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EFFECTS OF COLCHICINE ON THE TRANSCRIPTION RATE OF β-CASEIN AND 28 S-RIBOSOMAL RNA GENES IN THE RABBIT MAMMARY GLAND

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SUMMARY : The transcription rate of β-casein and 28S ribosomal RNA genes was evaluated using isolated mammary nuclei incubated with HgCTP. The β-casein mRNA and 28S rRNA sequences in the neosynthesized mercurated RNA and specifically retained on a SH-column were quantified with the corresponding ^{3}H-cDNA probes. Prolactin injected into pseudo-pregnant rabbits provoked an accumulation of β-casein mRNA and 28S rRNA in the mammary cell by increasing the transcription rate of both genes and by stabilizing selectively the β-casein mRNA. Colchicine injected with prolactin totally prevented the acceleration of the β-casein gene transcription whereas it did not prevent the activation of the ribosomal gene and the enhancement of the β-casein mRNA stability. These data suggest that prolactin sends multiple messages to the mammary cell and that the transfer of the prolactin information from the peripheral receptor to the β-casein gene is selectively interrupted when the integrity of microtubules is affected.

INTRODUCTION : The initiation of casein synthesis by prolactin is accompanied by an accumulation of casein mRNA (1-3). This accumulation of casein mRNA is the result of a stabilization of these molecules and of an enhancement of their gene transcription rate (4-7). The action of prolactin in vivo is coincident with an enrichment of the mammary cell in ribosomal RNA (8). The intracellular relay carrying the prolactin information from the hormone receptor on the plasma membrane to genes is presently unknown (6). Recent works demonstrated that the down-regulation of prolactin receptor (9), is not strickly required for prolactin action (10-12). It was also observed that colchicine (10-12) and related drugs (13) inhibit prolactin action without altering greatly the down-regulation of the receptor. These facts suggested that microtubules or at least cellular structures which contain tubulin are involved in the transmission of prolactin message to genes. The present work aims at examining the effect of colchicine on the transcription rate of β-casein and 28S rRNA genes.

MATERIALS AND METHODS

Pseudopregnant rabbits were injected with prolactin or colchicine for one day between days 12 and 14 after mating with a vasectomized male, essentially as described in previous work (8).

In all cases, ovine prolactin PS-13, kindly provided by NIH, was injected at 9.00 a.m. and 7.00 p.m., intramuscularly (100 IU per injection) and subcutaneously (25 and 50 IU per injection). Colchicine (Prolabo) dissolved in water was injected subcutaneously, (2 or 4 mg per injection), on mornings and evenings one hour before prolactin.

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Lactating rabbits were used around dav 20 of lactation. They were separated from the pups all day long except on mornings at 8.30 for 30 minutes when they were milked (7). Unless stated, all the treatments were of one day. In all cases, mammary gland was collected 90 minutes after milking.

Total mammary β-casein mRNA and 28S rRNA were evaluated by an hybridization with ^{3}H cDNA probes synthesized using AMV reverse transcriptase kindly provided by Dr J.W. Beard. The hybridization curves, not shown here, give the concentration of the two RNA species in the total mammary RNA. The total amount of these RNAs per mammary cell was calculated from the data of the hybridization curves and the content of the mammary cell in total RNA (8).

Nuclei were obtained from frozen tissue after a centrifugation through 2.2M sucrose. They were incubated in the presence of Hg CTP. The resulting neosynthesized Hg RNA was retained selectively on a SH-Sepharose column and eluted with β-mercapto-ethanol. The eluted RNA was precipitated by EtOH with E. coli tRNA as carrier. The β-casein mRNA and 28S rRNA sequences were evaluated by hybridizations with the cDNA probes. All these techniques have been described in detail in an earlier work (7).

In all cases, at least four pseudopregnant and two lactating rabbits were used for each determination.

