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The Effect of Polyamines on the Synthesis of Ribonucleic Acid by *Drosophila melanogaster* Larvae

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1. To elucidate further the possible role of polyamines in the synthesis of nucleic acids, a study of the effect of exogenously administered amines on the synthesis of RNA by *Drosophila melanogaster* larvae was undertaken. This system was chosen because of the previous investigations [Dion, A. S. & Herbst, E. J. (1967) *Proc. Natl. Acad. Sci. U.S.A.* **58**, 2367–2371; Herbst, E. J. & Dion, A. S. (1970) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **29**, 1563–1567] relating putrescine and spermidine to growth and development of *Drosophila*. 2. Larvae cultured on a defined medium containing variable concentrations of spermidine or putrescine accumulated the amines from the media and were enriched with respect to control animals incubated in amine-free cultures. 3. The addition of 1–5mM-spermidine to the liquid culture medium resulted in a 30–250% increase in the incorporation of [^{3}H]-uridine into 4S, 18S and 28S RNA from 72h *Drosophila* larvae incubated for 20min–12h; 1–100mM-putrescine and 120mM-spermidine inhibited the incorporation of [^{3}H]uridine into RNA. Spermidine was specific among related polyamines, since only spermidine, and not putrescine, spermine, cadaverine or ethylenediamine, increased the incorporation of [^{3}H]uridine into RNA. 4. This effect of the polyamine on the synthesis of RNA was not due to an elevated uptake and conversion of [^{3}H]uridine into [^{3}H]UTP or to an alteration in the rate of turnover of RNA.

Polyamines have been found ubiquitously throughout the plant and animal world (Tabor & Tabor, 1964). The early observation that polyamines are essential for the growth of *Hemophilus parainfluenzae* (Herbst & Snell, 1949) stimulated investigations directed towards their possible biological role in other prokaryotic organisms and in eukaryotic growth processes.

It is now recognized that during periods of rapid growth in a variety of organisms, an increase in the net accumulation or rate of synthesis of RNA is accompanied by an increase in the concentration of polyamines. These relationships have been established during embryonic development (Raina, 1962; Caldarera *et al.*, 1965; Snyder *et al.*, 1970) or during the cellular response to specific growth stimuli such as hormones (Jänne *et al.*, 1968; Russell & Taylor, 1971; Wyatt *et al.*, 1973), drugs (Seiler & Askar, 1972), or the regenerative response elicited by organ excision (Dykstra & Herbst, 1965). Similar correlations between polyamine concentrations and nucleic acid synthesis during the development of *D. melanogaster* have been reported (Dion & Herbst, 1967; Herbst & Dion, 1970). In *Drosophila*, an increase in the concentration of spermidine was found to occur concurrently with increased RNA and protein synthesis during embryonic, larval, pupal and adult development. Putrescine was present at low concentrations throughout development, whereas spermine was barely detectable. A study of polyamine variations during the development of the blowfly *Calliphora erythrocephala* (Heby, 1972) also indicates that in this insect spermidine is the dominant polyamine and that the highest concentration is attained during the rapid growth associated with early larval development.

To extend the indirect evidence previously obtained in our laboratory relating spermidine to nucleic acid metabolism in *D. melanogaster*, we have studied the effect of exogenously administered polyamines on RNA synthesis by intact developing larvae. A procedure for the labelling of larval RNA by allowing the animals to feed on liquid culture medium containing [^{3}H]uridine was developed as an alternative to cumbersome micro-injection techniques (Greenberg, 1969).

Experimental

Larval culture

D. melanogaster used for all experiments were wild-type flies which had been maintained in our laboratory for 10 years by repeated sib-matings. The flies were propagated on a synthetic sucrose/salts culture medium adapted from Pearl *et al.* (1926) by the addition of 1.5ml of propionic acid per litre of media. Large numbers of larvae of similar age were obtained via synchronized egg-laying on Petri dishes of larval culture medium containing 200mg of penicillin and 50mg of streptomycin sulphate per litre. Larval age was determined by assuming the standard 21h of embryonic development and was recorded as the number of hours of development after hatching plus or minus half of the interval for which egg-laying was allowed to proceed (for example, 72 ± 3h).

