THE INFLUENCE OF COBALT AND VITAMIN B_{12} DEFICIENCY ON REPRODUCTIVE, ADRENAL AND THYROID FUNCTION OF THE FEMALE GOAT.

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by
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D E C L A R A T I O N

This thesis is my original work and has not been presented for a degree in any other University.

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SUMMARY.

Twenty normocyclic female East African short horned goats were divided into four groups of 5 each and were made Vitamin B_{12} deficient (3 groups) by feeding them cobalt deficient rhode grass hay from the Rift Valley of Kenya. Maximal reduction in plasma B_{12} (determined by competitive inhibition radioassay kit) occured after 8 weeks parallel with reduction in body weight, and onset of macrocytic normochromic (i.e. pernicious) anaemia. The oestrous cycles became progressively irregular and finally ceased after the fifth oestrus. Blood was collected at 2 day intervals during the first month and at 3 day intervals thereafter up to 23 weeks (except at oestrus where collection was at 3 hourly intervals). Plasma progesterone, oestrogens, LH and pituitary LH, plasma corticosteroid and thyroid hormones (T_4 and T_3) were determined by respective radioimmunoassays. Peak plasma progesterone (Day + 9 to Day - 4) and oestrogens (Day - 1 to Day + 1) remained comparable to controls at 6 to 7 ng/ml for progesterone, and 60 to 80 pg/ml oestrogens for the first cycle, rising in the second cycle and thereafter declining sharply and progressively until cessation of cyclic activity.

Oestrous Day 0 LH surge showed an initial rise to peak values during the first,second and third oestrus before declining to values comparable to controls. There was pituitary acidophil hyperplasia concomitant with hypoplasia of delta basophils ; there was hyperplasia of beta basophils . Total pituitery LH was less than one third of control values at the end of 23 weeks (30 vs 101 ug/gland for deficient and control, respectively). Pituitary weights for deficient animals were, however, higher.

The primordial follicles as well as the cells of secondary and tertially follicles in the ovary showed marked atrophy. The adrenal cortex was markedly hypertrophic and there was consequently elevated corticosteroid levels (2.2 $\pm$ 0.6 ng/ml for controls vs 7.5 $\pm$ 1.5 ng/ml for deficient).

Similarly the thyroid gland was hypertrophic and hyperplastic and was mirrored by increase in plasma T_4 and T_3 values and free thyroxine index (4.7 $\pm$ 0.6 vs 11.5 $\pm$ 1.4 for control and deficient, respectively). After 15 weeks, energy supplementation to group 3 and protein to group 4 for 8 weeks reduced the above changes but did not lead to recovery.

It is concluded that vitamin B_{12} deficiency probably acts by an initial overproduction of hypothalamic liberins (LRH, CRH and TRH) which in turn cause initial increases in LH, ACTH and TSH in plasma before pituitary exhaustion leads to decreases in the trophic hormones.

Vitamin B_{12} deficiency may, however, have an additional direct effect on the ovary as it has been shown in this study that decrease in ovarian functions precedes a decline in plasma LH.

The observed symptoms, however, were essentially those of severe malnutrition.

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A C K N O W L E D G E M E N T S

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1:0. GENERAL INTRODUCTION.

Ruminants are capable of deriving their "total" Vitamin B_{12} (cyanocobalamin) from the endogenous synthesis of the vitamin by the ruminal microorganisms (Marston, 1952; Lee & Marston, 1969; Underwood, 1971; Marston, Allen & Smith, 1972). The microorganisms, however, must be supplied with an adequate and regular amount of cobalt to be able to effect the synthesis (Smith and Marston, 1970).

Cobalt deficiency is common in many parts of the world (Underwood, 1971; Jensen, 1974; Nicholas & Egan, 1975). Where the deficiency is extreme large areas of land are unsuitable for raising ruminants (Underwood, 1971). In East Africa, many important ranching areas are cobalt deficient thereby posing productivity problems especially to the peasant farmer who cannot afford mineral supplements. In Kenya for example, most of the areas of the Rift Valley around Nakuru, Molo, Muguga and along the escarpment are cobalt deficient ; while in Tanzania large areas around Arusha, Singida, Mbulu and Mpwapwa are deficient (Mwakatundu, 1978).

Ruminants, especially sheep and goats, when fed a cobalt deficient diet, become Vitamin B_{12} deficient very rapidly. Infact experience in East Africa shows that feeding goats on rhode grass hay from cobalt deficient areas results into deficiency within a period of about two months (Gombe & Verjee, 1976). Furthermore, ruminants are unable to store cobalt and vitamin B_{12} in the body, and supply therefore needs to be regular (Underwood, 1971). Thus when compared to other species, the requirements for cobalt is much higher in ruminants. Cobalt and Vitamin B_{12} deficiency produce in animals the same inhibitory effects on reproductive functions as reduced feed intake (Underwood, 1971; Wiessner, 1972; Lotthammer & Ahlswede, 1973; Jensen, 1974; Nicholas & Egan, 1975). Like the latter the B_{12} deficient animals show progressive loss of weight, cessation of cyclic activity and increase in embryonic mortality. Furthermore, during reduced feed intake there is either a reduced or increased secretion of gonadotrophins, oestrogens, progesterone, and other hormones (Setchell, Waites, and Lindner, 1965; Leathem, 1966; Donaldson, Bassett and Thorburn, 1970; Hill, Lamond, Henricks, Dickey and Niswender, 1970; Gombe, 1972; Howland, 1975; Beal, Short, Staigmiller, Bellows, Kaltenbach and Dunn, 1978).

In ruminants, the primary metabolic defect in Vitamin B_{12} deficiency is an impaired capacity to metabolize propionate (Somers, 1969; Underwood, 1971; Marston, Allen and Smith, 1972). This then leads to loss of appetite, a form of energy metabolic inefficiency (Marston, 1970).

I:I. Objective of the study.

It was the intention of this investigation to determine whether the observed effects of cobalt deficiency on reproductive functions were in fact due to poor food utilization or were exerted by a different mechanism of its own. Thus in this study plasma oestrogens, progesterone, corticosteroid, thyroid hormones and luteinizing hormone were monitored in cobalt deficient goats. Furthermore, this study was designed to determine whether a reversal feeding by supplementation of readily available energy or protein rich feed, could alleviate the reproductive inefficiency caused by Vit. B_{12} deficiency, since the limitation imposed on available nutrients by a decrease in ruminant microorganisms could be by-passed.

2:0. LITERATURE REVIEW

2:1. Cobalt deficiency: Introduction

Cobalt deficiency is scattered world wide in isolated areas where soils and pastures contain less than one part per million on dry matter basis (Underwood, 1971; Nicholas & Egan, 1975). Thus the disease due to cobalt deficiency is known by many names in different parts of the world. For example, in New Zealand the disease is called Bush sickness; in Scandinavia, wasting disease and in Kenya, Nakuruitis (Underwood, 1971).

Cobalt which is an essential trace element in ruminants is stored in the body in limited amounts only and supply therefore need be regular and continuous. The need for cobalt is for the synthesis of Vitamin B_{12} and to support ruminal microorganisms. Microorganisms assist to convert cellulose and other carbohydrate feeds into easily assimilated volatile fatty acids (Marston et al., 1972). Therefore in deficiency, there is a decrease in microbial population and in feed utilization efficiency.

The first clinical symptoms observed in cobalt deficiency are decrease in appetite and dull demeanor.

Lack of appetite then leads to a decrease in growth and gradual loss of body condition (Underwood, 1971; Blood & Henderson, 1974). The animal becomes emanciated, rheumy-eyed and listless. These clinical features are undistinguishable from those of inanition (Underwood, 1971). As deficiency progresses, severe inappetence, macrocytic anaemia, profuse lacrimation and pica supervene, exacerbated by intermittent diarrhoea with constipation (Blood & Henderson, 1974; Nicholas & Egan, 1975; Gombe & Verjee, 1976). Vitamin B_{12} deficiency in pre-ruminant calves, pigs, rats and man leads to loss of appetite, poor growth, and muscular incordination associated with demyelination of the peripheral nerves (Underwood, 1975; Church & Pond, 1976).

2:2. The Role of Cobalt in Ruminant Metabolism

The final pathway of volatile fatty acids from the rumen involves their entry into the Kreb's cycle after intermediate reactions. For example, propionic acid is metabolised as depicted below (Cannata, Focesi, Mazumder, Warner & Ochoa, 1965):-

Propionic Acid in presence of
Coenzyme A and ATP
↓
Propionyl CoA
↓
D - methylmalonyl CoA
↓
L - methylmalonyl CoA
In presence of L -
methylmalonyl CoA mutase (B_{12}
↓
Coenzyme).
↓
Succinyl- CoA
↓
Kreb's cycle.

The conversion of L-methylmalonyl CoA to succinyl-CoA utilizes a cobamide (Vit. B_{12}) coenzyme. The increase in urinary excretion of methylmalonic acid during Vit. B_{12} deficiency in humans (Gawthorne, 1968), ruminants (Marston, 1970), and the East African shorthorned goats (Gombe & Verjee, 1974) confirms the decrease in the activity of methyl-malonyl CoA isomerase, and a stemming back of other metabolic products.

The accumulation of metabolic by-products leads to reduced feed intake (Smith & Marston, 1970; Baile & Forbes, 1974). Thus the loss in body weight and other cobalt deficiency symptoms are due to loss of appetite - an energy metabolic inefficiency. In summary, the clinical cobalt deficiency is due to an inadequate supply of Vit. B_{12} *per-see* and not a consequence of the decreased voluntary feed intake which is part of the deficiency syndrome.

Anaemia and neurological symptoms during cobalt deficiency are due to a derangement in protein metabolism, especially that of methionine, thiamine and glutathione (Underwood, 1971; Gombe & Verjee, 1975; Frenkel, Mukherjee, Hackenbrock & Srere, 1976; Nishino & Itokawa, 1977). Furthermore, neurological features are due to spinal cord degeneration, lack of nerve cell permeability and unusual incorporation of methylmalonyl CoA into fatty acids of nerve cells (Frenkel, 1973). This leads to a derangement of neurotransmitters in the brain and especially catecholamines (Deana, Vincenti and Deana, 1977).

2:3. The Role of Cobalt and Vitamin B_{12} on Reproductive functions.

Search for relevant literature revealed scant researches specifically designed to investigate the effect of dietary cobalt on reproductive systems of the female ruminant. However, Marston (1952) in his review pointed out that cobalt and Vitamin B_{12} deficiency inhibits reproductive functions. In later reviews Bentley, Quicke, Kastelic & Phillips (1951), Roberts (1971), Underwood (1971), Wiessner (1972), Lotthammer & Ahlswede (1973) and Hafez (1974) report of abortions, abnormal oestrous cycles, silent oestrus, poor growth with delayed puberty, low conception rates and increased repeat breeders, birth of weak young ones as being common in ruminants fed on cobalt deficient pastures. These authors stress that cobalt deficiency symptoms are due to lack of appetite and progressive loss of body weights rather than to lack of Vit. B_{12} per se.
In 1959 Newberne & O'Dell suspected involvement of B_{12} deficiency in congenital abnormalities in rats. They reported that there was hydrocephallic irreversible brain damage and that the adrenal gland showed degeneration with the zone arcuata more pronounced while cytoplasm of the fasciculata cells was greatly distended with lipid.

In 1952, Marston had reported that there was thyroid gland hyperplasia in B_{12} deficient rats.

In his review in 1961, Leathem suggested that during B_{12} deficiency there are various forms of gonadal dysfunction with impaired secretion of gonadotrophins and sex hormones, This was later supported by findings in man and bulls that B_{12} deficiency lead to semen abnormalities and especially oligospermia and azoospermia (Busch, 1957; Watson, 1962; Tomaszewsk, Zmudzka and Nadwarny, 1963; Lotthammer and Ahlswede, 1973).

Administration of B_{12} to rats expedites puberty (Erofleeva & Mikhlin, 1968), and to man leads to improved maturation of spermatozoa (Watson, 1962).

2:4. The Role of Nutrition on Reproductive Functions

Both undernutrition and malnutrition selectively inhibit reproductive functions in preference to other essential body functions (Asdell, 1964; Leathem, 1966). The role of malnutrition to reproductive functions is heterogenous in that a deficiency of a specific dietary component, whether it is energy (Gombe & Hensel, 1973), protein (Gupta & Anand, 1971 & 1973) minerals (Gombe, 1972; Nicholas & Egan, 1975), Vitamins (Lutwak - Mann, 1958) or total nutrients (Donaldson, Bassett & Thorburn, 1970),

does interfere with reproductive functions in more or less the same manner, though there are many other factors such as experimental designs, duration of nutritional stress, animal species, age, sex, stage of reproductive cycle, composition and palatability of the feed, and previous history of management that are also important (Howland, 1972; Boone, Hill, Kennedy and Henricks, 1975; Nakanishi, Mori & Nagasawa, 1976).

In their reviews Asdell (1964), Leathem (1961 & 1966); Lamming (1966), Lamond (1970), McClure (1970), Rattray (1977) suggested that malnutrition affects reproductive functions by either inhibiting the metabolism, release and synthesis of hormones, responsiveness of the target organs or on the neural control of the hypothalamo - hypophyseal target organ axis.

Several early studies showed that malnutrition could be corrected by administration of either whole pituitary extracts (Moore & Samuel, 1931); or oestrogens and progesterone (Kinsey & Srebnik, 1963; Leathem, 1966), or improved nutrition. Furthermore, findings by Mullinos and Pomerantz (1940 and 1941) that defects due to malnutrition resembled those of hypophysectomy, led to the adoption of the theory of "pseudohypophysectomy" to describe the effects of malnutrition.

Mullinos and Pomerantz, however, did not determine levels of peripheral hormones. Until recently many of the early studies showed evidence to support the theory. Leathem (1966), Piacsek & Meites (1967), Srebnik (1970) had found that pituitary gonadotrophins were decreased in rats deprived of protein. Hill, Lamond, Henricks, Dickey and Niswender (1970) while feeding heifers on 85% the maintenance requirements for energy and protein, found that progesterone values, number of follicles and corpora lutea were decreased. Similar results were observed for oestrogens (Lamming, 1966; Leathem, 1966). These results agree with those found during hypophysectomy and gonadectomy (Loraine & Bell, 1971).

The "pseudohypophysectomy" theory was based upon the negative feedback mechanism to control hormonal output. However, recent findings have shown that the theory is inadequate. Donaldson et al.(1970) noted an initial significant increase ($P<0.05$) in plasma progesterone during the first cycle, and a decline in subsequent cycles. Gombe (1972) while feeding rats to a zinc deficient diet observed an initial increase in plasma progesterone ($P<0.100$) followed by a decrease ($P<0.050$ to $P>0.010$).

LH values during the early times of study were higher than those of control values. Cumming, Mole, Obst, Blockey, Winfield & Goding (1971) found similar results for progesterone and gonadotrophins when ewes were fed submaintenance diet. Further findings in male hyrax (Miller & Fairall, 1976), cattle, sheep and goats (Erb, Garverick, Patton, Randel, Monk, Udo-Aka & Callahan, 1976; Beal, Short, Staigmiller, Bellows, Kaltenbach . and Dunn, 1978) support these observations. The fact that malnutrition effects on reproductive functions lack specificity, and the inadequacy of the "pseudohypophysectomy" theory to explain the decrease and increase in progesterone when LH is increased, led Srebnik (1970) and Gombe (1972) suggest "stress" causing a general effect on the hypothalmus as the main route of action.

2:5 The Effect of malnutrition on body weights

Lamming (1966), Lamond (1970) reviewed that there are genetically controlled ranges of body weights for each animal breed and species, on which to expect efficient reproduction. For example, breeding cows can lose 10 to 15% (Lamond, 1970), rats 20 to 30 % of their body weights (Leathem, 1966; Howland, 1971 & 1975) without adverse effects on reproduction.

Leathem (1966), Downie and Gelman (1976) further stress that there is a correlation between body weights, plasma glucose and reproduction; a decrease results into infertility. Hill et al., (1970) suggested that compensatory growth, age, and other factors are, however, important when analysing effects of body weights on reproductive functions.

2:6 Malnutrition and the adrenal gland

Both malnutrition and hunger lead to variable degrees of stress. Early studies of adrenocortical function in malnutrition generally agree that levels of adrenocorticotrophic hormone (ACTH) and adrenocorticosteroids are either normal or elevated (Srebnik & Nelson, 1962; Tomkins & Maxwell, 1963; Leathem, 1966; Platt and Stewart, 1967; Stott and Thomas, 1971; Pimstone 1976). Associated with this is an initial hypertrophy and hyperplasia of the adrenal cortex (Platt & Stewart, 1967), and later atrophy (Chatterji and Sen Gupta, 1960). The increased production of corticosteroids from a compensatory hypertrophied adrenal cortex helps in alleviating the nutritional stress by inducing an efficient but temporary mobilization of the defficient feed (Leathem, 1961; Tomkins and Maxwell, 1963). On the other hand stress increases animal nutrient requirements, and also alters other endocrine systems (Marston, 1952), leading to reproductive inefficiency (Van Rensburg, 1965 and 1971).

Evidence shows that the adrenal gland functions are necessary for efficient reproductive functions. In female rats (Cupps, 1955), rabbits, man and domestic animals (Lamming, 1966; Leathem, 1966; Moustgard, 1969) adrenalectomy

Leads to infertility. When such adrenalectomized animals are on an adequate diet, however, normal reproduction can be restored by administration of corticosteroids (Fisher & Leathem, 1965). But when such animals are on a malnutrition diet administration of both sex hormones and corticosteroid are necessary for restoring reproductive functions.

Further evidence from studies on stress and administration of ACTH leading to high corticosteroid concentrations shows that reproductive functions are first improved (Stott & Thomas, 1971) and later inhibited with increase in the steroids.

Thus VanRensburg (1965 & 1971) Moustgard (1969) stated that a total lack or decrease in corticosteroids inhibits reproductive functions, whereas normal and physiological high concentrations stimulate reproductive functions and large concentrations have inhibitory effects. All the above cited adrenal functions are from the direct effects of the adrenal gland (Leathem, 1966) and are due to the influence via the hypothalamus and pituitary (VanRensburg, 1971).

2:7. The pituitary gland in malnutrition

Pituitary gonadotrophins are either increased, decreased or normal in malnutrition (Leathem, 1966; Lamond, 1970; Pimstone, 1976). In malnourished animals, cytology of the anterior pituitary indicates that there is atrophy and degranulation of acidophilic cells as Pimstone (1976) reported in rats, Heard & Sterwart (1971) in dogs and pigs, Lamming (1966) Leathem, (1966), and Jubb & Kennedy (1970) in cattle, sheep and goats. Heard & Stewart (1971) explained the atrophy to be due to loss in cytoplasm causing a decrease in pituitary cell size, crowding of the nuclei, and in more severe cases cell necrosis may be observed.

Work in the guinea pig (Heard & Stewart, 1971) showed that the basophils respond to malnutrition by compensatory increase in size, but later decrease and degenerate. Similarly Srebnik & Nelson (1962) had the same explanation in rat pituitary from protein-free diet. Depending on the degree of deficiency pituitary functions may not be impaired, for Gupta & Anand (1973) did not find castration cells in the pituitary gland of protein-deprived rats, and that reversal feeding led to recovery.

2:8. The Ovary in Malnutrition

Malnutrition leads to atrophy, and degeneration of the ovary, uterus, and mammary gland. The ovarian atrophy is due to follicular atresia (Hill et al., 1970), and decrease in corpora lutea size (Roberts, 1971). Conversely a high plane of nutrition increases numbers and sizes of follicles (Elsely and Macpherson, 1970; Hafez, 1974), and therefore increase in fecundity.

Recent studies show that inanition prevents follicular development to the antral stage (Gupta & Anand, 1973), whilst stimulating interstitial follicular hypertrophy (Hill et al., 1970). Furthermore inanition leads to reduced responsiveness of the ovary to gonadotrophins (Lamond, 1970; Gupta & Anand, 1973). On the other hand the lack of either prolactin, FSH or LH has been suggested to explain such malfunctions of the ovary (Leathem, 1961). The work of Gombe and Hansel (1973) and that of Beal at al., (1978), however, show that ovarian dysfunctions occur despite high plasma LH values.

2:9. The thyroid gland and other Endocrine organs

Platt & McCance (1960) first reported a decrease of growth hormone (GH) in malnourished patients; a similar picture was observed during hypophysectomy in rats, and was corrected by administration of GH (Srebnik & Nelson, 1962). In starved rats, deficiency of GH- releasing factor has been held responsible for a decrease in the GH as hypothalamic extracts from these animals added to normal pituitary incubates released only one fifth of the amount of GH as the amount that was released with the control extracts. (Meites & Fiel, 1965). Conversely in children with kwashiorkor and/or kwashiorkor with marasmus it is now generally agreed that plasma GH is elevated (Pimstone, Barbezat, Hansen & Murray, 1967). This is contrary to what is expected from the "pseudohypophysectomy" theory.

Studies on the role of the thyroid gland in malnutrition show that the gland initially increases in size of follicles and epithelia, and later decreases (Srebnik & Nelson, 1962; Platt & Stewart, 1967; Heard & Stewart 1971). Regression in thyroid morphology with a decrease in thyroid weight parallels a decrease in body weight (Leatham, 1961).

In severe protein depleted rats Srebnik, Evans & Rosenberg (1963), and Cowan & Margossian (1966) reported that there was reduced thyroid growth: this reduction was accompanied by suppressed thyroid to serum radioiodide concentration. The reduced thyroidal activity suggested therefore, a reduced concentration of Thyroid Stimulating Hormone (TSH) in these animals (Leathem, 1961).

Srebnik et al. (1963), however, found that the decreased thyroid function was not consistent with change in thyroid morphology and therefore suggested that it was not due to TSH function of the pituitary. Infact they found no change in TSH levels in pituitary glands of adult female rats fed a protein free diet. This was supported by the findings that injection of either 5 ug L- Thyroxine or TSH to protein deprived rats did not restore the metabolic rates to normal levels (Srebnik et al., 1963; Cowan & Margossian, 1966). Harland & Parkin (1972), however, reported that plasma levels of immunoassayable TSH were low in chronic malnourished Ugandan children and recovery was achieved by reversal feeding. In their reviews Lamming (1966), Leathem (1966) and Pimstone (1976) further reported low and normal plasma TSH, and high thyroid releasing hormone in malnutrition.

Most workers generally agree that Protein Bound Iodine (PBI) increases in malnutrition (Florsheim, Suhr, Mirise & Williams, 1970; Godard, Lemarchand-Beraud, 1973; Pimstone, 1976), the increase being possibly due to an accumulation of iodinated serum albumin (Florsheim et al,,1970). A few studies in protein deprived rats show that T_4 and T_3 values are normal (Srebnik, Evans & Rosenberg, 1963; Lamming, 1966; Lamond, 1970; Graham, Baerti, Claeyssen, Suskind, Greenberg, Thompson & Blizzard, 1973) possibly due to low thyroxine binding globulin and pre-albumin (Pimstone, 1976).

There have also been reports of low T_4 and T_3 values in malnutrition (Godard & Lemarchand - Beraud, 1973). Similar to findings on progesterone and LH during malnutrition (Donaldson et al., 1970) T_4 values have been reported to be elevated when TSH values are also high (Graham et al.,1973).

Several observations indicate that total lack of thyroid hormones cause reproductive ineffeciency (Thorsöe, 1964). Normal and physiological high thyroid hormones stimulate reproductive functions and large concentrations have inhibiting effect.

In thyroidectomized animals there is a reduction in the excretion of gonadotrophins (Balze, Arrillaga, Mancini, Janches, Davidson & Gurtman, 1962), and sex hormones (Leathem, 1966).

Conversely in rams Griffin, Henneman & Reineke (1962) found that semen quality was improved with increase in thyroid hormones.

Some investigators hold the view that the thyroid effects are due to a generalized change in metabolic activities that accompany thyroid gland alterations (Underwood, 1971), while others think that specific endocrine metabolisms i.e. that of oestrogens are affected (Fishman, Hellman, Zumoff, Gallagher, 1962 & 1965; Fukuda, Greer, Roberts, Allen, Critchlow & Wilson, 1975).

2:10. The hypothalamus in Malnutrition

Observations that Growth hormone-releasing factor and gonadotrophin - releasing factors are either decreased or increased during malnutrion suggest the hypothalamus as the main control centre. Negro-Vilar, Dickerman and Meites, (1971), found a decrease in FSH-releasing factor in protein deficient rats while Gombe (1972) found an increase in LH-releasing factor in rats deprived of zinc, and Meites and Fiel (1965) found an increase in GH-releasing factor in rats starved for 5 to 7 days.

The role of neural factors in malnutrition-induced endocrine changes was shown by Piacsek and Meites (1967), who found that rats placed on 50% of normal food intake ceased to cycle but when placed under constant illumination resumed cyclic activity inspite of the feed restriction. Similar findings have been observed by Cooper and Haynes (1967) that abnormal cycles in underfed rats became normal in presence of the male. Furthermore, oestrous cycles in underfed rats were modified by altering the time of feeding (Cooper & Haynes, 1969), and other external factors such as light (Ganong, 1977).

2:11. The Role of Stress in Malnutrition

The foregoing results show that malnutrition is a form of stress. Various forms of stress have been shown to give the same endocrine profile as of malnutrition. Surgical stress to rats on oestrous Day 5 led to an increase in oestrogen, progesterone and LH (Neguin, Alvarez, Campbell, 1975). Cold stress to rats have also been observed to increase thyroid stimulating hormone (Ranta, Mannisto & Tuomisto, 1977) and ACTH (Pimstone, 1976). Exposure of cattle to cold temperatures tended to increase circulatory GH levels (Olsen & Trenkle, 1973). Heat stress of 48°C and relative humidity of 30% for 10 to 11 hours per day for the first 11 days of the oestrous cycle leads to increase in plasma prolactin but not LH (Hooley, Stephenson & Findlay, 1977).

All these stress effects are mediated through the brain and the hypothalamus in particular by neurotransmitters (Ahran, Fuxe,, Hamberger, and Hokfelt, 1971; Ajika, Kalra, Fawcett, Krulich & McCann, 1972).

Brain catecholamines and indoleamines have been shown to be neurotransmitters involved in the control of gonadotrophins and prolactin secretions (Kamberi & McCann, 1969; Ajika et al., 1972). They evoke the gonadotrophin discharge by stimulating the release of either releasing or inhibiting factors (Neguin et al., 1975). It is the release of neurotransmitters and their control of trophic hormones during "stress" that led Gombe (1972) to suggest that "stress" was responsible for malnutritional effects.

3. MATERIALS AND METHODS

3:1. Experimental Animals. Twenty five male East African shorthorned goats purchased from several individual farmers of Machakos were used in this study. The female goats weighed between 16 and 28 kilograms, and were one to two years old. They were checked for pregnancy, and were observed through three oestrous cycles to ensure that they were cycling normally before being used in the study. The five bucks (after penis deviation) were used to detect oestrus.

Goats, though rarely affected by psychological, social and aggressive behaviours, lack the ability of adapting fast to experimental procedures (Davendra & Marca Burns, 1970; Pearson & Mellor, 1976). In this work therefore, does were bled a few drops daily during adaptation in order to accustom them to experimental procedures. During the first week, and regularly thereafter animals were drenched against parasites with 10 to 20 ml Thibendazole (Pfizer Lab), and 300 mg Amprol (Merck Sharp & Dohme), they were also injected with Berenil(Hoechst, Germany) at dosage rate of 7mg/kg intramascularly.

The rooms were daily cleaned and dressed with cobalt deficient rhode grass hay. Faecal and blood samples were regularly examined for evidence of parasitism.

3:2 Experimental Layout

All goats were housed in groups of five in large sheltered rooms during the adaptation period of two and half months. The rooms contained feeding troughs. The male goats were housed in a similar room adjacent to the female goats. During this period all animals were given 0.5 mg Vit. B_{12} as catosal (Bayer, Germany) at weekly intervals.

Dried rhode grass hay from cobalt deficient areas of Nakuru formed 80% of the diet. All the animals were given 1.5 kg per goat per day of Ration A (See Table 1) and a small quantity of lucerne hay when available. This ration prepared according to Morrison standards (Morrison, 1961), exceeded maintenance requirements for digestible protein (DP) and total digestible nutrients (TDN) and minerals except for cobalt.

Since adequate cobalt concentration is above 0.07 parts per million on dry matter basis, the cobalt concentration of 0.01 parts per million on dry matter basis fed to the experimental animals was regarded as inadequate (Underwood, 1971). Abundant supplies of clean, fresh drinking water and salt bricks (Pfizer Lab., Nairobi) were available to the animals in adjoining paved yard. The salt bricks were removed from the diet at the end of the adaptation period.

Each group of 5 animals was allowed out into the yard at least five times a day (8 a.m., 10 a.m., 12 a.m., 3 p.m., and 5 p.m.) for exercise, heat detection, bleeding, and to enable them to drink. During this time teasers were observed constantly while with the does, and were removed after about fifteen minutes. Does were regarded in oestrus only when mounted by more than one teaser as suggested by Shelton (1960). The first 20 does to show regular cycles with intervals of 18 to 23 days during this adaptation period were selected and randomly assigned to new groups for different treatments as explained below:

Group 1. Control A

Does numbers 82, 83, 91, 94 and 99 were allocated to this group. These were fed the same diet as during adaptation. In addition they were given by mouth one gram of cobalt in a form of cobalt oxide bullets. Since regurgitation of bullets is common (Connolly and Poole, 1967; Somers & Gawthorne, 1969) additional bullets were given every three weeks.

Group 2. Control B - Cobalt deficient

Does numbers 86, 87, 88, 89 and 90 were allocated to this study group. These were on ration A throughout the study.

Text Table 1

DAILY FEED INTAKE (per animal per day)

RATION A - CONTROL DIET

Item	DM %	DM (gm)	CP %	CP (gm)	DP %	DP (gm)	CF %	CF (gm)	TDN %	TDN (gm)
Rhode grass hay	80	1200	5.0	75	1.8	27	29.4	441	44.4	666
Wheat bran	7	105	1.1	16.5	0.9	13.5	0.7	10.5	4.2	63
Maize grain	12	180	1.1	16.5	0.7	10.5	0.3	4.5	9.7	145.5
Beans Seed	1	15	0.4	6.	0.2	3	0.1	1.5	0.7	10.5
Total	100	1500	7.6	114	3.6	54	30.5	457.50	59	885
Requirements Morrison		1500		99		54		450		820
RATION B - ENERGY RICH DIET(a)										
Rhode grass Hay	70	1050.	4.3	64.5	1.6	24.0	25.70	385.5	38.9	583.5
Wheat bran	3	45	0.5	7.5	0.4	6.0	0.3	4.5	1.8	27.0
Maize grain	25.5	383	2.3	30.0	1.6	24.0	0.6	9.0	20.7	310.5
Beans seed	1.5	23	0.6	9.0	0.3	4.5	0.1	1.5	1.0	15.0
Total	100	1501	7.7	111	3.9	58.5	26.7	400.5	62.4	936.

(a) The protein was kept constant, the energy increased by about 8%

RATION C - PROTEIN RICH DIET[a]

Item	DM		CP		DP		CF		TDN	
	%	(gm)	%	(gm)	%	(gm)	%	(gm)	%	(gm)
Rhode grass hay	70	1050	4.3	64.5	1.6	24	25.7	385.5	38.9	583.5
Wheat bran	5	75	0.8	12	0.6	9	0.5	7.5	3	45
Maize grain	6	90	0.5	7.5	0.4	6	0.1	1.5	4.9	73.5
Bean seed	19	285	7.1	106.5	3.1	46.5	0.7	10.5	13.1	196.5
Total	100	1500	12.7	190.7	5.7	85.5	27.	405	59.9	898.5

(a) Protein was increased by about 69 % while energy was kept constant.

Composition of the FEEDS on dry matter basis.

Rhode grass hay	100.00	6.19	2.30	36.70	55.50
Wheat bran	90.10	16.20	12.20	10.20	60.00
Maize grain	88.60	8.90	6.20	2.20	81.00
Bean seed	89.00	37.50	16.50	3.70	69.00

N.B. 1. All analysis for feedstuffs were according to the methods of analysis of analytical chemists (AOAC, 1970)

2.* The DP and TDN were estimated as according to formulae given by Church and Pond (1976).

3. Cobalt content was less than 0.01 parts per million on dry matter basis determined as according to AOAC (1970).

4. The feeds exceeded maintenance levels for CP, DP, TDN and minerals except for cobalt.

5. Key. DM = Dry matter
CP = Crude protein
*DP = Digestible Protein
CF = Crude fibre
*TDN = Total Digestible Nutrients.

* Estimated.

Group 3 and 4: Energy and Protein groups, respectively.

Numbers 80, 84, 85, 92 and 93 were in group 3 and numbers 95, 96, 97, 98 and 100 were in group 4. These received ration A for an initial period of 15 weeks and thereafter group 3 animals were fed 1.5 kg of a supplement ration B per day per animal, and group 4 animals received 1.5 kg per day per animal of ration C for another 8 weeks.

