RESEARCH METHODS

Construction and maintenance of stocks

Note by editors The material by H. J. Muller for the following sections was prepared in the form of pictorial diagrams on large wall charts, and was thus exhibited by him at the Sixth International Congress of Genetics at Ithaca in 1932. Photographs of these charts were supplied by Muller as the material for the following accounts. In order to reduce the charts to forms suitable for reproduction by typewriting on stencils, the editors have transcribed them, with as few as possible changes in the basic plan of each chart. With respect to details, they have made minor changes, to wit:- (1) recasting the nomenclature current in 1932 into that of 1935, (2) employing "f" as the marker for a "foreign" chromosome to be introduced into a given "homozygous" or "host" stock, which is marked by "h", and (3) substituting Pm for S, and cy^D for cy, as markers. (Star can not easily be told in flies with other eye shapes, while Plum is excellent; the use of cy left the emergent stock encumbered with this character, while the use of dominant markers (cy^D and ci^D, available since 1932) for chromosome 4 gives a clean stock.

Muller, H. J. Construction of Homozygous Stocks.
(See also similar technique by Bridges, Carn. Inst. Wash. Yr. Bk. 28:338, 1929.)

P_1 φ^h = donor for desired homozygote (or donor may be σ; if so, mate to $\underline{Y}$;Pm/$\neq$;H/$\neq$ φ)

X

Pm/$\neq$;H/$\neq$ $\sigma\sigma$ (Dominant markers in 2 and 3 may be replaced by T(2-3) with one dominant marker).

F_1 one σ 1^h;Pm/2^h;H/3^h (save for remating!) X $\underline{Cl}B/\neq$;Cy/$\neq$;CC,D/$\neq$ $\varphi\varphi$ (different dominant markers)

F_2 Father (remated) X $\underline{Cl}B/1^h$;Cy/2^h;CC,D/3^h $\varphi\varphi$ (crossing-over prevented)

F_3 $1^h;2^h;3^h$ completely homozygous $\varphi\varphi$ X $\sigma\sigma$

For controlling 4 also, the 4th chromosome dominant cy^D may be used together with Pm/$\neq$;H/$\neq$ (or with $\underline{Y}$;Pm/$\neq$;H/$\neq$), and ci^D with $\underline{Cl}B/\neq$;Cy/$\neq$;CC,D/$\neq$. For controlling Y also, mate donor σ to $\underline{Cl}B/\neq$;Pm/$\neq$;H/$\neq$;cy^D/$\neq$ φ; save donor σ and remate to $\underline{Cl}B/1^h$; Pm/2^h;H/3^h;cy^D/4^h daughter; use one son (1^hY^h;Pm/2^h;H/3^h;cy^D/4^h) to mate to $\underline{Cl}B/\neq$;Cy/$\neq$;CC,D/$\neq$;ci^D/$\neq\varphi\varphi$; save him and proceed as above for F_2 and F_3.

F_3 yields also all possible combinations of chromosomes of P_1 donor, homo- and heterozygously, and distinguishable by markers, for analysis of contributions of all the chromosomes (whether recessive or dominant) to multiple factor effects, and for tracing sources of variability. F_3 reveals also all recessive lethal and visible mutant genes of P_1.

Chromosomes of desired "h" stock need not carry any visible mutant.

Muller, H.J. Insertion of Foreign Chromosome into Homozygous Host Stock

(Stocks f and h need not contain any visible mutants)

(1) For substituting 1^f for 1^h in $1^h;2^h;3^h;4^h$ stock

P_1 f ♂♂ X ClB/+;T(2-3)S Cy/+,+;ey^D/+ ♀♀

F_1 1^f/ClB;2^f,3^f/T(2-3)S Cy;4^f/ey^D ♀♀ X h ♂♂

F_2 one ♂ 1^f;2^h,3^h/T(2-3)S Cy;4^h/ey^D X ClB/+;Cy/+;CC D/+;ci^D/+ ♀♀ (Different markers; Inversions)

(save for remating!)

F_3 Father (remated) X 1^f/ClB;2^h/Cy;3^h/CC,D;4^h/ci^D ♀

F_4 1^f;2^h;3^h;4^h completely homozygous ♀♀ X ♂♂

(2) For substituting 2^f for 2^h in $1^h;2^h;3^h;4^h$ stock

P_1 f ♀♀ X Pm/+;H/+;ey^D/+ ♂♂ AND h ♂♂ X ClB/+;Cy/+; CC,D/+;ci^D/+ ♀♀

F_1 1^f;2^f/Pm;3^f/H;4^f/ey^D ♂♂ X 1^h/ClB;2^h/Cy;3^h/CC,D;4^h/ci^D ♀♀

F_2 one ♂ 1^h;2^f/Cy;3^h/H;4^h/ey^D X 1^f/ClB;2^f/Cy;3^f/CC,D;4^f/ci^D ♀♀ (Recombination; save!)

F_3 Father X 1^h/ClB;2^f/Cy;3^h/CC,D;4^h/ci^D ♀♀

F_4 1^h;2^f;3^h;4^h completely homozygous ♀♀ and ♂♂

(3,4) Analogous schemes apply for 3 and 4 (selecting appropriate F_2 ♂)

If desired, foreign autosome may first be made like that in homozygous stock except in regard to particular region. Method for this is shown below in scheme for combining invisible genes in the same chromosome.

Muller, H. J. Insertion of Desired Genes into Attached X's

P_1 y ♀♀ X $T(1-4)B^S$ ♂♂ (Austin T; left part X attached to 4; rt end X carrying "Bar-super")

F_1 y/XR,B^S ♀♀ X m g^2 sd f ♂♂ (Let m, g^2, sd, f be the genes to be inserted)

F_2 (Crossing-over) $y \cdot B^S$/m g^2 sd f ♀ X m g^2 sd f ♂♂

F_3 (Crossing-over) m g^2 sd f·B^S /m g^2 sd f ♀ X + ♂♂

F_4 (Crossing-over) m g^2 sd f Y ♀

In earlier method, used by L.V. Morgan et. al, genes were inserted by crossing-over, from separate X-chromosome containing them, into attached X's, in triploids.

Muller, H. J. Combination of Invisible Genes

Nearly same methods apply for getting desired chromosome combinations of recessives that always must be worked with in the heterozygous condition.

1. Two invisibles in different autosomes (a in 2; b in 3)

P_1 a/2 ♂ X S/+;CC,D/+ ♀ AND b/3 ♂ X Cy/+;CC,Dfd/+ ♀

F_1 a/S;CC,D/+ ♀ X Cy/+;b/CC,Dfd ♀

F_2 a/Cy;b/CC,D ♂ X a/Cy;b/CC,D ♀ (balanced stock, if recessives have low viability or low fertility)

F_3 a/a;b/b ♂ and ♀ (if homozygotes are viable and fertile)

(for chromosome 4, markers ci^D and ey^D may be used in same way.

2. Getting combination of invisibles in 1 and 2 (a in 1;b in 2)
P_1 a/1 ♀ X Cy/+ ♂ AND b/2 ♂ X ClB/+;Pm/+ ♀.
(double mated) y;Pm/+ ♀
F_1 a;Cy/+ ♂ (double mated) X ClB/+;b/Pm ♀ and Xy;b/Pm ♀
F_2 a/ClB;b/Cy ♀ X a;b/Cy ♂ (balanced ♂)
F_3 a/a;b/b ♀ and a;b/b ♂ (if homo. viab. and fert.)
Analogous scheme using CC;D and CC,Dfd or Mc-2 for 3 applies for chromosomes 1 and 3.

3. Getting combination of two invisible genes (a and b) in same chromosome (in known positions). Let map order be a, v_1, v_2, b where v_1 and v_2 are visible genes between a and b.
P_1 a/+ X v_1 v_2 AND b/+ X v_1 v_2
F_1 a+/+v_1 v_2 ♀ X v_1 v_2 ♂ AND +/b/v_1 v_2 + ♂ × v_1 v_2 ♂
F_2 (by crossing-over) a + v_2/+ v_1 v_2 X v_1 + b/v_1 v_2 +
(chance of losing a or b from ♀ obviable by interpolating cross of ♂ by individual containing inversions)
F_3 a + v_2 +/+ v_1 + b ♀ X v_1 v_2 ♂
F_4 (by crossing-over) a+/+b/v_1 v_2 X D_1 v ♀
(where D_1 is dominant linked and where v is either v_1 or v_2)
F_5 a b/D_1 v ♂ X C,D_2 ♀ (where D_2 is a different linked dominant)
(lethal when homozygous, and C is an inversion preventing crossing-over).
F_6 a b/C,D_2 ♂ and ♀ (balanced ♂)
F_7 a b/a b ♂ and ♀ (homozygous ♂)
Combination of one invisible with one visible is correspondingly simpler.

Muller, H. J. and C. B. Bridges Balanced Stocks

Given "1", a gene (or combination of linked genes) with low or no viability or fertility when homozygous in ♂ or ♀ (or both, and "C"- a genetic complex that is non-perpetuating or lethal when homozygous, that contains gene rearrangements which prevent crossing-over between "1" and "C" and that preferably contains a dominant marker, then the most usual balanced stock for maintenance of "1" without selection is:

1/C ♂ X 1/C ♀

1/1 (fails to perpetuate); C/C (dies); 1/C and C/1 (continue as before.

To construct simple balanced autosomal stocks (1/C) Given 1 a/+ (or 1/a) ♂, where a = recessive marker and M = a dominant linked marker subject to crossing-over. Then:
P_1 M/+ X a/a
F_1 M/a ♀ X a/a ♂
F_2 M a/a ♀ X 1 a/+ ♂ (or 1/a ♂)
F_3 M a/1 a ♂ X C/+ ♀ (or M a/1 X C/+ ♀)
F_4 1 a/C ♂ and ♀ (or 1/C ♂ and ♀)
The more used complexes represented above by "C" are as follows:

Chromosome	Complex
1	ClB (Bar eyes), very little crossing-over. (Muller)
"	dl-49, sc lz^s (only recessive markers), crosses over right of garnet (Muller)
"	dl-49, y Hw (Hairy wing), crosses over right of garnet (Muller, Redfield)
2	Cy (Curly wings), with 2 inversions and very little crossing-over (Ward)
"	C2L C2R sp (only recessive marker, speck), 2 inversions, some crossing-over in middle. (Sturtevant)
"	Pm (Plum eye) inversions involving 2L and 2R. (Muller)
3	CC, D (Dichaete) little crossing-over except near ends (Oliver, Stone)
"	CC, Dfd (Deformed eye) 2 inversions, moderate crossing-over in middle (Payne, Bridges)
"	C3X, M3X (Minute bristles) 2 inversions, moderate crossing-over in middle (Muller)
"	Mc-2 (Moire eye) inversions and translocation with 2, crossing-over only near right end (Muller)
2 & 3	T(2-3) S Cy (Star eye, Curly wings) inversions and translocation 2-3, moderate crossing-over (Muller)
4	ci^D (Cubitus-interruptus - Dominant - venation) (inversion not needed for 4) (Sturtevant)
"	ey^D (eyeless - Dominant) (inversion not needed for 4) (Muller)

Many other usable complexes, some lacking dominant markers and many affecting a limited part of a chromosome, are available. (See list of symbols in DIS-3).

Muller, H. J. To Balance Sex-linked Genes

1. General method for genes to left of garnet.

Symbols:
l = sex-linked gene or combination of genes with low or no viability or fertility in ♂, or else in homozygous ♀.
sc = scute
lz^s = lozenge-spectacled (sterile in homozygous ♀, not in ♂)
dl-49 = inversion (left break about 11, right about 44 on map)

P_1 l/sc ♀ X dl-49, sc lz^s ♂
F_1 l/dl-49, sc lz^s ♀ X dl-49, sc lz^s ♂
F_2 l/dl-49, sc lz^s ♀ - like mother, fertile and continues ⚥
dl-49, sc lz^s/dl-49, sc lz^s ♀ - sterile
l ♂ - dies or is sterile or yields lethal or sterile l/l daughter
dl-49, sc lz^s ♂ - like father, fertile and continues ⚥

2. Genes to right of garnet may be balanced similarly, using CR ♀ s B in place of dl-49, sc lz^s. CR is rearrangement preventing crossing-over to right of garnet, ♀ s makes homozygous ♀ sterile (but not ♂) and B is Bar eye.

3. If "l" is itself associated with arrangement preventing crossing-over, then "C" in chromosome balancing it is unnecessary. Example:
Cl/lz^s ♀ X lz^s ♂ is balanced stock, though lz^s here is in normally arranged chromosome instead of with dl-49 inversion.
4. Other genes like lz^s which do not kill or sterlize ♂ but only homozygous ♀, may be balanced also with ClB. Example:
P$_1$ ClB ♀ X sn ♂ (sn = singed; sn ♂ fertile; sn/sn ♀ sterile)
F$_1$ ClB/sn ♀ X sn ♂ (balanced stock)
F$_2$ ClB/sn ♀ (fertile) sn/sn ♀ (sterile) ClB ♂ dies; sn ♂ fertile
5. Genes in class 4 also may be balanced against attached X's
y ♀ X sn ♂
y/sn ♀ (3X's - sterile) y ♀ sn ♂ no-X(dies)
Method 5 is inferior to others whenever ♀♀ with "l" may be required.
Methods 4 and 5 are inferior to 1 and 2 where "l" ♂ is hard to obtain or to breed.

Austin, Tex. laboratory Balancers Chromosome 1: ClB - Good, except for extreme ends.
sc^8 sn - good for chromosomes not crossing-over in the middle region (e.g. Translocations &c)
99b sn - Ditto.
dl-49 - Balances middle region.
In-Am - Balances right end (f-bb) (probably left as far as y)
Chromosome 2:
Cy - Balances all of chromosome; Crosses over but seldom.
Pm -
NS -
Chromosome 3:
CMc - Inversion in left arm
T2,3-C e - Translocation with inversions; balances all of chromosome except the ca region.
Dcx - Double inversion including D; balances middle of chromosome (all of chromosome except ru-h, & ca)
C3X - Balances most of chromosome, but crosses over too frequently.
C3c -
Payne -

Oliver, C. P. Balancers X-chromosomes with genes which cause sterility or poor viability in males and with which ClB cannot be used, can be balanced with the Dl inversion (Muller's) that has connected with it the visible spectacle eye. The spectacle female is sterile. Only a small per cent of crossing-over occurs to the left of forked. If spectacle-forked males are used to balance, no or only occasional selection is required. Punch eye, dominant, is a useful balancer for inviable genes in the left arm of 3 except for a small amount near the left end. The viability of punch is good.

Zuitin, A.I. A Third Chromosome Balancer

The lines carrying numerous mutant genes in the third chromosome may be balanced by means of the chromosome ru h D (Fiodorova), carrying the inversion which almost completely suppresses crossing over (to be described in one of the following issues of C.R. Ac. Sc. U.S.S.R.). The stock is : ru h D inv/ru cu ca; Cy sp/L^2.

Muller, H.J. Labor-Saving Method of Starting Homozygous or Balanced Stocks of ♂-fertile Sex-linked Genes

(1) Balancer stock "Patroclinous" ("Pat")
P_1 ClB, sc v/sc v lx f bb/Y ♀ (non-disjunction)

X dl-49, lz^s/Y ♂
Eggs: ClB, sc v (half carry Y) sc v lx f bb (half carry Y)
ClB sc v/sc v lx f bb (non-disjunction) Y (non-dis.)
Sperm: lz^s dl-49 Y
F_1 ClB, sc v/dl-49, lz^s ♀ (1/2 carry Y) ClB, sc v/Y (1/2 YY) (dies)
sc v lx f bb/dl-49, lz^s ♀ (" " ") sc v lx f bb/Y (" " ")
ClB, sc v/sc v lx f bb/dl-49, lz^s ♀ (dies) ClB, sc v/sc v lx f bb/Y ♀
dl-49, lz^s/Y ♂ Y/Y (dies)
To perpetuate, breed sc v B ♀♀ (need not be virgin) by dl-49, lz^s brothers or fathers (Selection needed to retain sc v lx f bb).
(2) Let "a" represent sex-linked gene or combination of genes. Then:
P_1 ClB, sc v/sc v lx f bb/Y ♀ X a/Y ♂
Eggs: ClB, sc v (1/2 carry Y) sc v lx f bb (1/2 carry Y)
ClB, sc v/sc v lx f bb (non-disjunction) Y (non-dis.)
Sperm: a Y
F_1 ClB sc v/a ♀ (1/2 carry Y) ClB, sc v/Y ♂ (1/2 YY) (dies)
sc v lx f bb/a ♀ (" " ") sc v lx f bb/Y ♂ (1/2 YY) (dies)
ClB, sc v/sc v lx f bb/a ♀ ClB, sc v/sc v lx f bb/Y
(3X ♀, dies or sterile) Y/Y (no X- dies)
a/Y ♂
To form stock of "a", breed B (not sc or v) ♀♀ (need not be virgin) by any brothers or fathers: ClB, sc v/a ♀ (1/2 carry Y) X a ♂.
If "a" has good viability and fertility when homozygous, this stock will automatically become a/a/(Y) ♀ X a/Y/(Y) ♂ after a few generations; otherwise, it will remain balanced and this will then be the more desirable condition to have it in. If presence of supernumerary Y in stock is undesirable, a stock of "Patroclinous" should be used in which the Y is marked by Cy owing to a translocation between Y and a chromosome 2 containing Cy. In this case only the non-Cy B F_1 ♀♀ are used for breeding. This stock is called "Curly Pat".

Muller, H.J. Balancing of Duplications by Deficiencies or Lethals and Vice versa -(Bridges, Muller)

Example: P, y w/sc^{jl} ljl ♀ X y/del-24/Y ♂ (del-24 is a deleted-X = duplication covering

locus of ljl, y and sc).
F_1 sc^{jl} ljl/del-24/Y ♂ X y ♀

Sperm: sc^{jl} $\underline{l}jl$/del-24 : sc^{jl} $\underline{l}jl$/Y : sc^{jl} $\underline{l}jl$: del-24 : Y

Eggs

	y	Y
F_2	y/sc^{jl} $\underline{l}jl$/del-24 ♀ (sterile)	Y/sc^{jl} $\underline{l}jl$/del-24 ♂ (fertile
	y/sc^{jl} $\underline{l}jl$/Y ♀ (sterile)	Y/sc^{jl} $\underline{l}jl$/Y ♂ (dies)
	y/sc^{jl} $\underline{l}jl$ ♀ (sterile)	Y/sc^{jl} $\underline{l}jl$ ♂ (dies)
	y/del-24/Y ♀ (fertile)	Y/del-24/Y ♂ (dies)
	y/del-24 ♀ (fertile)	Y/del-24 ♂ (dies)
	y/Y ♀ (fertile)	Y/Y (dies)

Fertile F_2 ♀♀ bred with fertile F_2 ♂♂ give same classes as before; hence stock is now self-perpetuating.

Muller, H.J. Detection of Mutations

1. Visibles arising in X of ♂: (Cross ♂ to y/Y ♀ and examine sons)

P y/Y ♀ X X/Y ♂ (donor)
F_1 y/X ♀ (3X, sterile) y/Y ♀ X/Y ♂ (examine) Y/Y (dies)

2. Lethals and visibles arising in X of ♂ (ClB method)

Key: B = Bar eye; C = inversion preventing crossing-over; n = new mutant, lethal, or visible; sc v f and car are recessive markers.

P_1 sc v f car/Cl B, sc v ♀ X X/Y ♂ (donor)
F_1 ClB, sc v/n? ♀ X sc v f car ♂ (breed in individual ♀ cultures)
F_2 sc ClB v/sc v f car ♀ (discard) ClB, sc v/Y ♂ (dies)
n?/sc v f car ♀ n?/Y ♂ (if lethal, di

Let "n" represent possible newly arisen mutant gene (lethal or visible). No crossing-over in F_1 ♀ because of inversion. If there is no "n" in given culture, ♂♂ will appear ♂. If there is a visible "n", all ♂♂ will manifest it. If there is a lethal "n", no ♂♂ will hatch (except occasional non-disjunctional sc v f car); this is determinable without etherization. If "n" proves to be present in a culture, make mass culture of F_2 n/sc v f car ♂♂ X brothers (visible), or, if "n" is lethal X sc v f car ♂♂; either way provides stock of "n" and count of F_3 ♂♂ shows locus of "n".

3. Visibles arising in X of ♀ are directly evident in sons.

4. Lethals arising in X of ♀

lz - lozenge-spectacled eye, sterile in homozygous ♀
dl-49 - inversion, preventing crossing-over to left of garnet.

P1 ♂/♂ ♀ (donor) X dl-49, lz^s B/Y ♂
Obtain virgin F_1 ♀♀ and breed in individual ♀ cultures to dl-49,B ♂♂ (cross by brothers would yield some ♂ F_2 ♂♂ by non-disjunction.) If $\underline{l}$ is present, no ♂♂ appear; determinable without etherization. (If $\underline{l}$ is to right of garnet, a few ♂♂ appear).

F_1 $\underline{l}$/dl-49, lz^s B ♀ X dl-49,lz^s B/Y ♂
F_2 $\underline{l}$/dl-49, lz^s B ♀ $\underline{l}$/Y ♂ (dies) dl-49, lz^s B/dl-49,lz^s B ♀ (lz^s B ♀ sterile) dl-49, lz^s B/Y ♂

If $\underline{l}$ is present, F_2 provides balanced stock of it unless to right of garnet.

5. Detection of mutations in autosomes same as method for getting homozygous stock. Method there given may be split for use with 2 alone or 3 alone.

Agol, I.J. New Method of Detecting Lethals in the X-chromosome.

Up to the present time Muller's method ClB has been used to detect lethals in the X-chromosome. At our laboratory we have adapted the method of y B sc^4 on the basis of the following considerations:

(1) y B sc^4 represents an inversion involving almost the whole length of the X-chromosome (from sc to bb) while the ClB inversion is far shorter (approximately from bi to Bx). Therefore y B sc^4 excludes the possibility of getting single cross-overs on both sides of the X-chromosomes.

(2) By the ClB method half of the chromosomes tested are lost, as only half of the progeny get the ClB chromosome in F_1; while by crossing the X-chromosomes tested (males) with homozygous y B sc^4 females nothing is lost.

