EFFECTS OF OESTROGEN AND PROGESTAGEN ON THE COMPOSITION OF HUMAN MILK

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Summary. Healthy nursing mothers were treated orally with an oestrogen (ethinyloestradiol, 0.3 mg daily) or a progestagen (6- α -methyl-17- α -hydroxyprogesterone acetate, 30 mg daily) for 5 full days after birth. Milk yield was not affected. A highly significant increase in protein content was observed in the milk of oestrogen-treated mothers. Theoretical implications of these results are discussed.

INTRODUCTION

Animal experiments, mostly on cows, have shown that milk composition may be modified by hormones to a limited extent (Meites, 1961). Feeding of thyroprotein to cows increases the percentage of butterfat (Reineke, 1942), with no changes in other milk components (Blaxter, Reineke, Crampton & Petersen, 1949). A wider 'enrichment' effect was observed by Folley (1936) and Folley, Scott Watson & Bottomley (1941) in the milk of lactating cows injected with moderate doses of oestrogens, which increased both fat and non-fatty solids. Spielman, Ludwick & Petersen (1941) and Hutton (1958) confirmed these results by injecting diethylstilboestrol and oestradiolbenzoate respectively. No parallel observations on nursing mothers have been reported so far. The present investigation, therefore, was designed to establish a possible influence of oestrogen and progestagen on human milk composition.

The inhibitory properties of both oestrogens and progestagens on lactogenesis in the human seemed to constitute a serious obstacle to such a study.

It has been shown (Toaff & Jewelewicz, 1963) that high doses of progestagen-oestrogen combinations are most effective inhibitors of lactogenesis in non-nursing mothers. The same doses also reduce lactation drastically in nursing mothers, as was observed in the preparatory stages of the present study. At the lower ovulation-inhibiting doses currently used in oral contraception, lactation may be diminished but rarely stops altogether (Rice-Wray, Goldzieher & Aranda-Rosell, 1963). Usually it is unaffected (Garcia & Pincus, 1964), particularly if the contraceptive therapy is not started before the appearance of breast milk (Kistner, 1967) and breast feeding continues.

Oestrogens have been widely used for 30 years for inhibition of lactation in

non-nursing mothers, but while they effectively prevent breast engorgement and pain, they do not prevent lactogenesis. This has been proved (Toaff & Jewelewicz, 1963) for ethinyloestradiol in the large dose of 0.3 mg daily. It seemed likely, therefore, that the effect of this drug on the composition of human milk could be studied even in non-nursing mothers, but with more ease and without harm in the presence of the powerful stimulus of suckling.

Progestagens, such as the orally active synthetic progestagen 6-alpha-methyl-17-hydroxyprogesterone acetate, which is devoid of oestrogenic action, have been shown to inhibit lactogenesis quite effectively when administered in high doses in the immediate *post partum* period to non-nursing mothers (Toaff & Jewelewicz, 1963). It was assumed that counteraction of the inhibitory action of this progestagen by the stimulus of suckling, might make it possible to study the modifications in milk composition resulting from the effects of the steroid. Clinical trials confirmed this assumption.

MATERIAL AND METHODS

Healthy mothers delivered in the Obstetric Department A of the Hakiryah Hospital, Tel Aviv, participated in the present investigation if their histories and conditions fulfilled the following requirements.

1. An uncomplicated second to fifth delivery at term.
2. The patient was not excessively fat.
3. Breast feeding was planned.

Of the patients selected, 175 completed the investigation. They were divided at random into three groups:

Group 1—comprising sixty-six women, treated with tablets of ethinyloestradiol (EO), 300 µg daily, in three equal doses;

Group 2—comprising sixty-three women, treated orally with 6-alpha-methyl-17-alpha-hydroxyprogesterone acetate (MAP), 30 mg daily, in three equal doses;

Control group—of forty-six women, treated with a placebo in the same way.