The culture of the mammary explants was carried in 199 medium in the presenc of insulin (5 µg/ml) essentially as previously described (11). The concentration of β-casein mRNA was evaluated using the cDNA probe (1).

α-amanitin was from Boehringer, 5,6 dichloro-β-ribofuranosylbenzimidazole from Calbiochem, and actinomycin D from Rhône-Poulenc.

RESULTS :

1) Action of colchicine during induction by prolactin

The transcription of β-casein and 28S ribosomal RNA gene is easily detectable in the pseudopregnant untreated rabbit. The specificity of these measurements is established by the fact that α-amanitin and actinomycin D added to the incubation medium of the nuclei reduced to undetectable levels the sequence of β-casein mRNA and 28S rRNA respectively in the fractions eluted from the SH-Sepharose column (Fig. 1A and 1B).

Colchicine injected for one day into pseudopregnant rabbits, at the dose of 4 mg or 8 mg per day did not significantly alter the transcription rate of both genes. Prolactin injected for one day accelerated significantly the transcription of the β-casein and 28S rRNA genes. Colchicine injected with prolactin totally prevented the effect of the hormone on the activation of β-casein gene whereas it did not hamper the enhancement of 28S rRNA synthesis (Fig. 1A and 1B).

The acceleration of the transcription of both genes was accompanied by an accumulation of the transcription products in the mammary cell. In agreement with a previous work (7) it can be observed that during the prolactin treatment,the content of the mammary cell in β-casein mRNA and 28S rRNA was increased about 40 and 2 fold respectively whereas their transcription rate was 4.5 and 2 times higher than before the hormone action (Fig. 2A and 2B). This suggests that prolactin acts on both genes but stabilizes specifically the casein mRNA.

Colchicine injected with prolactin totally and partially prevented the increase of the casein gene transcription and the accumulation of the casein mRNA

