PROGRESS REPORT

for

MUTAGENIC EFFECT OF RADIONUCLIDES INCORPORATED INTO DNA OF DROSOPHILA MELANOGASTER

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Introduction:

Previous work on this project, as reported in our Progress Report 1978-1979, has shown a significant difference in the mutation frequency of sex-linked recessive lethals associated with the position in the DNA molecule in which tritium decay occurs (see Table 1, reproduced from Table 3 of previous report). The local effect of tritium decay has been interpreted as being due to the transmutation of tritium to helium-3 at specific sites of incorporation; the radiation effects at various sites within the DNA molecule are presumed to be equivalent. Work during the past year has been in two areas: first, to show that the tritium precursors listed in Table 1, column 1, are metabolized and that the form(s) found in the DNA molecule are actually those listed in column 2; and second, to develop the alcohol dehydrogenase system of recovering mutations so that it can be used to distinguish between base changes and chain breakage events that may lead to formation of deletions. The rationale behind the use of, and need for, this system is described in the Renewal proposal.

Fidelity of incorporation of tritium-labeled precursors:

DNA from adult Drosophila males who were fed as larvae with 8-^{3}H-deoxyguanosine was analyzed chromatographically to determine the fidelity of the tritium label. In the first series of experiments seminal vesicles and testes from approximately 25 treated males were hydrolyzed in a solution of 60% $HClO_4$ spiked with adenine and guanine standards for one hour at 100°C. The hydrolysate was spotted onto a two dimensional paper chromatogram (Whatman 3mm) and chromatographed as follows: solvent system (I) 1-butanol-NH_3 (specific gravity 0.88) - H_2O (85:2:13,v/v) and solvent system (II) methanol-concentrated HCl-H_2O (70:20:10, v/v). Following elution of the guanine and adenine spots in 0.1N HCl and spectrophotometric

determination of the percent recovery of these standards, eluants of the spots were analyzed by liquid scintillation spectroscopy. From three successive experiments the ratio of tritium cochromatographing with guanine/adenine was determined to be 14.4 $\pm$5.3. Of the total counts collected from the adenine and guanine spots it appears that 93 $\pm$2% of the tritium cochromatographed with the guanine while 7 $\pm$ 2% of the tritium cochromatographed with the adenine.

In the second series of experiments DNA was extracted from the seminal vesicles and testes of about 75 treated males using extraction procedures of Aaron and Lee (1978). The DNA was lyophilized, and hydrolyzed for one hour at 100°C in a solution of 60% $HClO_4$ spiked with adenine and guanine standards. The hydrolysate was chromatographed and analyzed in a similar manner as described for the first series of experiments, although only two replicates were performed. The mean ratio of tritium cochromatographing with guanine/adenine was determined to be 59.1. From the total counts collected from the adenine and guanine spots it appears that 98.3% of the tritium cochromatographed with the guanine while 1.7% of the tritium cochromatographed with the adenine. The difference in the amount of tritium cochromatographing with the guanine versus adenine standards observed when comparing the two series of experiments, may be explained by a slight diffusion of standards in the tissue hydrolysis procedure (34% recovery of guanine; 55% recovery of adenine) while the procedure in which DNA was first extracted gave a cleaner separation (60% recovery of guanine; 82% recovery of adenine). The importance of these results is that they indicate that tritium introduced as 8-^{3}H-deoxyguanosine is apparently not metabolized to a large extent to 8-^{3}H-adenine, or is not incorporated in this form into the germ cell DNA. We would therefore have to associate the mutagenic activity following exposure to 8-^{3}H deoxy-guanosine with the 8-^{3}H-guanine alone (cf Table 1, column 2). The protocol described is applicable to analysis of all DNA precursors by changing the chromatographic solvents. Systems have been devised for recovery of all bases suspected of being labelled with tritium. During the coming year, males from all incorporation experiments will be analyzed.