(3) The y B sc^4 method facilitates the establishment of balanced cultures with lethals, because 1/y B sc^4 obtained in F_1 and inbred individually with their y B sc^4 brothers will give two categories of females: 1/y B sc^4 and y B sc^4/y B sc^4 which are easily distinguished one from the other, while crossing 1/ClB with any male gives two kinds of females, which it is impossible to differentiate phenotypically.

In case a new lethal has arisen in a cross with y B sc^4 we do not get maleless cultures in F_2, but all the males instead of being of two categories will be of one kind only, namely y B sc^4.

The method is used as follows. The chromosome tested (male) is crossed with a virgin homozygous female y B sc^4/y B sc^4. In F_1 all females are picked out and inbred individually with their brothers. If only one class of males is obtained in F_2, this indicates the presence of a lethal in the X-chromosome under investigation.

Muller, H.J. Accumulation of Mutations (Negativing of Natural Selection.

1. In chromosome 1 (possible in ♀♀ only) (C's B method). Key: Cs is inversion preventing crossing-over in right portion of 1 and containing gene making ♂♂ sterile. B = Bar eye. Dl-49 is inversion, preventing crossing-over to left of g in 1. lz^s = lozenge-spectacled eye, sterile in homozygous ♀. w = white eye.

P_1 Cs,B/ dl-49, w lz^s ♀ X dl-49, w lz^s♂♂ (many single-♀ cultures).

F_1 Cs,B/dl-49, w lz^s ♀ dl-49, w lz^s/dl-49, w lz^s ♀ (sterile)
Cs,B/Y ♂ (sterile) dl-49, w lz^s/Y ♂

Verify presence of B ♂♂ in each F_1 culture as preliminary test. Then breed F_1 ♀♀ in mass cultures X brothers, keeping those from different P_1 cultures separate.

F_2 offspring as before and so on to F_n (balanced ♂).

Final test: single ♀ from each culture^n of F_n is bred to her brothers. If lethal is present in Cs B chromosome, no B sons will appear in F_n / 1.

2. In chromosome 2 (using rotund as sterilizer)
rn - rotund wing; sterile in homozygous ♂ and ♀ (found by Glass)
sp - speck (a marker)
P_1 rn sp/Cy ♂ X S sp/Cy ♀ (in many single ♂ cultures)
F_1 rn sp/Cy (breed ♂ and ♀) (discard other types of F_1)
F_2 rn sp/Cy (breeds; balanced ♂) rn sp/rn sp (sterile
Cy/Cy (dies)
Make mass cultures of F_1, F_2, etc. to F_n corresponding to each P_1 culture. Therefore, $^1F_1 = ^2F_2 = F_3 = \ldots\ldots\ldots\ldots F_n$
Preliminary test: Examine F_2 for presence and normality of homozygous rn to insure against lethal or visible present at start of experiment.
Final test: Test single ♂ from each F_n culture, making same cross as of P_1 ♂; breed $F_n \neq 2$ in same way as F_1 and ascertain presence and normality of homozygous rn in $F_n \neq 3$ as in F_2.

3. In chromosome 3 (using inserted piece of 2 as temporary lethal). Key: Homozygous chromosome 3 carrying duplicating piece of 2 from T(2-3) Pale is lethal in hyperploid having normal 2/2 but non-lethal in fly having one normal and one Pale-deficient 2. 2^- = chromosome 2 deficient for fragment translocated to 3. 3^{+2} = chromosome 3 bearing fragment of 2.
Balanced Stock: Cy/2^-; CC,Dfd/3^{+2}
P_1 Cy/2^-; CC,Dfd/3^{+2} ♂ X CC,D/H ♀♀ in many individual ♂ cultures.
F_1 (a) $2^-/\neq$; CC,D/3^{+2} ♂♂ and ♀♀ (one mass "test culture" from each P_1)
(b) Cy/$\neq$; CC,D/3^{+2} ♂♂ and ♀♀ (one mass "stock cultures" from each P_1)
F_2 (a) Examine offspring of test cultures for presence of non-D flies ($2^-/\neq$; $3^{+2}/3^{+2}$) indicating that no lethal was present in 3^{+2} of P_1 ♂. If lethal was present discard sister stock culture. (Possible visibles in 3 are discovered simultaneously).
(b) Cy/$\neq$; CC,D/3^{+2} - Continue breeding from cultures whose sister test cultures showed no lethal (i.e. produced non-D flies).
F_n Same as F_2 (b)
Final test: Test single ♂ from each F_n "stock culture" crossing it by ♀ from stock Cy/2^-; CC,Dfd/3^{+2}
F_{n+1}: Cy/$\neq$; CC,D/3^{+2} (in single-♂ cultures) X Cy/2^-/CC,Dfd/3^{+2} ♀
F_{n+2}: (Select non-Cy, non-D, Dfd) $2^-/\neq$; CC,Dfd/3^{+2} ♂ and ♀
F_{n+3}: Examine for absence of non-Dfd ($2^-/\neq$; $3^{+2}/3^{+2}$) to discover if lethal is present. (Also examine to discover visibles).

4. In chromosome 2, with automatic selection of required offspring. Symbols: ab^2 = abrupt^2 (" parted"); homozygous ♂ sterile; mr = morula; homozygous ♀ sterile; "n" indicates possible newly arisen mutant; L^2 = dominant marker, Lobe eye.
P_1 ab^2 mr/L^2 ♂ X Cy/$\neq$ ♀ (many single-♂ cultures)
F_1 ab^2 mr/Cy ♂♂ and ♀♀ (Breed F_1 in mass cultures, corresponding to each P_1 male)

F_2 ab^2 mr/Cy (balanced ♂♂; examine each to see that ab^2 mr is present)

F_n Continue mass cultures of each non-lethal line to F_n

Test ab^2 mr chromosome of one ♂ from each F_n culture by fly from tester balanced stock ab^2 L^2 mr/Cy as follows:

$F_{n\neq1}$ Cy/ab^2 "n" mr ♂ X Cy/ab^2 L^2 mr ♀

$F_{n\neq2}$ ab^2 "n" mr/Cy (breed ♂♂ and ♀♀; balanced ♂ of "n"

$F_{n\neq3}$ Look for "ab^2 mr" appearing flies. If absent, "n" is lethal. If abnormal, "n" is a visible. If present and normal, no detectable mutation has occurred.

Muller, H.J. Accumulation of Mutations in Given Sex

1. In 1 of ♀: see "Cs,B" method above.

2. In 2 of ♂: by use of "Blond", a mutual translocation of 1 and 2, viable when homozygous and containing a dominant marker, the Blond bristles. (Found by Burkart with Stern). Key: 1 carrying right end of of 2 and deficient for left end of 1 = $Bld^{-1L/2R}$. 2 carrying left end of 1 and deficient for right end of 2 = $2^{-2R/1L}$. "all" = a series of 7 recessive markers scattered through 2.

P_1 $Bld^{-1L/2R}$; $2^{-2R/1L}$/Cy ♂ (many single ♂ cultures) X X ; Cy/"all" ♀

F_1 $Bld^{-1L/2R}$; $2^{-2R/1L}$/Cy(or "all") ♂ X X ; Cy/"all" ♀ (ballanced ♂). One mass culture and line from each P_1 ♂. (other combinations die or are highly infertile; if in latter crossing-over occurs, it is revealed by genes of "all").

Preliminary test for preexisting lethals and visibles in each P_1 ♂, and final test for new mutants is made as follows:

P_1 and $F_{n\neq1}$ $Bld^{-1L/2R}$; $2^{-2R/1L}$ 1(?)/Cy ♂ (single ♂ from each F_n line) X sc v 1x f bb/ClB, sc v/Y; S/Cy ♀ (virgin ♀♀ of "Curly Pat" stock)

F_1 and $F_{n\neq2}$ $Bld^{-1L/2R}$; $2^{-2R/1L}$ 1(?)/S (or Cy) ♂ (only ♂♂ which live) X $Bld^{-1L/2R}$/ClB, sc v (or sc v 1x f bb) ; $2^{-2R/1L}$ 1(?)/Cy ♀. (Choose non-sc, non-v, Cy orthoploid ♀♀, need not be virgin).

F_2 and $F_{n\neq2}$ Look for non-S, non-Cy ♀: $Bld^{-1L/2R}$/$Bld^{-1L/2R}$; $2^{-2R/1L}$ 1(?)/$2^{-2R/1L}$ 1(?) If absent, lethal is present; if they are abnormal (except Blond character) visible is present.

3. In 2 of ♂ and 2 of ♀ simultaneously; also in two 2's which alternate between ♂ and ♀. (By use of "abrupt2", sterile in homozygous ♂, of "morula", sterile in homozygous ♀, and of "rotund", sterile in both; crossing-over to be prevented by use of homozygous C3G, or inversions in "ab^2 pr mr" chromosome.)

P_1 rn mr/ab^2 pr mr ♂ X ab^2 rn/ab^2 pr mr ♀ (single ♂ and ♀ in many individual cultures)

F_1 (and F_{2-5} etc.) rn mr/ab^2 pr mr (♀ sterile; ♂ breeds) rn mr/ab^2rn (♂ and ♀ sterile) ab^2 pr mr/ab^2 pr mr (♂ and ♀ sterile) ab^2 pr mr/ab^2 rn (♂ sterile; ♀ breeds)

F_{2-4-6} etc. rn mr/ab^2 pr mr (only ♂ breeds) rn mr/ab^2 rn (steril
ab^2 pr mr/ab^2 pr mr (sterile) ab^2 pr mr/ab^2 rn
(only ♀ breeds)

Test for lethals and visibles in P_1 or F_n:

P_1 or F_{n+1} 1(?) rn mr/ab^2 1(?) pr mr ♂ X Cy,pr/S ♀
(Same method used for testing ab^2 pr mr/ab^2 rn ♀)

F_1 or F_{n+2}	(Mate Cy, non-pr together	(Mate Cy,pr together)
	1(?) rn mr/Cy,pr ♂ and	ab^2 1(?) pr mr/Cy,pr ♂ & ♀
F_2 or F_{n+3}	1(?) rn mr/1(?) rn mr	ab^2 1(?) pr mr/ab^2 1(?) pr mr
	(Look for presence and normality of rn mr flies in F_2 or F_{n+3}.	(Look for presence and normality of ab^2 pr mr flies in F_2 or F_{n+3}.

Stern, Curt Technique for obtaining large numbers of unfertilized females. Following a request by the editors of DIS a description is furnished of a genetic method published in 1929, Zeitschr. Abstgsl. 51: 315-316. A stock of the following constitution is maintained (Pasadena, Cold Spring Harbor, Rochester and other laboratories): g^2 B XY' Y"/y Y". The males thus possess the long arm of the Y-chromosome (Y') attached to the X-chromosome and a Y-fragment (Y") consisting of the short Y-arm plus part of the long arm. Y' carries the factor (or complex) K_1, Y" carries K_2, both of which have to be present to permit male fertility. The females have attached X-chromosomes and the Y" fragment. The stock keeps constant without selection.

1) In order to obtain unfertilized females with attached X-chromosomes virgin y Y" females of the stock are mated to males from any normal stock. The F_1 females will be yY and the F_1 males XY". If the P-individuals of such a culture have been removed before the hatching of the F_1, all males present will be XY" and sterile. All F_1 females, in spite of the presence of their brothers, will be unfertilized accordingly.

2) In order to obtain unfertilized females without attached X-chromosomes, XY'Y" males from the original stock are mated to virgin females from a normal stock (in order to exclude the accidental use of XXY females it is advisable to take short bristled females from a bobbed stock). The F_1 males being XY" will be sterile and the F_1 females (XY') will be unfertilized again.

The original stock should occasionally be tested for the occurrence of the very rare cross-overs in the XY' Y" males which lead to the reconstruction of a normal Y-chromosome. Test method: Mate in one bottle 1 female and 1 male from the stock and add females from a bobbed stock. Test the sons of the bobbed females for fertility. If sterile, continue the stock from the offspring of the test culture.

Observations on eggs, larvae, pupae and flies

Beadle, G.W. Collection of eggs. For the collection of eggs for measures of egg or larval-pupal mortality, small paper spoons containing food have commonly been used. They have the disadvantages of giving a food mass of unequal thickness and usually with a rounded surface. Detection of all the eggs is often difficult. Small nickel boxes made of sheet material about 0.3 mm. thick and of the dimensions 15 x 40 x 4 mm. with a strip 45 x 10 mm. soldered to the bottom so as to project about 30 mm. have been found to be very useful for egg counts and for collecting larvae of known ages. Standard cornmeal agar (containing animal charcoal, if desired, to increase the contrast) is pipetted into these boxes, filling them level full. They can be used in 20 x 100 mm. vials very conveniently. Examinations under a binocular can be made very rapidly. Experiments with different media with and without yeast indicate that yeast is a very important factor in stimulating rapid egg-laying. Standard food "painted" with a rather heavy suspension of yeast gives very satisfactory results. If it is necessary to have the eggs develop into adults, it is easy to slide the food mass out of the box on a cardboard strip 9 x 70 mm. It can then be transferred with eggs or larvae to a standard culture bottle containing food. With care, no eggs or larvae need be lost in the transfer. (Copied from DIS-4:64)

Marshak, A. Collection of eggs. Modification of Beadle's method for collecting eggs. Instead of using the metal containers for the agar-bone black medium, as suggested by Beadle, I have used strips of wood which can be easily cut and grooved with a buzz saw. The ends are then closed with plastic wood and the whole affair paraffined. The groove is filled with the agar medium till flush with the top and either seeded with yeast or moistened with acetic acid, and placed in a shell vial that is slightly longer than the wood strip. Egg counts can be rapidly and accurately made since almost the whole width of the groove is in the low power field of the binocular dissection scope. The strips may be used repeatedly by cleaning, autoclaving, and reparaffining.

Beadle, G.W. and Boris Ephrussi Collecting eggs and larvae. For collecting reasonably large numbers of eggs over known periods of time, 500 cc Berzelius beakers (without lip), stoppered with cotton enclosed in cheese-cloth, have been found convenient as containers for the adult flies. Eggs are collected on ordinary food heavily seeded with fresh yeast and held in boxes made of 26 gauge "Monel" metal. These are easily made from 2 by 3 inch pieces cut and folded to form a box 1-1/4 by 2-1/4 inches and 3/8 inches deep. A handle projecting 1 inch at one end is useful for handling these boxes with forceps. Young larvae can

conveniently be removed at desired intervals from the food surface and be transferred to appropriate containers. Straight-sided finger bowls containing about 130 cc of food and closed with cheese-cloth held on with a wide rubber band, can be satisfactorily used for growing up to 100 larvae. The food surface should be heavily seeded with fresh yeast.

Crew, F. A. E. Egg Counts. For counting daily egg output of single females a modification of the spoon technique is used. Small slices of cornmeal agar food cut from suitable blocks are placed on the spoons with a seeding of yeast. These have the advantages of being easily examined, readily detached and possessing a rough surface.

Mickey, George H. Collecting eggs. Our (Austin, Texas) routine method of getting eggs is to keep a single female and two or three males together for a few hours in a vial containing a spoon of food. About 4:30 or 5:00 P.M. the spoon is replaced with a fresh one in which the food has been poured and sprayed four or five hours earlier (in order to allow the yeast to start its growth) with a heavy suspension of fresh Fleishman's yeast containing a drop or two of glacial acetic acid. The food should be more fluid than that ordinarily used in food vials and is improved for the purpose of seeing the eggs by adding enough powdered lamp black to color it a dark gray. The flies lay better if they have not been etherized for at least 24 hours; they also lay more rapidly in the dark about 6 P.M. The vials should lie on their sides to give a horizontal laying surface.

The spoons are cardboard picnic spoons with the handles clipped off, the sides trimmed to fit the vials and the tips pointed in order to reduce the surface which may touch the bottom of the vial and crush the flies.

Schweitzer, Morton D. Collecting eggs. During the past year various techniques of collecting eggs have been tried. The following method has regularly yielded 100-600 eggs per culture per four hour period, with an average of 300. Not infrequently, on the first day of collection, the yield has been as high as 800-1300 in a four-hour egg-laying period. (D. melanogaster, pseudo-obscura, and to a small extent affinis and miranda)

The important precautions to be observed for optimum yield of eggs are:

(a) The females should not be etherized at any time prior to use for this purpose.
(b) The medium should be seeded with yeast at least 6 hours and not over 24 hours before use.
(c) The surface of the medium shoudl be slightly roughened just before being placed with the flies.

(d) The surface on which eggs are to be collected must be ventral to the flies.

The details of the procedure I have followed are as follows: Young flies, not over 24 hours old, are transferred to fresh food without etherization (20-40 ♀♀ and ♂♂). Two or three days later they are transferred to fresh food. At this time the medium on which the eggs are to be collected is prepared. It consists of ordinary cornmeal-molasses- agar with lampblack added to give contrast to the white eggs. The cornmeal is sifted before cooking. The food mixture is poured onto the ordinary type of paper milk bottle caps, leaving a margin of 1 cm. all around. When cool, the surface is uniformly seeded with fresh yeast. (Caps for 24 hours are prepared at one time.) The next morning the surface of the food on the caps is scraped with a metal tissue lifter. The flies are transferred to empty half-pint bottles which are capped with the prepared paper caps. The bottles stand with the caps down. New caps are substituted at appropriate intervals.

Eggs have been collected by this method continuously for a week or more at intervals of 2, 4, 6, 8, 12 hours. If the rate of oviposition falls off after a few days it may sometimes be renewed by transferring the flies to regular food bottles for 2-3 days. Strains that do not reach their optimum rate of egg-laying as early as the fourth day may be kept on regular food longer before beginning the experiment. (D. pseudo-obscura does well after 7-10 days from hatching, affinis and miranda even later.

If properly fitting caps are used (diam. = 1.625" for Bridges-type bottle, and 1.640" for most others), they may be washed and reused indefinitely. (Copied from DIS-4:65-66)

Schweitzer, Morton D. Handling eggs and larvae. When eggs are collected in the manner outlined above the usual high mortality due to handling and yeast overgrowth may be minimized by several precautions. After counting, the entire slab of food (or a segment containing an appropriate number of eggs) may be transferred to the surface of regular unyeasted food. If the surface of the food on the cap is sliced off with a scalpel just before use, the danger of yeast overgrowth is much reduced. An alternative method of transfer, that has given high percentages of imagines, is to allow the eggs to hatch on the food while it is still attached to the cap. The young larvae are transferred with a fine scalpel. In transferring larvae, an efficient method is to gently touch the scalpel to a larva, then touch the larva to a second one, etc. until 25-75 are adhering to each other. In this way the larvae are subject to a minimum of direct handling. (Copied from DIS-4: 66-67)

Hoover, Margaret E. Eggs for larval observations. In studies involving the embryological stages of Drosophila, the use of a synthetic medium may be found useful; especially if it is necessary to watch growing larvae day by day, a transparent food

becomes essential. We have found the following technique satisfactory for egg-larvae counts.

Eggs collected from spoons containing a level amount of cornmeal-agar food blackened by finely powdered charcoal and painted with yeast are placed in a row upon the surface of Pearl's synthetic medium in a watchglass. If each individual is to be accounted for throughout larval life, 10-12 eggs in each watchglass will probably be found a sufficient number. The eggs are easily transferred by needle to the watchglass without injury. Enough yeast is carried along in the transfer so that the addition of yeast is unnecessary. Each day every watchglass can be inspected as often as desired and each larva can be accounted for. We have most satisfactorily used a Greenough binocular with 9 X oculars, 2.3 X objectives, 150 Watt light placed horizontally to the mirror so that the reflected light passes up through the glass stage and through the transparent food giving intense illumination.

If it is necessary to study the individuals further, the pupae can be transferred from the watchglasses to regular food vials where pupation will occur normally and the adult flies can be collected in routine fashion.

Danner, Edwin C. Methods for obtaining Drosophila eggs for embryological study. In obtaining Drosophila eggs for fixation and sectioning or for study in vitro, the homopathic vials (3/4 X 3-1/2 inch) were found to give good results. (Shell vials of similar size may be better.) Approximately one half inch of banana agar medium is placed in each sterilized vial and allowed to solidify. Immediately upon solidifying, a strip of toweling (1 X 4 inch) with one end cut rounded to fit the vial is inserted so that the rounded end lies upon the medium and at right angles to the remainder of the strip which is pressed against the side of the vial. Moisture from the medium is absorbed by the toweling. Powdered yeast is then sprinkled upon the moist toweling and allowed to stand twelve to twenty-four hours.

In preliminary experiments, single pair matings resulted in good egg production. Virgin females approximately four and a half to five days old, when mated, usually laid eggs shortly after being fertilized. The eggs were laid on the growth of yeast on the strips of toweling. The parents may easily be shaken from the vial and the strip of toweling with the eggs upon it easily removed. Upon the removeal of the eggs, the strip may be reinserted and the parents returned to the vial for further egg laying.

In collectin the eggs for study, the moist toweling was made to adhere to a piece of cardboard or filing card to facilitate handling. With the aid of a binocular microscope, the eggs were easily removed with a needle and were free from medium.

To remove the opaque chorion, the eggs were placed in a drop of distilled water upon a glass slide. The excess water

was drained off and the slide placed under the direct light from a desk lamp with a fifty watt bulb to evaporate the remaining water and cause the eggs to adhere to the slide. This slight drying aids in the removal of the chorion with a sharp needle, the egg itself not shrinking from loss of moisture unless allowed to dry too long. For dissection purposes a #40 objective and #10 X oculars were used on a Spencer binocular microscope.

Altenburg, Edgar Eggs for ultra-violet treatment. Get eggs 3 to 3-1/2 hours after flies have been placed in bottle for egg laying. Keep them while laying in a dark place at about 26° C. and see that females are good layers, viz. young and fat, and that they are kept at about 25° C. for a day or two previous to laying. The flies should be removed from bottle in which they were placed for egg laying 1 hour and 10 minutes after they were put in the bottle. The eggs should be kept at 24° C. With a sharp razor blade cut a sharp edge on a blotter (preferably blue), the cut surface being at an angle of about 45° to the surface of the blotter. Moisten the blotter, and slide the eggs with the aid of a blunt needle to the edge of the blotter, so that only the polar cap projects beyond the cut edge. Treat so that rays strike polar cap only, rest of the egg being shielded by blotter. About thirty eggs can be arranged along the edge of one blotter and treated.