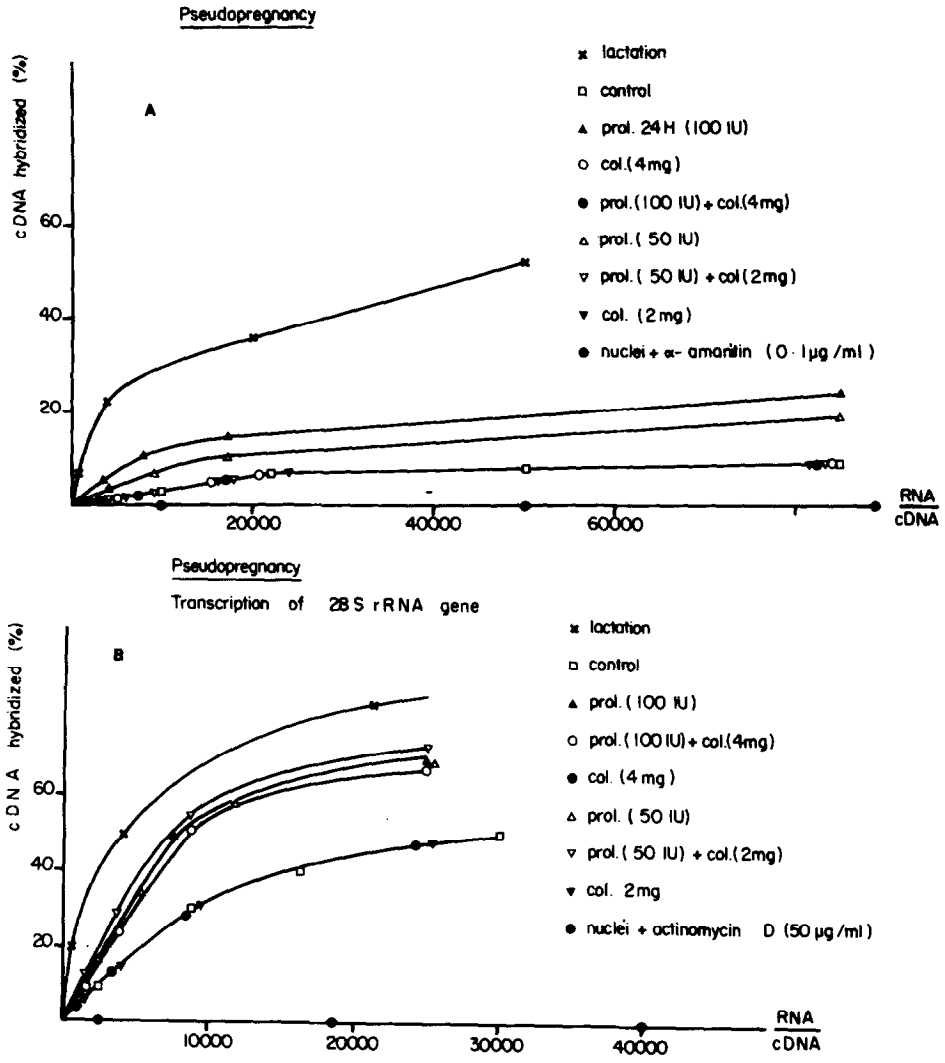

Fig. 1 - Transcription rate β-casein and 28S rRNA genes in nuclei from mammary glands of the pseudopregnant rabbits after treatments by prolactin and colchicine. Data are kinetic hybridization curves of the cDNA probes with the RNA eluted from the SH-Sepharose column. Results are expressed in % of the cDNA hybridized with the corresponding RNA sequence, thus resistant to S_1 nuclease as a function of the $\frac{RNA}{cDNA}$ ratio in the hybridization incubate.

(□) Untreated pseudopregnant rabbit
(▼) Colchicine 2 mg/injection
(○) Colchicine 4 mg/injection
(△) Prolactin 50 IU/injection
(▲) Prolactin 100 IU/injection
(▽) Prolactin 50 IU + colchicine 2 mg/injection
(●) Prolactin 100 IU + colchicine 4 mg/injection
(×) Lactating rabbit
(⊙) Lactating rabbit, nuclei incubated in the presence of 0.1 µg/ml α-amanitin
(⊙) Lactating rabbit, nuclei incubated in the presence of 50 µg/ml actinomycin D
A β-casein mRNA
B 28S rRNA

