

Table 1. Composition of the modified Shields and Sang medium (in mg/100 ml)

MgSO ₄ · 7H ₂ O	513	monosodium malate · 2H ₂ O	95
CaCl ₂ · 2H ₂ O	116	monosodium α-ketoglutarate	42
KCL	313	disodium fumarate	8
NaCl	86	disodium succinate · 6H ₂ O	14
NaH ₂ PO ₄ · 2H ₂ O	88	monosodium pyruvate	2
KHCO ₃	18		
monosodium L-glutamate	76	L-isoleucine	5
L-aspartic acid	9	L-leucine	9
L-threonine	15	L-tryosine	3
L-serine	17.5	L-phenylalanine	3
L-asparagine	15	L-β-alanine	12
L-glutamine	30	L-histidine	19
L-proline	40	L-tryptophan	10
L-glycine	17.5	L-arginine	21
L-α-alanine	75	L-lysine	7
L-valine	9	L-cystine	1
L-methionine	15	L-cystine	6
glucose	30	TC yeastolate (Difco)	200
trehalose	80		

Double distilled water ad 85 ml. 10 ml foetal bovine serum (Flow Laboratories) was added and the pH brought to 6.9 with 1 N NaOH. The volume was made 100 ml with A.bidest and aliquots were frozen on dry-ice-acetone and stored at -20°C.

incubating glands after various periods of preincubation, for 15 min in medium containing 0.05 μ Ci/ μ l of the precursor. All glands tested after 10 min, 6 hours and 18 hours preincubation displayed thymidine incorporation in 10-15% of the nuclei (Fig. 2) among which the various labeling patterns from only a few regions per chromosome labeled, to massive continuous labeling of the entire complement, are represented.

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There is no convincing evidence that EMS induces base substitutions at substantial frequency in *Drosophila*, even though it appears to do so in T₄ (Krieg, 1963 Genetics 48:561), whereas there is evidence that it produces chromosome breaks

(Fristrom, 1970 Ann. Rev. Gen. 4:325). Although temperature-sensitive mutations are produced in high frequency by EMS in *Drosophila* (Suzuki et al., 1967 Proc. Nat. Acad. Sci. 57:907), temperature-sensitivity per se is not sufficient to conclude a base substitution that renders a polypeptide inactive at restrictive conditions in *Drosophila*.

A simple genetic test has been used which identifies deficiencies in part or all of the white locus (Green, 1959 Zeitschrift für Vererbungslehre 90:375). w^{SP} homozygotes have mottled yellowish-brown eyes; w deficiency/w^{SP} trans heterozygotes are similar to w^{SP}/w^{SP}; but w point mutation/w^{SP} heterozygotes exhibit complementation to give a uniform reddish-brown color (Lindsley and Grell, 1967 Gen. Var. of *D. mel.*). Thus w deficiencies are clearly distinguishable from w point mutations.

4,340 EMS treated chromosomes (0.025 M EMS fed to males) which survived over w⁺y⁺Y were tested heterozygous to Df(1)N⁸ for mutations in the w - N region. 26 mutants were recovered as follows: 19 recessive lethals at the Notch locus, none of which included white; one rst semi-lethal; and six white recessive visibles, including three w, two w^a, and one w^e. All six whites when tested with w^{SP} were complementing, producing the uniform reddish-brown eye color. This test identifies all six white mutations induced by EMS as point mutations. Critical to this argument are 1) that the collection of mutants is not biased against large deficiencies of one or more bands, and 2) the technique can resolve true base substitutions as opposed to small deletions of part of the white gene. The extent to which the second holds is unknown to this author.