CCCXVI. THE EFFECT OF OESTROGENIC HORMONES ON LACTATION AND ON THE PHOSPHATASE¹ OF THE BLOOD AND MILK OF THE LACTATING COW.

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THE numerous papers which have appeared on the endocrine control of lactation since the review by Turner [1932] have been admirably reviewed, and the present position summarized, by Nelson [1936]. It now seems clear that mammary development is promoted by the oestrogenic hormones of the ovary and the corpus luteum hormone (though the respective roles of the two types of hormone appear to differ among different species), while the possibility remains that the hypophysis may exercise some direct influence on the development of the mammary gland. The initiation and maintenance of lactation in developed mammary glands is almost certainly due to the action of the lactation-producing hormone of the anterior pituitary, usually called prolactin, which was discovered by Stricker & Grüter [1928].

In order to explain why the beginning of lactation coincides with parturition, Nelson [1932; 1933; 1934; 1935; see also Nelson & Smelser, 1933] supposes that the ovarian hormones which are known to be present in relatively high concentration in the blood during pregnancy inhibit lactation, (a) by inhibiting the production of prolactin by the anterior lobe of the pituitary, and possibly (b) by a direct inhibitory influence on the mammary gland. With the fall in the level of the ovarian hormones in the blood which occurs at parturition, the inhibitory influences are removed and milk secretion begins.

The experiments of Parkes & Bellerby [1926-27], who showed that the growth rates of litters of lactating mice receiving injections of ovarian extracts were smaller than those of controls, are in harmony with the theory of Nelson. Similar results with mice were reported by de Jongh [1933] and Robson [1934-35], though Hain [1935], working with rats, attributed the diminished growth rate to an adverse effect on maternal behaviour rather than to a physiological inhibition of lactation. Smith & Smith [1933] for the rabbit and Nelson [1934] for the guinea-pig also obtained evidence that oestrogenic hormone administration inhibits milk secretion. Results pointing in the same direction are those of Copeland [1929], who from a statistical examination of a large number of bovine milk records concluded that a slight decrease in milk yield occurs during oestrus, and those of Staffe [1930] who found conversely that the milk yields of ovariectomized cows exceeded that of controls.

The above experiments on oestrogenic hormone administration suffer from the defect that the small experimental animals used in them are not well adapted to quantitative studies on milk secretion, since with such animals the effect of the experimental treatment can only be indirectly inferred from observations on the well-being of the young.

¹ The word "phosphatase" is used throughout this paper to denote the phosphomonoesterase A₁ [see Folley & Kay, 1936, 1].

The present work had two main objects, one of which was to study quantitatively the effect of oestrogenic hormone administration on lactation. For this work the cow was chosen as experimental animal on account of its suitability for accurate lactation studies, notwithstanding the fact that the high cost of both animals and hormones precluded the use of more than a few animals. It was hoped that by this means reliable information would be obtained on the extent to which oestrogenic hormones inhibit lactation if at all.

The second object of this work was to obtain some information on the effect of oestrogenic hormones on milk composition as part of a general programme of work now in progress at this Institute, which aims at elucidating the role of endocrine factors in controlling the chemical composition of milk (for work on thyroxine see Folley & White [1936]).

Practically no reliable information on the effect of oestrogenic hormone administration on milk composition exists, though it is reported by de Fremery [1936] that on administering oestradiol benzoate to goats which have been brought into artificial lactation by successive treatment with oestradiol benzoate and prolactin, a change from the secretion of milk to that of colostrum takes place.

Studies of the effects of oestrus and of castration on milk composition have contributed very little to this question. Copeland [1929] found that the fat content of cow's milk was slightly increased during oestrus, while the work of Staffe [1930] indicates that, apart from the fat content, which he reports to be increased, castration has practically no effect on milk composition.

EXPERIMENTAL.

Experimental animals. Four Guernsey cows belonging to the Institute herd, comprising registered and "grading up" cattle, were available for experimentation at different times. Table I gives various data relating to the experimental animals.

Table I.

