XXII. THE EFFECT OF CONTINUED TREATMENT WITH ANTERIOR PITUITARY EXTRACTS ON MILK VOLUME AND MILK-FAT PRODUCTION IN THE LACTATING COW

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IN a previous paper [Folley & Young, 1938] it was shown that single injections into cows in declining lactation of crude preparations of prolactin and of thyrotropic hormone obtained from fresh ox anterior pituitary tissue, resulted in a substantial temporary increase in milk yield. On the other hand, a similar single injection of a prolactin preparation obtained from acetone-desiccated, commercial anterior lobe powder by a slight modification of the method of Bates & Riddle [1935], had no significant effect on the milk yield, although repeated daily injections of the same prolactin preparation caused a substantial increase in milk yield. In general it was found that in the cow the lactogenic action of the anterior lobe extracts used was paralleled by their glycotropic (anti-insulin) activity rather than by their prolactin (pigeon crop-gland stimulating) activity.

The present paper records the results of experiments in which the influence of repeated injections of prolactin and thyrotropic preparations with high glycotropic activity was investigated.

METHODS

Animals. Thirteen dairy Shorthorn cows, belonging to the herd of the Reading University farm, were used in these experiments. Three groups, each of 4 animals, were selected so as to be as comparable as possible with regard to stage of lactation, time since mating if pregnant etc. A single cow was used in one experiment. The cows were allowed out to grass except when they came indoors for milking at approximately 8 a.m. and 4 p.m. each day. Their management was that usual for first-class dairy herds in this country.

Milk analyses. In addition to daily milk yield, percentages of fat and non-fatty solids in group-composite samples were determined twice daily throughout the experiment on the 3 main groups. The content of lactose, total chlorides and phosphatase was determined at intervals. The methods used for these determinations were those previously described [Folley & Young, 1938].

Anterior lobe extracts. Preparations "Prolactin-C" and "Thyrotropic-C" [Folley & Young, 1938] were used in this investigation. The method of preparation of these extracts and their general properties have already been described [Young, 1938, 1; Folley & Young, 1938]. In brief, the method of preparation consisted in the extraction of absolutely fresh ox anterior pituitary lobes with saline at pH 8 in the cold, followed by separation at 0° into the fractions soluble at pH 5.5 and those insoluble at this pH. The former constituted "Thyrotropic-C" and the latter "Prolactin-C".

Injection of animals. After a suitable control period, each of the 4 cows in Group A was given, on alternate days, an injection of 30 ml. of "Prolactin-C" equivalent to 10 g. of fresh anterior lobe tissue. Each cow in Group B (4 animals) received similar injections of "Thyrotropic-C". Group C served as a control group and each of these 4 animals was injected on alternate days with 30 ml. of a saline suspension of fresh ox liver equivalent to 10 g. of fresh tissue. These injections provided an adequate control for the possible influence on milk secretion of the introduction of non-specific protein into the circulation of lactating cows.

Repeated injections of "Prolactin-C", but not of the other tissue extracts used in the present investigation, produced local reactions of the type produced by injections of a crude saline extract of ox anterior lobe tissue [Folley & Young, 1938]. It was found that centrifuging the "Prolactin-C" preparation at pH 8 threw down material that was normally in suspension [cf. Young, 1938, 1] leaving a clear fluid. One cow received injections of 30 ml. of this fluid (equivalent to 10 g. of fresh tissue) on alternate days for 25 days and showed no local reactions.

RESULTS

Milk yield. The curves for aggregate milk yield in the 3 main groups are shown in Fig. 1. The daily yield of the cows receiving 5 injections of Prolactin-C was very substantially increased during the period of injections. Although the

Fig. 1. Aggregate milk yields of groups of 4 cows receiving injections of pituitary extracts on alternate days. The amount of extract injected on each occasion was equivalent to 10 g. of fresh ox anterior lobe. ↓ signifies the first injection. ↑ signifies the last injection. Curve A: animals receiving Prolactin-C. Curve B: animals receiving Thyrotropic-C. Curve C: control animals receiving liver extract. Curve D: uninjected control animals.

milk yield did not continue to increase over the whole period of the injections, there was no sign of a decline from the highest level reached, until after the injections were discontinued.

