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Effects of Colchicine on Nucleic Acid Metabolism during Metamorphosis of *Tenebrio molitor* L. and in some Mammalian Tissues

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1. Administration of 10 μg. of colchicine/pupa of the beetle *Tenebrio molitor* L. arrests its differentiation, the pupa remaining alive for 2–3 weeks. 2. The same concentration of colchicine inhibits DNA synthesis and stimulates RNA synthesis (as shown by incorporation into the nucleic acids of labelled adenine, labelled uridine and labelled thymidine). The effects of colchicine on nucleic acid metabolism are first detected 3 days after its administration to first-day pupae. 3. No effects of colchicine are seen on [1-^{14}C]glycine incorporation into protein *in vivo*. 4. Relatively high concentrations of colchicine (e.g. 10mM) suppress incorporation of [8-^{14}C]adenine into RNA in dorsal abdominal wall *in vitro*. Such concentrations have no effect on its incorporation into acid-soluble nucleotides. 5. Colchicine (1mM) suppresses incorporation of [8-^{14}C]adenine into DNA to a greater extent than into RNA in various mammalian tissues *in vitro* (e.g. rat spleen, regenerating rat liver, rat embryo, guinea-pig intestinal mucosa, Ehrlich ascites cells). Colchicine (1mM) has no effect on the rate of respiration of, or on incorporation of radioactivity into acid-soluble nucleotides in, the mammalian tissues tested. 6. Further evidence indicates complex-formation between colchicine and DNA, and it is suggested that the effect of colchicine in suppressing DNA synthesis is due to its combination with the DNA primer (template).

Colchicine is a classical mitotic inhibitor effective at low concentrations, *in vitro* or *in vivo*, in most plant and animal cells. It is effective in insects (Gaulden & Carlsen, 1951), and Voget (1947) has observed that treatment with colchicine modifies post-embryonic differentiation of insect antennae. It is reported (Tamano, 1960) that treatment of the fifth larval instar of *Bombyx* with colchicine brings about development of giant pupae.

Although the use of the drug was first mentioned in the Ebers Papyrus in Egypt about 1550 B.C. and the effect of colchicine on mitosis has been very extensively studied, there is a surprising lack of data on its biochemical mode of action. Its site of action is not yet defined. Lettré (1951, 1952) maintains that colchicine acts by preventing the utilization of ATP for chromosome movements. However, Bass (1959) states that there is insufficient evidence for this conclusion.

Swann (1957) suggests that colchicine affects nuclear RNA synthesis and thereby mitosis, but Taylor (1960) and Prescott & Bender (1962) have shown that the syntheses of protein and RNA are minimal during mitosis.

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Benitez, Murray & Chargaff (1954) report that three substances, *meso*-inositol, ATP and tropolone, can reverse the metaphase arrest of rat fibroblasts produced by colchicine. These authors have found that when ATP is added with colchicine to tissue cultures there is a decrease in the degree of mitotic arrest. However, the percentage of cells in the various phases of division is the same in the presence of added ATP as that found in its absence.

Wang, Greenbaum & Harkness (1963) have shown that treating rats *in vivo* with colchicine has little effect on most nucleotides in the acid-soluble pool of nucleotides in normal and regenerating rat liver. They observed that colchicine increases the concentration of NAD and AMP and decreases the concentration of ATP, GTP and UTP.

Using the radioautographic technique, Hell & Cox (1963) have shown that colchicine depresses the number of radioactive grains in guinea-pig epidermal tissues that have been incubated with tritiated thymidine. This, together with the observations that colchicine changes post-embryonic differentiation in insects (Voget, 1947), that it is a mutagenic agent for coliform bacteria (Parr, 1938) and for *Aspergillus* spp. (Steinberg & Thom, 1940) and that it brings about malformation in the chick embryo

(Lallemand, 1938), suggest that colchicine may act primarily on some aspect of nucleic acid metabolism. Creasey & Markiw (1964) have shown that colchicine administration causes marked inhibition of synthesis of RNA in Ehrlich ascites-carcinoma cells growing in the peritoneal cavities of mice.

The lethal dose of colchicine for mice is 5mg./kg. body wt., but with this dose the toxic effect appears only a week after administration.

We have used larger concentrations of colchicine to observe effects *in vitro* within a short period of time (e.g. 1hr.).

MATERIALS AND METHODS

T. molitor was maintained at room temperature on a diet of wheat-bran and oat-flakes (2:1, w/w) (Patterson, 1957). Prepupae were collected from the culture and kept in separate containers. The pupae that emerged daily between 5 p.m. and 10 a.m. were considered as first-day pupae. These were incubated in a highly humid atmosphere at 28°. Under these conditions pupation lasted 7 days, the adults emerging on the eighth day.

Pupae weighing 110–120mg. were used. For experiments *in vitro* the head and thorax were removed. Dorsal abdominal slices were obtained by means of a Stadie–Riggs tissue slicer. Three such slices were used per incubation flask. Pupae were injected by means of Hamilton microsyringes.

The incubation medium for insect tissues was Krebs–Ringer phosphate medium, pH7·4 (Ilan, Ilan & Quastel, 1966). Investigations (J. Ilan & J. H. Quastel, unpublished work) indicated that other saline media, approximating in composition to the amounts of salts present in insect haemolymph, showed no advantage over Krebs–Ringer phosphate medium in promoting incorporation of radioactivity from labelled adenine, uridine or glucose into nucleic acids.

