in the hormone-dependent milk protein synthesis in mouse mammary epithelium in vitro. It is noteworthy that the largest accumulation of spermidine occurs in the presence of insulin, hydrocortisone, and prolactin, the combination of hormones that is necessary for the maximal stimulation of casein and α-lactalbumin in vitro (*5*, *6*). Correspondingly, in the presence of insulin and prolactin, small increases in milk protein synthesis were accompanied by small increases in the spermidine. Recently, Russell and McVicker (*2*) reported that the spermidine concentration increased to 5 μmole per gram of tissue during lactation in the rat mammary gland. The consistency between their observation and the data presented here affords confidence that enhancement of spermidine accumulation by insulin, hydrocortisone, and prolactin in vitro may reflect physiological changes in mammary epithelium during lactogenesis.

The ability of exogenously added spermidine to stimulate milk protein synthesis in cultured mammary cells provides more direct evidence for its involvement in lactogenesis. Spermidine is effective at a concentration as low as $4 \times 10^{-4} M$, which is in the range found in the cells that are actively synthesizing milk proteins under the influence of insulin, hydrocortisone, and prolactin. This effect of spermidine is specific for hydrocortisone, since spermidine did not replace the requirement for insulin or prolactin. At present, however, little is known about the mechanism of spermidine action on milk protein synthesis. Moreover, it is yet to be determined whether spermidine itself is the active agent or whether its effect is mediated through its metabolites.

Finally, it should be mentioned that spermidine is not quite as effective as hydrocortisone with respect to milk protein synthesis. This difference may simply be due to some unknown side effects of the polyamine or due to its limited entry into the cells. However, more interesting is an alternative possibility that the steroid hormone may exert some critical effects which cannot be fulfilled by the exogenous addition of the polyamine in the culture system.

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5-Methylcytidylic Acid: Absence from Mitochondrial DNA of Frogs and HeLa Cells

Abstract. *The content of 5-methylcytidylic acid in nuclear DNA and mitochondrial DNA of* Xenopus laevis *and HeLa cells has been determined. Both nuclear DNA's contain 5-methylcytidylic acid. The 5-methylcytidylic acid content of* X. laevis *DNA is 1.7 mole percent of total nucleotides, and that of HeLa cell DNA is 0.7 mole percent. In neither mitochondrial DNA could any 5-methylcytidylic acid be detected; the limit of sensitivity was judged at below 0.1 mole percent for* X. laevis *DNA and below 0.05 mole percent for HeLa cell DNA.*

Nuclear DNA from many animals contains 5-methylcytidylic acid (Me-C) in addition to the four major nucleotides (*1*). The presence of this minor constituent has been suggested in the mitochondrial DNA (mtDNA) of *Physarum* (*2*), but only recently has a report appeared on the methylation of animal mtDNA (*3*).

In the course of studies on the Me-C content of ribosomal DNA my colleagues and I have adapted a highly sensitive method for its detection (*4*). The method utilizes ^{32}P-labeled DNA, which is digested to either 3′ or 5′ mononucleotides by a suitable combination of nuclease and phosphodiesterase. The nucleotides are separated by two-dimensional thin-layer chromatography and visualized by radioautography. By increasing the exposure times, small amounts of ^{32}P-labeled

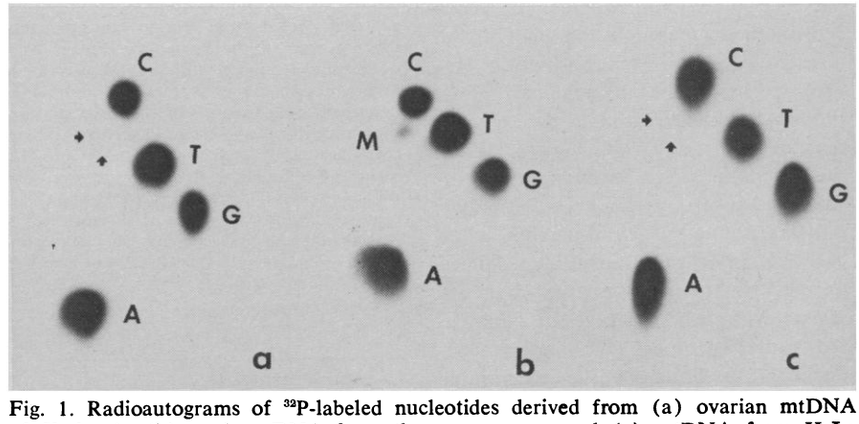

Fig. 1. Radioautograms of ^{32}P-labeled nucleotides derived from (a) ovarian mtDNA of *X. laevis*, (b) nuclear DNA from the same ovary, and (c) mtDNA from HeLa cells. A female *X. laevis* was injected with 1 mc of [^{32}P]phosphate; 3 days later the ovary was removed, and mtDNA was prepared as described (*8*); nuclear DNA was prepared from the connective tissue of the same ovary (*4*, *8*). HeLa cells were cultured for 3 days in the presence of [^{32}P]phosphate (10 μc/ml). The closed-circular component of mtDNA was prepared with the use of ethidium bromide–CsCl gradients (*9*). All DNA's were digested with micrococcal nuclease and spleen phosphodiesterase under the conditions described previously and separated by two-dimensional thin-layer chromatography (*4*). The individual nucleotides are indicated, with *M* standing for Me-C; *A*, adenylic acid; *C*, cytidylic acid; *G*, guanylic acid; and *T*, thymidylic acid. In (a) and (c) the position of carrier unlabeled Me-C in the chromatogram is indicated by two arrows.

nucleotides can be detected. The amount of label present is quantified either by cutting out each spot and counting in a liquid scintillation spectrometer or by densitometric evaluation of the radioautogram. The latter method is particularly suited for the determination of relative quantities of small amounts of a nucleotide. This method has now been applied to mtDNA of HeLa cells and to cells of the frog *Xenopus laevis*.

