Aust. J. Biol. Sci., 1978, **31**, 183–6

The Effect of 2-Deoxy-D-glucose on Ovarian Function of Cattle

T. J. McClure,[A] *C. D. Nancarrow*[B] *and H. M. Radford*[B]

[A] Department of Veterinary Clinical Studies, University of Sydney, Camden, N.S.W. 2570.
[B] Division of Animal Production, CSIRO, P.O. Box 239, Blacktown, N.S.W. 2148.

Abstract

The administration of the metabolic inhibitor, 2-deoxy-D-glucose (2DG), to four well fed heifers just before and during the time of expected oestrus, and to another heifer following the removal of the corpus luteum, prevented both the occurrence of oestrus and the formation of corpora lutea in all animals. Plasma progesterone concentrations remained low for at least 10–21 days after the last dose of 2DG. The results suggest that the inhibition of glycolysis is associated with the failure of both oestrus and formation of functional corpora lutea, and they support the hypothesis that hypoglycaemia is the primary biochemical change responsible for infertility induced by acute energy deficiency in lactating cattle.

Introduction

The association between hypoglycaemia and infertility in lactating cattle is well documented (Arzumanjan and Dorotjuk 1964; Blakely 1965; Payne *et al.* 1970; Oxenreider and Wagner 1971; Hunter 1976; McClure and Payne 1978) and McClure (1965, 1970) has suggested that the hypoglycaemia itself was a causal agent in this regard. This hypothesis was tested by administering protamine zinc insulin to lactating cows at pro-oestrus (McClure 1968). The treatment delayed the onset of oestrus in some cows and lowered the pregnancy rates following insemination of others in which oestrus occurred at the normal time.

Insulin, besides causing hypoglycaemia, alters other biochemical parameters (Fain 1974), thus it seemed desirable to evaluate the hypothesis further by using a drug which inhibits glucose metabolism. This paper reports the effects of administering the glucose metabolic inhibitor 2-deoxy-D-glucose (2DG) to well fed non-lactating heifers exhibiting normal oestrous cycles.

Materials and Methods

Animals and Husbandry

The experimental animals consisted of seven Hereford × Devon heifers which were approximately 15 months of age. They grazed good quality mixed improved pasture prior to and during the experiment which lasted from 3 February to 21 March 1975. The live weights of the animals ranged from 260 to 310 kg.

Determination of Reproductive Function

The occurrence of oestrus was assessed initially by making twice daily inspection of the animals, then on 21 February they were joined with a bull fitted with a chin ball marker. Thereafter the presence of chin ball marks indicative of mating was also recorded. The heifers were also yarded three times a week when the reproductive tract was examined manually *per rectum* to assess uterine tone, ovarian size, and the presence of Graafian follicles and corpora lutea. On these occasions

jugular blood samples were collected into heparinized tubes. The samples were packed in ice until the plasma was separated by centrifugation within 2 h. The plasma was stored at −18°C until assayed for progesterone using the method of Bassett and Hinks (1969) as modified by Thorburn and Schneider (1972). The within and between assay coefficients of variation were less than 16% over the range 1–8 ng progesterone.

Treatment with 2-Deoxy-D-glucose

Four of the heifers were treated when the clinical examination and earlier records of oestrus indicated that they were about to experience their second oestrous period following the start of observations. 50 g of 2DG (Calbiochem, San Diego, California, USA) were dissolved in 300 ml distilled water and injected subcutaneously on the predicted day of pro-oestrus, and again 24 h

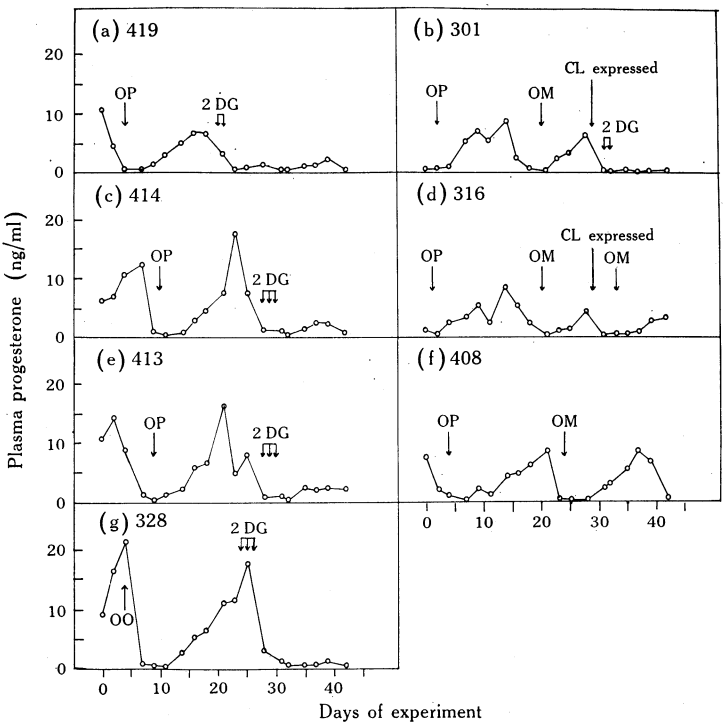

Fig. 1. Changes in peripheral progesterone concentration of seven individual heifers throughout the period of experimentation. OP, Oestrus presumed on clinical evidence; OO, oestrus observed; OM, oestrus with mating; ⇩⇩ days of treatment with 2-deoxy-D-glucose; CL, corpus luteum.

later. A third dose was given 24 h later again to three of these heifers. The decision to give the third dose was made on clinical grounds when it appeared that a large follicle was persisting. 300 ml of distilled water were injected subcutaneously into the control cows at comparable times. The dose of 2DG used was the maximal amount that could be given safely to the animals without causing clinical signs in systems other than the reproductive system.

