EFFECT OF SUBCUTANEOUS IMPLANTATION OF ADRENALIN TABLETS ON BLOOD-SUGAR AND MILK COMPOSITION IN LACTATING RUMINANTS

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(Received 17 August 1939)

A very prolonged effect of certain hormones can be obtained by the subcutaneous implantation of a compressed tablet of undiluted, crystalline substance [Deanesly and Parkes, 1937]. This technique has proved to be specially useful with oestrone and testosterone, or testosterone propionate, and effects lasting many months can be obtained from a single administration. The great efficiency of the method seems to be due to slow and even absorption over a long period, so that the alternation of wasteful excess and sub-threshold concentrations, such as is often produced by the daily injection of solutions, is avoided. In considering whether such a technique could be usefully applied to other hormones it was reasonable to take account of adrenalin, the most rapidly acting and quickly destroyed of the hormones. Further, of the various physiological responses to adrenalin, that of the blood-sugar seemed the most promising for investigation of the effects of prolonged action. It is well known that a single intravenous injection of adrenalin produces only a small and temporary increase in blood-sugar as compared with that which can be obtained by the continuous intravenous perfusion of the same total amount [Cori, Cori, and Buchwald, 1930a]. Subcutaneous administration, admitting of slower absorption and more prolonged action, results in a considerable increase in the blood-sugar (and lactic acid) which may last for several hours [Cori and Cori, 1929]. The possibility of further retarding the absorption of subcutaneously administered adrenalin has been investigated by Kohn and Bulger [1937], who found that the addition of small amounts of zinc sulphate to the solution decreased the maximum blood-sugar resulting from the injection, but increased the time over which an increase could be detected. With a larger quantity of zinc sulphate, absorption was so delayed that little effect was observed.

In view of these results we have investigated the possibility of administering large amounts of adrenalin in the form of a compressed tablet of the pure substance inserted subcutaneously, in the hope that an effective supply would be absorbed from such a depot, not merely for a few hours,
but for several days. About a year after the experiments recorded in this paper began, Keeney, Pierce, and Gay [1939] reported prolonged hyper-glycaemia (8 or 9 hours duration) in humans following injections of suspensions of adrenalin in oil.

The possibility of maintaining, by implantation of the solid hormone, a high blood-sugar over a prolonged period suggested an extension of the experiment. It is known that in lactating cows a low blood-sugar over a long period is associated with a decrease both in the amount and the lactose content of the milk [Gowen and Tobey, 1931] and, conversely, it has often been assumed that during a long period of raised blood-sugar the yield and the lactose content of the milk would rise. As yet, however, a satisfactory method of raising the blood-sugar in farm animals over a long period does not seem to have been evolved. The temporary increase in blood-sugar by oral or intravenous administration of glucose to cows sometimes causes a very temporary increase in milk lactose, but has not been shown to increase the milk yield [Whitnah, Riddell, and Hodgson, 1933; see also Nitzescu's [1925] work on the goat]. Indeed, Brown, Petersen, and Gortner [1936] found that intravenous injections of sugars into lactating cows caused hypoglycaemia possibly due to increased secretion of insulin. These authors concluded that intravenous injections of sugars were of doubtful value in studies of lactose synthesis. It seemed, therefore, that while experiments were being carried out on massive dosage with adrenalin in tablet form, it would be useful to use lactating farm animals in which both the yield and composition of the milk could be determined simultaneously with blood-sugar changes. Experiments have, therefore, been carried out on lactating goats and one cow.

**Methods**

Adrenalin was used in the form of the free base (B.D.H. Adrenaline). The physical properties of this powder seemed at first to be quite unsuited to the preparation of tablets of sufficient size and solidity. It was found, however, that jamming of the pellet press by the powder could be prevented by coating the die with a thin film of oil; in this way hard, polished tablets, up to 200 mg. in weight, were produced from a standard hand pellet press. Implantation into the animals was carried out under local anaesthesia (Percaine—5 c.c. of a 1/1000 solution intradermally and subcutaneously) by making a small incision through the skin, the wound being closed with two stitches. Doses up to 496 mg. of adrenalin were administered at one time by this method. Contrary to expectations, there was no local reaction, and despite the probable occurrence of local vasoconstriction the material was absorbed in the course of time without
any complications except in one case in which there was sloughing from a small area.