Larvae were collected by rinsing them on to a small filter apparatus and rapidly washing them with sterile liquid culture medium. During the incubation period, larvae (25–50mg wet wt.) were gently shaken at 25°C in 5ml beakers containing a total volume of 0.25ml of liquid culture medium (a volume sufficient to keep the larvae wet without totally immersing them). At the conclusion of the incubation, larvae were thoroughly washed with 100ml of water, weighed, and transferred to a homogenizer.

Analysis of polyamines

Larval polyamine concentrations were determined by using a modification of Seiler's dansyl procedure (Seiler & Wiechmann, 1967) as previously described (Herbst & Dion, 1970; Wyatt *et al.*, 1973).

RNA extraction

RNA was extracted from [^{3}H]uridine-labelled larvae by using a procedure similar to that used by Greenberg (1969) designed for *Drosophila*. After incubation and washing, the larvae (50–100mg wet wt.) were transferred to a Duall homogenizer (Kontes Glass Co., Vineland, N.J., U.S.A.) and homogenized at 0°C with 0.5–1.0ml of buffer [0.1M-NaCl, 0.01M-sodium acetate, pH5.1, 1% (w/v) sodium dodecyl sulphate] and 0.5–1.0ml of redistilled buffer-saturated phenol. Phenol extractions at temperatures higher than 0°C led to substantial degradation of the RNA. The larval homogenate was extracted three times with fresh phenol and the aqueous layer was extracted with 2vol. of anhydrous diethyl ether to remove residual phenol. The RNA in the aqueous layer was precipitated by the addition of 2.5vol. of 80% (v/v) ethanol containing 0.1M-NaCl and 0.01M-sodium acetate, pH6.0, and left at −25°C for 2–4h.

Sucrose-gradient analysis of labelled RNA

The ethanol-precipitated RNA was centrifuged at 1500*g* for 10min at 0°C and the RNA was dissolved in 0.3ml of NET/SDS buffer (Millette & Trotter, 1970) consisting of 0.1M-NaCl, 1mM-EDTA, 0.02M-Tris/HCl, pH7.5, and 0.5% (w/v) sodium dodecyl sulphate. This was layered on 17ml of 5–30% (w/v) sucrose (Schwarz/Mann, Orangeburg, N.Y., U.S.A.) gradients in the same buffer. Centrifugation was performed in the SW 27.1 rotor (Beckman Instruments, Palo Alto, Calif., U.S.A.) for 12–13h at 27000rev./min and 22°C.

Fractions (1ml) were collected from the gradients and the E_{254} was continuously monitored. Bovine serum albumin (200μg) was added to each fraction and the RNA was precipitated with 10% (w/v) trichloroacetic acid and filtered on Whatman GF/A glass-fibre filters as described by Birnboim (1970). The filters were dried and counted for radioactivity in toluene/Omnifluor (New England Nuclear Corp., Boston, Mass., U.S.A.) yielding a counting efficiency for ^{3}H of 34 ± 3%.

The area of the absorbance tracing and of the radioactivity profiles contributed by each species of RNA (4S, 18S and 28S) was determined by planimetry (Emerson, 1971), and the specific radioactivity of the RNA (c.p.m./E_{254} unit) was calculated.

Addition of the ribonuclease inhibitors Bentonite (Fraenkel-Conrat *et al.*, 1961) or diethyl pyrocarbonate (Weiner *et al.*, 1972) during RNA extraction did not alter the specific radioactivities of the RNA compared with RNA extracted without the inhibitors. Deoxyribonuclease treatment of the RNA after ethanol precipitation also did not alter the radioactivity profiles.

In a mixing experiment to determine the effectiveness of the extraction procedure, larvae were incubated with [^{3}H]uridine and the RNA was extracted. Half of the RNA was re-homogenized with unlabelled larvae. Both RNA samples were centrifuged on separate gradients and 90% of the added RNA was recovered after extraction. The relative specific radioactivities of the 4S, 18S and 28S RNA species in the two gradients were unchanged.

The gradient conditions described were capable of separating 16S and 23S bacterial rRNA from 18S and 28S larval rRNA. No labelled bacterial RNA was detected even when larvae were incubated with [^{3}H]uridine for 12h.