3:3 Blood sampling

The animals were already accustomed to experimental procedures before the beginning of the experiment. Blood samples were drawn between 9 and 10 a.m. every two days during the first month, and every three days thereafter except during oestrus when bleeding was at 3 hourly intervals.

About 6 ml. blood was collected from the external jugular vein into a universal bottle (25 ml) containing 2 mg EDTA as an anti-coagulant. Blood samples were then kept in an icebath until centrifuged at 1500 g for about 15 to 20 minutes, and plasma aspirated under sterile conditions and kept frozen at $-20^{o}C$.

3:4 Haematology and Body weights

Every two weeks each animal was weighed and 2 ml. blood was collected into heparinized receptacles and immediately analysed for the Haematocrit (Packed Cell Volume %), Haemoglobin (mg%), Red Blood Cells ($10^{6}/mm^{3}$) by the "coulter" counter haemoglobinometer. (Coulter Electronic Inc. Hialeah, Florida USA)

A differential leukocyte count was separately determined. Then the Mean Corpuscular Volume (MCV), and the Mean Corpuscular Haemoglobin Concentration % (MCHC) were calculated. From these data the type and degree of anaemia was characterized. Total plasma proteins were determined by the biuret method (Varley, 1969; Benjamin, 1974).

3:5. Histology

At termination of the experiment after 23 weeks, the pituitary gland, thyroid gland, a portion of the mammary gland, the adrenal gland and ovaries were removed within 15 minutes of death, trimmed, rolled on paper towel, and weighed as soon as possible. The pituitary gland was split mid sagitally and one half portion was frozen for hormone assay. All samples were dehydrated, impregnated with wax and sectioned at 7 µm on a sliding microtome. Erhlich's Haematoxylin and Eosin, Orange G, Periodic Acid Schiff, Haematoxylin - Azan and Goldner were used for staining, and samples were examined under a light microscope. Measurements for the different parts of the adrenal and thyroid glands were done by a binocular micrometer (Leitz Wetzlar Germany).

3:6. RADIOASSAYS

3:6:1. Solvents and Reagents

All reagents used were of analytical grade, and were obtained locally except the ones listed below:

(a) Dextran T-70 (mol. wt. 20,000) and Norit-A Charcoal. Sigma, Sweden.

(b) (i) Crystalline steroid for standards progesterone and oestradiol 17β.

(ii) Antiserum progesterone S ≠257
Antiserum oestradiol S ≠ 52
Guy-E, Abrahams, California, U.S.A.

The oestradiol 17β monohemisuccinated-HSA antiserum cross-reacts with oestradiol 17α (40%) oestrone (35%) and oestriol (8%).

(c) (i) 1α, 2α(n)$^3H_2$) progesterone (49ci/ mmol). Batch 14 TRK 341.

(ii) (6, 7, 3H) Oestradiol (58 ci/mmol) Batch 43 TRK 125

(iii) (1α, 2α(n)^{3}H) Corticosterone, Radiochemical centre, Amersham, England.

These were diluted to give a working solution of about 12,000 cpm per 100 ul, which was stored at 4^{0}C.

(d) Thyopac 4 for Thyroxine (T_4) Radioassay, (Batch D 92513 of 40 Microcurie). The kit comprising of:

(i) Freeze dried human serum as standards at 1.4 ug and 15.3 ug/100ml.

(ii) Extraction tubes

(iii) Assay tubes with buffer, absorbent granules and Binding protein.

(iv) Charcoal.

Radiochemical Centre, Amersham, England.

(e) Thyropac 3 - Triiodothyronine (T_3) Radioassay kit. (Batch 14566 of 50 Microcurie) Comprising:-

(i) Assay tubes with absorbent granules.

(ii) Standard at 10 units.

(iii) Charcoal.

Radiochemical Centre, Amersham.

(f) Cyanocobalamin (57Co) Radioassay kit. Catalog No. NEA 065.

New England Nuclear, Massachussets, U.S.A.

Comprised of:-

(i) Standards range 0, 50, 100, 200, 400, 800, 1600 pg/ml.

(ii) 0.1M Acetate buffer (PH 3.6)

(iii) Potassium cyanide

(iv) Vit. B_{12} Binder: Intrinsic factor.

(v) B_{12} (57Co) in acetate buffer (PH 4.8).

(g) Iodine 125 for LH iodination.

Batch No. D 27811.

Radiochemical Centre, Amersham.

3:7. VITAMIN B_{12} RADIOASSAY

In a review of cobalt and Vit. B_{12} metabolism in ruminants, Smith & Loosli (1957) and Andrews & Stephenson (1966), Somers & Gawthorne (1969) emphasized the potential value of plasma Vit. B_{12} levels as a specific aid in the rapid diagnosis of cobalt deficiency. The purpose of this assay therefore, was to provide such an information.

Vit. B_{12} was measured using Radioisotopic competitive inhibition method by a Vit. B_{12} (57 Co) Radioassay kit. The initial step in the assay was the inactivation of Vit. B_{12} serum protein binders by boiling (for about 15 minutes at 95°C) 100 ul of sample plasma in 1000 µl of 0.1M acetate buffer and containing 0.002% potassium cyanide (Glass tube, Pyrex 12 x 100mm). The cyanide was for the conversion and liberation of protein-bound B_{12} to free cyanocobalamin.

After cooling to room temperature for 30 minutes, 100 ul of B_{12} (57Co) trace solution in 0.1M acetate buffer (PH4.8) providing about 10,000 cpm,and 100ul of B_{12} Binder were added. The mixture was then incubated at room temperature for about 30minutes. The free B_{12} was separated from the bound ligand by adding 500ul of charcoal suspension, whirlmixing, incubating for additional 10 minutes, and centrifuging at 1400xg for 15 minutes.

The supernatant was decanted into new tubes and counted for 100 seconds in a mini-Gamma Counter (Mini Instruments, London, Type 6.20).

Finally the B_{12} concentration was determined from a standard curve made from standards assayed together with the samples.

All the samples were determined in triplicates. One tube was labelled "extract control" and served as a check upon completeness of extraction as suggested by Frenkel, McCall and White (1970), and no standard Vit. B_{12} binder was added subsequently. All the samples extracted had none specific B_{12} binding of less than 1%.

3:8. CORTICOSTEROIDS DETERMINATION

The competitive protein binding assay as described by Murphy (1967), Bassett and Hinks (1969) was adapted and used for the determination of corticosteroid concentration in peripheral plasma. Thus the determination was basically employing the same procedures described elsewhere for gonadal steroids.

3:8:1. Extraction. The extraction procedure on 1 ml.plasma involved the removal of progesterone and more polar steroids with 10 ml diethylether first, followed by the extraction of corticosteroids for 20 minutes with 10 ml ethanol. The latter was evaporated to dryness in vacuo and reconstituted in 1 ml ethanol.

3:8:2. Assay

Only one assay was done for all the samples in triplicates: 300 ul of ethanol sample aliquots were dried in vacuo, and 500 ul phosphate buffer (PH 7.0) was added. Cortisol Binding Globulin (CBG) was obtained from third trimester serum. 1ml. of CBG at 1:3000 dilution and 100 ul of $3H^+$ Corticosterone (12000cpm) were then added. This was followed by a two-hour incubation at 37^oC (waterbath) and a 30 min. cooling in ice-bath. The free ligand were seperated by adding 500ul of Dextran coated charcoal (250mg charcoal 25mg Dextran T-70 in 100ml phosphate buffer PH 7.0), and centrifuging. The supernatant was tipped off into vials containing 10 ml scintillation fluid (1000 ml Toluene/4mg PPO). Counting was done in a Packard 3320 liquid scintillation counter for one minute after overnight equilibration of the vials at room temperature.

Standards prepared in phosphate buffer (PH 7.0) and in the range of 0 to 1000 pg were used. 500 ul of standard and 500 ul buffer were likewise treated as for the samples, and the results obtained plotted on a logit - transform paper (Codex Book Co., Mass). to give the standard line.

Procedural losses were determined as explained later for oestrogens. Percentage recovery of this procedure was at 85 ± 0.50%. This method determines both cortisol and corticosterone concentration; therefore in this study all values have been given as corticosteroid concentration per ml. plasma. Coefficient of variation (intrassay) was determined by analyses of pooled plasma samples. The intrassay variation was below 6% (for 10 determinations).

3:9. THYROID HORMONES DETERMINATION

3:9:1. THYROXINE (T_4) RADIOASSAY

Thyopac 4 Radioassay kit utilising the competitive protein binding assay of Ekins (1960) as modified by Murphy & Pattee (1964) was used to determine Thyroxine (T_4) values.

Duplicates of 0.5ml plasma were extracted with 1.0ml ethanol for about 2 minutes and centrifuged at 2000g for about 5 minutes. Aliquots (0.5ml) of the supernatant were transferred to an appropriate "Thyopac 4" vial containing 125 Iodine labelled L-thyroxine, binding globulin and absorbent granules. The contents in each vial were mixed for about 30 minutes, then allowed to settle for about 2 minutes. Thereafter, 1.0ml supernatant liquid was transferred to a counting tube, and was counted in a Mini Gamma counter for 100 seconds. Likewise, two reference standards containing 1.4 ug per 100ml and 15.3 ug per 100 ml (T_4) were reconstituted in 1.0ml. distilled water and assayed.

T_4 of unknown sera were directly calculated by the following procedure:

(a)
$$S = \frac{\left(\frac{\text{Counts of low std}}{\text{counts of high std}}\right) - 1}{\text{value of high std} - \text{Value of low std.}}$$

(b)
$$\text{Unknown sera} = \text{low std. value} + \frac{\left(\frac{\text{Counts of low std}}{\text{counts of unknown}}\right) - 1}{S}$$

Values given in ug T_4/100ml.

In order to recheck the calculation, a calibration graph was also used.

3:9:2. Thyopac 3 kit was used to assay Triiodothyronine (T_3) absorbent granules uptake. All operations were conducted at room temperature. Aliquots of 0.1ml sample were added to respective test vials containing buffer, Liothyronine-I_{125} (T_3-125) and solid absorbent granules, mixed for about 15 minutes and allowed to settle for 3 minutes. 1.0ml supernatant was aspirated into a counting tube and counted on a mini Gamma counter. for 100 seconds. Likewise the two reconstituted standard human serum were analysed. The thyopac 3 value for each test plasma was calculated by the following procedure:-

$$\text{Thyopac 3 value} = \left(\frac{\text{Counts of unknown}}{\text{counts of std}}\right) \times \text{Thyopac 3 value of std.}$$

3:9:3. FREE THYROXINE (THYOPAC) INDEX

The values from Thyopac 4 and 3 were used to calculate the Free Thyroxine Index (FTI) by the following formula (Clark & Brown, 1971): $\frac{\text{Thyopac 4 value}}{\text{Thyopac 3 values}} \times 100 = \text{FTI}$

Also Thyopac 4 values (y axis) were mapped against Thyopac 3 values (x axis).

3:10. PROGESTERONE AND OESTROGENS RADIO-IMMUNOASSAYS (RIA)

3:10:1. Extraction

An aliquot of thawed plasma (1.0 ml) was extracted thrice with 5 mls of diethyl-ether and the pooled ethereal extracts evaporated to dryness in vacuo. The steroid residue was reconstituted in 2 ml. ethanol, and stored at -20°C until assayed for either progesterone or oestrogens.

3:10:2. Assay

The RIA previously described by Gombe & Kayanja (1974) for progesterone, and Gombe (1977) for oestrogens were used to quantitate total unconjugated oestrogens and progesterone. In brief, triplicates of 100 µl aliquots of the respective extracts were transferred to glass tubes and evaporated to dryness in vacuo (in case of progesterone 500 ul phosphate buffer was then added).

100 ul of antiserum (dilution for progesterone 1: 1000; for oestrogens 1:3000) was added to respective tubes, followed by whirl-mixing and incubation for a minimum of twenty minutes. 100 ul of respective hot steroid (giving 12000 cpm) was added, whirl mixed, and incubated over night at 4°C. Free steroids were separated by dextran coated charcoal (charcoal 250 mg Dextran T-70 25 mg in 100 ml 0.1M phosphate buffer for oestrogens, charcoal 625 mg Dextran T-70 62.5 mg in 100 ml 0.1M phosphate buffer for progesterone). After centrifugation at 1770 g for 1.0 min., the supernatant were tipped into counting vials containing scintillation fluid (10 ml of Toluene 1000 ml/4mg PPO). The vials were then shaked and equilibrated overnight at room temperature before counting. The samples were twice counted at 1.0 min. in the 3320 Packard liquid scintillation counter.

The results obtained from standards assayed together with the samples were plotted on a logit - transformed paper (Codex Book Co., Mass). The unknown concentration was read off the standard curve and multiplied by the appropriate dilution and recovery factor to give the original value.

Because oestrogen antiserum cross reacts with oestradiol17β(40%), Oestrone (35%) and Oestriol (8%) the results have been expressed as total ether-extractable (unconjugated) oestrogens per ml. of plasma.

Three pooled plasma samples were assayed in all the assays for the determination of interassay and intraassay coefficient of variations. Procedural losses were monitored by the addition of respective hot steroid (50,000 cpm) to 1 ml plasma before extraction. The overall recovery of the hot steroid was estimated by using 50 ul, 100ul and 300ul of the final extract.

The percentage recovery for progesterone procedures were at 78 ± 0.5% with coefficient of variation (inter - and intraassay) of 8.8% (n = 25). For oestrogens percentage recovery were at 82 ± 0.60% with coefficient of variations of 9.6% (n = 20).

3:11. LUTEINIZING HORMONE RADIOIMMUNOASSAY

Purified ovine LH-NIH-LH was available in the Department of Animal Physiology and had been supplied from the endocrine study section of the National Institute of Health. NIH-LH-ovine was used as standard. The anti-bovine LH antiserum which was used was prepared in the donkey and had been supplied by Dr. R.B. Heap, of Babraham, Cambridge. Before use the antiserum curve showed that 50% binding was at a dilution of 1:1600.

3:11:1. LH Radioiodination

Carrier-free Iodine 125 dissolved in Sodium hydroxide was used for the radioiodination. Purified LH was iodinated by a modification of the method of Greenwood, Hunter & Glover (1963). A disposable plastic tube with 5 ug LH in 10 µl buffer was thawed. 10 ul of 0.1M phosphate buffer (PH 7.4) containing 0.15 M sodium Chloride and 0.02% Merthiolate was added. Using a Hamilton Syringe 10ul of 1 mCi of Iodine 125 was added, followed immediately by 50 ug of chloramine T. The contents were mixed gently for 35 seconds. Iodination was stopped by adding 120 ug of sodium metabisulphite.

Purification of the iodinated mixture was accomplished by gel filtration using 10 cm sephadex G-25 column that had been equilibrated over night with phosphate buffer PH 7.4 - 0.1% Bovine Serum Albumin (PBS - 0.1% BSA). Half ml. fractions were eluted using 0.1M phosphate buffer. Prior to storage at $4^{o}C$, the labelled LH was diluted with 0.1M phosphate buffer so that 100 ul of a working solution would give about 35,000 cpm.

When labelled LH was more than 5 days old, a new iodination was done. Thus three iodinations were done in total.

3:11:2. RIA of LH

The procedure was based on the solid phase RIA modified by Hobson and Hansel (1972), and adpated for use in the Department of Animal Physiology. New polystyrene tubes. (20 x 100mm) were used in the assay without additional washing. One millilitre aliquots of the antiserum (1:1600) were dispersed into the tubes and incubated at 4°C for a minimum of 18 hours. Following this the tubes were twice washed with 1.5 ml aliquots of 0.9% saline, and lastly with 1.2 ml of PBS-0.1%BSA. (In few instances, 1.0ml aliquots of PBS-0.1%BSA was added in tubes and kept frozen at -20°C until used for the assay). The reagents were then added to empty coated tubes in the following order.

(A) Samples

(i) 800 ul of PBS - 0.1% BSA. Triplicates, incubated at room temperature for 30 minutes.

(ii) 200 ul of plasma (unknown). Whirl mixed and incubated for 6 to 8 hours in a moist cupboard.

(iii) 100 ul of LH_I-125(35000cpm) whirl mixed, and continued with incubation for an extra 18 hours.

(iv) Tip off and washing with tap water twice, drying with paper towels and counting on a Gamma Mini counter for 100 seconds.

(B) Standards

(i) 1.0ml of standards (range 0 to 25ng) incubated for 6 to 8 hours in moist cupboard.

(ii) The rest of the procedure as for the sample. The within and between assay coefficient of variations were less than 7% (n=12).

3:12 EXTRACTION OF THE PITUITARY FOR LH

LH extraction from the pituitary gland was according to the procedures of Ellis (1961) as modified by Reichert & Midgley (1968). The glands were thoroughly homogenised with 2.0ml distilled water at PH 5.5; then subjected to two 6 hours extraction. After centrifugation (2950g), the residue was dissolved in 6ml of 0.1M Ammonium sulphate and extracted first for 4 hours and then for 2 hours at PH 4.0. After centrifugation, corresponding supernates were combined, assayed for LH and measured for the total protein extracted. Protein concentrations were determined spectrometrically on an ultra-violet beckman spectrometer at 280nm. and also by the micro-Kjeldahl Nesslerization method as outlined by the official methods of Analysis of the Association of Analytical Chemists (AOAC, 1970).

3:13. Statistical analyses

All the data were analysed by the method of one way analysis of variance of a completely randomized design. Analyses of plasma progesterone and LH data were on the basis of (a) values during Day 0 of oestrous (b) values from various stages of the cycle (c) combined oestrus and cycle values throughout the cycle (d) on mean values throughout the cycle. Plasma oestrogen values were analysed on the basis of values from Day- 1 to Day $\pm$ 1 of cycle and on basis of values on various stages of the cycle. The t-test, Duncan's multiple range test and least significant difference tests were performed on all sets of values showing significances on analysis of variance as according to Snedecor & Cochran (1975).

Correlation (r) between body weights, Vit. B_{12} values and haematology values were calculated. Linear regression with equation $y=a + bx$ was also used to find the rate of decrease of data values.

Haematological parameter values and body weight values were changed into percent values before being analysed statistically.

4:0. RESULTS

4:1. CHARACTERIZATION OF THE VITAMIN B_{12} DEFICIENT ANIMAL.

4:1:1. Body weights.

Results are recorded on Text Table 2, 3A and 3B, and Appendix Table 1. The body weight values were changed into percentages so all the goats had the same percent body weights at the start of the experiment, and for the first 2 months. Thereafter animals on cobalt deficient diets showed a significant progressive decrease in body weights ($P < 0.001$). At the end of the study all deficient animals whether supplemented with energy or protein, had significantly ($P < 0.001$) lower body weights than those of control animals. Figures A & B show that the rate of loss in weights was at 1.71 kgs per month in group 2, 1.07 kgs per month in group 3, and 1.14 kgs per month in group 4. On the other hand control animals gained at average 0.19 kgs per month per animal during the same time of study.

Animals in group 2 with initial high body weights lost weights much faster at 7.02% per month per animal than for group 3 and 4 which were at 5.30% and 5.39% per month per animal, respectively.

TEXT TABLE 2 MEAN BODY WEIGHTS OF COBALT DEFICIENT GOATS (kgs) (a)

Group Number (b)		July 1977	Aug.	Sept.	Oct.	Nov.	Dec.	Jan. 1978
1(Control)	Mean	18.80	18.20	18.20	18.00	19.10	19.80	19.40
	SEM	±0.78	±0.58	±0.73	±0.55	±0.84	±1.53	±1.25
2 (Deficient)	Mean	24.60	22.20	19.40	17.00	16.75	15.50	14.00
	SEM	±1.21	±1.50	±1.40	±1.22	±0.75	±0.65	±0.41
3 (Energy)	Mean	20.00	18.60	16.40	14.60	14.40	14.20	13.60
	SEM	±1.38	±1.44	±0.68	±0.68	±0.51	±0.58	±0.51
4 (Protein)	Mean	21.00	20.20	19.20	17.00	15.40	15.40	14.80
	SEM	±1.26	±0.97	±1.20	±0.77	±0.68	±0.68	±1.52

(a) All values given as MEAN ± S.E.M. kgs.

(b) Duration of deficiency in months.

TEXT TABLE 3A

(i) Analysis of variance of the effect of feeding cobalt deficient diets on percent body weights of goats.

Source	df.	TSS.	MS	F	P
Total	116	25398.09			
Treatment	3	13910.34	4636.78		
Error	113	11487.75	101.66	45.61**	0.001

Comparison among Means

Group	1	2	3	4
Mean % Weight	100.34	71.69	76.28	81.19

LSD $P < 0.001$ = 12.81

LSD $P < 0.05$ = 7.52

Percent body weights are significantly different in group 2, 3 and 4 compared to control group 1: Group 4 is significantly higher than group 2.

(ii) Analysis of variance of the effect of feeding cobalt deficient diets on percent body weights on subsequent months.

August

Source	df.	TSS.	MS	F	P
Total	19	679.59			
Treatment	3	153.06	51.02		
Error	16	526.53	32.91	1.55	N.S.

All groups had the same % body weights in the 2nd month.

TEXT TABLE 3A Cont'd

September

Source	df.	TSS	MS	F	P
Total	19	1671.75			
Treatment	3	1419.05	473.02		
Error	16	252.70	15.79	29.96**	0.001

Comparison among Means

Group	1	2	3	4
% Body weight	100.00	78.63	81.64	91.41

LSD $P < 0.01 = 10.09$

LSD $P < 0.05 = 8.16$

Groups 2, 3 and 4 were by the 3rd month having significantly lower body weights. However, group 4 was significantly higher than group 2. ($P > 0.01$) and from group 3 ($P < 0.05$).

October

Source	df.	TSS	MS	F	P
Total	19	2725.33			
Treatment	3	2125.55	708.52		
Error	16	599.78	37.49	18.88**	0.001

Comparison Among Means

Group	1	2	3	4
% Body weight	95.94	69.20	72.71	81.23

LSD $P < 0.01 = 15.55$

LSD $P < 0.05 = 8.20$

Group 2, 3 and 4 were having lower % body weight than Group 1 (Control).

TEXT TABLE 3A cont'd

November

Source	df.	TSS.	MS	F	P
Total	18	3814.06			
Treatment	3	3486.27	1162.09		
Error	15	327.79	21.85	53.18**	P 0.001

Comparison among Means

Group	1	2	3	4
% Body weight	101.56	67.43	71.80	73.58

LSD P<0.01 = 18.51

LSD P<0.05 = 9.82

By the 5th month group 2, 3 and 4 were having lower % body weights, and that there was no difference among the three groups but all were lower than group 1 (P < 0.01).

December

Source	df.	TSS.	MS	F	P
Total	18	5524.87			
Treatment	3	4892.02	1630.67		
Error	15	632.85	42.19	38.65**	0.001

Comparison among Means

Group	1	2	3	4
% Body weight	104.74	62.49	70.69	73.64

LSD P<0.01 = 24.76

LSD P<0.05 = 12.96

Groups 2, 3 and 4 were having lower % body weights.

TEXT TABLE 3A Cont'd

January 1978

Source	df.	TSS	MS	F	P
Total	18	6113.94			
Treatment	3	5581.26	1860.42		
Error	15	532.68	35.51	52.39**	0.001

Comparison Among Means

Group	1	2	3	4
% Body Weight	102.86	56.59	67.78	70.81

LSD $P < 0.01$ = 23.12

LSD $P < 0.05$ = 12.10

(iii) Regression for body weights against duration of time (FIG. B. with Y = body weights . x = duration) Group 1. $Y = 18.11 + 0.19x$ ($r^2=0.42$) df = 4. t = 1.90 (N.S.)

Group 2. $Y = 25.33 - 1.17x$ ($r^2 = 0.90$) df = 4. t = 9.50 ($P < 0.001$).

Group 3. $Y = 20.25 - 1.07x$ ($r^2= 0.88$) df = 4. t = 5.94 ($P < 0.005$).

Group 4. $Y = 22.14 - 1.14x$. ($r^2= 0.94$) df = 4. t = 8.77 ($P < 0.005$).

TEXT TABLE 3B

(i) Analysis of variance of the effect of feeding cobalt deficient diets on percent body weights on group I.

Source	df	TSS	MS	F	P
Total	29	945.88			
Treatment	6	291.64	48.61		
Error	23	654.24	28.45	1.71	NS

Control (group 1) % body weights did not significantly very all through the study.

(ii) Group 2

Source	df	TSS	MS	F	P
Total	31	6025.94			
Treatment	6	4851.03	808.51		
Error	25	1174.91	47.00	17.20**	0.005

Comparison among Means

Month	July 1977	Aug.	Sept.	Oct.	Nov.	Dec.	Jan. 1978
% Body weight	100.00	90.10	78.65	69.20	67.43	62.49	56.59

In the third month, the % Body weights were significantly lower than the initial weight.

LSD $P < 0.050 = 13.72$

FIGA Effect of cobalt deficiency on % body weights changes

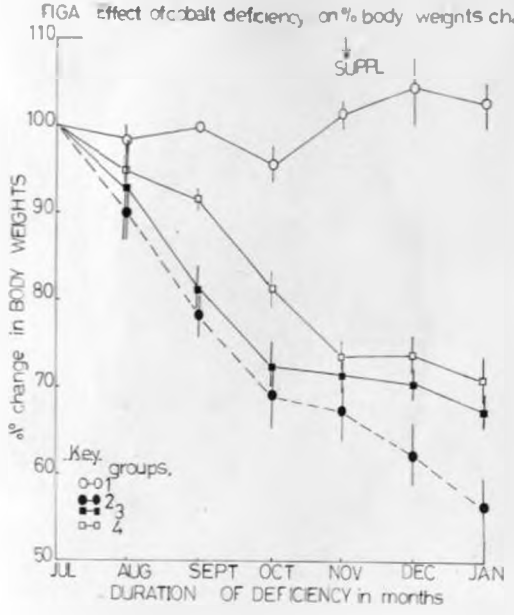
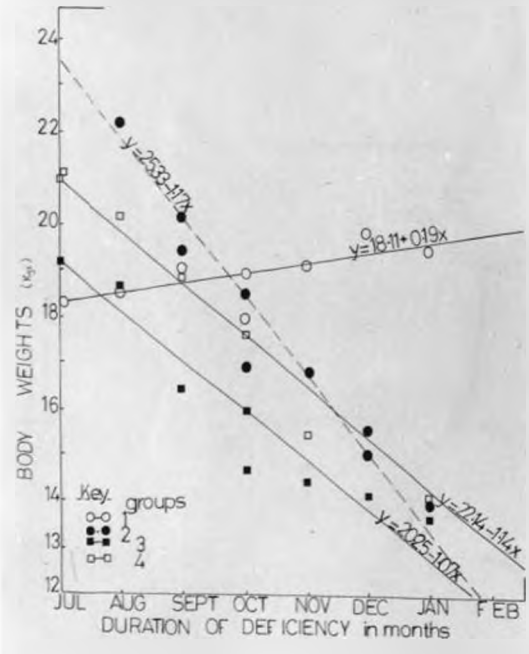


FIG B Regression of body weights with time.



4:1:2. Haematological Parameters.

Anaemia in all the cobalt deficient animals occurred after 8 weeks of feeding the cobalt deficient diet. The anaemia was characterized by a decrease in the number of Red Blood Cells (RBC) and Haemoglobin concentration ($P<0.05$). RBC values in the cobalt deficient groups 2, 3 and 4 were 9.87 ± 0.31, 10.01 ± 0.34, $10.39 \pm 0.30 \times 10^6/mm^3$, respectively, after 23 weeks as compared with the normal goats $11.37 \pm 0.22 \times 10^6/mm^3$ ($P<0.05$). The decrease in RBC values was from (1st to 23rd week) by 34.40% in group 2, 39.50% in group 3, and 33.80% in group 4.

Haemoglobin concentration was correspondingly reduced (see Tables 4 to 13, Appendix Table 3 to 7 and Fig C,D,E & F). The decrease in RBC and Hb values were correlated to the loss in body weights ($r = 0.90$ $P<0.005$). There was no significant difference in the Mean Corpuscular Haemoglobin Concentration in all the four groups. The Mean Corpuscular Volume (%) increased during this study. This was used to interpret the type of anaemia as macrocytic. Furthermore, Text Table 9 shows that there was a decrease in the packed cell volume (%) values in group 2, 3 and 4.

There was also a highly significant increase in the number of neutrophils and a reduction in the number of lymphocytes. Differential White Blood Cell count revealed that neutrophils in group 2, 3 and 4 were 49.56 ± 1.70%, 54.71 ± 1.74% and 50.83 ± 2.01%, respectively as compared with the normal goats (41.41 ± 0.76%). Lymphocytes were similarly altered to 49.00 ± 1.63%, 43.97 ± 1.72%, 46.86 ± 2.05% for group 2, 3 and 4 as compared to control values of 56.71 ± 0.86% ($P < 0.05$). These leukocyte alterations were more pronounced in group 3 than group 2 and 4 and were interpreted as being suggestive of adrenal hyperactivity. There was also no significant difference in total plasma proteins. Total protein values were(MEAN ± SEM gm%) 7.35 ± 0.07, 7.38 ± 0.07, 7.34 ± 0.06 and 7.30 ± 0.04 for group 1, 2, 3 and 4, respectively. The decrease in body weights that accompanied the normal values for plasma proteins may indicate an absolute hypoproteinemia as suggested by Schalm, Jain and Carroll (1975).

N.B. In the Text Tables that are presented in the following pages, the duration of deficiency is presented as follows:-

July (1st month), August (2nd month), September (3rd month), October (4th month) November (5th month), December (6th month), and January 1971 as the 7th month.

TEXT TABLE 4

THE EFFECT OF COBALT DEFICIENCY ON RED BLOOD CELL VALUES IN THE GOAT (a)

Group Number		DURATION OF DEFICIENCY IN MONTHS						
		1	2	3	4	5	6	7
1(control)	MEAN	11.49	11.25	11.41	11.73	11.09	10.90	11.72
	SEM	0.66	0.77	0.57	0.69	0.63	0.57	0.52
2(Defi - cient)	MEAN	12.38	11.66	10.29	8.66.	8.71	8.44	8.12
	SEM	0.09	0.45	0.58	0.36	0.19	0.25	0.10
3(Energy)	MEAN	12.96	12.06	11.16	9.33	8.52	8.31	7.84
	SEM	0.23	0.45	0.68	0.23	0.27	0.24	0.29
4(Protein)	MEAN	12 .62	12.12	11.65	10.19	9.18	8.65	8.36
	SEM	0.64	0.23	0.43	0.33	0.36	0.28	0.17

(a) All values given as MEAN $\pm$ SEM $\times 10^6/mm^3$.

TEXT TABLE 5A

Analysis of variance of the effect of cobalt deficiency on % RBC values.

Source	df.	TSS	MS	F	P
Total	136	32825.00			
Treatment	3	10125.53	3375.18		
Error	133	22699.47	170.67	19.78**	0.001

Comparison among mean

Group	3	2	4	1
% RBC	77.36	79.02	82.56	98.89

LSD P $<$ 0.05 = 8.76

LSD P $<$ 0.01 = 14.70

Group 1 - 3 =	21.53**	Group 3 - 4 =	3.54
Group 1 - 2 =	19.87**	Group 2 - 3 =	1.66
Group 1 - 4 =	16.33**	Group 3 - 4 =	5.20

Group 2, 3, and 4 were significantly lower than group 1 ($P < 0.01$).

TEXT TABLE 58

Analysis of variance of the effect of cobalt deficient on % RBC values in the: Control Group I.

Source	df.	TSS	MS	F	P
Total	34	828.03			
Treatment	6	128.94	21.35		
Error	28	699.54	24.98	0.85	NS

There was no change in RBC values during all the time of study.

Deficient Group 2

Source	df.	TSS	MS	F	P
Total	31	6454.43			
Treatment	6	5424.81	904.13		
Error	25	1029.62	41.18	21.96**	0.001

Comparison among means

Month	7	6	4	5	3
RBC %	64.84	67.40	69.09	69.58	82.13
Month	2	1			
RBC%	93.04	100			

LSD $P<0.01$ = 22.27

LSD $P<0.05$ = 16.46

1st - 2nd = 6.96 1st - 5th = 31.42**
1st - 3rd = 17.87* 1st - 6th = 32.60**
1st - 4th = 30.91** 1st - 7th = 35.16**

By the 3rd month, RBC significantly decreased in group 2 ($P<0.01$).

TEXT TABLE 5B cont'd.

