources definitely increases the survival of both 1308 and wild-type larvae. A purified RNA preparation (type XI from Sigma Chemical Company, prepared according to Crestfield et al., 1955) also stimulates the growth of both wild-type and 1308 larvae. Ribosomal core RNA, a pancreatic ribonuclease resistant product of ribosomal RNA, decreases survival of wild-type but increases survival of 1308 compared with casein medium without RNA. 1308 larvae will not survive with s-RNA or DNA added to the medium and the survival of wild-type larvae is reduced (table 6).

The 1308 larvae on casein medium with RNA, or on yeast medium develop as rapidly as wild-type on casein medium with RNA. The development rate of wild-type larvae on casein medium without RNA is greatly decreased (table 7).

The effect of increasing RNA concentration on survival of 1308 and wild-type larvae is shown in figure 1. The concentration of 4 mg/ml was used by Sang (1956) and does not affect the growth of 1308 or wild-type larvae. Bases, nucleosides and nucleotides supplied at concentrations based on a 4 mg/ml concentration of RNA should give a nutritional requiring mutant the requirement in adequate supply.

Table 5

SURVIVAL OF 1308 AND WILD-TYPE LARVAE ON YEAST MEDIUM

	No. of Larvae	No. of adults emerged	% survival
Wild-type	810	571	70.5
1308	760	485	63.8

Table 6

SURVIVAL OF 1308 AND WILD-TYPE LARVAE ON CASEIN MEDIUM SUPPLEMENTED WITH NUCLEIC ACIDS

		Wild-type			1308		
Nucleic Acid Added to Casein Medium[1]	Suppliers	No. of larvæ	No. of adults emerged	% adults	No. of larvæ	No. of adults emerged	% adults
Nil	--	950	391	41.2	1105	0	0.0
RNA	2	160	132	82.5	480	71	14.8
RNA	3	645	414	64.2	455	85	18.7
r-RNA (core)	4	240	39	16.2	240	7	2.9
s-RNA	5	240	11	4.6	240	0	0.0
DNA	6	100	18	18.00	125	0	0.0

(1) Nucleic acid added at concentration of 4 mg/ml.

(2) Sigma Chemical Company Type XI.

(3) Nutritional Biochemical Corporation, British Drug Houses, or Calbiochem.

(4) Sigma Chemical Company Type II-C

(5) Sigma Chemical Company Type III

(6) Nutritional Biochemical Corporation.

Table 7

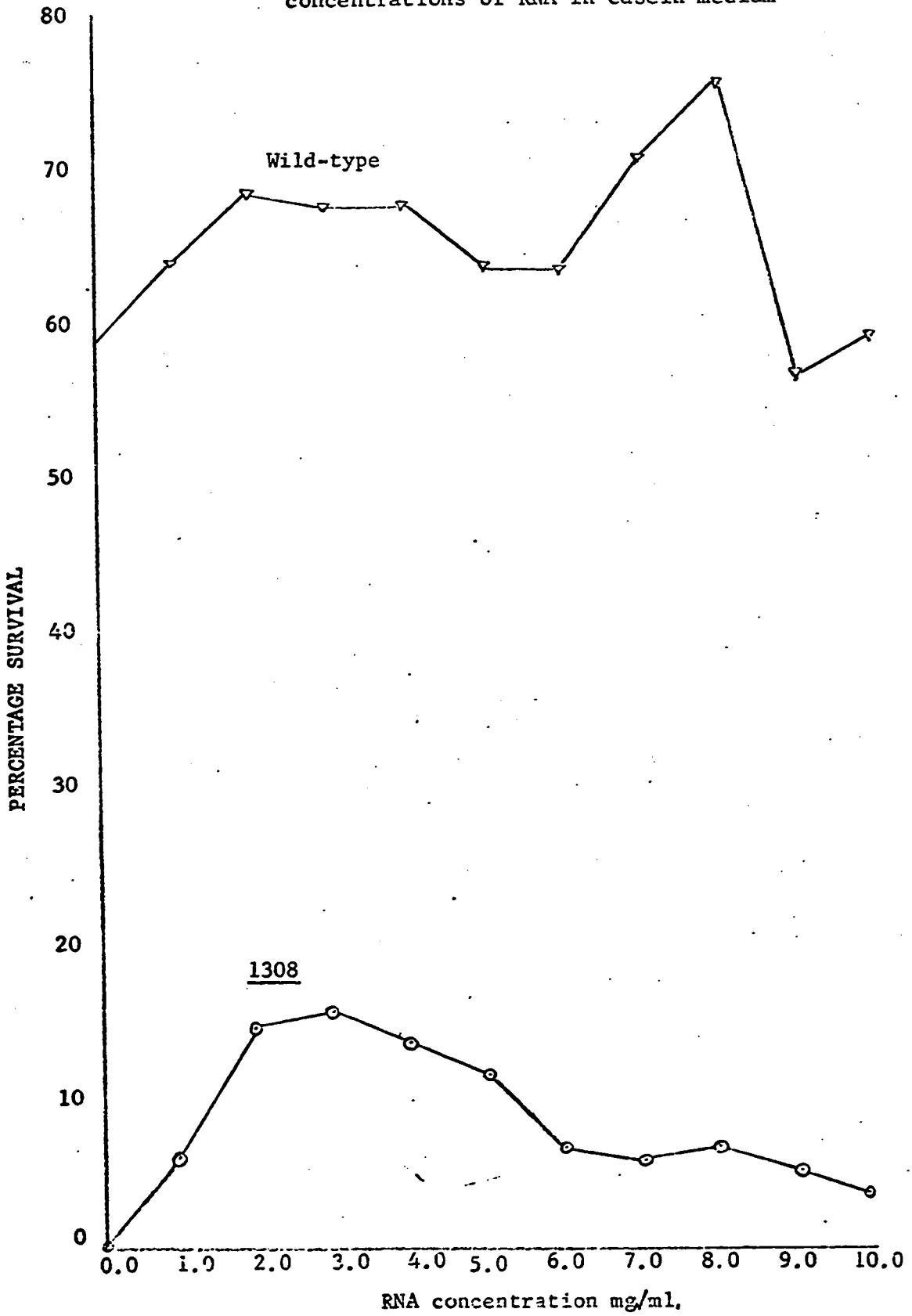
DEVELOPMENT TIME TO ADULTS AND SURVIVAL OF 1308 AND WILD-TYPE LARVAE ON DIFFERENT MEDIA AT 24-25°C

	Medium	No. of larvae	No. of adults	% survival	Days until first adult emerges	Average time to adult[1]	Standard deviation
1308	Yeast	75	53	70.6	10	12.1	± 1.26
	Casein	120	0	0.0	-	-	-
	Casein + RNA (4 mg/ml)	90	18	20.0	11	12.7	± 0.73
Wild-type	Yeast	120	95	79.2	9	10.4	± 0.56
	Casein	160	94	58.7	16	19.3	± 2.10
	Casein + RNA (4 mg/ml)	160	124	77.5	11	12.1	± 1.03

1 An estimate of the average time to pupation may be arrived at by subtracting 4.3 days from the average time to emergence of adults (Sang, 1956).

Figure 1

Survival of 1308 and wild-type larvae with increasing concentrations of RNA in casein medium



Extensive testing has not revealed the RNA component responsible for the increase in survival of 1308 on RNA supplemented medium. Wild-type larvae were used as a control to check the medium on which 1308 larvæ were to be tested. In tables 8 - 13, as an adjunct to the 1308 data, the effects of RNA components on the growth of wild-type larvae are included.

The response of wild-type larvae to the addition of RNA components to defined media varies widely with the strain used (Hinton, 1956; Sang, 1957; Geer, 1963). The wild-type strain used here was inhibited by the addition of adenine, adenosine or adenylic acid. Guanine, guanosine and guanylic acid produced less stimulation than the pyrimidine derivatives. Mixtures of the bases, nucleosides or nucleoside-5'-monophosphates inhibited growth of wild-type larvae, but the mixture of nucleoside-2'- and-3'-monophosphates stimulated growth. The inhibition of wild-type larvae on medium supplemented with adenosine was at first thought to be an artefact resulting from breakdown of the nucleoside during autoclaving. The addition of filter sterilized purine nucleosides after autoclaving did not improve the growth of wild-type larvae (table 9). The addition of the homopolymers to casein medium increased the viability of wild-type larvae by various amounts (table 12). This variation may partly result from the small sample size used. The addition of excess folic acid did not improve the growth of wild-type larvae, in the absence of RNA, as suggested by Sang (1956) (table 13).