Determination of specific radioactivity of [^{3}*H*]*UTP pool*

Acid-soluble nucleotides were extracted from larval tissue by using a modification of the procedure outlined by Tsuboi & Price (1959). Washed larvae (50–100mg) were homogenized in 0.5ml of 0.5M-$HClO_4$ at 0°C and the acid-insoluble material was removed by centrifugation. 10mg of acid-washed Norit (Fisher Scientific Co., Pittsburgh, Pa., U.S.A.) was added to the $HClO_4$ supernatant and the nucleotides were allowed to adsorb for 30min. The charcoal was sedimented by centrifugation, washed with water, and the nucleotides were eluted by the addition of 0.5ml of 0.1M-NH_3/50% (v/v) ethanol (1:1, v/v) followed by shaking for 2h at room temperature. After centrifugation, the supernatant was evaporated to dryness under N_2.

UTP was separated from the other mono-, di- and tri-nucleotides by using high-voltage paper electrophoresis as described by Silver *et al.* (1970). Purified UTP was included during electrophoresis so that the UTP spot could be observed with u.v. light and cut out. The [^{3}H]UTP was eluted from the paper with water and counted for radioactivity in Aquasol counting fluid (New England Nuclear Corp.).

Materials

The polyamines used in all of the investigations were the hydrochloride salts, purchased from Schwarz/Mann. 5-[^{3}H]Uridine (10.5Ci/mmol) and L-[*Me*-^{14}C]methionine (0.1mCi/1.1mg) were obtained from New England Nuclear Corp.

Results

Initially we determined whether the endogenous polyamine concentrations in *Drosophila* larvae could be enriched by incubation in amine-supplemented media. Larvae were incubated with spermidine or putrescine and the polyamine concentrations were determined after 4h (Table 1). Larval spermidine was enhanced by incubation with 1 and 10mM-spermidine. Larvae reared on synthetic sucrose/salts media contain minimal concentrations of putrescine detectable only when many animals are analysed (Herbst & Dion, 1970). Larvae incubated in the same medium supplemented with 10mM-putrescine, however, showed a large increase in putrescine.

Table 1. *Enrichment of Drosophila larvae with polyamines*

Larvae (72±3h) were incubated for 4h at 25°C in a total volume of 0.25ml of liquid sucrose/salts culture medium in which the concentration of putrescine or spermidine was varied. The incubation medium contained 0.225ml of culture medium plus 0.025ml of water (control) or of the amino dissolved in water. The animals were washed, homogenized and analysed by the fluorescence procedure cited in the Experimental section. Not detectable (N.D.) indicates values below 0.05nmol per animal. The values shown are means±S.E.M. for three determinations.

Polyamine in medium	Polyamine in larvae (nmol per animal)	
	Spermidine	Putrescine
None	0.34±0.05	N.D.
Spermidine		
(1mM)	0.54±0.08	N.D.
(10mM)	0.91±0.10	N.D.
Putrescine		
(1mM)	0.34±0.06	N.D.
(10mM)	0.25±0.06	0.6±0.11

To ascertain whether exogenous polyamine was entering the cells, larvae were incubated with [^{3}H]-putrescine and its uptake and subsequent conversion into [^{3}H]spermidine were monitored for 12h (Fig. 1). If putrescine were entering cellular metabolism it would be converted into spermidine by *S*-adenosyl-L-methionine decarboxylase and spermidine synthase. The uptake of [^{3}H]putrescine by the larvae increased rapidly for 2h, remained constant until 4h of incubation, and again increased until 12h. There was a delay of 40min before the appearance of [^{3}H]spermidine, after which its concentration increased for the entire 12h of incubation.

Having established that intracellular larval polyamine concentration could be elevated by incubation with exogenous amine, the effect of this increased concentration of polyamine on the incorporation of [^{3}H]uridine into RNA was studied.

The effect of spermidine on the incorporation of [^{3}H]uridine into RNA is shown in Fig. 2 and Table 2. The sucrose-gradient conditions used (see the

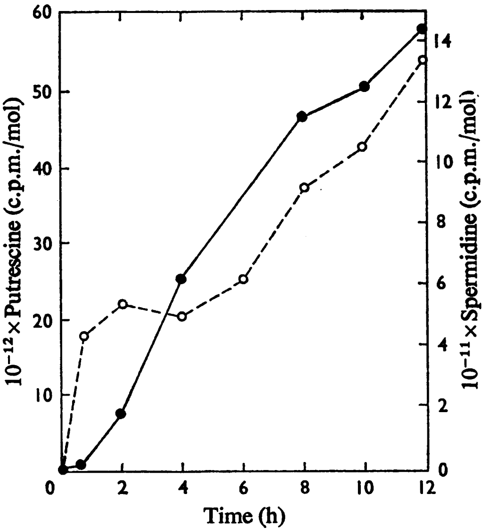

Fig. 1. *Rate of uptake of putrescine and its conversion into spermidine by Drosophila larvae*