Ehrlich ascites-carcinoma cells were grown in mice according to standard procedures (e.g. Tenenhouse & Quastel, 1960). Packed cells were suspended in 9vol. of iso-osmotic Krebs–Ringer phosphate medium; 1ml. of such a suspension was used per 3ml. of incubation medium.

Rat-spleen slices and slices of regenerating rat liver were prepared from male Wistar albino rats weighing about 250g. Partial hepatectomy was performed under ether anaesthesia according to the procedure of Higgins & Anderson (1931). At 24hr. after the operation, the animals were killed by a blow on the head and the liver was excised, chilled rapidly on crushed ice and sliced.

The methods used for measurement of respiration and incorporation of radioactive precursors into RNA and DNA were the same as those described in the preceding paper (Ilan *et al.* 1966).

Nucleotides were separated from nucleosides and bases by butanol extraction (Thomson, Smellie & Davidson, 1958).

Mono-, di- and tri-phosphonucleotides were separated by paper chromatography with 95% (v/v) ethanol–M-ammonium acetate, pH7·5 (15:6, v/v) (Bergkrist, 1957).

Protein from dorsal abdominal slices was prepared according to the procedure of Quastel & Bickis (1959) with a modification as it was necessary to dispose of the exoskeleton before determining the specific activity of the protein. The tissue was homogenized in 5ml. of 6% (w/v) trichloroacetic acid and washed with 5ml. of ether. The protein was dissolved by adding 2ml. of 0·5N-KOH and keeping it for half an hour at room temperature. The exoskeleton was separated by centrifugation. A 0·2ml. portion of 70% (v/v) $HClO_4$ was added to precipitate the protein, which was then washed twice with 5ml. of 6% trichloroacetic acid. The specific activity of the protein was then determined by the procedure quoted above.

The determination of [1-^{14}C]glycine incorporation into protein *in vivo* was as described in the preceding paper (Ilan *et al.* 1966).

[8-^{14}C]Adenine sulphate hemihydrate, [1-^{14}C]glycine and [2-^{14}C]thymidine were purchased from The Radiochemical Centre, Amersham, Bucks. [2-^{14}C]Uridine was obtained from New England Nuclear Corp., Boston, Mass., U.S.A., and colchicine from the Abbott Laboratories, Montreal, Canada. Sarcomycin was a gift from Banyu Pharmaceutical Co. Ltd., Tokyo, Japan. Mescaline hydrochloride was a product of the Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A. The colchicine was dissolved in water for experiments *in vitro* and in 0·9% NaCl for experiments *in vivo*. Fresh solutions were prepared for each experiment.

Values for studies *in vitro* are expressed as means of at least six separate experiments each of which was run in duplicate. Values for studies *in vivo* are given as means of six separate experiments in each of which four to six replicates were used.

RESULTS

Biological effects of colchicine on pupae of T. molitor. Colchicine dissolved in sterile 0·9% sodium chloride was injected into first-day pupae at concentrations in the range 10–400μg./pupa, in a volume of 10μl. A concentration of 10μg./pupa is approximately 0·27mM-colchicine in the tissue water. Control pupae were given injections of 0·9% sodium chloride. The insects were incubated at 28°, at which temperature pupation in the control group lasted 7 days, adults emerging on the eighth day. Colchicine-treated animals remained in the pupal stage, at all concentrations tried, for 2–3 weeks, at the end of which they died without having matured. In pupae given 10μg. of colchicine there was almost no mortality in 3 weeks, but in those that had received 400μg. of colchicine the mortality reached 30% within 2 weeks. The criteria for deciding whether the pupae were alive were reflex movements when touched, and heart beat (microscopic observation).

Effects of colchicine on the incorporation of ^{14}C *from various labelled precursors into RNA and DNA during pupation* in vivo. (*a*) Effects of colchicine on the incorporation of [8-^{14}C]adenine into RNA and DNA *in vivo*. First-day pupae were injected with 10μg. of colchicine and [8-^{14}C]adenine and incubated at 28°. Control pupae were injected on the first day with only 0·9% sodium chloride and [8-^{14}C]adenine. Samples were removed after 4hr. and then at intervals of 48hr. for determination

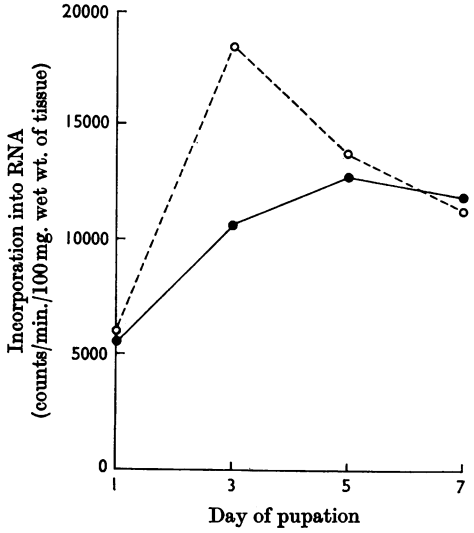

Fig. 1. Effect of colchicine on [8-^{14}C]adenine incorporation into RNA *in vivo*. First-day pupae were injected with 10μg. of colchicine and [8-^{14}C]adenine (specific activity 1·47μc/mg.)(220000 counts/min./pupa). Control pupae received 0·9% NaCl and [8-^{14}C]adenine. Samples of dorsal and ventral abdominal walls were taken 4hr. after injection. Remaining samples were taken at 48hr. intervals. The results represent the means of six experiments, the standard deviations from the means being approximately the same as those given in Table 1. ●, [8-^{14}C]Adenine; ○, [8-^{14}C]adenine + colchicine (10μg.).

