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The influence of cobalt/vitamin B_{12} deficiency as a « stressor » affecting adrenal cortex and ovarian activities in goats

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Summary. Twenty 1.5 to 2-year old goats were made vitamin B_{12} deficient by feeding them cobalt-deficient diets for 23 weeks in order to determine the effects of a progressive deficiency on ovarian and adrenal cortex activities. At 1-day intervals, blood samples were collected for haematological study and plasma for vitamin B_{12}, progesterone and corticosteroid radioimmunoassays. At 23 weeks, the adrenal cortex was taken for histological measurement and the pituitary gland and corpus luteum for LH and progesterone determinations, respectively. The regularity of the oestrous cycles was studied using teaser bucks. The goats receiving a cobalt-deficient diet presented irregular oestrous cycles (22.6 $\pm$ 0.8 days ; maximum deviation : 12 to 38 days), while those of the controls were 18 $\pm$ 0.3 days (maximum deviation : 16 to 21 days). Low vitamin B_{12} concentration led to macrocytic and normochromic anaemia. The concentration of plasma progesterone augmented, but it decreased during the third and subsequent cycles in cobalt-deficient goats as compared to the controls. Plasma corticosteroids were persistently high in cobalt-deficient goats as compared to the controls, and pituitary LH was low in the deficient goats. It is suggested that an erratic endocrinological control mechanism led to irregular cycles, the action site being located in the hypothalamo-pituitary axis.

Introduction.

The effects of a restricted dietary energy intake on the concentration of peripheral blood progesterone in domestic ruminants have already been reported (Gombe and Hansel, 1973 ; Beal *et al.*, 1978 ; Imakawa *et al.*, 1983) ; however, the results obtained were inconsistent, showing either an increase, a reduction, or no change in those levels. The inconsistency was partly explained by differences in age, species, sex, animal health, sexual cycle stage or the severity of the dietary energy restriction (Apgar *et al.*, 1975 ; Boone *et al.*, 1975). Some authors noted that a restricted energy intake led to an alteration in adrenal cortex production which then directly or indirectly affected ovarian activity and the concentration of peripheral plasma progesterone (Wagner *et al.*, 1972 ; First, 1979).

Few studies have been made of the endocrinology of abnormally long oestrous cycles during the transitory period from the cyclic to the anoestrous state in cows, ewes or goats on restricted dietary energy intake. Recently, Imakawa *et al.* (1983) suggested that the endocrinological factors, and not ovarian function, were responsible for the development of anoestrus.

Cobalt deficiency in ruminants is a vitamin B_{12} deficiency as well as a slow-developing energy deficiency (Underwood, 1977). It is therefore interesting to carry out studies in ruminants concerning the effects on reproduction and endocrinology of a very slow, but progressive, energy deficiency (Mgongo *et al.*, 1981).

The aim of the present study was to determine the effects of a low cobalt diet on stress in goats and to point out the role of adrenal cortex activity on ovarian function and oestrus cyclicity.

Material and methods.

Twenty East African short-horned normocyclic goats (1.5 to 2 years old) weighing 21 to 24 kg were divided randomly into two equal groups, a control group (A) and an experimental group (B). The goats were adapted to the experimental environment for 2 1/2 months. For a period of 23 weeks, they were given a cobalt-deficient diet (less than 0.01 mg/kg dry matter) containing mainly Rhodes grass (*Chloris gayana*) hay (Boone *et al.*, 1975). This diet provided 885 g of total digestible nutrients and 54 g of digestible protein per goat and per day. To prevent cobalt deficiency, the control animals received 1-g cobalt oxide bullets every 3 weeks. This ration, prepared according to Morrison standards (Morrison, 1961), exceeded maintenance requirements, and the amounts of the other dietary components (cobalt excepted) were approximately the same in both diets. Water was offered *ad libitum.*

All feed analyses, including that of the cobalt levels, were made according to the methods recommended by the Association of Analytical Chemists (AOAC, 1970).