Several hundred 72±4h larvae were incubated in sucrose/salts liquid culture medium containing 100μCi of [^{3}H]-putrescine and unlabelled 1mM-putrescine. At time-intervals up to 12h, 30 animals were removed and $HClO_4$ extracts were prepared. The amines in the extract were converted into dansyl derivatives which were separated and quantified by t.l.c. and fluorescence scanning. The fluorescent areas were scraped from the plates and the silica gel was extracted with 0.5ml of dioxan. The radioactivity associated with the dansyl-putrescine and dansyl-spermidine areas of the plate was determined by liquid-scintillation analysis in 10ml of Aquasol. ○, Putrescine; ●, spermidine.

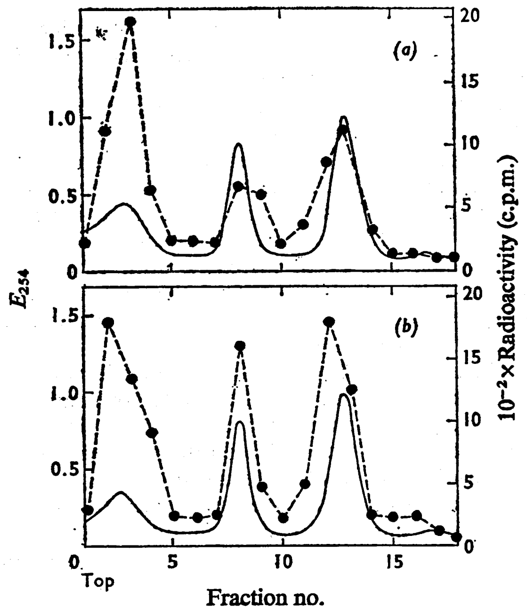

Fig. 2. *Effect of spermidine on the incorporation of* [^{3}*H*]-*uridine into RNA by Drosophila larvae*

Drosophila larvae (72±4h) were preincubated for 1h in sucrose/salts liquid culture medium containing (*a*) no and (*b*) 1mM-spermidine. Incubation was continued for 3.5h after the addition of 25μCi of [^{3}H]uridine. The larvae were washed and RNA was extracted and layered on 5–30% sucrose gradients as described in the Experimental section. Centrifugation was for 12h at 27000rev./min and 22°C in the SW27.1 rotor. Fractions (1ml) were collected and the E_{254} was continuously monitored. The RNA was precipitated and collected on glass-fibre filters, and the radioactivity was measured by liquid-scintillation analysis in toluene/Omnifluor counting fluid (see the Experimental section). ——, E_{254}; ●, c.p.m.

Experimental section) were capable of resolving 4S, 18S and 28S RNA and permitted the calculation of the specific radioactivity (c.p.m./E_{254} unit) of each species of RNA (Fig. 2). There was stimulation of the incorporation of [^{3}H]uridine into RNA of larvae incubated with 1mM- and 5mM-spermidine (Table 2). The maximum stimulation occurred with 5mM-spermidine after both 3.5 and 12h of incubation. The degree of stimulation by spermidine increased with the length of incubation. All species of larval RNA had increased specific radioactivities in the presence of exogenous spermidine (the '4S' RNA separated by sucrose-gradient centrifugation is composed of tRNA and 5S RNA). Higher concentrations of spermidine (120mM) in the incubation medium caused a decrease in the specific radioactivity of RNA relative to larvae incubated without amine (Table 2).

Table 3 shows the effects of other naturally occurring amines on the incorporation of [^{3}H]-uridine into RNA. Putrescine inhibited the incorporation of [^{3}H]uridine even at the low concentration of 0.1mM. This inhibitory effect was perhaps due to the large amount of putrescine (relative to spermidine) taken up by the larvae (Table 1). Spermine, which is found in the chick embryo (Caldarera *et al.*, 1965) but is virtually undetectable in *Drosophila* (Herbst & Dion, 1970), caused a small increase in the relative specific radioactivity of the RNA at low concentrations, whereas higher concentrations were inhibitory. The specific radioactivity of RNA from larvae incubated with 0.1–100mM-cadaverine was very similar to RNA from larvae incubated in the absence of amine. Ethylenediamine inhibited the incorporation of [^{3}H]uridine into RNA at almost all of the concentrations studied.

