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Replication in *Drosophila* Chromosomes : Part V—Polytene Chromosome Replication after *in vitro* Culture of Larval Salivary Glands

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Salivary glands from mid and late 3rd instar larvae of *D. nasuta* have been cultured *in vitro* in a modified Poels' medium [Poels, C.L.M., Cell Differentiation, 1 (1972), 63] and the progression of polytene replication under different *in vitro* culture conditions have been analysed autoradiographically. Results show that mass culture (30 glands/300 µl medium) of salivary glands from mid 3rd instar larvae in presence of larval fat bodies provides optimal conditions. Under these conditions, even 48 hr after *in vitro* culture, a large number of polytene nuclei continue to replicate and in many nuclei, new cycles of polytene replication are initiated during this period. The patterns of ^{3}H-thymidine incorporation in different regions of polytene nuclei under these *in vitro* conditions are closely comparable to those seen in freshly excised glands from late 3rd instar larvae of *D. nasuta*. The feasibility of maintaining and initiating new cycles of polytene replication under the *in vitro* condition opens scope for a detailed analysis of the factors regulating polytene replication.

THE polytene nuclei in salivary glands of *Drosophila* larvae provide an elegant material for the analysis of chromosome replication and transcription. These nuclei undergo a fixed number of cyclic DNA synthesis without any intervening mitotic division phase so that the consecutive S-periods are separated only by inter-synthetic or G-periods[1]. The last polytenic replication in different nuclei occurs during late larval and early prepupal stages after which the glands are histolysed. Inspite of a large number of studies on polytene chromosome replication, some of the basic questions like the duration of polytene S-period or the precise sequence of replication of different chromosomal segments, have not been adequately answered. The major difficulty in obtaining information about these aspects is the asynchrony in the replication of different polytene nuclei of a salivary gland; this *in vivo* asynchrony is possibly due to the achievement of different levels of polyteny by different nuclei and their different rates of progression through the S-period. *In vivo* synchronisation of the replicating polytene nuclei at certain stages of replication cycle has been achieved by feeding the third instar larvae on food containing inhibitors of DNA or RNA synthesis[2,3]. However, soon after the release of the block, asynchrony in replication cycles rapidly sets in and also the larvae rapidly pupate, as a result of which it is difficult to determine the exact time sequence of different stages of the polytene replication cycle under these conditions. One possible way to circumvent the *in vivo* asynchrony is to establish an *in vitro* system which will allow one to synchronise the DNA synthesis in the polytene nuclei and to study the factors involved in its control. Attempts to maintain salivary glands of *Drosophila in vitro* have been made earlier[4-6]. However, detailed information about the *in vitro* culture conditions for maintaining polytene replication in salivary glands and the patterns of *in vitro* replication in polytene chromosomes has been lacking. We, therefore, felt that it will be worthwhile to establish the *in vitro* conditions under which a satisfactory level of polytene replication can be maintained. In this study we have used a relatively simple medium for *in vitro* culture of larval salivary glands and we show that under these conditions of culture, polytene replication proceeds normally. A preliminary account of this work was presented earlier[7].

Materials and Methods

A wild strain of *Drosophila nasuta* has been used for these studies.

Larval selection and culture — The larvae of *D. nasuta* were grown in standard cornmeal-agar-brown sugar-yeast food at 24° ± 1°C. The eggs were collected over brief intervals of 30 min. Under the culture conditions used, pupation in *D. nasuta* occurs at about 156h after oviposition. About 30 hr prior to pupation (mid third instar larvae) a black stripe appears at the base of the posterior pair of spiracles and the larvae at this stage will be referred to as the early black spiracle stage larvae. About 5-6 hr prior to spiracle eversion, the anterior pair of spiracles become brownish and these late larvae will be referred to as the late brown spiracle stage larvae. For initiating the *in vitro* cultures, salivary glands from larvae of either of these two ages were dissected out.

Culture medium and culture conditions — Poels' medium[8], which is a modification of Sheilds and Sang's[9] medium was used for the present study.