The RNA components added to the casein medium, singly or in combination, do not convincingly support the growth of 1308 larvae. The bases or ribose-5-phosphate did not supplement the 1308 deficiency (table 18). Five 1308 larvae reached the adult stage in the presence of filter

Table 8

SURVIVAL OF WILD-TYPE AND 1308 LARVAE ON CASEIN MEDIUM SUPPLEMENTED WITH BASES OR RIBOSE-5-PHOSPHATE

Base Added[1,2,3]	Wild-type			1308		
	No. larvae	No. of adults	% survival	No. larvae	No. of adults	% survival
u	280	134	47.8	160	0	0
c	280	153	55.0	160	0	0
a	280	88	31.4	160	0	0
g	280	158	56.4	160	0	0
u c a g	280	75	26.8	160	0	0
ribose-5-phosphate	---	---	----	160	0	0
Nil	230	72	31.3	160	0	0

1 Bases are designated as follows: u - uracil, c - cytosine, a - adenine and g - guanine.

2 Final concentration of bases and ribose-5-phosphate was 0.5 mg/ml.

3 Bases supplied by Nutritional Biochemical Corporation. Ribose-5-phosphate supplied by Sigma Chemical Company.

Table 9

SURVIVAL OF WILD-TYPE AND 1308 LARVAE ON CASEIN MEDIUM SUPPLEMENTED WITH NUCLEOSIDES

Nucleoside Added [1, 2,4]	Wild-type			1308		
	No. of larvæ	No. of adults	% survival	No. of larvæ	No. of adults	% survival
U	300	192	64.0	150	0	0.0
C	325	197	60.6	100	0	0.0
A	300	83	27.6	125	0	0.0
G	325	159	47.9	125	0	0.0
Nil	150	57	38.0	150	0	0.0
A + G[3]	200	24	12.0	200	4	2.0
A[3]	80	20	25.0	120	0	0.0
G[3]	160	54	33.8	240	1	0.4
Nil	40	12	30.0	40	0	0.0

1 Nucleosides are designated as follows: U - Uridine, C - Cytosine, A - Adenosine, G - Guanosine.

2 Nucleosides were supplied by Sigma Chemical Company.

3 Filter sterilized nucleosides added to medium after autoclaving.

4 Final concentration of each nucleotide was 1 mg/ml.

Table 10

SURVIVAL OF WILD-TYPE AND 1308 LARVAE ON CASEIN MEDIUM SUPPLEMENTED WITH A MIXTURE OF NUCLEOSIDE-3'- and-2'- MONOPHOSPHATES[1,2]

	Wild-type			1308		
Nucleotide added	No. of larvae	No. of adults	% survival	No. of larvae	No. of adults	% survival
Up[3]	125	62	49.6	75	0	0.0
Cp	125	94	75.2	75	0	0.0
Ap	125	41	32.8	75	1	1.3
Gp	125	74	59.2	50	0	0.0
Up + Cp + Ap + Gp	125	96	76.8	50	0	0.0
Nil						

1 Mixture of nucleoside 2'-and 3'-monophosphates supplied by Calbiochem.

2 Final concentration of 2'-and 3'-monophosphates was 1 mg/ml.

3 Mixtures of nucleoside 3'-and 2'-monophosphates are designated as follows: Up - Uridine 2'-and 3'-monophosphates, Cp - Cytidine 2'-and 3'-monophosphates, Ap - Adenosine 2'-and 3'-monophosphates, Gp - Guanosine 2'-and 3'-monophosphates.

Table 11

SURVIVAL OF WILD-TYPE AND 1308 LARVAE ON CASEIN MEDIUM SUPPLEMENTED WITH NUCLEOSIDE-5'-MONOPHOSPHATES[1,2]

	Wild-type			1308		
Nucleotide added[3]	No. of larvae	No. of adults	% survival	No. of larvae	No. of adults	% survival
UMP	280	129	46.1	160	0	0.0
CMP	400	266	66.5	440	0	0.0
AMP	300	48	13.3	440	1	0.2
GMP	240	70	29.1	320	3	0.9
IMP	280	91	32.8	680	7	1.0
XMP	200	71	35.5	400	3	0.7
UMP + CMP + GMP + AMP	80	21	26.2	360	0	0.0
UMP + CMP	80	40	50.0	440	0	0.0
GMP + AMP	80	17	21.2	440	0	0.0
UMP + CMP + AMP	80	30	37.5	440	1	0.2
UMP + CMP + GMP	80	25	31.2	400	0	0.0
UMP + CMP + IMP	40	5	12.5	360	2	0.5
Nil	280	73	26.1	600	0	0.0

1 Nucleoside-5'-monophosphates supplied by Sigma Chemical Company.

2 The final concentration of the nucleoside-5'-monophosphates was 1 mg/ml.

3 Nucleotides are designated as follows: UMP - Uridine-5'-monophosphate, CMP - Cytidine-5'-monophosphate, AMP - Adenosine-5'-monophosphate, GMP - Guanosine-5'-monophosphate, XMP - Xanthosine-5'-monophosphate, IMP - Inosine-5'-monophosphate.

Table 12

SURVIVAL OF WILD-TYPE AND 1308 LARVAE ON CASEIN MEDIUM SUPPLEMENTED WITH HOMOPOLYMERS OF RNA NUCLEOTIDES[1,2]

	Wild-type			1308		
Homopolymer added	No. of larvae	No. of adults	% survival	No. of larvae	No. of adults	% survival
Poly U[3]	40	18	45.0	120	0	0.0
Poly C	40	14	35.0	240	0	0.0
Poly A	40	15	37.5	200	0	0.0
Poly G	40	36	90.0	160	0	0.0

1 The homopolymers were supplied by Sigma Chemical Company.

2 The homopolymers are designated as follows: poly U - polyuridylic acid, poly C - polycytodylic acid, poly A - poly adenylic acid and poly G - polyguanylic acid.

3 The final concentration of the homopolymers was 1 mg/ml.

Table 13

SURVIVAL OF WILD-TYPE AND 1308 LARVAE WITH INCREASING AMOUNTS OF FOLIC ACID IN CASEIN MEDIUM

	Wild-type			1308		
Folic Acid[1] Conc. mg/ml	No. of larvae	No. of adults	% survival	No. of larvae	No. of adults	% survival
3.0	160	36	22.5	240	0	0.0
30.0	80	28	35.0	200	0	0.0
300.0	200	14	7.0	280	0	0.0

1 Folic Acid supplied by Nutritional Biochemical

sterilized purine nucleosides, but the sample sizes used do not permit a comparison between the two experiments. One 1308 larva survived out of 75 tested on the adenosine-2'- and -3'-monophosphates and none of the other -2'- and -3'-monophosphates stimulated growth.

There may be a significant growth stimulation of 1308 by the purine nucleoside-5'-monophosphates, but it is inconsistant. Adenosine, guanosine, xanthosine and inosine-5'-monophosphates produced 1/440, 3/320, 7/680 and 3/400 adults respectively. In combination with the pyrimidine-5'-monophosphates these purine-5'-monophosphates did not increase viability. The homopolymers did not support the growth of 1308 larvae. The role of folic acid in the synthesis of purines suggests as a possibility a defect in folic acid synthesis and that RNA partially alleviated the stress placed on the mutant. Folic acid is normally added to the medium; increasing the concentration 10, 100 and 1000 times did not increase viability of 1308 larvae.

III. Strain 11523

Mutant strain 11523 was isolated as a recessive nutritional conditional lethal on amino acid medium and as a non-lethal on the yeast-agar medium. In the retest using the casein medium 11523 was also lethal.

The multimarked stock y cv v f was used to locate 11523. The back cross data under restrictive and permissive conditions is shown in table 14. The control data is the same as that used for mutant 1308 because the linkage experiments were done simultaneously. The deficiency of males in the y + + + and y cv + + classes on the restrictive medium indicate that 11523 is not located between y and v. The ratio of males in the y cv v + and + + + f classes show 11523 to lie between v and f

Table 14

11523/y cv v f LINKAGE TEST

The percentage of phenotypes within each sex on different media is presented

	11523				Wild-type			
Progeny classes	Casein medium		Yeast medium		Casein medium		Yeast medium	
	♀	♂	♀	♂	♀	♂	♀	♂
y cv v f	32.8	68.5	21.3	31.7	18.4	18.0	23.7	20.4
+ + + +[1]	25.3	2.4	32.4	18.6	32.6	34.0	34.3	39.0
y + + +	2.9	.5	4.3	2.1	4.8	4.8	3.3	3.0
+ cv v f	2.8	4.8	4.4	4.3	2.5	2.6	1.3	1.5
y cv + +	8.9	.3	8.3	2.2	8.4	6.2	5.3	5.6
+ + v f	7.5	8.3	9.0	16.0	13.0	11.0	9.8	9.3
y cv v +	9.4	1.8	4.5	1.3	4.5	8.8	7.3	7.1
+ + + f	10.3	10.7	11.4	18.7	11.0	9.9	12.9	11.8
Multiple Recombinants	4.1	2.9	4.5	5.0	4.6	4.5	2.2	2.2
Totals	1914	796	1792	1072	1036	726	604	602

1 + indicates wild-type alleles for markers.

