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# EFFECT OF COLCHICINE ON THE SYNTHESIS OF RIBONUCLEIC ACID IN MOUSE INTESTINAL MUCOSA*

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**Abstract**—The effect of colchicine on the synthesis of different fraction of ribonucleic acid in the mouse intestinal mucosa was studied. Two functionally different parts from small intestine—villi and crypts—were separated and the incorporation of [$^{14}$C]uridine into RNA was measured. Treatment with colchicine obscures the difference in total specific activities between nuclear and cytoplasmic RNA, which exists in control animals.

The results of separation of RNA in a sucrose density gradient show a stimulation of [$^{14}$C]uridine incorporation into cytoplasmic RNA from the villi (region 18 S to 4–5 S), and an inhibition of the incorporation in the same region in the crypts. The significance of these findings for the problem of small intestine function is discussed.

COLCHICINE is a classical mitotic inhibitor which produces a metaphase arrest in dividing cell populations[1] and causes dissolution of the mitotic spindle,[2] perhaps due to contamination with microtubule protein.[3]

Colchicine administration results in diarrhoea and intestinal malabsorption even when used in less than antimitotic doses. Small doses of colchicine also have been found to depress oxidative enzyme activity in the intestinal mucosa,[4] and in aerobic glucose metabolism in phagocyte.[5] It is possible that altered absorption could be explained on this basis rather than as a result of interference with cell division.

Previous experiments in this laboratory indicated that relatively high concentrations of colchicine (1·5–10 mg/kg body wt.) suppressed the incorporation of [$^{14}$C]orotic acid into RNA which has a DNA-like base composition extracted from the whole mouse small intestine at 60°, pH 8·3.[6]

This work examines the effect of colchicine on the synthesis of different RNA fractions in the villi and crypts of the mouse intestinal mucosa.

## MATERIALS AND METHODS

*Animals.* Adult, male mice about 3 months old, weighing 35–40 g were fasted for 18 hr before being used in these experiments. Colchicine was administered (1·5 mg/kg body wt.) intraperitoneally 6 hr before the mice were sacrificed. RNA was labelled *in vivo* 3 hr later by giving another i.p. injection of 50 $\mu$c[$^{14}$C] uridine/100 g body wt. dissolved in 0·2 ml saline.

*Fractionation of the tissue.* The animals were killed by a blow on the head and the

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small intestine was removed, from duodenum to caecum, the bowel was well rinsed with 100 ml of ice-cold saline containing 1% (w/v) bentonite, opened along the mesentery side and placed on a chilled glass plate under a microscope. The villi were then scraped off and separated from the remaining mucosa containing crypts by gentle movement of a tongue depressor. All subsequent stages were preformed at 4°. The villi were added to 20 ml of a chilled saline and collected after centrifugation for 10 min at 3000 *g*, weighed and homogenized with 15 vol. of buffer (0·05 M tris–HCl pH 7·6, 0·003 M $CaCl_2$, 0·25 M sucrose containing 1% bentonite) in a Dounce tissue homogenizer with a loose pestle (six strokes).[7] The rest of the mucosa (including mainly crypts and lamina propria) was removed from the wall by another scraping, weighed and homogenized in the same way. The homogenate was centrifuged for 10 min at 1000 *g*. The sediment was resuspended in 5 ml of the above buffer and recentrifuged. Both supernates were combined and the cytoplasmic RNA extracted from them. Nuclear RNA was extracted from the sediment.

*Cytoplasmic RNA.* Sodium dodecylsulfate was added to the supernate to a final concentration of 5 mg/ml, and the mixture was then extracted at 0° for 15 min with an equal volume of water-saturated phenol containing 0·1% (w/v) 8-hydroxyquinoline. The emulsion was separated by centrifugation for 10 min at 2000 *g* and the aqueous layer removed and re-extracted twice with phenol. The remaining phenol was removed from the aqueous phase with chloroform. To the aqueous layer was then added 0·1 vol. of a 20% (w/v) solution of potassium acetate of pH 5·0 and the nucleic acids were precipitated by adding two volumes of 96% ethanol at −15°. After 16 hr the nucleic acids precipitate was centrifuged and dissolved in a small volume of distilled water. The amount of nucleic acid was estimated by their absorbance at 260 nm.