The investigation was conducted as a double-blind trial. The three groups were studied at the same time. Only the nurse in charge knew which tablets each patient received. The patients themselves were told that treatment was given as a routine to influence lactation. Treatment was started 1 to 4 hr after delivery and continued for 5 days. After 5 full days of treatment and following the 10 a.m. breast-feed, a 5-ml specimen of milk was obtained with the aid of a breast-pump. The specimen was collected in a sterile test-tube and kept refrigerated at 4° C up to the time of examination, which was carried out within 24 hr of collection. The specimens reaching the laboratory were only identified by a serial number.

While aware of the limitations and errors inherent in the method of estimating changes in milk composition by 'spot' samples (Hyttén, 1954b; Hyttén & Thompson, 1961), it proved to be impossible to persuade mothers to interrupt the nursing of their babies for a complete 24-hr period on the day preceding their discharge from hospital. In the absence of exact values for the 24-hr milk

yield, any quantitative estimation of changes in milk composition is bound to be arbitrary. If a rough estimate of milk yield, obtained by test-weighing of babies before and after the same feed rules out conspicuous changes in lactopoiesis, then statistically significant changes in milk composition, demonstrated by the method of 'spot' sampling, may be assumed to be fairly representative of actual quantitative changes in the composition of milk for the day investigated. For this reason, milk samples were obtained for examination at the end of a feed controlled by test-weighing.

A second reason for obtaining the milk specimen at the end of a feed was the well-known variation which occurs in the major milk constituents during a feed. The increase in fat content, constantly present at the end of a feed (Hytten, 1954a; Whittlestone & Perrin, 1954), is generally associated with a small decrease in the concentration of lactose and, after the 5th day, a small increase in protein concentration (Hytten, 1954a). It seemed, therefore, that the optimal conditions for examination of 'spot' specimens of milk were present at the end of the 10 a.m. feed, when the fat content is the highest for the day (Hytten, 1954b).

The specimens thus obtained were analysed for lactose, protein and fat content.

The lactose content of milk was estimated by the ortho-toluidine method for determination of aldohexoses according to Hultman (1959). The milk protein was estimated by the biuret reaction according to Natelson (1963). The fat content was estimated by an adaptation of the method of van de Kamer, Ten Bokkel & Weyers (1947), which allows the estimation to be carried out on small quantities (1 ml) of milk. The results are comparable with those obtained by methods based on extraction, evaporation and weighing of the fat residue of larger (5- to 10-ml) samples of milk. All estimations were done in duplicate.

RESULTS

In the presence of suckling, lactation is not inhibited by relatively high doses of EO (0.3 mg) or MAP (30 mg). Using the rough estimation of the amount of milk ingested based on test-weighing the baby before and after a test-feed on the 6th day after birth, the influence of the above treatment on the milk yield appeared negligible (Table 1).

The concentrations of lactose, protein and fat in the milk of the control group, Group 1 (EO) and Group 2 (MAP) are shown in Table 2.

TABLE 1
INFLUENCE OF 5 DAYS OF EO AND MAP TREATMENT
ON THE MILK YIELD OF NURSING MOTHERS

<i>Weight of milk (g)</i>	<i>Control group</i>	<i>EO group</i>	<i>MAP group</i>
Up to 30	8.7%	12.3%	7.9%
30 to 40	17.3%	24.3%	16%
45 to 55	67.3%	53%	60%
60+	6.5%	10.5%	16%

The values obtained for the control group were very similar to the average values for transitional milk (6 to 10 days) calculated by the National Research Council of the United States (1953) from sources selected from among more than 1500 reports of investigations on the composition of the mammary secretion of 'average', 'normal' or 'healthy' subjects (Macy & Kelly, 1961). A comparison is shown in Table 3.