Name of cow	Duration of experiment	Wt. kg.	Age in years	No. of calf	Weeks in milk at start	Remarks
Orange 4	21. ii. 36– 14. vii. 36	465	5	3	5½	Aborted 34 days before term
Valven 3	21. ii. 36– 27. iv. 36	425	3	2	6½	Aborted 66 days before term
Venus 5	29. iv. 36– 1. x. 36	458	4¾	3	8½	Normal parturition
Valven 2	29. iv. 36– 5. ix. 36	345	3½	2	6½	Normal parturition

During the experiment each cow was kept indoors and given, as far as possible, a constant "winter" ration. All cows were milked twice daily at about 7 a.m. and 4 p.m., the total yield at each milking from each cow being determined on a spring balance to 0.25 lb.

Injections. Two forms of oestrogenic hormone were used. In the first experiment, with the object of administering the highest dosage of oestrogenic hormone which was available at the time, a sample of crystalline oestrone (Organon Ltd.) was injected as a part solution and part suspension in warm castor oil supplemented by a small amount of a solution of "dihydro-follicular-hormone benzoate" in sesame oil (Progynon B oleosum forte (Schering)). This latter

preparation contains a mixture of isomerides, 6.7 mg. of which are equivalent in biological activity to 5.0 mg. of oestradiol benzoate. In the later experiments dihydro-follicular-hormone benzoate was used solely, either in the form of the above-named preparation or as Provetan (Schering). The prolactin (Allen and Hanbury) used in one experiment was a highly active (tested by pigeon crop gland response) 1% suspension of crude prolactin, prepared by the method of Lyons & Catchpole [1933], in 0.3% cresol.

Each cow received different treatment, but in each case injections were intramuscular and preceded by a control period of at least 14 days. Details are given below.

Orange 4. A total of 485 mg. oestrone and 67 mg. dihydro-follicular-hormone benzoate was injected in 5 doses spaced over 2½ days. Simultaneously the control cow, Valven 3, received similar injections of castor oil.

Venus 5. (a) This cow was given 5 daily injections of 20.1 mg. dihydro-follicular-hormone benzoate, Valven 2 serving as control and receiving injections of sesame oil.

(b) After the lapse of a month, she was given 120.6 mg. dihydro-follicular-hormone benzoate in 4 injections over 2 days, each injection being accompanied by an injection of 20 ml. prolactin. The prolactin treatment was continued as above for a further 3 days, ending on the 4th day after the cessation of the oestrogenic hormone injections with 2 injections of 30 ml. each.

Valven 2. 15 days after serving as control for Venus 5, this cow was given 87.1 mg. dihydro-follicular hormone benzoate in 4 injections spread over 4 days.

Blood analyses. At suitable intervals during the experiments, blood samples were taken from the subcutaneous abdominal veins of the cows. This was always done at approximately 10 a.m., the sera being separated the same day and preserved overnight in the refrigerator.

Total serum Ca was determined by the method of Clark & Collip [1925] using *N*/200 permanganate and with the modification that the oxalate precipitation was given 24 hours; serum inorganic P by the Briggs [1922] method; and serum phosphatase by the procedure of King & Armstrong [1934], the incubation time being 30 min. at 37°.

Milk analyses. At each milking, in addition to the determinations of milk yield, samples were taken for the determination of milk fat content by the Gerber method and of the total dry matter by evaporation. From the data thus obtained the percentage of non-fatty solids in each sample could be calculated, the correction for fat volume [Bartlett, 1933-34] being omitted.

Determinations of the phosphatase content of samples of morning milk were made from time to time, at first by the method of Kay & Graham [1933-34] and later by the King & Armstrong [1934] method. At similar intervals in the later experiments, determinations of Ca, ash and of N distribution were made on morning milk samples. The ash content was determined gravimetrically and the Ca therein estimated by permanganate titration of the oxalate. N distribution was determined by a semimicro-method developed by Rowland [1936]. The Parnas-Wagner micro-apparatus was used for all N determinations. Total N was determined on whole milk and on a trichloroacetic acid filtrate. Caseinogen was removed by isoelectric precipitation in presence of acetic acid and sodium acetate and the N in the filtrate estimated. Globulin was precipitated from an aliquot of the caseinogen filtrate by saturation with MgSO_4 at neutrality and the N of the precipitate found. From the data thus obtained the percentage of the milk total N as caseinogen N, albumin N, globulin N and non-protein N could be calculated.

RESULTS.

