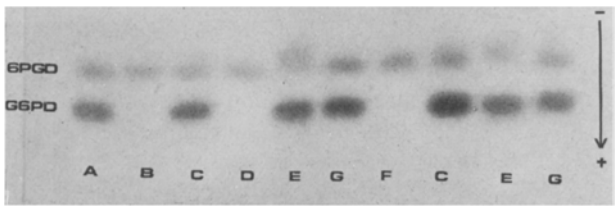
slight loss of activity was present, although not significantly different from the decrease found in the membrane-free lysate (Table II, B-D). This result is in contrast with the findings obtained on red blood cell lysates, in which erythrocyte membranes induce an activation on cytoplasmic GSSGR[2].

Our results show that incubation of whole platelet lysate in presence of added NADP induces changes in activity and in structure of 6PGD. This result indicates that the interaction between NAD(P)ase and 6PGD is similar in platelets and in red cells.

On the other hand, ultracentrifugation with complete removal of corpuscolate particles known to contain NAD(P)ase is necessary to prevent minor structural changes in 6-PGD.



Electrophoretic pattern of G6PD and 6PGD from platelet lysates after various treatments. Slot symbols from A to G correspond to the sample preparations symbol described under Materials and Methods. A, untreated, membrane free lysate; B, membrane free lysate, after incubation at 45°C without NADP; C, same as B, with NADP; D, whole lysate, after incubation at 45°C without NADP; E, same as D, with NADP; F, same as B, but after centrifugation of the lysate at 105,000 × *g*; G, same as B, but after centrifugation of the lysate at 105,000 × *g* + NADP.

NAD(P)ase activity and location in the cell could therefore be relevant in the control of the activity of some cytoplasmic enzymes. Although NAD(P)ase is present on the outer surface of the red blood cell[11], indirect evidence suggests that the enzyme is active also in the inner surface of the membrane[4]. Furthermore in other cells, NAD(P)ase is bound to the corpuscolate parts (mitochondria, microsomes) and its activity has been related to the regulation of the activity of several glycolytic enzymes, with specific differences in Ehrlich ascites tumor cells[12,13]. Our results on platelets further strengthens the hypothesis of a regulatory role for NAD(P)ase. Finally, activation of GSSGR by cell membranes is probably unrelated to their NAD(P)ase activity, and other mechanisms seem to be involved[14].

Riassunto. L'incubazione di lisati piastrinici con NADP provoca inattivazione della 6PGD e ne modifica la migrazione elettroforetica. Non si osserva invece attivazione della GSSG-R.

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Effects of 2-Br-α-ergocryptine on Plasma Prolactin Level and Milk Yield in Cows

The ergot alkaloids ergocornine and 2-Br-α-ergocryptine (CB 154) inhibit certain reproductive functions dependent on prolactin in rats[1–4] and also reduce serum and pituitary prolactin levels in rats and mice[5–7]. The anterior pituitary is considered the target of the inhibitory action on prolactin release[6]. Furthermore first clinical studies with CB 154 gave successful inhibition of galactorrhoea in several patients[8].

These results motivated our studies using the bovine species, which is distinct concerning reproductive physiology in the following points: a) In contrast to the rat LH rather than prolactin is assumed to be luteotropic. b) Our breeds are specially selected for milk yield. c) Prolactin blood levels and milk yield are exactly and continuously measurable over longer period in the same individuals, but the prolactin level has not been convincingly correlated with the stage of lactation or the milk yield[9]. There are two most distinct phenomena of the cows' prolactin blood level, i.e. the short increase during the milking stimulus and the longer lasting high peak before parturition[9–11]. In our first experiments we tried to examine the action of 2-Br-α-ergocryptine on these phenomena.

Material and Method. Animals. We used 4 non-pregnant cows and 1 pregnant cow around parturition. 4 cows were of the Brown Swiss and 1 cow of the Holstein-Frisian breed. The animals were 6–13 years old and they were kept in an open stable with pasturing.

Blood Collection. Blood plasma was collected in centrifuge tubes from the jugular vein; in experiment No. 1 by means of an inserted catheter and in the other experiments by needle puncture; the heparin preparation 'Liquemin' (Hoffmann-La Roche) was applied as an anticoagulant and the plasma samples were kept frozen (−18°C) until assay.

Inhibitor substance. The ergot alkaloid 2-Br-α-ergocryptine-methane-sulfonate = CB 154 (kindly supplied by SANDOZ, Basel) was used. The substance was dissolved

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in 40% ethanol and then diluted with saline. The compound was applied in 3 ml s.c. or i.m.

Prolactin assay. Prolactin was measured by the radioimmunoassay technique described by SCHAMS and KARG[10]. A highly specific antiserum to bovine prolactin, obtained by immunization of guinea-pigs, was used. This antiserum showed no cross-reaction with bovine growth hormone, luteinizing hormone and thyroid stimulating hormone, sheep follicle stimulating hormone, ACTH and oxytocine. NIH-P-B_2 19.9 IU/mg, kindly supplied by the National Institute of Health (USA), was used as antigen and reference preparation. The separation of the antigen antibody complex was done by the double antibody technique.