TABLE 2
VALUES OF LACTOSE, PROTEIN AND FAT (G/100 ML MEAN $\pm$ S.D.) IN
TRANSITIONAL MILK OF HEALTHY NURSING MOTHERS

Milk component	Control	Group 1—EO	Group 2—MAP
Lactose	5.73 $\pm$ 0.11	5.54 $\pm$ 0.12	5.62 $\pm$ 0.14
<i>t</i>		1.05	0.55
<i>P</i>		<i>P</i> > 0.05	<i>P</i> > 0.05
Protein	1.76 $\pm$ 0.07	2.20 $\pm$ 0.09	1.88 $\pm$ 0.06
<i>t</i>		3.13	1.32
<i>P</i>		<i>P</i> < 0.01	<i>P</i> > 0.05
Fat	3.55 $\pm$ 0.28	3.77 $\pm$ 0.19	3.06 $\pm$ 0.14
<i>t</i>		0.68	1.66
<i>P</i>		<i>P</i> > 0.05	<i>P</i> = 0.1
Total solids	11.03 $\pm$ 0.29	11.53 $\pm$ 0.23	10.59 $\pm$ 0.20
<i>t</i>		1.89	1.85
<i>P</i>		0.1 > <i>P</i> > 0.05	0.1 > <i>P</i> > 0.05

TABLE 3
COMPARISON BETWEEN AVERAGE VALUES CALCULATED IN G/100 ML OF WHOLE TRANSITIONAL MILK (6 TO 10 DAYS) BY THE NATIONAL RESEARCH COUNCIL OF THE U.S.A. (1953) AND MEAN VALUES IN G/100 ML OF WHOLE MILK, 5 FULL DAYS AFTER DELIVERY, IN THE PRESENT STUDY

Composition of milk	National Research Council	Present study
Lactose	6.6	5.73 $\pm$ 0.11
Protein	1.6	1.76 $\pm$ 0.07
Fat	3.6	3.55 $\pm$ 0.28

Statistical evaluation (Student's *t*-test) of the results obtained for Groups 1 and 2 showed a highly significant increase in the protein content of the milk of mothers treated with the oestrogen (*P* < 0.01).

The frequency distributions of values for protein content in the three groups are shown in Text-fig. 1, together with their normal curves. While the distribution of values in Group 2 (MAP) is practically the same as in the control group, the distribution of values in Group 1 (EO) is obviously skewed to the right, owing to the prevalence of high values following oestrogenic treatment.

TEXT-FIG. 1. The frequency distribution of the protein content of milk compared in (a) a control group of healthy nursing mothers 5 days after birth, (b) Group 1—EO-treated mothers, and (c) Group 2—MAP-treated mothers. The normal curves are superimposed.

DISCUSSION

The mammary glands reach full development during pregnancy. Following delivery, the minimal requirements for initiating or maintaining lactation appear to be adequate amounts of prolactin or adrenal cortical hormones (Meites, 1966). Meites believes that during pregnancy there is insufficient prolactin or adrenal corticoids, or both, to initiate lactation. He considers the lack of adequate amounts of lactogenic hormones more important in the prevention of lactation during pregnancy than the relative refractoriness of the mammary glands to lactogenic stimulation caused by the prevailing high levels of oestrogen, and progesterone. A commonly accepted view (Folley, 1956; Cowie &

Folley, 1961; Meites, 1966) is that the high levels of progesterone present during pregnancy partially or completely block the release of prolactin, otherwise stimulated by the oestrogen.

The ability of MAP, which is devoid of any oestrogenic action, in doses which may be considered equivalent to the large amounts of progesterone secreted by the placenta into the maternal blood stream at term, to inhibit lactogenesis in non-nursing mothers, is compatible with the view of a central inhibitory action of progesterone. The results obtained in the present investigation by treating nursing mothers in the same way, may be considered as evidence supporting that view: suckling, which has been proved to stimulate a rapid release of prolactin, ACTH and oxytocin (Meites, 1959; Cowie & Folley, 1961), successfully overcomes the inhibitory effect of the progestagen. Lactogenesis develops, practically unimpaired, and the composition of milk is only slightly affected (reduction of fat and total solids of borderline significance).