Detection of alcohol dehydrogenase (ADH) deficient mutations in both F_1 and F_2:

Our objective during the past year has been to develop a system for use in our laboratory for recovering forward mutations, both transitions and deletions, at the alcohol dehydrogenase (Adh) locus on the second chromosome of Drosophila melanogaster. Since we anticipate that a portion of the mutations induced as local effects of transmutation of ^3H incorporated in the DNA will be propogated as mosaic F_1 individuals, a method of recovering mutations that are F_1 mosaic must be devised. It is also desireable to incorporate in the experimental design a secondary dosimeter in the form of a mutagenic test with known response to different doses of mutagen; in our case the sex-linked recessive lethal test was selected.

An experimental design which encompasses all these features has been worked out during the past year in a series of four preliminary experiments involving seven independent treatments of our (stock-C) males (see Figure 1) with ethyl methanesulfonate (EMS). In this scheme, males from each treatment vial are randomly selected for mating to one of three types of virgins. Males crossed to stock-Q^{n4} virgins yield automatic virgins in the F_1, each of which carries a haploid complement of treated chromosomes. The F_1 ♀♀ are individually outcrossed following the protocol for detection of sex-linked recessive lethals. Males crossed to stock-O^{n4} virgins yield only ♀♀ in the F_1, all of which are heterozygous for one of the treated second chromosomes and the b Adh^{n4} pr chromosome; the sex-chromosome complement is irrelevant. For these preliminary experiments the F_1 ♀♀ progeny of the O^{n4} cross are aged at least 4 days before being screened. Exposure to the selective agent 1-pentene-3-ol was by flushing the vial for 4 minutes with air that had passed through a 1% aqueous solution of the alcohol (Aaron, 1979). Homozygous b Adh^{n4} pr flies survive this screening procedure, but the relation of degree of mosaicism for the Adh gene to survival has yet to be established. Similarly, the level of ADH activity throughout the organism (as for instance 5% for Adh^{n5}) which will lead to lethal accumulation

of the toxic ketone product of pentenol metabolism needs to be determined. For these experiments the parameters of the screening will have to be carefully controlled (see proposal for renewal).

Males crossed to stock-M ($M) virgins produce only males in the F_1, due to bobbed-deficiencies of both the attached-X and the Y^{Lc}. These males are heterozygous for the treated second chromosome and for one of the $M second chromosomes. Neither the treated second nor the second chromosome derived from the $M parental female carried any visible phenotypic markers in these preliminary experiments; therefore, the origin of the chromosome which carries a mutation allelic to $\underline{Adh^{n4}}$ (recovered in the F_2) is unknown. Females to be used in place of the $M females in this scheme are being produced as shown in Figure 2. These females will be homozygous for the brown-Dominant ($\underline{bw^D}$) allele, and the second chromosome derived from them will be distinguishable from the unmarked treated second of the $C males.

Production of the $\$Q^{n4}$ and $\$O^{n4}$ automatic virgins for use in these experiments is outlined in Figure 3. The stocks are more fragile than those previously used for routine mutagenicity testing, and some difficulty has been encountered in expanding them to yield the large numbers of vigorous females needed for experiments of this size. In order to cope with some of these difficulties we are working on humidity and age control as well as modifying collection procedures so as to reduce mating of virgins with males other than those treated. The least fertile crosses have been those with $M virgins. As mentioned above, these are being replaced in the design by brotherless females.

Our immediate objectives in these preliminary experiments were to recover mutants in both the F_1 and the F_2 and establish the use of the sex-linked recessive lethal test as a secondary dosimeter, as well as to determine the optimal cross size for these population-density sensitive flies and examine their brooding potential for storage experiments. Of the 24,153 F_1 individuals heterozygous for $\underline{Adh^{n4}}$ and

the treated second chromosome, 8 survived two days following screening; 4 died before producing progeny, and the remaining 4 are being placed in stock over $\underline{CyO^{nB}}$.