Injections of Thyrotropic-C also increased the aggregate milk yield, although to a much less extent than those of Prolactin-C (Fig. 1), and again

there was no definite diminution of the increased milk yield until the injections had ceased.

In the group receiving liver extract a slight increase in milk yield occurred immediately after the initial injection. Unfortunately, on the day on which injections began it was found necessary to turn all the cows used in this experiment into a new pasture. In a normal year when grass is plentiful this would have made very little difference to the milk yield, but this experiment was carried out in the summer of 1938 when grass was very scarce owing to drought. Under these conditions a change to a new pasture might be expected to exert a stimulating effect on milk yield. That this was so is shown by the aggregate milk yield curve for the same period for a group of 4 untreated cows of the same herd (Fig. 1). The slight increase in milk yield which occurred following the injections of the liver extract cannot therefore be ascribed to a lactogenic action of this extract.

The milk yield curve for the cow which received injections of the centrifuged Prolactin-C is shown in Fig. 2. In this experiment a total of 12 injections was

Fig. 2. Milk yield of a cow receiving injections of centrifuged Prolactin-C on alternate days. The amount of extract injected on each occasion was equivalent to 10 g. of fresh ox anterior lobe. ↓ signifies the first injection. ↑ signifies the last injection.

given, material being injected on alternate days. The milk yield rose to a maximum on the 10th day of the experiment, after which it steadily decreased, despite continued treatment.

Milk-fat percentage. The percentage of fat in the milk of the cows in Groups B and C (receiving injections of Thyrotropic-C and liver extract respectively) was unchanged during the experimental period (Fig. 3). The milk-fat percentage in the animals in Group A (receiving injections of Prolactin-C) was, on the other hand, very substantially increased as the result of the injections. As both the milk yield and the percentage of milk-fat were substantially raised, the total amount of fat secreted in the milk of these animals was very greatly increased as the result of treatment (Fig. 4). The total daily production of milk-fat by the cows in this group averaged 4.48 lb. for the 7 days before the injections were begun, and 6.63 lb. for the 10 days covering the period of the injections. Thus the daily production of milk-fat was increased by 48 %, on the average, following the institution of daily injections of Prolactin C.

Fig. 3. Percentage milk-fat in pooled samples of the milk from groups of 4 cows receiving injections of pituitary extracts on alternate days. The amount of extract injected on each occasion was equivalent to 10 g. of fresh ox anterior lobe. ↓ signifies the first injection. ↑ signifies the last injection. Curve A: animals receiving Prolactin-C. Curve B: animals receiving Thyrotropic-C. Curve C: animals receiving liver extract.

Fig. 4. Aggregate yields of milk-fat from groups of 4 cows receiving injections of pituitary extracts on alternate days. ↓ signifies the first injection. ↑ signifies the last injection. Curve A: animals receiving Prolactin-C. Curve B: animals receiving Thyrotropic-C. Curve C: animals receiving liver extract.

Fig. 5. Percentage non-fatty solid in pooled samples of the milk from groups of 4 cows receiving injections of pituitary extracts on alternate days. ↓ signifies the first injection. ↑ signifies the last injection. Curve A: animals receiving Prolactin-C. Curve B: animals receiving Thyrotropic-C. Curve C: animals receiving liver extract.

Non-fatty solid content of milk. The milk from the cows receiving injections of anterior lobe extract showed no clear indication of a significant change in non-fatty solid content (Fig. 5), there being a slight but probably insignificant fall. Lactose, chloride and phosphatase contents showed no regular alterations during treatment.

DISCUSSION

These experiments show that the rate of milk secretion of cows in declining lactation can be increased by repeated injections of preparations of prolactin and of the thyrotropic substance, both possessing marked glycotropic activity, to a level much higher than was attained with single injections of these preparations. The single experiment in which 12 successive injections of prolactin were given showed that with prolonged treatment the milk yield reaches a maximum value and then declines, despite repeated injections. It does not seem probable that this decline was due to the production of an "antihormone" in the injected animal, as daily injections of ox-prolactin for 12-15 weeks were required to induce the appearance of substantial "anti-prolactin" activity in rabbits [Young, 1938, 2].