Using calculations described in the materials and methods the map position of 11523 may be estimated as follows:

$$E_1 = \frac{(C_1)\ (M)}{P} \qquad E_2 = \frac{(C_2)\ (M)}{P}$$

$$X = 1 - \frac{O_1}{E_1} \qquad Y = 1 - \frac{O_2}{E_2}$$

$$= 1 - \frac{14}{\frac{64}{131}\ (545)} \qquad = 1 - \frac{85}{\frac{72}{131}\ (545)}$$

$$= 1 - 0.05 = 0.95 \qquad = 1 - 0.28 = 0.72$$

$$L = \frac{(X)\ (D)}{X + Y} = \frac{0.95\ (23.7)}{0.72 + 0.95} = 13.5$$

Map position of 11523 is 33.0 + 13.5 = 46.5

but close to f. Using the calculations described in materials and methods and included with table 14, 11523 is estimated to be at map position 46.5. In the data presented there are several factors which modify the results. There is a viability factor associated with cv in the control data; when this factor is combined with 11523 or another factor near forked, a synthetic lethal is produced which greatly reduces both males and females in the y cv v f class on yeast medium. In females on the restrictive casein medium the effect is not so pronounced. The males in the + + + + y + + + and y cv + + classes are either lethal free multiple recombinants or escapees from the lethal condition, possibly resulting from cannibalism of dead larvae and flies in the crowded cultures from which these flies were obtained. Cytological examination of 11523 wild-type heterozygous females did not show any evidence of a chromosomal aberration. These results at least limit 11523 within a small region of the X chromosome but proof of a point mutation is not yet available.

The only other mutant characteristic associated with the nutritional lethality of 11523 is female sterility. Attempts to isolate a homozygous strain have failed, so the Basc chromosome was retained as a balancer.

In yeast there are many metabolites not found in the casein medium. All of these metabolites are the potential nutrient or nutrients which permit survival of 11523. The determination of the essential requirement for 11523 is necessarily hit and miss. The nutrients which have been tried without success are shown in table 15.

At least, in the concentrations used, Para-amino benzoic acid, inositol, DNA and yeast extract do not supplement mutant 11523. The lone surviving male in each test may be the result of an escapee or a rare double recombinant within the Basc chromosome.

Table 15

SURVIVAL OF PROGENY FROM 11523/Basc X Basc CROSS ON SUPPLEMENTED CASEIN MEDIUM

Nutrients added to casein medium	♀ 11523/Basc	♀ Basc/Basc	♂ 11523	♂ Basc
DNA, Inositol PABA[1]	123	130	1	98
Yeast extract[2]	41	49	1	50

1 DNA (4 mg/ml), Inositol (42 mg/ml), Para-amino benzoic acid (2 mg/ml), supplied by Nutritional Biochemical Corporation.

2 Yeast extract (10 mg/ml) supplied by Nutritional Biochemical Corporation.

IV. Strain 1625

The mutant 1625 was isolated as an RNA requiring strain, which produced wild-type males on casein medium with RNA and on yeast medium, but only Basc males on casein medium without RNA. This strain would not grow initially as a homozygote, but after several attempts, a homozygous strain was isolated. The initial growth rate was very slow, but growth improved with subsequent transfers. The requirement for RNA has been subsequently lost, probably as a result of selection during the isolation of a homozygous strain.

In a recombination test with y cv v f, less than 5% recombinants were recovered, as compared to 51% in the control cultures (table 16). A large inversion encompassing about three-quarters of the X-chromosome was found in this strain.

In dipteran salivary glands homologous chromosomes undergo "somatic pairing", giving an inversion loop in an inversion heterozygote. The inversion loop formed by wild-type/1625 heterozygotes is shown in plate 1. From plates 1 - 4 the distal breakpoint of the inverted segment can be estimated to be about 3D5 - 6 and the proximal breakpoint about 20C2 (Bridges (1942) chromosome map reprinted in Lindsley and Grell, 1968). The mutant strain 1625 would therefore be designated In(1)1625, and cytologically described as In(1)3D5 - 6; 20C2, following the procedure of Lindsley and Grell (1968).

The nucleolus organizer region 20C1-2 (Cooper, 1959) is included in the 1625 inversion. Other X chromosome inversions which include the nucleolus organizer region are sc^{v2}, sc^{8}, sc^{s1} and sc^{L8} (Lindsley and Grell, 1968). Of these inversions, In(1)sc^{8} has a proximal breakpoint close to the nucleolus organizer at 20C1-2 (Cooper, 1959). In(1)sc^{8}

Table 16

1625/y cv v f LINKAGE TEST[1]

Progeny classes	1625 Casein Medium ♀	1625 Casein Medium ♂	Wild-type Casein Medium ♀	Wild-type Casein Medium ♂
y cv v f	296	366	191	131
+ + + +[2]	310	209	338	247
y + + +	1	0	50	35
+ cv v f	2	0	26	19
y cv + +	4	2	87	45
+ + v f	6	10	135	80
y cv v +	2	2	47	64
+ + + f	1	5	114	72
Multiple Recomb.	4	8	48	88

1 RNA requirement no longer present.

2 + indicates wild-type alleles of markers.

Plate 1 Somatically paired polytene X-chromosomes from an In(1)1625/+ heterozygous female. The cytological map positions of the breakpoints are shown.

Proximal heterochromatin (that region closer to the centromere than the proximal inversion breakpoint) of the wild-type-X (+P) and, possibly, that of the inverted X (InP) is indicated.

The wild-type sequence of the X-chromosome is indicated thus $\xrightarrow{+}$, where the arrow head points toward the centromere and away from the tip. This label will be used on all In(1)1625/+ heterozygous chromosomes. The cytological regions 2A and 10A are identified where present on all photographs.

Magnification X3000.


10A
8A
1nP
3D
+P
20C

Plate 2 Portion of a nucleus from an In(1)1625 heterozygote. The nucleolus (N) is shown attached to the X chromosome. Chromosomal material (M) is present in the nucleolus. The chromosomes are unpaired between 3D, the inversion breakpoint, and 7A. The proximal heterochromatin of the inverted chromosome is shown (InP). The proximal chromatin of the wild-type chromosome is not identifiable in this preparation but may be represented by (M).

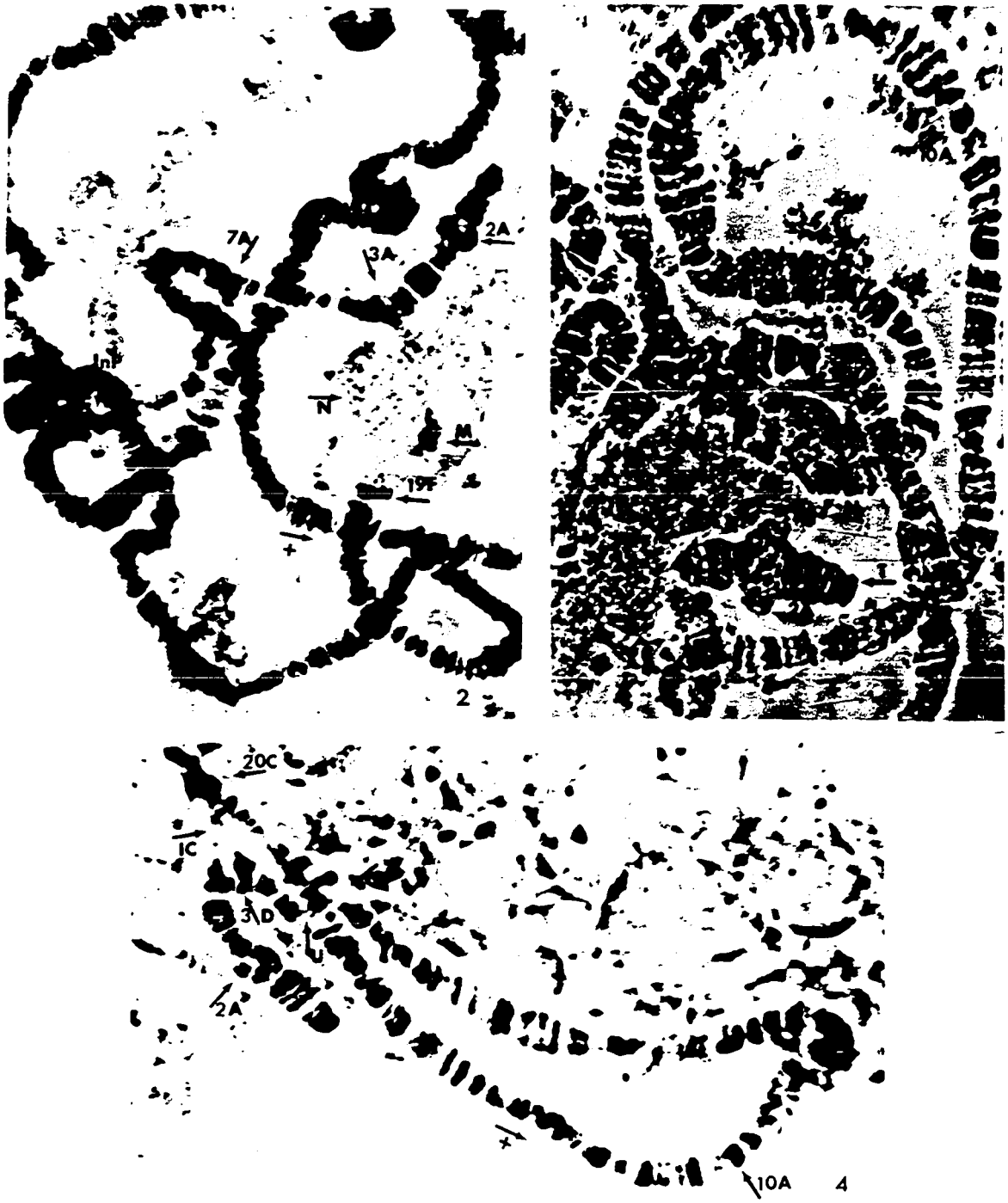
Magnification X1700

Plate 3 Portion of a nucleus from an In(1)1625 heterozygote. The nucleolus (N) is present. (+P) indicates proximal heterochromatin of the wild-type chromosome. The distal end of the inversion is stretched from under the X chromosome tip (T) to across the nucleolus. The proximal end of the inverted chromosome (InP) is stretched across the nucleolus and chromosome (4) to the chromocenter.