Aaron (1979) has noted that the frequency of recovered mutants is not affected by the method of performing the experiment (i.e., accumulation versus single large experiment). The frequency observed in the four combined experiments reported here (0.016%) is in line with that observed by Sofer and Hatkoff (1972) following EMS treatment (0.012%). The frequency of spontaneous mutations at this locus was not measured in these experiments, but has been reported as 0.00043% (Aaron, 1979) and 0.00065% (Mukai and Cocherham, 1977).

Two mutants have been recovered in the F_2. The number of chromosomes tested in the F_2 is uncertain, since we do not know the probability-factor of a male mating in the F_1 cross. It would be safe to say that the probability of detecting a mutation in the F_2 of experiment 1 through 3 was less than that probability in experiment four, since the average number of F_2 progeny per F_1 male in the last experiment was about three times that in any of the first three experiments. We will standardize on the mating conditions used for the fourth experiment, and do reconstruction experiments to determine the reproduction efficiency of individual males under these conditions.

The sex-linked recessive lethal frequencies in the triple treatments of experiment 2 were 26.1, 22.4 and 29.6 per cent after the first retest generation. These frequencies would be expected to drop slightly in the second retest and approach even more closely the value of 20.4% expected based on our EMS dose-response curve (Aaron and Lee, 1978). This comparison was made using treated males with the same X-chromosome as those used in the paper referenced, and females with the same X's. The males and females used here differ from those used earlier in their autosomal genetic constitution, and the treated male carries the unmarked Y^{Lc} in

place of $y^{+}Y^{L}B^{s}$. The influences of genomic variance not directly associated with the sex-linked recessive lethal test do not appear to have a large effect on the results of this test. We plan to do a controlled experiment assessing the influence of the homozygous b Adh^{n4} pr chromosome on the outcome of this test.

Conditions in the preliminary experiments have been varied with regard to the parental population density and extent of brooding. While we have been able to optimize conditions for the F_1 cross which yields mutants for screening in the F_2, new conditions for the parental crosses are being investigated to compensate for their lower fertility as well as their humidity and age sensitivity. The fifth experiment, which will include a substitute female in the least fertile of the P-crosses, is underway.

The scheme which has been devised for placing Adh^{n} mutants into stock is shown in Figure 4. Once the mutants are in stock, they will be analyzed as to the specific nature of the induced mutation (see Renewal Proposal).

LITERATURE CITED

Aaron, Charles S. 1979. X-ray induced mutations affecting the level of the enzyme alcohol dehydrogenase in Drosophila melanogaster: Frequency and genetic analysis of null-enzyme mutants. Mutation Res., 63:127-137.

Aaron, C. S., and W. R. Lee. 1978. Molecular dosimetry of the mutagen ethyl methanesulfonate in Drosophila melanogaster spermatozoa: Linear relation of DNA alkylation per sperm cell (dose) to sex-linked recessive lethals. Mutation Res., 49:27-44.

Sofer, W. H., and M. A. Hatkoff. 1972. Chemical selection of alcohol dehydrogenase negative mutants in Drosophila. Genetics, 72:545-549.

Mukai, T., and C. C. Cockerham. 1977. Spontaneous mutation rates at enzymes loci in Drosophila melanogaster. Proc. Natl. Acad. Sci. (USA), 74:2514-2517.

Table 1. Mutation frequency per decay of tritium incorporated in specific sites of the germ cell DNA (from Progress Report 1978-79).