In the case of each cow the following quantities were calculated for successive three-day periods: mean daily milk yield, mean daily production of milk fat, mean daily production of milk non-fatty solids, mean percentage of fat in milk and mean percentage of non-fatty solids in milk. The results for the 3 injected cows are plotted in Figs. 1, 2 and 3.

Effect of oestrogenic hormones on milk yield.

The curves for the control cow, Valven 3 (not shown), and the appropriate portion of Fig. 3 show that injections of the solvent oils had no effect on lactation. From Figs. 1, 2 and 3 it is apparent that the oestrogenic hormone injections were always followed by a considerable decrease in milk yield. The inhibition was in all cases temporary and was obviously correlated with a transient but considerable increase in the level of oestrogenic hormone in the blood.

The occurrence of an inhibition is confirmed and additional information as to its duration in each case provided by the behaviour of the milk phosphatase content. Fig. 4 shows that in each experiment oestrogenic hormone treatment was accompanied by a tremendous increase in the milk phosphatase concentration, quickly followed by a fall to more or less normal values. It may be noted that whereas the change in milk yield in these experiments was never more than approximately 20 %, the increase in the milk phosphatase concentration varied from 300 to 1200 %.

The studies of Folley & Kay [1936, 2] on normal lactation and of Folley & White [1936] on lactation in experimental hyperthyroidism have shown that in general an increase in the level of milk secretion is accompanied by a decrease in milk phosphatase concentration and *vice versa*. In the light of these findings the present results must be taken as additional proof that oestrogenic hormones, when injected in sufficient amount, can cause an inhibition of lactation and that this inhibition only lasts as long as a high concentration of oestrogenic hormone is maintained in the blood.

An attempt was made to decide the question whether the inhibition was exerted primarily on the anterior pituitary or whether it was a direct effect on the mammary gland by giving Venus 5 a series of simultaneous injections of dihydro-follicular-hormone benzoate and prolactin. It was considered that if Nelson's theory were true, prolactin administration should partially or wholly nullify the effect of oestrogenic hormone. The result of this single experiment, in which simultaneous treatment with prolactin had no action whatever on the results of oestrogenic hormone administration (see Fig. 2) is unfortunately inconclusive. It does not support Nelson's theory but, on the other hand, it does not provide evidence against it since the prolactin dosage may have been suboptimum. Unfortunately no facilities were available at the time for doing further experiments on this point.

Effect of oestrogenic hormones on milk composition.

In the cases of all 3 cows it is evident from Figs. 1, 2 and 3 that the injections of oestrogenic hormones caused an immediate and striking increase in the non-fatty solids content of the milk. In the 2 experiments (Orange 4 and Venus 5 (b)) in which the total dosages were largest and spread over comparatively short periods of time, the level of non-fatty solids in the milk remained considerably increased above normal for very prolonged periods,

Fig. 1. I, percentage of non-fatty solids in milk; II, percentage of fat in milk; III, daily production of milk non-fatty solids; IV, daily production of milk fat; V, daily milk yield.

Fig. 2. I, percentage of non-fatty solids in milk; II, percentage of fat in milk; III, daily production of milk non-fatty solids; IV, daily production of milk fat; V, daily milk yield.

amounting to over 2 months in the case of Orange 4 and over 3 months in the case of Venus 5. In the 2 experiments where the dosages were smaller, and more-

Fig. 3. I, percentage of non-fatty solids in milk; II, percentage of fat in milk; III, daily production of milk non-fatty solids; IV, daily production of milk fat; V, daily milk yield.

Fig. 4. In the case of Orange 4 the ordinates represent Kay and Graham [1933-34] phosphatase units in 1 ml. milk, while in the case of Venus 5 and Valven 2 they represent King and Armstrong [1934] phosphatase units in 100 ml. milk.

overspread over longer periods, the effect though striking at first, quickly decreased, but even here a small effect was evident for a long time (see Fig. 3, curve I).

Similarly, the curves in Figs. 1, 2 and 3 show clearly that the experimental treatment resulted in a considerable and prolonged increase in milk fat content.

In these experiments, therefore, administration of oestrogenic hormone caused, in addition to a temporary inhibition of milk secretion in the sense of a lessened milk volume, a very prolonged increase in concentration of solids in the milk secreted. The latter finding is consistent with the possibility that oestrogenic hormones may regulate the passage into the milk of the "isotonic diluent", the existence of which was suggested by Davies [1933] as a result of a study of the composition of cow's milk poor in non-fatty solids.