In the experiments on goats, arterial blood samples were obtained from exteriorized carotid loops. In the cow, arterial and venous blood samples were taken approximately simultaneously from the internal iliac artery and the mammary vein by the method of Graham, Kay, and McIntosh [1936]. In all experiments save one, a series of samples was obtained without difficulty from the carotid loops, but only three successive samples could be obtained from the iliac artery (see below). Since fasting blood-sugar values could not be measured because (a) of the difficulty of emptying the alimentary tract of a ruminant, and (b) starvation would interfere with lactation, blood samples were, as far as possible, taken at the same time of day throughout any one experiment, except that in some experiments an additional sample was taken shortly after the tablet was implanted. Determinations made during the control periods show that in our animals the blood-sugar values so determined varied very little from day to day.

All animals were milked twice daily, at 8.0 a.m. and 3.30 p.m.

Blood-sugar in experiments 1, 3, 4, and 5, and milk lactose, fat, and non-fatty solids, were estimated by the various methods listed by Folley and Young [1938]. In experiments 2, 6, and 7 blood-sugar was determined in tungstic acid filtrates by what was essentially the Hagedorn-Jensen method. Since it is well known that blood lactic acid is increased by adrenalin treatment, and since recent evidence suggests that lactic acid may be a precursor of lactose (see below), blood lactic acid was determined in experiments 4 and 5 by the method of Avery and Hastings [1931] and in experiments 2, 6, and 7 by Lohmann’s [1928] method.

Results

Experiment 1. Experiment 1 was carried out on goat Ar, which had been 6 months in milk and weighed 90 lb. The milk yield was approximately constant in the period preceding the experiment at about 27 oz. daily. Observations were made on blood-sugar, lactose (morning milk only), fat, and non-fatty solids, and milk yield. A tablet of adrenalin weighing 48.6 mg. was implanted at 12.30 p.m. on 6 January 1938. The observations showed a sharp rise in blood-sugar from the control level of 62–5 mg. %, so that by 3.30 p.m. on 7 January nearly 100 mg. % was found (Fig. 1). On the following day, 46 hours after implantation of the tablet, the value, 83 mg. %, was still high. At 68 hours after implantation the blood-sugar value was nearly down to normal. The lactose percentage in the milk showed a definite but temporary rise corresponding to that of the blood-sugar. The milk yield was steady for 2 days after the
implantation, but was afterwards irregular with a tendency to fall. The results indicate that there was no significant change in the daily fat percentage, but that the non-fatty solids showed a slight rise after the implantation.

Fig. 1. Goat Ar. (experiment 1). Adrenalin tablet implanted at ↑.

Note.—In all figures the following conventions have been adopted:

(a) Each day begins and ends at midnight indicated by the numerals.
(b) The results of blood analyses are shown at the hours at which the samples were taken.
(c) Except where otherwise stated, daily milk yields are entered at noon, i.e. midway between the a.m. and p.m. milkings.

Experiment 2. Experiment 2 was carried out on goat Ar. when 10½ months in milk. The daily milk yield was rising slowly at the time of the experiment. Lactose, fat, and non-fatty solids were determined in composite samples made each day by combining samples of milk obtained that morning and the previous evening in proportion to the yields. Blood-sugar and lactic acid were also estimated at suitable intervals, except that on
the second day after adrenalin administration it was unfortunately not possible to obtain a blood sample. After a control period of 6 days, a 55 mg. tablet of adrenalin was implanted at 4.30 p.m. on 19 June 1939. Eighteen hours later the blood-sugar was markedly raised above normal but had returned to normal 48 hours later (Fig. 2). Blood lactic acid showed no rise in this experiment. It is possible, however, that the blood lactic acid had risen and returned to normal before
the second post-implantation blood sample was taken, since Cori et al. [1930 b], in the rat, reported that the rise in blood lactic acid following adrenalin injection had disappeared when the blood-sugar was still high. Milk lactose content showed a temporary rise coincident with the high blood-sugar. The milk yield dropped sharply after the implantation, to such a degree that the total daily output of lactose was reduced, and slowly returned to its previous level. There was a slight post-implantation rise in non-fatty solids but no significant change in milk fat content.

Similar results as to the temporary rise in milk lactose and non-fatty solids and fall in milk yield were obtained in another experiment with the same goat (experiment 3), but on this occasion blood samples could not be obtained from the carotid loop.