Oestrous activity was observed daily and teaser bucks were used to aid in oestrus detection. The goats were weighed weekly and blood samples were collected every 2 days during the first month and at 2-day intervals thereafter. To show the severity of vitamin B_{12} deficiency, red blood cells, haemoglobin and packed cell volume were measured using an haemoglobinometer (Mgongo *et al.*, 1981).

Plasma vitamin B_{12} was determined using a competitive inhibition radioimmunoassay kit (Batch N° NEA 065, New England Nuclear, USA).

The competitive protein binding assay originally described by Murphy (1967) and Bassett and Hinks (1969) and validated by Mgongo (1979) was adapted and used for determination of the plasma corticosteroid concentration. Cortisol binding globulin (CBG) was obtained from third trimeter serum. In our tests, one ml of CBG (1 : 3 000 dilution) cross-reacted with cortisol (35 %) and corticosterone (42 %) ; all values have been given in total plasma corticosteroids.

Standard corticosterone and tritiated corticosterone (1α, 2α, ^3H), were obtained from Guy E. Abrahams, California and the Radiochemical Centre, Amersham, UK, respectively. The intra and inter-assay coefficients of variations determined were below 8 and 10 %, respectively.

Progesterone was determined using the radioimmunoassay reported by Gombe (1977) for our laboratory. The intra and inter-assay coefficients of variation were below 9 %.

At 23 weeks, all the goats were slaughtered and the ovaries, adrenal and pituitary glands were collected and weighed. The corpora lutea, isolated after removal from the ovaries according to Simmons *et al.* (1976), were later analysed to determine their progesterone content. The pituitary glands were homogenized separately in ice-cold, double-distilled water and a small aliquot of each homogenate was diluted for LH assay. The adrenal glands were processed for histologic examination ; the cells and different zones of the adrenal gland were measured with a calibrated binocular micrometer (Leitz, Wetzlar, Germany). To limit inherent error when selecting the sections, 200 different sections were made. The measurements as well as the statistical mean and standard error of the mean were taken as true values of the thickness of a particular zone.

Statistical analysis was carried out using the analysis of variance and Student's t-test according to Snedecor and Cochran (1967). Hormone concentrations were compared by peak values and by areas below the curve. Progesterone concentration in ng/ml was plotted as the ordinate and the duration of the oestrous cycles as the abcissa. The correlations were calculated to evaluate the relationship between weight loss in goats and the number of days before cessation of cyclic activity during the experiment.

Results.

Feeding a cobalt-deficient diet to goats resulted in a decrease in body weight and a macrocytic and normochromic anaemia. More details of the results are reported on table 1 and figures 1 and 2.

The distribution of the duration of the oestrous cycles (fig. 3) showed frequent irregular cycle length in cobalt-deficient goats. The mean ($\pm$ SEM) cycle length for the control goats in the 80 cycles exhibited during the 23 weeks of the experiment was 18.52 $\pm$ 0.23 days with a range of 16 to 21 days, whereas the cobalt-deficient goats in 69 cycles showed a mean of 22.57 $\pm$ 0.78 days with a range of 12 to 38 days. The 35 cycles occurring from the 9th to 23rd weeks of the experiment (*i.e.* immediately before cessation of cyclicity) showed a mean of 25.67 $\pm$ 5.61 days and irregular duration ranging from 15 to 48 days. 55 % of the cobalt-deficient goats had cycles longer than 21 days and 40 % had cycles of less than 10 days ; cycle length was normal in only 5 % of the goats. The cycle lengths of the control animals and those exhibited by deficient goats in the first 9 weeks showed no difference. In both groups only 25 % of the animals had cycle lengths of 21 days or longer and, unlike during cobalt deficiency (9 to 23 weeks), unusually prolonged cycles were virtually absent.

TABLE 1

Mean ovarian weight, corpus luteum progesterone and adrenal and pituitary gland measurements in control (A) and cobalt-deficient (B) goats (*).