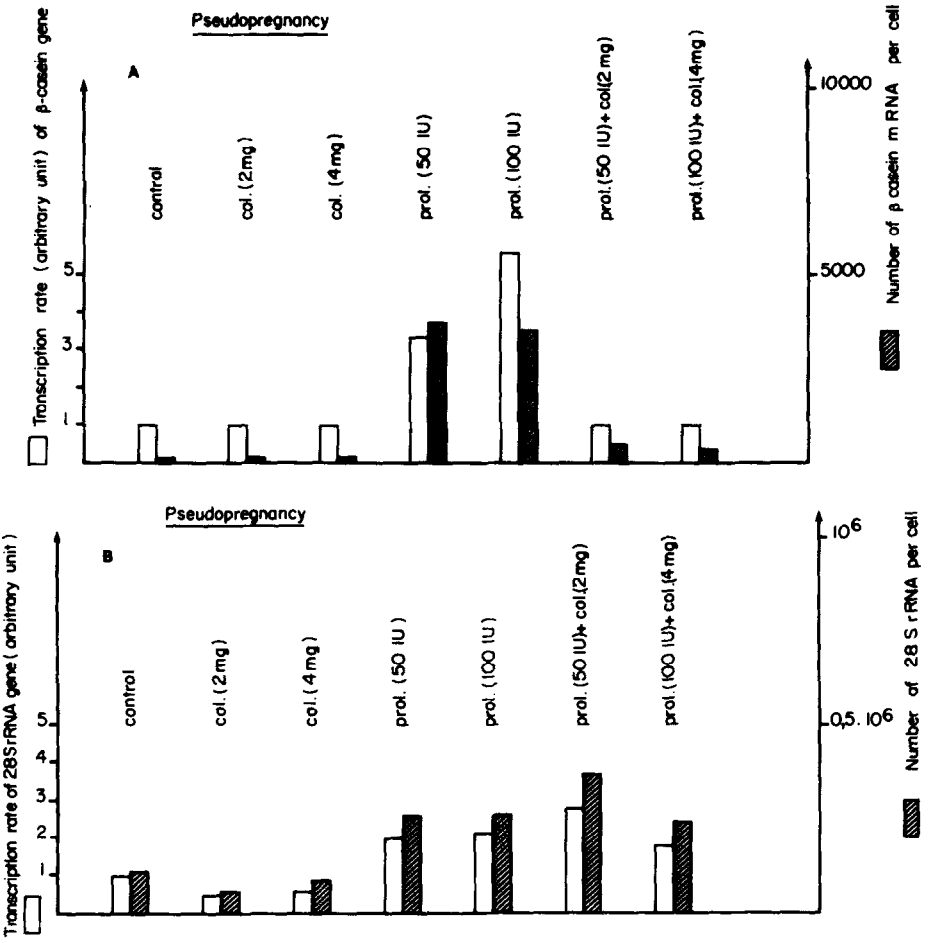

Fig. 2 - Variation of the transcription rates and of the amount of specific RNA per mammary cell. Results of the transcription rate are deduced from the hybridization curves of Fig.1 taking as a basis the value 1 obtained in the untreated pseudopregnant rabbit. The amount of the specific RNAs per mammary cell is deduced from hybridization curves not shown here which give the concentration of the RNA and from the total mammary RNA per cell given by the $\frac{RNA}{DNA}$ ratio (8).

A β-casein mRNA
B 28S rRNA

respectively, as though the capacity of the hormone to stabilize the casein mRNA but not its capacity to enhance the casein gene transcription was maintained in the presence of the drug (Fig.2A). Colchicine did not alter the response of the 28S rRNA gene (Fig. 2B). All these observations were recorded using two doses of prolactin (100 IU and 50 IU/injection) and also two doses of colchicine (2 mg or 4 mg/injection)

2) Action of colchicine in the lactating rabbit

Colchicine injected into lactating rabbits for one day reduced milk production by half. This effect was essentially reversible at the dose of 2 X 2 mg or 2 x 4 mg per day, since the day following cessation of the treatment, milk production returned to its original level (result not shown). This effect may be attributed to at least two actions of colchicine : a drop of prolactin secretion by hypophysis (14), and a direct inhibition of milk secretion from the mammary cell (15).

After a colchicine treatment for one day, the transcription rate of the β-casein gene was only about half of that observed in the untreated animal (Fig.3A). The same treatment by colchicine affected only very slightly the transcription rate of the 28S rRNA gene (Fig. 3B). Prolactin, injected with colchicine to maintain a high circulating hormone level despite the action of the drug on hypophysis, was unable to restore the transcription of β-casein gene to its normal value (Fig.3A). By contrast the injections of prolactin prevented largely the slight inhibitory effect of colchicine on the transcription of the 28S rRNA gene (Fig. 3B).

During the treatment of the lactating rabbits by colchicine for one day, the amount of β-casein mRNA was reduced to about half of its value (Fig. 4A). This drop may be ascribed to the slowing down of the gene transcription, but also possibly to a decrease of the casein mRNA stability which is highly dependent on prolactin, as shown earlier after a withdrawal of prolactin by a CB 154 treatment (7). Injections of prolactin with colchicine in the lactating rabbits almost totally restored the level of β-casein mRNA in the mammary cell, despite the reduction of the transcription rate of the gene(Fig.4A).

The amounts of 28S rRNA in the mammary cell during the colchicine and prolactin treatments in the lactating animal were essentially coincident with the transcription rate of the gene. Prolactin was able to suppress the slight colchicine action (Fig.4B).