The results obtained by treating nursing mothers with the potent oestrogen, ethinyloestradiol, seem to be explained by a direct, pharmacological action on the mammary glands. We found that a highly significant increase in the protein content of milk followed the use of relatively high doses of oestrogen (0.3 mg daily), while the milk yield remained unaffected. Animal experiments, reviewed by Meites (1961), show that the only effect of prolactin, or other components of the pituitary lactogenic complex, on lactating animals is an increase in milk yield with no changes in the composition of the milk except for an increase in percentage of milk fat after ACTH (Shaw, Chung & Bunding, 1955) or oxytocin (Adams & Allen, 1952). No central action of the exogenous oestrogen may therefore be postulated to explain our findings.

Villee and co-workers (Hagerman & Villee, 1952; Villee & Hagerman, 1953; Villee, Hagerman & Joel, 1960; Villee, 1963) produced evidence that oestrogens promote growth and increase the functional capacity of tissues which are usually considered target organs of oestrogens, through the activation of a specific enzyme, an oestrogen-dependent pyridine nucleotide transhydrogenase, which catalyses the reaction $\text{DPN}^+ + \text{TPNH} \rightarrow \text{DPNH} + \text{TPN}^+$. This oestrogen-dependent transhydrogenase has been shown to be present in human endometrium, myometrium, placenta, pituitary and mammary gland. According to Villee (1963), the increased activity of the enzyme resulting from the addition of oestrogens, both natural and synthetic, leads to an increase in the supply of biologically useful energy and thereby to an increase in the biosynthesis of proteins, nucleic acid and fats which are fundamental for growth. The same steps may lead to the biosynthesis of proteins and fat secreted by the mammary gland into the milk with the resulting 'enrichment' observed by us.

This action of ethinyloestradiol on the mammary glands should be considered a pharmacological one as, following delivery and removal of the placental source, the oestrogen levels in the maternal body fall rapidly. The urinary output of oestrone and oestradiol reaches non-pregnant levels within 5 days after delivery and remains low during the period of lactation-amenorrhoea (Brown, 1956). The high doses of ethinyloestradiol administered to influence the composition of milk establish a level of oestrogenic activity which is non-physiological during the lactation period.

