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.

IGOR B. DAWID
Department of Embryology,
Carnegie Institution of Washington,
115 West University Parkway,
Baltimore, Maryland 21210

References and Notes

1. H. S. Shapiro, in *Handbook of Biochemistry*, H. A. Sober, Ed. (Chemical Rubber Co., Cleveland, 1968), pp. H-30.
2. H. H. Evans and T. E. Evans, *J. Biol. Chem.* **245**, 6436 (1970).
3. M. M. K. Nass, *J. Mol. Biol.* **80**, 155 (1973).
4. I. B. Dawid, D. D. Brown, R. H. Reeder, *ibid.* **51**, 341 (1970).
5. I. B. Dawid, *ibid.* **12**, 581 (1965).
6. G. M. Brown and G. Attardi, *Biochem. Biophys. Res. Commun.* **20**, 298 (1965).
7. P. M. M. Rae, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 1141 (1973).
8. I. B. Dawid and D. R. Wolstenholme, *J. Mol. Biol.* **28**, 233 (1967); I. B. Dawid, *ibid.* **63**, 201 (1972).
9. R. Radloff, B. Bauer, J. Vinograd, *Proc. Natl. Acad. Sci. U.S.A.* **57**, 1514 (1967).
10. I thank M. Rebbert for technical assistance.

19 October 1973; revised 28 January 1974

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

by physical examination and a positive pregnancy test (Prognosticon, Organon). All had a negative personal and family history of thyroid disease and were free of goiter.

A suppository containing 50 mg of prostaglandin $F_{2\alpha}$ ($PgF_{2\alpha}$) (Upjohn, Kalamazoo, Michigan) was inserted intravaginally every 2 hours for three doses, and a dose of 100 mg was inserted every 2 hours thereafter for 12 to 24 hours until vaginal bleeding was induced. Peripheral venous blood was collected before initiation of prostaglandin administration and every 4 hours thereafter, and was analyzed for triiodothyronine (T3) (*5*), thyroxine (T4) (*5*), and thyrotropin (TSH) (*6*) by radioimmunoassay. Eleven of the fourteen women passed the conceptus within 24 hours, while the remaining three required suction curettage of the uterus for completion of the abortion. During prostaglandin administration, all subjects demonstrated systemic effects consisting of nausea, vomiting, diarrhea, or abdominal cramps.

After intravaginal administration of prostaglandins, T3 concentrations rose from a basal value of 156 ± 24 ng/100 ml (mean ± standard error) to a peak value of 256 ± 38 ng/100 ml; T4 concentrations rose from 7.6 ± 0.9 μg/100 ml to 10.4 ± 1.8 μg/100 ml. These differences were significant ($P < .01$). Peak T3 concentrations were observed at 4 hours in seven subjects, at 8 hours in one subject, and at 24 hours in two others. Concentrations of T4 reached a peak at 4 hours in four subjects, at 8 hours in four subjects, and at 24 hours in three others. There was no significant rise in T3 values in four subjects, or in T4 values in three subjects. Basal TSH concentrations were normal, 2.8 ± 1.3 microunit/ml, and failed to rise after prostaglandin administration.

Twelve normal pregnant women at term were also studied. All had a negative personal and family history of thyroid disease. They were admitted to the hospital at the estimated due date, and labor was induced by intravenous infusion of $PgF_{2\alpha}$ (Upjohn). The infusion rate was 2.5 μg/min for the first hour, 5 μg/min for the second hour, and 10 μg/min thereafter for up to 10 hours. Blood samples were obtained before initiation of prostaglandin infusion and hourly thereafter. All women underwent normal delivery within 12 hours.

Basal T3 and T4 concentrations were 179 ± 24 ng/100 ml and 9.8 ± 1.0 μg/100 ml, respectively, normal values for women in the third trimester of pregnancy (*7*). After $PgF_{2\alpha}$ infusion, T3 concentrations rose to 210 ± 26 ng/100 ml, while T4 values rose to 12.6 ± 1.7 μg/100 ml, both significant changes ($P < .01$). Concentrations of T3 were highest after 2 hours of $PgF_{2\alpha}$ infusion in six women, after 4 hours in one woman, and after 6 hours in another. Concentrations of T4 reached peak values after 2 hours in three subjects, after 4 hours in three others, and after 6 hours in two others. In one subject, there was no rise in either T3 or T4 values during prostaglandin infusion. In three subjects only T3 values rose, and in three others only T4 concentrations increased. Concentrations of TSH were unaltered (3.2 ± 1.2 microunit/ml before infusion; 2.9 ± 1.3 microunit/ml during infusion).

In view of the well-documented negative feedback of thyroid hormones on TSH secretion, a fall in TSH values might have been anticipated in these subjects (*8*). That this was not observed might be due to the lack of sensitivity of the TSH assay for hormone amounts at the lower limits of the normal range. Alternatively, prostaglandins could also be acting at the pituitary or hypothalamic level to enhance TSH secretion, thereby blunting the expected fall in TSH.