Group	Corpus luteum progesterone (μg/g)	Cell measurements		
		Adrenal fasciculata and reticularis (mm)	Adrenal cortex (mm)	Cytoplasm/ Nucleus ratio
A	65.8 ± 1.25	1.30 ± 0.15	1.54 ± 0.20	0.99 ± 0.15
B	18.6 ± 0.98a	1.50 ± 0.20a	1.85 ± 0.11a	1.44 ± 0.15a

		Pituitary gland		
	Ovary weight (mg)	Weight (mg)	LH concentration (ng LH/μg protein extracted)	LH content (μg)
A	17.50 ± 65.28	295.50 ± 25.05	5.77 ± 1.44	101.18 ± 11.00
B	790.50 ± 28.67a	402.00 ± 54.71a	1.44 ± 0.26a	46.71 ± 4.22a

(*) All values are given as means (± SEM). A = Control goats (n = 10) ; B = Cobalt-deficient goats (n = 10). The superscript signifies $P < 0.05$.

Peripheral progesterone concentrations in control goats were low during oestrus. Values between 0.64 ± 0.09 and 0.98 ± 0.20 ng/ml were obtained. From days 1 to 4 of this cycle, subsequent progesterone concentration increased, reaching maximal levels between 9 and 14 days (mean between 11 and 13 days) with a mean of 6.81 ± 0.31 ng/ml. Thereafter, there was an abrupt decline in the concentrations to oestrus values 3 to 1 days before oestrus.

Plasma progesterone concentration showed a similar pattern in cobalt-deficient goats. In the first cycle there was no difference between the two groups, but in the second cycle plasma progesterone concentrations were higher in cobalt-deficient than in control goats. In the third and subsequent oestrous cycles, peak plasma (t-test : $P < 0.05$) progesterone declined to values lower than those of the control goats. The changes in progesterone values were particularly marked in peak values, but little or no change in basal values was noted.

Ovarian weights and corpus luteum progesterone levels were also lower ($P < 0.05$) in cobalt-deficient than in control goats, and the values in the former were similar to those of goats which had stopped cycling. This observation was made when progesterone concentration did not exceed 1 ng/ml in the rest of the study. Details on the progesterone values are shown on figure 4.

Corticosteroid concentration during pretreatment and after the onset of treatment did not differ significantly between control and cobalt-deficient goats. However, the latter showed significantly higher ($P < 0.01$) corticosteroid levels

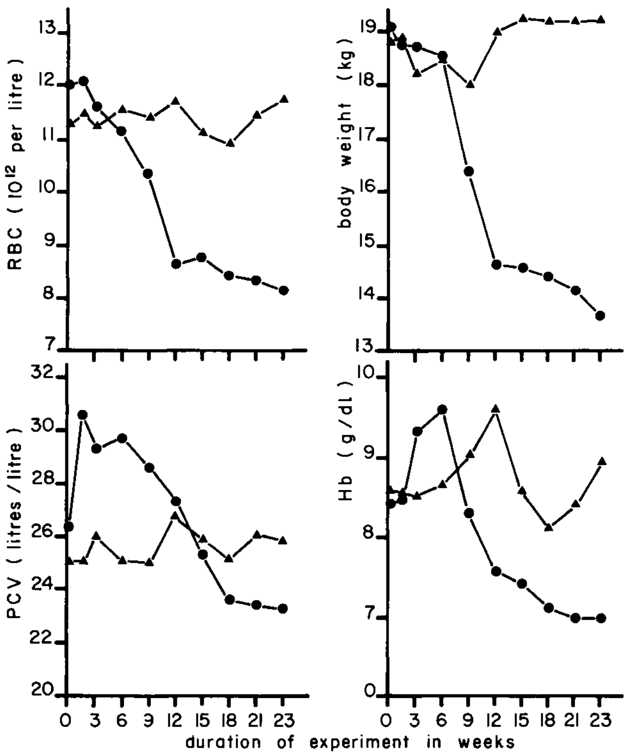

FIG. 1. — *Mean plasma haematological values and body weights of control* (▲: n = 10) *and cobalt-deficient* (●: n = 10) *goats.*