To ascertain if the degree of incorporation of [^{3}H]uridine into RNA was a measure of the net rate of larval RNA synthesis, the effect of the exogenous polyamines on the incorporation of [^{3}H]uridine into [^{3}H]UTP was determined (Table 4). The specific radioactivity of the [^{3}H]UTP pool was measured in the presence of spermidine and the related amines. Because of the large amount of material required to obtain pool information related to the actual concentration of a specific nucleotide triphosphate (Kijma & Witt, 1969; Emerson & Humphreys, 1971) the data presented are in terms of c.p.m./g wet wt. of larvae. None of the amines investigated substantially altered the specific radioactivity of UTP relative to the control values from larvae incubated without amines.

We have attempted to determine the mechanism by which spermidine apparently increases the net rate of synthesis of RNA. Polyamines have been reported to inhibit the degradation of rRNA by nucleases (Erdmann *et al.*, 1968; Khawaja, 1971). For this reason, the effect of spermidine on the turnover of 4S, 18S and 28S larval RNA was determined (Table 5). Larvae were incubated with L-[*Me*-^{14}C]methionine instead of [^{3}H]uridine, in view of the observation made by Weber (1972) and McElhone *et al.* (1971) that nucleotides released from the turnover of RNA in the cell appear to be preferentially re-incorporated into newly synthesized RNA and give an inaccurate estimation of RNA half-life.

Larvae previously incubated in media containing L-[*Me*-^{14}C]methionine were washed and re-incubated in the presence and absence of spermidine for 3 or 6h in fresh medium containing unlabelled L-methionine (Table 5). The relative specific radioactivities of RNA species isolated from the larvae were very similar to those reported by Greenberg (1969) for methyl-labelled RNA from *Drosophila virulis* larvae. There was no evidence of turnover of the cytoplasmic RNA, in the absence or in the presence of spermidine, at either time-period. It is unlikely therefore that the

Table 2. *Effect of spermidine on RNA synthesis by Drosophila larvae*

RNA was isolated from 72±4h *Drosophila* larvae which were preincubated for 1h in 0.25ml of liquid culture medium containing variable concentrations of spermidine, followed by incubation in the presence of 20μCi of [^{3}H]uridine (12h), 25μCi of [^{3}H]uridine (3.5h) or 100μCi of [^{3}H]uridine (20min). The RNA was analysed by sucrose-gradient centrifugation, and the areas of E_{254} peaks and of radioactivity profiles on 1ml gradient fractions were determined by planimetry. Specific radioactivity is expressed as c.p.m. per E_{254} unit for 4S, 18S and 28S RNA; relative specific radioactivity to zero-spermidine controls = 100 was also determined.

Labelling time	Spermidine (mM)	Specific radioactivity			Relative specific radioactivity		
		4S	18S	28S	4S	18S	28S
20min	0	5080	456	452	100	100	100
	1	9350	612	663	190	134	146
3.5h	0	7200	1392	1400	100	100	100
	1	8560	2256	2280	118	162	162
	5	11430	2487	2672	159	178	190
	10	6680	1896	2112	93	136	150
12h	0	3396	3707	5504	100	100	100
	1	5430	8455	12000	160	228	215
	5	10170	9682	12242	291	261	220
	120	2220	653	1588	65	17	28

Table 3. *Effect of related amines on the incorporation of* [^{3}*H*]*uridine into RNA of Drosophila larvae*

Drosophila larvae (72±3h) were preincubated for 1h in 0.25ml of liquid culture medium containing the indicated amine at the concentration specified. Then 25μCi of [^{3}H]uridine was added and the larvae were incubated for an additional 3h. The larvae were washed, and the RNA was extracted and analysed on sucrose gradients as described in the Experimental section and in Table 2. Relative specific radioactivity is calculated from zero-amine controls = 100. Specific radioactivity is expressed as c.p.m./E_{254} units.