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This medium was further modified by the addition of yeast extract, bactopeptone (Centron, India), insulin (Boots, India) and substitution of the amino acids by lactalbumin hydrolysate (Sigma, USA). The composition of the medium is given in Table 1. To ascertain the optimal conditions for maintenance of polytene replication cycles, the following cultures were set up :

(1) Glands were dissected from the black spiracle stage larvae as above; sister glands were either mass cultured (30 glands/300 μl of medium) in a single cavity block or cultured individually in depression slides (1 gland/10 μl medium). The glands were pulse labelled for 10 min with ^{3}H-thymidine after 24 hr of *in vitro* culture.

(2) Glands dissected from early black spiracle larvae were transferred to medium with or without 10% bovine serum and mass cultured for 24 hr prior to pulse labelling with ^{3}H-thymidine for 10 min.

(3) The medium was preconditioned for 24hr with larval fat bodies along with brain ganglia complex from early black spiracle larvae. Freshly dissected glands from black spiracle stage larvae were transferred to this preconditioned medium and mass cultured for 24 hr prior to ^{3}H-thymidine pulse labelling.

(4) Glands from early black spiracle stage larvae were co-cultured in mass with larval fat bodies for 24, 48 or 72 hr, prior to ^{3}H-thymidine pulse labelling In such long term cultures the medium was replaced every 24hr.

(5) Glands from late brown spiracle stage larvae were co-cultured with larval fat bodies for 24 or 48hr prior to ^{3}H-thymidine pulse labelling.

The cavity blocks or the depression slides were covered with coverglasses, tightly sealed with vaselene and kept in a moist chamber at 24°±1°C.

To determine the frequency of replicating nuclei and the patterns of replication under different culture conditions, the glands were pulse labelled (10 min) with 500 μCi/ml ^{3}H-thymidine (sp. act. 26 Ci/mM, BARC, India) in an incomplete medium (devoid of yeast extract, bactopetone and lactalbumin hydrolysate). After labelling, the glands were squashed and processed for autoradiography with Ilford L4 emulsion, exposed in dark at 4°-6°C for 5 days and developed with Kodak D19B developer in the usual manner[10]. For each experiment, preparations from at least 5 pairs of glands were examined. Before scoring the ^{3}H-thymidine labelling patterns, the autoradiographic preparations from different experiments were coded to avoid any bias in scoring.

To estimate the proportion of polytene nuclei which continue to replicate and initiate new cycles of replication under the culture conditions used, another set of experiment was done with glands dissected out either from early black spiracle larvae or from late brown spiracle larvae. In each case, the glands from a large number of larvae of one age were dissected out and made into different groups. From early black spiracle stage the following groups were made.

(i) *0 hr pulse labelling* — Soon after dissection glands were pulse labelled with ^{3}H-thymidine (500 μCi/ml) for 10 min in the incomplete medium and processed for autoradiography as above.

(ii) *0 hr–10 hr chronic labelling* — A group of glands were transferred to complete medium (Table 1) supplemented with 2 μCi/ml ^{3}H-thymidine (sp. act. 26 Ci/mM). After 10 hr chronic labelling, the glands were fixed, squashed and autoradiographed.

(iii) *0 hr–24 hr chronic labelling* — In this case the glands were chronically labelled with ^{3}H-thymidine (2 μCi/ml) as above for 24 hr beginning from the initiation of culture.

(iv) *24 hr pulse labelling* — Glands cultured *in vitro* for 24 hr were pulse labelled with ^{3}H-thymidine (500 μCi/ml) for 10 min in the incomplete medium as above.

(v) *24 hr–48 hr chronic labelling* — Glands cultured *in vitro* for 24 hr were transferred to fresh medium containing 2 μCi/ml ^{3}H-thymidine and after chronic labelling for 24 hr, they were fixed and autoradiographed.

(vi) *48 hr pulse labelling* — The final group of glands in this set was cultured for 48 hr *in vitro* and then pulse labelled with ^{3}H-thymidine (500 μCi/ml) for 10 min and autoradiographed.