*Crude nuclear RNA.* The nuclear sediment was suspended in 10 ml 0·1 M sodium acetate pH 6·0 and was shaken for 15 min at 60° with an equal volume of water-saturated phenol containing 0·1% 8-hydroxyquinoline. The suspension was then cooled to 0° centrifuged and the aqueous phase carefully removed with a pipette. This phase was twice re-extracted with phenol at 0°. The subsequent procedure was the same as with cytoplasmic RNA.

*Separation of RNA in a sucrose gradient.* We used a linear sucrose density gradient [5–22% (w/v) sucrose] in 0·05 M tris–HCl pH 7·6.[8] To remove a possible contamination with ribonuclease a suspension of DEAE cellulose equilibrated with the same buffer was added (10 ml of 5% suspension/100 ml sucrose solution). After 30 min the DEAE cellulose was removed by filtration.[8] 0·1–0·2 ml solution of nucleic acid (5–10 mg/ml) was carefully layered on top of 4·6 ml of sucrose gradient solution, which was then centrifuged in a Spinco model L ultracentrifuge, rotor SW 39, at 37,000 rev./min for 245 min at 4°. After centrifugation the bottom of the tube was punctured and the content of each tube was divided into 38 fractions. To each fraction was added 1 ml distilled water before reading the absorbance at 260 nm, and measuring the radioactivity.

*Determination of radioactivity.* Radioactivity was measured in a Nuclear Chicago Scintillation Counter. The sample of 1 ml was mixed with 15 ml scintillation mixture (PPO 7 g, POPOP 0·5 g, naphthalene 70 g/l. dioxane).

*Materials.* Uridine-2-$^{14}$C, specific activity 27 mc/m-mole was obtained from Schwarz BioResearch Inc. Colchicine ampoule 1 mg/2 ml was a product of Eli Lilly Co. All other chemicals were Anal. R. grade.

## RESULTS

The effect of colchicine on the incorporation of [$^{14}$C]uridine into RNA by the different parts of mouse intestinal mucosa is shown in Table 1. The distribution of RNA among the crude nuclear and cytoplasmic fractions reflected the inability of the

TABLE 1. EFFECT OF COLCHICINE ON INCORPORATION OF [$^{14}$C]URIDINE INTO RNA IN DIFFERENT PARTS OF MOUSE INTESTINAL MUCOSA

| | Control | | | | Treated with colchicine | | | |
|---|---|---|---|---|---|---|---|---|
| | Villi | | Crypts | | Villi | | Crypts | |
| | Nuclear RNA | Cytopl. RNA | Nuclear RNA | Cytopl. RNA | Nuclear RNA | Cytopl. RNA | Nuclear RNA | Cytopl. RNA |
| mg RNA* per g tissue | 0·62 | 1·63 | 0·70 | 2·36 | 0·74 | 2·00 | 0·49 | 2·25 |
| dis./min per g tissue | 3923 | 1721 | 4560 | 5000 | 1371 | 4532 | 2189 | 5293 |
| dis./min per mg RNA | 6300 | 1055 | 6480 | 2120 | 1856 | 2264 | 4470 | 2350 |

Details concerning isolation procedure are given in Methods section. Average of four experiments.
* Calculation was made on the base of 32 absorbance units = 1 mg RNA.