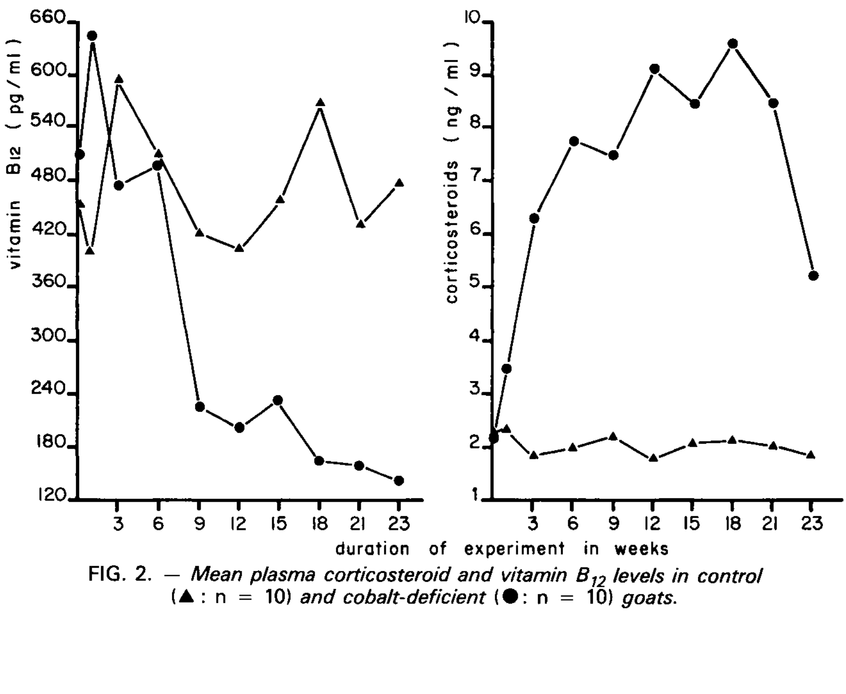

FIG. 2. — *Mean plasma corticosteroid and vitamin B_{12} levels in control* (▲: n = 10) *and cobalt-deficient* (●: n = 10) *goats.*

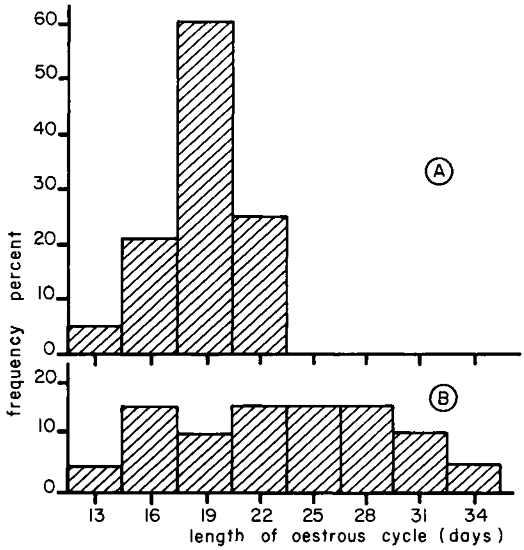

FIG. 3. — *Frequency of oestrous cycle length in goats.*
(control A, n = 10 ; cobalt-deficient B, n = 10).

after the 6th week of treatment. The large standard error of the mean observed in the increase of corticosteroid concentration reveals the wide variation among goats.

The histology of the adrenal cortex showed a marked hypertrophy of the cells in the zona fasciculata and reticularis.

Changes in body weight were more closely correlated with corticosteroid values in control ($r^2 = 0.47$; $P < 0.05$) than cobalt-deficient ($r^2 = 0.24$; $P < 0.05$) goats. The percentage of weight loss and the number of days before cessation of cyclic activity were also correlated ($r^2 = 0.65$; $P < 0.05$).