With glands dissected out from late brown spiracle stage larvae, the following 3 types of labelling were done :

(i) 0 hr pulse, (ii) 0 hr–10 hr chronic and (iii) 0 hr–24 hr chronic labelling. These three categories corresponded to the first three categories above from early black spiracle stage larvae. For autoradiography, the pulse as well as the chronic labelled preparations were exposed for 5 days and developed as usual. It may be noted that in other experiments (not included here), we have ascertained that a 5 day autoradiographic exposure in preparations from glands labelled for long periods with a low dose

TABLE 1 — COMPOSITION OF MEDIUM USED FOR *in vitro* CULTURE OF LARVAL SALIVARY GLANDS OF *D. nasuta*. (MODIFIED AFTER POELS 1972)

Component	mg/100 ml dist. water
NaCl	86
$NaH_2PO_4.2H_2O$	88
KCl	313
$KHCO_3$	18
$CaCl_2.2H_2O$	116
$MgSO_4.7H_2O$	513
Malic acid	95
α-ketoglutaric acid	42
Fumaric acid	8
Succinic acid	14
Pyruvic acid	2 (µl)
Glucose	30
Trehalose	80
Lactalbumin hydrolysate	812
Bactopeptone	500
Yeast extract paste	200
Streptopenicillin (Dicrysticin-S, Sarabhai Chemicals, India)	100
Insulin	0.04 unit
*p*H	7.0

(2 μCi/ml) of ^{3}H-thymidine, can adequately register all the labelling patterns.

Results

Patterns of in vitro polytene replication — The salivary glands of 3rd instar larvae of *D. nasuta* maintain their integrity for at least 3–4 days in the culture medium used here and continue to actively incorporate ^{3}H-uridine as in freshly excised glands (data not presented). During the first 2 days of *in vitro* culture, the explanted glands incorporate ^{3}H-thymidine in different nuclei and the autoradiographic labelling patterns seen in these nuclei are qualitatively similar to those seen in freshly dissected glands from 3rd instar larvae of *D. nasuta* which have been described earlier from this laboratory[10,11]. As in polytene nuclei of other species of *Drosophila*[11-14] in *D. nasuta* also, a given polytene replication cycle is believed to be initiated with replication of puffs and interbands (Interband, IB-labelling patterns), followed by replication of all bands, puffs and interbands (Continuous, C-labelling patterns) and finally the dense bands and heterochromatic segments continue to replicate in the late S (Discontinuous, D-labelling patterns). As described earlier[11] in *D. nasuta*, the IB patterns are divisible into low (LIB), mid (MIB) and heavy types (HIB); the C patterns into 2C and 3C types and the D-patterns into heavy (3D), mid (2D) and late or low (1D) discontinuous types. A distinctive feature of polytene replication in freshly dissected salivary glands of *D. nasuta* is the prolonged replication of the puff 48A on 2R; this puff initiates a polytene replication cycle and also continues to incorporate ^{3}H-thymidine even in very late discontinuous (1D) patterns[11]. We have seen all these patterns of ^{3}H-thymidine incorporation in the *in vitro* cultured glands also. The labelling patterns of 48A puff in cultured glands are closely comparable to those seen in freshly dissected glands. Some examples of labelling patterns seen *in vitro* are presented in Fig. 1. The faster completion of replication cycle by the X-chromosome in male polytene nuclei[11,15] is also characteristically seen in the *in vitro* cultured glands (Fig. 1c). Furthermore, the asynchrony and slower replication of the intranucleolar DNA in polytene nuclei of *D. nasuta*[10] is also maintained in the *in vitro* cultured glands. It has been observed in the *in vitro* cultured glands that the degree of ^{3}H-thymidine incorporation in the intranucleolar DNA is not correlated with the pattern of chromosomal labelling in the nucleus and also in many nuclei, the nucleolus was seen to be labelled even though the chromosomes were unlabelled (Fig. 2). Thus it may be concluded that the patterns of ^{3}H-thymidine incorporation observed in different nuclei in glands cultured *in vitro* under the present conditions are similar in all respects to those seen in freshly dissected glands and this implies that in general, the polytene replication cycles proceed in a normal manner in the cultured glands.