Gottschewski, G. Collecting eggs from weak stocks. Aus Stämmen, die im Hinblick auf Sterblichkeit und Legefreudigkeit durch Pärchenkultur über mehrere Generationen selektioniert sind, werden X 10 frischgeschlupfte Drosophilapärchen ausgesucht, die drei Tage in gewöhnlichen Kulturgläsern beisammen bleiben. Die 3-4 Tage alten Fliegen werden dann ohne Ätherbetäubung in einen einseitig geschlossenen Glaszylinder (12,5 x 6 cm) gebracht, den auf der unteren Seite ein Deckel einer Petrischale abschliesst. Auf dieser Schale ist Futter, das durch schwarzes Fliesspapier verdeckt ist. Das Fliesspapier wird mit 3% igem Eisessig und ausgequetschtem Futtersaft stark angefeuchtet. Die Schale wird jede Stunde durch eine neue ersetzt; das Gelege ausgezählt und nach ca. 19 Stunden beginnt die Hauptmasse der Larven zu schlüpfen. Die Daten gelten fur 25° C.. Durch einstündiges Ablesen der Larven erhalten wir 0-1 stündige Larvengelege. Bei guter Behandlung - beim Ablesen feuchter dunner Haarpinsel, gleichmässige Temperatur usw. - schlüpfen aus dem Eigelege 85-90% der Larven. Ich verwende gleichzeitig 6-8 Cylinder und erhalte durchschnittlich 2-300 gleichaltrige Larven, deren Variationsbreite bei dieser Methode auf das geringstmögliche Mass eingeengt ist.

Clancy, C. W. Two methods of ligating Drosophila larvae. Larvae of Drosophila may be ligated with fine, human hair at any desired segment by placing two surgical-dressing forceps on a table under a wide field binocular microscope in such a manner that the jaws support between them a previously prepared loop of hair. The loop, into which the etherized larva is placed by means of a small camel's hair brush moistened with saline solution, lies in the center of the binocular field dipping into a small drop of saline lying on a thick glass slide. If the larva has been properly etherized, i.e., quickly and sufficiently, it will relax and extend itself the moment the saline solution touches it. Orientation with respect to the segment at which ligature is desired is made with the brush or by moving the forceps holding the loop. The surface tension of the drop of water tends to hold the larva and aids in placing the loop.

In case the exact position of the ligature need not be determined at the time of tying, an even simpler and more rapid procedure may be carried out that eliminates both the binocular and the forceps. A heavy glass rod (1.0 cm. in diameter and 25.0 cm. long) is clamped to a ring stand by one end so that the opposite or free end extends horizontally toward the hands of the operator can rest comfortably on the table and at the same time hold the loop of hair at the same level as the rod. The larva, etherized, relaxed, and extended, is first placed in a small drop of saline on the upper surface of the rod. Surface tension of the water holds the larva and enables one to slip the loop around it and tighten the knot in the desired position.

In connection with a preliminary study of pupation, several hundred larvae have been tied by the above two methods. When tied shortly before pupation the larvae survive and go through preliminary pupation changes.

Ephrussi, Boris and G. W. Beadle A technic of transplantation for Drosophila anlagen. A technic of transplantation in Drosophila has been elaborated. By means of this technic imaginal discs of eyes, legs, wings and ovaries can be successfully implanted into larvae shortly before pupation.

The actual technic consists of the injection of the desired organ into the body cavity of a larva by means of a glass micro-pipette connected with the capillary tube and syringe of the standard Chambers' micro-manipulator. The pipette is made in drawing out with a micro-burner a glass capillary with an external diameter of about 0.7 mm. and a wall thickness of about 0.1 mm. to a finer capillary shaft of an external diameter from 0.1 to 0.16 mm. The bore of the shaft should be from 0.06 to 0.12 mm. The length of the shaft should be about 2-3 mm. At its base a constriction is made in the bore by heating with a horizontal micro-flame. The function of this

constriction is to block the tissue in the shaft, thus preventing its entering the larger part of the pipette, and also to act as resistance to the flow of liquid through the pipette. The pipette should have a very sharp point, which can be obtained by grinding on a fine-grained hone.

In our experiments the dissection of the donor-larva is made free-hand in a drop of 0.7% physiological salt solution. The host larvae are anesthetized on a glass slide in a simple glass vessel. After they are etherized and extended, they are moistened with a drip of Ringer, which tends to produce more nearly complete extension. They are then dried with filter paper and after a few minutes adhere to the slide.

The injection is made free-hand under a binocular microscope. Our equipment consists of two binoculars arranged so that they can be used from opposite sides of a narrow table. Two persons cooperate in the operation: one holding the larvae with a blunt curved metal needle, the other making the actual injection. (The complete description of the technic will appear in the American Naturalist.)

Beadle, G. W. Pigmentation of Malpighian tubes in larvae of D. melanogaster.

During the course of work on transplantation, it has been noticed that the Malpighian tubes of larvae of certain eye-color mutants are pale yellow or practically colorless, while those of wild-type larvae are distinctly yellow. Thus, ca, car, cm, g^2, lt, pp, rb, and w larvae appear to have very little or no pigment in the tubes. It is clear that these, and probably many more mutants, can be classified in the larval stage. To do this, it is not ordinarily necessary to dissect the larvae; the color can be seen through the body wall. The ability to identify particular mutant types in the larval stages obviously can be used to advantage in cytological studies, transplantation, etc.

Hoover, Margaret E. Some uses of Beadle's Malpighian tubes technique.

Although we have worked in a preliminary way for only a short time with Beadle's suggested technique utilizing the colors of the Malpighian tubes, it seems to offer interesting and important possibilities. The difference between yellow and white tubes can be easily seen in three-day old larvae and with practice the distinction can be made in younger ones. Careful examination with good strong lighting is necessary but the difference can be seen with accuracy. Such a technique can be put to good use in cases where an X-chromosome carrying a deficiency is balanced against dl-49 carrying garnet eye color. If ♂ carrying the deficiency die during larval life, the exact extent of survival can be accurately determined by isolating and observing the ♂ larvae with yellow Malpighian tubes. Moreover, by first observing the Malpighian tubes of the ♀ larvae used in making salivary gland chromosome preparations of such deficiencies, the use of ♂♀ not carrying the deficiency can be avoided.

Whittinghill, Maurice An aid in arranging flies for separating or counting them. To facilitate sorting and counting flies it is advantageous to have them arranged approximately in a line, which may be easily formed as follows. The flies are emptied from the etherizing bottle upon a card which has along its middle a flexible fold by which the card may be bent to form a trough. A few strokes of the brush distribute the pile of flies evenly along this groove. The card is now held flat with one hand while the other goes down the line casting each fly quickly to one side or the other with a brush handle or needle, thus sorting into two groups. If further classification is necessary, these two groups may be swept to opposite ends of the card, or to different cards, and each group easily arranged for the next sorting. After such a sorting each of the groups is found to lie in approximately linear order, which makes counting simple. Finally the card may be bent again to make a chute for dispensing the flies accurately into the morgue.

Stern, Curt Technique for the study of certain genetic constitutions in hypodermis spots. One tool in studies on gene action is the production of mosaic spots. Their appearance is based primarily on the occurrence of somatic segregation. A heterozygous cell Aa segregates into two cells AA and aa. If aa represents the genetic constitution to be tested and if the cell aa is viable and divides, a spot will appear. Often the genetic constitution to be tested in spots leads to inviability of the segregate thus not resulting in a visible aberrant area. The frequency of somatic segregation is rather variable so that conclusions as to inviability of certain constitutions can be drawn with reservation only, considering the possibility of absence of segregation. However, the following method furnishes reliable controls. Let a be the gene to be tested and b and c genes in the same linkage group effecting hypodermal characteristics in small spots. By mating flies of the constitution abC/ABc are produced. Somatic segregation will lead to abC/abC and ABc/ABc cells. The latter, known to be viable, will be able to produce a spot; the former, if viable will appear in direct contact with the ABc/ABc spot as an abC/abC twin spot. If aa leads to inviability no twin spot will be formed. In case of sex-linked genes yellow (y) may be used for b and singed^3 (sn^3) for c, so that either only single sn^3 spots or y next to sn^3 twin spots will be found (both y and sn^3 being recognizable as characteristics of even-single setae). Somatic segregation is caused by four strand crossing-over. If it occurs to the right of all loci studied, the foregoing holds true completely except for developmental reasons which may make the mosaic areas so small as not to cover at least one seta per single spot. Somatic crossing-over between the genes studied will not lead to twin spots. However, the frequency of somatic crossing-over at the spindle fibre region is high enough to produce a sufficient number of potential twin

spots.

Frequency of spots: Variable in different experiments from one spot on fractions of a percent to one spot on ten and more percent of all individuals. The frequency is increased considerably if the individuals carry an autosomal Minute (use stocks like Rochester #68, DIS-5).

Size of spots: From one seta to whole imaginal disk, very rarely larger.

Location of spots: Variable. The smaller spots, which in most experiments are the frequent ones, occur preferably on the abdominal tergites. Careful inspection under about 30x magnification is necessary for detection. (See note in DIS-5 on "foot-focusing device").

Harnly, Morris H. Wing measurements. The following method has been found satisfactory for making wing measurements: The Spencer Drawing Apparatus No. 345 MS (list price $62.00) on which a compound microscope can be mounted is used to project the wings. A 16 mm. objective and 10 X ocular are used. The size of the projected wing is determined by the distance of the microscope above the drawing board. When first setting up the apparatus it is advisable to project a wing and determine a height that will place the entire wild-type wing on the drawing paper. Ordinary 8 x 11 paper can be used for the drawings.

Having established the proper height of the microscope above the drawing board, a ruled 2 mm. slide is placed on the microscope stage and projected. This distance of 2 mm. can be marked off on a straight line on a permanent record sheet. Thereafter, whenever the apparatus is set up exactly the same magnification can be obtained by a proper adjustment of the height of the microscope above the drawing board (a slight movement of the draw tube may aid in this) using the 2 mm. slide and the record sheet as checks. The wing is removed from the fly with a McClure's angular-or-flat Iridectomy Scissors #c991 figure 2 (list price $9.00, Standard Scientific Supply Co.), mounted in 95% alcohol, projected and drawn. The length can be determined directly by projecting the 2 mm. ruled slide onto the drawings. An area equivalent to 4 sq. mm. can be obtained at the same magnification by projecting the ruled 2 mm. slide and measuring the square drawn with a Keuffel and Esser Compensating Polar Planimeter No. 4242. This will give by division the value in sq. mm. of one unit on the vernier. Measurements with the planimeter of the area of the wing drawings can then be converted into sq. mm.

Timofeeff-Ressovsky, N.W. and K.G. Zimmer. On the technique of radiation-genetic experiments. From both the genetic and physical points of view we want to lay stress on the following rules, the observation of which will be of great help for comparing and analysing the results of radiation-genetic experi-

ments in Drosophila species obtained by different authors.

1. The biological genetic conditions and methods of the experiment must be taken into consideration, and, if specifically deviating from the generally known and used standard (in respect to age and stage of irradiated flies, developmental stage of germ-cells at the time of treatment, methods and accuracy of detection of mutations, etc.), should be mentioned.

2. In experiments upon mutation rates of single genes uniform and selected material should be used.

3. When using X-rays, ionometric measurements of the tube output and its steadiness ought to be carried out before irradiating the flies. The conditions of measurement and irradiation must be exactly the same in respect to tube current, voltage, presence or absence of scattering substances in the neighborhood, and distance.

4. It is preferable to use the international r-units of X-radiation instead of other units not generally adopted.

5. It is necessary to state, besides the dosage applied, also: a) the time and intensity of irradiation (i.e. whole time of exposure, r/min, and whether fractioned or not), b) kilovoltage, c) filtration, and, if possible, d) halve-value-layer of the X-ray beam. For very soft X-rays (so-called Grenzrays) cellophane is the best material for halve-value-layer determinations; for all other qualities of X- and gamma radiations copper ought to be used rather than aluminium.

6. For soft radiations absorption within the flies becomes very important, and proper allowance for dosage reduction in deeper layers of the flies has to be made; this point, which can easily introduce errors as great as 100 per cent, has frequently been overlooked in the past. Thus, in the case of soft rays, beta- and cathode- radiations, the absorption in fly-tissues must be measured, and the mean depth of gonads during irradiation must be determined.

7. When planning experiments under special conditions (e.g. with extra soft or extra hard radiation, very high or very low intensities, in combination with other physical or chemical factors or stimuli, etc.) advice of a competent physicist, preferably one who is interested in such problems, ought to be asked for and followed.

8. Experiments with gamma-rays from radium require considerable amounts of radium or Radon. Such work is, therefore, rather dangerous if special measures of protection are not arranged and followed. During the last few years it has been shown that gamma-rays can be measured in r-units ionometrically. It is of course desirable to use these units in the future. If exact determination of the dosage output in r-units of the radium applicator used cannot be carried out, the following must be stated: a) the number of mgrs. of radium element, b) filtration (thickness and material), c) time of exposure. and d) distance. The radium (in needles, tubes, or other form) must be so arranged in the applicator as to give a large enough homogeneous field of gamma-rays. All details of the experimental set up and of the radium applicator (preferably with

diagrams giving all lengths, distances, etc. in mm's) have to be described.

9. During irradiation the flies ought to be kept in a container of light-atomic material, such as cellophane, gelatine, or bakelite, which has a wall-thickness equivalent to that of the ionisation chamber of the dosimeter.

Dobzhansky, Th. Collecting, transporting, and shipping wild species of Drosophila.

Usual Drosophila culture bottles can serve as traps. A sturdy string is tied around the bottle neck to facilitate its hanging in a convenient position on branches of trees, bushes etc. A layer of bait a few centimetens thick is placed on the bottom of the bottle, and covered with a piece of a paper towel or filter paper to absorb excess moisture. Fermenting banana mush is most satisfactory as bait. Ripe bananas are mashed with the aid of a spoon or a fork; some drops of fresh yeast solution is added, and the bottle is left standing for about 24 hours before use (if dry yeast is used this time is considerably lengthened). The bait remains good for at least four or five days after first used. The traps are exposed in such a way as to be readily accessible, and left undisturbed for a few hours; no useful purpose whatever is accomplished by leaving them exposed for days. In the case of some species, notably pseudoobscura and its relatives, it is important to expose the traps in the late afternoon, since these flies do not come into traps on hot days before sunset. On cloudy days they may be caught any time. The above bait attracts quite a number of species of Drosophila, including some, though probably not all, feeding on fungi. When a sufficient number of flies are in a trap, it is closed by the usual cotton stopper, and transported to camp (if properly packed such bottles can be transported for miles even on pack saddles without harm to the flies). For further transportation and shipping the flies should be placed in shell vials containing solid banana-agar food. Banana agar is prepared in the laboratory, poured into the vials, sterilized in an autoclave under pressure, and cooled in a slanting position. The openings of the vials are stoppered with sterilized cotton and wrapped in wax paper to prevent excess evaporation. Vials so prepared can be then carried for a month before use. Before the flies are put in them, a drop of yeast suspension is placed on the food, and a piece of filter paper inserted. Then the flies are transferred from the traps to the vials (a glass funnel of a proper size facilitates this operation greatly). Some wild species are exceedingly sensitive to heat. If they have to be carried around for any length of time in hot weather, vials with the flies are placed between layers of cotton in a metallic box, and this box is placed into a larger one, the space between the two being filled with cracked ice; changing the ice in this improvised ice box once a day is sufficient in any summer heat. If no ice is obtainable, the box with the flies should be wrapped in a moist towel and exposed to the wind. It

is desirable to ship the material collected to the laboratory as soon as possible. During the summer heat shipment via air mail has to be resorted to despite the expense involved. For this purpose the vials with flies are placed in cardboard boxes with cotton and wrapped like any other parcel. In some states the importation of living insects is prohibited, so that special permits may be necessary.

Cytological technique

Painter, T.S. Aceto-carmine technique for salivary chromosomes. The writer has worked almost exclusively with temporary aceto-carmine mounts and the commments given below apply only to that type of preparation.

Much difficulty has been experienced in getting a good iron aceto-carmine stain. Powdered carmines, from many different sources, have been tried including the certified product of Coleman and Bell, Grübler's Karmin Rubrum Opticum, and Carmine 40 from several sources. The trouble has been either that the stain would not take or was not selective enough or else it would not keep, at ordinary room temperature here in Texas for more than a few days without going bad. At present we are using some uncertified carmine manufactured by Coleman and Bell and are obtaining a satisfactory stain in the following way. In one flask an excess of carmine is simmered, under a reflex condenser, in 40 per cent acetic acid, for an hour or two, when it is cooled and filtered. In another flask carmine is boiled in 60 per cent acetic acid similarly, and after cooling is filtered. A trace of ferric acetate is added to both stock solutions. From time to time, as needed, the two stains are mixed in equal proportions. We are unable to explain why this procedure gives a good stain but it works.

The salivary glands are dissected out in Ringer's solution (cold-blooded or frog formula) and transferred to a clean slide with a pipette. The Ringer's is quickly removed with a pipette and iron-aceto-carmine is flowed over the glands from one side. After a few moments the first stain is removed and fresh stain is added in considerable excess. The slide is put to one side and allowed to stand until a little of the carmine begins to precipitate around the edge, a matter of 15 to 25 minutes depending on atmospheric conditions. A cover slip is now placed over the glands, and the excess stain removed with a pipette and filter paper. Being careful not to let the coverslip move, the preparation is next blotted with a good deal of pressure, a process which usually frees the individual nuclei from the surrounding cytoplasm. Under a dissecting binocular, the individual nuclei are crushed with a blunt needle, by the pressure applied locally to the cover slip, and then after blotting the slide once more to remove all traces of the stain the coverslip is sealed with vaseline or melted paraffin.

The type of light filter used for the examination of preparations is the BG 7 optical glass filter put out by Zeiss.

Marshak, A. A rapid method for making permanent mounts of Drosophila salivary gland chromosomes. (1) A saturated solution of aceto-carmine is prepared by boiling carmine in a 45 per cent aqueous solution of glacial acetic acid for several hours. A reflux condenser is attached to the flask containing the solution in order to prevent changes in concentration by evaporation. A clear dark red solution is ob-

tained, either by sedimentation or filtration. No iron is added at any time.

(2) The glands are dissected out and stained in deep depression slides for not longer than fifteen to twenty minutes. If left for a longer period the chromosomes become fragile and cannot be well stretched.

(3) The glands are mounted on a microscope slide and washed with fresh aceto-carmine to remove any debris that may be present. They are then covered with a square coverslip and the excess fluid taken up with blotting paper.

(4) The chromosomes are then spread by pressing on the coverslip with a dissecting needly. The coverslip must not be allowed to slide or the nuclei will be rolled into dense useless masses. This is easily prevented by pressing firmly on one corner of the coverslip with the finger. This step is executed under a dissecting microscope so that each nucleus may be observed and adequately spread.

(5) A saturated solution of carmine in glycerine is then put around the coverslip and a piece of absorbent paper placed against one edge of the coverslip. The slide is then put away until the glycerine has displaced all the aceto-carmine under the coverslip, usually over night. The glycero-carmine solution is prepared by dissolving the carmine in warm glycerine and then filtering. If ordinary glycerine is used in this step, the preparation will fade after a few days.

(6) The slide is immersed in alcohol to remove the excess glycerine and then blotted. It is then sealed with balsam, gum mastic-paraffin or any other suitable seal.

By this technique it has been possible to mount three hundred glands in two days and have them all preserved in excellent condition for observation. After one month there has been no noticeable change in the chromosomes. Furthermore, it is possible by this technique to stretch the chromosomes a great deal without breaking them.

If it is desired to mount the glands in balsam, the slides may be allowed to stand with the glycerine for a day or two longer. The coverslip can then be readily slipped off, or pried off with a fine needle, or floated off in absolute alcohol. It is then cleared in clove oil and xylol and mounted in balsam. The loss of material by this technique is much less than with the method of removing the coverslip in aceto-carmine.

Bridges, Calvin B. Current method for permanent aceto-carmine smears. Trial of various modifications of the methods for making temporary and then permanent preparations of smeared cells from salivary glands and other tissues have been carried out by various workers here. The method is so uniformly reliable and yields permanents of such high quality that it is no longer customary to carry out even preliminary examinations on temporary mounts. Permanents are essential for continual rechecking of the banding in each case as new information or material raises questions.

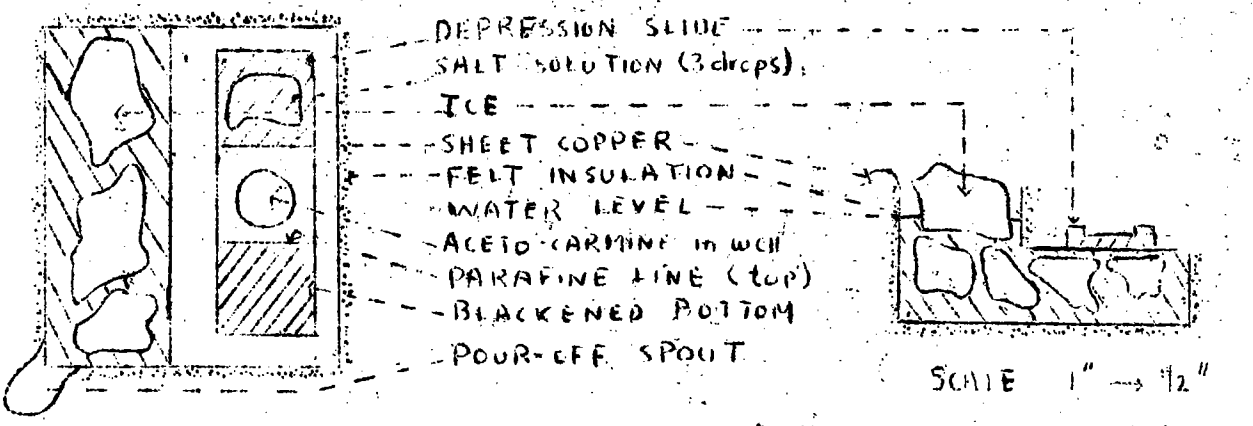
A prime condition of success is that the larvae be uniformly and thoroughly overfed with yeast and raised at a low temperature (see notes by J. Schultz). Fully adult but still unpupated larvae should be used, since degeneration of the cells starts immediately with pupation. Female larvae should be selected and males discarded, since in males the chromosomes, especially the X, are paler in stain and less turgid than in females. Best are females with one or two extra Y-chromosomes, where the extra "inert" material leads to plump easily-stained chromosomes. Attached-XY females are used as the source of an extra Y whenever it is otherwise desirable to make an outcross of a balanced autosomal stock (to get heterozygotes freed from the balancer). The giant attached-X females, formerly recommended, are no longer used in preference to other attached-X females, since the extra large chromosomes found in giant females are too resistant and elastic to stretch well in the smearing. Female larvae can be distinguished from males by their tiny colorless transparent spherical ovaries attached to the yellowish-opaque lateral fat-bodies between the third and fourth branches of the trachae, counting from the rear. Male larvae have large ellipsoidal testes each embedded in the fat body.