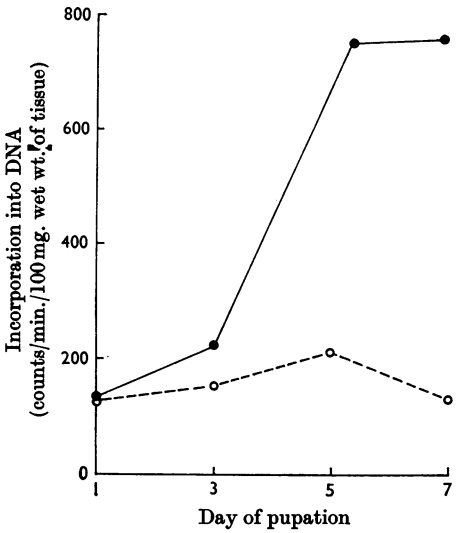

Fig. 2. Effect of colchicine on [8-^{14}C]adenine incorporation into DNA *in vivo*. Experimental conditions were as given in Fig. 1. ●, [8-^{14}C]Adenine; ○, [8-^{14}C]adenine + colchicine (10μg.).

of incorporation of adenine into RNA and DNA in dorsal and ventral abdominal slices. The results given in Fig. 1 show that on the third day after injection there was an increased incorporation of [8-^{14}C]adenine into RNA of pupae that had received colchicine. After the third day the amount of ^{14}C incorporated into tissue RNA decreased, indicating breakdown of the tissue RNA.

An injection of 10μg. of colchicine inhibited the incorporation of [8-^{14}C]adenine into DNA. The effect appeared after the third day. By the fifth day the extent of inhibition of incorporation of radioactivity into DNA was considerable (Fig. 2).

(*b*) Effects of colchicine on the incorporation of [2-^{14}C]uridine into RNA *in vivo*. First-day pupae were injected with colchicine and incubated at 28°. A group of these was injected also with [2-^{14}C]-uridine. Groups of the colchicine-injected insects were taken at 48hr. intervals, injected with [2-^{14}C]uridine and incubated at 28° for an additional 24hr. The amount of radioactivity in RNA of whole pupae was then determined.

The results (Table 1) show that with an injection of 200μg. of colchicine there was no effect on incorporation of radioactivity into RNA on the first day. If the [2-^{14}C]uridine was injected after the third day a stimulation of incorporation occurred, the counts in the injected pupae being 120% higher than in the controls. In pupae injected with [2-^{14}C]uridine on the seventh day there was an inhibition of almost 50% in the incorporation of radioactivity into RNA. Injection into first-day pupae of 400μg. of colchicine caused an inhibition in incorporation of [2-^{14}C]uridine into RNA from the first day (Table 1).

(*c*) Effects of colchicine on the incorporation of [2-^{14}C]thymidine into DNA *in vivo*. When [2-^{14}C]-thymidine was injected in the presence of 10μg. of colchicine, the inhibitory effect on uptake of thymidine by DNA was apparent after the third day of incubation (Fig. 3). After the fifth day the amount of radioactivity incorporated into DNA *in vivo* decreased, indicating breakdown of tissue DNA.

In all cases the first pronounced effect of colchicine on the incorporation of precursors into RNA or DNA was seen 3 days after the colchicine was injected.

Effects of colchicine on the incorporation of radioactivity from [8-^{14}C]*adenine*, [2-^{14}C]*uridine and* [U-^{14}C]*glucose into RNA of the dorsal abdominal wall of* T. molitor *in vitro*. Experiments were carried out to observe the effects of colchicine on RNA synthesis in the dorsal abdominal wall *in vitro*.

Table 1. *Effects of colchicine on* [2-^{14}C]*uridine incorporation* in vivo *into RNA of dorsal abdominal body wall at different intervals of incubation in the pupal stage*

Colchicine was injected in a volume of 10μl. into first-day pupae. Control insects received 10μl. of 0·9% NaCl. [2-^{14}C]Uridine (88000 counts/min./pupa) in 2μl. was injected on the first day or at 48hr. intervals after the colchicine injection. Pupae were incubated at 28°. Radioactivity in RNA was determined 24hr. after injection of uridine. The results are means ± S.D. of six independent determinations, each carried out on a group of six animals.

Time of injection of uridine / Colchicine injected ...	Incorporation into RNA (counts/min./100mg. wet wt. of tissue) None (control)	200μg./pupa	400μg./pupa
First day	12660±325	14000±350	9870±285
Third day	15300±355	36700±500	15570±362
Fifth day	30000±450	36700±525	17130±413
Seventh day	45170±570	24150±415	9500±250

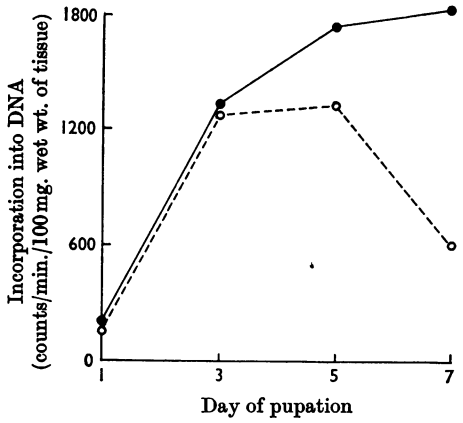

Fig. 3. Effect of colchicine on [2-^{14}C]thymidine incorporation into DNA *in vivo*. Experimental conditions were as given in Fig. 1, except that [2-^{14}C]thymidine (specific activity 0·12μc/mg.) (200000 counts/min./pupa) replaced [8-^{14}C]adenine. ●, [2-^{14}C]Thymidine; ○, [2-^{14}C]thymidine + colchicine (10μg.).