The great increase in milk-fat content as the result of treatment with prolactin was unexpected in view of the simultaneous increase in milk yield which occurred. The total production of milk-fat by each cow was increased by over 0.5 lb. a day as the result of this treatment. This increase is particularly interesting when it is remembered that the daily injection of a prolactin preparation relatively poor in glycotropic activity resulted in no significant increase in milk-fat content, although the milk yield was substantially increased thereby [Folley & Young, 1938]. There is no evidence whether or not the glycotropic substance influences fat metabolism, but it should be recalled that Marks & Young [1938] found that treatment of young rabbits with prolactin and thyrotropic preparations, both high in glycotropic activity, resulted in a substantial increase in liver fat. The glycogen content of the livers of these animals was also abnormally high. Similar results have been obtained with adult rabbits and with other species of animals (unpublished observations). It is not known at the present time how many active substances are present in anterior pituitary extracts of the type used in the present investigations. Until further fractionation of the active substances has been effected one can only attempt to correlate the observed physiological activities of the different fractions used. Table I summarizes the different physiological activities of the prolactin and thyrotropic preparations used in the present investigation. There is no clear indication of any correlation of the activity in increasing milk-fat content with any of the other activities investigated.

Table I. *Summary of physiological activities of different extracts used*

Physiological activity	Reference	Pro-lactin-C	Thyro-tropic-C
Glycotropic (anti-insulin) activity	Young [1938, 1]; Marks & Young [1938]	+++	++
Increased liver fat content of fasting rabbits	Marks & Young [1938]; and unpublished data	+++	++
Increased liver glycogen content in fasting rabbits	Young [1937]; Marks & Young [1938]	++	+++
Milk yield increase in cows in declining lactation	Folley & Young [1938]; and present communication	+++	++
Increase in milk-fat content in cows in declining lactation (repeated injection of extract)	Present communication	+++	0

The injection of thyroxine into cows in declining lactation results in an immediate increase in milk yield, together with a delayed increase in the fat content of the milk [Folley & White, 1936]. The above results cannot be due to the thyrotropic activity of the pituitary extracts used, as the prolactin-C was most potent in influencing milk yield and fat content and this preparation has only a very slight thyrotropic activity [cf. Young, 1938, 1].

The results recorded in the present paper emphasize the undesirability of attempting to ascribe to any single substance of the pituitary gland stimulation of all the processes concerned with the secretion of milk. It is clear that in cows in declining lactation, neither the increase in milk yield nor the increase in milk-fat content, both of which are found to follow the repeated injection of a suitable pituitary extract, are related to the activity of the extract in causing growth of the pigeon crop-gland. The determination of how many different factors are involved in the control and stimulation of the complex processes of milk formation must await further fractionation of the extracts used.

SUMMARY

1. Repeated injections of thyrotropic and prolactin preparations into cows in declining lactation resulted in a pronounced increase in milk yield, the increase being more marked with the prolactin preparation.

2. The increase in milk yield following injections of the prolactin preparation was accompanied by a substantial increase in milk-fat content. The average daily production of milk-fat was increased by nearly 50 % during the period of 5 successive injections of prolactin.

3. Under prolonged treatment with prolactin the milk yield did not continue to increase, but reached a steady level and then declined, despite continued treatment.

4. The increase in milk yield and milk-fat content of cows in declining lactation, following the injection of pituitary extracts, cannot be ascribed to the action of a single substance.

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REFERENCES

- Bates & Riddle (1935). *J. Pharmacol.* **55**, 365.
Folley & White (1936). *Proc. roy. Soc. B*, **120**, 346.
— & Young (1938). *Proc. roy. Soc. B*, **126**, 45.
Marks & Young (1938). *J. Physiol.* **93**, 61.
Young (1937). *J. Physiol.* **90**, 20P.
— (1938, 1). *Biochem. J.* **32**, 524.
— (1938, 2). *Biochem. J.* **32**, 656.