Dissection and fixation of the salivary glands at near-zero temperatures seems to give better detail and less capsulation of the banding (see notes by Schultz). For piercing the larvae and other materials, a large low-sided basin or tray is filled with cracked ice. In the ice are bedded salt cellars (black) for prechilling the larvae, bottles of physiological salt solution and of concentrated aceto-carmine and special depression slides for the dissection and fixing. The depressing slide (see figure below) is 1" x 3" x 1/4", with a 5/8" circular well, with vertical walls, 1/8" deep. The upper surface of the slide is finely ground. The end regions are marked off for dissecting spaces by lines drawn in melted paraffin to keep the salt solution from running into the well of the slide. The bottom of the slide, under these end regions, not under the well, is painted black for contrast to the larvae.

The larvae are put in the dry depressions of the chilled saltcellars for about five minutes before dissection. Because of the chilling they stop crawling about and are much easier to handle and dissect. For keeping the larvae and the liquids chilled during dissection and the first stages of fixing, a special insulated iced platform (see figure below) can be made of soldered sheet copper with felt covering, of a size to fit conveniently on the stage of a dissecting microscope.

The interior of the dissecting platform is filled with ice at the time the tray is filled. The platform is put on the dissecting microscope stage so that the light comes from the far side along the ledge. The well is filled nearly level full with the chilled aceto-carmine. (Aceto-carmine can be made by boiling for an hour 45% acetic acid with an excess of carmine powder (Coleman and Bell is good) in a flask with reflux condenser, and

decanting for use the clear liquid after cooling and thorough settling). On the ground glass surface at the far end of the slide are put three drops of salt solution for the dissecting bath.



In dissecting (at 15 diameters magnification) cut off anterior end of larva, as close behind mouth hooks as possible, by pressure with side of needle tip against non-slip ground glass surface, while holding larva with another needle. From cut-open end emerge first the pair of salivary glands and then other organs. The glands (transparent club-shaped bodies attached to each other by the short branched duct which led to the base of the mouth hooks) are cleared from the fat bodies and picked up together on the tip of a needle and immersed under the surface of the aceto-carmine in the well.

In case the glands break apart they can be made to stick together on the needle tip, turning this upside down and then immersing under the aceto-carmine for 30 seconds before scraping them off. Up to about 16 pairs of glands may be fixed together in one well. Avoid excessive iron (from the needles) in the early stages of fixation. After the required number are in the well, the slide may be set aside at room temperature or higher for continuing the fixation at a faster rate. Short fixations before the smearing are liable to result in pale chromosomes and distortion and tearing through adhesions of the still soft material of the strands. Fixation from 1 to 2 hours seems best. Longer than about 4 hours may lead to fragmentation of the chromosomes upon smearing. With long fixation refill the well as the acetic acid evaporates.

To make mounts, put albuminized slide (see notes of Baur) on white plate on stage of dissecting scope. Put 3 drops concentrated aceto-carmine on slide and spread evenly over albumin surface. Four pairs of glands may be put in a row on the slide and covered with a 22 x 40 mm. cover. Slides should not be over 1.20 mm. thick and covers not over .16 mm. Avoid bubles in lowering cover in place! The albuminized slides must have been protected against dust and lint while drying and during storage and the covers carefully cleaned, otherwise any particle would

prevent the cells being crushed properly.

Place slide, with cover glass well centered, on lower half of folded paper towel and drop upper half over the cover. Gentle pressure on top half, without side slip, removes excess of aceto-carmine, leaving only faint tinge of pink on slide and the glands partly spread and crushed. Complete the spreading and crushing, with slide on white plate on stage, by vigorous sweeps with side of needle tip, held nearly parallel to surface, holding corners of cover with fingers to avoid all side slippage. If too much fluid has been left in slide the spread chromosomes will later change their place rather than stick where they are spread. The thin film of albumin makes a soft bed which saves the chromosomes from being crushed, as would easily occur between bare slide and cover glass, and causes practically 100% adherence to the slide.

The fresh preparations are now "ripened" by storage in saturated 95% alcohol vapor over night. A large glass vessel 6" deep is lined on bottom and sides with several layers of filter or towel paper, 95% alcohol poured in until the paper is melted and a little excess liquid is left over on the bottom. The slides are stood on end around the sides and the whole covered tightly. This vapor method of replacement of aceto-carmine (or other liquid under a cover glass) by alcohol gives a slow and perfect dehydration with a minimum of shrinkage. Vapor condenses on the upper end of the slide and the displaced liquid seeps off at the bottom.

After ripening and dehydration (the material also sticks to slide better after ripening in alcohol vapor) the slides are immersed in 95% alcohol, where they may be left indefinitely. The covers may detach spontaneously; but they can be easily removed by placing the side in a shallow Petrie dish (J.C. Li), covering with 95% alcohol and holding 2 rear corners of cover (against slippage) with fingers, while a flat thin point of bent needle is slipped like a wedge under opposite edge. The slide is left in the alcohol while the cover is cleaned of traces of tissue. The slide is drained briefly, laid on its back and 2 drips of Euparal (thinned slightly with thinner or with 100% alcohol) dropped on the glands. The cover is put on swiftly (to avoid drying and also condensation of moisture) and air bubbles avoided. The excess of thin Euparal is squeezed out by folded paper which absorbs it as it emerges (avoid slippage while pressing). After drying of slide, clean off excess of Euparal with cloth barely moistened in 95% alcohol. Slides continue to clear and improve, and are fully equal to balsam slides, without the time and expense and shrinkage involved in treatments with absolute and Xylol.

Schultz, J. Notes on methods for salivary chromosomes. Larvae used for salivary gland preparations should optimally be from single females which are transferred to fresh 1/2 pint culture-bottles daily. Several such transferred pairs provide the required numbers of uniformly well-developed larvae. The larvae should be

raised in a cool place, preferably a 19° cold room, and the culture medium should be enriched with yeast (sterilized) or extra live yeast (1/8-1/16 cake of Fleishmans yeast) sprinkled on the food surface when the larvae are half grown. Absorbent paper should be provided for the adult larvae to emerge upon, and the easiest way to collect larvae is to pull out this paper and pick off the fully grown individuals.

Glands dissected at practically zero temperature and with the fixation started at that temperature seem to give sharper detail and less "capsulation" of the banding than material dissected and fixed at room temperature. The Ringers solution (or 0.73% Na Cl solution) and the aceto-carmine should first be chilled to near zero by standing on ice. The larvae also should have an ice treatment of five or more minutes before dissection. The dissection and first stages of fixation should be carried out on a depression slide held at low temperature (for details of procedure see notes by Bridges). After the fixation has proceeded for five or more minutes at the low temperature, the remainder of the fixation can be carried out at either room temperature or higher. With higher temperatures less time is needed for the fixation and staining to reach a suitable stage.

Mounting in Euparal, directly from 95% alcohol, has been found perfectly satisfactory if done rapidly in a dry atmosphere.

Bauer, Hans Notes on permanent preparations of salivary gland chromosomes.

Perfect attachment of smeared cells and chromosomes to the mounting slide can be obtained by use of a film of albumen on the slide. The albumen solution is made by mixing together 100 cc distilled water, 25 gm powdered egg albumen (Merck) and 0.5 gm thymol. After the mixture has stood several days and the undissolved albumen has settled, the top clear portion is decanted for use. A drop of albumen solution is spread evenly and thinly over the whole slide by scraping with the end of a second slide whose edge should be unchipped. The albumen film must be thoroughly dry before use! For use, the dried albuminized slide is put on a level place and three drops of aceto-carmine spread evely over its entire surface. The stained glands are placed in this fluid film, covered with a cover glass (air bubbles must be avoided) and crushed by pressure.

The gradual replacement of the aceto-carmine by 95% alcohol can be made by the vapor method of Bridges (see above) or by putting the slide in a staining jar filled with alcohol only high enough to cover 1 or 2 mm of the lower edge of the cover glass. After half an hour the slides are ready to be transferred to another jar filled completely with 95% alcohol. Here the cover slip usually detaches itself after a short time; otherwise it can be removed by needles. Mounting is done according to the methods described above by Bridges.

In cases of too strong stainability of the cytoplasm by aceto-carmine, the Feulgen method is advisable (Feulgen, R.,

1926, Handbuch der biol. Arbeitsmethoden 5: 2,2 Hälfte. - Bauer, H., 1932, Zeits.f.Zellf.u.mikr.Anat. 15. - The receipts are to found in the last edition of Lee's Vademecum.) Slides are prepared by the aceto-carmine method to the stage of detached cover glasses; or slides already mounted in euparal can be soaked in 95% alcohol till the cover glasses detach. The slides are then transferred through 70% alcohol and distilled water into chrom-formol for postfixation (3 parts of 1% chromic acid and 1 part of formalin); duration 10-20 hours (overnight). Thereafter the slides are washed for 10 minutes in running water and, after transfer through distilled water and cold normal H Cl solution, hydrolyzed for 30-40 minutes in normal H Cl warmed to 60° C. on a water bath. After hydrolysis they are transferred through cold normal H Cl and distilled water into the fuchsin-sulphurous acid for 1-3 hours. After the staining the slides are washed in a large jar with tap water, moving them backward and forward several times (washing in SO_2-water is not necessary); the side of the slide carrying the objects should always be turned in the direction of the movement. Then they are transferred through distilled water and 70% alcohol into 95% alcohol. There they should remain until the red color in the albumen layer, caused by the decomposition of the fuchsin-sulphurous acid, is completely removed. Then the slides are mounted in euparal, or, through absolute alcohol and xylol, in Canada balsam. Only the chromatic structures (containing thymus nucleic acid) are stained; the staining is not quite as intense as that by aceto-carmine and does not seem any sharper or less diffuse in the demonstration of the banding. During the postfixation the chromosomes, due to shrinkage, become thinner.

These methods are applicable also to ganglia, ovaries and testes.

Lawrence, Elizabeth Gay (Carnegie Institution of Washington, Baltimore, Md.) Note on the use of dioxan in making permanent aceto-carmine preparations.

At the suggestion of Mr. C. H. Miller of this laboratory, dioxan has been tested as a medium to replace alcohol and xylol in making permanent smears. Only preliminary tests have been made so no detailed method has yet been worked out. The general procedure, however, seems simple and the preliminary results very satisfactory.

Cover slips are soaked off in dioxan, then the material is rinsed in dioxan for a few seconds and mounted directly in balsam. Dioxan is relatively inexpensive. It may be procured from the Carbide & Carbon Chemicals Corporation, Carbide & Carbon Building, 30 E. 42nd Street, New York City, and presumably from Grübler in Germany. Its use has been described in the following papers:

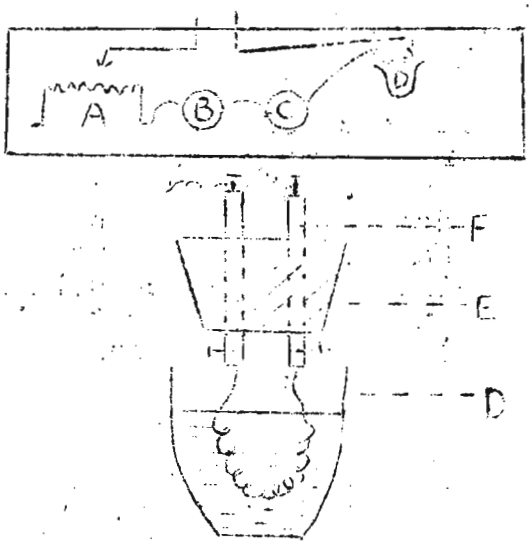
"The Use of Dioxan in the Embedding of Microscopic Objects," by Heinz Graupner and Arnold Weissberger, Zool. Instit. & Chem. Lab. of the Univ. of Leipzig; Zool. Anzeiger, Bd. 96:204-206,1931.

"The Use of Solutions in Dioxan as Fixatives for Frozen Sections", by Heinz Graupner and Arnold Weissberger; Zool.Anzeiger, Bd. 102:39-44, 4 abb., 1933.

Kaufmann, B. P. Technique for spreading salivary chromosomes. (1) In making aceto-carmine smears of ganglia and salivary glands we have secured more uniform distribution of cells and less breakage by flattening with a weighted roller rather than with a needle or similar instrument, where the results depend on the pressure exerted by the technician. For this purpose we use pieces of glass tubing, filled with about 150-200 grams of mercury, and securely corked. A horseshoe-shaped wire with the ends inserted in the corks may be used as a handle in drawing the roller across the cover.

Griffen, A. B. Sealing slides with paraffin. In the preparation of salivary chromosome slides for rapid checking of breaks and translocations, it is seldom desirable to spend long hours in making permanent mounts of all the preparations; it is far more practicable to make well-sealed temporary slides which may be made into permanent records as the worker sees fit. Paraffin, applied at smoking heat with a small brush, forms a neat, rigid and easily removable seal for such slides; after the initial use of the material the longitudinal paraffin strips may be flicked away easily from the cover-slip and the slide treated for permanency by Bridges' technique. For providing paraffin the apparatus described below is very handy.

A small 15 cc crucible is used as the melting pot; into this vessel, filled with paraffin, is suspended a crescent spiral of Chromel-A-22 resistance wire with a 22.5 ohm rheostat, a safety fuse, and a toggle switch in the circuit. After the initial adjustment of the rheostat a flip of the switch instantly produces smoking paraffin.



A - rheostat
B - fuse
C - switch
D - crucible
E - two-hole rubber stopper
F - attachment post

Bridges, Calvin B. The Examination of salivary chromosomes. The detail present in salivary chromosomes extends in fineness beyond the resolving power and definition of the best microscopes. Aside from the use of very expensive and cumbersome ultra-

violet apparatus with photography, the greatest amount of this detail can be seen by the following procedure, according to my experience. For objective and ocular I prefer 90 X apochromatic 1.40 N.A. with 10 X compensating ocular. Second choice is 120 X apochromatic objective with 10 X or 7 X ocular, and third choice is 90 X 1.30 N.A. with 10 X ocular. Oculars higher than 10 X seem inferior in results, though 12.5 X offers possibilities. For substage condenser 1.40 N.A. achromatic is best, and any achromatic is better than any aplanatic or other type of condenser. It is as imperative that the condenser be used oil-immersed and focused as it is for the objective to be so used. A front surfaced aluminized mirror (vapor deposited) is definitely superior to other mirrors and is more flexible in its setting angles than is the total-reflecting prism, which is otherwise the best. For examinations a binocular body with inclined ocular tubes is to be preferred. For camera-lucida drawings a monocular straight tube should be substituted.

Too much attention cannot be paid to the illumination of the chromosomes. For critical examinations one can employ a Bausch & Lomb thirty-nine Dollar lamp (nearly as good as the "research" lamp). The light is from a 6-volt, 18-ampere ribbon-filament tungsten incandescent lamp, of projection type, with bayonet (not screw) base and brazed (not soldered) center contact. This lamp is run on alternated current transformed from 110 to 6 volts. Intensity of the light is controlled by a variable resistance, of not less than 175 ohms and not less than 1.5 amperes capacity, in the primary 110-volt circuit to the transformer. Use of this resistance is imperative for exact adjustment of light under all conditions of filters, illumination and eye-fatigue. Also it increases the life of the lamp many times its rated life.

The light from the ribbon filament is brought to a sharp focus in air as near as practical to the front of the condensing lens. This is accomplished by turning the loop of the filament toward the back of the housing and moving the lamp mounting back in the housing and racking the condenser to its limit forward. After the filament is carefully aligned with the center of the condenser, an iris diaphragm is mounted in the plane of the image of the filament. The mounting support may be made as a right-angles wooden trough extending forward from the cylindrical lens casing below, and wired tightly to it. The iris is mounted to a smaller trough which slides in the support trough. To find the exact setting for the iris, put on the stage (condenser oiled) a slide and focus on it with the oil immersion objective. Next place the lamp with the field iris about 15 cm. from the mirror face and center the beam carefully on the mirror. With open field iris focus the edges of the ribbon filament sharply in the field by racking the condenser screw. Next close down the iris to about 2 mm. and slide it along its trough until its edge is prefectly sharp in focus. The iris carrier can thus be screwed permanently to its supporting trough. In critical use this field iris is closed to give a lighted area about half the diameter of the field of the ob-

jective, and its edge (as well as the ribbon at its midpoint) should be clearly in focus in the plane of the preparation. The iris beneath the condenser should then be closed down until the light haze in the marginal field narrows until it disappears by coinciding with the edge of the field iris image. This procedure does not use the full aperture to the condenser (approximately 0.6) but increases definition by contrast.

The carmine stain absorbs a maximum in the green at about 530. Hence to get greatest contrast a green filter should be used whose transmission is entirely within the absorption band of carmine. The best filter for maximum contrast is Wratten No. 62 (mercury Green). This is a dense filter and is recommended for cataloguing and being certain of the existence of the very faintest lines. For general use Wratten filter No. 61 (N) is the best, since it allows discrimination of the relative intensities of the lines through having a broader transmission (on both sides) than is the absorption band of carmine. Nearly as good as 61 for general use is 58A-B2 (dark). Filters 61 and 62 should both be obtained and none others are needed for aceto-carmine work. They should be mounted as close to the substage condenser as possible, to avoid fading of a spot where light is concentrated.

In checking details of banding search for places where the chromosome (instead of lying lax) is stretched to two or four times its normal length. The stretch comes almost exclusively between bands and the bands are thus moved apart sufficiently to be distinctly resolvable as separate entities. Many apparently single bands are thus seen as doublets and an astonishing number of faint lines are brought to view. Final checking of bands is made by oblique light, cast along the axis of the chromosome. The oblique light is best obtained by sliding a black card partly over (below) the intake face of the substage condenser and as close to it as possible.

Microscopical examinations should always be done in a brilliantly and uniformly lighted room, never in a darkened room, cubicle or dark corner. When the general outside illumination and the microscope field exactly match in intensity there is a minimum of eye fatigue and dazzle by sudden entry of light through an expanded (dark-adapted) iris! Spotty sidelights and highlights should be cut out by a semi-cylindrical shield, made of Bristol board and wood and mounted on the microscope body. The interior of this shield and the tops of the objective and draw tubes should be painted neutral gray of very light color and flat (non-gloss) tone, instead of the customary glossy black.

It is recommended that camera-lucida drawings be made at a standard magnification of 5,000 diameters, which is large enough to represent the detail seen. This exact magnification can be obtained by trial of projection of a ruled stage-micrometer upon the drawing surface and adjustment of the length of path of projection until 10u on the stage micrometer becomes exactly 50 mm on the drawing board. The microscope and lamp can be mounted together on a board and this board raised above the

regular table by blocks to an amount exactly determined by trial. With 90 X objective and 30 X ocular and with mirror arm fully extended, the microscope base needs raising approximately 1-1/2 inches above the drawing level on the table.

Anthes, E.H. Critical illumination for microscopic research

The need for a correctly designed illuminating unit for use with a research microscope is no less than the need for carefully constructed optics. The Bausch & Lomb Research Lamp is specially designed to meet the requirements of the critical microscopist. Its source, enclosed in a light tight, yet well ventilated housing, consists of a 6 Volt 108 Watt Tungsten Ribbon Filament bulb which is used with a transformer on 110 Volt A.C. The bulb is of the prefocused type which eliminates tedious centering and alignment with the condensing system when lamps are changed. The lamp housing is fitted with a highly corrected condenser with iris diaphragm. The condenser can be focused by means of a lever. A support protruding from the front of the lamp house carries a water cooling cell and filter holder. Since it is necessary to control the intensity of the illumination to meet specific conditions a set of four neutral glass filters are supplied, having densities of 0.3, 0.6, 0.9 and 1.5 respectively. These can be used alone or in conjunction with Wratten or other filters. Best results are obtained when the source or Ribbon filament is focused sharply on the mirror of the microscope. The condenser of the microscope will then form a uniformly illuminated image of the front surface of the condensing system of the lamp in the plane of the object. When the specimen on the microscope is properly illuminated, the field of view will be completely and evenly filled with light if the object is viewed through the eyepiece. In addition, the light entering the microscope objective must completely fill the aperture of the objective. This may be checked by observing the back lens of the objective by removing the eyepiece and by viewing the back lens through a pin hole cap. It will be found that in order to secure the best results the condenser of the microscope should be carefully focused for each objective. In the case of an oil immersion lens, the back lens of the oil immersion should be completely filled with light, thus making certain that its numerical aperature is fully utilized. In the case of a dry objective (4 or 3 mm) the back lens need not be fully illuminated when working with lightly stained specimens as too much light tends to obliterate fine details in the specimen. It is suggested to reduce the iris diaphragm of the condenser of the microscope so that only 2/3 of the aperature of the back lens of such an objective is filled with light. When working with oil immersion lenses, care should be taken that the iris diaphragm in front of the condensing system of the research lamp is reduced as otherwise too much of the object is illuminated resulting in "glare". This field diaphragm should be wide open when low power objectives are employed. The full numerical aperture of an oil

immersion objective is utilized only when cedar wood oil is put between the front of the microscope condenser and the back of the slide. If this condition is not fulfilled, the numerical aperture of the observing objective is reduced to 1 as light leaving the condenser passes through air whose refractive index is 1 as against 1.5 of cedarwood oil.
(This contribution by Mr. E. H. Anthes of the Bausch & Lomb Optical Co. was prepared upon request of the editors.)