The effects of colchicine *in vitro* in the dorsal abdominal wall during pupation on [8-^{14}C]adenine incorporation into RNA and into the acid-soluble fraction are shown in Table 2. Effects were only found with large concentrations of colchicine. As can be seen, 10mM-colchicine inhibited the incorporation of [8-^{14}C]adenine into RNA by 42–75% during pupation, whereas 1mM-colchicine had no significant effect. Respiration of the pupae *in vitro* was not affected by 10mM-colchicine.

Neither the incorporation of [8-^{14}C]adenine into the acid-soluble fraction nor the amount of nucleotide formed (counted after extraction with water-saturated butanol) was inhibited by 10mM-colchicine.

Examination of the acid-soluble fraction showed that more than 80% of the radioactivity in this fraction was present in ATP, 5–10% in ADP and only trace amounts of label in AMP, adenine or adenosine. This result was not affected by the addition of colchicine (10mM) to the incubation medium.

Colchicine was tested for its effect *in vitro* on [8-^{14}C]adenine, [2-^{14}C]uridine and [U-^{14}C]glucose incorporation into RNA of dorsal abdominal body-wall slices obtained during metamorphosis (Table 3). 10mM-Colchicine inhibited the incorporation of [8-^{14}C]adenine into RNA by 51–65%, whereas 5mM-colchicine inhibited by only 29–37%. The extent of inhibition of uptake of radioactivity into RNA from [2-^{14}C]uridine and [U-^{14}C]glucose was greater than from [8-^{14}C]adenine. 10mM-Colchicine inhibited the incorporation of ^{14}C from [2-^{14}C]uridine into tissue RNA by 83–88% and from [U-^{14}C]glucose by 77–81% into tissue RNA, whereas 5mM-colchicine inhibited the incorporation of ^{14}C from [2-^{14}C]uridine into RNA by 57–71% and that from [U-^{14}C]glucose by 56–67%.

Time-course studies. A study was made of the effects of colchicine on the incorporation of radioactivity from [8-^{14}C]adenine, [2-^{14}C]uridine and [U-^{14}C]glucose into RNA of dorsal abdominal body wall *in vitro* as a function of time (Figs. 4, 5 and 6). Incorporation of radioactivity from all three precursors into RNA *in vitro* both in the absence and presence of colchicine (10mM) increased linearly with time for a period of 2hr. There was no lag period in the inhibition caused by colchicine. The velocities of incorporation of radioactivity from [8-^{14}C]adenine, [2-^{14}C]uridine and [U-^{14}C]glucose were inhibited by 50, 80 and 74% respectively in the presence of 10mM-colchicine.

Effects of injection of colchicine into pupae on [1-^{14}C]*glycine incorporation into protein* in vivo.

Table 2. *Effects of colchicine on* [8-^{14}C]*adenine incorporation into RNA and the acid-soluble fraction in dorsal abdominal body wall* in vitro

Tissue was incubated in Krebs–Ringer phosphate buffer, pH 7·4, at 37° for 1 hr. in the presence of 10 mM-glucose and 18 μg. of [8-^{14}C]adenine (222000 counts/min./ml.) in a total volume of 1 ml. The gas phase was O_2. The results are means ± S.D. (where appropriate) of at least six independent determinations, each run in duplicate.

Day of pupation	1			3			5			7		
Concn. of colchicine (mM)	0	1	10	0	1	10	0	1	10	0	1	10
Incorporation into RNA (counts/min./100 mg. wet wt. of tissue)	1600 ±110	1600 ±125	825 ±81	1650 ±112	1390 ±98	530 ±35	1700 ±135	1600 ±117	980 ±47	1750 ±102	1500 ±93	730 ±47
Incorporation into acid-soluble fraction (counts/min./100 mg. wet wt. of tissue)	45000	45000	45000	44000	45000	45000	41000	43000	42000	43000	42000	45000
Nucleotides in acid-soluble fractions (after extraction with water-saturated butanol) (%)	92	96	91	95	92	94	90	89	89	90	89	92

Table 3. *Effects of colchicine* in vitro *on the incorporation of radioactivity from* [8-^{14}C]*adenine,* [2-^{14}C]*uridine or* [U-^{14}C]*glucose into RNA of dorsal abdominal body-wall slices obtained during pupation*

The amounts of radioactive precursors used were: [8-^{14}C]adenine, 400000 counts/min. (16·8 μg.)/vessel; [2-^{14}C]uridine, 312400 counts/min. (12·2 μg.)/vessel; [U-^{14}C]glucose, 524600 counts/min. (28·0 μg.)/vessel. Tissue was incubated in Krebs–Ringer phosphate buffer, pH 7·4, at 37° for 1 hr. in an atmosphere of O_2. 10 mM-Glucose was present together with adenine or uridine. The total volume was 1 ml. The results are means ± S.D. of at least six independent determinations, each run in duplicate.