Discussion.

The unexpected findings of this study, *i.e.* that plasma progesterone was lower during the third oestrous cycle in goats receiving a cobalt-deficient diet, are consistent with those reported in cattle by Donaldson *et al.* (1970), Hill *et al.* (1970), Gombe and Hansel (1973) and Beal *et al.* (1978) following energy, protein, or total nutrient restriction. However, only a few data are available on the biphasic pattern consisting of initial increases in plasma progesterone before decreases in the subsequent cycles (Wagner *et al.,* 1972 ; Dunn *et al.,* 1974). There is thus a remarkable similarity between the progesterone profile following cobalt (vitamin B_{12}) deficiency and that after restricted food intake. It is our contention that the mode of action of both parameters is the same, namely, that

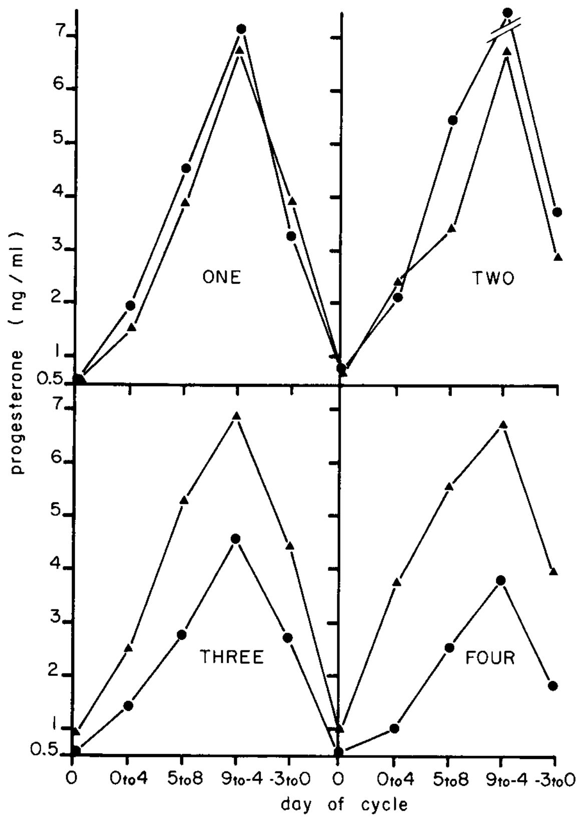

FIG. 4. — *Mean plasma progesterone concentrations in control* (▲ : n = 10) *and cobalt-deficient* (● : n = 10) *goats during cycles 1, 2, 3 and 4.*

the effect of cobalt/vitamin B_{12} deficiency is that of chronic malnutrition. These results indicate that a slowly-induced nutritional stress, as that caused in the present study, leads to a biphasic pattern in progesterone concentration, and that acute or severe nutritional effect might only be responsible for the monophasic decreases in peripheral hormones. These statements must be confirmed by further research because some investigators have been unable to obtain the same results in repeated experiments, indicating a multifactor involvement (Apgar *et al.,* 1975 ; Beal *et al.,* 1978 ; Imakawa *et al.,* 1983).