Lebedeff, G.A. · Method of mounting the reproductive organs of Drosophila. The fly is placed in a drop of physiological solution and the abdomen is separated from the rest of the body. With two needles the reproductive organs (duct and gonads) are cleared from the rest of the abdominal organs. At this time it is advisable to transfer the organs to another drop of physiological solution (on the same slide) in order to eliminate small bits of foreign tissue which may adhere to the organs. Next, the organs are transferred into a drop of white of egg on to another clean slide (with shallow concavity) where the organs are placed in the desired position. Excess of albumen is removed by means of blotting paper. This transfer helps the organs to adhere to the slide and prevents their drying. After leaving the organs in the white of egg for about 12 to 24 hours, the slide is then placed into absolute alcohol, then transferred into solutions of 85, 50, 35, 15 percent alcohol for about 30 minutes or less in each. Then the slide is ready to be placed in tap water. If it is desired to have the colorless parts of the duct and the ovary differentiated, the slide may be stained with "light green". The testes, however, will preserve natural color. The preparation then is ready for dehydration and is passed through alcohol solution, ending with absolute xylol. Thirty minutes or less is a sufficient period of time for keeping the preparation in each of the alcohol solutions. Finally, the preparation is mounted in balsam or euparal.

EQUIPMENT

Thermal Control

Bridges, C. B. Incubators and thermal control. For incubators and cold-rooms of room size the least expensive and best engineered installation I have seen is that built at Stanford University, Palo Alto, under designs of Prof. Douglas Whittaker in cooperation with the Frigidaire Company. This controls against both rise and fall of temperature and its range of settings is from slightly above freezing to above 40° C.

Detailed descriptions of incubators and thermal controls used at the Pasadena laboratory and also at several other laboratories in the U.S.A. are given in: "Apparatus and methods for Drosophila culture". Am. Nat. 66: 250-273, 1932.

For trouble-proof inexpensive relay that described by Koepf and Mezen in Science 83: 109-110 would seem to be the best available.

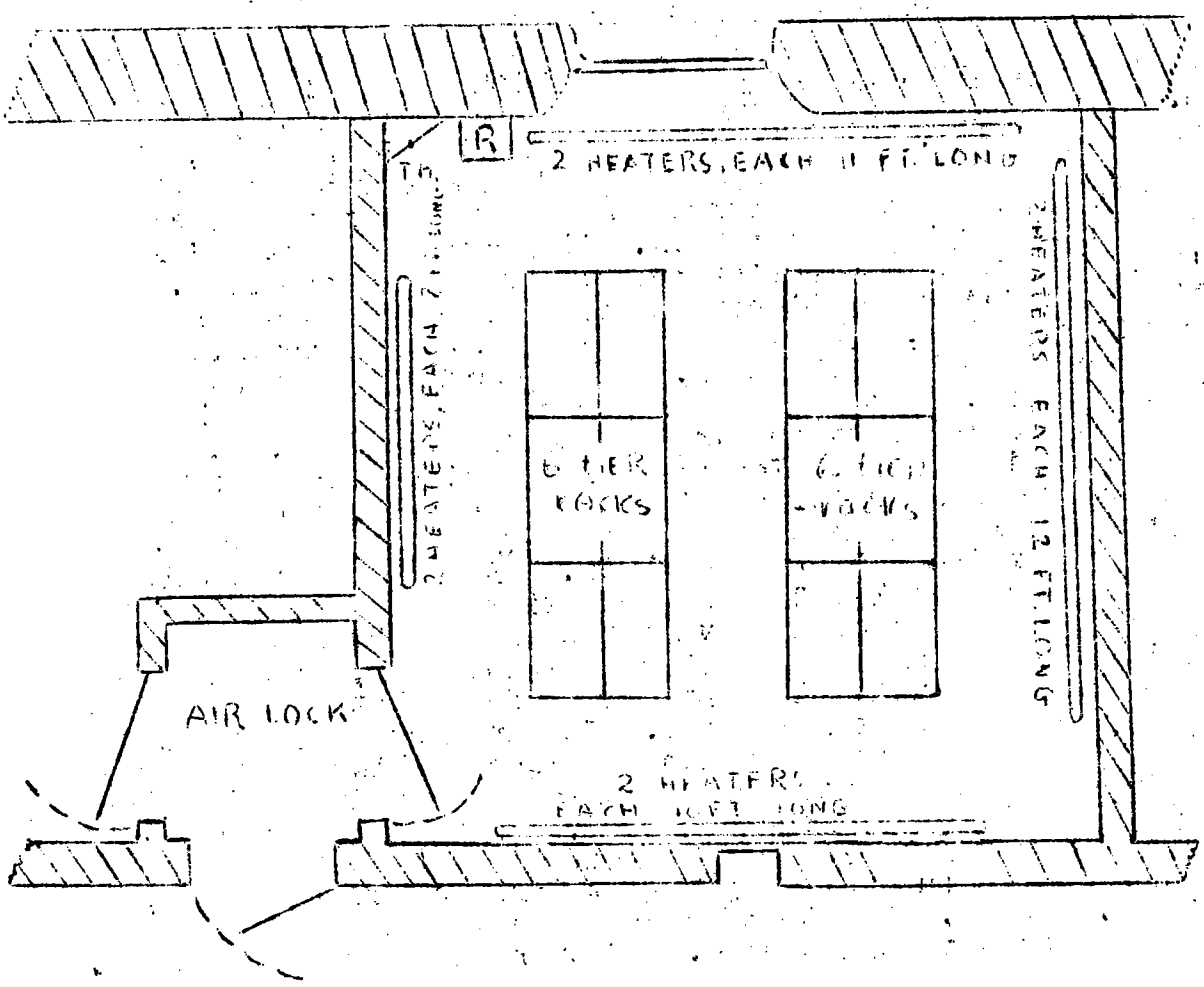
Crew, F.A.E. Constant temperature room. The room measures 15' x 14' 9" x 8' 8" high. Three of the walls are inside ones and the outside one is to the South and has a thickness of 2'. The north wall is of 10" thickness having a corridor on the other side. The remaining walls are of 6" thickness. The rooms on either side and also the corridor have an average temperature of 14° C. The floor and ceiling are of aerro-concrete construction, and although the actual thickness was not ascertained for the purpose of arriving at probable heat losses, a value of 12" was taken. The window in the room is in the south wall and has a glass area of approximately 16 square feet.

It was estimated that 82 feet of 2" diameter tubular heaters loaded at 60 watts per foot run would be sufficient to maintain the temperature of the room at any predetermined value up to 28° C. with the outside temperature at 5° C.

The heaters are mounted horizontally in pairs on each of the walls at a height of 15" from floor level. At time of erection provision was made for adding an extra tube to each or any pair should it be desired to run at higher temperatures. As the supply available was 230 volts, 50 cycles single phase alternating current, it was possible to adopt a thermionic relay switch for the automatic temperature control of the room. The relay switch comprises a thermionic valve supplied by a small transformer with filament anode and grid potentials operating the triple pole main contactor switch by means of the anode current. The thermostat is connected in the grid circuit and effects control by the application or non-application of a suitable potential to the grid. The thermostat is similar in construction to a glass mercury thermometer but in addition contains two contacts one of which is in permanent contact with the column of mercury. The other is connected to a small spiral of platinum wire. A small glass capsule containing a short piece

of iron is located at the top of the thermostat tube to which is attached a length of platinum wire passing through and making contact with the platinum spiral already mentioned. The capsule can be moved up and down in the tube by means of a permanent magnet applied externally and hence the thermostat can be arranged to make contact and "open" the main contactor switch at any desired temperature. With this type of thermostat it is possible to obtain temperature regulation within extremely fine limits.

On test it was found that in the event of the filament of the thermionic valve failing the main contactor switch would remain "on" and the room temperature rise to excessively high level. A small relay was therefore included in the filament circuit and so long as current is flowing in the filament the armature of the relay is held away from a pair of contacts, which when bridged by the falling armature completes a battery circuit to an alarm bell fixed in the corridor. In the event of an interruption of the supply, this relay also operates the alarm bell. (Messrs. Round & Robertson, Edinburgh).
See sketch



Gravett, Howard L. Thermal control system. Temperature is controlled with great exactness in the Experimental Zoology Laboratory at the University of Illinois. Electric incubators are housed in especially constructed high- and low-temperature rooms. While experiments are being run it is possible to keep these rooms from varying more than one degree from 25° and 15° C. Under these conditions the incubators housed in them can be held constant to within 0.3° of the required temperature.

The incubators used are made by the Chicago Surgical and Electrical Company. The heat unit is a high resistance wire coil and contact is accomplished by the bending of a diaphragm. A twelve-inch General Electric dish fan with lengthened shaft so that it could be installed with the motor outside and the blades inside of the chamber has acted in a satisfactory manner in keeping the air stirred. The only attempt to control the humidity is to keep an open vessel of water in each incubator. A Thycos, ribbon type, bi-record thermometer made by the Taylor Instrument Company of Rochester, New York, is used for a continuous record of the temperature.

The constant temperature rooms are cooled by air blown over brine coils and the high-temperature room is varied by steam coils. Both systems are under automatic control installed by the Johnson Service Company of Milwaukee.

Department of Biology, Amherst College. Constant temperature and humidity control. With the assistance of a grant from the Rockefeller Foundation, the laboratory has now completed the installation of a constant temperature room especially desigened for Drosophila work. The room is about 15' x 8' x 8' and is built into a storeroom on the basement floor. The walls and ceiling are 3" cork insulation attached on the inside of 4" studding, and two coats of water-proof cement plaster are applied over the cork. The outside is covered with fir sheathing, so that a 4" air space is enclosed. The room is air conditioned by an air duct on the end wall, and outlets on the side walls. The air conditioning apparatus was furnished by the Carrier Co. and is capable of maintaining the room at any temperature from 5° C to 50° ± 2° C with a relative humidity of from 20% to 100% ± 5%. At present the room is being maintained at 15° C with a relative humidity of 65%. Water and electric current are available within the room.

Within the room are placed 12 unit incubators, similar to the two shelf type developed by Bridges and Plumkett. Each contains an 8" fan run at low speed. Temperatures from 16° C to 40° C can be maintained over long periods within any of these units. The heating elements are electric bulbs and the thermoregulators are toluol or alcohol filled, controlling the heating elements by single relays amplified by a vacuum tube. The relay apparatus was furnished by Lovett Garceau, Diamond Hill, R.I., at $20.00 each. The temperature at any point in these incubators varies not more than 0.2° C over an indefinite period.

The humidity is controlled by the percentage humidity in the room itself.

In addition to the incubator chambers maintaining temperatures above that of the room, there has been installed a large four door Frigidaire. Within this have been introduced five distinct insulated compartments with double glass doors (about 14" x 10" x 15") each with a heating element enclosed in lamp cylinder of asbestos. The temperature is controlled by toluol filled thermo-regulators operating the heating element through Dunco Relays (Struthers Dunn, Inc., Phila. #CS-1022-$11.00). The Frigidaire cooling system maintains a temperature of 4°-8°C around the insulated chambers, and temperatures up to 15° C are maintained within the chambers with a variation of less than 0.2° C over long periods.

The system is somewhat unique in that the room as a whole is held at an intermediate temperature with a series of 17 compartments at accurately controlled temperature above and below that point. Such a system is much less expensive than that of keeping the room at the lowest point, and the various intervals are more easily maintained. It has now been in operation for three months and seems to be entirely satisfactory.

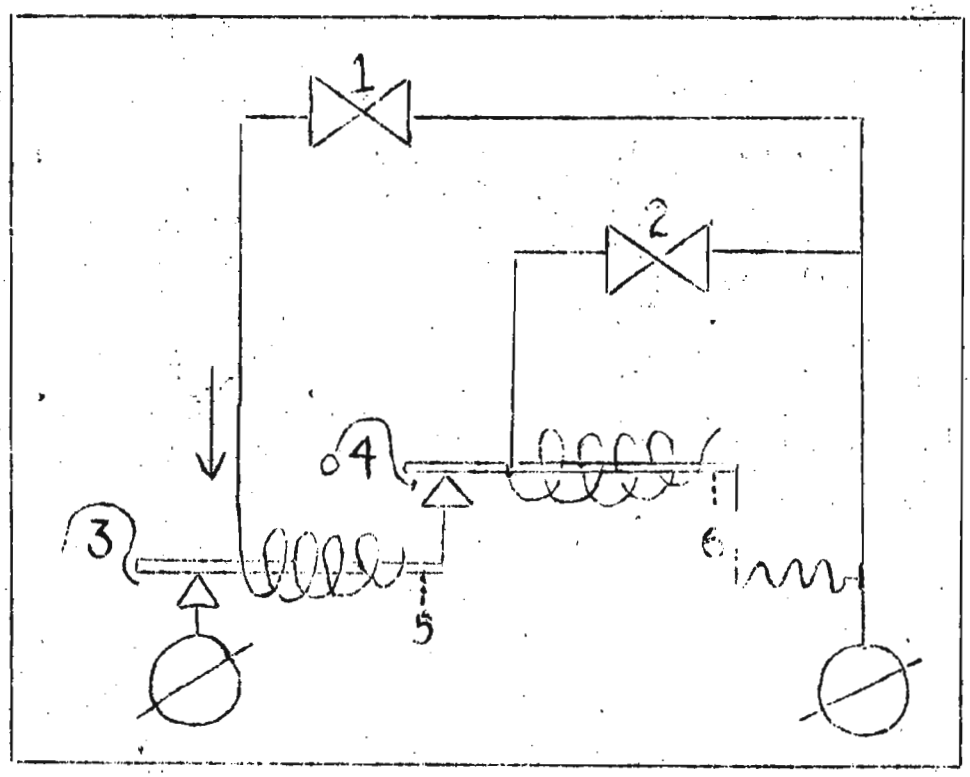
A series of Wheatstone Bridge Resistance Thermometers which will make a printed record of temperatures and relative humidities of six separate compartments simultaneously (with and accuracy of #0.25° C) is now being installed.

The room is large enough for a series of shelves and a table for microscopic work, so that observation as well as other types of work can be carried on.

The total cost of the whole installation was close to $4000.00. The room and equipment were planned by the members of the department. Further details can be furnished by writing to H. H. Plough.

Medvedev, N.N. Thermal control As experience shows, the best solution of the incubator problem is the construction of one big room-like thermostat instead of a number of them for personal use or for the use of some workers. The small incubators are used only in special work, when high or low temperatures are desirable.

The thermal control system of Frank that we adopted, after much experience, may be generally recommended as a very precise and cheap one. It consists of two regulators (see figure), one of which (2) keeps the desirable temperature (e.g. 26° C); the second regulator (1) is an extra one and breaks all contact when the temperature in the incubator accidently reaches 2-3 degrees above the point desired. This complete breakage of the current is brought about by the spring (3), being so designed that it breaks the current at a definite temperature but does not close it again automatically. The spring (4) on the other hand, breaks and closes contact automatically. Both regulators (1,2) as well as the breaking parts (5,6) are made from bimetallic plates.



Use of similar thermostat rooms and thermal control systems is reported by R. A. Masing and A. I. Zuitin.

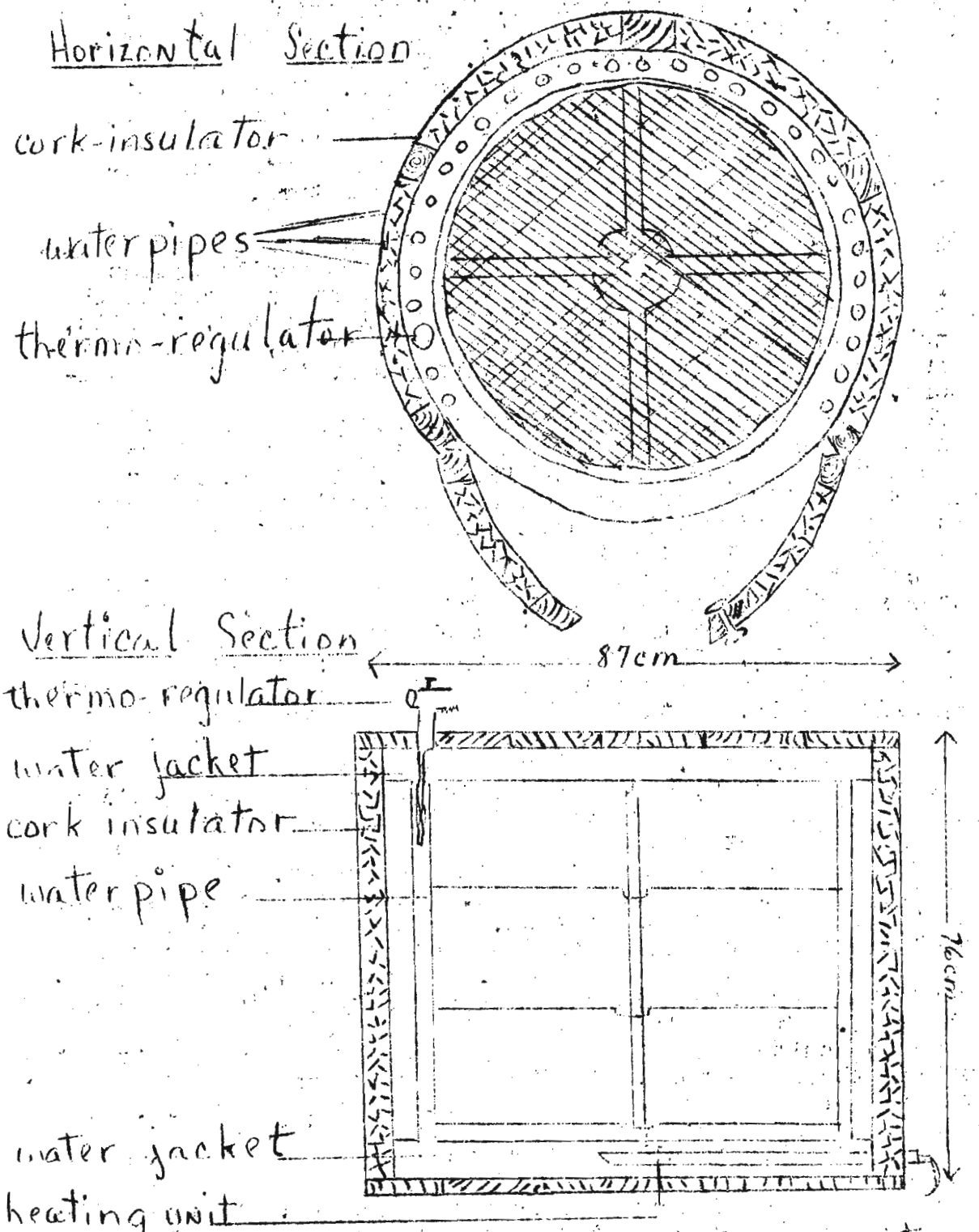
Mossige, Jeanne Constant temperature arrangement. Constant temperature water baths up to 29° are used at the Zoology Laboratory of Columbia University with great success. The flies are placed in six inch vials in wire holders, the upper two inches above the water level, with deep cotton plugs. This gives a temperature accurate to within 0.1° and a larger working space than any of the usual small laboratory incubators.

Shull, A.F. Heating units and thermal control. Heating units for temperature cabinets made up in any form, similar to those used in poultry brooders, may be obtained from Oakes Manufacturing Co. Inc., Tipton, Indiana. A small-sized unit costs $1.20.

Temperature control for these cabinets may be obtained with Circuit Breakers, made by the above company, at a cost of $1.30 each.

Camara, A. Incubators. We use Common incubators of Charles Hearson of London and Hugersboff of Leipzig. We have also some incubators built in this Institute.

Gottschewski, G. Incubator. The following figure shows the design of the incubator used at our laboratory. This incubator is built at our Institute. The capacity is 150 culture bottles. It costs about 600 R.M. including thermo-regulator which is obtained from Leitz, Cat. 32A no. 10190, Neuer Elektro-Präzisions-Thermo-regulator grosses Modell and costs 98 R.M.



Hersh, A. H. Incubators. A multiple temperature incubator, total length about 15 feet including an icebox at one end and at the other an electrically heated water tank fitted with thermoregulator. A long copper trough insulated with glass wool serves for heat conduction. This apparatus is slightly modified from model #3 described by C. B. Williams and T. W. Kirkpatrick (1924, Technical and Scientific bulletin # 38, Ministry of Agriculture, The Government Press, Cairo, Egypt). The thick copper trough is deep enough to accommodate 8 oz. bottles. When the hot tank is kept at about 40° C, a temperature gradient over the interval 10° to 35° can be maintained with sufficient constancy for any investigation which does not require any extensive amount of space at any single temperature.

Spencer, W. P. Incubators In culturing many species of Drosophila optimum temperature conditions are extremely important and even in the case of easily cultured species such as melanogaster, virilis, or funebris much of the trouble with mold, mites, and infertility would be obviated by starting cultures at the optimum temperature.

The incubators described below are not presumed to take the place of the accurate and elaborate temperature control systems developed by Bridges and Plunkett. They do, however, supply at very little expense a means of rearing large numbers of stock and experimental cultures at a temperature fluctuating not more than a degree centigrade.