Energy - Group 3

Source	df.	TSS	MS	F	P
Total	34	8169.16			
Treatment	6	7241.78	1206.96		
Error	28	927.38	33.12	36.44**	0.001

Comparison among means

Month	7	6	5	4	3
RBC%	60.50	64.15	65.79	72.07	86.43
Month	2	1			
RBC%	92.62	100.00			

LSD $P < 0.05$ = 18.59
LSD $P < 0.01$ = 33.36

1st - 2nd = 7.38 1st - 5th = 34.21**
1st - 3rd = 13.57 1st - 6th = 35.85**
1st - 4th = 27.93* 1st - 7th = 39.50**

By the 4th month, RBC significantly decreased in group 3 ($P < 0.01$).

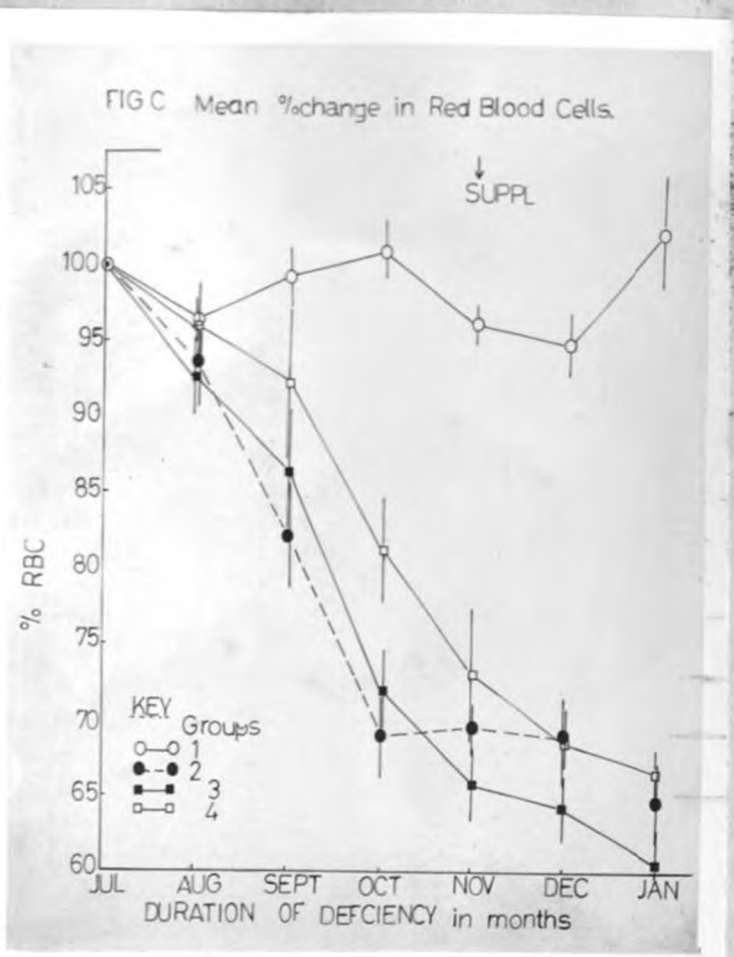


FIG C Mean %change in Red Blood Cells.
SUPPL
105
100
95
90
85
80
75
70
65
60
% RBC
KEY
Groups
1
2
3
4
JUL
AUG
SEPT
OCT
NOV
DEC
JAN
DURATION OF DEFCIENCY in months

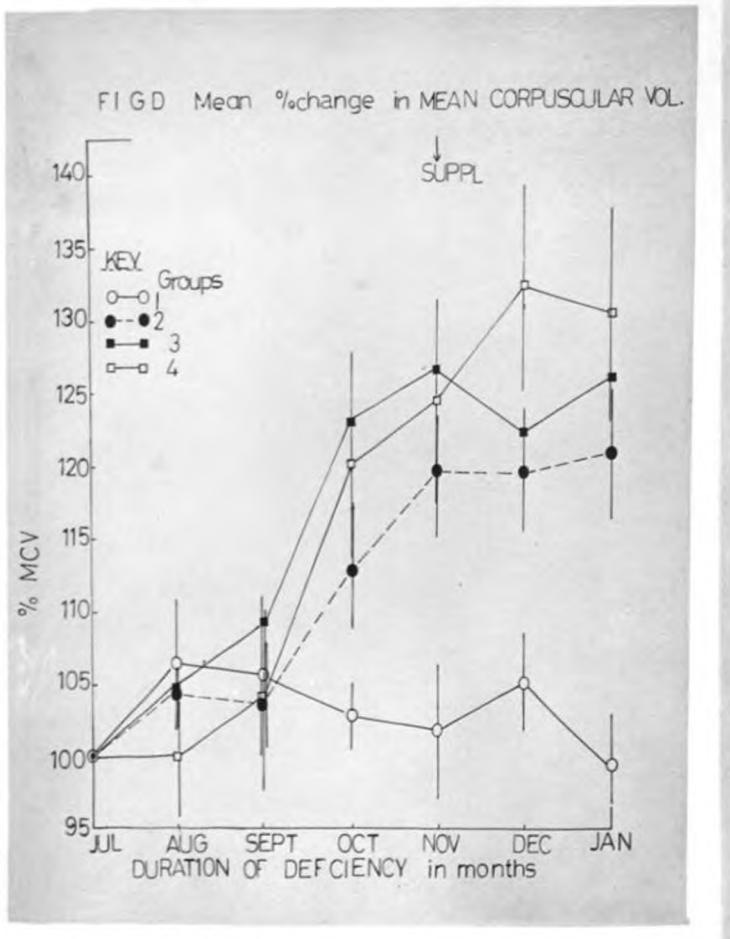


FIG D Mean %change in MEAN CORPUSCULAR VOL.
SUPPL
140
135
130
125
120
115
110
105
100
95
% MCV
KEY
Groups
1
2
3
4
JUL
AUG
SEPT
OCT
NOV
DEC
JAN
DURATION OF DEFCIENCY in months

TEXT TABLE 6.

THE EFFECT OF COBALT DEFICIENCY ON HAEMOGLOBIN VALUES IN GOATS

DURATION OF DEFICIENCY IN MONTHS

GROUP NUMBER		JULY 1977	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	JANUARY 1978
I (Control)	Mean	8.58	8.50	9.02	9.60	8.56	8.12	8.94
	SEM	0.52	0.37	0.64	0.45	0.46	0.40	0.68
2 (Deficient)	Mean	7.82	7.72	7.32	6.74	6.55	6.18	6.00
	SEM	0.48	0.56	0.52	0.65	0.66	0.56	0.48
3 (Energy)	Mean	8.50	9.48	8.44	7.56	7.42	7.10	7.00
	SEM	0.41	0.30	0.14	0.31	0.30	0.27	0.27
4 (Protein)	Mean	9.30	9.26	8.52	8.04	7.25	6.98	6.82
	SEM	0.53	0.31	0.66	0.30	0.43	0.33	0.23

N.B. All values given as MEAN ± SEM gm/100 ml

TEXT TABLE 7

Analysis of variance of the effect of cobalt deficiency on Haemoglobin values of the goat.

Source	df.	TSS	MS	F	P
Total	135	27636.45			
Treatment	3	12973.93	4324.64		
Error	132	14662.52	111.08	38.93**	0.001

Comparison among means

Group	2	4	3	1
% Mean Hb.	85.68	86.87	93.71	101.53

LSD $P < 0.05 = 7.15$

LSD $P < 0.01 = 9.40$

Group 1 - 2	= 15.85**	Group 3 - 4	= 6.84
Group 1 - 3	= 7.82*	Group 2 - 3	= 8.03*
Group 1 - 4	= 14.66**	Group 2 - 4	= 1.19

Group 2, 3 and 4 had a decrease in Hb concentration ($P < 0.10$ to > 0.50)

TEXT TABLE 8 THE INFLUENCE OF COBALT DEFICIENCY ON PACKED CELL VOLUME (%)

Group Number		DURATION OF DEFICIENCY IN MONTHS						
		Jul. 1977	Aug.	Sept.	Oct.	Nov.	Dec.	Jan. 1978
1 (Control)	Mean	25.40	26.40	25.30	26.80	24.90	25.20	25.80
	SEM	2.20	2.38	2.06	2.85	2.29	1.82	2.56
2 (Deficient)	Mean	30.80	29.80	26.00	24.40	24.88	24.00	23.38
	SEM	1.77	0.97	0.84	1.21	2.05	1.47	1.57
3 (Energy)	Mean	30.70	29.40	28.68	27.30	25.40	23.70	23.30
	SEM	0.80	1.67	0.40	0.49	0.40	0.30	0.83
4 (Protein)	Mean	29.60	28.40	28.20	27.80	26.80	26.40	25.40
	SEM	0.68	0.75	0.86	1.46	0.86	0.51	0.93

N.B. All values given as MEAN ± SEM (PCV%).

TEXT TABLE 9

Analysis of variance on the effect of cobalt deficiency on % packed cell volume.

Source	df.	TSS	MS	F	P
Total	135	28773.68			
Treatment	3	10223.88	3408.96		
Error	132	18549.80	140.53	24.25**	0.001

Comparison among means

Group	2	3	4	1
% PCV	77.26	84.66	90.27	101.10

LSD $P < 0.05 = 8.06$

LSD $P < 0.01 = 13.51$

Group 1 - 2 = 23.84** Group 3 - 4 = 5.61

Group 1 - 3 = 16.44** Group 2 - 3 = 7.40

Group 1 - 4 = 10.83** Group 2 - 4 = 13.01*

Group 2, 3 and 4 had a decrease in PCV %

TEXT TABLE 10

THE INFLUENCE OF COBALT DEFICIENCY ON MEAN CORPUSCULAR VOLUME (%) (a)

GROUP NUMBER		July 1977	August	September	October	November	December	January 1978
1(Control)	MEAN	22.03	23.46	22.10	22.70	22.46	23.16	21.96
	SEM	1.07	1.16	1.06	1.46	1.65	1.23	1.71
2(Deficient)	MEAN	**24.91**	**25.66**	25.61	28.24	28.49	28.45	28.78
	SEM	1.59	0.94	1.75	1.26	1.80	1.19	1.76
3(Energy)	MEAN	23.73	24.59	26.00	29.32	29.94	28.60	29.77
	SEM	0.86	0.99	1.59	0.89	1.03	0.64	0.85
4(Protein)	MEAN	23.52	23.49	24.42	28.20	29.45	30.68	30.49
	SEM	0.86	0.97	1.48	2.36	1.79	1.42	1.50

(a) All values given as MEAN ± SEM %

TEXT TABLE 11A

Analysis of variance of the effect of cobalt deficiency on percent MCV %

Source	df.	TSS	MS	F	P
Total	136	30368.27			
Treatment	3	4227.01	1409.00		
Error	133	26141.26	196.55	7.17**	0.001

Comparison among means

Group	1	2	3	4
Mean %	102.30%	110.93%	115.62%	115.88%

LSD $P < 0.05 = 9.52$

LSD $P < 0.01 = 12.51$

Groups 2, 3 and 4 had higher MCV% values.

TEXT TABLE II B

Analysis of variance of the effect of cobalt deficiency on percent mean corpuscular volume in Group 1.

Source	df.	TSS	MS	F	P
Total	34	2166.19			
Treatment	6	214.20	35.70		
Error	28	1951.99	67.71	0.51	NS

There was no change in the control group.

Group 2

Source	df.	TSS	MS	F	P
Total	31	5196.40			
Treatment	6	2095.59	349.27		
Error	25	3100.81	124.03	2.82*	0.050

Comparison among means

Month	1	3	2	4
% MCV	100%	103.70%	104.38%	114.03%
Month	6	5	7	
% MCV	119.47%	119.54%	120.76%	

LSD $P < 0.05$ = 18.50

LSD $P < 0.01$ = 15.34

By 5th month MCV% was significantly higher than the initial value.

TEXT TABLE 118 Cont'd.

Group 3

Source	df.	TSS	MS	F	P
Total	34	6463.39			
Treatment	6	3627.45	604.58		
Error	28	2835.94	105.28	5.97**	0.001

Comparison among Means

Month	1	2	3	6
% MCV	100%	103.59%	109.20%	120.90%

Month	4	7	5
% MCV	123.09%	125.93%	126.55%

LSD $P < 0.05$ = 13.03

LSD $P < 0.01$ = 17.57

LSD $P < 0.001$ = 23.37

By 4th month MCV% was significantly higher than the initial value.

Group 4

Source	df.	TSS	MS	F	P
Total	34	12601.46			
Treatment	6	5945.36	990.88		
Error	28	6656.16	237.72	4.17**	0.025

Comparison among means

Month	1	2	3	4
% MCV	100%	100.12%	104.02%	120.11%

Month	5	7	6
%MCV	125.82%	130.14%	130.97%

LSD $P < 0.05$ = 19.97

LSD $P < 0.01$ = 26.94

By 3rd month MCV% was significantly higher than the initial value.

TEXT TABLE 12A THE EFFECT OF COBALT DEFICIENCY ON MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION (%)(a)

Group Number		DURATION OF DEFICIENCY IN MONTHS						
		Jul. 1977	Aug.	Sept.	Oct.	Nov.	Dec.	Jan. 1978
1 (Control)	Mean	33.50	32.10	35.97	34.39	34.99	32.43	34.98
	SEM	1.23	1.77	1.81	2.54	1.81	0.85	1.07
2 (Deficient)	Mean	25.70	25.90	28.31	27.76	26.48	25.84	25.95
	SEM	2.04	1.70	2.85	2.03	2.15	2.10	2.31
3 (Energy)	Mean	27.66	32.31	29.52	27.65	29.18	30.02	30.34
	SEM	0.91	1.20	0.33	0.76	0.89	1.43	2.25
4 (Protein)	Mean	31.61	32.62	30.24	29.06	27.06	26.40	26.82
	SEM	1.53	0.74	1.73	0.77	0.68	0.83	0.85

(a) All values given as MEAN ± SEM %.

TEXT TABLE 12B

Analysis of variance of the effect of cobalt deficiency on the percent Mean Corpuscular Haemoglobin Concentration (MCHC).

Source	df.	TSS	MS	F	P
Total	134	16190.93			
Treatment	3	3555.85	1185.28		
Error	131	12635.08	96.45	12.29**	0.001

Comparison among means

Group	4	1	2	3
Mean % MCHC	92.82	100.51	102.21	106.96

LSD P < 0.01 = 10.23

LSD P < 0.05 = 6.10

Group 1-2 = 1.70 Group 3-4 = 14.14**

Group 1-3 = 6.45* Group 2-3 = 4.75

Group 1-4 = 7.69* Group 2-4 = 10.39**

There is an increase in MCHC in Group 2 and 3 which is interpreted as hyperchromic, and a decrease in Group 4 which is interpreted as hypochromic. Benjamin (1974) and Schalm et al. (1975), however, report of no condition whereby the MCHC is increased above normal values, since the erythrocyte cannot be supersaturated with haemoglobin. Benjamin suggests that the hyperchromic anaemia is an increase in the weight of haemoglobin in the average erythrocyte. Thus the hyperchromic anaemia of Group 2, and 3 is interpreted as normochromic anaemia in this study.

TEXT TABLE 13A

(i) Analysis of variance of the effect of cobalt deficiency on % total WBC

Source	df.	TSS	MS	F	P
Total	27	2963.00			
Treatment	3	428.28	142.76		
Error	24	2534.72	105.72	1.35	NS

(ii) Analysis of variance of the effect of cobalt deficiency on % neutrophils.

Source	df.	TSS	MS	F	P
Total	27	10936.99			
Treatment	3	3629.36	1209.79		
Error	24	7307.63	304.48	3.97*	0.025

Comparison among means

Group	1	4	2	3
% Neutrophils	104.30	120.82	126.87	135.43

LSD $P < 0.05$ = 19.26

(iii) Analysis of variance of the effect of cobalt deficiency on % Lymphocytes.

Source	df.	TSS	MS	F	P
Total	27	5114.81			
Treatment	3	1334.94	444.98		
Error	24	3779.87	157.49	2.83	0.025

Comparison among means

Group	3	4	2	1
% Lymphocyte	75.81	82.79	83.82	93.08

LSD $P < 0.05$ = 13.85

TEXT TABLE 13B

MEAN LEUKOCYTE VALUES IN NORMAL AND COBALT DEFICIENT GOATS

DURATION OF DEFICIENCY IN MONTHS	GROUP I	GROUP 2	GROUP 3	GROUP 4
1st	14.18±1.42	12.04±0.39	11.72±0.71	14.28±0.52
2nd	14.78±1.60	11.81±0.98	11.43±0.64	13.54±0.64
3rd	13.92±1.41	12.42±2.27	11.30±0.60	13.16±0.65
4th	13.76±1.10	10.88±1.28	11.02±1.34	14.40±0.40
5th	13.86±2.01	13.25±1.99	12.32±1.34	16.06±1.16
6th	13.58±1.35	11.63±1.45	8.96±0.68	10.74±1.97
7th	14.78±1.35	12.30±1.64	8.80±0.84	9.72±1.40

* All values given as MEAN ± SEM X 10^3 per mm^3.

TEXT TABLE 138

Mean Leukocyte Values

	Total neutrophils %			
DURATION OF DEFICIENCY IN MONTHS	GROUP I	GROUP 2	GROUP 3	GROUP 4
1st	40.00±2.02	39.40±1.63	40.40±2.11	42.40±2.77
2nd	41.20±2.56	41.00±2.86	47.80±3.90	39.60±1.94
3rd	41.60±2.34	45.80±0.68	52.00±2.39	50.60±2.54
4th	40.25±1.89	60.20±5.20	58.40±7.36	62.00±6.16
5th	42.60±3.37	55.00±3.49	58.80±4.12	58.80±5.55
6th	45.00±1.22	55.25±1.70	61.80±3.25	52.60±4.08
7th	41.40±2.38	53.25±0.75	63.80±3.25	52.60±5.97

* All values given as MEAN ± SEM % X 10^3 per mm^3.

TEXT TABLE 13 B

Mean leukocyte values

Duration of deficiency In month	Total Lymphocytes % Group I	Group 2	Group 3	Group 4
1st	59.20±1.91	58.00±2.17	58.00±2.10	56.60±2.62
2nd	56.20±2.71	56.60±2.56	50.20±4.09	60.00±1.84
3rd	56.80±2.87	54.00±2.88	47.20±2.78	46.80±2.06
4th	57.20±2.01	38.20±4.45	41.40±3.44	35.60±5.86
5th	56.60±3.83	43.75±3.25	38.20±3.73	38.00±5.00
6th	53.00±1.26	43.50±2.22	37.60±3.17	44.60±6.60
7th	55.00±2.61	46.25±0.85	35.20±3.25	46.40±3.75

* All values given as MEAN ± S.E.M. %

FIG E %change in HAEMOGLOBIN

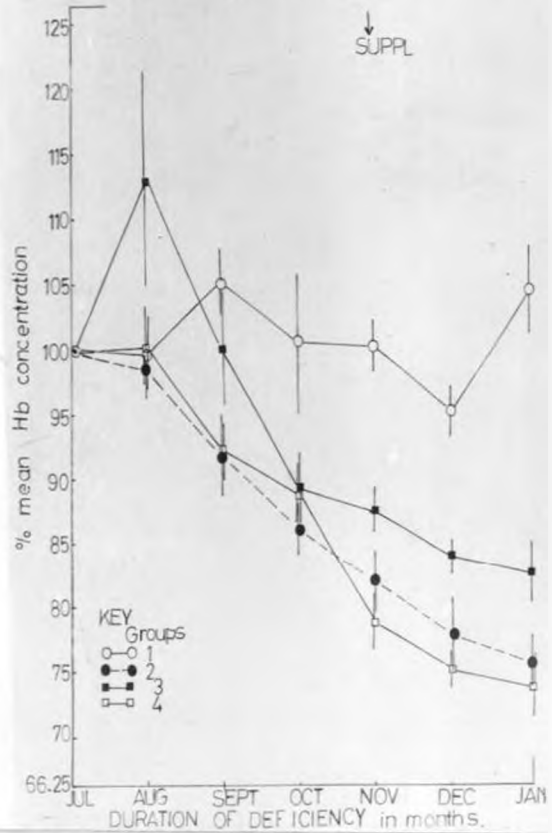
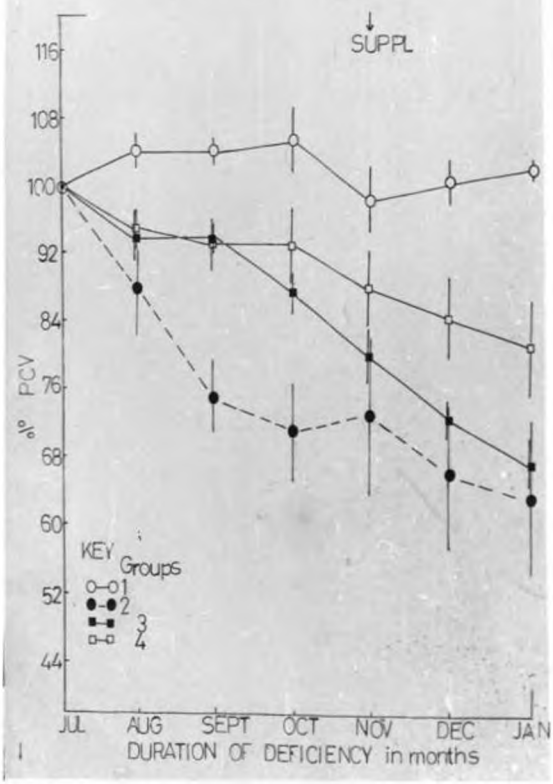


FIG F Mean PACKED CELL VOLUME



4:1:3. VITAMIN B_{12} (CYANOCOBALAMIN) VALUES.

Plasma Vit. B_{12} concentration decreased significantly ($P < 0.05$), during the period of study with groups 2, 3 and 4 values of 289.55 ± 31.92, 238.83 ± 23.44, and 217.03 ± 23.94 pg/ml. as compared to 475.65 ± 20.37 pg/ml for control animals. The decline became significant after 8 weeks ($P < 0.050$) when Vit. B_{12} values decreased to 25% of the initial concentration. Further results are shown on Text Table 14 to 15, Figs. G, H & I and Appendix Table 8.

The decline in Vit. B_{12} was correlated to loss in body weight ($r = 0.94$, $P < 0.005$). Animals with initially high body weights lost Vit. B_{12} at a faster rate, thus group 2 lost 22.21 pg/ml per week (3.42%), whereas group 3 and 4 lost 21.17 and 14.16 pg/ml per week (3.41% and 2.15%, respectively.)

Fig. H.

The Regression $Y = a + bx$.

where Y = Vit. B_{12} . x = duration

Group 1 (Control) $Y = 466.36 \pm 0.50x$.

Group 2 (Deficient) $Y = 500.65 - 22.21x$.

Group 3 (Energy) $Y = 458.53 - 21.17x$.

Group 4 (Protein) $Y = 357.21 - 14.16x$.

FIG G Time course of CYANOCOBALAMIN

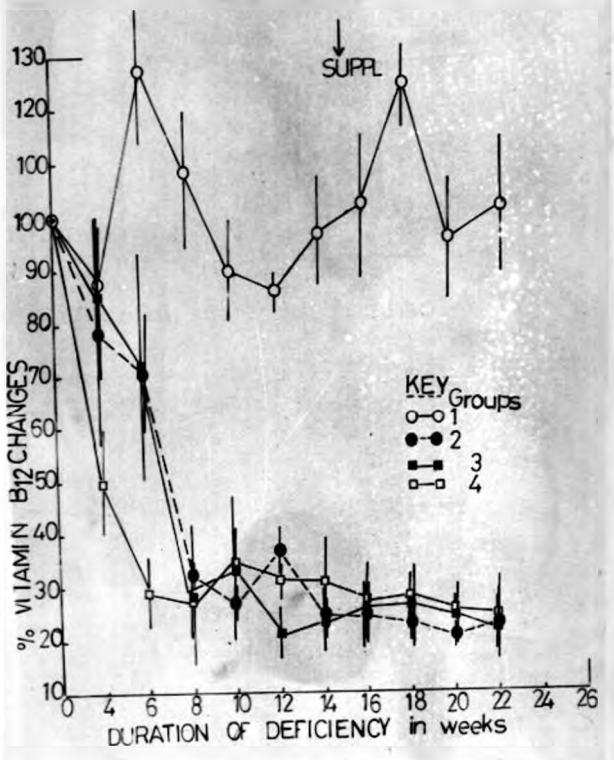


FIG H Regressin of Vit.B_{12} with time.

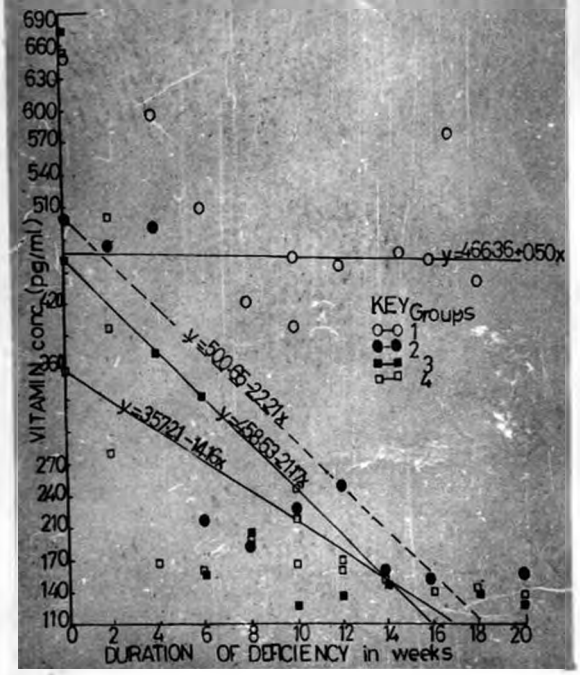


TABLE 14 MEAN CYANOCOBALAMIN CONCENTRATION IN NORMAL AND COBALT DEFICIENT GOATS

Date of Sample Collection	Group I		Group II		Group III		Group IV	
	MEAN	S.E.M.	MEAN	S.E.M.	MEAN	S.E.M.	MEAN	S.E.M.
August 8, 1977	462.00	34.70	675.00	93.89	648.00	121.84	653.20	129.95
August 16	397.00	38.78	472.80	94.99	502.00	52.17	281.20	46.85
September 1	598.00	94.36	491.60	122.18	374.00	64.08	168.00	28.79
September 16	507.00	83.18	215.80	34.75	155.40	16.71	155.00	18.86
October 1	422.00	68.22	184.60	38.59	194.00	52.04	186.52	65.75
October 16	396.00	25.42	227.25	84.15	126.00	18.12	166.40	14.85
November 1	454.00	76.79	161.25	11.30	136.60	11.14	169.20	19.70
November 16	460.00	53.94	160.00	12.08	150.00	13.51	148.60	15.13
December 1	574.00	49.25	155.50	13.85	141.60	12.04	152.20	17.70
December 16	436.00	55.37	140.50	15.63	138.20	26.93	142.80	9.88
January 10 (1978)	480.00	89.67	157.75	37.47	128.00	10.58	134.90	19.91

NB: All values given as MEAN ± SEM pg/ml.

TEXT TABLE 15A

Analysis of variance of the effect of feeding cobalt deficient feeds on cyanocobalamin values.

Source	df.	TSS.	MS	F	P
Total	213	9752561.08			
Treatment	3	2127213.66	709071.22		
Error	210	7625347.42	36311.18	19.53**	0.005

Comparison among means

Group	1	2	3	4
B_{12} pg/ml	471.45	289.96	238.49	225.27

LSD $P < 0.05$ = 103.33

LSD $P < 0.01$ = 135.80

TEXT TABLE 15B

(i) Analysis of variance of the effect of cobalt deficiency on percent change in cyanocobalamin concentration per subsequent collection in Group I

Source	df.	TSS.	MS.	F	P
Total	54	35771.64			
Treatment	10	12396.09	1239.61		
Error	44	23375.55	531.26	2.33	NS

Thus variation in concentration (cyanocobalamin) did not significantly differ from one collection to the other in the control group.

TEXT TABLE 15 B Cont'd.

Group 2.

Source	df.	TSS	MS'	F	P
Total	43	40798.25			
Treatment	10	26877.77	2687.78		
Error	33	13920.48	421.83	6.37**	0.005

Comparison among means

No. collection	10	11	9	8	7	5
% change concentr.	20.33	22.99	23.02	23.88	24.02	27.19

No. Collection	4	6	3	2	1
% change concentr.	32.43	36.95	63.43	69.34	100

LSD $P < 0.001 = 51.11$

LSD $P < 0.05 = 29.25$

Group 3

Source	df.	TSS	MS	F	P
Total	54	59219.91			
Treatment	10	41427.09	4142.71		
Error	44	17792.82	404.38	10.24**	0.005

Comparison among means

No. collection	6	10	11	7	9	8
% change concentr.	21.21	21.34	22.75	23.80	26.01	27.18

No. collection	4	5	3	2	1
% change concentr.	24.45	33.17	70.82	85.26	100

LSD $P < 0.001 = 45.17$

LSD $P < 0.05 = 25.17$

That is energy supplementation to group 3 did not make a change in cyanocobalamin values, and the animals had significantly low values by the 3rd month.

Group 4

Source	df.	TSS	MS	F	P
Total	54	37846.65			
Treatment	10	24401.68	2440.17		
Error	44	13444.97	305.57	7.99**	0.005

Comparison among Means

No. collection	11	10	8	9	
% change in concentration	24.40	25.30	25.44	27.23	
No Collection	4	5	3	7	6
% change concent.	27.93	28.40	29.05	30.51	31.07
No. Collection	2	1			
% Change Concentration	49.63	100			

LSD $P < 0.01 = 38.44$

LSD $P < 0.05 = 22.16$

That is energy supplementation did not make a change in cyanocobalamin values, and the animals had significantly low values by 3rd month.

4:2. REPRODUCTIVE FUNCTIONS

4:2:1. The Oestrous cycle lengths

The occurence of longer and irregular oestrous cycle lengths was characteristic of animals in group 2, 3 and 4. The cycles, became irregular and longer ($P < 0.05$) in the second oestrous cycle and later ceased in the fourth or fifth cycle. Analysis of a total of 40 cycles in control animals and 78 cycles in the deficient animals showed the MEAN ± S.E.M. was 18.52 ± 0.33 days with range of 16 to 21 days in controls, as compared to that of deficient animals (22.57 ± 0.78 days with range of 12 to 38 days). Further results are shown in Text Table 16 & 17, and Fig J.

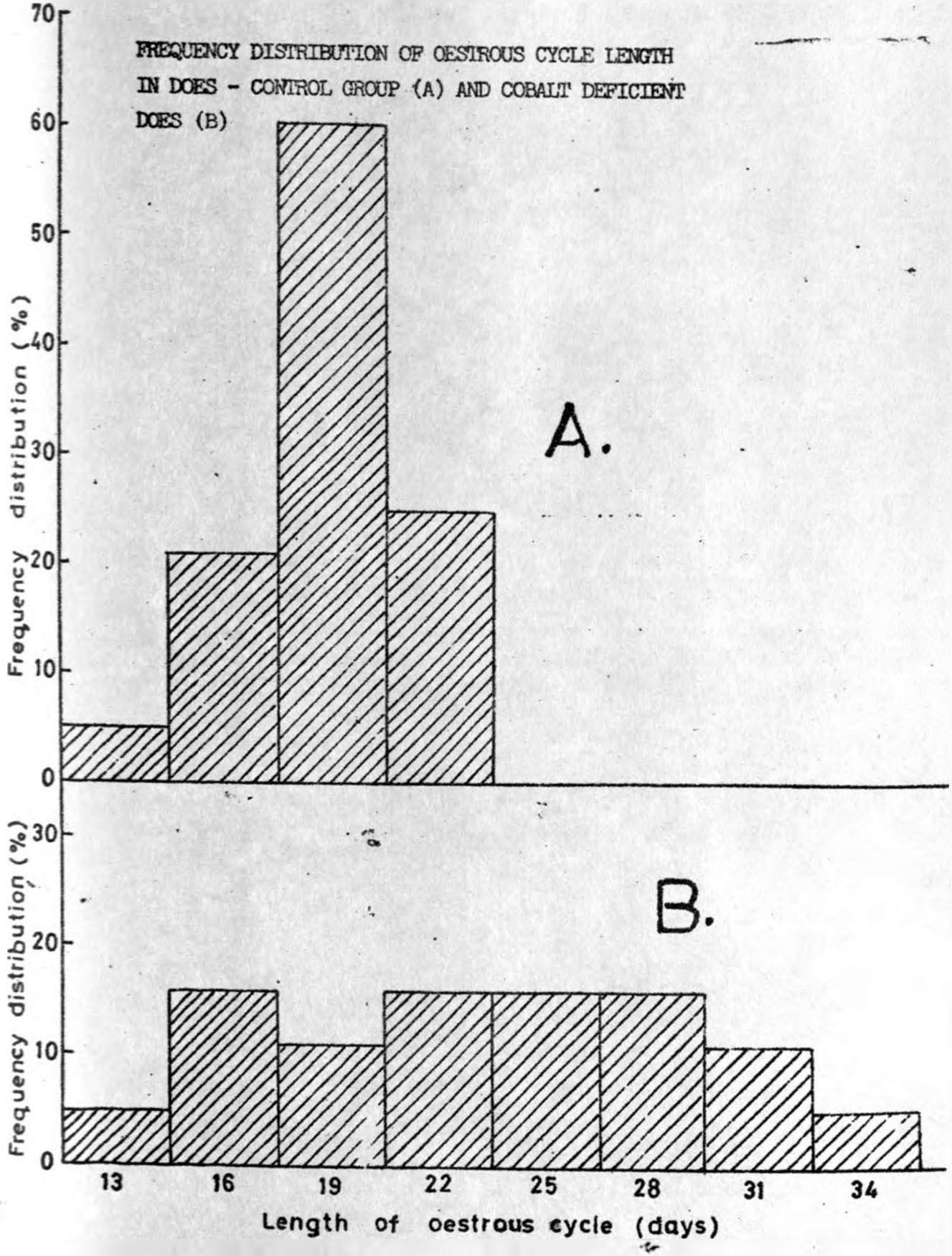
The distribution of the oestrous cycle lengths shown on Fig. J. show that only 25% of the control animals had cycle lengths of 21 days or longer as compared to 64% in the cobalt deficient animals. However, shorter cycles were of the same distribution in both the normal and cobalt deficient animals (less than 5%). Unlike the cobalt deficient animals unusually prolonged cycles were virtually absent in the control animals.