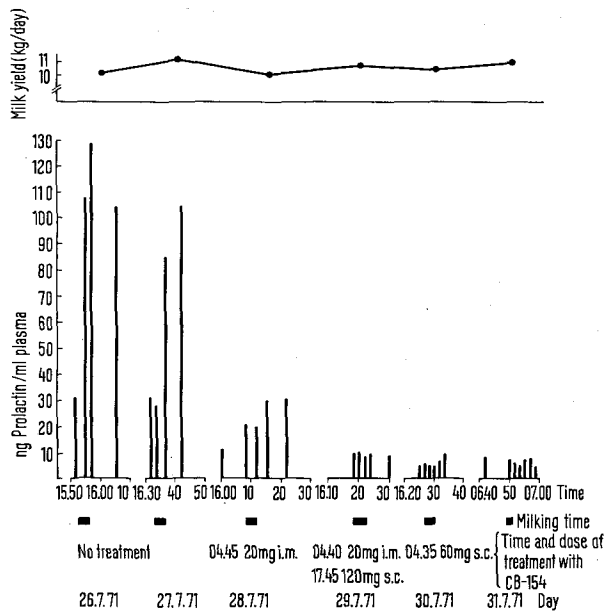

Fig. 1. Cow Kabine; 3 days treatment with CB 154, effect on plasma prolactin level and milk yield.

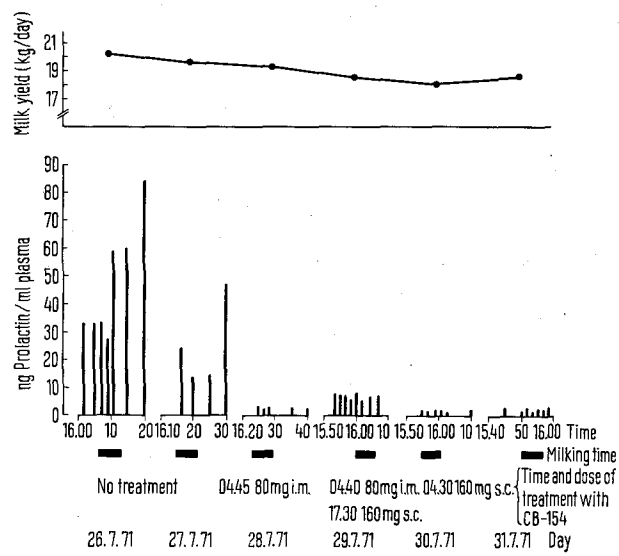

Fig. 2. Cow Adria; 3 days treatment with CB 154, effect on plasma prolactin level and milk yield.

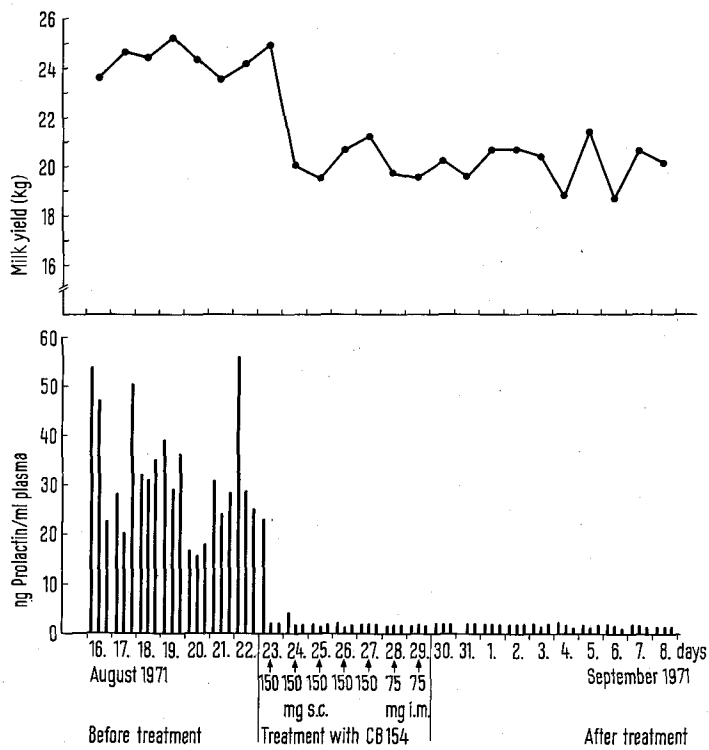

Fig. 3. Cow Kaido; 5 days 150 mg plus 2 days 75 mg CB 154; effect on plasma prolactin and milk yield.