Xenopus mtDNA was prepared from the ovary of a frog that had received an injection of [^{32}P]phosphate 3 days before dissection. Nuclear DNA was prepared from the same animal. HeLa cells were exposed to ^{32}P for about three cell generations and closed-circular mtDNA was prepared (Fig. 1). Nuclear DNA was obtained from the same cell culture. These DNA samples were digested to 3′ mononucleotides, separated on thin-layer chromatography plates, and radioautographed. Unlabeled authentic Me-C was added before chromatography to mark its location on the chromatogram. Figure 1 shows the Me-C spot in the digest of *Xenopus* nuclear DNA (Fig. 1b) and the absence of any spot at this position in the digests of both *Xenopus* and HeLa mtDNA (Fig. 1, a and c). HeLa cell nuclear DNA contained a Me-C spot.

Table 1 shows the Me-C content of the nuclear and mitochondrial DNA's determined in this way. The Me-C content of *Xenopus* nuclear DNA is in good agreement with that reported earlier (*4, 5*). HeLa cell nuclear DNA has a lower Me-C content in good agreement with that reported (*6*). Chromatographs of both HeLa and *Xenopus* mtDNA's did not show detectable Me-C spots. The sensitivity of the experiments was estimated from the size of the smallest peak that would have been detected unambiguously in densitometer tracings and comparing such "minimal" peaks with the peaks of the major nucleotides (Table 1). If Me-C were present below the detection limit there could be 15 to 30 residues in each molecule of mtDNA.

No minor spot was seen on the radioautograms of mtDNA hydrolyzates. This fact suggests that no minor nucleotide occurs in mtDNA, although it is possible that some modified nucleotides comigrate with one of the major nucleotides. This can only be tested by specifically looking for certain modified nucleotides. It has been shown that 5-hydroxymethylcytidylic acid separates from the four major nucleotides in the system used here (*7*); this nucleotide is therefore absent from mtDNA, as well as from nuclear DNA.

Table 1. 5-Methylcytidylic acid (Me-C) content in *Xenopus* and HeLa DNA's. The Me-C content was determined from densitometric traces of autoradiograms (Fig. 1 and similar experiments). The sensitivity of the procedure was estimated from the smallest densitometric peak that could have been observed.

Cells	Me-C, mole percent of total nucleotides	
	Nuclear DNA	mtDNA
X. laevis	1.7	< 0.1
HeLa	0.7	< 0.05

Recently, Nass reported the presence of Me-C in mtDNA from mouse and hamster cells (*3*). From 12 to 36 Me-C residues were reported to occur per molecule of mtDNA. These values are close to the range of detectability limit in my work. This fact, and possible species differences, may explain the discrepancy between Nass' conclusion and my result. Alternatively, the methylation of mouse and hamster mtDNA might have been overestimated. In the analyses of Nass, the amount of Me-C was determined from the radioactivity of a region of the chromatogram known to contain Me-C; however, the presence of a distinct spot of radioactive Me-C has not been established by either radioautography of rechromatography. The possibility might thus be considered that the observed low level of radioactivity was due to other bases which "smeared" into the Me-C region.

The only other report where an animal DNA has been analyzed for Me-C at similar sensitivity with a negative result concerns the amplified ribosomal DNA of *X. laevis* which contains less than 0.2 percent Me-C (*4*). Like mtDNA, amplified ribosomal DNA is extrachromosomal. Whether this fact is causally related to the absence (or very low content) of Me-C is not known.

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Prostaglandins Stimulate Thyroid Function in Pregnant Women

Abstract. *Intravaginal or intravenous administration of prostaglandin $F_{2\alpha}$ to pregnant women produced significant elevations of plasma triiodothyronine and thyroxine concentrations, but no rise of thyrotropin. These observations are consistent with those of previous in vitro studies indicating that prostaglandins can act directly on the thyroid gland to stimulate thyroid hormone synthesis or release, or both.*

Prostaglandins, a group of naturally occurring and widely distributed hydroxycarboxylic acids, have far-reaching physiologic effects, including stimulation of many indicators of thyroid cell activity. Several investigators have demonstrated that prostaglandin E_1 or E_2 can increase adenylate cyclase activity, adenosine 3′,5′-monophosphate concentration, glucose oxidation, and colloid droplet formation in thyroid slices or homogenates (*1, 2*). Iodine trapping, organification of iodine, and proteolysis have also been stimulated in thyroid slices by prostaglandin E_1 (*3*). Similar results have been reported for prostaglandins of the F series (*2, 4*). In view of these in vitro observations, we examined the effects of prostaglandins on thyroid function in women receiving prostaglandins either for termination of first-trimester pregnancy or for induction of labor.

Fourteen women in early pregnancy (7 to 14 days past their expected menstruation) who desired voluntary interruption of pregnancy were admitted to the Clinical Research Center at the New York University–Bellevue Hospital Center. Pregnancy was confirmed