TEXT TABLE 16

Group 1 Oestrous cycle lengths in Cobalt deficiency does

Animal Number	I	II	C III	Y IV	C V	L VI	E VII	VIII	MEAN	SEM
Control										
82	20	18	17	20	19	20	23	17	19.25	0.70
83	16	18	19	21	21	16	14	16	17.63	0.91
91	18	20	18	18	16	18	18	19	18.13	0.40
94	16	18	20	21	19	19	18	14	18.13	0.79
99	21	17	18	16	18	18	18	20	18.25	0.56
MEAN	18.20	18.20	18.40	19.20	18.60	18.20	18.20	17.20		
SEM	1.20	0.49	0.51	0.97	0.81	0.66	1.43	1.07		
Group 2										
86	15	27	20	30					23.00	3.39
87	24	28	48	-					33.33	7.43
88	23	23	31	30					26.75	2.17
89	19	29	24	-					24.00	2.89
90	20	19	-	-					19.50	0.50
MEAN	20.20	25.20	30.75	30.00						
SEM	1.59	1.85	6.18	0.00						
Group 3										
80	16	16	20	28					20.10	2.83
84	22	32	30	30					27.00	2.38
85	23	25	22	25					24.40	0.87
92	12	32	26	29					24.75	4.42
93	28	31	23	36					29.50	2.72
MEAN	20.20	27.20	24.20	29.60						
SEM	2.80	3.09	1.74	1.81						
Group 4										
95	15	26	15	26	12				18.80	2.99
96	27	20	37	-	-				28.00	4.93
97	12	21	17	24	-				18.50	2.60
98	22	17	17	18	16				18.00	1.05
100	22	25	27	-	-				24.67	1.45
MEAN	19.60	21.80	22.60	23.67	14.00					
SEM	2.69	6.02	4.17	2.85	2.00					

FIG J


FREQUENCY DISTRIBUTION OF OESTROUS CYCLE LENGTH IN DOES - CONTROL GROUP (A) AND COBALT DEFICIENT DOES (B)
A.
B.
Frequency distribution (%)
70
60
50
40
30
20
10
0
30
20
10
0
13
16
19
22
25
28
31
34
Length of oestrous cycle (days)

TEXT TABLE 17

Analysis of variance of the effect of cobalt deficiency on: The first oestrous cycle length.

Source	df.	TSS	MS	F	P
Total	19	386.95			
Treatment	3	13.35	4.45		
Error	16	373.60	23.35	0.19	NS

The Second oestrous cycle length

Source	df.	TSS	MS	F	P
Total	19	553.80			
Treatment	3	234.60	78.20		
Error	16	319.20	19.95	3.92*	0.05

Comparison among means

Group	1	4	2	3
Cycle length (days)	18.26	21.80	25.20	27.20

LSD $P < 0.05 = 5.99$

The third oestrous cycle length

Source	df.	TSS.	MS	F	P
Total	18	1218.42			
Treatment	3	346.47	115.49		
Error	15	871.95	58.13	1.99	NS

Shorter and longer (irregular) cycle lengths were characteristic of cycle three.

Text Table 17 cont'd

During the first - four oestrous cycle lengths

Source	df.	TSS	MS	F	P
Total	73	2696.71			
Treatment	3	564.43	188.15		
Error	70	2132.26	30.46	6.18*	0.001
Group	1	4	2	4	
cycle lengths (days)	18.50	21.56	24.63	25.30	

LSD $P < 0.05 = 5.14$

Mostly it was irregular oestrous cycle lengths, thus per group cycle lengths are longer in group 2, 3 and 4.

4:2:2 ADRENAL GLAND FUNCTION

Results for corticosteroid concentration are shown on Text Table 18 and 19, Appendix Table 9 and Figures K and L_1 to L_2. In all the groups corticosteroid concentration during pre-treatment and after the onset of treatment did not differ significantly. However, group 2, 3 and 4 on cobalt deficient diet had significantly higher ($P < 0.01$) corticosteroid concentration after 5 weeks of treatment. Maximum concentration was achieved during the 11th week in groups 2 and 4, and 6th week in group 3($P < 0.01$). Thereafter this maximal corticosteroid concentration decreased but not to pre-treatment levels. The large standard errors (Fig k) illustrate that the increase in corticosteroid concentration was different from one animal to another.

Individual animals corticosteroid values show that 44% achieve maximal levels during the 6th week, 22% achieve it either during the 11th or 13th week while only 11% achieve after 13th week.

Supplementing the animals with either energy or protein rich diets had no effect on corticosteroid concentration of groups 3 and 4. Thus during all the time of study group 2, 3 and 4 had corticosteroid concentrations of 6.74 ± 0.64, 6.09 ± 0.41 and 5.65 ± 0.33 ng/ml

respectively as compared to 2.03± 0.06 ng/ml in the control animals. Histology of the adrenal cortex showed a marked hypertrophy of cells of the zona fasciculata and reticularis suggesting hyperactivity of the gland (Fig L_2 and Text Table 198).

Text Table 18 cont'd

Duration of deficiency in weeks	Group 3		Group 4	
	MEAN	S.E.M.	MEAN	S.E.M.
0	20.82	1.14	22.75	1.42
2	43.08	6.14	45.58	11.64
4	60.52	6.34	74.37	7 .44
6	101.54	12.19	62.22	5.94
8	69.43	12.19	73.95	6.40
11	70.78	11.96	77.42	20.85
13	71.88	17.55	73.49	7.60
16	62.49	3.20	55.37	1.49
18	58.73	4.81	55.75	3.12
21	58.50	4.68	53.58	1.53
23	67.18	1.36	52.51	0.56

*All values in ng/10 ml.

Body weight and corticosteroid values

regression equations.

Control group one

$Y = -33.88 \pm 2.92 x.$ ($r^2 = 0.47$)

deficient group

$Y = 145.04 - 3.65 x$ ($r^2 = 0.24$).

TEXT TABLE 19 A

Measurements of the adrenal cortex and the respective zones in normal and cobalt deficient goats (a).

	Group 1	Group 2	Group 3	Group 4
Adrenal cortex	1.54±0.20	1.85±0.10	1.75±0.10	1.75±0.10
zona glomerulosa	0.23±0.09	0.25±0.10	0.23±0.10	0.23±0.05
Zona fasciculata and reticularis	1.30±0.15	1.50±0.21	1.52±0.10	1.52±0.12

(a) All values given as MEAN ± SEM mm.

The adrenal cortex was enlarged in groups 2, 3 and 4 ($P<0.05$). The zona glomerulosa was not different in all the groups. Zona fasciculata and reticularis were enlarged in groups 2, 3 and 4.

Hypertrophy of the cells of the zona fasciculata and reticularis was characterized by an increase in the cytoplasm to nucleus ratio. Thus the cytoplasm nucleus ratio was 1.44 ± 0.15 for groups 2, 3, and 4 ($P<0.05$) as compared to 0.99 ± 0.15 for control animals.

TEXT TABLE 19B

(i) Analysis of variance of the effect of cobalt deficiency on plasma corticosteroid concentration in goats.

Source	df.	TSS	MS	F	P
Total	131	127457.37			
Treatment	3	41983.58	13994.53		
Error	128	85473.79	667.76	20.96**	0.001

Comparison among means

Group number	1	4	3	2
Corticosteroid ng/10 ml	19.67	52.79	55.91	67.71

LSD $P < 0.01 = 23.19$

LSD $P < 0.05 = 12.99$

There was a difference in corticosteroid values: with an increase in groups 2, 3 and 4.

(ii) Analysis of variance of the effect of cobalt deficiency on plasma corticosteroid concentration as subsequently collected (23 weeks). In group 1

Source	df.	TSS	MS	F	P
Total	43	4748.69			
Treatment	10	688.66	68.87		
Error	33	4060.03	123.03	0.56	NS

TEXT TABLE 19C cont'd.

6th Week

Source	df.	TSS	MS	F	P
Total	12	14224.33			
Treatment	3	12546.02	4182.01		
Error	9	1678.31	186.48	22.43**	0.005

Comparison among means

Group	1	4	2	3
Cort. ng/10ml.	19.84	62.22	77.77	101.54

LSD $P < 0.05 = 32.15$

8th Week

Source	df.	TSS	MS	F	P
Total	12	9105.67			
Treatment	3	7298.10	2432.70		
Error	9	1807.57	200.84	12.11**	0.005

Comparison among means

Group	1	3	4	2
Cort. ng/10ml.	21.60	69.43	73.95	74.68

LSD $P < 0.05 = 33.37$

11th Week

Source	df.	TSS	MS	F	P
Total	12	26041.04			
Treatment	3	14686.25	4895.42		
Error	9	11354.79	1261.64	3.88**	0.05

Comparison among means

Group	1	3	4	2
Cort. ng/10ml.	21.48	70.78	77.42	111.83

LSD $P < 0.05 = 33.63$

TEXT TABLE 19C cont'd

13th Week

Source	df.	TSS	MS	F	P
Total	11	13535.04			
Treatment	3	9119.53	3039.84		
Error	8	4415.51	551.94	5.51*	0.025

Comparison among means

Group	1	3	4	2
Cort. ng/10ml.	17.65	71.88	73.49	91.21

LSD $P < 0.05$ = 44.23

16th Week

Source	df.	TSS	MS	F	P
Total	12	9485.61			
Treatment	3	7202.12	2400.71		
Error	9	2283.49	253.72	9.46**	0.005

Comparison among means

Group	1	4	3	2
Cort. ng/10ml.	21.66	53.49	62.49	84.63

LSD $P < 0.05$ = 37.50

18th Week

Source	df.	TSS	MS	F	P
Total	12	12685.00			
Treatment	3	9635.20	3211.73		
Error	9	3048.80	338.87	9.48**	0.005

Comparison among means

Group	1	4	3	2
Cort. ng/10ml.	21.34	55.75	58.73	96.07

LSD $P < 0.05$ = 43.34

Text Table 19C Cont'd.

21st Week

Source	df.	TSS	MS	F	P
Total	12	8477.71			
Treatment	3	7167.42	2389.14		
Error	9	1310.29	145.59	16.41**	0.005

Comparison among means

Group	1	4	3	2
Cort. ng/10ml.	20.62	53.58	58.50	84.12

LSD $P < 0.05$ = 28.41

23 week

Source	df.	TSS	MS	F	P
Total	12	4837.36			
Treatment	3	4301.73	1433.91		
Error	9	535.63	59.51	24.10**	0.005

Comparison among means

Group	1	2	4	3
Cort. ng/10ml.	17.80	51.24	52.51	63.85

LSD $P < 0.05$ = 18.15

FIG I BODY WEIGHTS, HAEMOGLOBIN AND B_{12}

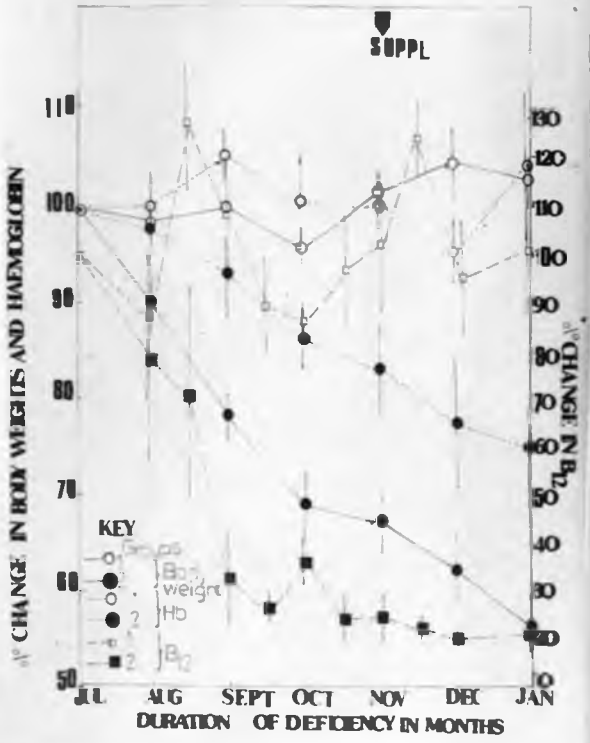


FIG K CORTICOSTEROIDS CONC IN PLASMA

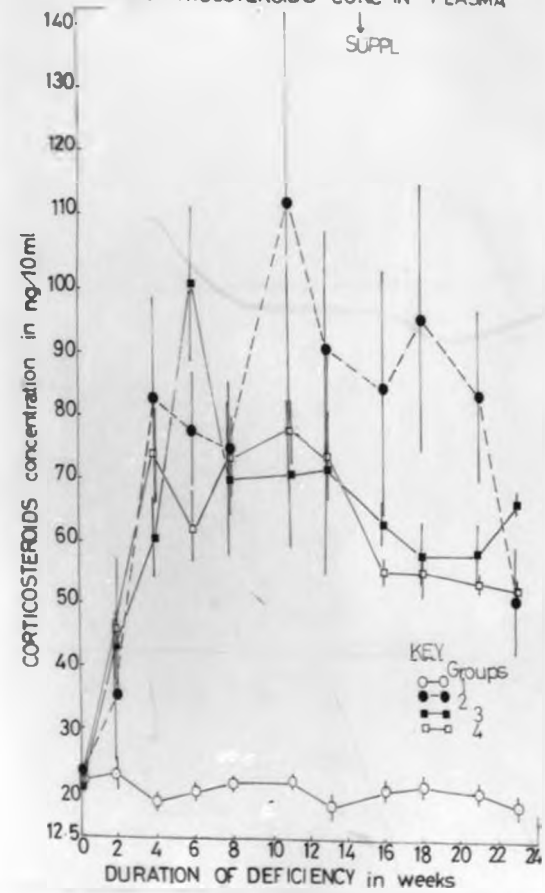


PLATE I. ADRENAL CORTEX.

Fig. L_1. Adrenal cortex for control group goat number 82 at x 4000 magnification. H. Azan stain. Zona fasciculata and reticularis. Cytoplasm slightly eosinophilic and small vesiculation at the periphery of the cytoplasm distinct. Few vesicular nuclei are present, many nuclei are dark staining showing a tendency towards pyknosis.

Fig. L_2. Adrenal cortex for deficient goat number 86 (group two - deficient) at x 4000 magnification. H. Azan stain. Hypertrophied adrenal cortex showing the zona fasciculata and reticularis with cells well defined and closely packed as indicated by reduced size of the intercellular space. Cytoplasm is plenty, finely granulated and extensively vacuolated. Nuclei are predominantly of the vesicular type. The foamy cytoplasm with plenty of Vacuoles are due to extraction of lipid during processing. Group 3 and 4 animals had a similar appearance in their adrenal glands as those for group 2.

Key: N = nucleus. C = cytoplasm.
V = cytoplasm vacuoles.

4:2:3. THE THYROID GLAND FUNCTION

Results of the thyroid gland functions are shown on Text Tables 20 and 21, Appendix Table 10 and 11, and individual values for respective animals are mapped in Figures P, O and Q.

Significant differences in Thyroxine (T_4) and Triiodothyronine (T_3) uptake were observed between group 1 and groups 2, 3 and 4, although there was considerable overlap between the groups. No significant differences were, however, found between T_4, T_3-uptake estimations in groups 2, 3 and 4. As depicted in Fig. P groups 2, 3 and 4 had 25 to 50% higher T_4 than controls. Similarly Free Thyopac Index (FTI) was higher in groups 2, 3 and 4 after about 6 weeks (Fig. Q).

Control group animals show little variations in T_4 and T_3 hormones as is shown by the heights of the SEM. Cobalt deficient animals, however, show great variations between individuals; the difference being more significant with increase in the duration of the deficiency. This result is shown by a linear regression (Fig N) and the heights of the SEM (Fig M).

Histology results are shown on Figs. R_1 to R_8. The thyroid gland in cobalt deficient animals was markedly enlarged ($P < 0.005$). The follicles in groups 2, 3 and 4 were smaller, more even and were bordered by thick basal lamina; the epithelia were of high columnar. (0.088 ± 0.0086 mm.). The epithelium in control animals was of simple squamous type (0.03± 0.004 mm). Furthermore the thyroid colloid was scantier and less easily stainable from goats on cobalt deficient diet. All the above changes were ameliorated but not obliterated by energy and protein supplementation (Figures M up to R).

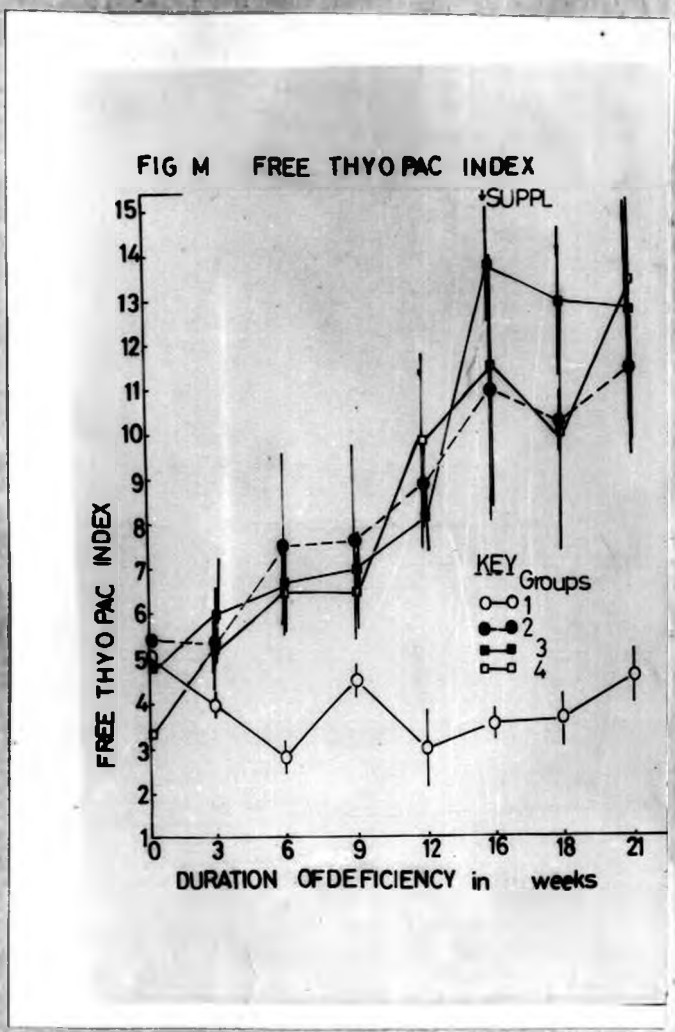


FIG M FREE THYO PAC INDEX
+SUPPL
FREE THYO PAC INDEX
DURATION OF DEFICIENCY in weeks
KEY Groups
1
2
3
4
0
3
6
9
12
16
18
21
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15

FIG N Regression of T_4 with T_3 Values

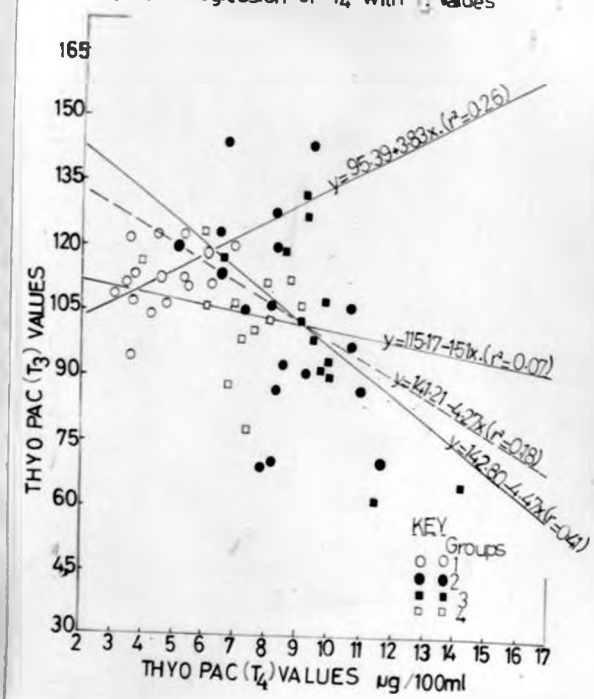


FIG O T_3 SEPHADEX UPTAKE/ T_4 VALUES

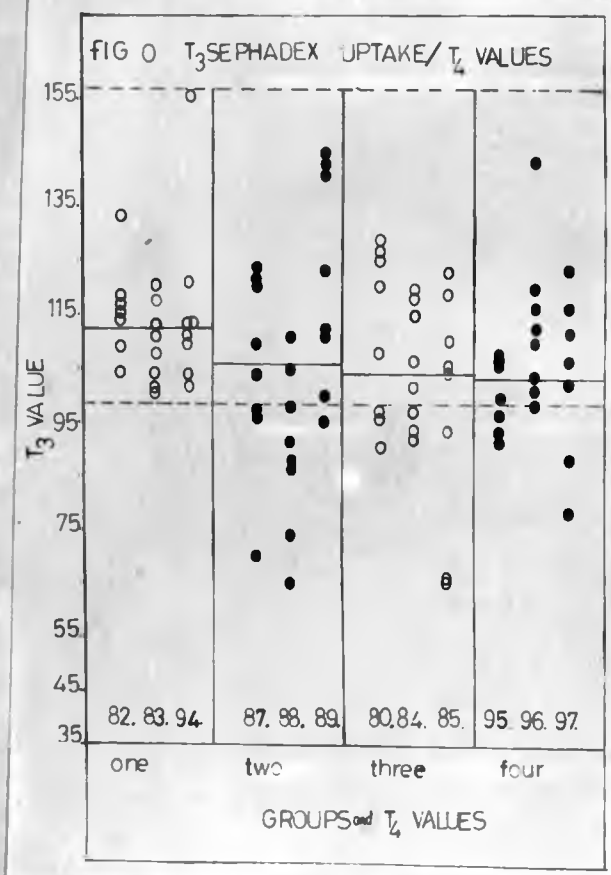


FIG P PLASMA THYROXINE (T_4).

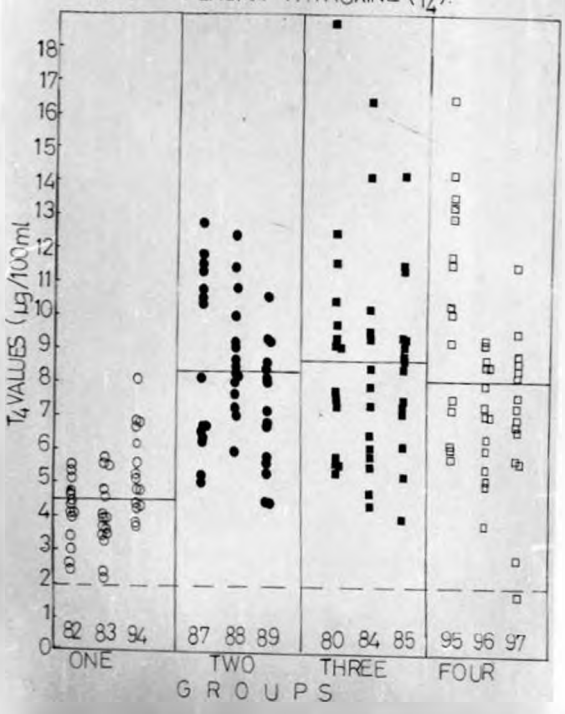


FIG Q. FREE THYO PAC INDECIS IN GOATS

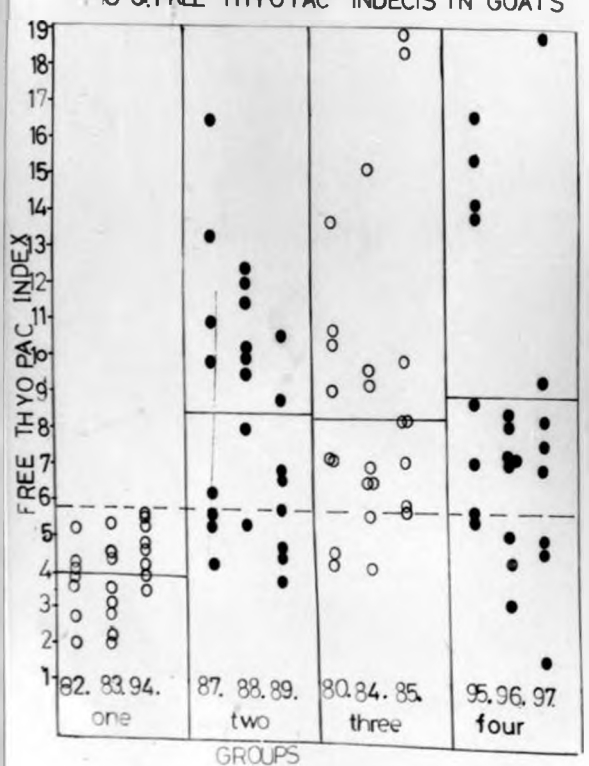
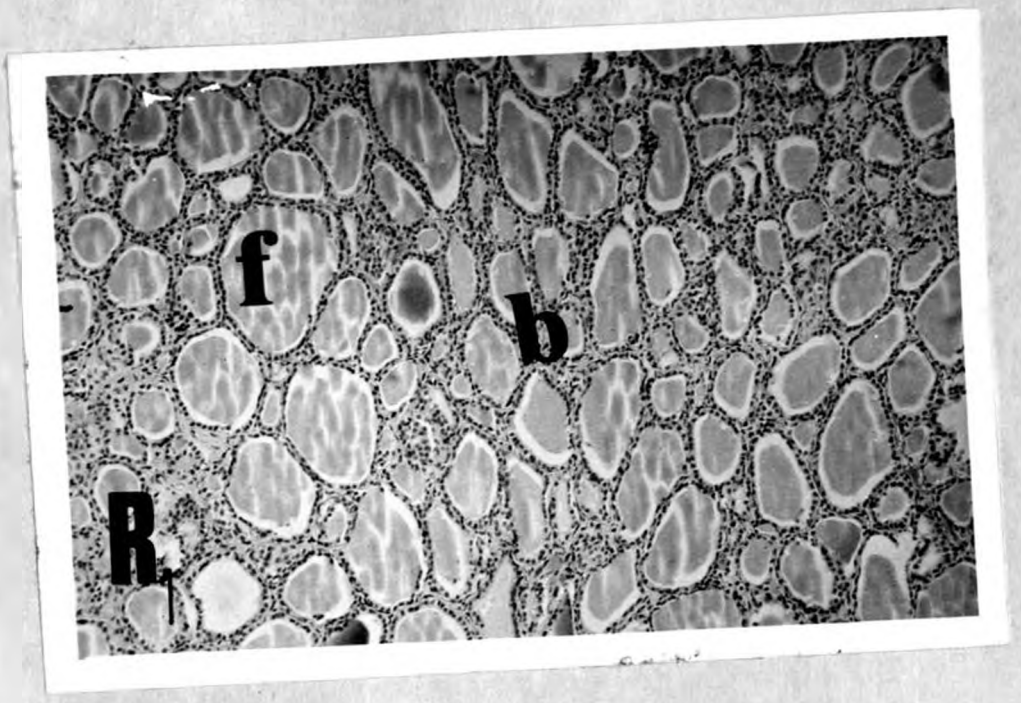


PLATE 2: THYROID GLAND

Fig. R_1 Control animal number 82 (x 1000 magnification) H.E. stain. Plate figure shows the presence of both large and small follicles, bordered by a thin basal lamina which was difficult to resolve by a light microscope. The small follicles of average diameter of 0.33 ± 0.02mm predominate over the large follicles of about 1.27 ± 0.14 mm. The nucleus is oval, and the cytoplasm is scarce. (All photos key:

f = follicular lumina. b = basal Lamina , n = nucleus.)

Fig. R_2 cobalt deficient animal number 88 (group 2) atx1000 magnification, H.E. stain. There is presence of even follicles of about 0.99 ± 0.08 mm for the largest and 0.64 ± 0.028 mm diameter for the smallest. These follicles are bordered by a thick basal lamina and interfollicular space. The nucleus is spherical and cytoplasm is plenty. The follicular lumina is small and the colloid is staining with reduced affinity.


f
b
R1

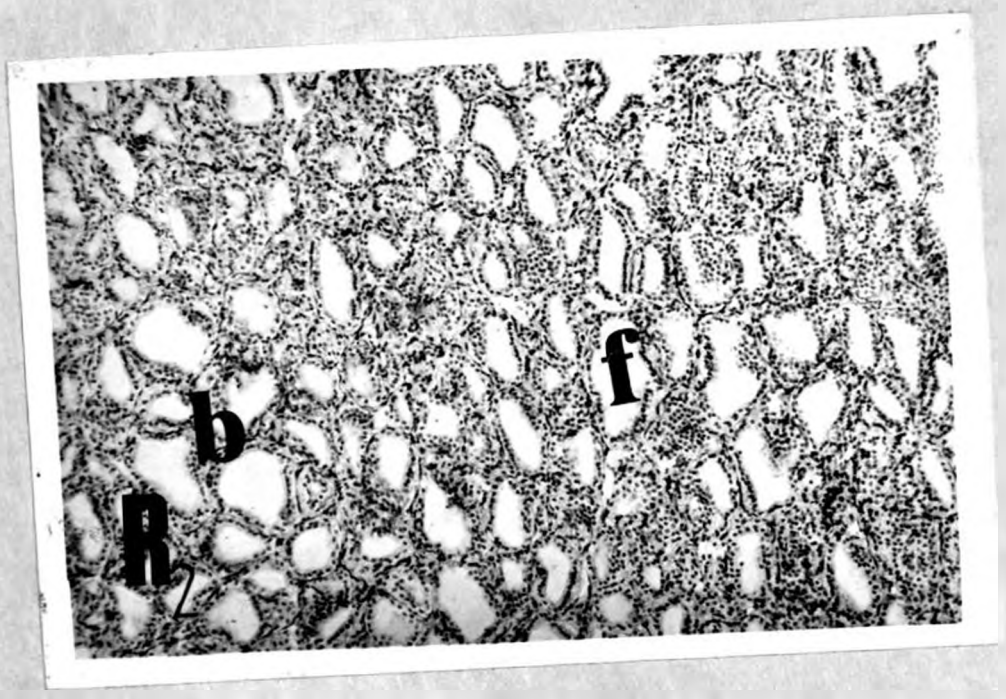

b
f
R2

PLATE 3: THYROID GLAND

Fig. R_3. Animal number 82 (x 2500 magnification) H.E. Stain. The epithelium is of simple squamous to low cuboidal of about 0.03 ± 0.004 mm high as compared to that of controls (Control group).

Fig. R_4. Animal number 88 (x 2500 magnification) H.E. stain. The epithelium is of tall columnar of about 0.088 ± 0.0086 mm high. Follicles have no stainable material when compared to that of control animals (Deficient group).

Fig. R_5. Animal number 97 at x 25000 magnification (Protein supplemented group four). H.E. stain. The figure shows that energy and protein supplementation reduced the degree of hypertrophy and hyperplasia. The figure shows the presence of both large (0.099 ± 0.08 mm) and small (0.33 ± 0.02 mm), follicles bordered by a thick interfollicular space and cytoplasm is plenty. The epithelium is of low columnar of about 0.056 ± 0.0034 mm high.

f
R3
b
f
R4
f
b
R5

PLATE 4: THYROID GLAND

Fig. 6. Animal number 82 (4000 magnification) H.E. stain. Thin squamous epithelium with dark staining nuclei, cytoplasm is scanty and follicles (f .) contain stainable colloid. (Control group).

Fig. 7. Animal number 88 (4000 magnification) H.E. stain. Thick cells with plenty of cytoplasm. The nuclei are pale and large. The basal lamina very distinct. In follicles there is no stainable colloid. Follicular lumina is small. The cells show appearance of vacuoles around the periphery of the nucleus. (Deficient group).