Optimal culture conditions — Frequencies of different types of labelling patterns vary under different culture conditions and after different periods of *in vitro* maintenance. An analysis of these frequencies provides information about the efficacy of a given culture condition. The data on the observed frequencies of different ^{3}H-thymidine labelling patterns in glands taken from early black spiracle larvae and cultured under different conditions for 24 hr are presented in Table 2. There is a slight increase in the frequency of the ^{3}H-thymidine labelled nuclei in glands cultured singly as compared to sister glands which were mass cultured. But, since the increase in the frequency of the labelled nuclei in individually cultured glands is only slight and since the handling of the mass cultured glands is more convenient than of a large number of individually cultured glands, we have preferred mass culture to isolated single gland culture.

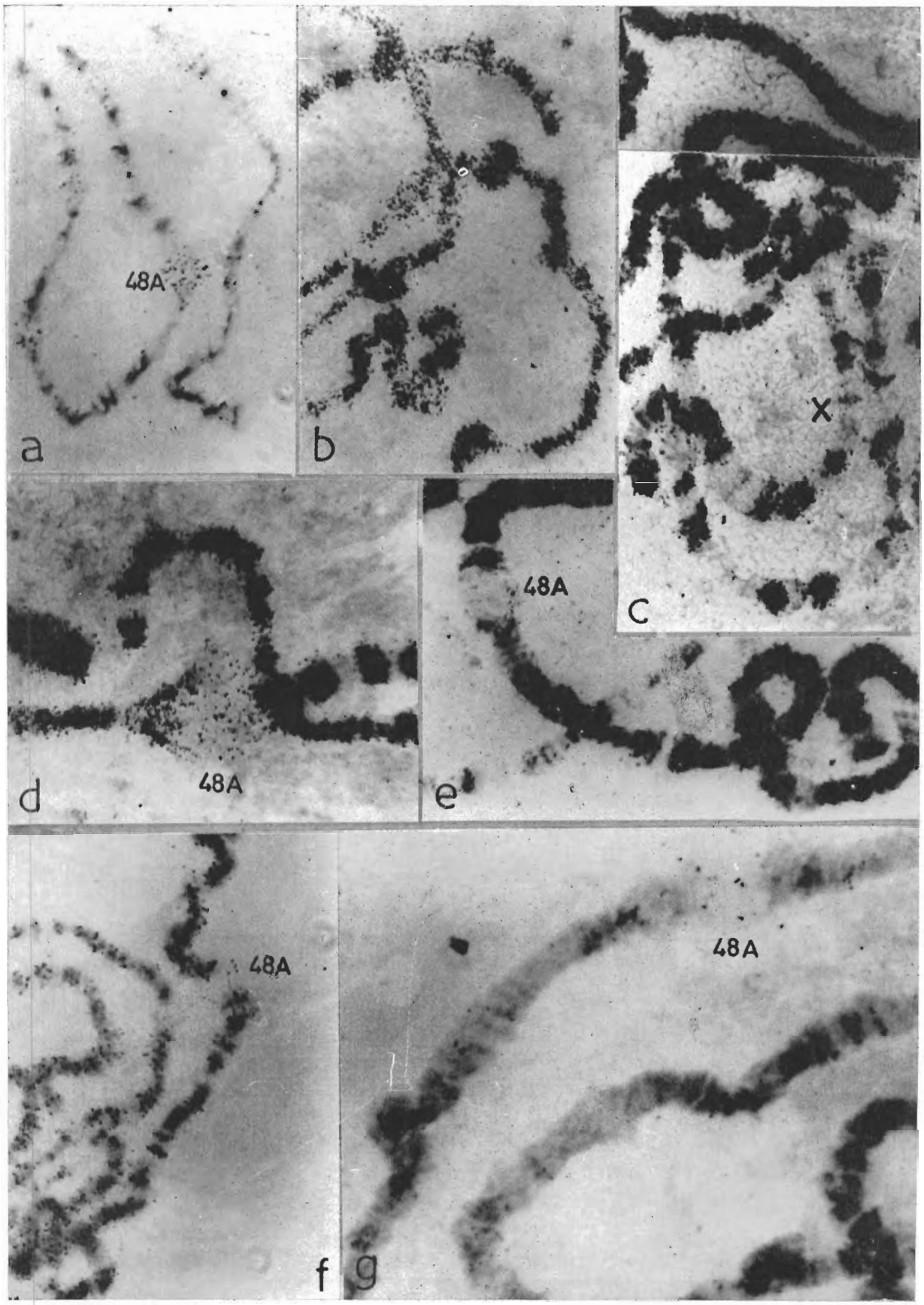
Serum supplement does not seem to improve *in vitro* polytene replication since the glands cultured *in vitro* in medium without serum show polytene replication nearly as well as the glands cultured in serum supplemented medium (Table 2). Also it was observed that certain batches of sera were positively toxic to glands. Therefore, we have not used serum supplemented medium for further studies.