Magnification X1400.

Plate 4 Polytene X chromosome from an In(1)1625/+ heterozygote. Cytological map positions of breakpoints are shown. The inverted chromosome stretched between the breakpoints is indicated by IC. The chromosome strand CS consists of paired homologues. Unpaired regions of the wild-type and the inverted chromosome are shown by a (U).

Magnification X1700.


7N
2A
3A
N
M
19
2
10A
I
20C
1C
3D
2A
10A
4

Plates 5, 6 and 7

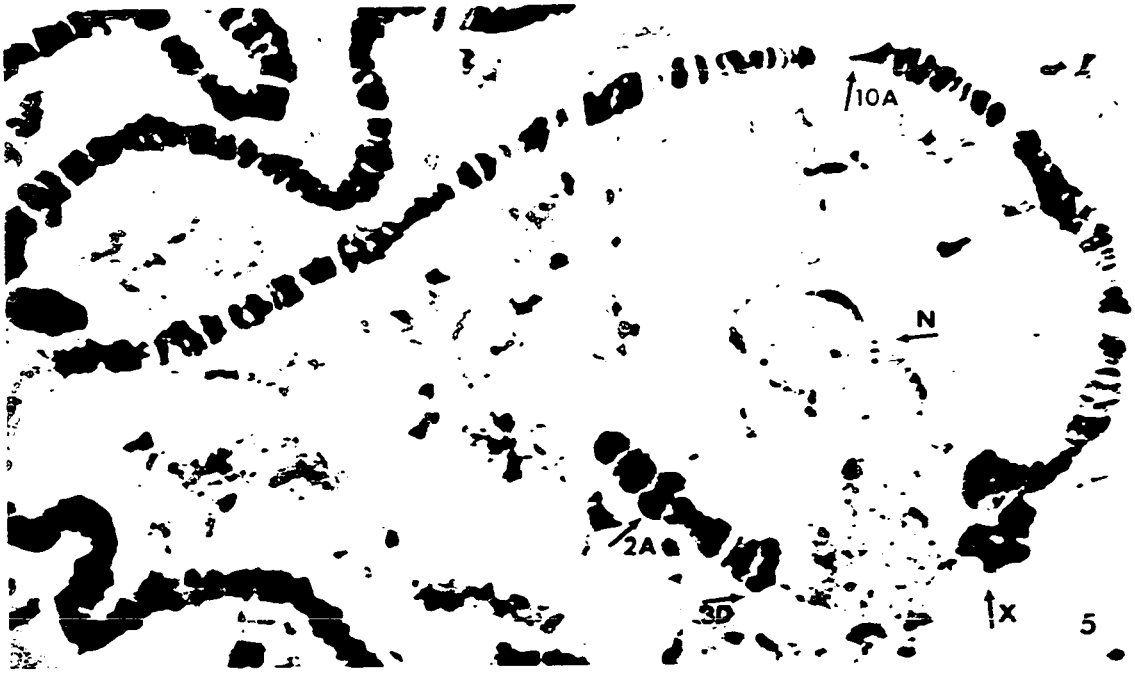
Portion of a nucleus from an In(1)1625 homozygote. The X chromosome has separated at the nucleolus (N).

(5) The cytological regions 19 and 20 are obscured by folding of the chromosome. This region is indicated with an X.

(6) The cytological region 19F next to the inverted proximal heterochromatin is indicated. The cytological region 3D of the proximal end of the inversion is shown.

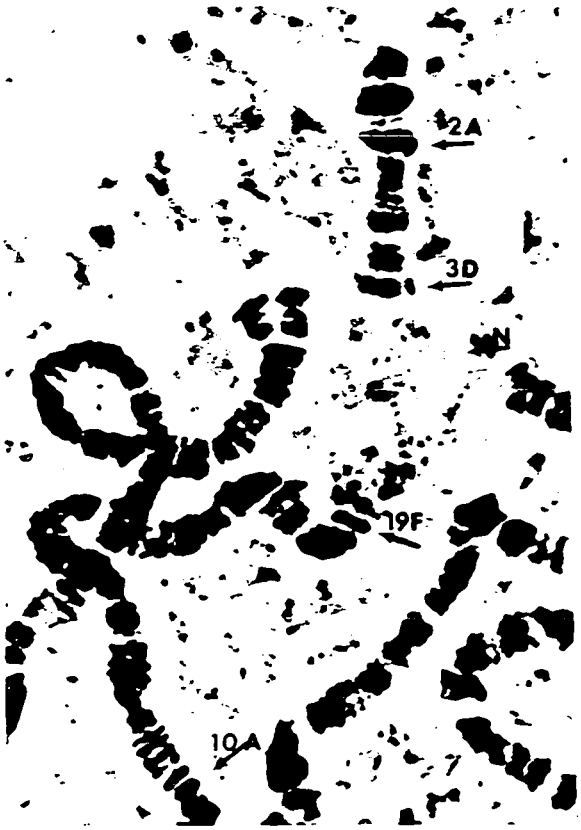
(7) The cytological region 19F next to the inverted proximal heterochromatin is indicated.

Magnification of Plates 5, 6 and 7 X1800.



5

6



7

Plate 8 Polytene X chromosome from In(1)sc^8 homozygote. Both ends of the inverted chromosome are attached to a portion of the chromocenter (C). Nucleolar material is indicated by (N).

Plate 9 Polytene X chromosome from In(1)sc^8 homozygote. Both ends of the X chromosome are attached to the chromocenter (C). Chromosome 3L is also attached to the chromocenter.

Plate 10 Polytene X chromosome from In(1)1625 homozygote. The X chromosome has separated at the nucleolus. Chromosomal material (M) is stretched between the two portions of the X chromosome. The proximal heterochromatin is shown (P). Compare the end of the chromosome in this plate with the one in Plate 11.

Plate 11 Tip of X chromosome of In(1)sc^8 in which the tip has broken away from chromocenter. The proximal heterochromatin to which this chromosome is normally attached has been removed. Compare the tip of this chromosome (T) with the tip in Plate 10.

Magnification of Plates 8, 9, 10 and 11 X1800.

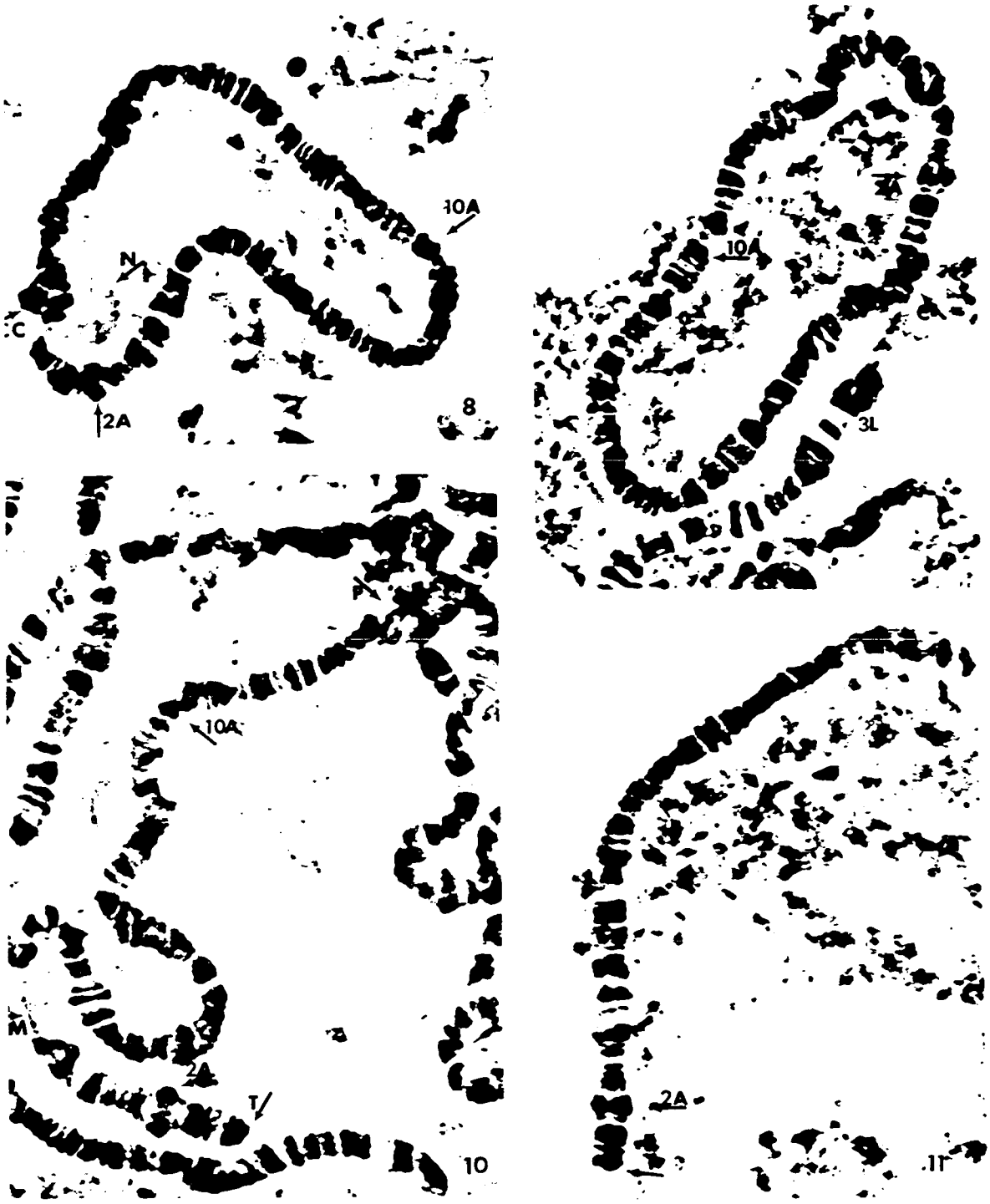

10A
N
C
2A
8
10A
3L
10A
M
2A
T
10
2A
11

Plate 12 Portion of a nucleus from In(1)1625-XO male showing free X chromosome and separated X tip.