Fig. R_8. Animal number 85 at 4000 magnification (Energy supplemented group three). H.E. Stain. As for Fig. R_5.

f
n
b
f
R6

b
f
n
f
R7

f
b
f
n
R8

TEXT TABLE 20A

MEAN THYROXINE (T_4) VALUES

Duration of deficiency in weeks	GROUP I	GROUP 2	GROUP 3	GROUP 4
0	5.66 ± 0.29	6.00 ±0.25	5.84 ± 0.64	3.87 ±1.20
2	4.74 ± 0.28	5.56 ± 0.82	4.56 ± 0.66	5.84 ± 1.65
3	4.28 ± 0.20	6.05 ± 1.23	7.26 ± 0.95	6.01 ± 0.11
5	4.38 ± 0.60	7.39 ± 0.88	6.22 ± 0.70	7.68 ± 2.02
6	2.91 ± 0.42	7.12 ± 0.49	7.42 ± 0.89	7.58 ± 1.09
8	5.43 ± 0.81	7.54 ± 0.61	6.16 ± 0.57	8.07 ± 1.48
9	5.24 ± 0.78	7.44 ± 0.57	8.13 ± 0.83	7.33 ± 0.82
11.	3.57 ± 0.66	6.89 ± 0.81	12.60± 4.38	7.87 ± 1.21
12	3.31 ± 0.99	9.30 ± 0.69	8.20 ± 1.09	8.96 ± 1.21
14	3.72 ± 0.23	9.55 ± 1.87	10.45± 0.61	8.21 ± 0.69
15	3.90 ± 0.24	10.32± 0.65	10.83± 1.73	10.88± 2.85
17	5.15 ± 1.01	10.71± 0.88	11.87± 2.60	10.48± 1.56
18	4.23 ± 0.52	9.80 ± 0.78	12.03± 1.41	9.67 ± 2.35
20	4.97 ± 0.27	9.59 ± 1.12	8.99 ± 0.84	11.00± 0.86
21	5.68 ± 1.34	10.68± 1.28	10.09± 0.65	9.15 ± 1.94
Mean	4.48	8.28	8.76	8.21

* All values in µg/100 ml.

TEXT TABLE 20 B

MEAN PLASMA THYOPAC 3 VALUES

Duration of deficiency in weeks	GROUP I	GROUP 2	GROUP 3	GROUP 4
0	113.96±4.54	111.16±4.27	119.36±0.73	109.33±3.59
3	108.70±3.62	114.87±4.87	121.05±2.00	114.50±3.70
6	103.97±2.62	111.76±21.63	113.13±6.92	120.24±11.49
9	115.44±6.08	110.20±23.28	115.70±6.57	113.92±4.76
12	115.26±2.38	105.80±8.10	101.55±4.66	99.64±1.96
15	109.34±2.77	103.62±14.02	82.98±8.69	93.59±7.81
18	113.76±4.20	94.65±4.10	92.89±0.69	96.75±6.51
21	119.14±16.06	93.65±7.80	83.67±11.06	76.11±19.07

All values given as MEAN ± SEM (THYOPAC -3 VALUE).

TEXT TABLE 20C

MEAN FREE THYOPAC INDEX

Duration of deficiency in weeks	GROUP I	GROUP 2	GROUP 3	GROUP 4
0.	5.00±0.41	5.43±0.43	4.89±0.52	3.56±1.15
3	3.94±0.20	5.38±1.35	6.03±0.87	5.26±0.23
6	2.79±0.39	7.52±1.96	6.52±0.40	6.57±1.43
9	4.54±0.60	7.61±2.16	7.06±0.78	6.50±0.90
12	2.91±0.94	8.93±1.05	8.00±0.74	9.87±2.04
15	3.58±0.26	11.08±2.88	13.87±3.91	11.51±2.56
18	3.75±0.52	10.32±0.42	12.95±1.53	10.13±2.68
21	4.66±0.60	11.49±1.36	12.79±2.79	13.48±3.36

All values given as MEAN ± SEM

TEXT TABLE 21

(i) Analysis of variance of the effect of cobalt deficiency on free thyopac index in goats.

Source	df.	TSS	MS	F	P
Total	95	1533.04			
Treatment	3	404.58	134.86		
Error	92	1128.48	12.27	10.99**	0.005

Comparison among means

Group	1	3	2	4
Mean	3.90	8.36	8.43	9.01

FTI

LSD $P < 0.01 = 2.66$

LSD $P < 0.005 = 2.91$

Group 1 - 2 = 4.53** Group 3 - 4 = 0.65

Group 1 - 3 = 4.46** Group 2 - 3 = 0.07

Group 1 - 4 = 5.11** Group 2 - 4 = 0.58

Free Thyopac Index was higher in groups 2,3, and 4 as compared to the controls.

TEXT TABLE 21 Cont'd.

(ii) Analysis of variance of the effect of cobalt deficiency on Thyroxine (T_4) values, in between individual animal.

Source	df.	TSS	MS	F	P
Total	178	2701.89			
Treatment	12	1692.02	141.00		
Error	167	1009.87	6.05	23.31**	0.005

Comparison among means

Animal number	83	82	94	96	97	89
T_4 ug/100ml.	3.94	4.06	5.43	7.06	7.09	7.27

Animal number	84	85	87	88	80	95
T_4 ug/100ml.	8.25	8.51	8.66	8.85	9.36	10.47

LSD (P 0.05 = 1.76

LSD (P 0.01 = 2.96

Text Table 21 Cont'd.

(iii) Analysis of variance of the effect of cobalt deficiency on T_3 Sephadex uptake in goats,

Source	df.	TSS	MS	F	P
Total	95	27543.20			
Treatment	3	1336.53	445.51		
Error	92	26206.67	284.86	1.56	NS

Comparison among means

Group	4	3	2	1
T_3 uptake	103.01	103.79	105.64	112.45

LSD $P < 0.05$ = 9.55

Group 1 - 2 = 6.81	Group 3 - 4 = 0.78
Group 1 - 3 = 8.66*	Group 2 - 3 = 1.85
Group 1 - 4 = 9.44**	Group 2 - 4 = 2.63

There was a decrease in T_3 uptake that was significant in groups 3 and 4. by LSD analysis.

The F test, however, did not show significant decrease in T_3 uptake. The direct t-test showed an increase in T_3 uptake ($P < 0.05$).

.4:2:4: MAMMARY GLAND FUNCTION

PLATE 5

Fig. S_1 Control animal number 82 (2500 magnification). Goldner stain. More than 50% of the field is of glandular tissue. Alveoli distended with large amounts of stainable colloid in the lumen. Plenty of alveoli with irregular sizes and little connective tissue mass. Fat tissue is abundant. Percent estimates were done by use of a binocular micrometer (Leitz Wetzlar, Germany)

Fig. S_2. Cobalt deficient animal number 86 (2500 magnification). Goldner stain. The figure shows little glandular tissue. Alveoli present are small with little unstainable colloid. There are few alveoli cells that are vacuolated. There is no adipose tissue.

Key: con = connective tissue.
alv. = alveoli.

alv
S_1

duct
con
S_2

PLATE 6: MAMMARY GLAND

Fig. S_3. Cobalt deficient animal number 89 (2500 magnification). Goldner stain. Disappearance of alveolar tissue and presence of large ducts.

Fig. S_4. Energy supplemented animal 80. (2500 magnification). Goldner stain. Figure shows only a few alveoli. Most of the mammary glands are transitional between normal and deficient animals (Group 2). There are sparse connective tissue with relatively more alveoli structure than deficient groups (about 40 to 60%).

Key: Con = connective tissue

Alv = alveoli


alv
duct
con
S3

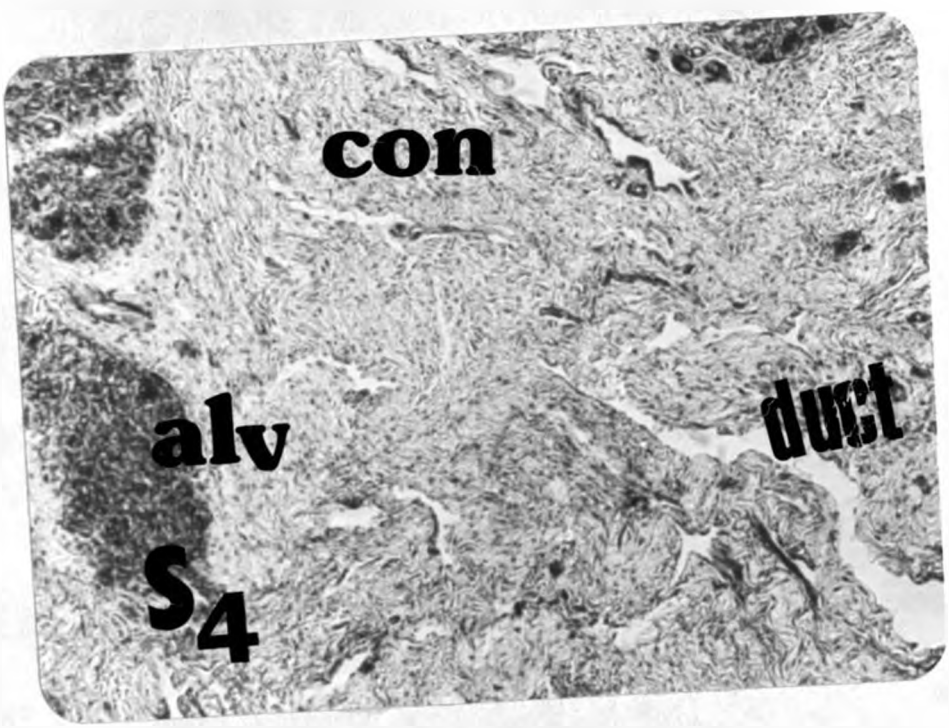
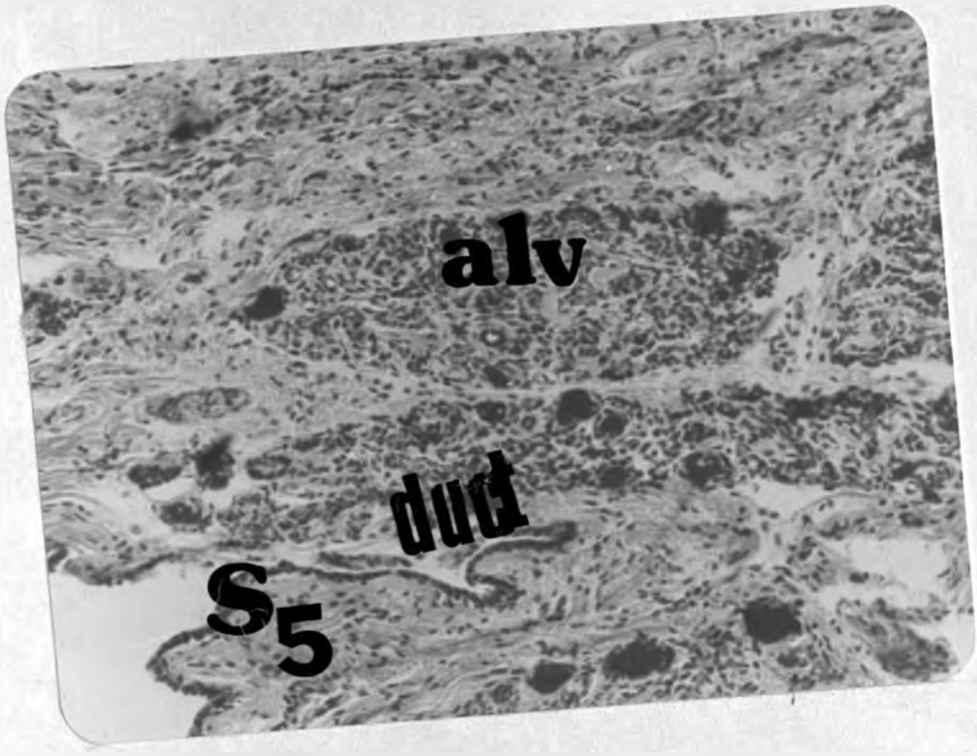

con
alv
duct
S4

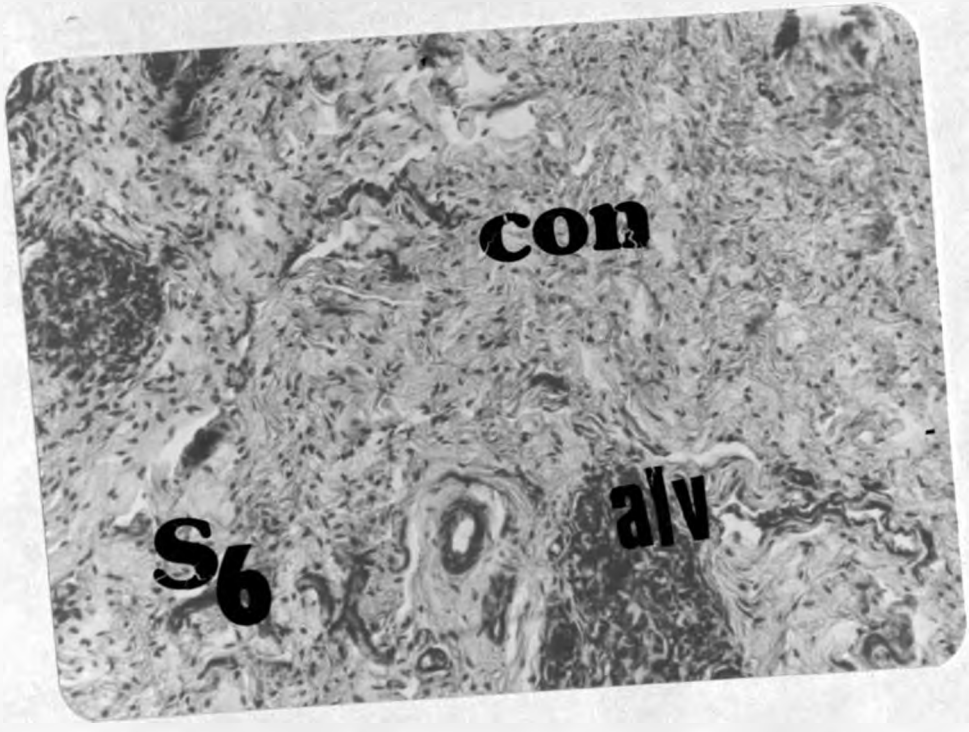
PLATE 7: MAMMARY GLAND

Fig. S_5. Animal number 97, protein supplemented (2500 magnification). Goldner stain. Absence of adipose tissues. Presence of few alveoli with stainable colloid. Large bands of connective tissue predominate.

Fig. S_6. Animal number 100. Protein supplemented. (2500 magnification). Goldner stain. Absence of adipose tissue, and alveolar structure and replaced by large connective tissue masses.

Key. Con = connective tissue
Alv. = alveoli


alv
dud
S5


con
alv
S6

4:2:5. PLASMA PROGESTERONE CONCENTRATION

Plasma progesterone values were subdivided into values according to days since oestrus thus with Day 0, Day 1 to Day 4, Day 5 to Day 8, Day 9 to Day-4, Day -3 to Day -1 values. Progesterone values in all the 5 control goats showed that peripheral progesterone values were low during oestrus, values of 0.64 ± 0.09 to 0.98 ± 0.20 ng/ml were obtained in this study. Subsequently the concentration increased during Day 1 to Day 4 of the cycle, and reached maximum values during Day 9 to Day -4 of the cycle, with (MEAN ± SEM) of 6.81 ± 0.31 ng/ml. Thereafter there was an abrupt decline during Day -3 to Day -1 of the cycle.

Plasma progesterone of Cobalt deficient goats showed the same cyclic pattern of changes as in controls. (groups 2, 3 and 4). Plasma progesterone concentration for the first oestrous cycle did not differ from those of the control group. However, in the second cycle peak plasma progesterone were higher than those for control animals. In the third and subsequent cycles peak plasma (t-test $P < 0.05$) progesterone declined to below controls.

Further results are shown on Text Tables 22 and 23, Appendix Table 12 and Figs T and V.

Although the changes in plasma progesterone values were initially seen on peak (Day + 9 to Day -4) values, later on decline at other stages of the cycle were observable in the cobalt deficient goats.

TEXT TABLE 22

MEAN PROGESTERONE VALUES IN NORMAL AND COBALT DEFICIENT GOATS: THE FIRST FOUR OESTROUS CYCLES

Number of cycle	Day since Oestrus(b)	Group 1 MEAN	S.E.M.	Group 2 MEAN	S.E.M.
Cycle 1	0	0.64	0.09	0.50	0.13
	1 to 4	1.57	0.29	1.98	0.21
	5 to 8	3.88	0.78	4.45	0.82
	9 to -4	6.77	0.58	7.03	0.43
	-3 to 0	3.91	0.32	3.20	0.42
Cycle 2	0	0.70	0.10	0.77	0.11
	1 to 4	2.39	0.28	1.86	0.36
	5 to 8	3.40	0.55	5.63	0.78
	9 to -4	6.74	0.59	9.83	0.87
	-3 to 0	2.87	0.26	3.84	0.91

		Group 3		Group 4	
Cycle 1	0	0.53	0.16	0.85	0.15
	1 to 4	2.18	0.39	1.68	0.27
	5 to 8	4.79	0.82	4.39	1.12
	9 to -4	7.53	0.57	7.34	0.97
	-3 to 0	3.94	0.74	3.15	0.50
Cycle 2	0	0.80	0.04	0.66	0.36
	1 to 4	2.42	0.65	2.05	0.32
	5 to 8	4.11	0.47	6.88	2.03
	9 to -4	8.05	0.49	8.37	0.82
	-3 to 0	4.05	0.65	3.37	1.03

(a) Number of goats is 5 per group

(b) Day in relation to oestrus - taken as Day 0

(c) All values in ng/ml plasma

TEXT TABLE 22

MEAN PROGESTERONE VALUES

Number of cycle	Day since Oestrus(b)	Group 1 MEAN	Group 1 S.E.M.	Group 2 MEAN	Group 2 S.E.M.
Cycle 3	0	0.85	0.04	0.34	0.08
	1 to 4	2.53	0.47	0.67	0.24
	5 to 8	5.34	1.18	1.53	0.32
	9 to -4	6.91	0.56	4.35	0.74
	-3 to 0	4.45	1.68	2.98	0.57
Cycle 4	0	0.98	0.20	0.37	0.19
	1 to 4	3.78	0.56	0.84	0.24
	5 to 8	5.66	0.69	1.94	0.57
	9 to -4	6.72	0.60	4.26	0.67
	-3 to 0	3.97	0.64	1.61	0.51

		Group 3		Group 4	
Cycle 3	0	0.73	0.08	0.41	0.12
	1 to 4	1.85	0.35	1.90	0.41
	5 to 8	3.48	0.45	3.64	0.16
	9 to -4	4.50	0.26	4.73	0.19
	-3 to 0	2.23	0.53	2.77	0.10
Cycle 4	0	0.40	0.11	0.44	0.13
	1 to 4	1.16	0.28	1.21	0.38
	5 to 8	2.74	0.38	2.98	0.79
	9 to -4	3.82	0.23	3.37	0.47
	-3 to 0	1.73	0.11	1.79	0.69

(a) Number of goats is 5 per group (b) Day in relation to Oestrus taken as Day 0. (c) All values in ng/ml.

TEXT TABLE 22

MEAN PROGESTERONE FOR DEFICIENT ANIMALS

		Groups 2, 3 and 4 Deficient	
Number of cycle	Day since Oestrus	MEAN	S.E.M. (ng/ml)
Cycle 1	0	0.63	0.10
	1 to 4	1.95	0.16
	5 to 8	4.54	0.42
	9 to -4	7.30	0.32
	-3 to 0	3.26	0.37
Cycle 2	0	0.74	0.10
	1 to 4	2.11	0.23
	5 to 8	5.54	0.77
	9 to -4	8.42	0.61
	-3 to 0	3.75	0.41
Cycle 3	0	0.49	0.09
	1 to 4	1.47	0.30
	5 to 8	2.88	0.45
	9 to -4	4.53	0.22
	-3 to 0	2.66	0.25
Cycle 4	0	0.38	0.07
	1 to 4	1.07	0.16
	5 to 8	2.55	0.34
	9 to -4	3.82	0.27
	-3 to 0	1.71	0.23

Day in relation to oestrus - taken as Day 0 . All values given as MEAN ± SEM ng/ml. (15 animals).

TEXT TABLE 23A

Analysis of variance of the effect of cobalt deficiency on peak plasma progesterone (Day 9 to Day - 4) values of the first four successive cycles. (deficient group 2, 3, and 4)

Source	df.	TSS	MS	F	P
Total	15	52.29			
Treatment	3	36.07	12.02		
Error	12	16.22	1.35	8.9**	0.025

Comparison among means

Cycle number	4	3	2	1
Prog.(ng/ml)	4.45	5.12	8.25	7.17

LSD $P \cdot 0.05 = 1.79$

Cycle 1 - 4 = 2.63* Cycle 2-4 = 5.71**

Cycle 1 - 3 = 2.05* Cycle 2-3 = 3.13 **

Cycle 1 - 2 = 1.08 Cycle 3-4 = 0.58

An increase in peak progesterone was observed in the second cycle, followed by decline in subsequent cycles ($P > 0.025 < 0.05$).

N.B. Direct analysis of individual peak plasma progesterone by the t test; however, show that there is an initial increase in progesterone ($P < 0.05$) followed by decline in the subsequent cycles ($P < 0.025$).

TEXT TABLE 23 B

Analysis of variance of the effect of cobalt deficiency on mean progesterone values of the first four cycles in:-

Group 1 - Control

Source	d.f.	TSS	MS	F	P
Total	114	794.47			
Treatment	3	12.29	4.10		
Error	111	782.18	7.05	0.58	NS

NS thus indicating in the control group plasma progesterone values did not differ during the first four oestrous cycles.

Group 2 - Deficient

Source	df.	TSS	MS	F	P
Total	115	1048.70			
Treatment	3	142.50	47.50		
Error	112	906.20	8.09	5.87**	0.05

Comparison among means

Cycle number	3	4	1	2
Progesterone (ng/ml)	2.08	2.25	4.23	4.58

LSD $P < 0.05 = 1.92$

Cycle 1 - 2 = 0.35 Cycle 1 - 4 = 1.98*

Cycle 2 - 3 = 2.50* Cycle 1 - 3 = 2.15*

Cycle 3. - 4 = 0.17 Cycle 2 - 4 = 2.33*

Text Table 238 Cont'd.

Group 3 - Energy

Source	df.	TSS	MS	F	P
Total	139	679.04			
Treatment	3	86.13	28.71		
Error	136	592.91	4.36	6.58*	0.01

Comparison among means

Cycle number	4	3	2	1
Progesterone ng/ml	2.54	2.88	4.23	4.26

LSD LSD P $\leq$ 0.05 = 1.42

LSD P $\leq$ 0.100 = 1.19

Cycle 1 - 2 = 0.03 Cycle 3 - 4 = 0.34

Cycle 1 - 3 = 1.38* Cycle 2 - 3 = 1.35*

Cycle 1 - 4 = 1.72* Cycle 2 - 4 = 1.69*

Group 4 - Protein

Source	df.	TSS	MS	F	P
Total	112	1394.38			
Treatment	3	113.37	37.39		
Error	109	1281.01	11.75	3.22*	0.05

Comparison among means

Cycle number	1	2	3	4
Progesterone ng/ml	3.95	5.13	3.14	2.26

LSD P < 0.01 = 1.79

LSD P < 0.05 = 1.35

Cycle 1 - 2 = 1.18 Cycle 3 - 4 = 0.88

Cycle 1 - 3 = 0.81 Cycle 2 - 3 = 1.99**

Cycle 1 - 4 = 1.69* Cycle 2 - 4 = 1.88**

In groups 2, 3 and 4, progesterone values decreased significantly in the fourth cycle, and thus a difference from the first cycle.

FIG T_1 MEAN PROGESTERONE VALUES.

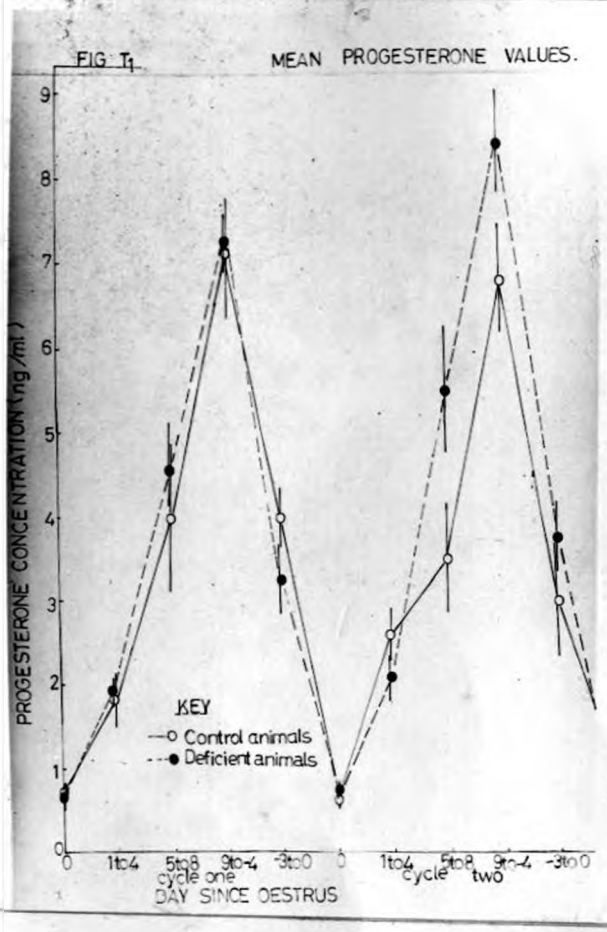
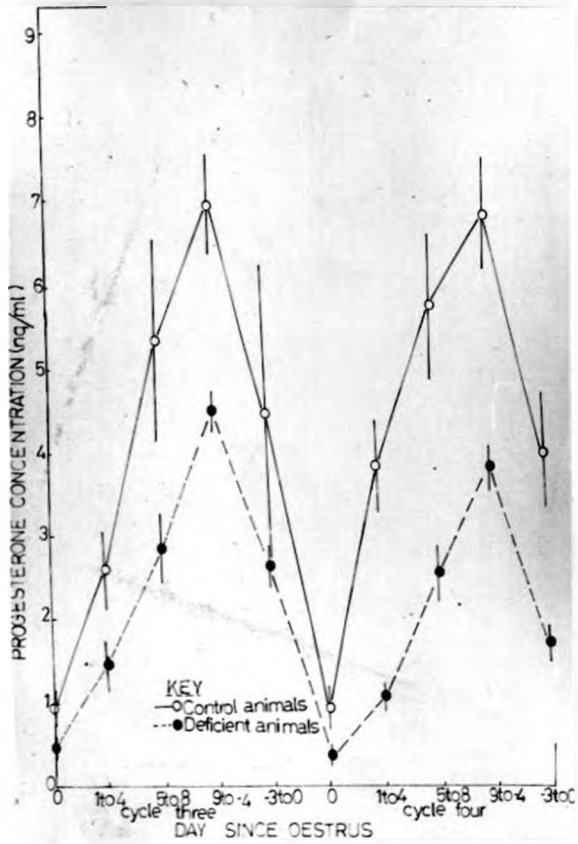


FIG T_2 MEAN PROGESTERONE VALUES



4:2:6. TOTAL UNCONJUGATED OESTROGENS IN PLASMA

As in the case of progesterone, total unconjugated oestrogens were analysed after dividing the values into rising, peak, declining and low stages of the cycle ie Day 10 to Day -2, Day-1 to Day +1, Day 2 to Day 6 and Day 7 to Day 10 of the oestrous cycle.

Oestrogen values in the control animals did not differ at the corresponding stages of the cycles during the experimental period. The results have been recorded on Text Table 24, 25 and 26, and Appendix Table 12.

Animals on cobalt deficient diets, however, had significant decrease in total unconjugated oestrogens in the third and subsequent cycles. The decrease was initially more marked in peak oestrogens values (Day -1 to Day 1, $P < 0.025$). Cyclic changes in oestrogens during the experimental period (of 23 weeks) were not, however, as marked as changes in progesterone values (See Fig. U).

TABLE 24

MEAN CONCENTRATION (a) OF OESTROGENS IN NORMAL AND COBALT DEFICIENT GOATS

Number of oestrous Cycle	Day since estrus (b)	Group 1 MEAN	Group 1 S.E.M.	Group 2 MEAN	Group 2 S.E.M.
Cycle 1	-1 to 1	64.18	6.77	71.07	5.68
	2 to 6	13.56	2.24	18.20	2.06
	7 to 10	11.73	2.10	10.30	1.61
	10 to -2	23.89	4.65	27.25	4.33
Cycle 2	-1 to 1	75.86	2.34	70.94	8.46
	2 to 6	15.39	1.80	25.85	4.06
	7 to 10	9.39	1.68	18.09	3.82
	10 to -2	17.84	4.96	33.20	5.17
Cycle 3	-1 to 1	63.77	10.84	58.33	7.68
	2 to 6	17.60	3.77	16.68	2.21
	7 to 10	13.46	2.92	12.30	3.09
	10 to -2	18.76	3.94	17.81	2.77
Cycle 4	-1 to 1	78.69	4.38	44.65	2.21
	2 to 6	12.13	2.39	14.51	3.73
	7 to 10	22.13	6.06	15.84	3.69
	10 to -2	29.12	7.53	18.28	5.38

(a) Concentration in pg/ml

(b) Day of oestrus being day 0

Table 24 cont'd

Number of oestrus cycle	Day since oestrus (b)	Group 3 MEAN	Group 3 S.E.M.	Group 4 MEAN	Group 4 S.E.M.
Cycle 1	-1 to 1	71.10	4.94	73.84	9.79
	2 to 6	17.52	3.35	25.11	8.08
	7 to 10	13.84	1.33	15.46	3.29
	10 to -2	33.10	4.88	29.81	7.55
Cycle 2	-1 to 1	76.04	8.05	76.68	9.69
	2 to 6	18.61	4.28	17.63	2.38
	7 to 10	13.83	3.08	10.28	3.08
	10 to -2	23.17	3.30	25.00	3.06
Cycle 3	-1 to 1	55.02	2.97	37.13	5.64
	2 to 6	9.61	1.13	12.67	0.89
	7 to 10	11.98	1.48	13.85	3.36
	10 to -2	15.03	1.25	13.67	2.31
Cycle 4	-1 to 1	45.39	1.31	42.47	4.13
	2 to 6	5.84	1.16	18.12	3.27
	7 to 10	13.30	3.70	10.78	1.96
	10 to -2	17.66	2.28	17.78	3.29

(a) Concentration in pg/ml.

(b) Day of oestrus being Day 0.

TEXT TABLE 24.
MEAN OESTROGENS VALUES FOR GROUPS 2, 3 and 4

Number of cycle	Day since Estrus -1 to 1	2 to 6	7 to 10	10 to -2
Cycle 1	72.01 ±3.24	20.28 ±2.78	13.20 ±1.39	30.05 ±2.79
Cycle 2	74.55 ±4.08	20.70 ±2.32	14.07 ±2.07	27.12 ±2.63
Cycle 3	50.16 ±4.90	12.99 ±1.46	12.71 ±1.29	15.50 ±1.25
Cycle 4	44.17 ±1.37	12.82 ±2.65	13.31 ±1.71	17.91 ±1.74

All values given as MEAN ± SEM (pg/ml).

Statistical analysis using the t-test shows that cycle 3, and 4 peak oestrogens (Day -1 to Day 1) were lower in cobalt deficient goats than controls. ($P < 0.050$). There was also a transient increase in cycle 2.

TEXT TABLE 25

Analysis of variance of the effect of cobalt and B_{12} deficiency on peak plasma oestrogens (Day - 1 to Day 1) of the first four successive cycles.

Source	df.	TSS	MS	F	P
Total	15	2904.55			
Treatment	3	1535.13	511.71		
Error	12	1369.42	114.12	4.48**	0.05

Comparison among means

Cycle number	4	3	1	2
Oestrogens pg/ml	52.80	69.48	70.05	74.88

Cycle 1 - 2 = 4.83 Cycle 2 - 3 = 5.4

Cycle 1 - 3 = 0.57 Cycle 2 - 4 = 22.08

Cycle 1 - 4 = 17.25* Cycle 3 - 4 = 16.68*

LSD P $<$ 0.05 = 16.45

In the fourth cycle plasma peak oestrogens declined significantly.