Presence of larval fat bodies in the medium during *in vitro* culture of larval organs has been reported

TABLE 2 — FREQUENCY OF DIFFERENT AUTORADIOGRAPHIC LABELLING PATTERNS IN SALIVARY GLANDS PULSE LABELLED WITH ^{3}H-THYMIDINE AFTER *in vitro* CULTURE FOR 24 HR UNDER DIFFERENT CONDITIONS

[In all cases, salivary glands were taken out from the early black spiracle stage larvae]

	Culture conditions	Total nuclei	Frequency of labelling patterns (%)					
			Interband IB	Continuous C	Discontinuous			Unlabelled
					3D	2D	1D	
I. 1.	Serum +	429	0.23	8.39	4.90	7.93	47.78	30.77
2.	Serum —	336	0.60	9.52	6.55	9.82	39.28	34.23
II. 1.	Isolated culture	324	0.00	1.54	1.54	4.94	41.98	50.00
2.	Mass culture	220	0.45	11.82	1.82	1.82	26.82	57.27
III. 1.	24 hr Preconditioning	766	1.50	13.80	10.44	23.27	25.27	25.72
2.	Fat body co-culture	711	1.13	10.13	8.44	16.17	26.86	37.27


48A
a
b
x
c
d
48A
48A
e
48A
f
48A
g

to be helpful[18] and accordingly we have tried either to precondition the medium for 24 hr with larval fat bodies, or to co-culture the salivary glands with fat bodies. Both these conditions appear to improve the progression of polytene replication *in vitro* since the frequencies of early patterns (i.e. IB and C types) are higher than under other conditions (Table 2). Moreover, the general morphology and spreading of the polytene chromosomes in squash preparations of glands cultured in presence of fat bodies is better than otherwise. In the preconditioned medium the frequency of unlabelled polytene nuclei is lower than in fat body co-cultured medium. Nevertheless, for routine cultures, we have only co-cultured the fat bodies since preconditioning introduces additional risks of infection of the culture.

Larval age and labelling frequency in vitro — Comparison of the ^{3}H-thymidine labelling patterns in glands taken out from younger and older larvae and cultured *in vitro* for different periods reveals (Table 3) that salivary glands from early black spiracle stages are more suited for *in vitro* polytene replication studies than those from older brown spiracle stage larvae. In glands from older larvae cultured for 24 hr and 48 hr (Table 3), not only the frequency of labelled nuclei is very low (10–20%), but also the IB and C patterns are completely absent. On the other hand, the glands from early black spiracle stage larvae show a much higher frequency of replicating nuclei and moreover after *in vitro* culture of 24 hr and 48 hr, the initial labelling patterns (IB and C types) are still present

TABLE 3 — FREQUENCY OF DIFFERENT AUTORADIOGRAPHIC LABELLING PATTERNS IN SALIVARY GLANDS FROM DIFFERENT LARVAL AGES PULSE LABELLED WITH ^{3}H-THYMIDINE AFTER DIFFERENT PERIODS OF *in vitro* CULTURE