Magnification X2000.

Plate 13 Polytene X chromosome from In(1)1625-homozygote showing relationship with nucleolus (N). Methyl green/pyronin staining.

Magnification X2000.

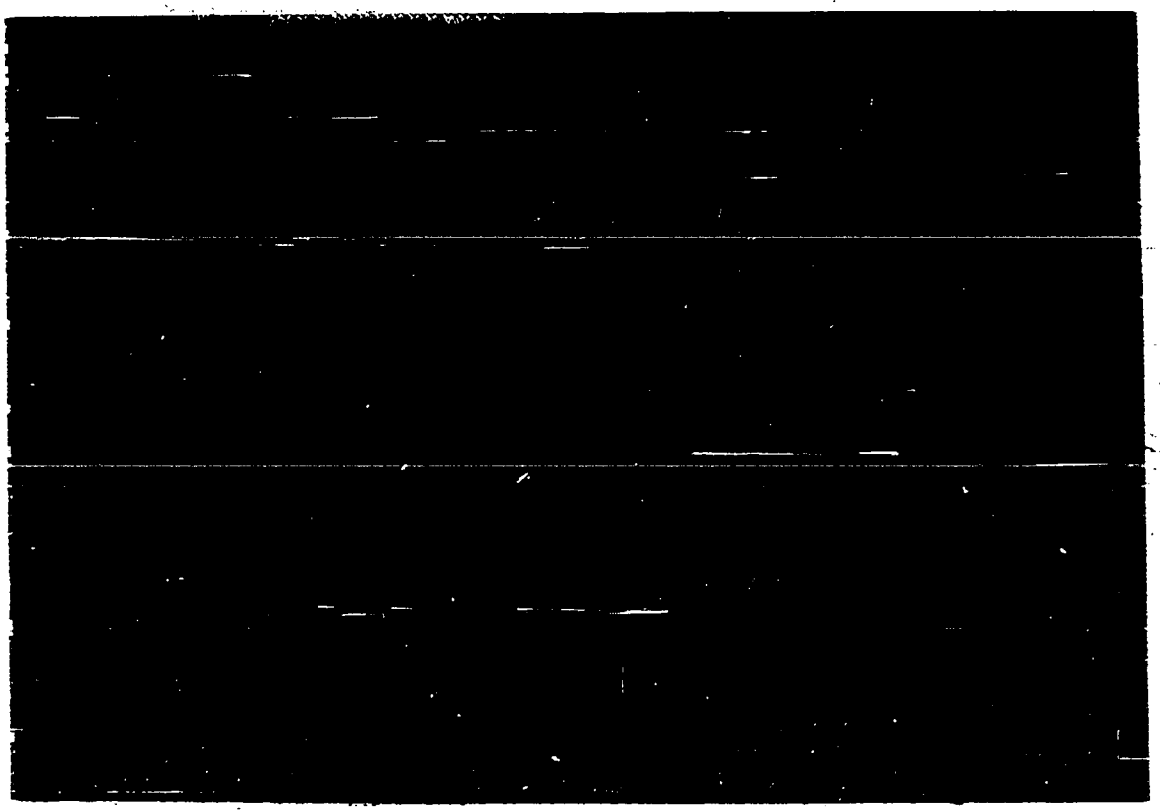
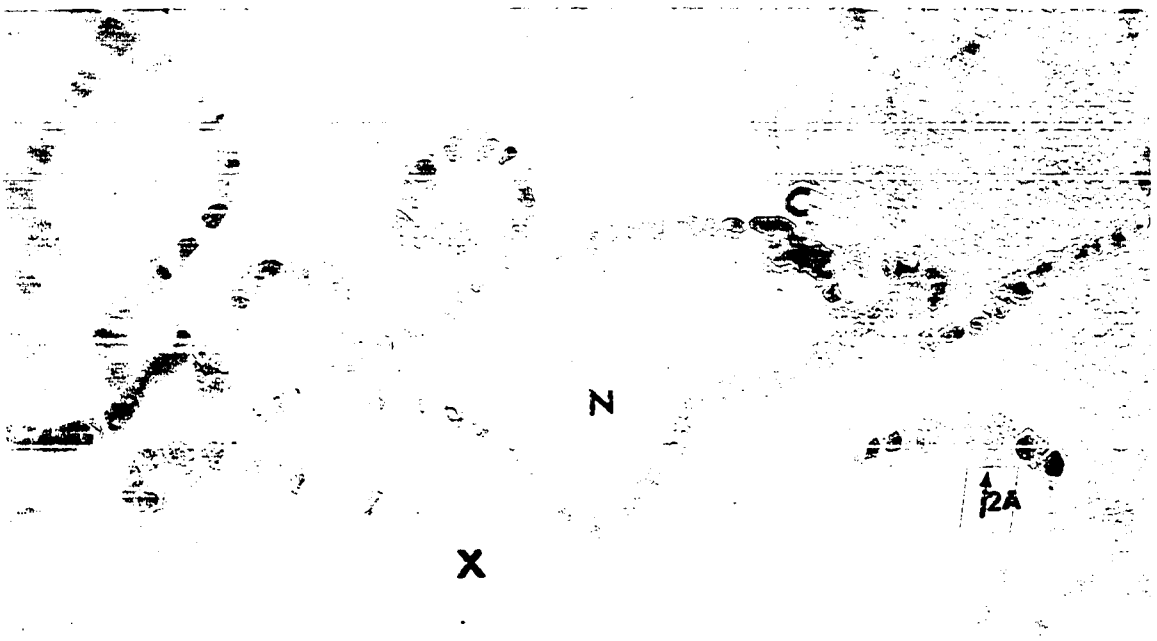


Plate 14 Portion of a nucleus from In(1)1625 male showing bilobed nucleolus (N) near chromocenter (C). Methyl green/pyronin staining. Separated proximal portion of X chromosome is indicated by (X). Magnification X2000.

Plate 15 Illustration similar to above except for DNA in nucleolus indicated with arrow (D).


C
N
12A
X

includes enough of the proximal heterochromatin to allow pairing of the inverted region with the chromocenter (plates 8 and 9). In some cells one end of the In(1)$\underline{sc}^{8}$ chromosome is broken away from the chromocenter, but the end which breaks free does not carry the nucleolus organizer region (plate 11). In In(1)$\underline{1625}$ homozygous females, the X chromosome always has a free tip with the nucleolus organizer region free from the chromocenter. The X chromosome frequently breaks at or near the nucleolus organizer site, this break is found in about 56% of the cells observed (plates 5, 6, 7 and 10).

The relationship of the nucleolus to the X chromosome is not easily photographed in orcein stained squashes because the nucleolus is not distinguishable from the cytoplasmic background. The methyl green/pyronin double stain for DNA and RNA (Brachet, 1952) gives a greater difference in staining intensity between the nucleolus and cytoplasm, because of the greater RNA concentration in the nucleolus (plate 13). The Y chromosome which forms part of the chromocenter carries a nucleolus organizer region. In In(1)$\underline{1625}$ males the nucleolus organizer of the X chromosome might be expected to pair with the homologous region in the Y. This pairing was not observed, but a bilobed nucleolus formed from the In(1)$\underline{1625}$ X chromosome and the chromocenter was observed (plates 14, 15).

In XO males resulting from a cross between attached XY males and In(1)$\underline{1625}$ females, the X chromosome tip was free of the chromocenter (plate 12).

Three unusual morphological characteristics are associated with the In(1)$\underline{1625}$. The wings are slightly crumpled or wavy giving the impression that the wings failed to unfold completely after emergence, and the eyes are slightly darker than wild-types. The eye and wing phenotypes

are highly variable and overlap wild-type. The third phenotype involves the absence of macrochaetae particularly on the head; this phenotype is highly variable but does not overlap wild-type.

Six of the seven macrochaetae on each side of the head are commonly affected. The six affected sites are the anterior orbital, median orbital, posterior orbital, anterior vertical, posterior vertical and the postvertical. The attachment of the macrochaetae appears to be weak and the macrochaetae fall off as the flies age (See table below).