3) Effect of colchicine added to culture medium

Previous work demonstrated that colchicine added to the culture medium of mammary explants prevented the induction of casein synthesis and the parallel accumulation of casein mRNA (10,11). In addition, colchicine and several other microtubule disrupting drugs, proved not to alter the rate of casein synthesis when added to the culture medium of mammary fragments explanted from lactating rabbits and cultured for one day in the absence of prolactin (11,13). Results of Fig.5 indicate that after one day of culture in the absence of prolactin, the mammary cell of the lactating rabbit has lost spontaneously a large amount of the casein mRNA. No additional loss was provoked by colchicine added to the culture medium. This suggests that colchicine per se does not affect the transcriptional process of the β-casein

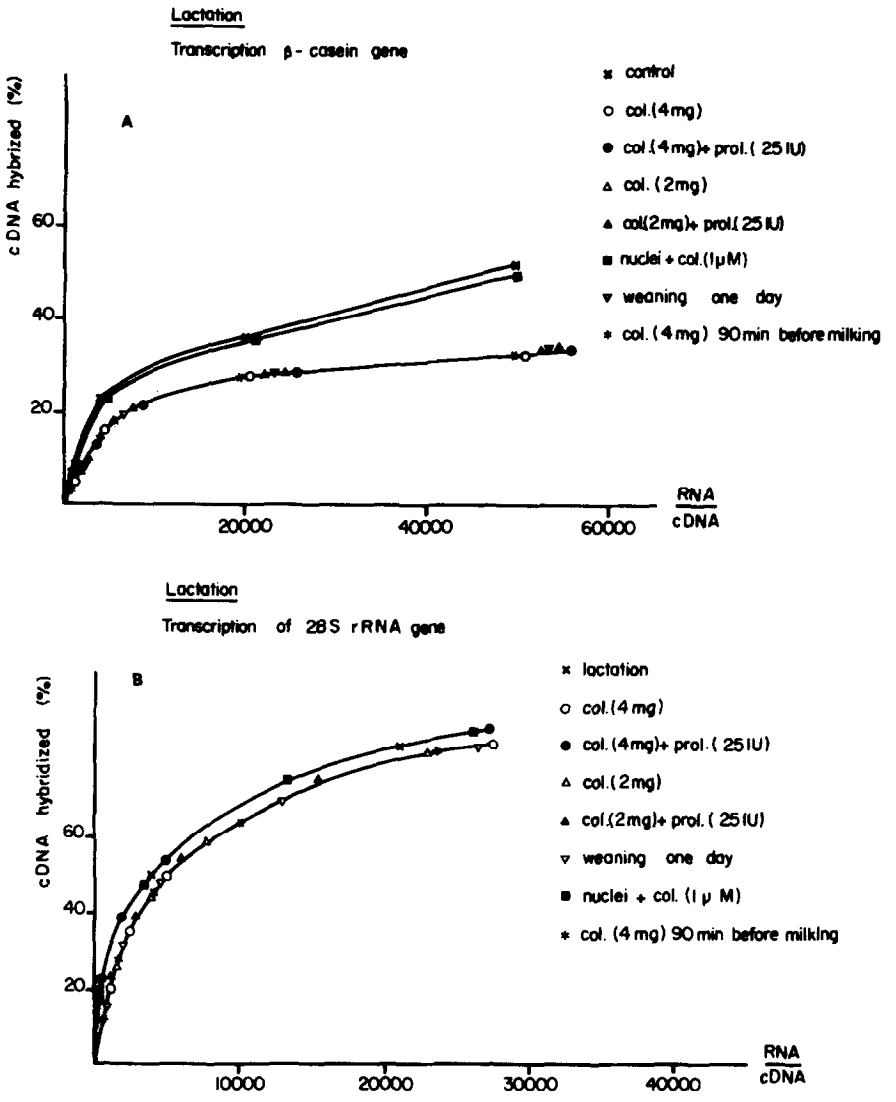

Fig. 3 - Transcription rate of β-casein and 28S genes in isolated nuclei from mammary glands of the lactating rabbit after treatment by prolactin and colchicine. Data are kinetic hybridization curves of the cDNA probes with the RNA eluted from the SH-Sepharose column. Results are expressed in % of the cDNA hybridized with the corresponding RNA sequences as a function of the $\frac{RNA}{cDNA}$ ratio in the hybridization incubate.