TABLE 1[b]

Treatment Compound	Form of ^{3}H incorporation in DNA	slrl per tritium disintegration ($X10^{-5}$)[a]
Deoxycytidine (G-^{3}H)	(G-^{3}H)dC (G-^{3}H)dT	0.64
Deoxythymidine (Me-^{3}H)	(Me-^{3}H)dT	1.45
Deoxycytidine (5-^{3}H)	(5-^{3}H)dC	3.18
Uridine (5-^{3}H)	(5-^{3}H)dC	3.08
Deoxyguanosine (8-^{3}H)	(8-^{3}H)dG (8-^{3}H)dA[c]	4.49
Uridine (6-^{3}H)	(6-^{3}H)dC (6-^{3}H)dT	5.30

[a] Sex-linked recessive lethal frequencies before and after storage were corrected for their appropriate control frequencies (See Table 1) and the difference between them was divided by the number of tritium disintegrations during storage.

[b] Reproduced from Table 3 of previous Progress Report, for reference.

[c] Recent work has shown this not to be a major form of incorporation (see text).

Figure 1. Experimental design of crosses made with treated males to allow 1) sex-linked recessive lethal test as a secondary dosimeter, 2) screening for Adh-negative mutations in the F_1, and 3) screening for Adh-negative mutations in the F_2 to allow recovery of mutations which were mosaic in the F_1.

Figure 3. Circle-crosses used to produce automatic virgins (♀♀) for scheme outlined in Figure 1.

Figure 2. Circle-cross used to produce brotherless females with the bobbed-deficient attached-X; for replacing $M ♀♀ in Figure 1.

Figure 4. Genetic scheme for placing chromosome carrying induced Adh-negative mutation into stock over $Cy0^{nB}$.

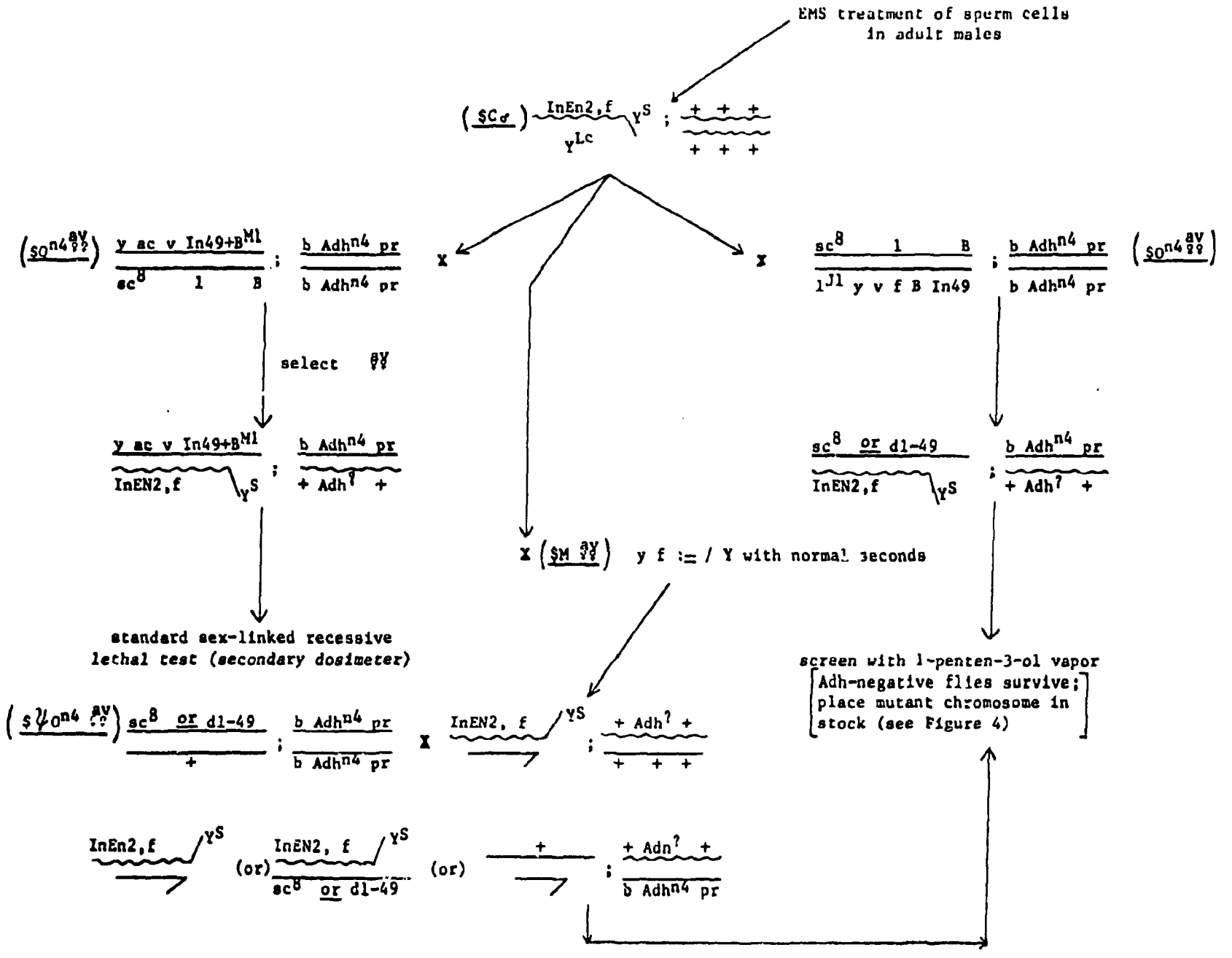
EMS treatment of sperm cells in adult males
select
standard sex-linked recessive lethal test (secondary dosimeter)
y f ⁄ Y with normal seconds
screen with 1-penten-3-ol vapor
[Adh-negative flies survive; place mutant chromosome in stock (see Figure 4)]
(or)
(or)