No. of Macrochaetae Missing/fly[1]

	1625		wild-type	
	♂	♀	♂	♀
First scoring	3.0	2.4	0.0	0.0
Second scoring	6.9	6.8	0.0	0.1

1 Macrochaetae missing were scored on 10 males and 10 females selected at random, flies were then stored for ten days in separate vials and scored again.

Flies homozygous for bobbed (bb) alleles have shorter and thinner bristles than normal, and the abdominal tergites are etched at the sides. Cooper (1959), from a review of the available cytological and genetic data, concluded that the nucleolus organizer region and the bb locus were closely linked. The bb locus is thought to be another name for the nucleolus organizer region and the bobbed phenotype, a manifestation of partial deficiencies of the nucleolus organizer region (Ritossa et al., 1966). Since the proximal breakpoint of In(1)1625 is near the nucleolus organizer and In(1)1625 flies show a bristle defect, allelism between bb and In(1)1625 seemed possible, although the phenotypes are not the same. Crosses between In(1)1625 and several bobbed alleles to test for In(1)1625/bb allelism, have shown that they are not allelic (See table below).

Cross	Progeny classification	
	♀	♂
1625 ♀ x Df(1)bb y v car bb ♂	wild-type	1625
1625 ♀ x In(1) dl-49, ty-1, bb ♂	wild-type	1625
1625 ♀ x w^e bb^1 ♂	wild-type	1625
1625 ♀ x Y^{-bb}/w s n bb ♂	wild-type	1625

DISCUSSION

I. Mutagenesis

The nutritional conditional lethals reported here are the first whole-body nutritional mutants induced in animals. The percentage of nutritional lethals found, 0.5% of all X chromosomes tested, has approximately the same frequency as induced in other organisms. For example, in Neurospora 0.55% of the single spore cultures checked were nutritional lethals (Beadle and Tatum, 1945). The close similarity is surprising when the mutagenic treatments, the screening processes, and the different synthetic capabilities of the two organisms are considered; indeed, it must be completely coincidental. The thiamine mutants in Arabidopsis reported by Redei (1965), while restricted to one biochemical pathway, represented 0.025% of the plants analyzed.

EMS is one of the most effective mutagenic agents used for studies on Drosophila, both when the adult feeding method described above is used and when adult males are injected (Epler et al., 1966). In the experimental results presented, 37% of the chromosomes tested carried sex linked recessive lethals, much lower than the 63% reported by Suzuki et al. (1967), using the same feeding method and the same concentration of EMS. This difference probably results from two factors, the greater infertility of F_2 cultures on the defined medium used and a difference in the amounts of the mutagenic solution applied to the Kleenex in each bottle; Suzuki et al. (1967) applied 10 ml of mutagenic solution whereas the Kleenex was saturated in these experiments.

One of the mutants described here, 1625, was found to have a large inversion of the X chromosome. The primary mutagenic action of EMS is thought to be the induction of point mutations through mispairing of

purine bases during DNA replication (Kreig, 1963). However, Freese (1963) suggests the possibility of the induction of chromosomal rearrangements with alkylating agents without providing any suggestion as to a mechanism. Suzuki et al. (1967) also found three rearrangements in 60 mutants tested in EMS induced sex linked recessive lethals. In(1)1625 was probably induced by the EMS treatment but may have resulted from a spontaneous mutation.

II. Genetics of Auxotrophy in Drosophila

In Drosophila, genes controlling certain biosynthetic pathways are known to be scattered throughout the genome. This is true of the genes controlling the biosynthesis of eye pigment and body pigment. An "operon" type of organization for the control of biosynthetic pathways has not been found although an "operon" model has been applied to explain the control of thorax development in Drosophila by the bithorax genes (Lewis, 1964).

Of the strains known in which a nutritional requirement has been demonstrated, only 1308 and 11523 are restricted to small regions of a chromosome. The adenine requirement of strain In(2LR)40d has been shown to be inseparable from inversions which include almost the entire second chromosome (Hinton, 1955). A multigenic control of the cytidine requirement in Drosophila has been suggested by Ellis (1959) because chromosome substitution has shown that the X, second and third chromosomes contribute equally to this requirement.

In the 1308/y cv v f data the main 1308 factor was shown to be located between cv and v. The excess of the + + + f males over the expected suggests that a modifying factor near forked also contributes to the nutritional lethality of 1308. The removal of this factor by recombination increases the viability of the + + + f males. This

factor is also found in the similar + + f male class of the 1308/w m f recombination test. The modifying factor may also play a role in the failure of the RNA precursors to stimulate growth of 1308. A detailed analysis of the influence of this factor will depend on the testing of large numbers of recombinant progeny containing the contributing factors within specific regions of the X chromosome in a manner outlined by Thoday (1961).

In the y cv v f/11523 recombination test, 11523 or a factor near 11523 in combination with cv produces a synthetic lethal which reduces viability of both males and females on yeast medium. On the restrictive medium the viability of females carrying the synthetic lethal is greatly increased, suggesting that the synthetic lethal is nutritionally influenced and an excess of a metabolite or metabolites found in yeast produces heterozygous lethality. Progeny tests of males in the + + + + and + + + f classes have shown that most males in these classes still carry the nutritional lethal, and hence must be considered escapees from the lethal condition. The small number showing loss of the lethal could be accounted for by multiple recombination.

III. Strain 1308

Strain 1308 was found in cultures derived from EMS treated Oregon wild-type males. Since wild-type strains of Drosophila show extreme variation in their response to RNA supplemented medium, the response to RNA of the wild-type strain used here serves as a control for the response of 1308

The Oregon S, K and R strains (Sang, 1957; Burnet and Sang, 1963; Hinton, 1956) show little requirement for RNA. In these strains the development rate is increased but survival is not affected by the addition of RNA to the medium. The effect of RNA on the development rate

can be analyzed by the addition of exogenous bases, nucleosides or nucleotides to the medium. For these strains adenosine or adenylic acid are the major contributing RNA components which increase rate of development most satisfactorily. In other wild-type strains, Oregon K (presumably from a different source than the Oregon K strain used above) (Ellis, 1959) Riverside and Canton-S (Geer, 1963), both the survival and development rate are increased in the presence of RNA. The pyrimidine components of RNA were found to contribute most to the stimulation found with RNA.

The wild-type strain used in these experiments responds to dietary RNA in a manner similar to the strains used by Geer (1963) and Ellis (1959). Pyrimidine nucleotides or nucleosides, particularly cytidine-2'- and -3'-monophosphate will partially replace RNA in the medium. Guanine, guanosine and guanosine-2'- and -3'-monophosphates also stimulate growth.

The variation in response of Drosophila wild-type strains to RNA and RNA precursors may reflect the ability to synthesize RNA precursors. This variation is not suprising when it is considered that Drosophila normally lives on yeast which supplies large quantities of exogenous RNA precursors.

The degree of degradation most satisfactory to growth stimulation should also be ascertainable by the addition of the bases, nucleosides and nucleotides singly or in combination to the medium. With the wild-type strain used here, the mixture of nucleoside-2'- and -3'-monophosphates will completely replace RNA in the medium, whereas mixtures of nucleoside-5'-monophosphates do not give the stimulation found with RNA. This suggests that RNA is degraded to the nucleoside-2'- and -3'-

monophosphates by the larvae, instead of the nucleoside-5'-monophosphates. Sang (1957) suggests that the nucleoside-2'- and -3'-monophosphates are further degraded to the nucleosides during assimilation by the larvae because the nucleosides and nucleotides are used equally well. This suggestion is supported by the results found with the wild-type strain in the present experiments where the pyrimidine nucleosides support growth to almost the same extent as the pyrimidine nucleoside-2'- and -3'-monophosphates. Since both bases and nucleosides stimulate growth, the larvae probably have the necessary enzymes for converting these precursors to utilizable nucleotides unless they are degraded and used as a carbon and/or nitrogen source. Labelled RNA precursors added to the medium have been shown to be incorporated into RNA and DNA of developing larvae (Sirlin and Jacob, 1960).

The inhibition found with adenine derivatives in this wild-type strain contrasts sharply with the results of Hinton (1956) and Sang (1957). A speculation can be made that the control mechanisms in this strain differ from the strains of Hinton (1956) and Sang (1957) by being more sensitive to inhibition by adenine derivatives, but there is no supporting evidence.

There is a 15% survival of 1308 larvae on casein medium supplemented with RNA, but surprisingly, no RNA precursors or degradation products tested, either singly or in combination, supports growth to the extent of RNA itself. Two possible reasons for the failure of RNA precursors to supplement growth could exist. Firstly, the supplementation may not be produced by RNA but by a contaminant common to all RNA preparations used, but not found in the RNA precursor

supplements. Secondly the supplement could be RNA but the supplementation can only occur when RNA of the specific kind used (yeast RNA) is present. This latter possibility would imply some other complex chemical properties which will be considered later.