Donor larval age	Period of *in vitro* culture (hr)	Total nuclei observed	Frequency of different labelling patterns (%)					
			IB	C	3D	2D	1D	Unlabelled
A. Early black spiracle	24*	711	1.13	10.13	8.44	16.17	26.86	37.27
	48	488	0.00	3.48	7.58	18.24	40.36	30.33
	72	322	0.00	0.00	0.00	0.00	6.80	93.20
B. Late brown spiracle	24	540	0.00	0.00	0.19	2.78	16.30	80.73
	48	356	0.00	0.00	0.00	0.28	10.39	89.33

*Data for this sample are same as for fat body co-culture condition in Table 2.

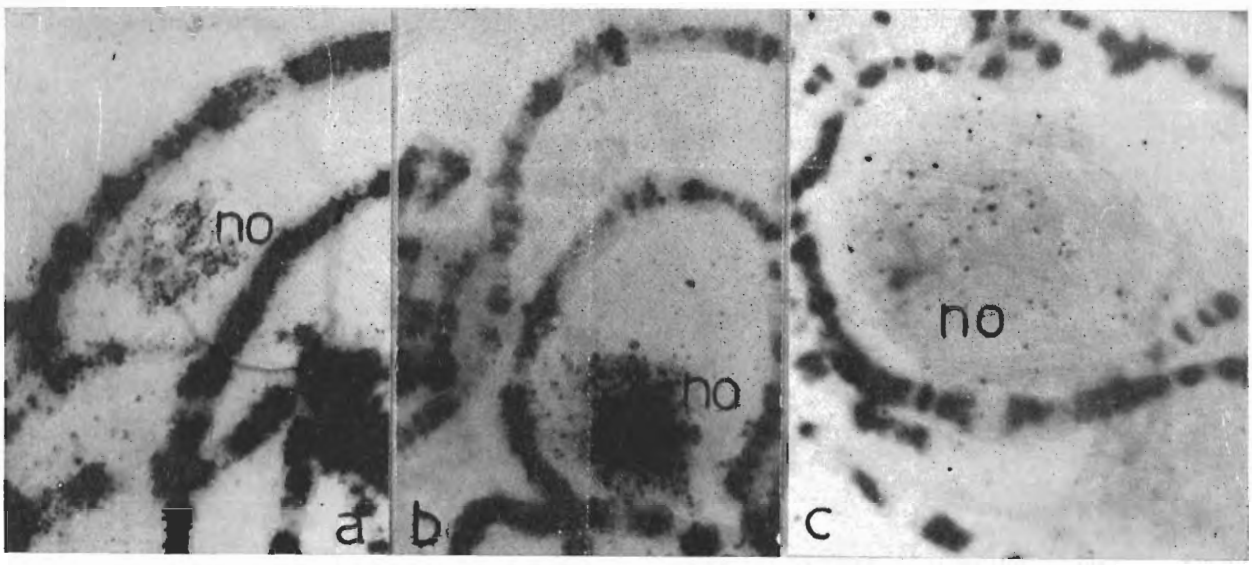


Fig. 2 — Examples to show persistence of autonomy of ^{3}H-thymidine incorporation in intranucleolar DNA in polytene nuclei in *in vitro* cultured salivary glands. In **a**, the chromosomes show 3C-3D pattern, while nucleolus (no) is moderately labelled; in **b**, the chromosomes show 1D pattern but the nucleolus is very heavily labelled; in **c**, the nucleolus is low labelled although the chromosomes are unlabelled. Magnification in **a-b** = 1200×; in **c** = 2000×.

Fig. 1 — Representative examples of autoradiographic patterns of ^{3}H-thymidine incorporation in polytene chromosomes of mid third instar larval salivary glands of *D. nasuta* after *in vitro* culture for 24 hr [**a** – mid interband; **b** – 2C; **c** – 3C on autosomes and 2D on male X-chromosome; **d** – 3D, **e** – 3D-2D transition; **f** – early 1D and **g** – very late 1D pattern with only a few bands labelled]. Note characteristic patterns of ^{3}H-thymidine incorporation on 48A puff on 2R in interband (**a**) and very late 1D (**g**) type labelled nuclei. Magnification in **a–f** = 1200×; in **g** = 2000×.