FIGURE 1

bw^D Circle Cross

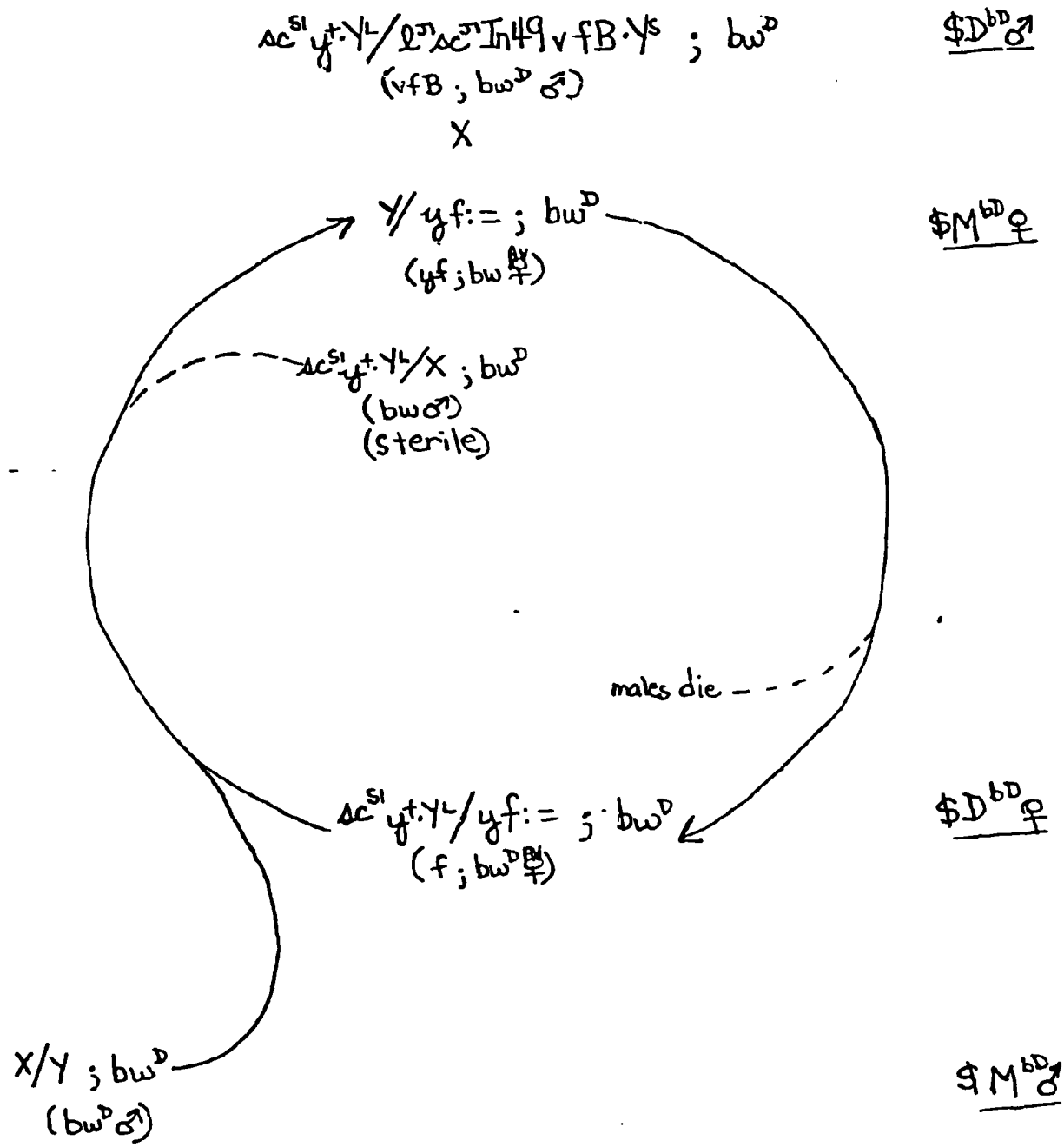

FIGURE 2

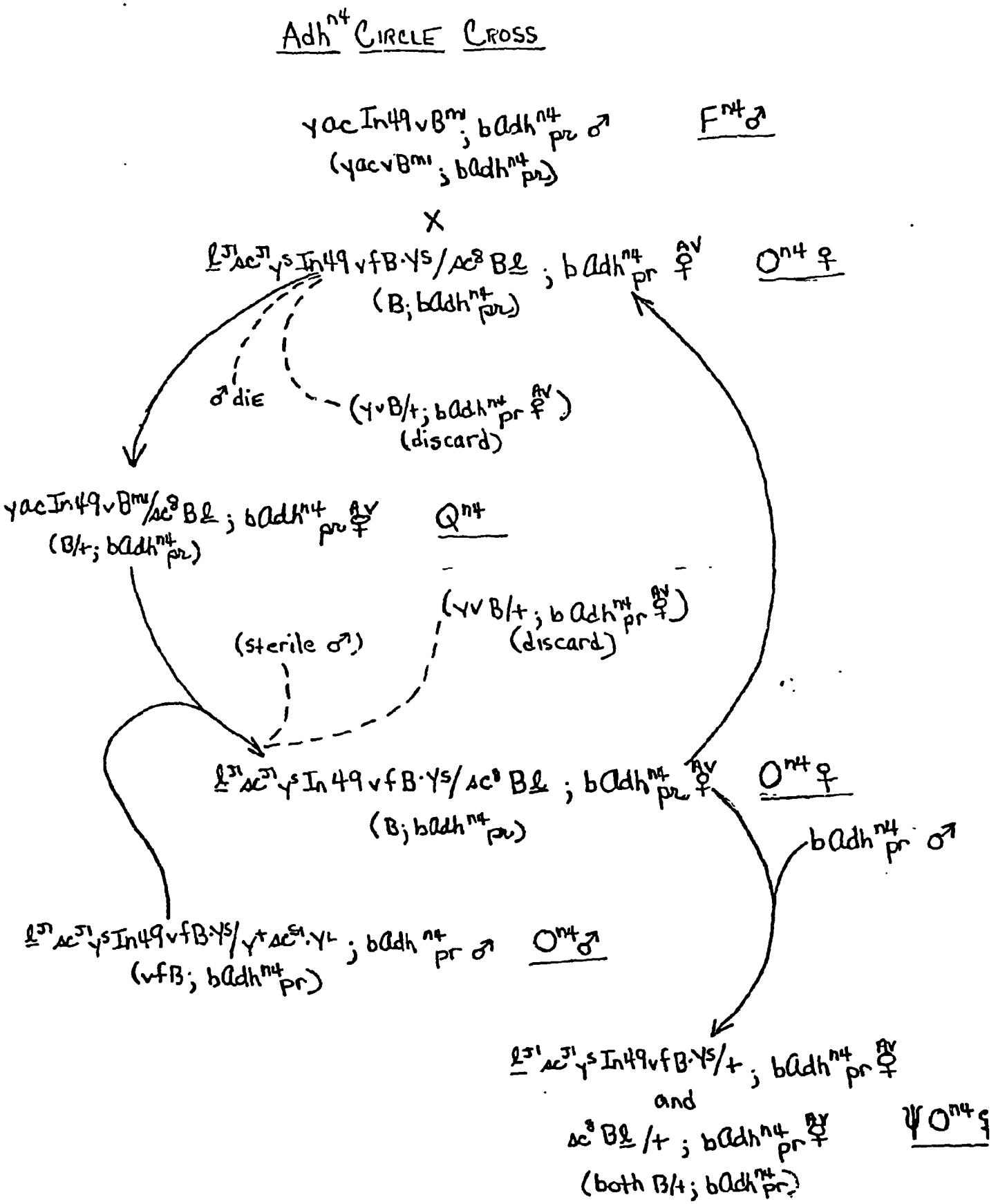
Adh^n4 CIRCLE CROSS
yacIn49vB^m1 ; bAdh^n4 pr ♂
(yacvB^m1 ; bAdh^n4 pr)
F^n4 ♂
X
l^J1 sc^J1 y^s In49 vfB·Y^s / sc^8 Bl ; bAdh^n4 pr ♀^AV
(B; bAdh^n4 pr)
O^n4 ♀
♂ die
(Y vB/+; bAdh^n4 pr ♀^AV)
(discard)
yacIn49vB^m1/sc^8 Bl ; bAdh^n4 pr ♀^AV
(B/+; bAdh^n4 pr)
Q^n4
(sterile ♂)
(Y vB/+; bAdh^n4 pr ♀^AV)
(discard)
l^J1 sc^J1 y^s In49 vfB·Y^s / sc^8 Bl ; bAdh^n4 pr ♀^AV
(B; bAdh^n4 pr)
O^n4 ♀
bAdh^n4 pr ♂
l^J1 sc^J1 y^s In49vfB·Y^s/y^+ sc^S1·Y^+ ; bAdh^n4 pr ♂
(vfB; bAdh^n4 pr)
O^n4 ♂
l^J1 sc^J1 y^s In49vfB·Y^s/+ ; bAdh^n4 pr ♀^AV
and
sc^8 Bl/+ ; bAdh^n4 pr ♀^AV
(both B/+; bAdh^n4 pr)
Ψ O^n4 ♀