(×) Untreated lactating rabbit
(Δ) Colchicine 2 mg/injection
(O) Colchicine 4 mg/injection
(▲) Colchicine 2 mg/injection + prolactin 25 IU/injection
(●) Colchicine 4 mg/injection + prolactin 25 IU/injection
(▽) Untreated lactating rabbit separated from pups for one day
(✱) Colchicine 4 mg, 90 minutes before milking
(■) Nuclei extracted from the mammary gland of an untreated lactating rabbit and incubated in the presence of 1 μM colchicine
A β-casein mRNA
B 28S rRNA

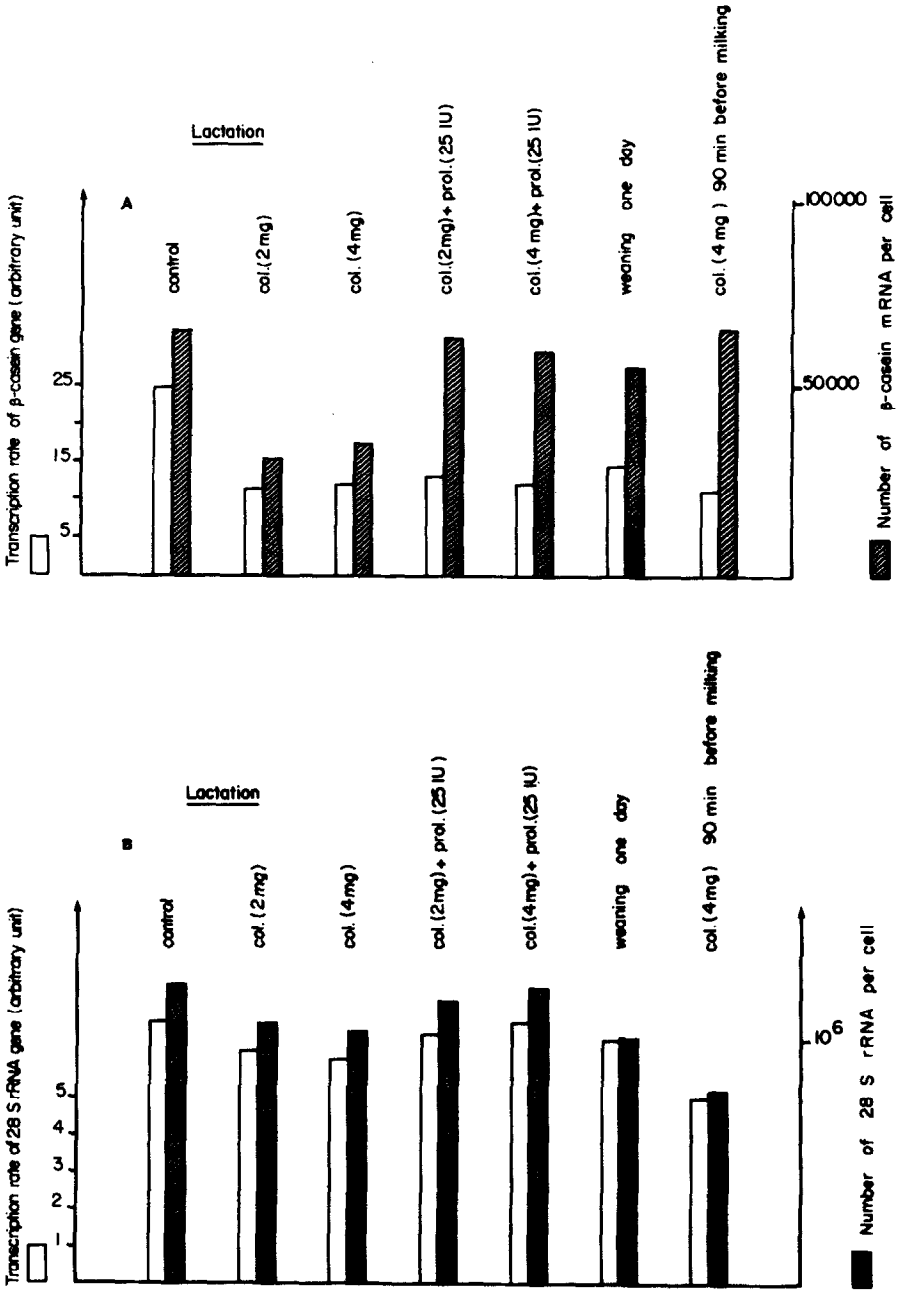

Fig. 4 - Variation of the transcription rates and of the amount of specific RNA per mammary cell. Results of transcription rate are deduced from the hybridization curves of Fig.3 taking as a basis the value 1 obtained in the untreated pseudopregnant rabbit. The amount of the specific RNA per mammary cell is deduced from hybridization curves not shown here, which give the concentration of the RNA and from the total mammary RNA per cell given by the $\frac{\text{RNA}}{\text{DNA}}$ ratio (8).