A casket pack (rough box) may be secured from any funeral director for from 25 to 50 cents. This consists of a strong light wooden framework to which is tacked three ply 1/8 inch wood veneer panels. These boxes vary in size; one of average size we are using has the following inside measurements: length 7' 4", width 2' 6", depth 2' 1". The lid is hinged to the box with three or four hinges, and hooks or other fasteners provided to hold this door shut. As the wood veneer is tacked on the inside of the framework, panels of cellotex or other composition insulating material are cut of a size to fit snugly into the framework on the outside. These are held in place by one or two light lath tacked over them and to the framework. The box is set on end and may be put on castors. The first shelf is placed at least two feet above the bottom. These shelves may be made of one-half inch wire mesh tacked on wooden frames which rest on supports nailed to the framework. Each shelf, for an incubator of the above dimensions, has a capacity of 100 half pint milk bottles. Five shelves can easily be used with ample room for removing culture bottles from the back of the shelf without disturbing those in front. The heating unit consists of electric light bulbs placed in or near the bottom of the incubator. A thermostat is placed on the back wall about middle of the incubator. One light bulb of 60 watts is capable of keeping this incubator with approximately 40 cubic feet of air space about three degrees C above the room temperature. We use a 60-watt bulb in series with a bi-metallic thermostat and other

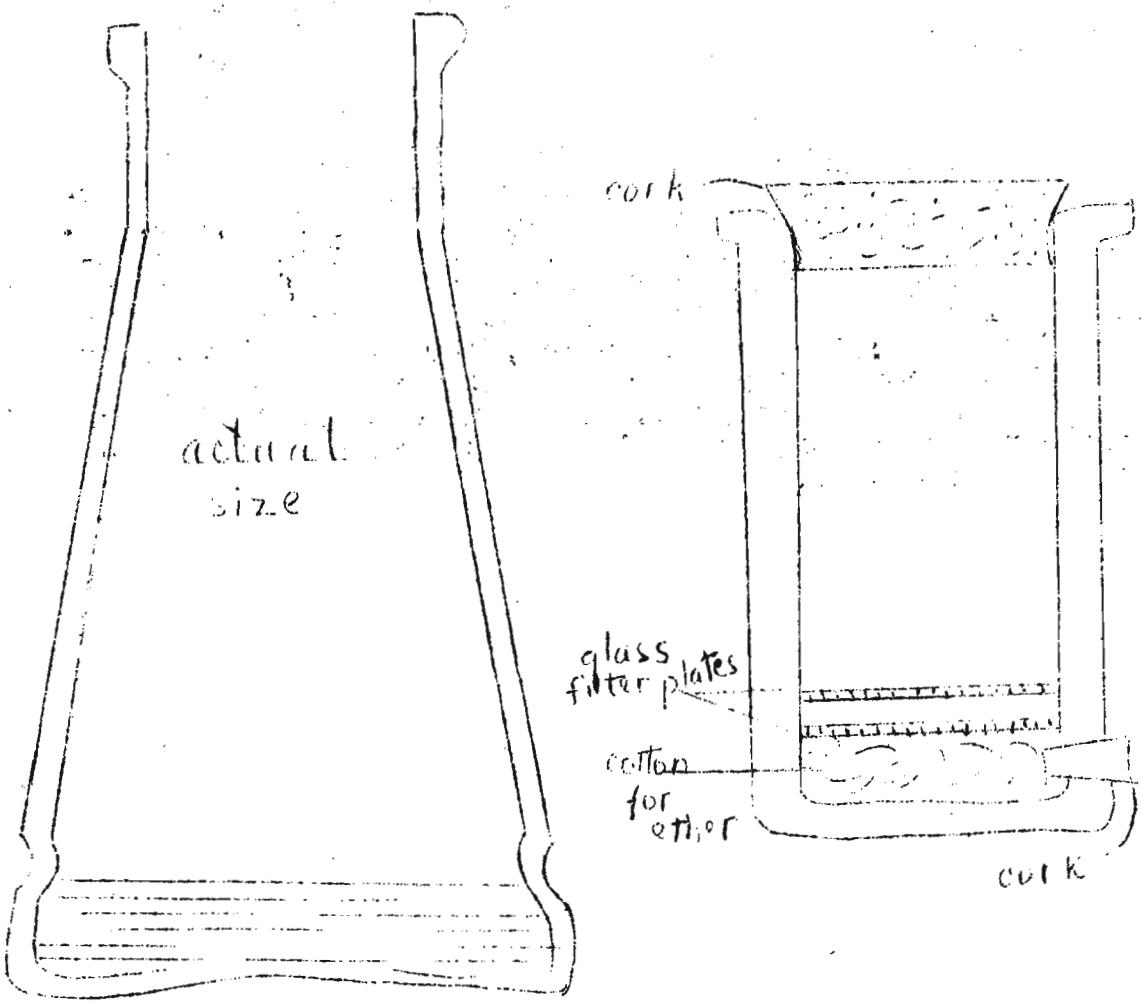
bulbs which may be turned on, but which are not in series with the thermostat. By carrying a light load on the thermostat danger of sparking and sticking is lessened; then if this does occur the temperature will not go so high that serious damage is done. It is of course desirable to use a room for fly culture where day and night temperature fluctuate to a minimum. A cellotex shelf is provided which may be fitted in at any level to cut down the size of the incubator when the full capacity is not needed. Such a 500 bottle incubator gives surprisingly little fluctuation in temperature from shelf to shelf corners. This may be due to the long distance from the heating units to the first shelf. A galvanized pan three inches deep covers the bottom of the incubator and is kept full of water. The incubator, when empty can easily be moved by one person. The total cost is less than $10.00. Such an incubator has been in use in our laboratory for two years, with no appreciable wear.

Stern, Curt Incubators. Two-shelf incubators with toluene mercury thermoregulator and Aminco relays as described by Bridges, 1932, Am.Nat. pg. 258-265. In order to keep temperature constant at or below outside temperatures (no cool rooms available!) we have installed cooling devices above the heating units at the height of the middle shelf: (a) copper tubing, through which running water circulates constantly is sufficient for keeping the temperature within a few degrees below the outside temperature, (b) for lower temperatures a grid connected with a refrigerator unit is being used.

Utensils

Crew, F.A.E. Culture bottles. Half-pint milk bottles are used for stock cultures. For convenience all experimental work is done in 4" x 1" vials.

Gottschewski, G. Culture bottle and etherizer. Culture bottles can be obtained from Bartsch, Quilitz u. Co., Berlin N.W. 40, Döberitzer Str. 3-4. Cost is 0.20 R.M. per piece. The etherizer is made by the Jenaer Glaswerk Schott u. Gen., Jena, and costs 8.50 R.M.



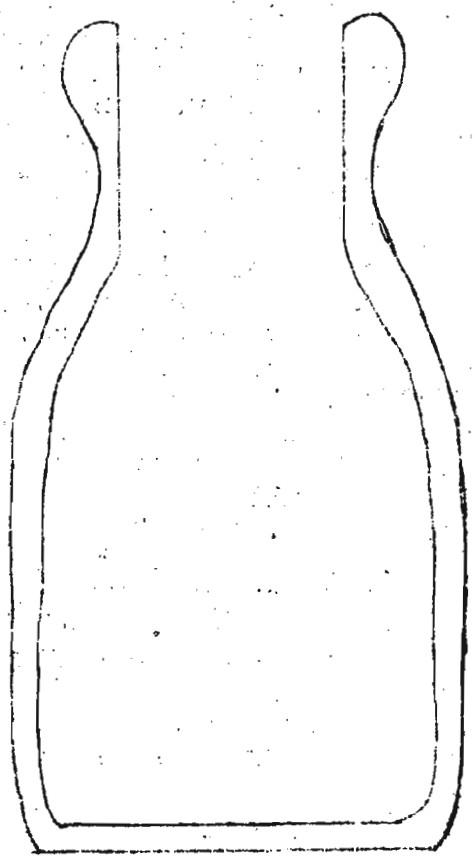
Kyoto University. Culture bottles Generally the bottles for pickling the scallion (allium bakeri) are used. The bottles are used widely in Japan, and can be purchased at a low price (about Y4 per 100 bottles). The size and the volume of the bottle are as follows: Height, about 18 cm; inside diameter of the mouth, about 3 cm; inside diameter of the bottom, about 6 cm; volume, about 350 cc.

For special works, the small milk bottles or other adequate bottles and tubes are used.

Gravett, Howard L. Culture bottles. The bottles used for the various stocks are wide-mouthed 250 c.c. bottles. The dimensions are as follows: height (including neck) 6 inches, width at base and below neck 2.5 inches, neck 1 inch and width of neck 1.25 inches.

Hemopathic vials (3/4 x 3.5 inches) are used for experimental work in which single pair matings are to be used.

Medvedev, N.N. Culture bottles For individual crosses and tests we use vials 80 x 20 mm in size. In order to get a large number of flies we use a special kind of bottle, adopted in our Institute over two years ago. They are made of thick glass, preventing the accidental breadage of them. The size and dimension of this kind of bottle is given in the following figure.



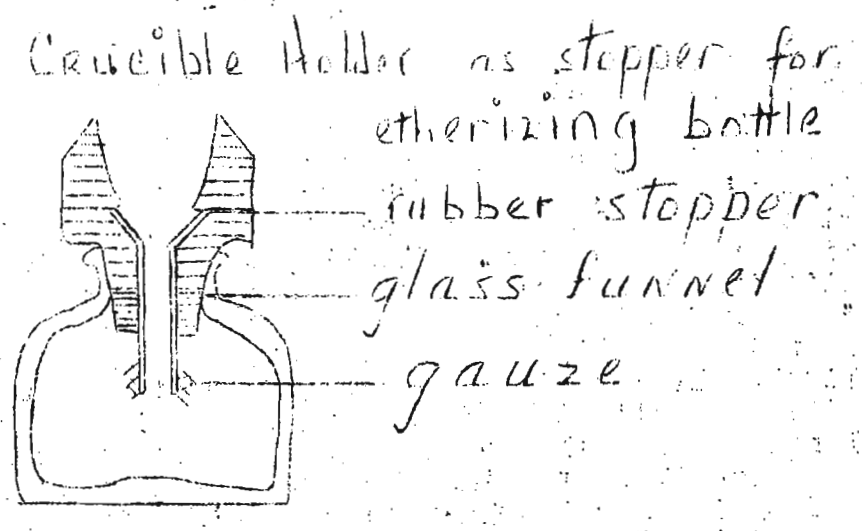
Shipman, E.E. Culture bottle. Due to the high cost of transportation on the bottles designed by Bridges and manufactured by the Owens-Illinois Pacific Coast Company at San Francisco, it was necessary to find a substitute bottle manufactured nearer home. The writer has found a Urine Specimen Bottle, No. 820, manufactured by the Glasco Products Company, Chicago, Illinois, quite satisfactory. The bottle is made of the same type of glass as milk bottles, has straight sloping sides, the inside top diameter is about 1/4 inch less than the inside bottom

diameter, and has milk bottle type opening so that paper caps may be used if desired. The writer handled three gross of them this summer with an average of about 35 offspring per bottle and had only four cases where the food cake shook completely loose, daily removals were made so that the danger of loose food cakes was much greater than in routine stock work. (Copied from DIS-3: 54).

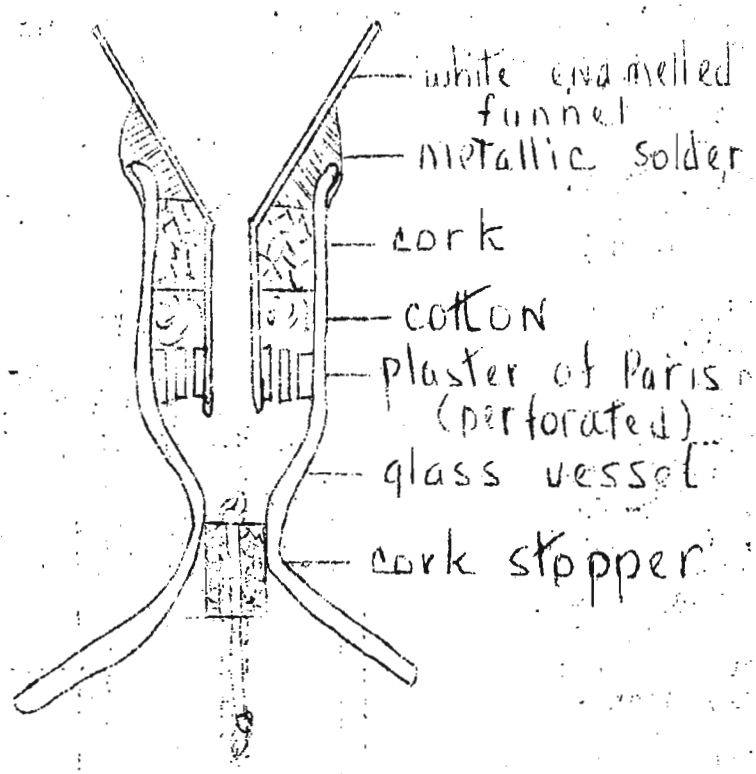
Spencer, W. P. Culture bottles. For some months I have been using small green and white glass salt and pepper shakers, with aluminum screw caps in place of glass vials for culturing flies. These shakers can be purchased for two for five cents in any 5 and 10 store in the U. S. They have a total capacity of 60 cc.; we use 15 cc. food medium in them as compared to 30 cc food medium in a quarter pint milk bottle and 50 cc. in a half pint bottle. As the holes in the screw caps are large enough to let small Drosophila through, circular disks are cut from library cards and fitted inside the caps, and then punched with needle holes. When once a cap is fitted with a card-board disk it can be sterilized and used many times before a new card-board disk is needed. An ordinary library card is cut in four pieces, 2-1/2" by 1-1/2", each piece is folded once and these are stuck, one into each culture bottle. The card serves a double purpose; it is sufficiently rigid to hold the food plug in place in case of CO_2 formation, as it pushes against the screw cap; then it furnishes pupation surface. A larger square bottle, with aluminum screw cap can be purchased for five cents each. This bottle occupies the same shelf space as a half pint milk bottle but has considerably more food surface. I am using the small shakers for rearing stock cultures of a number of the smaller Drosophila species as montium, bipectinata, and affinis, and for maturing flies of slow breeding species. Small tags made of strips of library card are used for numbering, and these tags are fitted under the edge of the screw cap. It is to be hoped that eventually square culture bottles, with aluminum screw caps punched with very fine holes may be placed on the market. Such bottles made of clear glass ought to sell for not more than five cents a piece for larger sizes and correspondingly less for small sizes. However, it is not likely that these prices can be secured without the cooperation of a number of the larger laboratories.

Amherst Laboratory Etherizing bottle. It has been found that a rubber Walter's Crucible Holder furnished by any laboratory supply house at thirty-five cents is an excellent stopper for the etherizing bottle (of sketch) and makes the use of more expensive and more complex bottles unnecessary. The rubber stopper fits around the lip of any bottle or vial, and flies shaken out fall through the glass funnel into the bottle. A few drops of ether from a dropping bottle on the gauze around the stem of the funnel is sufficient for several samples of flies. The outer lip of the rubber stopper should be ground

down to form a narrow upper lip all around. The stem of the glass funnel is cut to a suitable length and fire polished.



Demerec, M. Etherizing bottle.



The following drawing represents an actual size cross section of the etherizing bottle used in our laboratory. The design is a slight modification of an early Bridge's design. Ether is poured in through the bottom hole. A few drops suffice for one hour's work.

Specifications:

Funnel: White enamaled 1/8 quart improved funnel manufactured by the Vollrath Co., Sheboygan, Wis., obtainable in hardware stores or could be ordered through a hardware store. Top is cut off to fit the culture bottle and bottom cut off to fit the glass vessel. Cost 30-40 cents.

Glass vessel: Can be made by any glass-blower. We ordered it from Eck & Krebs, 131 West 24th Street, New York, at 50 cents a piece. (Copied from DIS-2: 62)

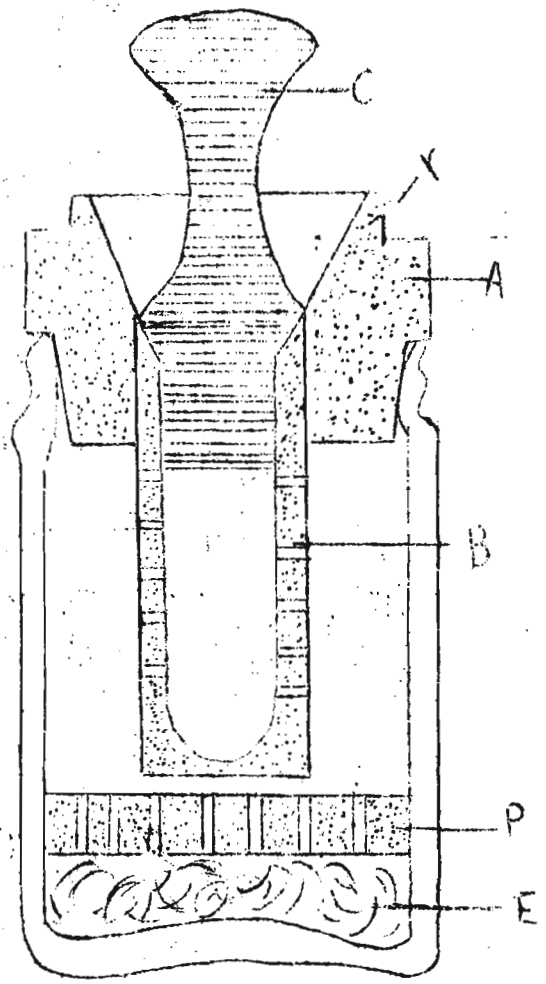
Miokey, George H. Etherizer.

A very simple type of etherizer may be constructed from a pure aluminum, seamless funnel, 2 in. across top, (may be purchased for about 75 cents per dozen at any hardware store) and a A.C. glass carburetor bowl (genuine A.C.

service part no. 854004, 15 cents each; A.C. Spark plug Company Flint, Michigan).

The top of the funnel must be trimmed down to a slightly larger size than the diameter of the cup then bent over the latter's edge. Around the funnel tube a mass of cotton covered with cheese cloth is wound tightly and held in place with fine wire or with string. Finally, the lower tip of the tube is slit in several places upward to the cotton and the resulting "fingers" bent up to help hold the cotton securely. This etherizer is probably best adapted to use in a laboratory where little fly work is done or to elementary students use, because of its simplicity and cheapness, but it is not suited to prolonged Drosophila work because of loss of ether.

Doubtless the improvement described by Stern in DIS-1 and by Demerec in DIS-2 on Bridge's original etherizer ('32, Amer.Nat. 66: 250-273), leave little to be desired in the way of an etherizer; nevertheless, through numerous experiments we have developed a new model based upon Muller's suggestions in DIS-2. The accompanying sketch represents an actual size section of our special design. The glass bottle is an ordinary specimen jar (Cenco no. 10373 wide mouth bottle, diam. 2-1/8", ht. 3-1/4"; screw cap size no.53; 40 cents a dozen) hence may be replaced very readily if broken. The upper portion, funnel and tube, is made of Bakelite turned on a small lathe. Part A is fashioned from Bakelite BT-45-005 at 60 cents a pound, and parts B and C are of Bakelite BT-61-893 at $2.75 a pound, (procured from the Bakelite Corporation, 247 Park Avenue, New York City). The latter grade is used for the tube and stopper because it can be polished to a highly transparent quality.



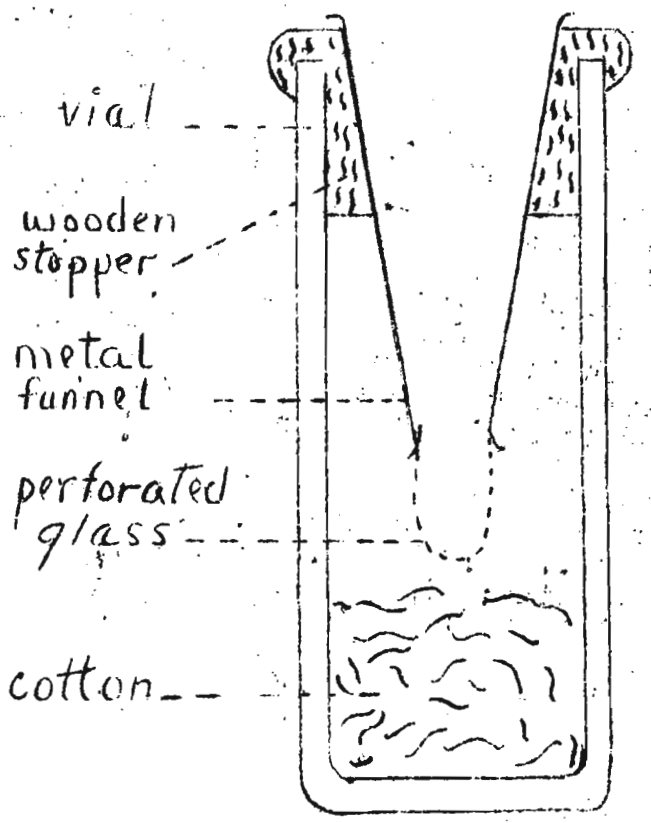
This screw cap jar has a diameter about equal to that of the mouth of a milk bottle and the threads give a nice grip, thereby making it convenient, after the milk bottle has been inverted over the rim (r), to hold it in place with one hand and knock the whole against a large rubber stopper.

Merely the friction of a tight joint is sufficient to hold the tube (B) in the top (A);

The bottom of the hole drilled in the tube should not be pointed but must be rounded so as to prevent flies being caught. Very small holes are drilled into the side of the tube (B) to allow ether vapor to enter from the glass jar. Cotton should be packed in the bottom of the glass jar (E) and held in place by plaster of paris (P) which should be perforated. If the tube is polished, it is possible to see the flies from the side and to tell when they are anaesthetized. Ether may be added either by pouring it into the tube or by dropping it onto the plaster after removing the top (A).

It is necessary to add ether only about once a day, or even at longer intervals if the stopper remains in place when not in use. Not only is the saving in ether remarkably economical but also valuable in regard to the comfort and well-being of the investigator. Indeed, this latter consideration was the chief reason for developing our new design.

Muller, H. J. Etherizing bottle. The type which I have found most practical all round (and at the same time the easiest to construct, repair and clean) consists of a glass containing-vessel (whose shape may be chosen according to the convenience of the operator), into the neck of which is firmly fitted, preferably through the mediation of a hollowed-out cork, a funnel, preferably of metal, with an upper end wide enough to fit against the mouth of the widest-mouthed culture vessel used. To the narrow end of the funnel, below, a large gelatine capsule of the same diameter, and containing numerous fine needle-holes, is glued on. In the space between the bottom of the suspended capsule and the bottom of the containing vessel is packed a mass of dense cotton, (which must not touch the capsule). Flies go in and out through the same opening, without manipulation oa any stoppers being necessary and are quickly etherized by the diffusing ether. The ether used need usually be added to the bottle but one a day, as a minimum is lost (an advantage both ecomonically and physiologically). If desired, glass can of course be substituted for the gelatine, but the latter has considerable durability and the ether can be poured in directly through the gelatine sieve. The latter is later wiped quite dry with a paint brush. (opied from DIS-2:62



Spencer, W. P. Etherizing bottle. The bottle I am now using is an adaptation of the one described by H. J. Muller in DIS-2.