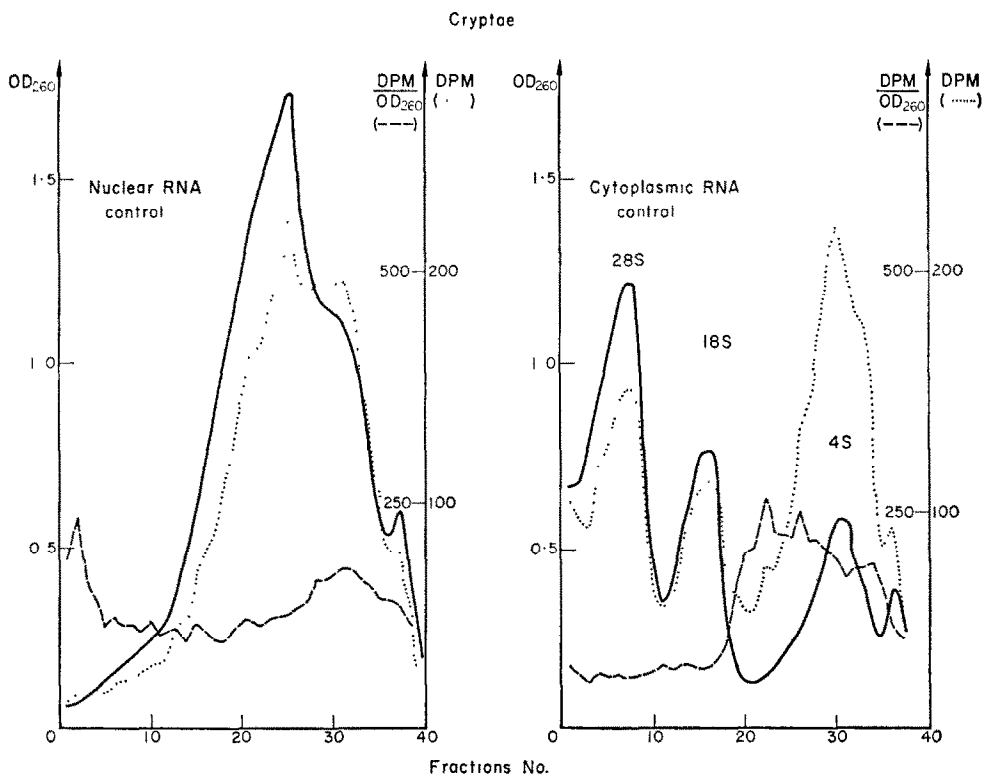


FIG. 1. Incorporation of [$^{14}$C]uridine into RNA of crypts in control animals. Sucrose density gradient as described in text. $OD_{260}$—absorbance at 260 nm, DPM—dis./min, dis./min/$OD_{260}$—specific activity of RNA, S—designation of RNA sedimentation constants.

usual centrifugation method to separate quantitatively the cell particles[9] but a comparison is always possible between these fractions. Colchicine reduced the specific activity of crude nuclear RNA extracted from intestinal villi and crypts. On the other hand, colchicine increased the specific activity of cytoplasmic RNA from villi and crypts. In general, treatment with colchicine minimized the difference in the specific activities between crude nuclear and cytoplasmic RNA as compared to the corresponding animals without treatment.

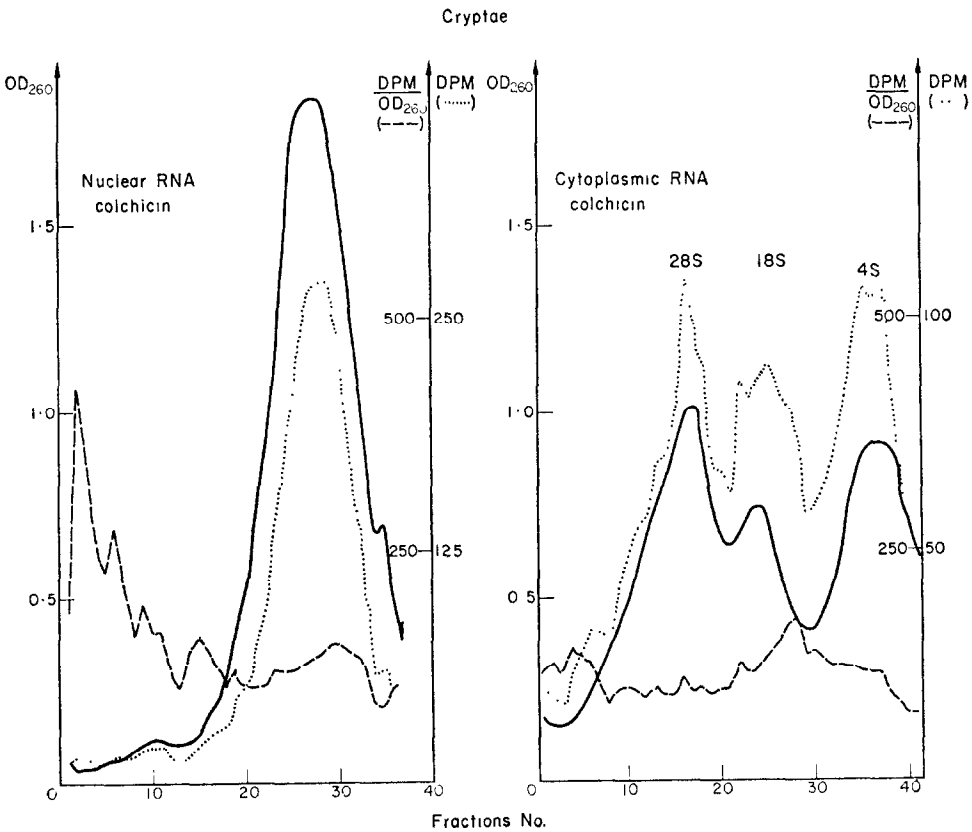


FIG. 2. Incorporation of [$^{14}$C]uridine into RNA of crypts in treated with colchicine animals. Same conditions as in Fig. 1.