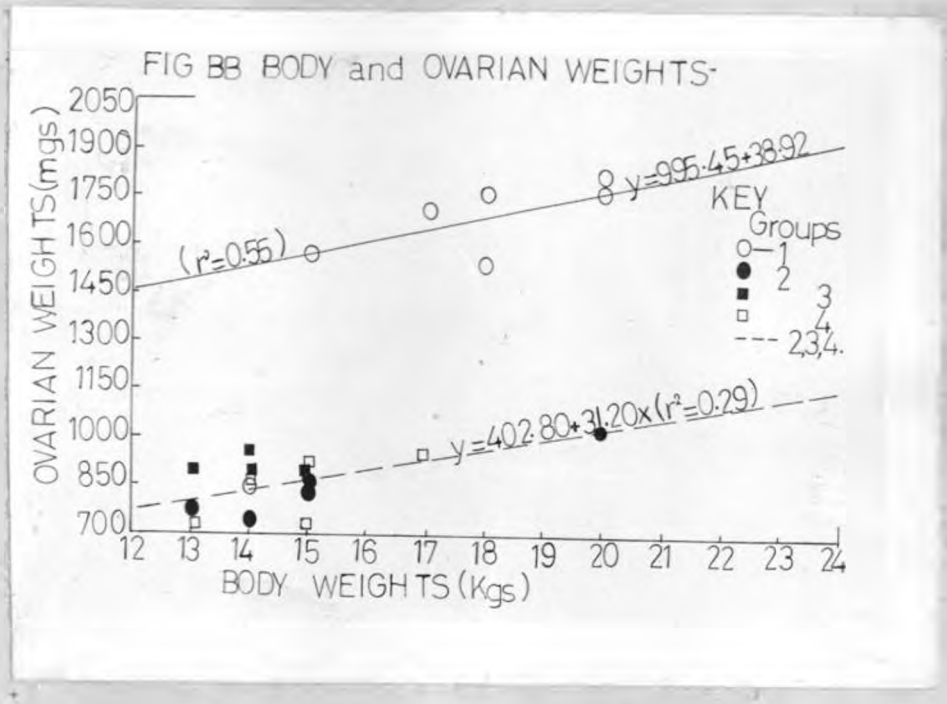


FIG BB BODY and OVARIAN WEIGHTS-
OVARIAN WEIGHTS(mgs)
2050
1900
1750
1600
1450
1300
1150
1000
850
700
y=995.45+38.92
(r²=0.55)
y=402.80+31.20x(r²=0.29)
KEY
Groups
1
2
3
4
2,3,4.
12 13 14 15 16 17 18 19 20 21 22 23 24
BODY WEIGHTS (Kgs)

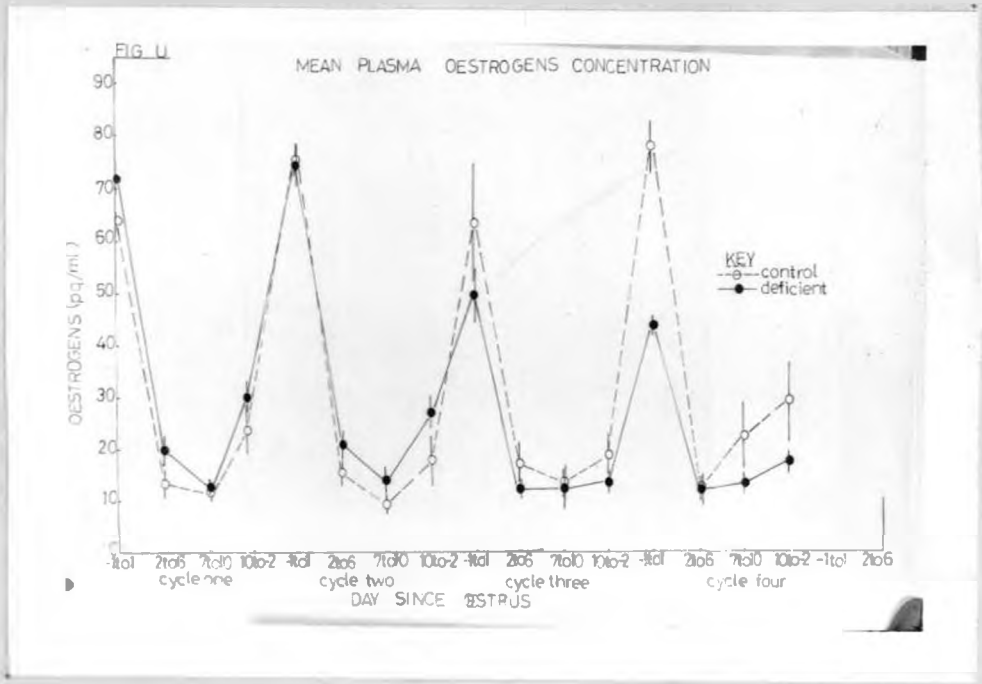


FIG U
MEAN PLASMA OESTROGENS CONCENTRATION
OESTROGENS (pg/ml)
90
80
70
60
50
40
30
20
10
KEY
control
deficient
-1to1 2to6 7to10 10to-2 -1to1 2to6 7to10 10to-2 -1to1 2to6 7to10 10to-2 -1to1 2to6 7to10 10to-2 -1to1 2to6
cycle one
cycle two
cycle three
cycle four
DAY SINCE OESTRUS

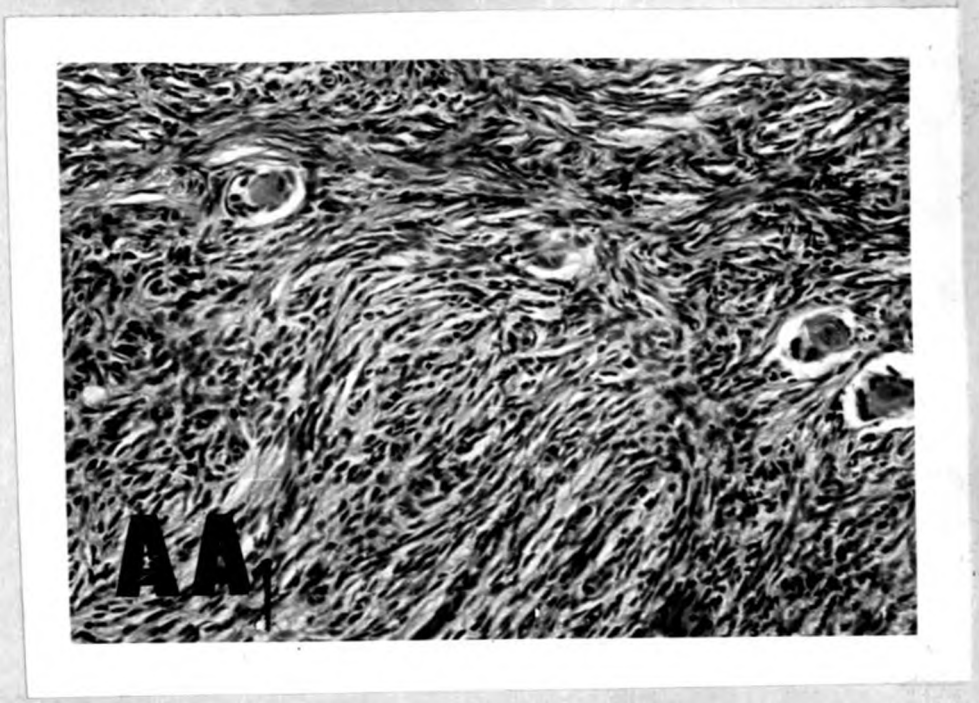
4:2:7 THE OVARY

Results of ovarian weights and histology are shown on Text Table 21 and 27 and Figures AA and BB. Deficient goats had markedly lower ovarian weights; ovarian weights were 790.5 ± 28.67, 869.80 ± 3.36, 861.06 ± 29.01 (MEAN + SEM gms) for groups 2, 3 and 4, respectively as compared to 1750.58± 65.28 in the control animals. The decrease was more marked when ovarian weights were expressed as ratios of body weights. ($P < 0.01$).

PLATE 8: OVARY

Fig. AA_2. Animal number 86; H.E. stain. x 4000 magnification. (group 2 - deficient). Ovaries of cobalt deficient goats from groups 2, 3 and 4 show absence of primordial and primary follicles as compared to controls.

Fig. AA_1. Animal number 82. H.E. stain. x 4000 Magnification. (Group 1 - control). Presence of primordial follicles and secondary follicles in different stages of development.


AA1

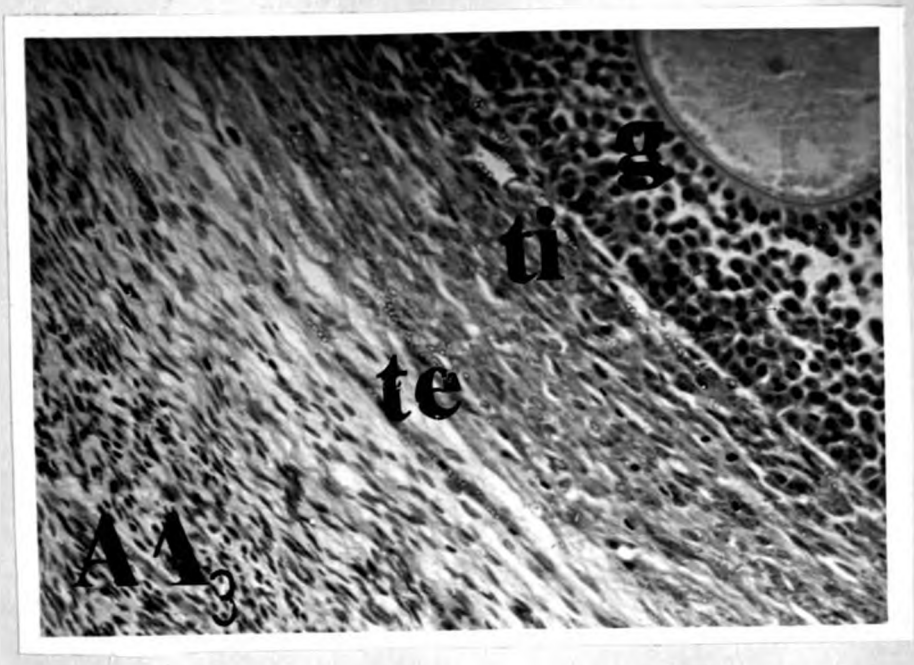

AA2

PLATE 9: OVARY

Fig. AA_4 Animal number 86; H.E. stain, 4000 magnification (Cobalt deficient group 2). Although few distorted primordial follicles are observed in the deficient group , there are multiple large follicles lined by sloughing, pyknotic granulosa cells; the theca interna and membrana granulosa are small. This suggests development of follicles up to the antral stage only.

Fig. AA_3 Animal number 82; H.E. stain, 4000 magnification (Control group 1). There are follicles with thick intact theca interna and externa, and granulosa cells.

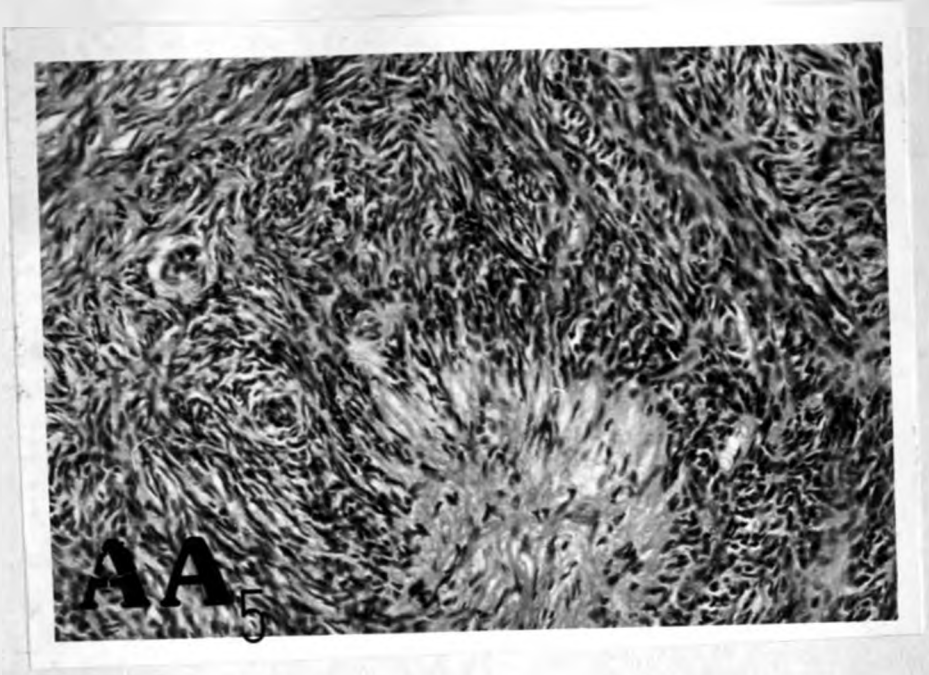
Key: (ti) theca interna
(te) theca externa.
(g) granulosa cells.

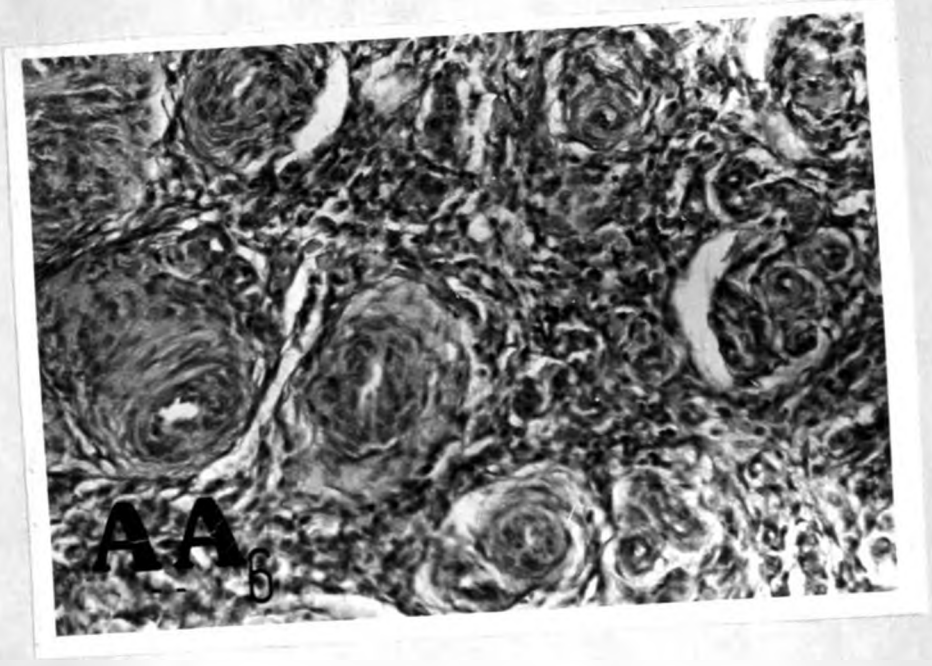

g
ti
te
A A3


g
ti
te
A A4

PLATE 10: OVARY.

Fig. AA4 Animal number 86; HE stain. 500 magnification (group 2, deficient). Shows hypertrophy of the ovarian strome. There was absence of corpora Lutea though in one group 4 animal, there was a CL from a past cycle. (See Animal number 100, H.E. stain, 500 magnification; Fig. AA5).


AA5


AA6

TEXT TABLE 27

(i) Analysis of variance of the effect of cobalt deficiency on ovarian/body weight ratios.

Source	df.	TSS	MS	F	P
Total	18	4187.81			
Treatment	3	3715.27	1238.42		
Error	15	472.54	31.50	39.31**	0.001

Comparison among means

Group	2	4	3	1
Ratio	56.51	58.40	64.04	91.01

LSD $P < 0.01 = 21.06$
LSD $P < 0.05 = 11.02$

Group 1 - 2 = 34.50** Group 3 - 4 = 5.64
Group 1 - 3 = 32.61** Group 2 - 3 = 7.53
Group 1 - 4 = 26.97** Group 2 - 4 = 1.89

(ii) Analysis of variance of the effect of cobalt deficiency on ovarian weights.

Source	df.	TSS	MS	F	P
Total	18	18418101.87			
Treatment	3	18283919.14	6094639.71		
Error	15	134182.73	8945.52	681.31**	0.001

Comparison among means

Group	2	4	3	1
Mean	790.50	861.00	869.80	1750.58

LSD $P < 0.01 = 355.17$
LSD $P < 0.05 = 185.82$

Group 1 - 2 = 960.08** Group 3 - 4 = 8.80
Group 1 - 3 = 889.58** Group 2 - 3 = 79.30
Group 1 - 4 = 880.78** Group 2 - 4 = 70.50

4: 2: 8. PERIPHERAL LUTEINIZING HORMONE VALUES. (PLASMA)

Peripheral LH values were analysed with similar consideration to plasma progesterone and oestrogen as shown on Text Table 28. Maximum LH values in the control animals were achieved on Day 0 of oestrus, with minimal levels occuring around Day 5 to Day -4 of the cycle. There was a small but insignificant rise on Day -3 to Day -1. Day 0 LH in cobalt deficient Groups 2, and 4 differed significantly from control right from cycle I ($P < 0.05$). This difference became more enhanced in cycles 2 and 3 following a general rise in peak LH in all the deficient goats (Groups 2, 3 and 4) ($P < 0.01$). Thereafter Day 0 LH of deficient groups declined to control levels. Further results on LH are shown on Figure V and W, Text Tables 28 and Appendix Table 12.

TEXT TABLE 28

MEAN LH VALUES IN NORMAL AND COBALT DEFICIENT GOATS

		GROUP NUMBER			
Number of cycle	Day since Oestrus (a)	Group 1 MEAN	S.E.M.	Group 2 MEAN	S.E.M
Cycle I	0	10.93	0.44	12.35	1.36
	1 to 4	2.33	0.29	1.54	0.41
	5 to 8	1.79	0.35	2.22	0.34
	9 to -4	1.67	0.25	1.50	0.29
	-3 to 0	2.63	0.27	3.24	0.34
Cycle 2	0	9.14	0.66	14.51	1.68
	1 to 4	1.69	0.37	3.02	0.40
	5 to 8	1.40	0.40	4.86	1.19
	9 to -4	1.56	0.36	2.58	0.41
	-3to 0	2.26	0.23	3.26	0.61
Cycle 3	0	8.45	0.63	17.60	3.96
	1 to 4	1.88	0.47	0.81	0.24
	5 to 8	1.36	0.27	1.23	0.35
	9 to -4	1.69	0.27	1.10	0.18
	-3 to 0	2.28	0.19	2.21	0.21
Cycle 4	0	9.06	0.29	11.13	2.15
	1 to 4	1.90	0.21	2.25	0.67
	5 to 8	1.46	0.32	2.83	1.14
	9 to -4	1.86	0.30	1.89	0.17
	-3 to 0	3.03	0.26	2.79	0.65

(a) Day of oestrus taken as Day 0

(b) All values given as MEAN ± SEM ng/ml plasma

Text Table 28 cont'd.

Number of cycle	Day since Oestrus (a)	Group 3 MEAN	Group 3 S.E.M.	Group 4 MEAN	Group 4 S.E.M.
	0	11.14	1.82	13.28	1.81
	1 to 4	2.11	0.47	2.57	0.30
Cycle 1	5 to 8	3.97	1.39	1.83	0.60
	9 to -4	3.06	0.73	3.01	0.43
	-3 to 0	3.29	0.50	3.64	0.68
	0	13.41	1.94	13.50	1.69
	1 to 4	2.15	0.66	3.10	0.20
Cycle 2	5 to 8	2.16	0.38	2.65	0.60
	9 to -4	2.88	0.48	2.67	0.84
	-3 to 0	3.78	0.53	3.84	0.44

(a) Day of oestrus taken as Day 0
(b) All values given as MEAN ± SEM ng/ml.

Text Table 28 cont'd

Number of cycle	Day since Oestrus (a)	Group 3 MEAN	Group 3 S.E.M.	Group 4 MEAN	Group 4 S.E.M.
Cycle 3	0	14.85	1.87	17.11	2.65
	1 to 4	1.38	0.29	2.83	0.60
	5 to 8	1.29	0.26	2.40	0.47
	9 to -4	2.23	0.29	3.16	0.79
	-3 to 0	2.74	0.34	3.68	0.61
Cycle 4	0	10.95	1.55	11.52	1.19
	1 to 4	2.04	0.44	1.48	0.30
	5 to 8	2.25	0.59	2.77	1.20
	9 to -4	2.48	0.59	1.65	0.32
	-3 to 0	2.83	0.35	3.83	1.19

(a) Day of oestrus taken as Day 0

(b) All values given as MEAN ± SEM ng/ml.

TEXT TABLE 28

MEAN L.H. FOR GROUPS 2, 3 AND 4

Number of cycle	Day since Oestrus 0	1 to 4	5 to 8	9 to-4	-3 to 0
Cycle I	12.26 ±0.85	2.07 ±0.26	2.67 ±0.58	2.52 ±0.40	3.38 ±0.25
Cycle II	13.81 ±0.82	2.76 ±0.28	3.22 ±0.64	2.71 ±0.28	3.63 ±0.26
Cycle III	16.52 ±1.43	1.67 ±0.42	1.64 ±0.29	2.16 ±0.44	2.88 ±0.33
Cycle IV	11.20 ±0.76	1.92 ±0.26	2.61 ±0.46	2.01 ±0.24	3.15 ±0.42

NB. All values given as MEAN ± SEM (ng/ml). Day of oestrus taken as Day 0.

TEXT TABLE 29

(i) Analysis of variance of the effect of cobalt deficiency on Day 0 Plasma LH

Source	df.	TSS	MS	F	P
Total	15	108.29			
Treatment	3	53.65	17.88		
Error	12	54.65	4.55	3.93*	0.05

Comparison among means

Group	1	3	4	2
Mean LH conc. ng/ml	9.40	12.59	13.85	13.90

LSD $P < 0.01 = 4.61$

LSD $P < 0.05 = 3.29$

Group 1 - 2 = 4.50* Group 3 - 4 = 1.26

Group 1 - 3 = 3.19 Group 2 - 3 = 1.31

Group 1 - 4 = 4.45 Group 2 - 4 = 0.05

(ii) Analysis of variance of the effect of cobalt deficiency on Day 0 LH values of control group against pooled values for deficient groups (2, 3 and 4). (cycles 1, 2, 3 and 4).

Source	df.	TSS	MS	F	P
Total	15	108.29			
Treatment	4	97.34	24.34		
Error	11	10.95	1.00	24.34*	0.001

Text Table 29 A cont'd

Comparison among means

Cycle number	control groups	Deficient 4	1	group cycles. 2	3
Mean LH ng/ml	9.40	11.20	12.26	13.81	16.52

LSD $P < 0.01$ = 3.57

LSD $P < 0.05$ = 2.54

Control - 1 = 2.86* Control - 4 = 1.80

Control - 2 = 4.41** Cycle 1 - 2 = 1.55

Control - 3 = 7.12** Cycle 1 - 3 = 4.26**

Cycle 1 - 4 = 1.06

Day 0 LH values were higher in the deficient group during cycles 1, 2, 3 than those of the control group, however, cycle 4 values are not different.

FIG V1 MEAN [redacted] AND LH VALUES

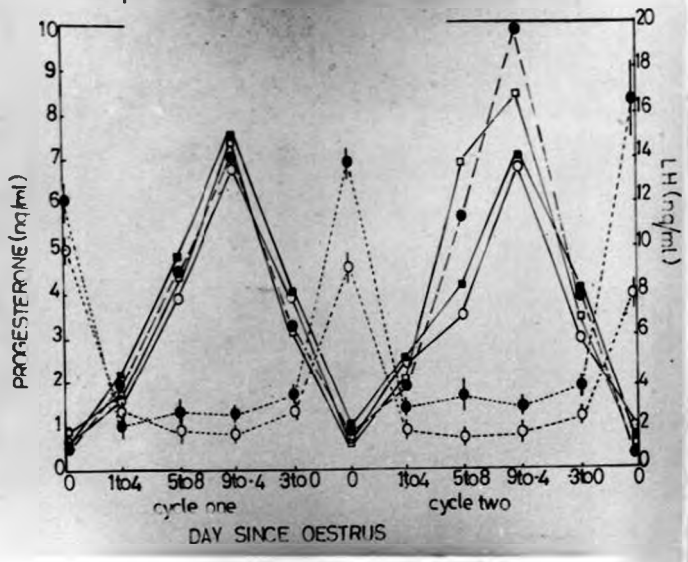


FIG V2 MEAN PROGESTERONE AND LH VALUES

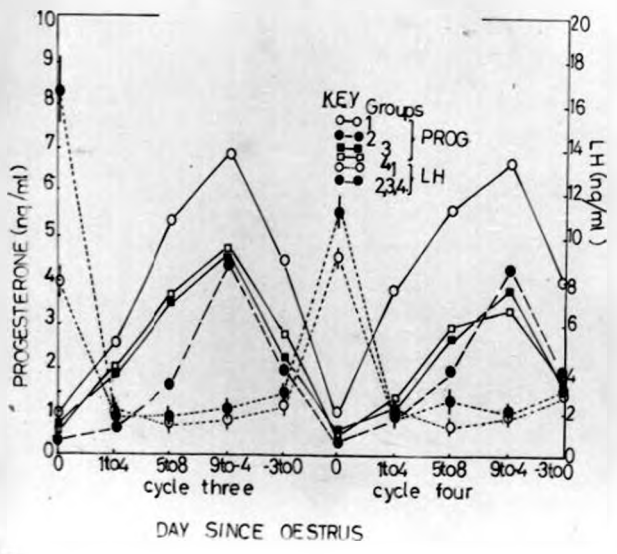


FIG V1 MEAN PROGESTERONE AND LH VALUES

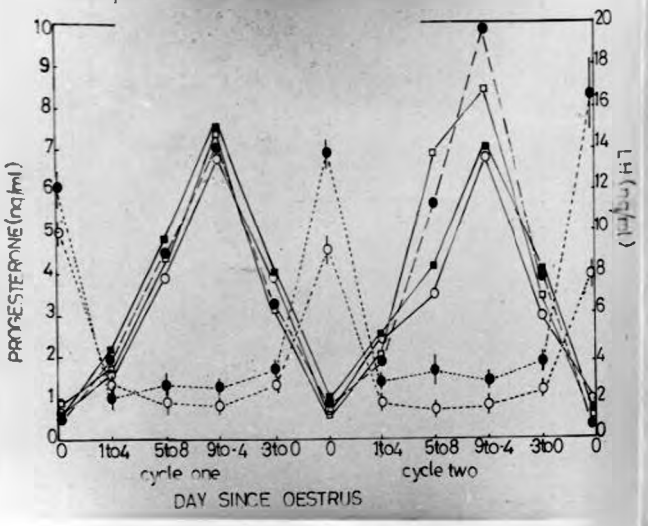
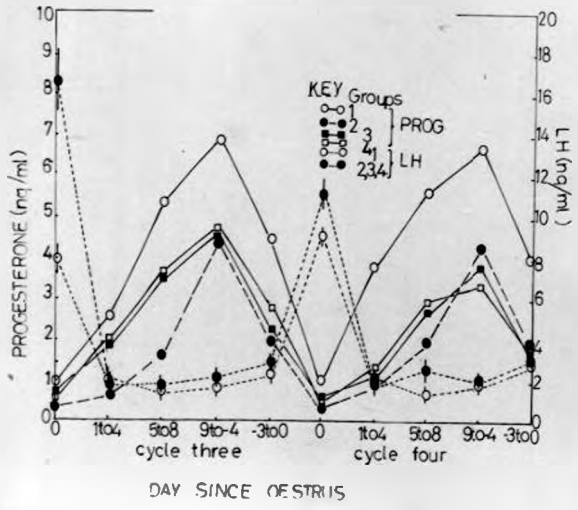


FIG V2 MEAN PROGESTERONE AND LH VALUES



4:2:9. THE PITUITARY GLAND

The purpose for examining the pituitary gland in this study was to try and observe whether release, synthesis and storage of LH was altered in cobalt deficient goats. The results have been recorded on Text Table 30 and 31 Appendix Table 13 and Figures W, X and Y.

It can be noted that in cobalt deficient goats the total content and concentration of pituitary LH decreased. The decline in pituirary LH was not dependent on body weight changes nor on pituitary weight (r^2 = 0.05 and 0.01, respectively). Pituitary weights were higher in cobalt deficient animals (by about 41 %), concomitant with the decrease in body weights, although these were not correlated (r^2 = 0.05). Histological examination showed that the increase in pituitary weights was due to acidophils hypertrophy; although in few cases both acidophils and basophils actually showed every sign of atrophy; with little cytoplasm and crowding of dark pyknotic nuclei. These can be seen on Figures Z_1 to Z_4.

TEXT TABLE 30

The effect of cobalt deficiency on pituitary weight and LH content.

Group number	Extracted Protein(mg)	Total LH (ug)	ngLH/ug protein	Pituitary wts.(mg).
1	20.15±3.58	101.18±11.40	5.77±1.44	295.50±25.05
11	22.95±4.30	30.00±2.51	1.44±0.26	402.00±54.71
III	23.31±4.12	46.71±4.22	2.34±0.56	411.60±39.75
IV	26.02±5.18	58.57±5.46	2.43±0.31	416.00±39.72

All values given as MEAN ± SEM respectively.

TEXT TABLE 31

(i) Analysis of variance of the effect of cobalt deficiency on pituitary LH content.

Source	df.	TSS	MS	F	P
Total	16	13579.69			
Treatment	3	11229.57	3743.19		
Error	13	2350.12	180.78	20.71**	0.001

Comparison among means

Group	2	3	4	1
LH/ug	30.00	46.71	58.57	101.18
protein	LSD P < 0.001 = 55.89			
	LSD P < 0.01 = 39.88			

Group 1 - 2	= 71.18*	Group 3 - 4	= 11.86
Group 1 - 3	= 54.47**	Group 2 - 3	= 16.71
Group 1 - 4	= 42.61**	Group 2 - 4	= 28.57

Group 2, 3 and 4 had low pituitary LH content than control group (1).

TEXT TABLE 31 Cont'd

(ii) Analysis of variance of the effect of cobalt deficiency on pituitary LH concentration.

Source	df.	TSS	MS	F	P
Total	16	76.91			
Treatment	3	43.94	14.65		
Error	13	32.97	2.54	5.77**	0.01

Comparison among means

Group	2	3	4	1
LH/conc. ng/ug protein	1.44	2.34	2.43	5.77

LSD P. $<$ 0.05 = 3.39

(iii) Pituitary weights were analysed by use of the t-test. Group 2, 3 and 4 pituitary glands were heavier ($P < 0.05$) than the control group (1). Groups 2, 3 and 4, however, did not differ in pituitary weights.