Experiment 4. Experiment 4 was carried out on goat Na. (approx. 100 lb. in weight) which was 1½ months in milk. The milk yield was rather irregular in the period preceding the experiment, but averaged about 60 oz. daily. Observations were made morning and evening on the milk yield and on the percentage of non-fatty solids, fat, and lactose. Over the greater part of the experiment blood-sugar and lactic acid were also estimated, and determinations of these quantities were begun 5 days before the experiment was started. A tablet of adrenalin weighing 51-2 mg. was implanted at 2.45 p.m. on 10 May 1938. As shown in Fig. 3, the observations indicated a great increase in the blood-sugar on the day after the implantation. The value was still raised slightly on the second day, 45½ hours after implantation. Blood lactic acid was also somewhat increased. There was a temporary increase in the percentage lactose in the milk, associated with the rise in blood-sugar and lactic acid. Since there was no immediate change in milk yield the implantation resulted in an increase in the total daily yield of lactose, a different result from that obtained in experiments 2 and 3. The percentage of milk fat was unchanged, while non-fatty solids showed a temporary rise.

Experiment 5. It seemed of interest to determine whether or not the results obtained with goats could be duplicated in a cow. Accordingly, experiment 5 was carried out on cow B1, weighing about 900 lb., and 3 months in milk. The cow was at the peak of the lactation curve with the milk yield constant at about 27 lb. daily. Milk fat, non-fatty solids, and lactose percentages were determined on samples from each milking, beginning 2 days before implantation of the adrenalin tablet. Blood-sugar and lactic acid were determined in arterial and mammary venous blood samples. At 4.30 p.m. on 2 February 1938 tablets of adrenalin amounting to 496 mg. were implanted. There was a rise in arterial and mammary
Fig. 3. Goat Na. (experiment 4). Adrenalin tablet implanted at ↑. Results of lactose determinations are entered at the time at which the samples were taken.
venous blood-sugar and lactic acid on the day after implantation (Fig. 4). The percentage milk lactose also showed a rise on the day after implantation (Fig. 5). There was an immediate fall in milk yield. The daily fat percentage rose, but there was no change in the daily percentage of non-fatty solids. There was a fall in both the daily yield of non-fatty solids, and also in that of lactose, due to the fall in milk yield. In other words, the decrease in the milk yield more than outweighed the rise in the percentage lactose. Further, the rise in fat percentage was not sufficient to cause a rise in total daily fat production.

It was hoped to obtain information about the uptake of glucose and lactic acid by the mammary gland from comparison of the analyses of the arterial and mammary-venous blood samples. Unfortunately, it was not found possible1 to take more than three arterial samples by the technique used, and of these three the last was obtained too long after the venous sample to permit satisfactory comparison [see Graham et al., 1936]. On the day after implantation the uptake of glucose was apparently almost unchanged, while that of lactic acid was trebled, but these findings require confirmation. The figures for venous blood, however, should be reliable.

Experiment 6 (Control). Control experiments with both goats were considered advisable in order to estimate the effect of the implantation technique on blood and milk composition. Therefore, after a suitable control period in which observations were made as before, a 44-mg. tablet of paraffin wax was subcutaneously implanted at 12.30 p.m. on 26 March 1939 into goat Ar. At this time she had been 7½ months in milk. The values for blood-sugar and lactic acid, milk yield and lactose content are given in Fig. 6. It is seen that in this experiment the implantation of an inert tablet was followed by a drop in milk yield of about the same magnitude as in two of the three adrenalin experiments with this goat, indicating that in this animal, which is rather excitable, the drop in milk yield following adrenalin was due to the disturbance associated with the implantation rather than to a specific effect of the hormone.

Experiment 7 (Control). The results of an experiment with goat Na. (5 weeks in milk) in which a 43-mg. wax tablet was implanted at 4.30 p.m. on 19 June 1939 are given in Fig. 7. In this experiment lactose was determined in composite milk samples as in experiment 2. This animal is more placid than goat Ar., and in this case it is clear that the implantation had no effect on milk secretion. This result is significant in view of the fact that adrenalin implantation was not followed by a drop in milk yield in

1 On successive occasions it became increasingly difficult to feel the internal iliac artery through the rectal wall, probably because of the formation of haematomata after previous punctures.
Fig. 4. Cow B1. (experiment 5). Adrenalin tablet implanted at ↑. In this figure the calculated lactose percentage in combined a.m. and p.m. milk is entered at noon each day. Since the milk fat rose during the experiment, the lactose figures are corrected for fat volume.
this goat and supports the view that adrenalin itself does not decrease milk yield.