The results of sucrose density gradient separation of RNA showed a difference between villi and crypts. As shown in Figs. 1 and 2 the incorporated radioactivity corresponded to the changes in absorbance at 260 nm for both nuclear and cytoplasmic fractions from the crypts. Ribosomal RNA newly synthesized during this time period is found in the cytoplasm. In the control experiments using crypts the maximum incorporated radioactivity was found in sRNA, and the maximum specific radioactivity appeared between 18 S and 4–5 S (Fig. 1). Treatment with colchicine abolished the difference in incorporation into 28, 18 and 4 S RNA.

The separation was not so good in the case of crude nuclear RNA possibly because of aggregation of ribosomal RNA during the hot phenol extraction procedure,[10] and

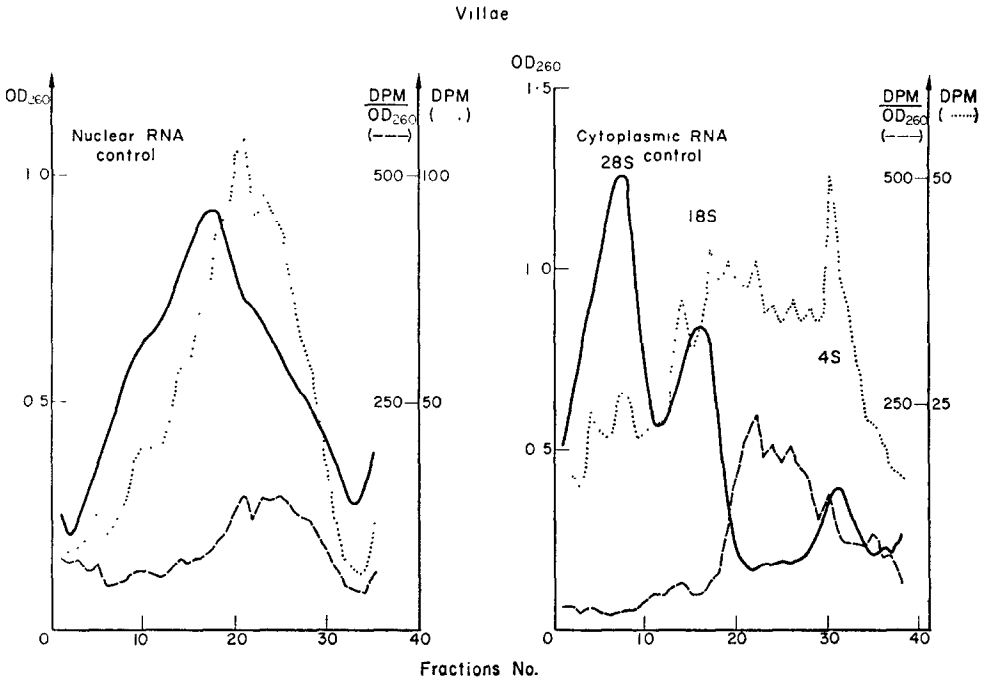


FIG. 3. Incorporation of [$^{14}$C]uridine into RNA of villi intestinales in control animals. Same conditions as in Fig. 1.

in the presence of cytoplasmic contaminants of this fraction.[9] After colchicine injection there may have been some increase in specific activity of nuclear RNA in its heavier fractions.

The incorporation of [$^{14}$C]uridine into cytoplasmic RNA from the villi did not parallel the absorbance at 260 nm and had a maximum value between 18 S and 4–5 S. A maximum specific activity was found in this same region in control mice. Colchicine exaggerated this tendency (Figs. 3 and 4). In the nuclear fraction, colchicine treatment also increased the specific activity mainly in the region with higher sedimentation constant. Although the explanation for these findings is unknown in the case of crude nuclear RNA, because of possible aggregation or contamination, one might still distinguish two different colchicine effects: (a) stimulation of uridine incorporation into cytoplasmic RNA of intestinal villi (region 18 S to 4–5 S) and (b) inhibition of incorporation of RNA of the same region by intestinal crypts. These data suggest a different action of colchicine upon various parts of intestinal mucosa.