	Incorporation into RNA (counts/min./100 mg. wet wt. of tissue)											
Day of pupation	1			3			5			7		
Concn. of colchicine (mM)	0	5	10	0	5	10	0	5	10	0	5	10
[8-^{14}C]Adenine	2245 ±130	1600 ±110	1090 ±47	2500 ±142	1750 ±115	1120 ±85	2485 ±142	1650 ±115	870 ±50	2530 ±145	1600 ±92	1000 ±47
[2-^{14}C]Uridine	1560 ±57	552 ±48	218 ±18	1510 ±68	532 ±36	232 ±19	1550 ±85	446 ±47	268 ±22	1580 ±112	680 ±9	186 ±13
[U-^{14}C]Glucose	3080 ±150	1040 ±48	585 ±37	2820 ±135	1115 ±85	577 ±48	2925 ±135	1160 ±114	602 ±52	2830 ±145	1250	645 ±65

Colchicine (10 μg. in 10 μl. of 0·9% sodium chloride) was injected into first-day pupae that were incubated at 28°. Samples of the pupae were injected on the first day or at 24 hr. intervals with [1-^{14}C]-glycine (0·16 μc, specific activity 71·4 μc/mg.) (each pupa receiving only one injection of glycine) in a volume of 2·5 μl. The specific activity of protein in whole pupae was determined 24 hr. after glycine had been injected and the pupae incubated at 28°. Colchicine did not interfere with the incorporation of glycine into protein during the first 6 days of pupation, the specific activity of protein being in the range 540–570 counts/min./mg. in the control group (in which 0·9% sodium chloride was injected instead of colchicine) as well as in the treated pupae.

Colchicine (25 μg., 50 μg. or 100 μg. in 10 μl.) and glycine (as given above) were injected into first-day pupae. The specific activity of the protein was determined 24 hr. later, after the pupae had been incubated at 28°. Again the specific activity of the protein was not affected and was in the range mentioned above.

The results are consistent with the fact that the concentration of ATP is not affected by colchicine.

Effects of colchicine on [8-^{14}C]*adenine incorporation into mammalian tissue constituents* in vitro. In

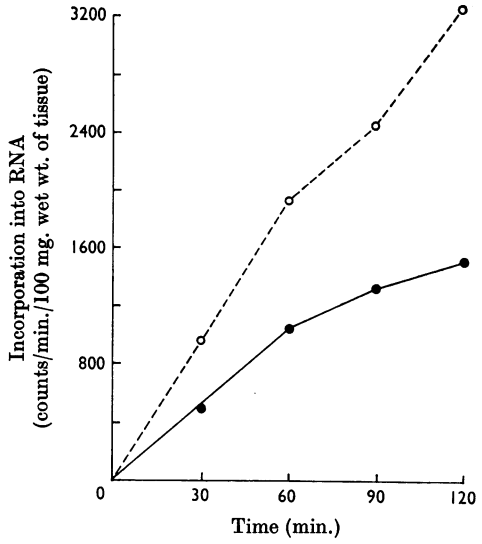

Fig. 4. Effect of colchicine on [8-^{14}C]adenine incorporation into RNA of dorsal abdominal body-wall slices *in vitro* as a function of time. The tissues of first-day pupae were incubated in Krebs–Ringer phosphate, pH 7·4, in the presence of 10 mM-glucose, [8-^{14}C]adenine (200000 counts/min., 3·4 μg.) and 10 mM-colchicine. The final volume was 1 ml. The gas phase was O_2. The temperature was 37°. ●, [8-^{14}C]Adenine + colchicine (10 mM); ○, [8-^{14}C]adenine.

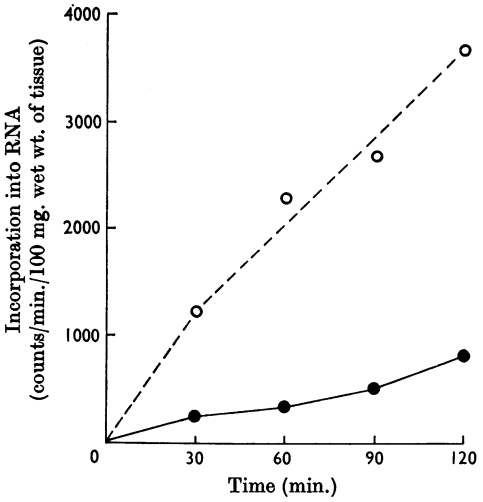

Fig. 5. Effect of colchicine on [2-^{14}C]uridine incorporation into RNA of dorsal abdominal body-wall slices *in vitro* as a function of time. Experimental conditions were as given in Fig. 4, except that [2-^{14}C]uridine (405800 counts/min., 12 μg.) replaced [8-^{14}C]adenine. ●, [2-^{14}C]Uridine + colchicine (10 mM); ○, [2-^{14}C]uridine.

view of the results obtained with insect tissue *in vitro* comparable experiments were carried out with mammalian tissues *in vitro*.