A β-casein mRNA
B 28S rRNA

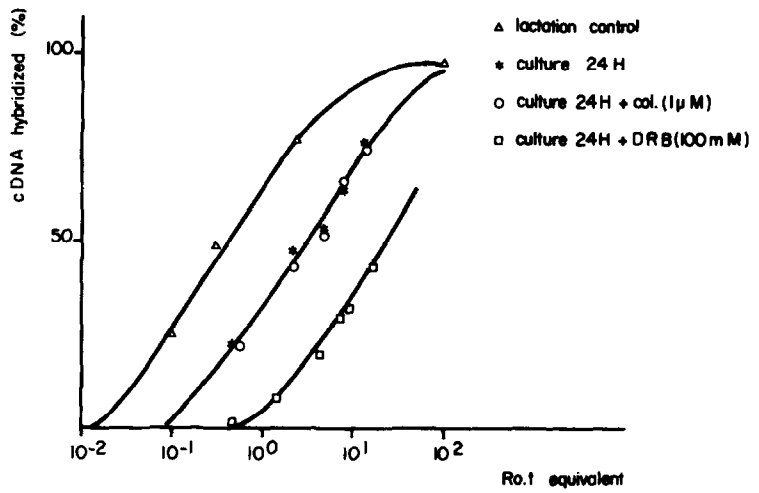

Fig. 5 - Effect of colchicine and 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) on the concentration of β-casein mRNA. The drugs were added to the culture medium which contained insulin (5 μg/ml). Duration of the culture was one day. Results are expressed in % of the cDNA hybridized as a function of the concentration of RNA in the hybridization incubate (R_o) and the time (t).

(△) Lactating rabbit before the culture
(✱) At the end of the culture without drug
(○) Colchicine 1 μM
(□) DRB 100 mM

gene. This conclusion is reinforced by the fact that the content of the mammary tissue in β-casein mRNA was greatly reduced when the transcription of genes coding for mRNAs was blocked by 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (Fig.5) (16). Moreover, colchicine added to the incubation medium of the isolated nuclei did not hamper the transcription of the β-casein and 28S rRNA genes (Fig. 3A and 3B).