(Tables 2 and 3), which indicates that some nuclei are still initiating new cycles of polytene replication. However, after 72 hr of *in vitro* culture, these glands show fewer replicating nuclei and all of them show terminal phase (1D-pattern) of polytene replication cycle (Table 3). After 96 hr of culture, ^{3}H-thymidine incorporation is not seen in any nucleus (data not presented) and also the morphology of the polytene nuclei shows deterioration since the chromosomes in squash preparation appear clumped and with poor band-interband differentiation. Nevertheless, the ^{3}H-uridine incorporation is still intense (data not presented) in these nuclei. The deterioration of chromosome morphology and accumulation of secretory granules in cell cytoplasm were more pronounced in glands taken from brown spiracle stage larvae.

Initiation of new replication cycles in cultured glands — The results of the above studies in which salivary glands were pulse labelled with ^{3}H-thymidine after different periods of culture suggests that some nuclei in glands from younger larvae continue to initiate new cycles of replication after *in vitro* culture for 24 hr and 48 hr while in those from older larvae, new cycles are probably not initiated. To ascertain this aspect, we have labelled the glands continuously with ^{3}H-thymidine during different time intervals of *in vitro* culture. Since, the IB and C type labelling patterns are believed to occur in early stages of a polytene replication cycle[10-12], it is expected that if new polytene replication cycles are being initiated in different nuclei of a gland during the *in vitro* culture period, the frequency of C-type labelled nuclei would increase with increasing duration of ^{3}H-thymidine labelling. The data on the frequencies of different patterns in samples pulse labelled or chronically labelled at different periods of *in vitro* culture, are presented in Table 4. It is seen that compared to 0 hr pulsed glands (from younger larvae), the frequency of C-pattern nuclei rises considerably in glands chronically labelled with ^{3}H-thymidine for 10 hr or 24 hr (Table 4). This shows that during this interval *in vitro*, new replication cycles have been initiated in a significant proportion of polytene nuclei. Similarly during the 24 hr and 48 hr interval of *in vitro* culture of glands from black spiracle stage larvae, new replication cycles are initiated in some nuclei since the frequency of C-patterns in chronically labelled (between 24 hr and 48 hr) glands is much higher than that seen in glands pulse labelled at 24 hr or at 48 hr. In contrast to these results in glands from black spiracle stage larvae, polytene nuclei in glands from brown spiracle stage do not initiate new cycles of replication during the *in vitro* culture period since the frequency of C-patterns remains nearly the same in 0 hr pulsed and 10 hr and 24 hr continuously labelled glands (Table 4).

Discussion

The salivary glands of late 3rd instar larvae of *Drosophila* are terminally differentiated and in the normal course of development are destined to be histolysed during early pupal stages[17]. There have been several attempts in the past to culture these larval glands *in vitro* for shorter or longer periods and these attempts have met with some success[6,16]. In some of the earlier studies the process of polytene replication in the *in vitro* cultured glands was also briefly looked into but, in these studies it was found that after some hours of *in vitro* culture only a very low proportion of polytene nuclei in the glands incorporate ^{3}H-thymidine[5,6]. Further, a precise information about the similarity of *in vitro* and *in vivo* polytene replication was also lacking. Due to these limitations, the advantages of the ability to maintain polytene replication cycles *in vitro* could not be exploited. In this respect the present culture conditions are promising for studying polytene replication *in vitro*, since during the first 48 hr of *in vitro* culture, a large number of polytene nuclei are seen to incorporate ^{3}H-thymidine. And since the patterns of ^{3}H-thymidine incorporation in different regions (including the 48 A puff, male X-chromosome and intra-nucleolar DNA) of nuclei in the *in vitro* cultured glands are closely comparable to those seen in freshly dissected glands[10,11], the progression of polytene replication under the present *in vitro* culture conditions appears to be normal. Moreover, our results also show that under the *in vitro* culture conditions used, new polytene replication cycles are initiated during the period of culture. This is primarily evidenced by the presence of IB