Amine	Specific radioactivity			Relative specific radioactivity		
	4S	18S	28S	4S	18S	28S
None	23140	3240	3342	100	100	100
Putrescine						
(100mM)	10800	1500	1844	45	46	55
(10mM)	13580	1780	2300	58	55	68
(1mM)	13940	1850	1934	60	57	57
(0.1mM)	17420	2620	3280	75	80	98
None	17490	3936	4708	100	100	100
Spermine						
(100mM)	8930	508	1072	51	13	23
(10mM)	16100	3912	4000	92	71	85
(1mM)	16750	4200	5171	104	106	110
(0.1mM)	19020	4750	5972	118	121	127
None	22160	3120	3272	100	100	100
Cadaverine						
(100mM)	22820	2780	3178	103	89	97
(10mM)	20370	2932	2879	92	94	88
(1mM)	21560	3307	3236	97	106	99
(0.1mM)	21970	3235	3431	99	103	105
None	23580	2520	2440	100	100	100
Ethylenediamine						
(100mM)	17490	1030	1164	74	41	47
(10mM)	19780	2190	2464	79	87	100
(1mM)	17500	1908	1832	74	75	75
(0.1mM)	22042	2740	2462	93	108	100

Table 4. *Effect of amines on the incorporation of* [^{3}*H*]*uridine into UTP*

Drosophila larvae (72±6h) were incubated for 3h in 0.25ml of liquid culture medium containing 25μCi of [^{3}H]uridine and 1mM of the indicated amine. Free nucleotides were extracted with 0.5ml of 0.5M-$HClO_4$ and adsorbed on 10mg of Norit as described in the Experimental section. The dried nucleotides were dissolved in 50μl of electrophoresis buffer (0.015M-trisodium citrate, pH4.05; 0.04% EDTA) containing 0.1mg of UTP and separated by high-voltage electrophoresis on Whatman 3MM paper at 3250V for 60–65min. The UTP spot was detected under u.v. light, cut out and extracted in a scintillation vial by shaking with 2.0ml of water at 37°C for 3h; it was counted for radioactivity in Aquasol (New England Nuclear Corp.). Specific radioactivity = c.p.m./g of larvae. The values shown are means±S.E.M. for four determinations.

Amine	Specific radioactivity	Relative specific radioactivity
None	4451±391	100±6
Spermidine (1mM)	4418±362	99±8
Putrescine (1mM)	4796±208	107±4
Spermine (1mM)	3992±356	89±8
Cadaverine (1mM)	4125±409	92±10
Ethylenediamine (1mM)	4411±161	99±4

Table 5. Turnover of Me-^{14}*C-labelled Drosophila RNA*

Drosophila larvae (72±6h) were incubated with 5μCi of L-[*Me*-^{14}C]methionine in 0.25ml of culture medium for 3.5h. The larvae were washed thoroughly and placed into beakers containing fresh liquid culture medium with unlabelled 1mM-L-methionine and left for 60min. Spermidine or water was added to yield the indicated amine concentration, and the larvae were incubated for the specified time. The larvae were thoroughly washed and the RNA was extracted (see the Experimental section). The purified RNA was separated on 5–30% sucrose gradients in 0.1M-NaCl/1mM-EDTA/0.02M-Tris/HCl (pH 7.5)/0.5% sodium dodecyl sulphate buffer and 1.0ml fractions were collected. The radioactivity was counted after acid precipitation on glass-fibre filters, and the specific radioactivity (c.p.m./E_{254} unit) was obtained by relating the areas of the E_{254} peaks to the corresponding c.p.m.

Amine	Time (h)	Specific radioactivity		
		4S	18S	28S
None	0	2200	524	429
None	3	2420	552	496
Spermidine (1mM)	3	2550	468	510
None	3	1920	452	416
Spermidine (1mM)	3	1895	460	425
None	6	2290	594	586
Spermidine (1mM)	6	2640	584	600
None	6	2040	614	570
Spermidine (1mM)	6	2350	602	506

stimulation of RNA synthesis by spermidine can be explained by the decreased turnover of mature RNA species in the presence of the polyamine.