The results in Table 4 show the effect of colchicine on the uptake of [8-^{14}C]adenine into DNA, RNA and the acid-soluble fraction of Ehrlich ascites-carcinoma cells, rat spleen, regenerating rat liver, rat embryo and guinea-pig mucosa *in vitro*. In all mammalian tissues examined, 1 mM-colchicine had no inhibitory effect on the respiratory rate. 1 mM-Colchicine inhibited the incorporation of [8-^{14}C]-adenine into DNA of Ehrlich ascites-carcinoma cells by 51% and into RNA by 18·5%. Incorporation into DNA and RNA in rat spleen was inhibited by 58 and 27·5% respectively. With rat embryo, this concentration of colchicine inhibited incorporation into DNA by 48% but there was no effect on incorporation into RNA. The rates of respiration and incorporation of [8-^{14}C]adenine into RNA and into the acid-soluble fraction of rat embryo were not inhibited even with 10 mM-colchicine. At the latter concentration uptake of radioactivity from [8-^{14}C]adenine by DNA was inhibited by 76% in this tissue. 1 mM-Colchicine inhibited incorporation of adenine into DNA and RNA in guinea-pig intestinal mucosa by 47·5 and 18% respectively.

Thus the results given in Table 4 show that

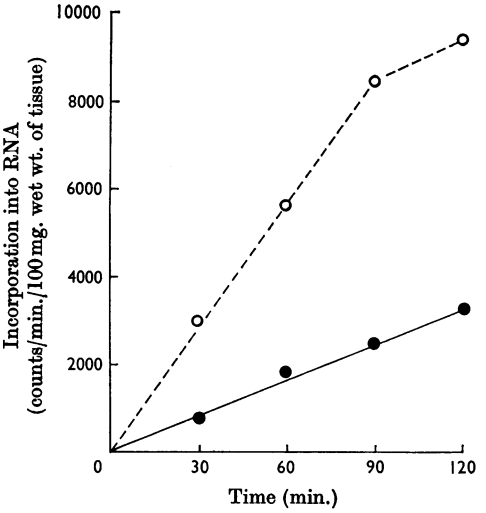

Fig. 6. Effect of colchicine on the incorporation of radioactivity from [U-^{14}C]glucose into RNA of dorsal abdominal body-wall slices *in vitro* as a function of time. Experimental conditions were as given for Fig. 4, except that [U-^{14}C]-glucose ($1{\cdot}49 \times 10^6$ counts/min./vessel, 56 μg.) with no glucose carrier was added as precursor, and [8-^{14}C]adenine was omitted. ●, [U-^{14}C]Glucose + colchicine (10 mM); ○, [U-^{14}C]glucose.

incorporation of [8-^{14}C]adenine into RNA and DNA was affected by colchicine and that 1 mM-colchicine inhibited incorporation into DNA to a

Table 4. *Effects of colchicine on* [8-^{14}C]*adenine incorporation into RNA, DNA and the acid-soluble fraction in mammalian tissues* in vitro

Tissues were incubated in Krebs–Ringer phosphate buffer, pH 7·4, in the presence of 10 mM-glucose. Ascites cells were incubated with 1 mM-[8-^{14}C]adenine (222000 counts/min./vessel). Normal tissues were incubated with 0·1 mM-[8-^{14}C]adenine (380000 counts/min./vessel). The total volume was 3 ml. Incubation was at 37° for 1 hr. The gas phase for normal tissues was O_2 and for ascites cells air. The results are means ± S.D. of at least six independent determinations, each run in duplicate. —, Not determined.

	Incorporation (counts/min./100 mg. wet wt. for normal tissues and counts/min./0·1 ml. of packed cells for Ehrlich ascites-carcinoma cells)								
	Incorporation into DNA			Incorporation into RNA			Incorporation into acid-soluble fraction		
Concn. of colchicine (mM)	0	0·1	1	0	0·1	1	0	0·1	1
Ehrlich ascites-carcinoma cells	428 ±21	—	211 ±14	7207 ±630	—	5865 ±610	47500 ±3300	—	47500 ±3300
Rat spleen	72 ±5	75 ±4	30 ±4	2500 ±142	2500 ±160	1800 ±125	27450 ±650	27500 ±610	25300 ±595
Regenerating rat liver	121 ±9	135 ±11	53 ±5	2000 ±104	2000 ±112	1700 ±110	55800 ±485	54600 ±515	55000 ±535
Rat embryo	200 ±15	150 ±13	104 ±5	1900 ±85	1880 ±81	1915 ±84	36000 ±385	35500 ±350	36200 ±355
Guinea-pig mucosa	143 ±12	145 ±11	75 ±5	5200 ±270	5150 ±250	4500 ±195	65000 ±4500	63450 ±4100	65500 ±4350

larger extent than into RNA. The uptake of radioactivity by the acid-soluble fraction and the respiration rate were not affected by 1 mM-colchicine *in vitro*.

When Ehrlich ascites-carcinoma cells were incubated with 1 mM-colchicine in the presence of thymus DNA the inhibitory effect of colchicine on the incorporation of [8-^{14}C]adenine into nucleic acid disappeared (Table 5). Incubation of Ehrlich ascites-carcinoma cells with 3 mM-colchicine resulted in an inhibition of the incorporation of [8-^{14}C]adenine into DNA and RNA by 70 and 32% respectively. When 2 mg. of DNA was also added to the medium, inhibition by colchicine was almost completely abolished for incorporation of [8-^{14}C]-adenine into DNA and completely abolished for incorporation into RNA (Table 5).

The results shown in Table 5 indicate that the addition of DNA to the medium abolished or greatly diminished the inhibitory effect of colchicine on the incorporation of [8-^{14}C]adenine into RNA and DNA. This fact points to a combination of colchicine with the DNA.