The RNA used for routine addition to the casein medium was supplied mainly by Nutritional Biochemical Company. This RNA is inexpensive and of low purity. When the RNA precursors did not support growth of 1308 larvae and the possibility of a supplementing contaminant in the RNA preparations was considered, a purified RNA preparation was obtained from Sigma Chemical Company. This purified RNA (type XI) also stimulated growth of 1308 larvae. Analysis of this RNA preparation by the supplier has shown a protein contaminant to be present, but no other contaminants. The protein casein is, in any case, a constituent of the medium so the protein fraction added with the RNA should not be a significant supplement. However, other contaminants have not been completely ruled out. Two other RNA preparations have also been used, s-RNA and RNA core (a pancreatic ribonuclease resistant product of ribosomal RNA). 1308 larvae did not survive in the presence of s-RNA in the casein medium. However, the s-RNA reduced the survival of the wild-type larvae well below that expected in the absence of the RNA preparation discussed above. The s-RNA is therefore not a satisfactory test of 1308 larvae supplementation by RNA. The RNA core reduced the survival of both 1308 and wild-type larvae below that expected with "whole" RNA preparations. The reduced survival of wild-type larvae in the presence of RNA core suggests that RNA which may not be easily degraded or further degraded, perhaps, because of secondary structure, is not easily utilized by Drosophila.

The failure of RNA precursors and homopolymers, to support growth of 1308 larvae, plus the possibility of supplementation by an RNA contaminant, suggests that RNA is not responsible for growth stimulation. However, several complicating factors could be responsible for the failure to obtain a definite stimulation with RNA precursors.

Animals normally acquire a major portion of their essential metabolites from their diet. The nutritional balance of the diet is demonstrably critical to the animal involved. In Drosophila Geer (1963) has shown that the utilization of RNA and RNA precursors is partially dependent on the amino acid source used. The dietary balance of the RNA precursors has also been shown to be important. Geer (1963) reported that adenylic acid in the presence of the pyrimidine nucleotides was inhibitory, but with all four nucleotides in combination the growth stimulation found was similar to that with RNA. Sang (1957) has shown that cytosine and uracil derivatives stimulate growth only in the presence of adenine and guanine derivatives. The balance of RNA precursors in the diet may be critical for 1308 larvae but the one to one ratios used in the present studies have not produced the growth stimulation found with RNA.

1308 in the recombination tests was shown to have at least one modifying factor near forked. If this modifying factor is also involved in the metabolism of RNA, 1308 probably requires more than one metabolite. Determining the proper nutritional balance of a multiple requirement may prove difficult.

Lowering the pH from pH 7.0 to pH 5.0 of casein medium has been shown to alleviate the requirements of a cytidine and an adenine

requiring strain in Drosophila (Ellis, 1959). The hypothesis that the pH change altered the assimilation and utilization of amino acids and other components in the medium was supported by the finding that a similar pH change did not alleviate the adenine requirement on amino acid medium. 1308 is not affected by a similar change in pH, so the pH influence hypothesized by Ellis (1959) is not implicated in the failure of 1308 to grow without RNA.

The possibility of a larger RNA degradation product (e.g. an oligonucleotide) instead of the bases, nucleosides or nucleotides supplementing the requirement should at least be mentioned. The lower charge over a larger mass may make easier transmission of a large molecule across membranes, thus allowing oligonucleotides passage where smaller RNA degradation products do not pass. This speculation does not have any known precedent in other organisms.

The only suggestion of the RNA precursors which may be involved in supplementation of 1308 is the observation that a few 1308 flies survive in the presence of purine derivatives. If the purine derivatives are responsible for supplementing the 1308 deficiency the role of purines may be direct or indirect. The glycine, hypoxanthine and thymine requiring mutant in a Chinese hamster cell line which is probably the result of a deficiency for a folic acid cofactor is an example of a requirement, supplemented indirectly by other metabolites. Folic acid derivatives contribute two carbon fragments to the purine biosynthetic pathway. A possible deficiency of folic acid in the mutant 1308 was checked by addition of excess folic acid to the medium but no growth stimulation was found. However, the possibility of the deficiency lying between folic acid and derivatives N^5, N^{10}-methenyl tetrahydrofolate,

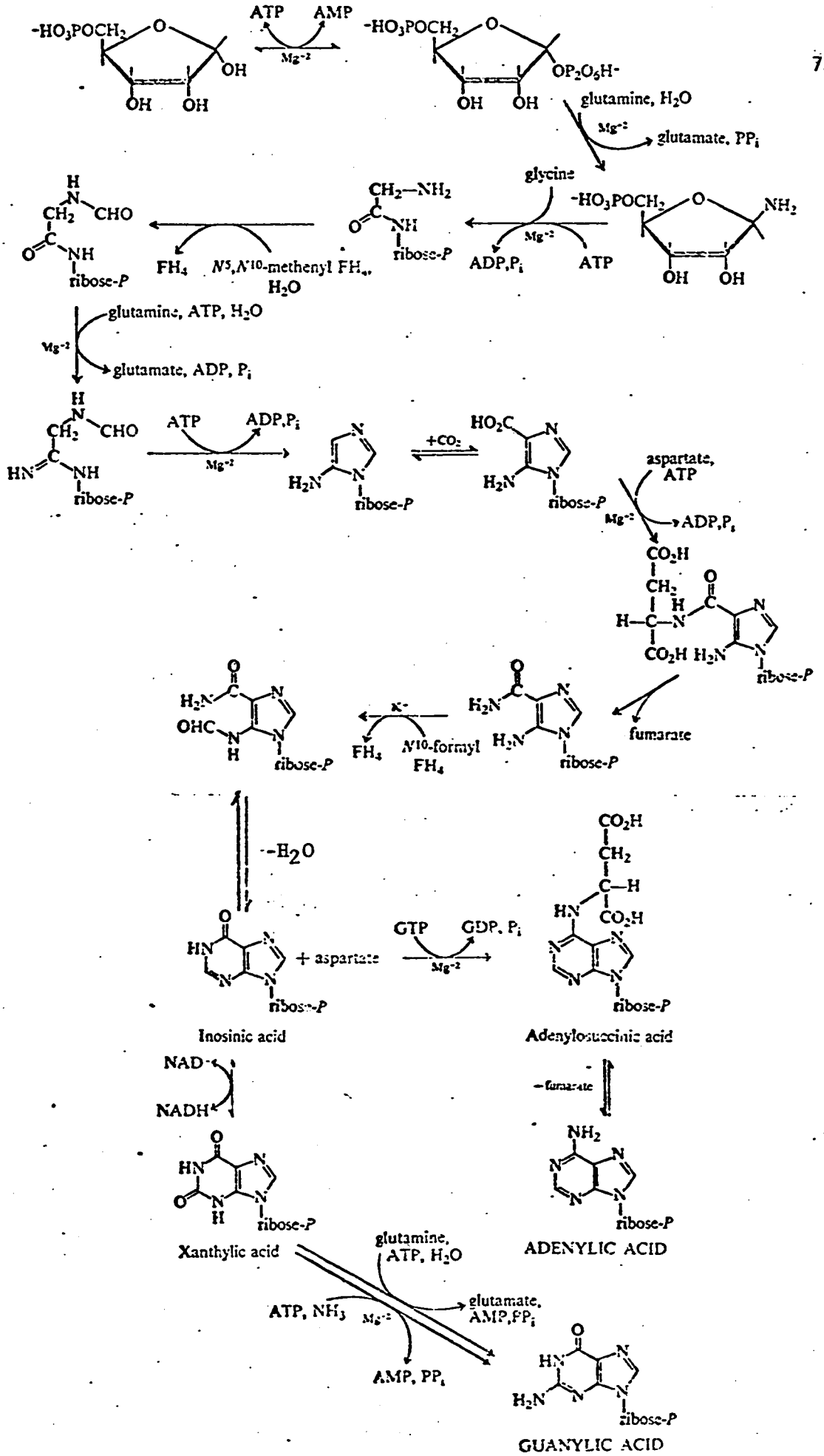
and N^{10}-formyltetrahydrofolate which actually donate the carbon fragments, has not been tested (see figure 2). Of the amino acids used in purine biosynthesis only glutamine is not present in the casein added to the medium. The other amino acids, glycine and aspartic acid are provided in concentrations in excess of the amount supplied in the amino acid medium, but the ratio of the amino acids could be the important factor. The purine biosynthetic pathway as found in other organisms includes many steps, but only two branches are known, one branch to the histidine pathway and another to the thiamine pathway. Histidine and thiamine are required by Drosophila so metabolic blocks in the biosynthesis of these metabolites are not the basis for the 1308 deficiency.

The possibility that yeast supplies nutrients or a nutritional balance which is not present in the RNA casein medium was demonstrated with 1308 larvae where about 60% survive on yeast and about 15% on RNA casein medium. The interactions of the metabolites are subtle and depend on different concentrations and ratios,, but the evidence to date suggests that RNA (possibly the purine precursors) or an RNA contaminant is involved and it is hoped that a more rigorous analysis of possible factors will establish the biochemical basis for the deficiency of 1308.

IV. Strain 11523

The difference in nutritional supplements supplied by the yeast-agar medium and the casein medium are potentially large so the determination of the genetic lesion in 11523 is expected to be difficult. Several obvious supplements such as para-amino benzoic acid, DNA, inositol and yeast extract (containing amino acids and vitamins) have

Figure 2. Purine biosynthetic pathway.
Adapted from Mahler and Cordes, (1966).