**DISCUSSION**

These experiments in the first place indicate that the tablet implantation technique of Deanesly and Parkes [1937] is useful in the case of adrenalin. In all cases the results showed that implantation of solid adrenalin was followed by hyperglycaemia of long duration which, in the most favour-
able cases (experiments 1 and 4), amounted to at least 42 hours. Unfortunately, close estimates of the duration of the hyperglycaemia can in no case be made since it was not considered advisable to puncture the exteriorized carotid arteries more frequently than once a day. None of the animals
died from 'adrenalin shock' [see Wyman and tum Suden, 1939], though in a preliminary experiment to test this point, implantation of two 25-mg. tablets at intervals of an hour resulted in the death of the goat (a virgin female) 6 days later.

Graham, Jones, and Kay [1936] found that, in normal cows, the fall in sugar content of blood on passing through the mammary gland was positively correlated with the sugar-level of the arterial blood and also,
though this is less certain, with the milk yield. The work of Gowen and Tobey [1931] and others on the effects of inanition, and administration of insulin or phloridzin indicates that a lowering of the blood-sugar level, however produced, leads to a fall in milk yield and a diminished lactose content. It is, therefore, attractive to speculate that blood-sugar is a limiting factor in milk secretion and that if the blood-sugar level could be raised for a sufficiently long period a rise in milk yield would result. At the same time an increased lactose content might be expected.

In our experiments, in which the blood-sugar level was raised for many hours, the second expectation was realized but not the first. In every case the lactose percentage rose, but the milk yield did not increase: in three experiments, in fact, it fell sufficiently to outweigh the increased lactose content, so that the total daily lactose output was reduced. The control experiments indicate that this was not due to some other physiological effect of adrenalin but rather to trauma associated with the implantation. There were no uniform changes in milk fat-content which could be ascribed to the adrenalin implantation, but in most experiments a temporary rise in non-fatty solids content occurred, which calculation showed was due to the increase in lactose content.

In two of the three experiments in which blood lactic acid was estimated it was found to increase coincidently with blood-sugar and milk-lactose percentages. The absolute lactose production also increased in experiment 4. These findings might be interpreted as giving indirect support to Graham’s [1937] view that both glucose and lactic acid are utilized by the mammary gland for the synthesis of lactose, though, on the other hand, it might be argued that they are equally consistent with the classical theory of the origin of lactose from the blood-sugar alone. In experiment 5 it would appear that, although the absolute output of lactose fell, the gland took up much more lactic acid after the adrenalin implantation, while the glucose uptake was unchanged. This anomalous result is open to suspicion, since the last arterial blood sample was not obtained within the requisite time. The venous samples, however, were obtained quickly, and these showed an appreciable rise in glucose and lactic acid, thus confirming the results of experiment 4.

In passing, it may be noted that the normal values for the arterial blood lactic acid of the lactating goat determined during the control periods in these experiments are very much lower than values reported by Houchin, Graham, Peterson, and Turner [1939], which for 28 samples ranged from 21.29 to 134.30 mg. % with a mean of 53.88 mg. %. Our 13 determinations ranged from 5.4 to 15.0 mg. % and averaged 8.56 mg. %, results of the same order as for human venous blood [e.g. Long, 1924].

It is of some interest to find that prolonged hyperglycaemia, under the
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conditions of these experiments, may, in favourable circumstances, i.e. when the trauma of implantation does not lower the milk yield, lead to an increase in lactose secretion but not to an increase in the volume of milk secreted. Blood-sugar and possibly lactic acid therefore appear to be limiting factors in lactation as far as lactose synthesis is concerned, but as regards the volume of fluid secreted in unit time by the mammary gland the position is probably complicated by alterations in the blood-supply due to the action of the adrenalin.

**Summary**

1. By the subcutaneous implantation of adrenalin tablets into goats and a cow, hyperglycaemia was maintained for many (in two cases for at least 42) hours.
2. Blood lactic acid rose appreciably during the same period.
3. Coincidently, the percentage of lactose in the milk was raised.
4. In no case was the milk yield increased, while in three experiments it fell, evidently because of trauma associated with the implantation.
5. No uniform effects on milk fat were found, but there was a tendency for the non-fatty solids content temporarily to increase.
6. It is incidentally recorded that the arterial blood lactic acid in the normal lactating goat ranged from 5·4 to 15·0 mg. %.

We are much indebted to Prof. H. D. Kay and Dr. A. S. Parkes for their interest in this work and to the latter for making and supplying the adrenalin tablets. We also desire to thank Dr. G. W. Scott Blair for determinations of milk fat and non-fatty solids.

**REFERENCES**