Since the optical rotation of colchicine in solution is strongly negative and that of DNA is strongly positive, we measured the optical activity of DNA and colchicine separately and as a mixture. Determinations were carried out at room temperature with a Zeiss Photoelectric Precision Polarimeter. Results (Table 6) are expressed as optical rotations at a wavelength of 589 mμ. The observed optical rotation of the mixture differed from the sum of the optical rotations of its components with different mixtures of DNA and colchicine. As a control, colchicine was replaced by fructose, which has a negative optical rotation. The observed values are almost identical with those expected

Table 5. *Effects of DNA on the inhibitory action of colchicine on* [8-^{14}C]*adenine incorporation into DNA and RNA* in vitro *in Ehrlich ascites-carcinoma cells*

Packed cells (0·1 ml.) were incubated in Krebs–Ringer phosphate buffer, pH 7·4, at 37° for 1 hr. in the presence of 10 mM-glucose and 0·1 mM-[8-^{14}C]adenine (380000 counts/min./vessel). The final volume was 3 ml. The gas phase was air. The results are means ± S.D. of six independent determinations, each run in duplicate.

Additions	Incorporation into DNA (counts/min./0·1 ml. of packed cells)	Incorporation into RNA (counts/min./0·1 ml. of packed cells)
None	630±52	11600±380
DNA (2 mg.)	656±58	11550±415
Colchicine (3 mM)	190±22	7900±650
Colchicine (3 mM)+DNA (2 mg.)	603±55	11650±410
Colchicine (1 mM)	310±22	9600±385
Colchicine (1 mM)+DNA (2 mg.)	645±60	11650±510

Table 6. *Optical rotations of colchicine, DNA and mixtures of both substances*

Expected value is the sum of readings obtained when optical rotation or each compound or mixture is measured separately.

	$[\alpha]_{589\,m\mu}$
Colchicine (1 mg./ml.)	−0·215°
DNA (1 mg./ml.)	+0·131
DNA (1 mg./ml.)+colchicine (1 mg./ml.) (observed value)	−0·183
DNA (1 mg./ml.)+colchicine (1 mg./ml.) (expected value)	−0·084
Colchicine (0·5 mg./ml.)	−0·107
DNA (1 mg./ml.)	+0·138
DNA (1 mg./ml.)+colchicine (0·5 mg./ml.) (observed value)	−0·031
DNA (1 mg./ml.)+colchicine (0·5 mg./ml.) (expected value)	+0·031
Colchicine (0·25 mg./ml.)	−0·0492
DNA (1 mg./ml.)	+0·138
DNA (1 mg./ml.)+colchicine (0·25 mg./ml.) (observed value)	+0·0483
DNA (1 mg./ml.)+colchicine (0·25 mg./ml.) (expected value)	+0·088
Fructose (1 mg./ml.)	−0·141
DNA (1 mg./ml.)	+0·138
DNA (1 mg./ml.)+fructose (1 mg./ml.) (observed value)	+0·003
DNA (1 mg./ml.)+fructose (1 mg./ml.) (expected value)	−0·003
Fructose (0·5 mg./ml.)	−0·0706
DNA (1 mg./ml.)	+0·138
DNA (1 mg./ml.)+fructose (0·5 mg./ml.) (observed value)	+0·063
DNA (1 mg./ml.)+fructose (0·5 mg./ml.) (expected value)	+0·067

from the sum of the individual components (Table 6).

Effects of mescaline on morphogenesis and incorporation of adenine into DNA and RNA of pupae. The chemical structure of mescaline resembles part of the colchicine molecule. H. Lettré (unpublished work, cited by Eigsti & Dustin, 1955) found no effect of mescaline on mitosis in fibroblasts in tissue culture.

We injected 100 μg. of mescaline hydrochloride (dissolved in 0·9% sodium chloride in a volume of 1 μl.) into each of 40 pupae. The pupae survived for 2–3 weeks without undergoing any differentiation and died after this period. Because of the similarity with the effects of colchicine we tested the action of mescaline hydrochloride on nucleic acid metabolism. First-day pupae were injected with mescaline hydrochloride and [8-^{14}C]adenine and incubated at 28° until their fifth day of pupation, when incorporation of radioactivity into RNA and DNA was measured. Results presented in Table 7 show that 160 and 80 μg. of drug inhibited

Table 7. *Effect of mescaline on* [8-^{14}C]*adenine incorporation into RNA and DNA of whole pupae* in vivo

First-day pupae were injected with [8-^{14}C]adenine (specific activity 53 μc/m-mole) (100 000 counts/min. in a volume of 10 μl.) and mescaline (1 μl.). Control pupae were injected with 0·9% NaCl. Radioactivity in DNA and RNA was determined after the pupae had been incubated for 5 days at 28°. The results are means ± S.D. of six independent determinations, each involving a group of six animals.

Injection	Incorporation into DNA (counts/min./100 mg. wet wt. of tissue)	Incorporation into RNA (counts/min./100 mg. wet wt. of tissue)
Control (0·9% NaCl)	6050 ± 280	37 000 ± 515
Mescaline (80 μg./pupa)	2840 ± 110	35 600 ± 565
Mescaline (160 μg./pupa)	2720 ± 103	41 000 ± 615

incorporation of [8-^{14}C]adenine into DNA by 55 and 53% respectively. These concentrations were without effect on the incorporation of [8-^{14}C]-adenine into RNA, indicating that the drug has a specific effect.