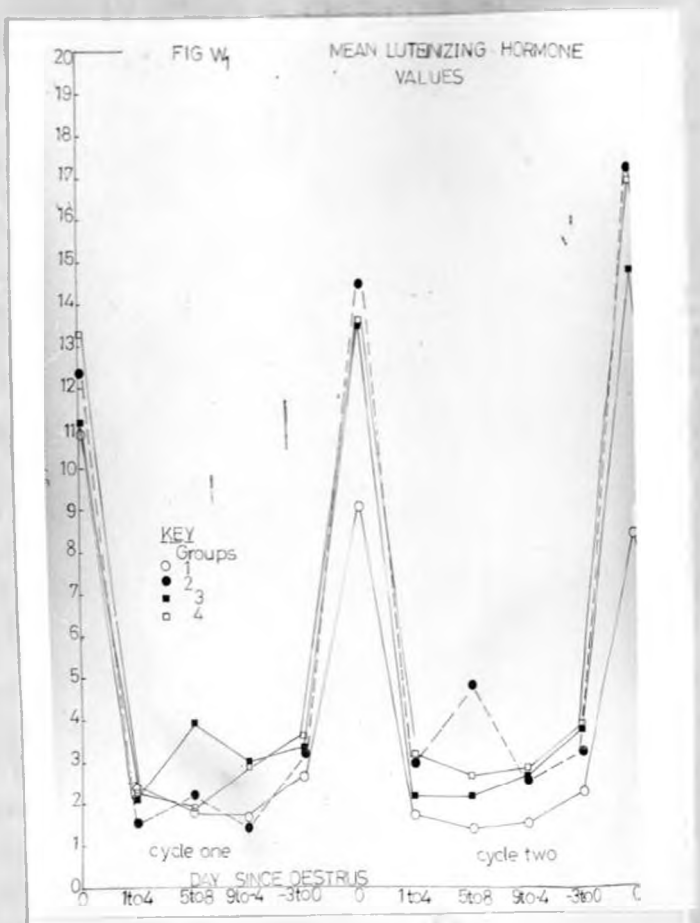


FIG W1
MEAN LUTEINIZING HORMONE VALUES
KEY
Groups
1
2
3
4
cycle one
cycle two
DAY SINCE OESTRUS
0 1to4 5to8 9to-4 -3to0 0 1to4 5to8 9to-4 -3to0

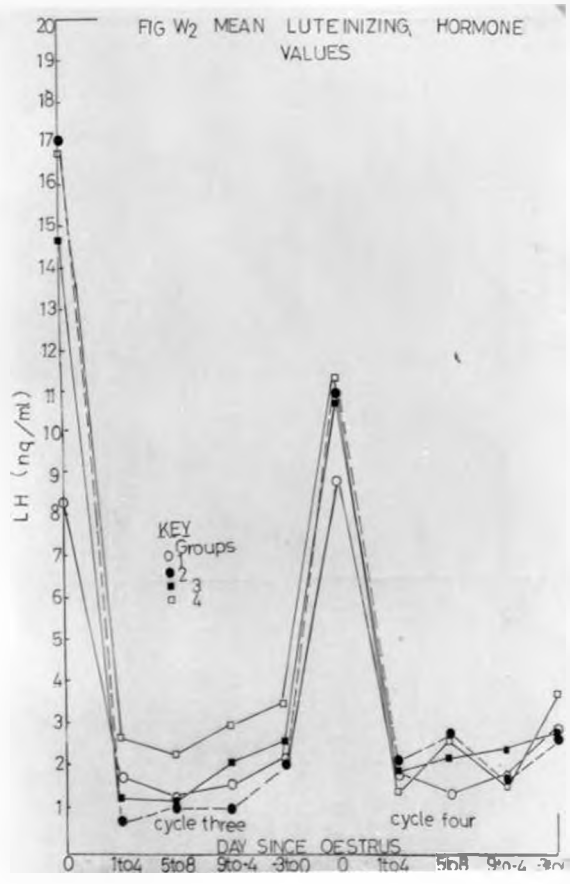


FIG W2 MEAN LUTEINIZING HORMONE VALUES
LH (nq/ml)
KEY
Groups
1
2
3
4
cycle three
cycle four
DAY SINCE OESTRUS
0 1to4 5to8 9to-4 -3to0 0 1to4 5to8

FIG Y. PITUITARY and BODY WEIGHTS

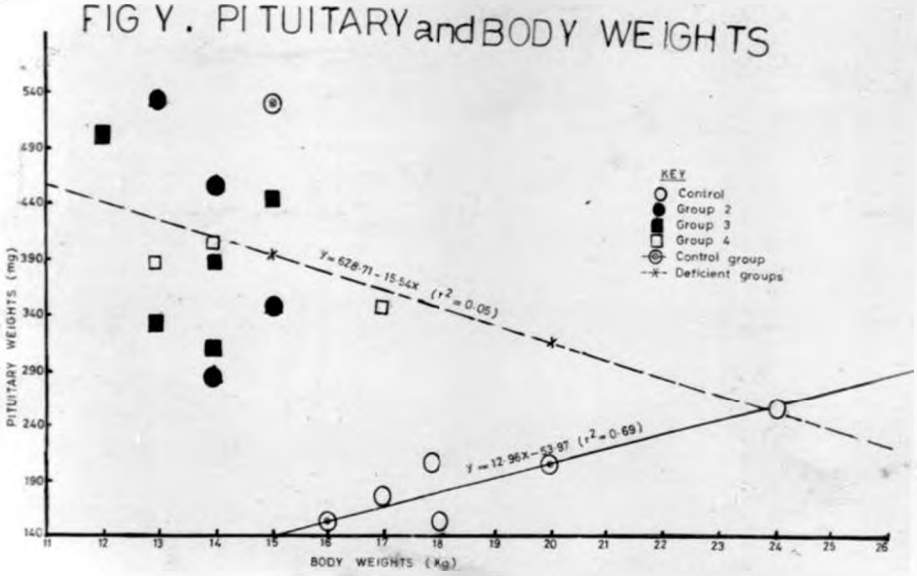


FIG X PITUITARY WEIGHTS and TOTAL LH.

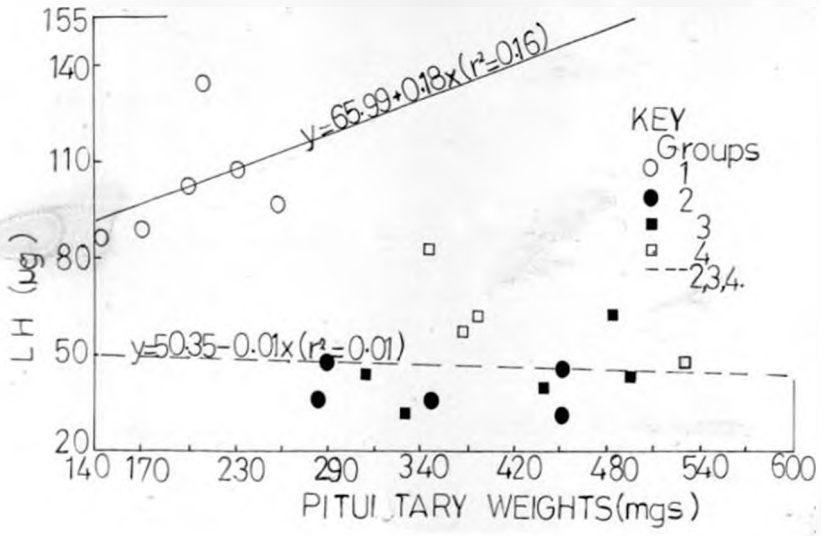
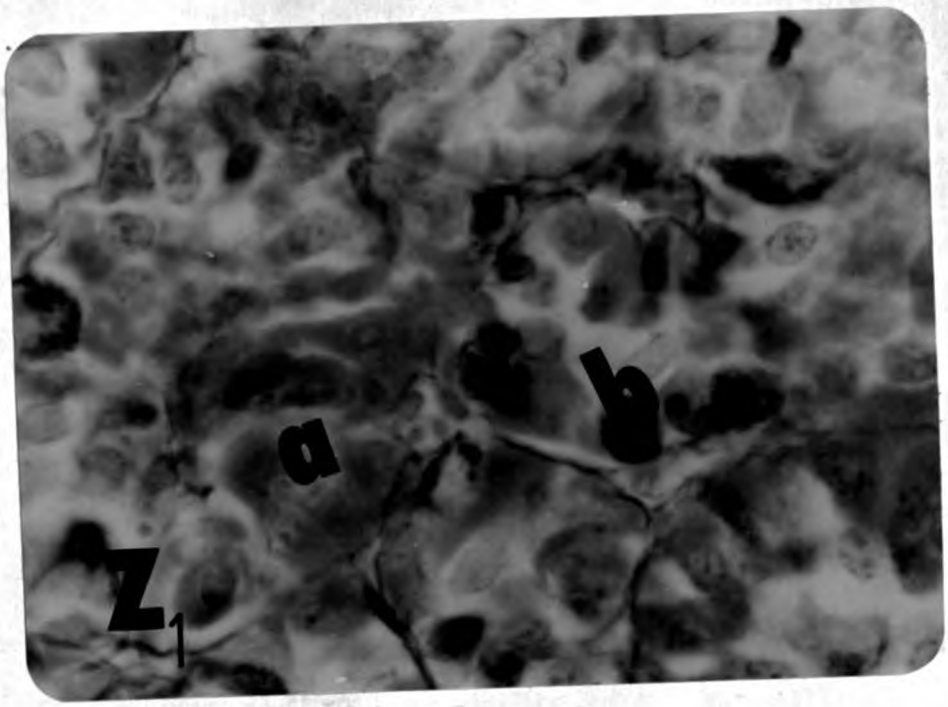


PLATE 11: PITUITARY GLAND.

Fig. Z_1. Animal number 82 (control) PAS stain at x 10000 magnification. In control group, PAS stain show granular basophils and numerous acidophils.

Fig. Z_2. Animal number 86 PAS stain at x 10000 magnification. (Deficient group 2) Basophils are few in deficient animals, agranular with little cytoplasm. But occasionally large irregular basophils can be seen; these are probably of the beta type. Acidophils are numerous, with plenty of cytoplasm.


a
b
Z_1

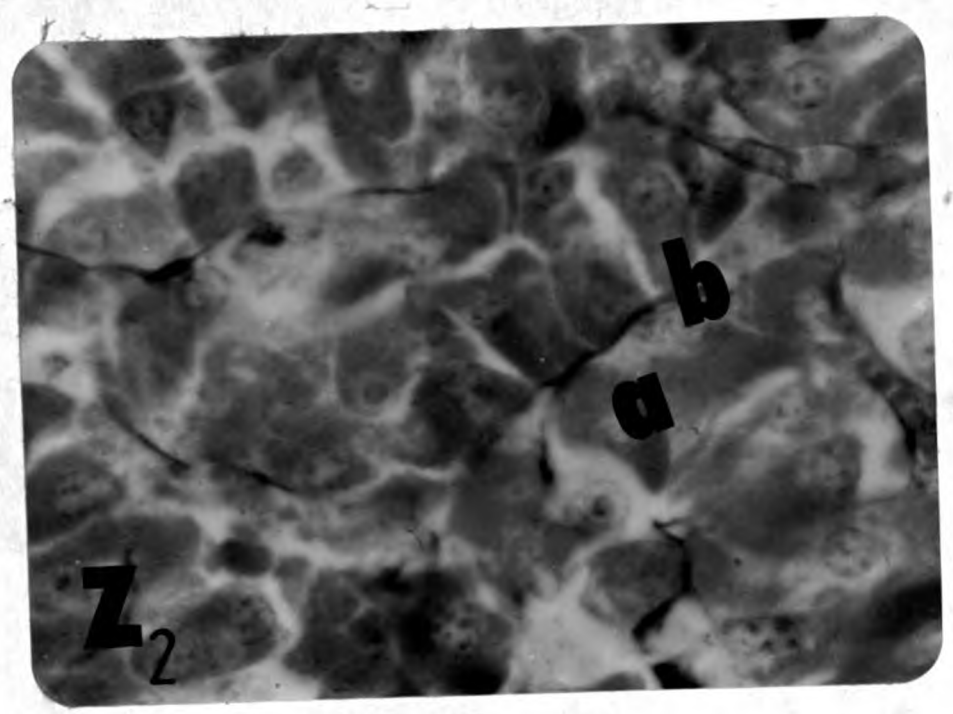

b
a
Z_2

PLATE 12: PITUITARY GLAND

Fig. Z_3. Energy and protein supplementation did not improve the effect of cobalt deficiency on the pituitary. Fig. Z_3. Animal Number 80 (Energy supplemented, PAS stain at x 10,000 magnification). PAS reaction indicate a decrease in basophils and as well as the nuclei i.e. suggesting atrophy. The crowding of the cells was observed in 6 animals.

Fig. Z_4. Animal number 98 (Protein supplemented - group 4). PAS stain at x 10,000 magnification. Note again the basophils. In some of the animals pituitary showed numerous acidophilic cells, highly granulated and hypertrophied. Acidophils nuclei are large, and less opaque, cytoplasm is abundant with large vacuoles.

a
b
Z_3
b

a
b
Z_4

5:0. DISCUSSION

5:1. Cobalt

Cobalt is an essential trace element which is continuously needed for vitamin B_{12} synthesis by goats, sheep and cattle, but storage in the body is very limited (Underwood, 1977). It is therefore, necessary that a daily supply of cobalt in the herbage should be at the level of 0.07 parts per million on dry matter basis (ppm DM) so as to supply 0.08 mg Cobalt per animal per day, which is the daily requirement for goats and sheep (Underwood, 1971; Church and Pond, 1976). The low content (0.01 ppm DM) of Cobalt fed to goats in the present study was therefore, not sufficient for adequate Vit. B_{12} synthesis, thus a deficiency in cobalt and Vit. B_{12} was rapidly established.

The values obtained for plasma vitamin B_{12} in normal and cobalt deficient goats of this study were comparable to those reported earlier by Marston (1952), Gawthorne (1968), Jones and Anthony (1970), Underwood (1971 and 1977), and Macpherson et al. (1973 and 1976), who reported levels of 400 to 900 pg/ml in normal goats and sheep while in B_{12} deficiency levels were below 100 pg/ml.

There is little doubt that the poor storage of B_{12} in the ruminant liver (Marston, 1970), and the rapid decline in the rumen of B_{12} synthesizing microorganisms in absence of cobalt (Marston & Lee, 1949; Keener, Percival, Ellis & Benson, 1950; Daubarn, Hine & Smith, 1957; Somers & Gawthorne, 1969; Macpherson et al., 1976) contributed to the rapidity of avitaminosis B_{12} observed in this study.

Furthermore, in this study control goats exhibited great variations in plasma B_{12} values. Similar variations have previously been explained to be due to the different rates of synthesis of cobalamines in the rumen resulting from differing diurnal eating patterns, and hence variations in amounts of B_{12} available for absorption into the blood system. The variations were more marked in animals fed cobalt replete than cobalt deficient diets (Somers and Garthorne, 1969).

In this study blood for B_{12} determination was drawn before the animals were fed in order to standardize the plasma B_{12} values; therefore the B_{12} values obtained represent pre-feeding B_{12} values.

The large fluctuations seen in control groups cannot therefore be explained along the lines suggested by Somers and Gawthorne (1969).

The change in B_{12} concentration in cobalt deficient goats in this study was initially fast, then stabilised before declining slowly. A similar pattern of change in B_{12} values during cobalt deficiency has also been observed by Dawbarn et al. (1957), Marston (1970), Jones and Anthony (1970), Findlay (1972), Macpherson et al. (1973 and 1976).

Dawbarn et al. (1957), Marston, Shirley, Allen and Smith (1961), Marston, Allen and Smith (1972), and Rickard, Bigger and Elliot (1975) reported that B_{12} activity of sheep and cattle jugular blood was almost entirely due to dimethyl-benzimidazole cobamide, one of the 9 cobamides and cobanamides detected in sheep kept on a cobalt deficient diet. They found that the concentration of cobamide increased markedly in the rumen contents of sheep kept on a cobalt deficient diet.

Although the individual cobamides were not determined in the present study, it is suggestive that the low stable B_{12} values observed after an initial rapid decline of 8 weeks was possibly due to the effect of the cobamides.

Infact some workers (Macpherson et al. 1976) have observed a slight recovery in plasma B_{12} levels in individual cobalt deficient subjects possibly due to the same mechanism. The limited B_{12} supply from the liver and other B_{12} storage organs as is common during deficiency and stress (Somers and Gawthorne, 1969; Smith and Marston, 1970a; Underwood, 1971), could also contribute to the stabilization of the low B_{12} levels during deficiency.

5:2 Signs of Cobalt and B_{12} deficiency

This study revealed that goats fed cobalt deficient rhode-grass hay show marked deficiency in B_{12} and decline in body weights as early as 8 weeks as previously reported by Gombe and Verjee (1976).

This is also in reasonable agreement with the findings of Somers and Gawthorne (1969), Marston (1970), Underwood (1971), Macpherson et al. (1973 and 1976), and several other workers who reported that B_{12} values decreased in ruminants within 14 weeks of cobalt deficiency. Lee & Marston (1969), Marston (1970), Underwood (1971), Nicholas and Egan (1975) and Underwood (1977) reported that cobalt and B_{12} deficiency occurred in sheep in the majority of cases, after 18 weeks of feeding a cobalt deficient diet.

Of the above cited studies, however, it is only Gombe and Verjee (1976) and Macpherson et al. (1976) who reported early loss in body weight (within 8 to 14 weeks) on feeding a cobalt deficient diet. Comparable changes in body weight were reported after 18 weeks by the other researchers. This difference in duration before observing a cobalt and B_{12} deficiency is probably due to species difference and the severity of cobalt deficiency in the diet fed.

The fact that in this study the rhode grass hay was from an area with the lowest known cobalt content in the herbage (Mwakatundu, 1978) may explain the rapidity of the onset of deficiency. Furthermore the difference found by the various researchers could be due to the influence of other minerals such as copper, iodine, molybdenum and iron that are associated with cobalt metabolism (Ammerman, 1970; Underwood, 1977).

The absence of pasture cobalt is therefore of particular concern to many ranchers in Kenya and Tanzania, most of whose farms are in these very cobalt deficient areas. This is particularly so for peasant farmers who cannot afford supplementing animals with minerals.

Benjamin (1974) and Schalm, Jain and Carroll (1975) have reported erythrocyte values for a normal goat as being 8 to 18 mill. per ml for RBC, 8 to 12 mg % for Hb, 22-38 % for PCV, 16 to 25 % for MCV and 30 to 36 % for the MCHC. Similar values were obtained for control goats of this study. Decrease in B_{12} values resulting from low cobalt in the feed was accompanied by loss in weight, low haemoglobin concentration (Hb), low packed cell volume (PCV %) and a higher mean corpuscular volume (MCV %). Similar findings have been reported by

Marston (1952), Lee and Marston (1959), Underwood (1971), Macpherson et al. (1973 and 1976).

The anaemia due to low B_{12} in humans is megaloblastic macrocytic (Chanarin, Myra, Bennett, 1962; Underwood, 1975). In animals Benjamin (1974), Schalm et al. (1975), Gombe and Verjee (1976) and Machperson et al. (1976) reported of macrocytic/normochromic anaemia; Gawthorne, Somers and Woodliff (1966), Somers and Gawthorne (1969) and Church and Pond (1976) observed normocytic/normochromic anaemia, while Underwood (1977) reported of normocytic/hypochromic in Lambs and microcytic/hypochromic in calves.

In the present study the anaemia was characterized as macrocytic and normochromic, further confirming the relationship of Cobalt and Vitamin B_{12} and their role in Red Blood Cell production as reported by Gombe and Verjee (1976) and Underwood (1977). Group four animals, however, indicated that probably there might be a macrocytic/hypochromic anaemia in severely affected goats.

5:3 The Adrenal Cortex function

Several endocrine gland functions are altered during malnutrition (Leathem, 1966). In this study the adrenocortical function was examined because of its role in stress and reproduction (Braden & Moule, 1964; Van-Rensburg, 1965; Catt, 1970; Macdonald, 1975).

Goats are normally known to have relatively low plasma corticosteroids and values of 1-2 ng/ml have been reported (Van Rensburg, 1971; Macdonald, 1975). In this study control goats had similar values of adrenocorticosteroid with little fluctuations as evidenced by the small standard error of mean (SEM See Figure K).

Feeding a cobalt deficient diet, however, led to an increase in adrenocortical activity. The adrenocortical hyperfunction was indicated by elevated plasma corticosteroid and cellular hyperplasia of the zona fasciculata and reticularis.

Adrenal hyperfucntion has also been observed in malnutrition of either protein (Pimstone, 1976), energy (Van Rensburgh, 1971; Stott and Thomas, 1971); or total nutrients (Srebnik and Nelson, 1962; Leathem, 1966; Catt, 1970; Marple, Judge & Aberle, 1972). Schonland, Shanley, Lo ning, Parent and Coovadia (1972), and Paisey, Angers, Frenke (1973), however, observed that

plasma corticosteroid values in malnourished children were not altered unless superimposed by an acute stress.

In this study all the goats were free from disease, parasitism and unnecessary disturbances, and were quite accustomed to handling as a result of two months of adaptation and training. The increase in corticosteroid values obtained in these goats therefore, reflects no influence of external stress besides that of malnutrition (B_{12} deficiency).

Goats have relatively high lymphocyte (50 to 70 %) and low neutrophil (30 to 48 %) counts. The marked lymphopenia and neutrophilia observed in cobalt deficient goats are consistent with effects of elevated corticosteroid levels in goats as reported by Van Rensburg (1971), Benjamin (1974) and Schalm et al. (1975).

Lunn et al. (1973), suggested that during malnutrition, increase in corticosteroids is evident only when there is loss in body weights ($r = 0.42$). This is likely to result from the anti-stress effects of corticosteroids which promote the utilization of body reserves. This was not found to be the case in this study. Infact the correlation was below that of control

animals (r = 0.1), further suggesting the decrease in body weights was not due to high corticosteroid levels. The increase in corticosteroid levels, however, may have contributed to loss in body weights.

Earlier data on adrenocortical function in malnutrition and other stresses have been reviewed by Van Rensburg (1965), Lamming (1966), Leathem (1966) and Pimstone (1976), Adrenal gland hypofunction occurs in severe cases of malnutrition (Lurie & Jackson, 1962; Chatterji & Sen Gupta, 1966; Stott & Thomas, 1971; Lunn, Whitehead, Hay and Baker, 1973). On the other hand mild prolonged malnutrition initially elevates the adrenocortical function (Srebnik & Nelson, 1962; Stott & Thomas, 1971; Christian, 1972; Pimstone, 1976) as shown by an increase in corticosteroid concentration (Alleyne & Young, 1966; Lunn et al. 1973), and hypertrophy of the adrenal cortex (Van Rensburg, 1965; Platt & Stewart, 1967; Campbell, Srebnik & Grindeland, 1970).

Subsequently the sustained elevation of Corticosteroids results in exhaustion and atrophy of the adrenal cortex (Upjohn Drug manual, 1974).

Recent studies of the role of corticosteroids during malnutrition suggest that corticosteroid binding globuline (CBG) decrease thereby increasing the blood levels of free biologically active corticosteroid. Samuel, Kadival, Patel, and Desai (1976) found that CBG were low in 35 malnourished children ($P < 0.05$), total corticosteroids normal while "free cortisol" was raised. Leonald and Mac William (1964) and Baldijao, Atinmo, Pond and Barness (1976) have reported similar findings. They all agree that during malnutrition the hypothelamo - pituitary - adrenal system is not impaired since malnutrition subjects are able to respond to other forms of stress such as disease and hypoglycaemia.

In this study total plasma corticosteroids were elevated due to adrenocortical hyperfunction. Longer duration of cobalt deficiency possibly could have led to a decrease in adrenocortical functions as suggested by the above authors.

5:4. THYROID GLAND FUNCTION

Earlier studies have shown that feeding thyroactive materials to rats increases Vitamin B_{12} requirements (Coates and Porter, 1959). Baby pigs deprived of B_{12} show enlarged liver and thyroids (Church and Pond, 1976). In B_{12} deficient chicks (Underwood, 1975), and rats (Newberne and O'Dell, 1959), the thyroid gland hypertrophies indicating need by the gland for B_{12}. Cobalt deficiency in ruminants increases the size of thyroid follicles and heights of epithelial cells, and that goitre in low iodine-cobalt areas is dependent on the iodine and cobalt ratio (Underwood, 1975). Other works on thyroid gland function show that manganese, iron, iodine and cobalt are also necessary for the synthesis of thyroid hormones (Blokhima, 1970; Underwood, 1971; Nicholas & Egan, 1975; Underwood, 1975).

The present study showed clearly that malnutrition due to cobalt deficiency leads to hyperplasia and hypertrophy of the thyroid gland and an increase in both thyroxine (T_4) and Triiodothyronine (T_3), which are characteristic of hyperthyroidism (Bustad and Fuller, 1970; Jubb and Kennedy 1970; Smith, Jones and Hunt, 1972; Hersch and Evered, 1973; Burke and Eastman, 1974).

There was also an increase in the Free Thyroxine (Thyopac) Indices (FTI) in agreement with earlier reports by Clark and Brown (1970), Evered, Ormston, Smith, Hall and Bird (1973) and Burke and Eastman (1974).

Changes in thyroid function are not, however, restricted to cobalt deficiency. Other types of malnutrition (energy, protein etc) cause increases in plasma T_4 and T_3, and thyroid hyperplasia. Thus Heard & Stewart (1971) observed increases in thyroid vesicular cell heights in animals receiving low protein diets. Reports by Lamming (1966) and Leathem (1966) in farm animals, Graham et al., (1970), Godard et al. (1973) in humans, and Florsheim et al. (1976) in rats show that T_4, and FTI are increased when these subjects are placed on low energy, low protein or/ and vitamin deficient diets.

There is evidence that the hyperthyroidism is due to an elevation of TRH and TSH in energy, protein and vitamin malnourished animals (Bustad and Fuller 1970; Mason & Wilkinson, 1973; Pimstone, 1976). This is contrary to the basic principle of negative feedback control of thyroid hormone production.

The continued hyperactivity of the thyroid gland should then basically be from a prolonged stimulation as occurs in tumor conditions (Bustad and Fuller, 1970; Jubb & Kennedy, 1970; Mason and Wilkinson, 1973).

Several workers have, however, obtained low TSH during malnutrition. Harland and Parkin (1972), Graham et al. (1973), Pimstone (1976) report of low TSH in malnourished children. Similar observations in rats and farm animals by Srebnik et al. (1963), Lamming (1966) and Leathem (1966) have been reported. It should be stressed that in the latter reports the low TSH is concomitant with low T_4 and T_3 indicating loss of negative feedback effects on T_4/T_3 on the hypothalamus (Srebnik & Nelson, 1962; Godard. et al.,. 1973; Pimstone, 1976).

The ease with which thyroid malfunctions are initiated by malnutrition is age dependent (Srebnik & Nelson, 1962). Srebnik & Nelson (1962) observed that low protein diets affected young rats more than the adults, and that lower TSH values were observed in the young animals.

The disparity of the research findings (whether or not TSH is elevated or decreased) is probably due to the severity of stress imposed, the ages of the animals used, their nutritional status and the stages during which these hormones were monitored.

It is probable that initially TRH and TSH are elevated in every case and that subsequently prolonged stress leads to depletion of TRH with consequent reduction in TSH and T_4/T_3 values. This would explain the loss of negative feedback effects during malnutrition. It would also explain why thyroid hyperplasia has been reported in malnourished animals, and why once glandular hyperplasia has occurred it takes some time to regress following decline of high TSH levels.

Other explanations have, however, been given for the low T_4 and Protein Bound Iodine (PBI) during hypothyroidism of protein-calorie malnutrition. Ingenbleek, Visscher & DeNayer, (1972) and Pimstone (1976) suggested decreases in plasma binding proteins in malnourishment from observations that "free T_4" in kwaehiokor patients is elevated concomitantly with low thyroid binding proteins. Since most of T_4 in blood is protein bound, and not active (Westphal, 1970; Mason and Wilkinson, 1973), and only the "free T_4"is active, normal and/or high thyroid activity might therefore, be indicated by an increase of the free form.

Negative feedback on TSH levels would then be achieved by the increase of the "free T_4" and possibly "free T_3" (Bustad and Fuller, 1970).

Direct measurements of T_4 and T_3 as were done in this study, would suggest that the above explanation is unlikely; as the increase in the Free Thyroxine Index would have led to a negative feedback which would be expected from a responsive hypothalamus.

Yet other workers (Srebnik & Nelson, 1962; Srebnik et al., 1963; Florsheim et al., 1970; Srebnik, 1970) explain the low T_4 and T_3 values during malnutrition as a reflection of some form of thyroid gland dysfunction; with the thyroid gland having a reduced sensitivity to high hormonal concentrations or impaired catabolism leading to impairment of hormonal synthesis. The thyroid gland dysfunction in presence of high TSH and TRH then leads to hypothyroidism that would trigger further reduction in TSH and possibly TRH, and finally regression of the thyroid vesicular cells (Pimstone, 1976).

This hypothesis is supported by findings of Cowan and Margossian (1966), Srebnik (1970), Pimstone et al. (1973), that the decreased thyroid activity during malnutrition was not due to lack of pituitary TSH for, administration of TSH to protein deficient children did not improve the condition. Infact Pimstone et al. (1973) observed that high TSH levels in malnourished children were reduced by administration of exogenous T_3, thus indicating that TSH is normally suppressible.

Srebnik et al. (1963) and Srebnik (1970) had found that while pituitary TSH in adult female rats was not changed by feeding a diet free of protein, thyroid gland activities (especially that of organification of iodine, but excluding that of hormonal synthesis since distribution of radioiodide among the iodinated aminoacids of thyroid digest was normal) were suppressed as was indicated by a decrease in the thyroidal to serum radioiodide. concentration, although Florsheim et al. (1970) observed that such plasma iodine increase might be partly from an impaired kidney excretion. Whatever the mechanism is for the increase in iodine, high blood iodine reduces TSH output (Mason & Wilkinson, 1973).

In my opinion the above theories are disingenous and do not take into account the observed responsiveness of the hypothalamus particularly the increases in neurotransmitters during stress. The decrease in plasma TSH following exogenous T_3 in Kwashiokor children was probably pharmacological. Similar decreases have been induced with other hormones. A mild disturbance of the thyroid gland in malnutrition, however, has not been excluded.

5:5. The oestrous cycle of the East African shorthorned goats

In this study the oestrous cycles for the East African shorthorned goats were comparable to those reported for other world breeds. Thus the mean cycle length of 18.52 ± 0.33 days in the control goats are in agreement with the average values of 18 to 23 days for 70% of the animals (Lyngset, 1964; Davendra and Burns, 1970; Jaroszy, Deans & Dukelow, 1971; Van Rensburg, 1971; Salama, 1972). Short oestrous cycle lengths of 4 days have been reported to be fairly common in goats (Lyngset, 1964). In this study about 5% of the cycles were of short duration. Feeding a cobalt deficient diet, however, led to increase in irregularities of cycle lengths before cessation. This trend has also been reported during cobalt deficiency (Lotthamer and Ahlswede, 1973), and for many different forms of malnutrition (Roberts, 1971; Hafez, 1974; Rattray, 1977), whereby the cycle lengths become abnormally long before cessation. Oestrus behavioral symptoms also become less marked with time of deficiency, terminally silent oestrus and anoestrous are common.

5:6. PROGESTERONE VALUES

Several workers (Heap & Linzell, 1966; Van Rensburg, 1971; Jones & Knifton, 1972; Thorburn & Schneider, 1972; Heap, Bedford and Linzell, 1975) employing radioimmunoassay techniques have reported progesterone values during the oestrous cycle of the goat. In a 21 Day oestrous cycle, mean progesterone concentration is lowest during oestrus and up to Day + 3 of the cycle (0.3ng/ml to 0.8 ng/ml). Subsequently progesterone values increase to between 5.7 ng/ml and 10.7 ng/ml by about oestrous Day 3 and Day 8. After the gradual increase, there is fairly constant levels (8.9 ng/ml to 10.7 ng/ml) from about Day 10 to Day 15 of the cycle. This period of highest progesterone coincides with the time when the Corpus Luteum (CL) is maximal and most active (Harrison, 1948; Baird et al. 1975; Hamidul Islam, 1976). This is then followed by an abrupt decrease in progesterone levels from Day 17 (Stabenfeldt, Holt and Ewing, 1969).

The above pattern and values agree with those obtained in this study for the control goats (0.64 $\pm$ 0.01 ng/ml for the lowest values and 6.91 $\pm$ 0.56 ng/ml for maximal values).

On the other hand Shevah, Black, Carr and Land (1975), Erb et al. (1976), Hendricks and Bailey (1976) Beal et al. (1978) and Hill et al. (1970) observed that beef heifers on deficient diet had an abrupt decline in plasma progesterone ($P < 0.01$) accompanied by decreases in the numbers and sizes of follicles and CL.

Snook, Saatman & Hansel (1971), Baird et al. (1976) have reported a positive correlation between progesterone and LH levels from Day 3 to Day 15 of normal cycle. Gombe's study, however, showed that during malnutrition the progesterone values from Day 3 to Day 15 of the cycle were negatively correlated ($P < 0.01$) to LH values. In the present study plasma progesterone values were significantly higher during the early stages in cobalt deficient than control goats. Similar Similar changes have been reported by Beal et al. (1978) and Chew et al. (1978) but Donaldson et al. (1970) and Gombe (1972) recorded less marked elevations. have been reported by Donaldson et al. (1970) and Gombe (1972) ($P < 0.05$ vs < 0.10).

In later stages of cobalt deficiency peak plasma progesterone declined to less than 2/3rds of control values. Since the changes in progesterone were more marked with peak

values, it indicates that the CL function is not maximal in B_{12} deficiency.

5:7. PLASMA OESTROGENS

Few studies have measured plasma oestrogen levels in cycling goats and also plasma oestrogen during malnutrition. The present results show that levels of plasma oestrogen in control goats are similar to those for sheep (Holst, Braden & Mattner, 1972; Scaramuzzi and Land, 1978), and the pattern of change in plasma levels is similar to changes in urinary oestrogens in goats (Lyngset & Lunaas, 1972).

In this study one observes a closer agreement in changes in oestrogen values with those for progesterone values during malnutrition. Thus oestrogens are high initially and decline with progress of the dietary restriction. This is in agreement with the findings reported in other species (Lamming, 1966; Leathem, 1966; Wentzel, Morgenthal and Van Nierkerk , 1975). As was the case with progesterone, changes in oestrogen values were initially more marked on peak values but decline occurred on other values with the duration of deficiency.

5:8. LUTEINIZING HORMONE

The patterns and levels of LH concentration during the oestrous cycle of control goats reported in the present study are similar to those described by others in goats including Pretorius (1972 and 1973). Pretorius observed in a 21 Day cycle that LH levels became maximum on oestrous Day 21 to early Oestrous Day 0. This then was followed by low levels throughout the oestrous cycle except for small increases between Day 1 and Day 6, and Day 12 and Day 18 of the oestrous cycle. In this study LH levels are characterized by similar variations between days of the oestrous cycle and within individual animals.