ATP
AMP
Mg+2
-HO3POCH2
OH
OH
OH
OP2O5H-
glutamine, H2O
glutamate, PPi
glycine
CH2—NH2
ribose-P
ADP,Pi
ATP
NH2
FH4
N5,N10-methenyl FH4
H2O
glutamine, ATP, H2O
glutamate, ADP, Pi
CHO
HN
CH2
NH
HO2C
+CO2
aspartate,
ADP,Pi
CO2H
H2N
OHC
K+
N10-formyl
fumarate
-H2O
+ aspartate
GTP
GDP, Pi
Inosinic acid
Adenylosuccinic acid
NAD+
NADH
-fumarate
Xanthylic acid
ADENYLIC ACID
glutamine,
ATP, H2O
ATP, NH3
glutamate,
AMP,PPi
AMP, PPi
GUANYLIC ACID

not supported the growth of 11523. However, many other potential nutrients e.g. sugars and other yeast extracts have not been tried. Two observations may lead to the identification of 11523 requirement; first, the sterility of 11523 females, if this is actually a pleiotropic effect, and second the reduction of 11523 flies under crowded conditions on yeast medium. B vitamins have been shown to be essential for female fertility (Sang and King, 1961) and these may be limiting in the restrictive medium. The reduction of 11523 flies in crowded yeast cultures may result from the exhaustion of the essential metabolite which is normally in a limited supply or from an accumulation of compounds which inhibit metabolism of 11523 flies.

V. In(1)1625 Strain

Unlike the other known inversions which include the nucleolus organizer region in Drosophila melanogaster In(1)1625 frees the nucleolus from the chromocenter. The analysis of the genetic, the physical and maybe the biochemical relationship of the nucleolus and the nucleolar organizer region may well be facilitated using this inversion strain. This relationship has not been determined despite the advances made in localizing the ribosomal RNA cistrons within the nucleolus organizer region (Ritossa and Spiegleman, 1965; Brown and Gurdon, 1964).

The nucleolus in salivary glands of Chironomids has been shown to resemble a puff or Balbiani ring arising from one or two bands (Pelling and Beerman, 1966; Kalinas et al., 1964). Pelling and Beerman (1966) claim that the nucleolar organizer is therefore similar to any other active locus, but this viewpoint should not be extended to other diptera without reservation. In Drosophila species the only connection between the nucleolus and nucleolus organizer region is a small strand (plate 12).

This strand has been shown to contain DNA (Barr and Plaut, 1966). Other workers have demonstrated chromosomal material projecting into the nucleolus in mammals and insects (Ashton and Schultz, 1964; Bernhard, 1966; Sotelo and Wettstein, 1966).

The X chromosome of In(1)1625 females is frequently broken in squash preparations. This break is presumed to be in the nucleolus organizer region, because the separated X chromosome tip and the broken stub are both attached to the nucleolus. The specific separation of the X chromosome at this point appears to be related to the nucleolus organizer, perhaps due to the expansion of chromosomal material into the nucleolus. Since a specific breakage is not found at other puffed loci, there appears to be a distinct difference between other active regions and the nucleolus organizer region. In In(1)1625 the nucleolus organizer region apparently alters the structural integrity of the chromosome permitting a specific breakage during squashing. In wild-type strains of Drosophila free X chromosomes are found in squash preparations, but at a much lower frequency than in In(1)1625. The nucleolus organizer region is very close to the centromere of the extreme acrocentric X chromosome, and breaks near the chromocenter are likely to be in proximal heterochromatin, perhaps, preferentially, in the nucleolus organizer region.

In(1)1625 with one breakpoint in proximal heterochromatin and the other breakpoint in distal euchromatin, has the structural rearrangements necessary for the production of position effects (Lewis, 1950; Baker, 1968). This suggests that some of the mutant characteristics associated with In(1)1625 may result from position effects.

Females heterozygous for the recessive allele ruby and the In(1)1625 have the variable eye phenotype found with In(1)1625 homozygotes in about

the same frequency. Ruby, at 4C3-7, is quite distant from the breakpoint, but the eye color phenotype found in In(1)1625 may result from a position effect on the ruby locus.

In(1)1625 was first selected as a nutritional conditional lethal on casein medium in the absence of RNA. In the first isolate of In(1)1625 males were viable in the presence of RNA in casein medium or on yeast medium. Initial matings between In(1)1625/Basc females and In(1)1625 males failed to produce homozygous In(1)1625 female progeny, and when a homozygous strain was isolated the strain was found to no longer require RNA.

Two possible explanations of the loss in RNA requirement have been considered. Firstly that the original mutant isolated in addition to the phenotypes already mentioned carried an undetected bobbed mutant, (bb mutants are considered to be deletions of a portion of the nucleolus organizer region) which in selection of a homozygous strain produced a viable complement of ribosomal RNA cistrons, by recombination as suggested by Ritossa et al. (1966). The second explanation involves a postulate of Baker (1968) that the removal of the nucleolus organizer region to distal euchromatin might produce a position effect on the nucleolar organizer region inhibiting ribosomal RNA synthesis and causing lethality in XO males. The In(1)1625 proximal breakpoint is closer to the nucleolus organizer than any other known inversion, but XO males, although visibly smaller than normal are viable. The original isolate was not tested for lethality in XO males, but the failure to produce homozygous females support this hypothesis. The loss of RNA requirement could be explained as the result of the accumulation of position effect

modifiers during the isolation of a homozygous strain. In both of these explanations viability found with RNA added to the medium is difficult to account for, but a nonspecific increase in RNA synthesis as a result of supplying exogenous RNA precursors is suggested.

VI. Present State of Auxotrophy in Drosophila

Nutritional conditional mutants have been produced in Drosophila for the first time. The step from wild-type, capable of growing on the casein medium, to auxotroph with a metabolic block has not been established for 1308 and 11523, but both mutants may well prove to be auxotrophs. The possibility must be considered that 1308 and 11523 will not yield to analysis as auxotrophs for the following reasons. First, in microorganisms many mutants capable of growing on complete medium but not on minimal medium have not yielded to analysis. Second, either or both of these strains may be double mutants with more than one requirement or single mutants with complex multiple requirements. Third, the complication of diploidy and fourth, the complex life cycle may be incompatible with the selection of auxotrophic mutants. In Arabidopsis, despite extensive studies, only thiamine auxotrophic mutants have been found, but the system in plants may be further complicated by chloroplast metabolism. Many metabolic blocks resulting in diseases in humans have been reported, some of which can be manipulated through the diet, therefore it may be expected that similar genetic lesions can be produced in Drosophila.

Auxotrophic mutants in Drosophila, if found, should yield a wealth of information on genetic fine structure analysis, metabolic pathways and genetic control mechanisms in animals. These mutants should also prove useful for studies on differentiation and development. Using auxotrophs or nutritional conditional lethals, the

selection of alleles for genetic fine structure analysis should be simplified. To select alleles, mutagen treated females would be mated to males on yeast medium. Progeny males are then selected and mated to females heterozygous for the original mutant and a balancer on restrictive medium. Alleles for the selected loci would be found in cultures failing to produce wild-type females, but producing a prescribed number of heterozygous females.

In previous intragenic recombination studies in Drosophila either a prodigious number of flies had to be counted or a complicated stock had to be synthesized with the appropriate lethals closely linked to the locus being studied (Carlson, 1959; Chovnick et al., 1964). The ease with which recombinants are selected for in intragenic studies with auxotrophs or nutritional conditional mutants makes studies on many loci feasible.

Biosynthetic pathways for the synthesis of essential metabolites are usually quite similar, but some striking differences are known. In the synthesis of lysine two pathways have been determined, one common to fungi and another found in bacteria, algae and higher plants. Auxotrophic mutants in Drosophila will, of course, help to establish biochemical pathways in that organism and should be useful for comparisons with other organisms.

A number of alleles of different genes controlling the same biosynthetic pathway would permit a search for the operon-type of controlling systems which are found in microorganisms. Glassman (1965) suggests that operon like organization of genetic material does not occur in Drosophila, although Lewis (1964) has evoked an operon model to explain the complex bithorax locus. Drosophila is the only diploid organism in

which genetic knowledge approaches the level of sophistication required for the analysis of operon like models.

Drosophila has many discrete stages in the life cycle for studies on differentiation and development. In tissue culture different tissues are found to have varying metabolic requirements, and some tissues require a specific concentration of metabolites before differentiation can take place. In addition, isozyme studies have shown that isozymes specific for a particular tissue or organ, are synthesized or shut off at prescribed times during development. Auxotrophic mutants in Drosophila should help to delineate the metabolic requirements of organisms during the process of differentiation and development.

SUMMARY

Three out of six hundred and four EMS treated X chromosomes tested were found to be nutritional conditional lethals. One of these mutants 11523 is viable on yeast medium, but lethal on casein medium. 11523 is female sterile when homozygous. The nutritional deficiency has not been determined for this mutant. The second mutant 1308 requires RNA for growth on casein medium, but the RNA precursors do not support growth. A few 1308 flies survive on casein medium in the presence of purine nucleosides or nucleotides. Both 1308 and 11523 have been localized within a small region of the X chromosome.. The third strain 1625 contains an inversion including a large portion of the X chromosome and the nucleolus organizer. This inversion always removes the nucleolus from the chromocenter with which it is otherwise associated. The use of these mutants for further research has been discussed.

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