The possibility that the observed effect was mediated by placental TSH cannot be excluded, since the placental hormone does not cross-react in the human TSH radioimmunoassay (*9*). Alternatively, labor itself might cause an elevation in thyroid hormone concentration, as described for T3 (*10*). If this were so, the women receiving intravenous prostaglandin at term would probably have shown the greater changes. In fact, the women receiving intravaginal prostaglandins in the first trimester of pregnancy demonstrated a more marked response. Definitive resolution of these possibilities awaits studies in human male subjects. However, our initial observations in male rats indicate that prostaglandins cause increased serum T3 and T4 concentrations, which suggests that neither labor nor the presence of a uterus or a placenta is a prerequisite for this response.

After either TSH administration (*11*) or a rise in TSH concentration induced by TSH-releasing hormone (*12*), there is a disproportionate rise in serum T3 compared to that of serum T4. That this was not observed in the women receiving prostaglandins is further evidence that the prostaglandin effect on thyroid hormone concentrations was not mediated by TSH. Prostaglandins might directly affect the ratio of thyroidal secretion of T3 and T4, or they might affect peripheral conversion of T4 to T3. These possibilities warrant further study. Whatever the mode of prostaglandin action, our observations raise the possibility that these compounds, which have been isolated from the thyroid of different species (*13*), including man (*14*), have a role in regulating thyroid function in vivo.

L. SHENKMAN
Y. IMAI
K. KATAOKA
C. S. HOLLANDER
Endocrine Group, Department of Medicine, New York University School of Medicine, New York 10016
L. WAN
Department of Obstetrics and Gynecology, New York University School of Medicine
S. C. TANG, T. AVRUSKIN
Division of Pediatric Endocrinology and Metabolism, Department of Pediatrics, Brookdale Hospital Medical Center, Brooklyn, New York 11212

References and Notes

1. T. Kaneko, U. Zor, J. B. Field, *Science* **163**, 1062 (1969); T. Onaya and D. H. Solomon, *Endocrinology* **86**, 423 (1970); U. Zor, G. Bloom, I. P. Lowe, J. B. Field, *ibid.* **84**, 1082 (1969).
2. U. Zor, T. Kaneko, I. P. Lowe, G. Bloom, J. B. Field, *J. Biol. Chem.* **244**, 5189 (1969).
3. G. Burke, K. Kowalski, D. Babiarz, *Life Sci.* **10**, 513 (1971); C. S. Ahn and I. N. Rosenberg, *Endocrinology* **86**, 396 (1970).
4. G. Burke, *J. Clin. Endocrinol. Metab.* **30**, 76 (1970); A. Dekker and J. B. Field, *Metab. (Clin. Exp.)* **19**, 453 (1970).
5. T. Mitsuma, J. Colucci, L. Shenkman, C. S. Hollander, *Biochem. Biophys. Res. Commun.* **46**, 2107 (1972).
6. W. D. Odell, J. F. Wilbur, R. D. Utiger, *Recent Prog. Horm. Res.* **23**, 47 (1967).
7. N. I. Robin, S. Refetoff, V. S. Fang, H. A. Selenkow, *J. Clin. Endocrinol. Metab.* **29**, 1276 (1969); T. Mitsuma, T. AvRuskin, L. Shenkman, K. Sau, J. Colucci, C. S. Hollander, *Excerpta Med. Int. Congr. Ser. No. 256* (1972), abstr. 522, p. 208.
8. S. Reichlin, in *Neuroendocrine-Pituitary Control in the Thyroid*, S. C. Werner and S. H. Ingbar, Eds. (Harper & Row, New York, ed. 3, 1971), pp. 95–111.
9. J. M. Hershman and W. R. Starnes, *J. Clin. Invest.* **48**, 923 (1969).
10. D. R. Hottelling and L. M. Sherwood, *J. Clin. Endocrinol. Metab.* **33**, 783 (1971).
11. P. R. Larsen, *J. Clin. Invest.* **51**, 1939 (1972).
12. C. S. Hollander, T. Mitsuma, L. Shenkman, P. Woolf, M. C. Gershengorn, *Science* **175**, 209 (1972).
13. S. M. M. Karim, K. Hillier, J. Devlin, *J. Pharm. Pharmacol.* **20**, 749 (1968).
14. E. D. Williams, S. M. M. Karim, M. Sandler, *Lancet* **1968-I**, 22 (1968); P. M. Grimley, E. J. Deftos, J. R. Weeks, A. S. Rabson, *J. Natl. Cancer Inst.* **42**, 663 (1969).
15. We thank the Upjohn Company for prostaglandins. Supported by PHS grants 2 RO1-AM 14303-03, RR 96, and 1 FO3-AM 51560-02.

6 August 1973; revised 9 November 1973