Wide-mouthed heavy glass, specimen bottle of about 60 cc. capacity; cork to fit. A hole 3/4 inch in diameter is cut through the cork with a large borer and then reamed out so that a large gelatine capsule, diameter 3/4 inch and length 1-7/8 inch can be fitted into the lower end of this hole. It is pushed in but not glued. With a hot needle numerous holes are punched in lower end of capsule. An aluminum funnel 2-1/2 inches long rests in the cork and sticks well down into the capsule. Cotton is packed in the bottom of the bottle and before using a pipette full of ether is introduced. Then the cork with capsule is inserted, the funnel laid in place and the flies jarred into the capsule. When they are etherized the funnel is removed and the flies poured out on the counting card. Etherization time is slightly longer than with the Bridges type etherizer. Simplicity of construction, ease of cleaning, and the fact that capsules are easily replaced are advantages. If the bottle is kept covered when not in use one pipette of ether lasts for hours.

Recently a workman in our laboratory constructed 15 of these etherizers in two hours time.

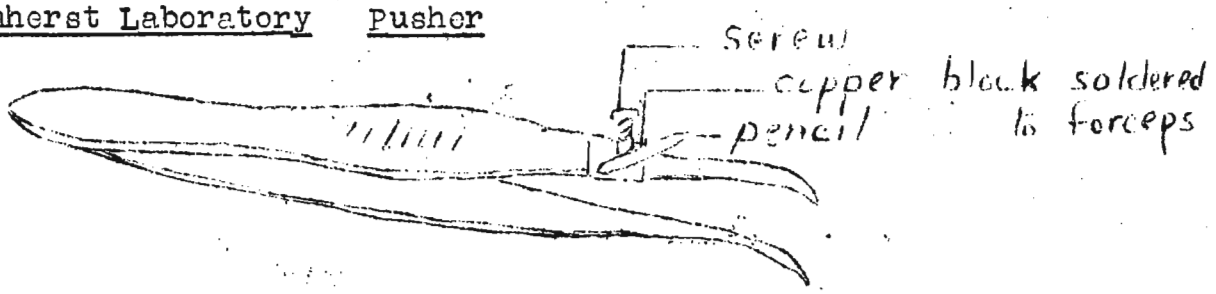
Total cost:

Construction time -- 10 minutes
1 large cork --------.02 cents
1 gelatine capsule -.02 "
1 alumin. funnel ---.10 "
1 specimen bottle --.15 "

Stern, Curt and etherizer. Food-filling funnel A funnel has been constructed which facilitates the filling of food-bottles. The funnel is made of metal and has a capacity sufficient for food for about 60 bottles. It has a metal faucet which is easy to handle. The funnel is mounted on a stand but can be detached for cleaning purposes. Below the funnel opening the stand has a hole through which extra drops of the food fall through, so that the bottles do not get dirty. The base of the stand has a rail which guides the bottles. This funnel can be obtained from the Will Corporation, Rochester, N.Y. A similar model has been in use for many years at the Kaiser Wilhelm Institut and has proven a great help in reducing the time and labor involved in the preparation of fly-food.

The Will Corporation has also on sale an etherizing glass following in general the design of C. B. Bridges (32) given in Amer. Nat., 66:250-273. An improvement consists of the following: Instead of a small funnel and a layer of plaster of Paris which have to be fitted and fastened into each glass individually, a one piece metal funnel and ether holder is used which fits in without special fastening devices. (Copied from DIS-1:57).

Amherst Laboratory Pusher



Forceps with pencil attached to speed up orienting fly and recording.

Mickey, George H. Pusher An excellent fly pusher may be constructed out of a metal needle holder (80 mm long, with screw chuck for holding needles; 20 cents each, no. 2978 Schaar and Company, Chicago), a steel spear point needle (40 cents doz., no. 3951 Central Scientific Company, Chicago), a piece of rubber tubing 1/8 in. inside diameter, and a no.3 or no. 4 red sable point brush (obtainable at any paoint store for 20-25 cents each). Red sable is little more expensive than camel's hair but is much more satisfactory in that it wears longer and has more "life" or spring. In place of the wooden handle of the brush one should substitute the metal needle holder, the back-end of which is held in place by any good metal cement. Then the piece of rubber tubing should be slipped over the handle to make a larger, firmer grip. The angle of the needle's spear point can be filed down to suit individual preference. When the needle is inserted, the pusher is ready for use.

Shipman, Emmet E. Pushers. In handling individual flies the writer has found a pair of regular dental forceps highly satisfactory. They are about 15 cm. long, the points being set at an angle are about 17 mm. long. The spring tension may be reduced by grinding on the inner part of the springs near the base. The writer has found them more comfortable for long use and the points stay in good alignment. They may be obtained from nearly any store handling dental and medical supplies.

Mickey, George H. Counting plate. Until recently at our Austin laboratories the most satisfactory plates on which to count flies has been old glass photographic or lantern slide plates which were out of date. At first they are rather too light in color, causing reflection and glare, but gradually become darker the longer they are exposed. Disdavantages are that the color finally turns too dark, the emulsion wears off through sliding on the table top, and the plates are broken too easily. We have just received some baked enamel plates from the Burdick Enamel Sign Company, 36 South State

Street, Chicago, which have none of these disadvantages. These new plates, made of 18 Ga. steel 3 x 5 inches in size, either have both sides covered with a medium dark gray baked enamel or have one side white and the other gray. In lots of 24 the price is 75 cents each.

(A plate of opaque glass was found to be a very satisfactory counting plate. It gives a rigid, smooth surface of good optical quality for examining flies. Such a plate can be cut to any size. Opal glass can be procurred from various supply houses. - Editors).

Muller, H. J. Fly morgue — In place of the usual method of having a jar of alcohol or other volatile fluid into which the flies to be discarded are dropped through a narrow slit, it is much more convenient to have a broad dish containing a non-volatile oil. The used oil from automobiles affords a conveniently obtained medium. The opening may be protected by a wide-mesh wire grating. The flies do not have to be brushed off in any exact manner, but may be merely jarred off by knocking the porcelain plate against the screen with one motion of one hand. Renewal is seldom necessary and there are no disturbing odors. This method was used independently in Texas and in the USSR.

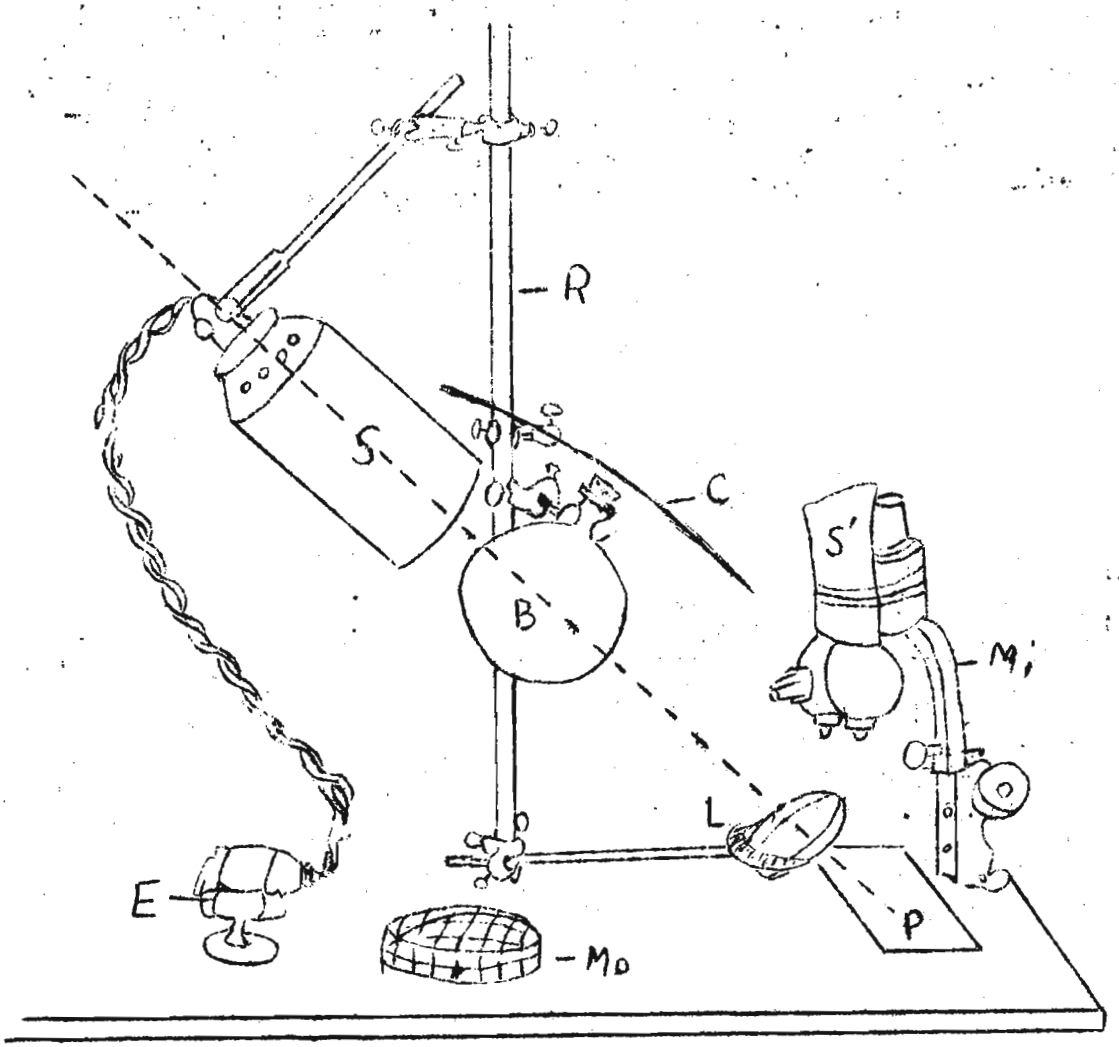
Mickey, George H. Trays for vials — A very satisfactory container which has not been reported to DIS but which has been in use for handling flat bottomed shell vials, is a round tray 11 in. across and 3 in. deep, made of 20 Ga. (or lighter) galvanized sheet iron. Such a tray can be washed and sterilized repeatedly and will last indefinitely. Since it holds approximately 100 vials, no more convenient method for handling and filling vials without actually counting them could be desired. Moreover, this container lends itself admirably to a method suggested by Altenberg of etherizing cultures "en masse". For this, the vials are inverted in one tray, covered with a towel half-saturated in ether, and then covered with another tray. There is very little danger of over-etherization and the flies do not stick in the food since the vials are inverted. These "tins", as they are called, were constructed by a local tinner at a cost of 50 cents each.

Bridges, C. B. Light — Light for the binocular should fall on the flies and white-glass plate as a broad-base converging beam whose axis is about 45° from the vertical, which angle gives least highlights and disturbing reflections. A frosted 100 W tungsten globe focused through a 1-liter globular water flask, placed very close to the flies, is excellent.

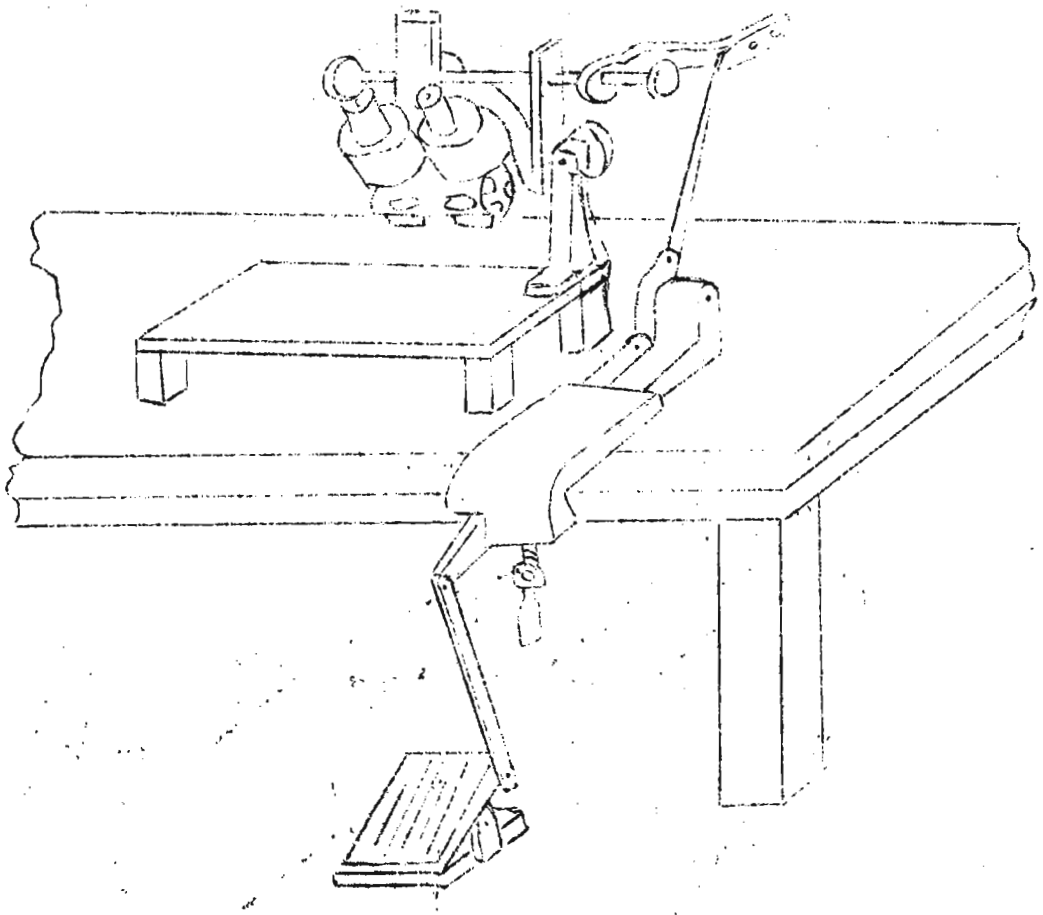
About 15 diameters seems to be optimum magnification for routine binocular examination of Drosophila, since higher magnifications have so little depth of focus that special or continual refocussing is necessary.

Mickey, George H. Lighting arrangement. Our present arrangement for dissecting binocular, lights, etc. is as illustrated in the following sketch. Mi, microscope; P, enameled counting plate; Mo, morgue, composed of finger bowl containing used crank case oil and covered with wire test tube holder cut cown; E, electric socket on table top; R, ring stand screwed into hole in table; B, 500 cc. round bottom pyrex flask with short neck, filled with glycerine (much better than water) for cooling and concentrating the light rays; S, shade of bright tin (enameled black on outside) inside of which is a 200 watt frosted light bulb; C, cardboard to throw light down; S', small black shade on microscope to shield eyes from light.

Parts S,B, and L should be perfectly aligned and adjusted to make a field of light about 2 in. in diameter immediately below binocular.



Stern, Curt Foot-focusing device for binocular. A special foot-focusing device for binoculars has been constructed by Bausch & Lomb, Rochester, N.Y. A simple system of levers connects a foot treadle with the focusing adjustment screw. It works with very little friction. This device proves highly useful whenever it is desired to use both hands for handling of the flies, e.g., in work with somatic segregation or in operative work with any object. The device can be adapted to different binoculars. The binocular used in Rochester is constructed, with certain changes, according to principles suggested by C. B. Bridges.



Oliver, C. P. A dry heat sterilizer for cotton plugs. Where sterilizing equipment is not handy or not available, a small dry-heat sterilizer can be made at little cost. Heat is supplied by a common electric hot-plate (600 watts). To keep the cotton from scorching and yet to keep most of the heat from being lost, put a ten-inch-deep bucket between the plate and the sterilizing chanber. The open end of the bucket should be placed on the plate and should have a diameter as large as that of the electric coil. A large hold should be cut into the bucket-

bottom, and it is better to have most of the hole covered with a strip of thin asbestos. Eight to ten small holes cut into the side of the bucket give the necessary circulation. A common, tin bread-box (10 x 12 inches) with the tip filled with numerous holes made with an ice pick serves as the sterilizing chamber. Cotton placed on the bottom of the box will scorch if the temperature goes too high. To eliminate this condition, cover the bottom of the chamber with a piece of thick (8 mm.) asbestos, supported at two ends by pieces of asbestos. Asbestos also lines the lower parts of the sides of the box, although this may not be necessary. Into a hole in the top of the box thrust a thermometer down into the mass of cotton. Heat easily reaches 65° C, and the cotton does not burn or scorch.

BREEDING METHODS

Culture media

Bridges, Calvin B. Food formula The food formula in use in the Pasadena laboratory by all workers is that published in Amer.Nat. 66: 268, 1932.

Water	75.0 c.c.
Molasses (free from SO_2	13.5 c.c.
Cornmeal (coarse yellow)	10.0 grams
Agar-Agar	1.5 grams

About 60 c.c. per 1/2 pint culture bottle.

Other recommendations in the above paper in Amer. Nat. still hold substantially correct.

Gottschewski, G. Culture medium. Normalfuttermenge für ca. 60 Kulturflaschen.

2000 ccm H_2O und 30 g Agar kochen lassen, bis Agar gelöst, dazu 125 ccm H_2O und 375 ccm Sirup. Schliesslich 600 ccm H_2O und 400 g Maismehl zusetzen, das Ganze zwei bis drei mal aufkochen lassen, beim letzten Mal werden 1-3/4 g Nipagin-M hinzugefügt (Nährmittelfabrik J. Penner A.G. Berlin-Schöneberg; konservierende Wirkung verhindert das Schimmeln der Kulturen). Das Futter wird dann inFlaschen (vgl. Fig. 1) abgefullt; dazu kommen einige Tropfen milchiger Emulsion von Bäckerhefe in H_2O gelöst, Krepp-Papier und Zillstoff-Wattekorken. Vor dem Abfüllen wird alles in 150° steriliziert (elektrischer Sterilisator). Koch- und Abfüllgefasse (über letztere vgl. **DIS-1:57**, Apparatus, C. Stern) sind so bemessen, dass gleichzeitig eine Futtermenge für 300 Flaschen gekocht werden kann. Bis zum Gebrauch werden die Flaschen in Eisschränken aufbewahrt.

Gravett, Howard L. Food formulae The banana-agar-yeast food is used for most of the experimental work. The formula for the food is as follows: 100 c.c. of soft water, 100 grams of well-ripened banana and 2 grams of agar-agar. When the food is cool, a small pinch of pulverized yeast foam is added. It is then allowed to stand at 25° for about 24 hours before using.

The cornmeal-sorghum-agar food is used for maintaining the fly stocks. The formula used in the laboratory is as follows: 1155 c.c. of water, 22.5 gm. of agar-agar, 210 c.c. of sorghum and 120 gm. of cornmeal. One thousand c.c. of water is boiled and the agar added. The remainder of the water is mixed with the cornmeal to which the sorghum has already been added. This latter mixture is then added to the water and agar, stirred well, and placed in bottles.

Wilbur M. Luce adds 5 c.c. of sorghum to the regular banana-yeast-agar food and finds it very satisfactory for experimental work. He also tilts his vials after placing food in them in order to allow it to harden on a slant. He claims this lessens the possibility of gas formation and also increases the surface for yeast growth. (University of Illinois)

Kyoto Laboratory Food formulae

Agar-agar	10 gr.
Koji (malted rice)	100 gr.
Brown sugar	40 gr.
Acid potassium phosphate (KH_2PO_4)	0.8 gr.
Water	800 cc.

Use 50 cc. per culture. Heat the agar in the water (about 600 cc.) to dissolve, add the Koji ground in a mortar, with the sugar, the phosphate and the remaining water. This medium developed by M. Chino gives satisfactory results for D. melanogaster, D. virilis, D. ananassae and many other species. But, for D. repleta, D. buskii and a few other species, it seems prper to add albuminous material such as peptone in the above medium.

Ludwig, W. Food Formulae

a) Standard-Nährboden für D. melanogaster:
Rüben-Sirup 125 / Wasser 710 : Agar pulv. 10 erhitzen bis z. Kochen; dann / Nipagin 1 g in 5 ccm Alkohol absol., dann Maismehl pulv. 133 / Wasser 200; weiter erhitzen bis z. Aufkochen. (Nipagin, erhältlich bei Julius Penner A.G.Berlin-Schoeneberg, verhindert in dieser Konzentration jede schimmelbildung).
b) Für D. funebris immer, für D. melanogaster gelegentlich, wenn Gläser mit Standard-Nährboden zufällig nicht vorrätig-sind: ein als Pulver Käuflicher Nährboden (bei Dr. P. Kuliga, Luisenkrankenhaus, Schumannstr. 78, Dusseldorf, Deutschland). In pulverflaschen 3 cm hoch auffüllen, mit wenig Hefosuspension, die 1% Nipagin enthalt, kalt anrühren. Gläser durch Gaze, nicht curch Watte verschliessen. (Zusammensetzung des Nährbodens ist Geheimnis des Herstellers). Gläser sofort gebrauchsfertig.

Masing, R. and A.I. Zuitin Food formulae

The food formula used in our laboratories is as follows:

Agar-agar	40 gr.
Yeast	150 "
Currant	~~40~~ " 40
Sugar	50 "
Wheat flour	50 "
Water	750 "

Medvedev, N.N. Food Formulae

Two kinds of fly food are used in our Institute. One of them is prepared for stocks exclusively; the formula of this kind of food is as follows: water 12.800; cornmeal 1.6000; syrup 1.400; agar-agar .200.

The second kind of food as reported by Offermann and Schmidt in DIS-3 gives the greatest number of flies per bottle. For the formulae see following article.

Offermann, C.A. and I.K. Schmidt

Culture media for Drosophila.

With the development of the Drosophila technique, not only a certain amount of sterilization of the culture medium during its preparation became necessary, but also an adaptation of it to different requirements. Productivity and duration of the media are the two main factors to be considered for our purpose, and they are to a certain degree in inverse relationship. By productivity we mean the quantity of flies produced in a given time. By means of overcrowding a certain food can yield a higher number of flies which are small in size, but this higher yield will usually be cancelled by a serious loss in the speed of development (in strongly overcrowded bottles in fact the cycle has proved to be as much as twice the usual length). Three functional types of media may be distinguished: 1) for the maintenance of parent flies, 2) for the maintenance of lines of stock cultures, 3) for the attainment of high productivity. 1) this type has proven to be extremely useful for the current work where we have to keep alive the flies from the moment we obtain them until the moment of their use. In this case offspring are not desired. Flies have been kept on such a medium for over a month (some over two months) at room temperature, without a transfer. The same vial or bottle can be used over again until the surface dries out, and etherized flies will not stick to its surface. - Water 90 cc., agar 2 gr., syrup 7 gr., Nipagin .15 gr. 2) Suitable media serving this purpose, such as the banana agar and the cornmeal syrup media, are already in use in all Drosophila laboratories and will not be described here. 3) The main characteristics of this type are: production of large quantity of flies, short cycle of development, and low selective level (preservation of individuals of low viability).