In this study, the corticosteroid levels in control goats were similar to those reported for normal goats (2-3 ng/ml), whereas cobalt-deficient goats had fourfold higher concentrations similar to the levels observed at parturition in normal goats. However, the duration of corticosteroid elevation was more prolonged (Van Rensburg, 1971 ; First, 1979 ; Mgongo *et al.,* 1983). The hypertrophy of the adrenal cortex and higher concentrations of plasma corticosteroids are consistent with stress, in the present case due to cobalt deficiency (Braden and Moule, 1964 ; Stott and Thomas, 1971 ; Moberg, 1976). Malnutrition or other forms of stress may cause the activity of the pituitary gland (indicated by changes in pituitary LH in this study) and the adrenal cortex to increase because of hypothalamic stress due to altered neuroendocrinology (Deaver and Dailey, 1983). Although the precise way in which corticosteroids influence reproduction is unknown (MacDiarmid, 1983), their moderating effect on levels of LH and progesterone has been reported (Tomasgard, 1976 ; First, 1979 ; Matteri and Moberg, 1982 ; Wagner and Li, 1983). Corticosteroids play a role in reproduction partly by their significant depression of circulating levels of gonadotrophins and of corpus luteum function and by the release of luteolytic substances (Tomasgard, 1976 ; Da Rosa, 1979). The biphasic pattern of progesterone in this study presumably originated from either over-production of the adrenal cortex or increased production of progesterone, and the decreases were due to the moderating effects of LH and the suppressing effects of corticosteroids on the corpus luteum (Matteri and Moberg, 1982). Subsequently, plasma corticosteroid levels declined from week 14 of our study, signifying that, as the cobalt deficiency progressed, the goats re-adjusted to stress.

Our results also suggest that there may be a transitory period during which the endocrinological factors are not regularly controlled. This would result in irregular oestrous cycles involving both longer and shorter cycles before the cessation of oestrus cyclicity. During this transitory period, progesterone concentrations are low, and the persistence of these low levels would indicate that the feedback mechanisms affected by progesterone have been altered (Yen, 1977).

During this same period, corticosteroid concentrations are constantly high and LH concentrations probably low. A similar situation was reported in cattle (Gombe and Hansel, 1973 ; Imakawa *et al.,* 1983). If our supposition is true, it confirms the suggestion of Imakawa *et al.* (1983) and Camp *et al.* (1983) that the endocrinological mechanisms in cattle leading to the development of a corpus luteum for the subsequent cycle fail before the cessation of cyclicity. The absence of normal corpus luteum activity and the altered endocrinological mechanisms in the goats of the present study could therefore explain the prolonged length of the oestrous cycle.

However, in our study, contrary to results obtained by Imakawa *et al.* (1983), oestrous cycle lengths, body weights and progesterone concentrations during the oestrous cycle prior to cessation of cyclic activity appear to be related to the onset of anoestrous.

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Résumé. *Déficience en cobalt et vitamine B_{12} chez la chèvre. Assimilation à un stress. Retentissement sur les activités ovariennes et cortico-surrénaliennes.*

Une carence en vitamine B_{12} a été provoquée chez 20 chèvres âgées de 18 à 24 mois, en leur distribuant un régime apportant 885 g de nutriments digestibles totaux et 54 g de protéines digestibles par chèvre et par jour, mais carencé en cobalt. Le but de ce travail était de déterminer l'effet d'un développement progressif d'une carence en cobalt sur les activités ovariennes et corticosurrénaliennes. Chaque jour de l'expérience, du sang fut prélevé pour les examens hématologiques et le dosage de la progestérone et des corticostéroïdes. A la 23e semaine, les corticosurrénales furent prélevées pour des examens histologiques, l'hypophyse pour le dosage de la LH et les corps jaunes pour la détermination des concentrations en progestérone. Les œstrus furent contrôlés régulièrement avec un bouc bout-en-train. Les résultats montrèrent l'irrégularité des cycles sexuels lors de carence en cobalt (18,5 $\pm$ 0,3 jours, écarts : 16 à 21 jours pour les témoins, contre 22,6 $\pm$ 0,8 jours, écarts 12 à 38, pour les animaux carencés). L'anémie macrocytique et normochromique se développe avec de faibles teneurs sériques en vitamine B_{12}. La sécrétion de progestérone fut prolongée, mais diminua au cours du 3e cycle et des cycles suivants chez les animaux carencés. Les niveaux de corticostéroïdes plasmatiques furent constamment élevés chez les chèvres déficientes en cobalt par rapport aux témoins. Les concentrations hypophysaires de LH furent basses chez les animaux carencés. Il est suggéré qu'un système de contrôle endocrinien perturbé est responsable des cycles irréguliers, sa localisalisation vraisemblable étant au niveau hypothalamo-hypophysaire.

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