Observations and blood sampling of these four treated and three control heifers continued for a total of 42 days. However, on the ninth day after the second oestrous period, corpora lutea of two of the control heifers were expressed by massage *per rectum* and one of these animals was treated twice with 2DG, at 2 and 3 days after the corpus luteum was expressed (Figs 1*b* and 1*d*). The experiment was terminated 11–22 days after the beginning of individual 2DG treatments.

Results and Discussion

The reproductive activity exhibited by each animal throughout the experiment is illustrated in Fig. 1. Six heifers exhibited normal ovarian function, as exemplified by behavioural signs, rectal palpation data, and changes in peripheral progesterone concentrations before the treatment with 2DG but one, No. 328, was observed in oestrus while the progesterone concentration was still high. Following the injection of 2DG just before and during the time of expected oestrus or following corpus luteum removal, oestrus did not occur, a corpus luteum did not form as judged from rectal palpation, and progesterone concentrations in the plasma remained at levels very much lower than those which were obtained during the previous oestrous cycle and in the control animals (Fig. 1). In the one animal where 2DG was given while progesterone concentrations were still high the treatment was still effective in inhibiting subsequent ovarian function. The inhibition of ovarian activity persisted to the end of the period of observations in all treated animals.

2-Deoxy-D-glucose is converted into 2-deoxy-D-glucose-6-phosphate which is a competitive inhibitor of the substrates for both glucosephosphate isomerase or glucose-6-phosphate dehydrogenase (Sols and Crane 1954; Hochster 1963). Notwithstanding other unrecognized metabolic effects of 2DG, the present results suggest that the inhibition of the glycolytic pathway can prevent both oestrus and the formation of functional corpora lutea in well fed non-lactating heifers.

In the mouse, 2DG induces ovarian dysfunction apparently by causing failure of pituitary gonadotrophic secretion which in turn is likely to have stemmed from hypothalamic failure (McClure 1967). The basic mechanism involved in ovarian failure in the present experiment is not known. However, the results are consistent with evidence from earlier field (McClure 1965, 1970) and pen experiments using protamine zinc insulin treatment (McClure 1968) which supports the hypothesis that hypoglycaemia is the primary biochemical change responsible for infertility induced by an acute energy deficiency in lactating cattle.

Acknowledgments

The authors thank Mr G. Chapple for performing the progesterone analyses.

References

Arzumanjan, E. A., and Dorotjuk, E. N. (1964). The importance of biochemical factors in controlling infertility in cows. *Vestn. Skh. Nauki (Moscow)* **9**, 54–9. (Abstr. *Anim. Breed. Abstr.* **33** 3327.

Bassett, J. M., and Hinks, N. T. (1969). Microdetermination of corticosteroids in ovine peripheral plasma: effects of venipuncture, corticotrophin, insulin and glucose. *J. Endocrinol.* **44**, 387–403.

Blakely, J. E. (1965). The effect of nutrition on reproductive physiology and growth in beef heifers. *Diss. Abstr.* **25**, 13283.

Fain, J. N. (1974). Mode of action of insulin. In 'MTP International Review of Science: Biochemistry Series One'. Vol. 8. 'Biochemistry of Hormones'. (Ed. R. V. Rickenberg.) (Butterworth: London.)

Hochster, R. M. (1963). Hexose and pentose analogues. In 'Metabolic Inhibitors'. (Eds R. M. Hochster and J. H. Quastel.) (Academic Press: New York.)

Hunter, A. P. (1976). Nutritional and other causes of infertility in dairy herds in the Illawarra district of the south coast of New South Wales. M.V.Sc. Thesis, University of Sydney.

McClure, T. J. (1965). A nutritional cause of low non-return rates in dairy herds. *Aust. Vet. J.* **44**, 119–22.

McClure, T. J. (1967). Infertility in mice caused by fasting at about the time of mating. III. Pathogenesis. *J. Reprod. Fertil.* **13**, 393–403.

McClure, T. J. (1968). Hypoglycaemia, an apparent cause of infertility of lactating cows. *Br. Vet. J.* **124**, 126–30.

McClure, T. J. (1970). An experimental study of the causes of a nutritional and lactational stress infertility of pasture-fed cows, associated with loss of bodyweight at about the time of mating. *Res. Vet. Sci.* **11**, 247–54.

McClure, T. J., and Payne, J. M. (1978). Observations on the first service non-return rates of some hypoglycaemic, concentrate-fed dairy herds. *Aust. Vet. J.* **54**, 7–9.

Oxenreider, S. L., and Wagner, W. C. (1971). Effect of lactation and energy intake on post partum ovarian activity in the cow. *J. Anim. Sci.* **33**, 1026–31.

Payne, J. M., Dew, S. M., Manston, R., and Faulks, M. (1970). The use of metabolic profile test in dairy herds. *Vet. Rec.* **87**, 150–8.

Sols, A., and Crane, R. K. (1954). Substrate specificity of brain hexokinase. *J. Biol. Chem.* **210**, 581–95.

Thorburn, G. D., and Schneider, W. (1972). The progesterone concentration in the plasma of the goat during the oestrous cycle and pregnancy. *J. Endocrinol.* **52**, 23–36.

Manuscript received 10 August 1977