The addition of killed yeast in large quantities to the ordinary food formulae was introduced a few years ago by Muller (in 1928), giving surprisingly good results. These media had, however, the inconvenience or requiring a constant supply of fresh ingredients. Dry yeast was used in place of fresh yeast by Winchester and by Gershenson. The authors have recently experimented with a systematic series of modifications of the Russian food mixture with the addition of dry or fresh yeast. Fifty different modifications have been tried, approximately twenty vials being employed for each trial and counts of the offspring made. Each ingredient was tested in different concentrations. As a result the following formulae have been found the best for obtaining high productivity. (A. with dry yeast) - Water 80 cc., Agar 1.5 gr., Dry yeast 1.5 gr., Raisins 4 gr., Syrup 5 gr., cornmeal 5 gr., Nipagin .15 gr. The agar is dissolved by bringing the water slowly to the boiling point, dry yeast (that has been disintegrated in a small part of water) is added and the mass is kept boiling for another ten minutes, so as to make sure that all the yeast cells are killed. Then the mashed raisins, syrup and cornmeal are added. with continuous stirring, and the food will be ready for distribution. The addition to the liquid mass of "Nipagin T" Naehmittelfabrik Julius Penner A.G. Berlin-Schoeneberg as found in Dr. Nachtsheim's laboratory, is important for cultures which

contain few larvae or develop slowly.

The layer of food should be somewhat deeper than 1/2 inch and its surface seeded with pure live yeast (fresh or dried). Adding paper and making the surface appetizing with fruit juice did not increase the yield in our case. 200 flies per vial and 1000 per half pint bottle should be considered a good average. This means that a vial can be employed where formerly a bottle was required, and a bottle can take the place of a group of bottles. Not only the number, but the size of flies is considerably increased. When fresh yeast is easily available it can be employed advantageously by substituting 15 grams fresh yeast for 1.5 grams of dry yeast in our formula.

The preceding formulae enable us to prepare food of each of the three types by the use of ingredients which will not spoil. A laboratory can thus provide itself with a year's supply at once, avoiding further trouble in this connection.

We desire to call special attention to the convenience offered by the new type of medium here described: The syrup-agar for the preservation of the P. flies, for the great elasticity it introduces in current laboratory work.

Parker, D.R. Food formula The food used in the University of Texas laboratory is made according to the following formula:

1 pound bananas (250 cc)
20 grams agar-agar
125 cc Karo syrup (white)
15 grams dried brewer's yeast (sterilized)
625 cc water
.15 gram Moldex A (dissolved in 95% alcohol)
Total 1 liter of food

The water and agar are heated until the agar is completely dissolved. When the bananas have been mashed thoroughly, they are added along with the other ingredients to the melted agar and the food is poured immediately, a glass funnel with rubber tube and spring stopcock being used for this purpose. Bottles are plugged with cotton stoppers, whereas the vials are covered with cloth towels until cool, after which they are sprayed with a thick suspension of fresh Fleischmann's yeast, punched, papered, and stoppered in the usual fashion. (According to suggestions by Muller in DIS-3: 52) Bottles are treated in the same manner; when they are cool, the stoppers are removed and paper toweling is added in the place of confetti. In both cases, by using a glass tube about 1/4" inside diameter and a large rubber bulb, a small hole is punched in the food at the side of the container in order to release gases formed in fermentation.

Spencer, W.P. Food formula

1 liter water
25 grams chopped agar
1000 grams mashed ripe or over-ripe banana.

It has been my experience that this medium is distinctly auperior to corn-meal-molasses-agar for many species of Drosophila, and equal to it for all species tested, which will grow on corn-meal.

Tanaka, Yoshimaro Food formula

Wheat flour	70 gms
Brown (or raw) sugar	60 gms
Agar-agar	15 gms
Water	1000 cc

Synthetic Food

(From Pearl, R. and U.B.D. Penniman, Am.Nat. 60: 357-366. 1926).

Solution A:

Cane sugar	500 gms
$KNaC_4O_6\ 4H_2O$	50 "
$(NH_4)_2\ SO_4$	12 "
$MgSO_4\ 7H_2O$	3 "
$CaCl_2$	1.5"
H_2O to make 3000 cc. of Solution	

Solution B is composed of:

Agar-agar	135 gms.
Tartaric acid ($C_4H_6O_6$)	30 "
KH_2PO_4	4 "
H_2O to make 3000 cc. of Solution.	

To prepare solution B, agar is thoroughly dissolved in water, to which the salts are added. To make the medium equal parts of solution A and B mixed. If a softer food is desirable the amount of agar should be decreased.

Gravett, H.L. Brewer's Yeast

The following were the most favorable prices from a series of letters sent out last May.

1. Standard Brands Incorporated, Dry Yeast Department, 595 Madison Avenue, New York, New York.

Fleischmann's Pure Dey Brewer's Yeast - powered - supplied to universityies and colleges for 30 cents per pound, delivered, in quantities from 1 to 99 pounds.

2. Northwestern Yeast Company, 1750 North Ashland Avenue, Chicago, Illinois.

Yeast Foam Tablet Powder to universityies for 20 cents per pound in quantities up to 10 pounds.

3. Vitamin Food Company, Inc., 122 Hudson Street, New York, New York.

Vita-Food Dried Brewer's Yeast - No. 2 Green Label, a straight dried brewers yeast with hop taste, 1 pound cans at $3.90 per dozen (approximately 32-1/2 cents per pound). Prices F.O.B. New York City in quantities of less than 100 pounds. The prices are relatively lower on larger quantities. (University of Illinois)

Muller, H.J. Seeding with yeast.

In place of the usual method of allowing drops of yeast to fall into the bottle from a pipette or sprinkling crumbs of yeast, it saves time and ensures more even distribution if one makes up a very thin suspension of the yeast in water, and then sprays this through a simple atomiser, such as is used for spraying fixative on charcoal drawings. In this way a great number of cultures may be seeded at once en masse.

Control of mites and molds

Spencer, W.P. Life history of the laboratory mite. The adults live in the culture medium and do not crawl over the inside of the culture bottle or on pupation paper as do the non-parasitic mites. The males are much smaller than the females, and may be seen mating with them; both have a squat appearance in contrast to the long, thinner non-parasitic mite. The eggs are laid in the culture medium and the young mites after several days to a week metamorphose into the migratory stage. These are brownish in color, just visible to the naked eye, and are extremely active. They crawl up out of the culture medium onto the sides of the bottle, and readily penetrate cotton plugs or other stoppers. They are constantly on the move and may travel at least several feet. During the course of this migration they attach to any insect with which they come in contact, and sink the mouth parts into the insect. On Drosophila they tend to attach themselves most frequently to the legs, particularly the proximal joints, to the wings, and about the genitalia. However, no part of the fly is immune and in heavy infestation literally hundreds of mites may attach to a single fly. Most of these mites leave the fly in a week or ten days after attachment, then grow to the adult stage in the culture medium and reproduce.

In fast breeding species of Drosophila such as melanogaster or simulans it is quite feasible to use mite infested parents and by taking their first offspring (providing temperature and other culture conditions have been optimum) get rid of the mites in one generation. This assumes that precautions have been taken to keep any new migrants from getting into the culture bottle. The life cycle of these species is more rapid than that of the mites and it seems that mites which have become firmly attached to one host do not leave for a second host.

In slower breeding species the best procedure is to transfer parents one to several times until they are finally free of the parasitic stage. One transfer is not always sufficient as a heavily infested fly may carry some of the parasitic stage for over two weeks before they leave, and in the meantime the mites which were first to leave will have produced a new generation of the migratory stage.

Spencer, W.P. Mite and mold control 1) The life cycle of mites seems to be less affected by low temperature than is that of flies. Hence during a mite infestation it is important to provide the optimum breeding temperature for each species of Drosophila and for all stock cultures suspected of being exposed to infestation. In general temperatures of 24 C to 26 C are optimum for most species, with certain exceptions noted in the literature. Funebris, hydei, immigrans do better at 24 C, melanogaster, simulans, carribea at 25 C or 26 C.

2) Rapid turnover of stocks with immediate discarding of all old stocks. During periods when mites are present all old

stocks are placed in a dry sterilizer and heated sufficiently to kill mites and eggs before bottles are washed. This seems a surer way of killing all mites than using a disinfecting solution on bottles.

3) Flies to be used as parents for new stocks are placed in small vials for one week to ten days, even though they appear to be free of mites. Then they are transferred to fresh stock culture bottles of standard size. During this period the mites which may have been present on the flies will have left them to breed in the culture medium, but a new generation of the migratory stage will not have come on.

4) All cultures, both the vials, and final stocks are kept standing in shallow metal trays containing Lysol solution, 1 part to 200 parts water. These trays are placed in incubators (see below) of 40 cubic foot capacity. As strong carbolic acid fumes are detrimental to flies it is is important to use as weak a Lysol or carbolic acid solution as will be effective. The above solution, 1:200 is not detrimental in enclosed incubators with evaporating surfaces of 1 square foot to two cubic feet of air space and no ventilating fan.

Tests of the following as possible liquid media for the control of mites were made; coal oil, 10% sulphuric acid, No. 10 light motor oil, 5% copper sulphate, Lysol. The migratory stage crawls readily through or on the surface of all of these except Lysol, which is very effective as a lethal agent. They also crawl readily through a band of vaseline. Many mites were then immersed in various concentrations of Lysol; the cessation of movement of all appendages was taken as the killing time.

Table

Dilution of Lysol	Killing time, All mites dead in
1: 40	5 minutes
1: 80	10 minutes
1: 160	15 minutes
1: 320	50 minutes

5) When a mite infestation seems to be entirely cleared up a fair sample of culture bottles from various incubators should be kept over a long period of time (isolated of course) as tests. The adults of the parasitic mite can readily be seen in such culture bottles and distinguished from the non-parasitic species of mites which may be present, but which can hardly be considered a pest. The parasitic mite in the adult stage is larger and with a squatty body, the non-parasitic mite is long and with long white hairs on the body.

6) Assume that any shipment of flies from another laboratory contains mites; of course this works both ways. It has been my experience that flies taken in nature seldom harbor the laboratory mite. In a considerable amount of collecting I have found them only once. Of course in mite-infested laboratories stray flies will likely be infested.

7) During a period of mite infestation all apparatus used in handling flies and all table tops etc. should be frequently wiped clean with Lysol or other sterilizing media, and it should

be kept in mind that mites can easily be passed from bottle to bottle by handling.

Most of the trouble with molds can be avoided by starting cultures at the optimum temperature until larvae are present. Of course in slow breeding species such as sulcata or repleta parent flies should be matured in small vials for a week to ten days. If a multiple mutant stock, or any one difficult to carry becomes infected with mold or harmful bacterial growths, flies to be used as parents from this stock may be kept for a few days in a vial with a hardy culture of some other species. Here the larvae of the second species keep down the mold and the flies of the first stock have a chance to mature in a mold-free environment. Assume that mold spores will be spread by infected etherizing bottle, or any other piece of apparatus used in manipulating flies and take necessary precautions to prevent this.

Demerec, M. Control of mites. As a preventative measure against the spread of mites we are keeping stock cultures (and also all other culture bottles which are used during a long period) standing in a weak soap solution. For this purpose shallow (2 inches or 5 cm high) galvanized iron trays are used. These are made to order to fit our shelves (usually 12 x 36 x 2 inches). In case any of the cultures is infected with mites the soap solution prevents their spread to adjacent cultures and keeps the infection under control. Some of our trays have been in use for over five years without any sign of wear. The initial cost for trays, therefore, is spread over a long period. Mites can also be controlled effectively by avoiding accumulation of old culture bottles and by wiping frequently, shelves and tables, with carbon tetrachloride or kerosene. (Copied from DIS 2:61).

Gottschewski, G. Control of mites. Beim Auftreten von Milben werden Kulturflaschen und Abstellregale regelmässig mit einer 5% igen Sagrotan-Lösung abgewaschen. Ausserdem werden die vermilbten Kulturen 2 Tage nach ihrem Ansetzen erneut in frische Flaschen umgesetzt. Die Milben bleiben fast vollständig im Futter der alten Kultur und die umgesetzen Fliegen sind Milbenfrei. Dadurch ist es gelungen, die Milben vollständig zu vertreiben.

Gowen, John W. Control of mites Three or four years ago I would have said that the control of mites was relatively simple. At the present time, however, in view of my experience of the past two years, I do not consider it quite so simple - although entirely possible. My method of control consists in using cotton-stoppered bottles and in transferring pairs as soon as they hatch. This method repeated three or four times has, in every case, freed the culture of mites. Using paper caps it does not seem possible to control mites by this

technique as they are apparently able to invade the bottle by way of the space left between the neck and the cap. The place where the bottles to be freed of mites are kept must, of course, be protected against contamination from cultures carrying mites.

Kyoto Laboratory Control of mites — As a preventive method against the spread of mites, several larvae and pupae in the culture are put into a sieve with fine meshes, and washed with rapid running water, After the measure, they are transferred to a fresh culture bottle.

Shipman, E.E. Ridding cultures of mites. — If not too many cultures are involved the following plan might be followed. Larvae are bathed in 70% alcohol for about one minute, dipped in water, and then put on fresh food. Most of the larvae survive the treatment. The writer used this method of eliminating mites from personal stocks three years ago and has seen no mites in the stocks since that time. (University of Illinois).

Columbia University Laboratory Mites and molds — For keeping down mold, we have been using 0.1% Nipagin-M with both banana and cornmeal formulae and have completely eliminated mold with no effect whatever on viability. This was determined by careful experimental counts.

For cleaning stocks of mites a piece of paper on which larvae have pupated may be completely immersed in alcohol (70%) for two minutes, dried, and placed in a clean bottle to hatch. This is much simpler and more efficacious than immersing individual flies.

Crew, F.A.E. Mites and mold — The addition of Nipagin M has proved a satisfactory protection against mold.

Two attacks of mites in vial cultures (but none in stocks) have been experienced. On such occasion the parasites were eliminated by segregating affected cultures and avoiding contamination. The mites were observed to enter clean cultures through the crevices often formed by the muslin coverings then used over cotton wool stoppers. When the use of muslin was discontinued, the spread of mites was rapidly reduced. Instruments coming in contact with affected cultures were sterilized after use.

Glass, B.H. Control of mold and mites. — In combatting a severe infection of mold in Drosophila cultures, it has been found helpful to hold individuals for two or three days in vials of food containing 0.2% formaldehyde, added when the food is prepared. Flies can live for several weeks in such

vials, inoculated in the usual manner with yeast; but larvae do not develop after hatching; and mold growth is inhibited. These vials are also very useful for holding individuals to br mated at some future time. Before transferring to fresh food, the flies are given a bath in a watch-glass of 70% alcohol for 2 to 3 minutes; then dried on filter-paper. Flies will stand a considerable immersion in alcohol with no permanent ill effects. They may be handled readily with brush and forceps. The alcohol bath treatment is also effective in freeing flies from mites. Larvae are especially easily cleaned in this way, the mites coming off at once; whereupon the larvae may be touched on filter-paper, and transferred at once to the food, using a long-handled needle, to which they gently adhere.

Schott, R. Mites and molds. When mites appeared in our cultures last summer all shelves and incubators were washed with phenol solution, pupae were isolated and brushed free of mites. Then rapid transfer of cultures followed for several generations. All old bottles were immediately soaked in phenol solution or boiled.

To prevent mold, cover surface of media with 10% alcohol, drain off and seed with yeast.

Parker, D.R. Moldex-A as a mold inhibitor. Tests were run recently to find a substance to inhibit the growth of mold. The compounds tried out were Moldex-A, Nipagin-M, and Nipagin-T. These were added to our regular banana food in the ratio of .15 grams of anti-mold substance to 100 c.c. of food. Twenty vials were made of each of the above compounds, as well as twenty vials of plain food.

One half of the vials were inoculated heavily with mold, and the other half left uninoculated. One pair of flies was placed in each vial. Moldex-A was the most efficient in the prevention of mold. However, in the uninoculated series, the Moldex vials gave a slightly lower yield of flies than did the plain food. Egg counts were then run to see the possible effect that Moldex might have on hatchability. Out of approximately 3000 eggs, 98.7% reached the adult stage. This is about 7% higher than the usual hatch on plain food at a cost of about $1.50 per pound. (Copied from DIS-4: 65).

Shipman, E.E. Mold Preventatives (Preservatives). Th. Goldschmidt Corporation, 147 Waverly Place, New York City, New York, has several different preservatives which would probably serve to prevent mold. Nipagin M has been reported in the literature but it is chemically pure and therefore more expensive than Nipagin T, the technical grade. Nipagin M is listed at $1.00 per ounce and $8.00 per pound. Nipagin T is listed at 60 cents per ounce and $4.70 per pound. They have both been reported as being used in food cultures for Drosophila in 0.15%. In a communication from the company it is recommended that Nipagin T

be used in the amount of not over 0.1% since a higher amount may prevent fermentation. The preservatives should be boiled for three minutes in the water used in making the preparation.

I have no personal experience with these preservatives but plan to test Nipagin T and Nipakombin A, another of their preservatives, as soon as possible. (University of Illinois).

Amherst Laboratory Restriction of use of Nipagin M. The use of Nipagin M to stop the growth of molds in culture bottles has been found, also, to delay the development of D. melanogaster. At 28° with 0.07 gm Nipagin to 60 cc. food, the duration of the egg-larval period, in a selected stock, was increased by more than three hours. In quantitative phenogenetic studies the use of Nipagin should, therefore, be restricted. It may be useful, however, for observing the effect of increased time of development on quantitative characters.

Miscellaneous methods

Parker, D.R. Method of carrying stocks. The early method of carrying stocks in this institution was to keep them in bottles, merely shaking them from the old one into the new one at each change, with occasional etherization and examination of them. Last year, however, we adopted a new method which seems to be more efficient. The stocks are now carried in vials, keeping one old vial and mating three new ones at each change. The four are fastened together by means of a rubber band to which is attached the tag label. The flies are etherized by means of the mass method of Altenburg.

The advantages of this system are: (1) The flies are examined at each change, and (2) by making 3 new vials the chances of loss by contamination are greatly reduced. It is possible by this method to practically rid all of the stocks of mites, provided there are no adverse conditions of temperature.

This method takes a bit more time than the older one, but it will perhaps repay the loss with better stocks. (Copied from DIS-4: 65).

Muller, H.J. Labelling of stock cultures. In place of the usual practice of Drosophila laboratories of pasting a label on each stock culture and writing the name of the stock anew at each transfer, I have for many years found it much quicker and less subject to error, if the designation of the stock is written once for all in ink or India ink on both sides of a cardboard tag which is affixed through its string to a rubber band that passes around the neck of the culture vessel. This tag is transferred to the new vessel when the flies are transferred, and it is best to have a separate tag for each culture vessel. (Copied from DIS-3: 52).

Hoover, Margaret E. Maintenance of stocks. Drosophila stock bottles are kept at Cold Spring Harbor in galvanized tin trays filled with soap solution to prevent spreading of mites, and are placed on wooden shelves constructed for this purpose. These shelves measure 36 x 11-1/4 inches. Four bottles can be placed in a row the width of the shelf and about fourteen bottles lengthwise without any crowding. Our cabinets have ten shelves each from the floor to the ceiling so that one cabinet will hold 140 cultures.

Following H.J. Muller's suggestion (DIS-3, 1935) stocks are labeled by using small celluloid or heavy cardboard tags on which the labels are written; an elastic band is looped through a hole punched in each tag and slipped over the neck of the bottle. With the transfer of cultures, the tags are dipped in carbon titrachloride to prevent the spread of mites and transferred to a new bottle. Heading each row of four bottles on

the shelves is a labeled celluloid tag hooked over the end of the tin tray by a card holder. The stocks are arranged on the shelves according to the listing in DIS.

Stocks are kept in 1/4 pint milk bottles. Paper milk caps with a flap are used as stoppers. Experience indicates that virilis stocks go better in small containers.

Muller, H.J. Supplying vials with paper. When numerous small vials have to be handled it is time consuming to prepare and insert paper for each one, although the presence of paper is helpful. For this purpose it is convenient to use white confetti, which can be purchased already prepared in considerable quantities. This is sifted between the fingers into the cultures en masse, as they stand still uncovered after having been seeded with yeast. (Copied from DIS-3: 52).

Brierley, Jean Method for handling vials in transfers of Active flies. I have found 30 by 100 mm. vials well suited to single pair matings. In my work it is necessary to transfer these pairs to fresh vials frequently, without etherization. The size of the vials makes it hard to hold their mouths in exact apposition during the transfer. To eliminate the chance of their slipping, I use a c-shaped metal band about 15 mm. wide, which slips over the end of the vial, protruding half its width beyond the mouth. The other vial fits into this half of the ring, and the two vials are held firmly together. Any metal will do, as long as it is fairly thin and quite flexible. It must be elastic enough to cling closely to the glass and adjust itself to the variations in sizes of vials. (University of Michigan).

Kaiser, S. Transfer of flies. We have obtained our best results in transfers by inserting into the food, cones made of towelling paper. Etherized flies come to in these cones without getting wet or dirty. The larvae creep up on the paper and pupate on it.

Stern, Curt Feeding flies kept temporarily in vials. Food (water-molasses-agar, filled into Petri-dishes, before cornmeal is added to the mixture used for bottles) is placed on a parallelogram-shaped piece of cardboard (size 42 x 25 mm. altitude 20 mm., thickness 1 mm.). These cardboard pieces are cheaper than paper spoons and are ordered per 5000 or 10,000 from stationery stores. The shape of the piece eliminates the danger of crushing flies (method suggested in 1931 by Dr. F. Koller, then in Berlin-Dahlem).

Oliver, C.P. Protection of cultures from mice. Mice have made necessary the use of a substitute for cotton plugs for cultures which are left outside a closed chamber. Paper milk