FIGURE 3

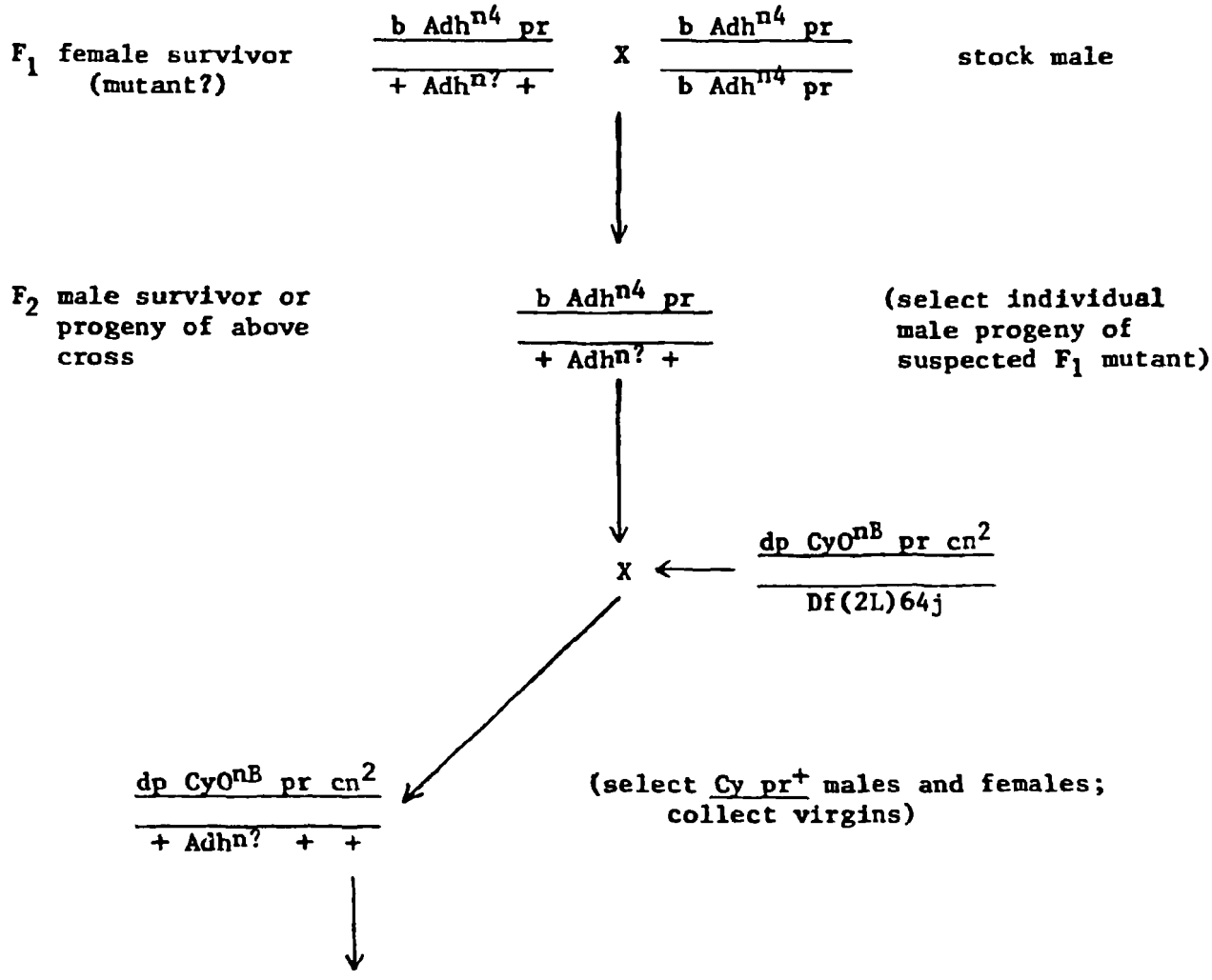
F1 female survivor (mutant?)
b Adhn4 pr / + Adhn? +
X
b Adhn4 pr / b Adhn4 pr
stock male
F2 male survivor or progeny of above cross
b Adhn4 pr / + Adhn? +
(select individual male progeny of suspected F1 mutant)
X
dp CyOnB pr cn2 / Df(2L)64j
dp CyOnB pr cn2 / + Adhn? + +
(select Cy pr+ males and females; collect virgins)
Sibling crosses will produce either a stock balanced over CyO or progeny which carry the homozygous mutation (Adhn?) and can be used to establish the homozygous stock.

FIGURE 4