CONCLUSION

In earlier works, it was shown that colchicine and several other microtubule disrupting drugs prevented the initiation of casein synthesis and the accumulation of casein mRNA (10,11,13). The present work demonstrates that colchicine in vivo prevents also the acceleration of the transcription rate of the β-casein gene by prolactin, as though the transmission of the prolactin signal from the receptor on the plasma membrane to genes was interrupted as soon as the integrity of the microtubule network is altered. The possibility that colchicine acts directly on the transcription mechanism is rendered unlikely by the fact that the drug has no effect when added to the cell-free transcription medium. However, clearly not all the prolactin signal is blocked by colchicine since the acceleration of the trans-

cription of the 28S rRNA gene takes place normally, as in the absence of the drug. Similarly, injections of colchicine in the lactating rabbit reduced the amount of casein mRNA in the mammary cell but the drug was unable to prevent prolactin to stabilize casein mRNA.

It may seem surprising that colchicine exhibits some inhibitory effect when it is injected into the lactating rabbit whereas this drug is inactive when added to the culture medium of tissue explanted from animals during lactation. This discrepancy is partly due to the fact that the culture was carried out in the absence of prolactin in order to evaluate the effect of colchicine independently of prolactin action. In the in vivo experiments, two events must be taken into consideration: the secretion of prolactin by hypophysis and milk removal. Indeed, the drop of casein mRNA in the mammary cell after colchicine injections into the lactating animal is probably due to the lack of prolactin in blood, since the level of casein mRNA could be maintained with moderate doses of injected prolactin. By contrast, the transcription rate of casein gene was not restored by the hormone. This lack of effect of the hormone on transcription under these conditions is not really surprising. Indeed, prolactin withdrawal during lactation was shown to reduce the transcription rate of casein gene only after several days of treatment by CB 154 (7). Thus, the drop of the transcription rate of casein gene induced in the lactating rabbit by colchicine is probably due neither to a lack of prolactin, nor to a direct effect of the drug on the transcriptional process. In a previous study, it was observed that the engorgement of the mammary gland by milk during weaning is an extremely potent signal which leads to an inhibition of casein gene transcription. This inhibitory effect of milk accumulation is not reversed by hormone injections (7). A daily variation of the transcription rate of the casein gene seems to reflect the normal working of the mammary gland, since the transcription was reduced by half when the rabbit was left one day without being milked (Fig. 3A and Ref.7), the natural interval between two milkings in this species. Thus, it looks as though milk removal was unable to elevate the transcription rate of casein gene when colchicine is present. It should be kept in mind that the daily variation of casein gene transcription is observed even under a treatment by CB 154 thus in the absence of prolactin (at least over a period of one or two days), as long as milk is normally withdrawn (7). A direct evaluation of this hypothesis was attempted by injecting colchicine into a lactating rabbit, only once 90 minutes before milking. This treatment by colchicine did not reduce the amount of milk sucked by the pups but it totally prevented the enhancement of casein gene transcription which normally takes place after milking (Fig. 3A). Earlier observations have shown that the progressive accumulation of milk in acini is accompanied by morphological changes of the mammary epithelial cells : the cells are cuboidal after milking and flattened when acini are filled with milk (17). Possibly, these changes of cell morphology during the

milking cycle require the integry of the cytoskeleton and they may be related to parallel variation of the casein gene transcription.

From the observations reported here and in previous papers (10,11,13), it can be concluded that prolactin gives a multiple information to the mammary cell leading to a cell multiplication, to an activation of casein gene transcription, to a stabilization of casein mRNA, to an activation of rRNA gene transcription and to variation of other cellular functions not considered here. Only the two first parameters are clearly affected by colchicine, suggesting that prolactin actions in the mammary cell are mediated through several independent mechanisms. Interestingly, it appears that the capacity of prolactin to stabilize casein mRNA, to activate rRNA gene transcription and to accumulate total nucleotides in the mammary cells is exerted simultaneously as though a common mechanism was involved for these three parameters (7). It remains to determine whether all the effects of prolactin result from a direct action on the mammary cell since for instance, no clear accumulation of rRNA in the mammary gland was observed in the explants during culture even in the presence of prolactin (1).

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