TABLE 4 — FREQUENCIES OF DIFFERENT AUTORADIOGRAPHIC LABELLING PATTERNS IN SALIVARY GLANDS FROM DIFFERENT LARVAL AGES AFTER PULSE OR CHRONIC LABELLING DURING DIFFERENT PERIODS OF *in vitro* CULTURE (FOR EXPERIMENTAL DETAILS, SEE TEXT)

	Larval age	Culture age (hr)	Labelling	No. of nuclei observed	Frequencies of different labelling patterns			
					IB	C	D	Unlabelled
A.	Early black spiracle stage	0	Pulse	567	6.95	20.86	55.26	16.93
		0 to 10	Chronic	774	4.13	31.91	55.43	8.53
		0 to 24	do	683	0.40	43.19	49.92	6.44
		24	Pulse	288	2.43	1.74	70.13	25.69
		24 to 48	Chromic	627	1.88	7.18	61.72	29.18
		48	Pulse	395	0.00	0.00	54.67	45.32
B.	Late brown spiracle stage	0	Pulse	490	1.43	10.82	47.09	40.61
		0 to 10	Chronic	613	0.00	7.50	58.50	33.90
		0 to 24	do	622	0.00	10.90	50.30	38.50

and C type labelling patterns in glands pulse labelled with ^{3}H-thymidine after 24 and 48 hr of *in vitro* culture and further supported by the observed increase in the frequency of C-patterns and decrease in the unlabelled nuclei when the glands were chronically labelled with ^{3}H-thymidine for longer durations. The occurrence of initiations of new replication cycles in explanted glands is very significant since this would permit a detailed analysis of the factors regulating initiation and progression of polytene replication about which so far very little is known.

Our *in vitro* culture conditions differ from the earlier attempts in the following important aspects and presumably, these have resulted in a better maintenance of polytene replication under the *in vitro* conditions. We have added insulin to the culture medium and have used larval fat bodies either to precondition the medium or in co-culture with salivary glands; the serum supplement has also been omitted. And finally, we have also examined the effect of larval age on the progression of polytene replication *in vitro*. Insulin has been described as a *Drosophila* hormone and has been shown to help in proliferation and differentiation of *Drosophila* cells *in vitro*[19], and therefore, might as well be helpful in polytene replication. Similarly, co-culture of larval fat bodies has also been seen to improve polytene chromosome morphology as well as replication under the *in vitro* condition. It would appear that some metabolic activities of larval fat bodies modify the culture medium in a favourable way. Davis and Shearn[18] have also found a positive role of larval fat bodies in the *in vitro* culture of larval imaginal discs. Further analysis of the role of fat body co-culture would be useful. Our results also indicate that preconditioning of the medium with larval fat bodies and brain complexes is further helpful in progression of polytene replication cycle *in vitro*.

The observed differences in the *in vitro* progression of polytene replication in glands taken from younger larvae and older larvae are interesting. Not only are new replication cycles not initiated in explanted glands from older larvae, but the frequency of ^{3}H-thymidine labelled nuclei after 24 hr and 48 hr of *in vitro* culture is much lower in these glands than in those taken from younger larvae. Presumably, this difference is related to the normal developmental programming of salivary glands. During normal development also, new cycles of replication are initiated in very few or none of the salivary glands cells in very late 3rd instar stages, while in younger larval salivary glands many nuclei enter fresh replication cycles[1,11,20]. Thus, the *in vitro* results obtained with salivary glands taken from early black spiracle larvae and from late brown spiracle larvae reflect the *in vivo* potential of the respective glands. It would be of interest to know if exposure of these explanted glands to ecdysone or juvenile hormones can modify the developmentally imprinted regulation of the polytene replication. These studies, which are currently in progress, should throw light on the hormonal regulation of polytene replication[1,16].

In conclusion, it may be said that the feasibility of studying the process of replication from initiation to termination of a replication cycle under *in vitro* conditions opens the possibility of studying this complex process in detail.

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