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Functional capacities of isolated salivary glands in a chemically defined medium.

Mid third instar salivary glands of Drosophila hydei were incubated for various periods up to three days in a chemically defined medium, the composition of which is given in Table 1. At various moments after the onset of incubation, the genome response to extracellular stimuli, the capacity for uridine and thymidine incorporation and the submicroscopic structure of the gland cells were studied and compared with that of glands under in vivo conditions.

Upon addition of 0.01 ug/ml ecdysterone after 12 hours of preincubation of the glands, a complete pattern of response, identical to

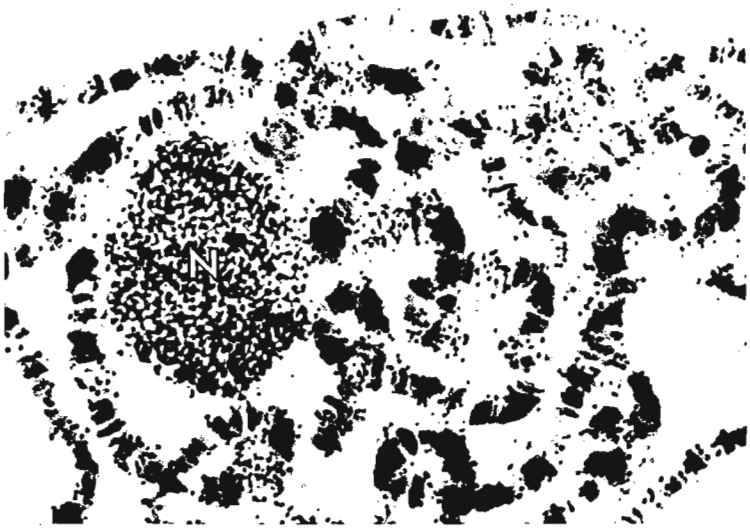

Fig. 1. Salivary glands were incubated in medium for 72 hours and subsequently transferred to medium containing 0.5 uCi ^{3}H-uridine/ul for 5 minutes. N, nucleolus.

that observed during normal development prior to puparium formation or after injection of the steroid into intact larvae, is observed. Within 15 min after the addition of the steroid, the first series of ecdysone specific changes in the puffing pattern are evident. At 4-6 hours after addition of the steroid to the medium the cells start secretion of the stored mucopolysaccharide product. The submicroscopic features of this process and the consequences for the structural organization of the cytoplasm of the gland cells are essentially equivalent to those observed to occur during normal development or after injection of the steroid into mid third in-

Fig. 2. Salivary glands were incubated in medium for 18 hours and subsequently transferred to medium containing 0.05 uCi ^{3}H-thymidine/ul for 15 min.

star larvae. The effect of ecdysterone has not been tested on gland kept in the medium for longer than 12 hours.

Autoradiographical and biochemical analysis of the incorporation of ^{3}H-uridine (spec.act.24 Ci/mM) following a 15 min pulse after 12 hours preincubation not only revealed a normal pattern of incorporation of the precursor into nuclear components, heavy labeling of puffs and nucleolus, but also a normal processing of the ribosomal precursor and an apparently normal spectrum of newly synthesized RNA species in sucrose gradients (Poels, in press). Autoradiographs of pulse labeled salivary glands after 72 hours of preincubation revealed heavy labeling of chromosomes and nucleoli (Fig. 1).

Incorporation of ^{3}H-thymidine (spec.act. 6.7 Ci/mM) was studied autoradiographically by

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

$MgSO_4 . 7H_2O$	513	monosodium malate. $2H_2O$	95
$CaCl_2 . 2H_2O$	116	monosodium α-ketoglutarate	42
KCL	313	disodium fumarate	8
NaCl	86	disodium succinate. $6H_2O$	14
$NaH_2PO_4 . 2H_2O$	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-phenylaline	3
L-asparagine	15	L-β-alanine	12
L-glutamine	30	L-histidine	19
L-proline	40	L-trytophan	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 uCi/ul 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.

Shellenbarger, D.L. University of Iowa, Iowa City, Iowa. Evidence that EMS induces point mutations at high frequency. There is no convincing evidence that EMS induces base substitutions at substantial frequency in Drosophila, even though it appears to do so in T_4 (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 $Df(1)N^8$ 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.