## DISCUSSION

In Erhlich ascites carcinoma cells treated with colchicine inhibition of sRNA synthesis has been observed.[11] Pretreatment with massive doses of glutamic acid prevented the inhibition of RNA synthesis produced by the alkaloid. Uptake of [$^{14}$C]valine into tumor cell proteins also was depressed by the treatment. It was suggested that the inhibition of synthesis of soluble RNA after colchicine treatment was sufficient to lead to a decreased formation of protein required specifically for the mitotic processes.

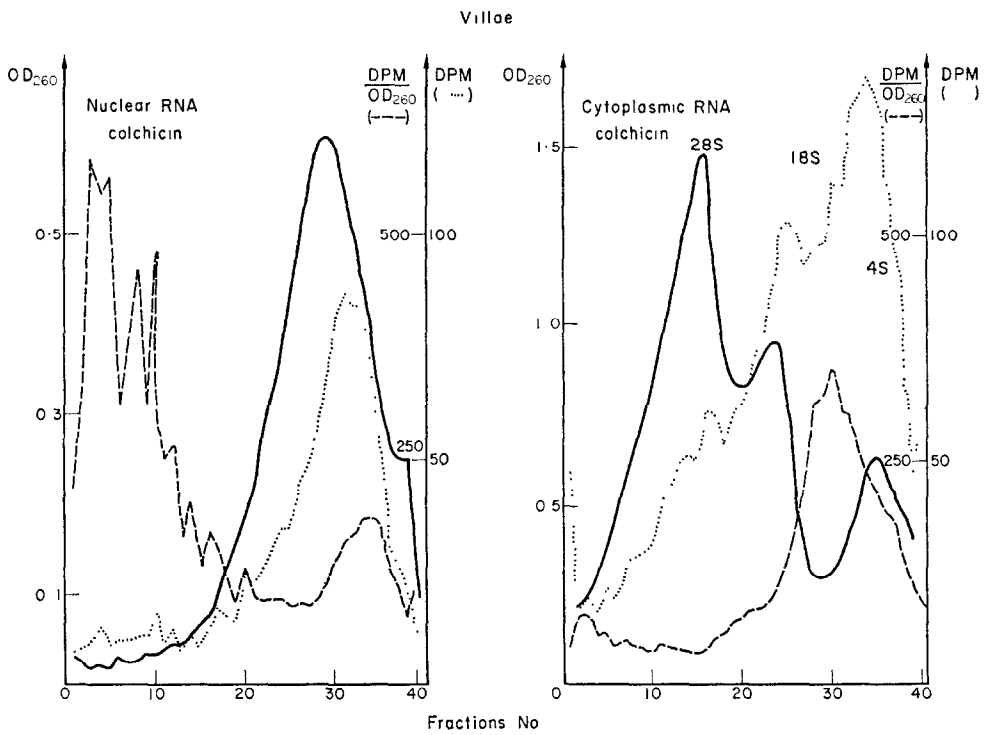


FIG. 4. Incorporation of [$^{14}$C]uridine into RNA of villi in treated with colchicine mice. Same conditions as in Fig. 1.

A similar conclusion was reached by studying effect of several mitotic inhibitors in leukemia human leucocytes[12] where there was inhibition of [$^{3}$H]uridine incorporation into all classes of RNA, but most strikingly by sRNA. Normal granulocytes are resistant to this inhibitory action.

If we assume that higher total specific activity of nuclear RNA in control mice (Table 1) indicates that RNA synthesis occured in the nuclei and the RNA diffused into cytoplasm, then decrease of difference in specific activities after treatment with colchicine means either suppressed synthesis of RNA in the nuclei or accelerated diffusion toward cytoplasm. There is also a clear difference in the behavior of villi and crypts to colchicine. The general effect of alkaloid in mouse small intestine represents a reduction of nuclear RNA synthesis.

Mice treated with colchicine show an impaired ability to supply new cells from the crypts, thereby altering the architecture of the villi. It seems that cells on the tips of the villi because of delayed replacement, survive longer and thus may become functionally inadequate.[4,6] On this basis, depression of absorption by the intestine appears to be a secondary effect of colchicine.

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