Further there is some indication in the results plotted in Figs. 1, 2 and 3 that oestrogenic hormone treatment causes an actual increase in the efficiency of the mammary gland cells as regards their ability to synthesize milk constituents. Thus curves III and IV in Fig. 1 show that in the case of Orange 4 the lactation inhibition was unaccompanied by any temporary decrease in the daily production of milk fat and milk non-fatty solids. Moreover the same curves distinctly suggest that for more than 2 months after the cessation of injections the normal decline in the daily output of fatty and non-fatty solids in the milk, a decline which normally should follow approximately the same course as the decline in milk yield, was not in evidence. Both of these facts indicate that oestrogenic hormone treatment had conferred upon the mammary gland of this cow the power to synthesize milk constituents at a higher rate than before the treatment. In the cases of the other 2 cows, whilst there was a decrease in the daily output of both types of milk solids during the period of lactation inhibition, thereafter there is some indication (curves III and IV, Figs. 2 and 3) that the rate of decline in each case was slightly lessened. Unfortunately, circumstances did not permit the use for this work of two well-matched groups of cows which would have enabled this question to be decided by the application of statistical methods.

As regards the mechanism of the prolonged effects just discussed, it was considered possible that whilst the anterior lobe of the pituitary might well be concerned in the lactation inhibition, the alterations in milk composition might be due to a direct effect of oestrogenic hormones on the mammary gland, perhaps connected with the power of these substances to promote mammary development. It was, however, not possible to separate the two phenomena in the one experiment in which dihydro-follicular-hormone benzoate and prolactin were injected simultaneously. Since neither hypophysectomized nor ovariectomized cows were used, it is conceivable that the above phenomena were due to an effect of oestrogenic hormones on the anterior pituitary, which may have in turn reacted upon the ovary. In this connexion the work of Fevold *et al.* [1936], who found that administration of oestrogenic hormones increases the secretion of the luteinizing hormone by the anterior pituitary, comes to mind.

In general, these results help to explain certain previously known phenomena. The effect of pregnancy in hastening the normal decline in bovine lactation has been studied by Gaines & Davidson [1925-26] who postulated the intervention of a then unknown depressant factor. Further, Bartlett [1933-34] has demonstrated that pregnancy causes an increase in the non-fatty solids content of cow's milk. On the basis of the present results, these phenomena are probably due to the gradual rise in the level of ovarian hormone in the blood which is characteristic of gestation.

Lack of effect of oestrogenic hormones on the relative proportions of the nitrogenous constituents of milk. Fig. 5 shows the effect of injections of dihydro-follicular hormone benzoate on the density and on the total N, caseinogen N,

ash and Ca contents of the milk of the two cows Venus 5 and Valven 2. The experimental treatment caused a considerable increase in the milk total N, partly due, as Fig. 5 shows, to an increase in the caseinogen N. As is to be expected, the latter phenomenon was associated with a rise in the Ca and ash contents of the milk.

Fig. 5. I, density of milk at 20°; II, calcium content of milk; III, ash content of milk; IV, total nitrogen of milk; V, caseinogen nitrogen of milk.

De Fremery [1936], working with goats which had been brought into artificial lactation, claimed that treatment with oestradiol benzoate caused a change from the secretion of true milk to that of colostrum. From the work of Engel & Schlag [1924-25] and of Crowther & Raistrick [1916] it is established that colostrum is richer than milk in all nitrogenous fractions, perhaps the most noteworthy feature of colostrum being its very high content of globulin. Further, Engel & Schlag have found that during the gradual transition from colostrum to milk there is a progressive fall in the dry matter and ash contents of the fluid accompanied by an increase in lactose content.

Certain of the present findings, such as the increase in density and in the total solids, total N, caseinogen and ash contents of the milk following oestrogenic hormone administration, at first sight appear to be in agreement with the claims of de Fremery. The results of determinations of the N partition in the milk of two of the cows, given in Tables II and III, show conclusively, however, that such is not the case. These results show clearly that the partition of N in the milk of the experimental cows was not appreciably affected by injections of dihydro-follicular-hormone benzoate, all nitrogenous fractions participating to proportionately equal extents in the rise in total N. Had the treatment caused a transition from milk to colostrum, it is evident from the work of Crowther & Raistrick that considerable alterations in N partition would have occurred. In particular the proportion of the total N as globulin N would have increased tremendously.