DISCUSSION

Administration of 10 μg. of colchicine/pupa arrests its differentiation into the adult insect, the pupa remaining alive for 2–3 weeks. The same concentration of colchicine inhibits DNA synthesis (Figs. 2 and 3) (measured as incorporation of radioactive precursors) and stimulates RNA synthesis (Fig. 1 and Table 1). The effects of colchicine on nucleic acid metabolism *in vivo* can first be detected 3 days after administration of the drug to first-day pupae. There is a stimulation of RNA synthesis even with 200 μg. of colchicine. With this dose of drug the stimulation occurs 3 days after the injection. An injection of 200 μg. of colchicine into first-day pupae causes an inhibition in RNA synthesis on the seventh day of pupation.

The stimulation in RNA synthesis occurring on the third day, when DNA synthesis was inhibited, could have been due to the fact that the pool of free nucleotides was shared by these two biochemical processes. Nygaard, Gruttes & Rusch (1960) studied the interrelationship between RNA and DNA synthesis in the slime mould, *Physarum polycephalum*, as a model system for growth and differentiation. During synchronous growth RNA synthesis was retarded only at the time of DNA synthesis. The investigators explained this finding as a competition for common precursors for synthesis of DNA and RNA. Such a competition was shown by Grant (1960) to occur in the early developing embryo of *Rana*. Sung & Quastel (1963)

reported a stimulation *in vitro* of [8-^{14}C]adenine incorporation into RNA when DNA synthesis was inhibited by sarcomycin in Ehrlich ascites-carcinoma cells.

In *Tenebrio* pupae the biochemical effects of colchicine *in vivo* are first detected 3 days after administration. The toxic effects of lethal doses of the drug in mammals are first apparent 1 week after administration. In both cases there is a lag period before the effects of the drug are observed. Therefore to demonstrate the biochemical effects of colchicine *in vitro* it is necessary to raise the concentration of the drug.

Though colchicine inhibits nucleic acid biosynthesis, the drug does not affect energy metabolism. This may be concluded from the fact that the concentration which inhibits DNA synthesis *in vivo* is without effect on the rate of incorporation of labelled glycine into protein. Moreover, when dorsal abdominal body-wall slices are incubated with [8-^{14}C]adenine and 10mM-colchicine, the uptake of label by the acid-soluble fraction as well as the amount of ATP formed were the same as in the control (Table 2).

In *Tenebrio* pupae colchicine primarily inhibits DNA synthesis and to a smaller extent RNA synthesis (Figs. 1 and 2). The same was found to be the case with mammalian tissues. 1mM-Colchicine had no effect on respiration, inhibiting mainly DNA synthesis and to a slighter extent RNA synthesis (measured as [8-^{14}C]adenine incorporated; Table 2). Since colchicine inhibits DNA synthesis and cell division without affecting energy metabolism, the question arises why it prevents differentiation in a tissue such as the dorsal abdominal wall, whose cells do not divide during metamorphosis (Wigglesworth, 1942) and in which no DNA synthesis occurs during this period (Ilan *et al.* 1966). When first-day pupae were injected with colchicine (200μg.) it was found that by the seventh day of pupation a 50% inhibition had occurred in RNA synthesis (incorporation of [2-^{14}C]-uridine). RNA synthesis was also inhibited when actinomycin D was administered to seventh-day pupae. Simultaneously with the inhibition in RNA synthesis, normal development of this tissue was arrested. It is therefore conceivable that in the dorsal abdominal body wall the inhibition in RNA synthesis is responsible for the arrest in differentiation.

If RNA synthesis on the seventh day is responsible for differentiation of this tissue, then the inhibition in RNA synthesis could explain why differentiation had not occurred. It is known that even ligated or isolated pupal abdomen can differentiate into the adult form. Gross & Cousineau (1963) have shown that incorporation of labelled amino acids into proteins of fertilized sea-urchin eggs continued for 7hr. after synthesis of RNA was stopped by addition of a relatively high concentration of actinomycin D. Under these conditions cell division continued but cell differentiation was suppressed.

When Ehrlich ascites-carcinoma cells were incubated *in vitro* with colchicine and thymus DNA, the latter provided full protection for [8-^{14}C]-adenine incorporation into RNA and DNA (Table 5). This result suggests that colchicine may inhibit DNA synthesis by combining with the DNA primer (template) in a specific manner, thereby preventing DNA from serving as a primer mainly for DNA synthesis and to a slight degree for RNA synthesis. Such a combination is known to occur with mitomycin C and DNA (Iyer & Szybalski, 1964). When DNA-dependent RNA synthesis is inhibited by actinomycin D it is believed that the latter inhibits RNA synthetase by combining with the DNA primer (template) (Reich, Franklin, Shatkin & Tatum, 1962; Bickis & Quastel, 1962). It has been suggested by Reich *et al.* (1962) that enzymes catalysing DNA biosynthesis and DNA-dependent RNA biosynthesis may differ significantly in their respective stereochemical relationship to DNA. Evidence for the interaction of colchicine with DNA is provided by the fact that when both substances are mixed there is a significant shift in the optical rotation from that expected by calculating the sum of the separate rotations of the two substances.

These results strongly suggest that colchicine under our experimental conditions inhibits DNA synthesis by combining with the DNA primer in a specific manner. However, they do not rule out the possibility that the observed inhibition in the incorporation of [8-^{14}C]adenine and [2-^{14}C]thymidine into DNA is due to the inhibition of one or more enzymes involved in DNA biosynthesis.

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