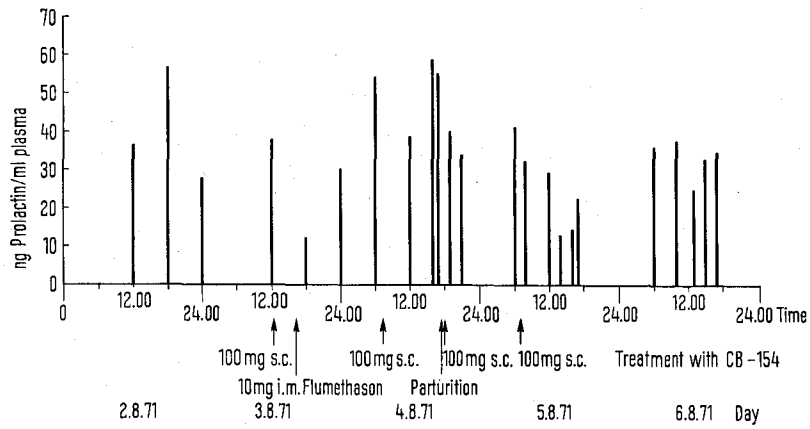

Fig. 4. Cow Kalihell; prevention of the blood prolactin peak before parturition with CB 154.

Experiments and results. Experiment 1. We tried to depress the prolactin peak released by the milking stimulus with CB 154. This experiment involved 2 cows over a period of 6 days (2 days before treatment, 3 days treatment and 1 day after treatment with CB 154). Blood was collected with frequent sampling during milking. For details and results see Figure 1 and 2; in these, results of experiments in which 20, 60, 80 or 160 mg CB 154 were applied, are demonstrated. It is clearly seen that 20 mg resulted in less depressed prolactin values than 80 mg. In both cases a reduction of prolactin levels was seen already on the first day of treatment.

Experiment 2. In this experiment we studied the influence on prolactin blood levels of CB 154 on 2 cows during 1 week's treatment. Blood was collected 3 times per day (in the morning and in the afternoon immediately before and 5 min after milking). The effect on prolactin level and milk yield is shown for one of these animals in Figure 3. Already, during the 1st day of treatment, the concentration of prolactin decreased to a very low basal level. The inhibitory effect of CB 154 on prolactin release lasted more than 8 days after the last application. At the beginning of treatment the milk yield decreased only by 10–20% and remained fairly constant at this lower level. The 2nd cow gave very similar results.

Experiment 3. This preliminary experiment in 1 cow was done to study the effect of CB 154 on parturition. 8 days before normal calving CB 154 was administered and parturition was induced by means of a corticoid injection. 24 h after corticoid treatment, parturition occurred. The effect on the prolactin level is shown in Figure 4. There is no obvious difference in prolactin levels before and after parturition.

Discussion. These experiments clearly demonstrate that CB 154 has a good blocking effect on plasma prolactin in the bovine species. Starting with doses of 80 mg i.m. or s.c. per day, there is a very effective inhibition. The blocking effect is not more pronounced with a higher dose of 160 mg CB 154.

Contrary to the drastic decrease of prolactin, the milk yield remained unchanged (Figures 1 and 2) or dropped only slightly (Figure 3). This means that, in the bovine, prolactin is not a main galactopoietic hormone; we conclude that, in this species, a complex of hormones is necessary for galactopoiesis, as has been suggested earlier on the basis of experiments with hypophysectomized goats[12].

The results in the second experiment (Figure 3) demonstrate that the blocking effect of CB 154 lasts more than 8 days after treatment, despite regular milking. The milk yield in the 2nd experiment increased 3 weeks after treatment with CB 154, but no blood was collected at that time. Further experiments concerning this question are in progress.

During the 3rd experiment, parturition was induced by corticoid injection[13] after administration of CB 154. Despite a completely normal delivery, the prolactin peak before parturition could not be seen. In contrast to the other experiments, in this case prolactin basic values were rather high. The average milk yield after treatment was about 13 l in contrast to 18–20 l in 2 previous lactation periods. This being a preliminary experiment on only 1 animal, we can only tentatively conclude: a) The prolactin peak in connection with parturition may have to be interpreted rather symptomatically than functionally; b) that the inhibitory action of CB 154 may have a stronger effect on the initiation of lactation than on the milk yield after the onset of lactation.

Zusammenfassung. An 5 Kühen wurde der Effekt eines spezifischen Prolaktininhibitors (2-Br-α-ergokryptin = CB 154, SANDOZ, Basel) auf den Plasmaprolaktinspiegel und die Milchleistung untersucht. Bereits mit einer Dosis von 80 mg CB 154 pro Tag konnte eine sichere Hemmwirkung der Prolaktinausschüttung erreicht werden. Im Gegensatz zu dem starken Prolaktinabfall war die bestehende Milchleistung überhaupt nicht oder nur um 10–20% vermindert. Prolaktin dürfte somit beim Rind nicht als maßgeblicher oder ausschließlicher galaktopoetischer Faktor anzusehen sein. Dagegen zeigte sich in einem vorläufigen Versuch, daß mit CB 154 nicht nur der bekannte Prolantinpeak um den Geburtstermin unterdrückt, sondern auch die einsetzende Laktation wesentlich beeinträchtigt werden kann.

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