Discussion

Administration of exogenous polyamines has been previously shown to increase the incorporation of labelled precursor into RNA of *D. melanogaster* salivary glands (Dion & Herbst, 1967), chick embryos (Caldarera *et al.*, 1971), perfused rat liver (Fausto, 1972), amphibian oocytes (Wylie & Russell, 1973) and cultured cells (Goldstein, 1965; Raina & Jänne, 1970). In addition to demonstrating a similar effect on the synthesis of RNA by *Drosophila* larvae, we have shown that added spermidine enriches the polyamine pool severalfold (Table 1, Fig. 1). We have also shown that the relative increase in specific radioactivity of RNA from larvae incubated in the presence of spermidine (Table 2) was a measure of the net rate of RNA synthesis and was not due to a greater uptake of [^{3}H]uridine by larvae incubated with amine and its increased conversion into [^{3}H]UTP (Table 4). The 30–150% increase in the synthesis of RNA caused by spermidine (Table 2) was similar to the extent of polyamine stimulation observed in rat liver (Fausto, 1972) and cultured cells (Raina & Jänne, 1970), but significantly less than the 300–1200% increase in incorporation of precursor observed in the chick embryo (Caldarera *et al.*, 1971) and amphibian oocytes (Wylie & Russell, 1973). A possible explanation for the lower degree of stimulation might be that RNA synthesis is already proceeding at a very rapid rate in 72h *Drosophila* larvae (Church & Robertson, 1966) and is only capable of being stimulated severalfold. Younger larvae (24h), in which there is an even more rapid rate of RNA synthesis, have a 4-fold greater amount of endogenous spermidine than do 72h larvae (Herbst & Dion, 1970) and exogenous spermidine does not increase RNA synthesis (results not shown).

Spermidine was specific among the related amines in its stimulatory effect on the synthesis of all RNA species (Tables 2 and 3). The rate of synthesis of spermidine also appears to be co-ordinated with RNA synthesis during the development of insects (Herbst & Dion, 1970; Heby, 1972). Although capable of stimulating RNA synthesis in *Drosophila*, 1–10mM-spermidine did not produce an increase in DNA or protein synthesis during 3–12 h of incubation (Byus, 1974) and did not cause any change in the rate of development of the flies as evidenced by the appearance of the white puparium stage. Thus although the concentration of spermidine seems to play an important role in the synthesis of RNA, it does not appear to control DNA synthesis and the rate of development of *Drosophila* larvae.

The inhibitory effect on RNA synthesis of high concentrations of spermidine, spermine, putrescine and ethylenediamine (Tables 2 and 3) could be due to the oxidation of these amines by *Drosophila* larvae. Oxidized amines have been observed to inhibit RNA and protein synthesis in both eukaryotic and prokaryotic cells (Otsuka, 1971; Bachrach, 1971). The sera of several animals have been found to contain sufficient amine oxidase to produce toxic concentrations of oxidized amines (Cohen, 1971), and it is possible that *Drosophila* haemolymph also has amine oxidase activity. The inhibition of RNA synthesis caused by 0.1–1 mM-putrescine might also be explained by the possible regulatory role of the cellular spermidine/putrescine ratio in controlling RNA synthesis during the development of *Drosophila* (Herbst *et al.*, 1973).

Attempts were made to determine if spermidine was stimulating the synthesis of the 38S rRNA precursor found in *Drosophila* larvae (Greenberg, 1969). We were unable to detect 38S rRNA precursor on sucrose gradients or polyacrylamide gels even when larvae were incubated for 15min with 150μCi of [^{3}H]uridine. This was probably due to the rapid rate of processing of *Drosophila* rRNA precursor (Greenberg, 1969) and the inability of the larvae to take up enough [^{3}H]uridine to label 38S RNA preferentially relative to the other species of RNA. Weinman (1972) was also unable to detect a distinct 38S rRNA precursor in adult *Drosophila*. Wylie & Russell (1973) and Fausto (1972) have shown that exogenously administered polyamines can stimulate the incorporation of [^{3}H]uridine into rRNA precursors of amphibian oocytes and perfused rat liver respectively. There have also been numerous reports of the stimulation of DNA-dependent RNA polymerase by spermidine (cited in Herbst & Tanguay, 1971).

Thus there is an abundance of evidence linking elevated concentrations of spermidine with periods of rapid growth and RNA synthesis. It is not inconsistent with these observations that spermidine could be functioning in *Drosophila* by increasing the synthesis of RNA by DNA-dependent RNA polymerase(s).

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