Table II. *Determinations of the N partition in the milk of Venus 5.*

Date (1936)	Before injections		After 1st series of injections						After 2nd series of injections	
	12. v.	18. v.	21. v.	25. v.	4. vi.	10. vi.	16. vi.		24. vi.	2. vii.
mg. N per 100 ml.	512	509	545	599	534	569	561		530	630
% of total N as										
Protein N	94.6	95.1	94.9	95.1	95.3	95.3	95.0		95.2	95.5
Caseinogen N	78.5	79.2	78.2	78.7	78.5	78.0	77.8		75.8	79.8
Albumin N	10.5	11.9	12.5	11.6	9.0	11.1	10.0		11.5	10.2
Globulin N	5.6	4.0	5.0	4.9	7.8	6.1	7.2		7.9	5.6
Non-protein N	5.4	4.8	5.1	4.9	4.7	4.7	5.1		4.8	4.6

Table III. *Determinations of the N partition in the milk of Valven 2.*

Date (1936)	Before injections				After injections			
	18. v.	21. v.	25. v.	4. vi.	10. vi.	16. vi.	24. vi.	2. vii.
mg. N per 100 ml.	544	544	540	566	642	597	580	586
% of total N as								
Protein N	95.1	95.4	95.3	95.7	96.0	95.4	95.9	95.3
Caseinogen N	77.6	77.6	76.8	77.5	77.5	77.0	78.3	76.0
Albumin N	12.9	12.8	12.9	10.8	12.1	10.4	10.0	13.3
Globulin N	4.6	5.1	5.6	7.4	6.3	8.1	7.6	6.1
Non-protein N	4.9	4.6	4.7	4.3	4.0	4.6	4.1	4.7

The slight rise in the proportion of total N as globulin N which was first observed on 4. vi. 36 can be explained as due to the development of a mild streptococcal infection of the udder, the existence of which in both cows was inferred from bacteriological examination of the milks carried out soon after. The milk of both cows was found to be free from streptococci before the experiment started.

The effect of oestrogenic hormones on Ca and P metabolism.

In Fig. 6 are plotted for all 3 experimental cows the results of determinations of serum Ca, serum inorganic P and serum phosphatase. In all experiments the oestrogenic hormone injections caused a temporary fall in the serum total Ca.

Fig. 6. I, blood serum phosphatase; II, total blood serum calcium; III, blood serum P as inorganic phosphate.

The most noteworthy feature of these results is the marked temporary rise in serum phosphatase which followed closely upon the drop in serum Ca. The results

for inorganic P were more variable, but in most cases the injections appeared to cause a transient rise in this quantity.

These results for serum Ca are in general agreement with previous work. Leites [1924] found that castration of bitches caused a slow but marked rise in serum Ca while a similar effect was reported by Frei & Emmerson [1930] to follow castration of cows. The latter workers also observed a temporary fall in serum Ca following injections of oestrogenic hormones into cows.

The rise of serum phosphatase observed in these experiments is unequivocal, and taken in conjunction with the changes in serum Ca, may indicate, in the light of what is known of serum phosphatase in bone disorders [see Folley & Kay, 1936, 1], that oestrogenic hormones are capable of exercising, either directly or indirectly, far-reaching effects on the skeletal system.

SUMMARY.

1. Administration of oestrogenic hormones to lactating cows has been found to cause a considerable inhibition of lactation in the sense of a lessened milk yield. The inhibition was temporary and appeared to depend on the attainment of a high level of oestrogenic hormone in the blood.

2. After treatment with oestrogenic hormones the milks of the cows so treated contained considerably higher percentages of fat and non-fatty solids than before treatment. With high doses this effect lasted for 2-3 months.

3. The N partition in the case of such "concentrated milk" was found to be normal showing that the experimental treatment had not caused the secretion of colostrum.

4. Oestrogenic hormone administration caused a temporary fall in serum Ca quickly followed by an equally transient though considerable increase in the serum phosphatase. This was taken to indicate that oestrogenic hormones are capable of exercising generalized effects on the skeletal system.

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