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REFERENCES

- ADAMS, H. P. & ALLEN, N. N. (1952) The effect of removal of residual milk by use of oxytocin upon the yield and fat content of subsequent milkings. *J. Dairy Sci.* **35**, 1121.
- BLAXTER, K. L., REINEKE, E. P., CRAMPTON, E. W. & PETERSEN, W. E. (1949) The role of thyroidal materials and of synthetic goitrogens in animal production and an appraisal of their practical use. *J. Anim. Sci.* **8**, 307.
- BROWN, J. B. (1956) Urinary excretion of oestrogens during pregnancy, lactation, and establishment of menstruation. *Lancet*, **i**, 704.
- COWIE, A. T. & FOLLEY, S. J. (1961) *The mammary gland and lactation*. In: Sex and Internal Secretions, 3rd edn, Vol. 1. Ed. W. C. Young. Williams & Wilkins, Baltimore.
- FOLLEY, S. J. (1936) The effect of oestrogenic hormones on lactation and on the phosphatase of the blood and milk of the lactating cow. *Biochem. J.* **30**, 2262.
- FOLLEY, S. J. (1956) *The physiology and biochemistry of lactation*. Thomas, Springfield, Illinois.
- FOLLEY, S. J., SCOTT WATSON, H. M. & BOTTOMLEY, A. C. (1941) Experiments on the chemical enrichment of cow's milk by the administration of diethylstilboestrol and its dipropionate. *J. Dairy Res.* **12**, 1.
- GARCIA, C. R. & PINCUS, G. (1964) Clinical considerations of oral hormonal control of human fertility. *Clin. Obstet. Gynec.* **7**, 844.
- HAGERMAN, D. D. & VILLEE, C. A. (1952) Effects of estradiol on the metabolism of human endometrium in vitro. *Archs Biochem. Biophys.* **40**, 481.
- HULTMAN, E. (1959) Rapid specific method for determination of aldosesaccharides in body fluids. *Nature, Lond.* **183**, 108.
- HUTTON, J. B. (1958) Oestrogen function in established lactation in the cow. *J. Endocr.* **17**, 121.
- HYTTEN, F. E. (1954a) Clinical and chemical studies in human lactation. II. Variation in major constituents during a feeding. *Br. med. J.* **i**, 176.
- HYTTEN, F. E. (1954b) Clinical and chemical studies in human lactation. III. Diurnal variation in major constituents of milk. *Br. med. J.* **i**, 179.
- HYTTEN, F. E. & THOMPSON, A. M. (1961) *Nutrition of the lactating woman*. In: Milk: the Mammary Gland and its Secretion, Vol. II. Eds. S. K. Kon and A. T. Cowie. Academic Press, New York and London.
- KISTNER, R. W. (1967) *Therapeutic application of progestational compounds in gynecology*. In: Advances in Obstetrics and Gynecology, Vol. I. Eds. S. L. Marcus and C. C. Marcus. Williams & Wilkins, Baltimore.
- MACY, I. G. & KELLY, H. J. (1961) *Human milk and cow's milk in infant nutrition*. In: Milk: the Mammary Gland and its Secretion, Vol. II. Eds. S. K. Kon and A. T. Cowie. Academic Press, New York and London.
- MEITES, J. (1959) *Mammary growth and lactation*. In: Reproduction in Domestic Animals, Vol. I. Eds. H. H. Cole and P. T. Cupps. Academic Press, New York and London.
- MEITES, J. (1961) *Farm animals: hormonal induction of lactation and lactopoiesis*. In: Milk: the Mammary Gland and its Secretion, Vol. I. Eds. S. K. Kon and A. T. Cowie. Academic Press, New York and London.
- MEITES, J. (1966) *Mammary growth and lactation*. In: Neuroendocrinology, Vol. I. Eds. L. Martini and W. F. Ganong. Academic Press, New York and London.
- NATELSON, S. (1963) *Microtechniques of clinical chemistry*, 2nd edn. Thomas, Springfield, Illinois.
- NATIONAL RESEARCH COUNCIL U.S.A. (1953) The composition of milk. *Bull. natn. Res. Coun., Wash.* **254**.
- REINEKE, E. P. (1942) cited by Meites (1961).
- RICE-WRAY, E., GOLDZIEHER, J. W. & ARANDA-ROSELL, A. (1963) Oral progestins in fertility control: a comparative study. *Fert. Steril.* **14**, 402.
- SHAW, J. C., CHUNG, A. C. & BUNDING, I. (1955) The effect of pituitary growth hormone and adreno-corticotrophic hormone on established lactation. *Endocrinology*, **56**, 327.
- SPIELMAN, A., LUDWICK, L. M. & PETERSEN, W. E. (1941) Effect of diethylstilbestrol in milk secretion. *J. Dairy Sci.* **24**, 499.
- TOAFF, R. & JEWELWICZ, R. (1963) Inhibition of lactogenesis by combined oral progestogens and oestrogens. *Lancet*, **ii**, 322.
- VAN DE KAMER, J. H., TEN BOKKEL, H. & WEYERS, H. A. (1947) Rapid method for the determination of fat in feces. *J. biol. Chem.* **177**, 347.

- VILLEE, C. A. (1963) *The influence of oestrogens on uterine and placental enzymes*. In: Modern Trends in Human Reproductive Physiology, Vol. I. Ed. H. M. Carey. Butterworths, London.
- VILLEE, C. A. & HAGERMAN, D. D. (1953) Effects of estradiol on the metabolism of human placenta in vitro. *J. biol. Chem.*, **205**, 873.
- VILLEE, C. A., HAGERMAN, D. D. & JOEL, P. B. (1960) An enzymatic basis for the physiologic functions of estrogens. *Recent Prog. Horm. Res.* **16**, 49.
- WHITTLESTONE, W. G. & PERRIN, D. R. (1954) Variations in fat content of human milk during suckling. *J. Dairy Res.* **21**, 204.