Work in Sheep and Cattle (Cumming et al. 1971; Findlay & Cumming, 1976, Beal et al. 1978), in heifers (Gombe, 1972), and in rats (Nakanishi, Mori & Nagasawa, 1976) showed that initial increases were found in both pituitary and peripheral LH before these declined with the duration of malnutrition. Changes in hypothalamic LH - releasing hormone (LRH) corresponded with, but preceded changes in pituitary LH.

A 50% restriction of food intake in rats lowered the concentration of LH in pituitary and hypothalamal LRH, with no change in pituitary FSH (Piacsek and Meites, 1967). Similarly Lamming (1966) and Leathem (1966) reported that rats on protein free diets had lower pituitary and plasma gonadotrophin. Leathem suggested that the decrease in pituitary LH was not caused by the type of food restriction; for rats on 0% protein and 100% calorie had lower gonadotrophin than rats on 20 or 0% protein and 200% calorie.

In proportion to body weight, pituitary glands of malnourished rats (Srebnik, 1970), and cattle (Lamming, 1966; Leathem, 1966), are heavier than those of control animals. This is in agreement with my findings in this study.

Other workers, however, have reported no changes in pituitary gland (see Review by Leathem, 1966 and Pimstone, 1976). Yet others have reported pituitary atrophy in malnutrition in rats (Mullinos and Pomerantz, 1940) and farm animals (Heard & Steward, 1971).

Ibrahim and Howland (1972) have obtained higher pituitary LH concentrations in starved ovariectomized rats than in those ovariectomized but on normal diets indicating that the effect of malnutrition is independent of ovarian feedback effects. Earlier Howland (1971, 1972 and 1975) had shown that rats restricted to less than 50% of their normal diets had more pituitary-LH concomitant with low plasma LH. He suggested impaired hormonal synthesis and release under malnutrition.

Increase in follicle stimulating hormone (FSH) with no change in LH has been reported in adult female rats following 30 to 35 days depletion of protein (Srebnik et al., 1961). This agrees with the work of Lamming and Krause (1963) who obtained an increase in pituitary FSH after restriction of rats on calorie deficient diet for 45 days. Several workers agree that in malnutrition FSH is affected before LH (Horn, 1955; Leathem, 1966).

On the other hand Srebnik and Nelson (1962) and Srebnik (1970) reported that feeding immature and adult rats protein free diets for 14 to 30 days led to decrease in pituitary and plasma FSH and LH.

Malnutrition has been shown to affect the histological appearance of the pituitary. In young guinea pigs acute inanition has been shown to result in increase in size and activity of pituitary basophils and decrease in activity of acidophils (Lamming, 1966). This is the opposite of what was noted in the present study where marked hyperplasia of acidophils concomitant with atrophy of few cells of the basophilic series was prominent. Other workers have reported atrophy and degranulation of pituitary cells in malnutrition (Mullinos and Pomerantz, 1940). Heard & Steward (1971) reported loss of cytoplasm as causing the decrease in pituitary cell size. Histological appearance of the pituitary in this study favor the contention that malnutrition initially leads to hypertrophy and later atrophy of the cells.

All the above studies collectively indicate that initially the ability of the pituitary to synthesize gonadotrophin is not impaired by restricted feed intake. Furthermore gonadotrophin at one time or another during restricted feeding initially increases before declining to low levels with the duration and severity of the dietary restriction.

Data from this study show that initially plasma and possibly pituitary LH were increased, and later returned to normal values when goats were fed a cobalt deficient diet for 23 weeks. The rise in LH is temporal and is for a short period only. The normal plasma LH values observed therefore would have declined with prolongation of the study while the animals were cycling. It is possible therefore in acute severe deficiency conditions to miss the temporal rise in LH especially when blood collection is spread on longer intervals.

5:9. ENDOCRINE PROFILE

The results from this study show that the hormonal changes during cobalt deficiency do not obey the positive or negative feedback mechanisms as suggested by the "pseudohypophysectomy" and "pseudogonadectomy" theories of Mullinos and Pomerantz (1940), Lamming (1966) and Leathem (1966). In the present study plasma progesterone and oestrogens initially increased and were accompanied with high LH, corticosteroid and thyroid hormones. In later stages of cobalt deficiency plasma progesterone and oestrogens declined accompanied by a decline of LH to normal values while corticosteroid and thyroid hormones(T_4 and T_3) remained elevated. These changes occurred when Vit. B_{12} levels were declining progressively in parallel with loss in body weight, anaemia and cessation of cyclic activity. Terminally, after 23 weeks, histological examination of the mammary gland showed complete absence of alveolar tissue. This is expected from the low oestrogens and progesterone values, for neither growth hormone, corticosteroids, prolactin or prostaglandins in absence of oestrogens and progesterone will support normal mammary gland function (Fulkerson, Hooley, Mcdowell and Fell 1976; Baldwin and Plucinski, 1977;

Field, Cow, Mcdowell and Gooden, 1977; Peel, Hooley and Finday, 1977; Taylor, Peel, Robinson and McCowan, 1977).

Gombe and Hansel (1973), Gupta and Anand (1973), Giannina and Leathem 1974, Apgar, Aspros, Hixon, Saatman and Hansel (1975) have suggested that restricting feed intake reduces responses of the ovary and Corpus Luteum (CL) to LH stimulation, and results in less progesterone synthesized and released by the Corpus Luteum. The negative feedback exerted by progesterone on LH secretion is decreased, leading to increase in plasma LH.

Whereas the above theory may be true for later stages of malnourishment when plasma oestrogen and progesterone are low despite reasonably high LH levels, it does not explain the earlier increases in LH when progesterone values are still elevated, nor does it explain subsequent decline in LH when all cyclic activities have ceased.

Recently Beal et al. (1978) have shown that in cows with ovaries removed, restriction of dietary energy intake still produced a greater release of LH. They suggested that the pituitary gland responsiveness to gonadotrophin releasing hormone (GnRH) during malnutrition was increased and that malnutrition had direct effects on the pituitary .

The general increase in the three trophic hormones (Thyroid, Adrenal and gonadotrophin releasing hormones) in this study would be more correctly explained if the hypothalamus was the primary site of action rather than the target glands or the pituitary. In my opinion the "pseudohypophysectomy" theory is inade - quate to explain the influence of malnutrition on reproductive functions. The hypothalamic stress syndrome suggested by Srebnik (1970), Gombe (1972), Nakanishi et al. (1976) and Pimstone (1976) seems to fit the facts more closely.

5:9:2. STRESS AND HYPOTHALAMIC MONOAMINES

Stress of whatever nature or origin is a neurological factor working via the hypothalamus and hippocampus to increase or decrease the levels of monoamines (McEwen, Weiss & Schwartz, 1970; Campbell & Spear, 1972). Malnutrition too is a form of stress, which is induced over a period of time (Marple, Judge and Aberle, 1972; Moberg, 1976). Several workers have even shown an increase in adrenocorticoids in cattle and sheep under submaintenance rations (Stott & Thomas, 1971; Moberg 1976); an evidence of stress. It is the contention of this thesis that malnutrition stress probably acts non-specifically towards the release of monoamines at different levels, which then accumulate in different parts of the brain.

Large quantities of monoamines (serotonin, dopamine, epinephrine and norepinephrine) have been isolated from the brain (especially the median eminence and hypothalamus) of rats, sheep and cattle. Depending on the concentration and duration of administration, they have been shown to stimulate or inhibit the release of trophic hormones (Donoso, Bishop, Fawcett, Krulich and McCann, 1971; Nequin et al.,1975; Jackson, 1977; Ranta, Mannisto and Tuomisto, 1977; Ganong, 1977).

The close topographical aggregation of dopaminergic and GnRH neurones (in the lateral external and medial external layers of the median eminence) and the norepinephrine and GnRH neurones (in the subependymal and medial external layers) would also suggest functional relationship (Fuxe & Hokfelt, 1969; Donoso et al. 1971; Kamberi, Mical & Porter, 1971; Ganong, 1977).

5:9:3. THE INFLUENCE OF MONOAMINES ON GONADOTROPHIN RELEASING HORMONE

Donoso et al. (1971) administered 1.25µg of dopamine intraventricularly and observed an increase in LH. Larger doses of over 19µg dopamine, however, inhibit ovulation in rats. On this basis Swanson & Stormshak (1975) and Jacksone (1977) argued that the high levels of dopamine during pregnancy, pseudopregnancy and lactation may explain the low levels of LH at these times.

On the other hand both low and high levels of norepinephrine have been shown to stimulate LRH secretion in rats, sheep, cattle and humans (Kamberi et al.,1971; Kalra, Kalra, Krulich, Fawcett and McCann, 1972; Sawyer, 1975; Jacksone, 1977; Yen, 1977).

The above workers also showed that low dopamine and low norepinephrine (NE) levels increase the release of prolactin releasing factor leading to increase in peripheral prolactin. High levels of dopamine and NE, however, stimulate release of prolactin inhibiting factor (PIF) with subsequent decrease in peripheral prolactin (Buttler, Willet & Malven, 1971; Beck & Wuttke, 1978). Beck & Wuttke (1978) have recently confirmed that low dopamine and NE levels increase both prolactin and LH, but at different concentration thresholds.

Donoso et al. (1971) after giving L-dihydrophenyl-alamine (DOPA) to elevate brain catecholamines following blockage by α-methyltyrosine for monoamine synthesis observed that plasma prolactin was decreased with no change in FSH and LH values. This is in agreement with findings by Ajika, Kalra, Fawcett, Krulich and McCann (1972), Fenske & Wuttke (1978) that high levels of dopamine and NE in presence of high prolactin increases LH output, while low prolactin in presence of high dopamine and NE decreases LH output. They suggest that the mechanism by which high prolactin levels cause dopamine to be less effective in reducing

LH may be the desensitization of some of the dopaminergic receptor mechanisms that are inhibitory to LH release. This then allows the release of LRH despite persistently high dopamine turnover.

All the above observations agree and show that low dopamine and norepinephrine levels stimulate releasing factor for prolactin, however, high levels inhibit prolactin release through PIF while stimulating LRH release, higher still doses inhibits LH and prolactin release.

Similarly FSH has been shown to have a lower threshold for dopamine and NE than LH, but higher than prolactin (Ganong, 1977; Yen, 1977). This will agree with the concept that FSH is released before LH for follicular development (Edwards, Fowler, Gore-Langton, Gosden, Jones, Readhead and Steptoe, 1977; Rao, Richards, Midgley & Reichert, 1977; Yen, 1977). Serotonin administration, however, have little or no effect on plasma prolactin, LH or FSH (Bergen, Barchas, Vernikos-Danellis, 1974).

5:9:4. THE INFLUENCE OF MONOAMINES ON ADRENAL TROPHIC HORMONE.

Several studies have shown an association of the hypothalamo - pituitary - adrenal system to serotonic neurotransmitter substances (Moberg, 1976; Ganong, 1977). High serotonin levels as occur during stress inhibit the negative feedback mechanism for corticosteroids. The absence of the negative feedback then leads to high CRF - ACTH levels accompanied by increased corticosteroids output (Bergen, Barchas, Vernikos - Danellis, 1974). The role of the hippocampus in the regulation of CRF-ACTH, originates in the mediation of serotonic neurological functions (McEwen et al.,1970; Campbell & Spear, 1972).

An increase of dopamine has been shown to increase corticosteroids. Also it has been shown that administration of high levels of glucocorticosteroids decreases levels of monoamine oxidase in the brain, thereby allowing further increases in monoamine, ie positive feedback (McEwen et al.,1970). The reverse of the above is also true.

5:9:5. THE INFLUENCE OF MONOAMINES ON THYROID TROPHIC HORMONE

Dopaminergic system complimented by the adrenergic system have also been shown to control the thyroid stimulating hormone (TSH) release. High dopamine levels inhibit, while high adrenergic levels stimulate the release of TSH (Ranta et al.,1977). The concentrations of neurotransmitter substances required to release both TSH and adrenal cortical hormone are, however, much higher than those for prolactin, LH and FSH (Ranta et al. 1977).

5:9:6. THE INFLUENCE OF OVARIAN STEROIDS ON HYPOTHALAMIC MONOAMINES

Convincing evidence is now available for a causal relationship between oestrogen and the changes in turnover of catecholamines in the hypothalamus which appears to be dose and time dependent. Administration of oestrogens to spayed rats induced a marked and selective acceleration in the dopamine turnover with concomitant lowering of LH and FSH serum levels.

An opposite trend occurs in the norepinephrine nerve terminals (Kalra et al.,1972; Yen, 1977). These changes show that the inhibitory feedback control of LRH secretion by oestrogens is partly at the hypothalamic level.

Similarly the interaction of catecholamines and prostaglandins(PGS) have been shown to regulate the release of gonadotrophin (Yen, 1977). Although the interaction effects of catecholamines and PGS to trigger LRH release is not well understood, it is however, believed that PGS may cause transient blood vessel dilation with consequent increase in LRH delivery from the hypothalamus to the pituitary without an actual increase in neural release of LRH. But increases in local brain PGS may also stimulate the LRH neurones directly to release LRH and thus contribute to the high LH levels observed during stress (Batta, Zanis & Martini, 1974; Ojeda, Jameson & McChan , 1974; Yen, 1977).

5:10. THE MODE OF ACTION OF COBALT DEFICIENCY

It was noted in the literature review that cobalt deficiency is more severe in younger animals, indicating that organs with greatest metabolic activity are affected faster than others.

Of the body tissues the brain has greatest metabolic activity. It would be expected therefore that cobalt deficiency would impair brain function first.

It is well documented that glucose is the only source for brain energy requirements (White, Handler & Smith, 1968). Goats have normally low blood glucose levels and utilize volatile fatty acids as a major source of energy (Baile & Forbes, 1974). Since the brain of goats cannot use volatile fatty acids (VFA) as source of energy, the glucose must come from gluconeogenesis with VFA as source.

In B_{12} deficiency the derangement in propionate metabolism (Coates and Porter, 1959; Cannata et al., 1965; Gawthorne, 1968; Smith & Marston, 1970b; Underwood, 1975) leads to less gluconeogenesis and therefore low plasma glucose (Macpherson et al., 1976). A deficiency in glucose leads to immediate brain malfunction which is interpreted as hunger, a form of chronic stress (Leathem, 1961; Marston, 1970; Baile & Forbes, 1974).

It is known that myelin lipid turnover is at a significant high rate (Smith, 1968), and that myelin renewal activity requires normal fatty acid synthesis (Hughes & Eliason, 1960). Furthermore, the intergrity and formation of myelin is dependent upon the quantity of specific fatty acids available (Frenkel, 1973; Deana et al. 1977).

In B_{12} deficiency there is accumulation of methylmalonyl CoA and methyl CoA substances that substitute acetyl CoA in the synthesis of fatty acids for neural tissue (Cannata et al., 1965; Smith & Marston, 1970b; Frenkel et al., 1976; Barley, Sato, Abeles, 1977). This therefore leads to incorporation in neural tissue of abnormal and branched fatty acids (Barley et al., 1977) with the result that nerve tissue intergrity, renewal capacity and myelination are altered (Underwood, 1975; Macpherson et al., 1976; Deana et al., 1977).

In B_{12} deficiency there is impaired protein synthesis necessary for neural tissue (Frenkel, Kitchens, Johnston, Frenkel, 1974; Macpherson et al., 1976; Frenkel et al., 1976; Deana et al., 1977).

Absence of proteins, vitamins (ie thiamine) and fatty acids also leads to decreased permeability of the nerve tissue

which in turn might be an accompaniment of neuropathy (Deana et al., 1977; Nishimo and Itokawa, 1977).

All the above workers observed that during malnutrition due to Vit. B_{12} deficiency, there is abnormal brain function with increases in nerve tissue impermeability. This suggests that brain neurotransmitter substances are altered. In fact recently Deana et al., (1977) have shown altered enzyme systems responsible for neurotransmitter substances and a decrease in neurotransmitters in chronic B_{12} deficient rats; thus further supporting that B_{12} leads to hypothalamic stress.

The role of neurotransmitters in neural function is to modify membrane permeability of the post-synaptic cells so as to allow the generation of excitatory post-synaptic potential or inhibitory post-synaptic potential. The altered neural function accompanied with altered neurotransmitters in B_{12} deficient leads to dysfunction of the neural tissue and many other important metabolic activities including those of reproduction.

It is the contention of this thesis therefore, that the observed changes in cobalt deficient goats were initially due to the influence of stress stimuli on the brain and especially hypothalamus, while in later stages it was due to the combined effects of and the defects in the neurotransmission brought about by the avitaminosis B_{12}.

5:11. Endocrine Profile in Cobalt and B_{12} deficient goats: The Influence on the ovary, pituitary, adrenal and thyroid functions.

It was noted in the previous discussions that cobalt deficiency leads to hypothalamal stress with an increase in monoamines which subsequently influences trophic hormone output. It was also noted that the concentration of monoamines affecting the various trophic hormones differed in time and magnitude. Prolactin seemsto be readily influenced, followed by FSH and later LH. ACTH and TSH, however, respond only to high levels of the neurotransmitters.

Prolonged stress due to malnutrition, however, leads to a decrease in prolactin, FSH and LH, hormones that are not essential for the survival of an individual. Corticosteroid as well as thyroid hormones increase probably to maintain metabolic rate, a compensatory mechanism against stress which partly contributes to body weight loss.

One can therefore explain the initial increase in LH, FSH and ovarian steroids in malnutrition as being due to an initial hyperactivity of the hypothalamus, pituitary and ovary.

The reduced levels of FSH that occur later in prolonged stress might lead, however, to a decrease in follicular growth. This is in agreement with the belief that FSH acts in presence of oestrogens and progesterone on granulosa cells of the pre-antral follicles to promote follicular development and to increase content of receptors for LH; subsequently LH acts on the same follicular cells to induce luteinization. Large quantities of LH on the other hand will decrease both FSH and LH receptors (Pickering and Fink, 1976; Richards & Midgley, 1976 and Rao, Richards, Midgley and Reichert, 1977; Stelmasiak and Galloway, 1977). One would therefore, be able to expect low peripheral oestrogens and possibly androgens due to lack of the follicular control mechanism. A fact that well agrees with earlier reports that malnutrition reduces the ovarian responses to gonadotrophin by reducing the numbers of receptors for both LH and FSH, thereby leading to low ovarian responses to high plasma LH.

The role of corticosteroids in reproduction has been previously reviewed. In short, corticosteroids influence reproductive functions partly through increase in LH. Thus it is not surprising that transport stress of short duration improves ovulation in ewes possibly through LH release (Braden & Moule, 1964; Lang, 1964; Brunners, Donaldson & Hansel, 1969; Van Resnsburg, 1971; Baird, Land, Scaramuzzi & Wheeler, 1976).

High corticosteroid values have also been shown to decrease Corpus Luteum size and function. It has been suggested that corticosteroids act either directly on CL to reduce protein synthesis and cell division (Brunners et al., 1969; Myles & Daly, 1974; Tomasgard, 1976 a & b), or through the release of Luteolytic substances i.e. high levels of LH and postaglandins (Carlson, Flynn and Lefer, 1977). For Brunners et al., (1969), Tomasgard (1976 a) showed that ACTH administration to heifers led to small CL in normal animals; the treatment was, however, ineffective after hysterectomy. This suggests that the influence of corticosteroids on CL function may be exerted through postaglandins. Corticosteroids release of postaglandins during parturition has now been well documented (Thorburn, Nicol, Bassett, Shutt and Cox, 1972; Flint and Hiller, 1975).

All in all, high corticosteroid and LH levels reduce CL - function thereby decreasing plasma progesterone as reported earlier (Hansel, Concannon and Lukaszewska, 1973).

It is interesting also to note that during the early stages of stress corticosteroid effects are potentiented by an increase in prolactin. Similarly elevated CRH - ACTH increases the steroidogenesis of corticosteroids, progesterone and androgens. (MacCann, Rodriques & Nallar, 1966; Christian, 1971; Wagner, Strohbehn & Harris, 1972; Makin, 1975).

Prolonged stimulation from CRH - ACTH, however, reduces the activity of the adrenal cortex cells through exhaustion. A similar picture would be expected to occur in the TRH-TSH overstimulation on the thyroid gland.

Malnutrition, thus has non specific effects on reproductive and endocrine glands. The specific defects due to B_{12} deficiency, as is for energy and protein deficiency or any lack of a dietary component, only influences the speed or degree by which the reproductive organs are affected.

5:12. CONCLUSION.

It is the conclusion of this thesis that cobalt deficiency leads to malnutrition which acts non-specifically by initial stimulation of hypothalamal activity (neurotransmitters in particular) leading to overproduction by the pituitary and target organs; subsequently over production of nuerotransmitters due to either prolonged or short but severe hypothalamal stress decreases activity in target organs, pituitary gland and hypothalmus.

The impairment of reproductive function in Vitamin B_{12} deficiency is exerted via the atrophy of pituitary delta basophils, and atrophy of ovarian primordial follicles and thecal cells. The destruction of primordial follicles within so short a time makes Vitamin B_{12} deficiency a major economic factor, as the return to full reproductivity is bound to be slow or even impossible.

The hypertrophy of the thyroid and adrenal gland is due to the stimulatory effects of hypothalamal stress and B_{12} deficiency on the pituitary and hypothalamus. It is suggested that Vitamin B_{12} is probably necessary for the regulation of sensitivity and metabolism of various endocrine cells, selectively.

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Appendix Table 1

Changes in Body weights of normal and cobalt deficient goats (All values in kilograms).

Animal Number and group	1977 July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
Group I							
82	18.0	17.0	18.0	17.0	18.0	18.0	18.0
83	17.0	17.0	17.0	17.0	17.0	16.0	17.0
91	20.0	19.0	20.0	18.0	19.50	21.0	20.0
94	18.0	18.0	18.0	18.0	19.0	19.0	18.0
99	21.0	20.0	21.0	20.0	22.0	25.0	24.0
Group II							
86	21.0	20.0	17.0	17.0	16.0	15.0	14.0
87	25.0	22.0	20.0	16.0	15.0	14.0	13.0
88	28.0	26.0	24.0	20.0	18.0	16.0	14.0
89	26.0	25.0	20.0	19.0	18.0	17.0	15.0
90	23.0	18.0	16.0	13.0			
Group III							
80	25.0	24.0	19.0	17.0	16.0	16.0	15.0
84	20.0	19.0	16.0	15.0	15.0	15.0	14.0
85	18.0	17.0	16.0	14.0	13.0	13.0	12.0
92	17.0	17.0	15.0	14.0	14.0	13.0	13.0
93	20.0	16.0	16.0	13.0	14.0	14.0	14.0
Group IV							
95	20.0	19.0	18.0	17.0	15.0	15.0	13.0
96	20.0	19.0	18.0	16.0	15.0	15.0	15.0
97	19.0	19.0	18.0	16.0	14.0	15.0	14.0
98	20.0	20.0	18.0	16.0	15.0	14.0	15.0
100	26.0	24.0	24.0	20.0	18.0	18.0	17.0

APPENDIX TABLE 2

RED BLOOD CELL COUNTS (millions per cu. mm)

ANIMAL NUMBER AND GROUP	DURATION OF DEFICIENCY IN MONTHS						
	JULY 1977	AUG.	SEPT.	OCT.	NOV.	DEC.	JAN. 1978
Group 1							
82	11.90	11.20	11.00	12.90	11.20	10.91	11.25
83	12.20	11.90	12.00	12.20	12.20	12.37	12.80
91	9.82	9.79	10.20	10.20	9.79	9.27	10.00
94	10.20	9.54	10.50	10.00	9.54	10.05	11.85
99	13.35	13.80	13.35	13.35	12.70	11.89	12.70
Group 2							
86	12.48	12.72	10.80	8.00	8.51	8.04	8.04
87	12.23	11.96	9.40	8.56	8.37	8.40	8.00
88	12.50	12.00	12.25	9.97	9.25	9.14	8.40
89	12.60	11.60	10.00	8.76	8.70	8.16	8.02
90	12.10	10.00	9.00	8.00	-	-	-
Group 3							
80	13.20	11.25	10.00	9.61	8.25	7.93	7.05
84	12.50	11.00	10.00	9.90	9.45	8.99	8.00
85	12.35	12.35	10.65	8.85	8.02	7.85	7.35
92	13.25	12.20	11.52	8.74	8.10	8.00	8.65
93	13.52	13.25	13.65	9.56	8.76	8.76	8.15
Group 4							
95	12.85	12.82	12.64	11.25	9.90	9.23	8.70
96	13.50	11.74	10.50	9.33	8.00	7.74	8.00
97	11.60	11.60	11.75	10.54	9.93	8.39	8.00
98	12.65	11.95	10.85	9.93	8.95	8.65	8.30
100	12.50	12.50	12.50	9.89	9.10	9.26	8.78

APPENDIX TABLE 3

HAEMOGLOBIN (gm/100 ml)

ANIMAL NUMBER AND GROUP	DURATION OF DEFICIENCY IN MONTHS						
	JULY 1977	AUG.	SEPT.	OCT.	NOV.	DEC.	JAN 1978
Group 1							
82	8.20	8.00	8.10	10.00	8.80	8.10	9.10
83	8.50	8.60	9.50	9.50	8.20	8.00	7.90
91	7.30	8.20	7.80	-	7.30	7.30	7.80
94	8.40	7.80	8.40	8.40	8.40	7.60	8.40
99	10.50	9.90	11.30	10.50	10.10	9.60	11.50
Group 2							
86	7.50	7.60	7.00	6.50	6.10	6.00	6.00
87	8.60	8.90	8.60	7.20	7.00	6.00	6.00
88	9.20	9.10	9.00	8.50	8.10	7.70	7.10
89	6.50	6.50	5.50	5.50	5.00	5.00	4.90
90.	7.30	6.50	6.50	6.00	-	-	-
Group 3							
80	9.70	9.80	8.90	8.20	8.20	7.90	7.70
84	8.70	8.80	8.20	8.00	7.70	7.20	7.50
85	8.20	9.70	8.60	7.70	7.60	7.20	7.00
92	8.70	8.80	8.20	7.50	7.20	7.00	6.50
93	7.20	10.30	8.30	6.40	6.40	6.20	6.30
Group 4							
95	8.20	8.60	8.00	7.80	7.00	6.50	6.60
96	9.50	9.20	8.90	8.60	-	7.40	6.90
97	8.50	8.70	8.00	7.20	6.60	6.10	6.00
98	11.20	10.30	9.50	9.00	8.50	8.00	7.50
100	9.10	9.50	8.20	7.60	6.90	6.90	7.00

APPENDIX TABLE 4

PACKED CELL VOLUME (%)

ANIMAL NUMBER AND GROUP	DURATION OF DEFICIENCY IN MONTHS						
	JULY 1977	AUG.	SEPT.	OCT.	NOV.	DEC.	JAN 1978
Group 1							
82	27.0	28.0	27.5	32.0	30.0	24.5	27.0
83	23.0	23.0	24.0	23.0	23.0	24.0	23.0
91	20.0	22.0	20.0	20.0	18.0	21.0	20.0
94	24.0	24.0	23.0	24.0	23.5	24.5	24.0
99	33.0	35.0	32.0	35.0	30.0	32.0	35.0
Group 2							
86	25.0	33.0	28.0	20.0	20.0	20.0	19.0
87	34.0	30.0	25.0	25.0	25.0	25.0	24.0
88	30.0	30.0	25.0	27.0	30.0	27.0	26.5
89	30.0	27.0	24.0	24.0	24.5	24.0	24.0
90	35.0	29.0	28.0	26.0	-	-	-
Group 3							
80	33.00	30.00	29.00	28.00	26.00	23.00	20.00
84	30.00	28.00	28.00	28.00	26.00	24.00	24.00
85	32.00	32.00	30.00	28.00	25.00	24.00	24.00
92	30.00	29.00	28.00	27.00	26.00	23.00	24.00
93	28.50	28.00	28.00	25.50	24.00	24.50	24.50
Group 4							
95	27.00	26.00	25.00	24.00	25.00	25.00	23.00
96	30.00	30.00	29.00	29.00	27.00	27.00	28.00
97	30.00	28.00	28.00	25.00	26.00	26.00	24.00
98	31.00	30.00	30.00	32.00	30.00	28.00	27.00
100	30.00	28.00	29.00	29.00	26.00	26.00	25.00

APPENDIX TABLE 5

MEAN CORPUSCULAR VOLUME (%)

ANIMAL NUMBER AND GROUP	DURATION OF DEFICIENCY IN MONTHS						
	JULY 1977	AUG.	SEPT.	OCT.	NOV.	DEC.	JAN. 1978
Group 1							
82	22.69	25.00	25.00	24.81	26.79	22.46	24.00
83	18.85	19.33	20.00	18.85	18.85	19.40	17.97
91	20.37	22.47	19.61	19.61	18.39	22.65	20.00
94	23.53	25.16	21.90	24.00	24.63	24.30	20.25
99	24.72	25.36	23.97	26.22	23.62.	26.91	27.56
Group 2							
86	20.03	25.94	25.93	25.00	23.50	24.88	23.63
87	27.80	25.08	26.60	29.21	29.87	29.76	30.00
88	24.00	25.00	20.41	27.08	32.43	29.54	31.55
89	23.81	23.28	24.00	27.40	28.16	29.41	29.93
90	28.93	29.00	31.11	32.50	-	-	-
Group 3							
80	25.00	26.67	29.00	29.14	31.52	29.00	28.37
84	24.00	25.45	28.00	28.28	27.51	26.70	30.00
85	25.91	25.91	28.17	31.64	31.17	30.57	32.65
92	22.64	23.77	24.31	30.89	32.10	28.75	27.75
93	21.08	21.13	20.31	23.67	27.40	27.92	30.08
Group 4							
95	21.01	20.28	19.78	21.33	25.25	27.09	26.44
96	22.22	25.55	27.62	31.08	33.75	34.88	35.00
97	25.86	24.14	23.83	23.72	26.18	30.99	30.00
98	24.51	25.10	27.65	32.23	33.52	32.37	32.53
100	24.00	22.40	23.20	32.62	28.57	28.08	28.47

APPENDIX TABLE 6

MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION (%)

ANIMAL NUMBER AND GROUP	DURATION OF DEFICIENCY IN MONTHS						
	JULY 1977	AUG.	SEPT.	OCT.	NOV.	DEC.	JAN 1978
Group 1							
82	30.37	28.57	29.45	31.25	29.33	33.06	33.70
83	36.96	37.39	39.58	41.30	35.65	33.33	34.35
91	36.50	37.27	39.00	-	40.56	34.76	39.00
94	35.00	32.50	36.52	35.00	35.74	31.02	35.00
99	31.82	28.29	35.31	30.00	33.67	30.00	32.86
Group 2							
86	30.00	25.03	25.00	32.50	30.50	30.00	31.58
87	25.29	29.67	34.40	28.80	26.00	24.00	25.00
88	30.67	30.33	36.00	31.48	27.00	28.52	26.79
89	21.67	24.07	22.92	22.92	20.41	20.83	20.42
90	20.83	22.41	23.21	23.08	-	-	-
Group 3							
80	29.39	32.67	30.69	29.29	31.34	34.35	38.50
84	29.00	31.43	29.29	28.57	29.62	30.00	31.25
85	25.63	30.31	28.67	27.50	30.40	30.00	29.17
92	29.00	30.34	29.29	27.78	27.69	30.43	27.08
93	25.26	36.79	29.64	25.10	26.67	25.31	25.71
Group 4							
95	30.37	33.08	32.00	32.50	28.00	26.00	28.70
96	31.67	30.67	30.69	29.66	-	27.41	24.64
97	28.33	31.07	28.57	28.80	25.38	23.46	25.00
98	37.33	34.33	31.67	28.13	28.33	28.57	27.78
100	30.33	33.93	28.28	26.21	26.54	26.54	28.00

APPENDIX TABLE 7A

LEUCOCYTE VALUES IN NORMAL AND COBALT DEFICIENT GOATS

GROUP ONE

	JULY			AUGUST		
82	WBC[a]	TN[b]	L[c]	WBC	TN	L
82	12.50	36.00	63.00	13.50	35.00	65.00
83	11.40	40.00	59.00	12.00	38.00	56.00
91	12.10	45.00	55.00	11.50	40.00	55.00
94	16.00	35.00	64.00	17.00	43.00	57.00
99	18.90	44.00	55.00	19.90	50.00	48.00
	SEPTEMBER					
82	11.98	50.00	46.00			
83	11.50	37.00	62.00			
91	11.50	43.00	56.00			
94	16.40	40.00	60.00			
99	18.20	38.00	60.00			

(a) WBC = Total white Blood Cells counts (10^3/cm)

(b) TN = Total Neutrophils %

(c) L = Lymphocytes %

APPENDIX TABLE 7A

TOTAL LEUKOCYTE VALUES

GROUP ONE

ANIMAL NUMBER	OCTOBER			NOVEMBER		
	WBC[a]	TN[b]	L[c]	WBC	TN	L
82	13.20	35.00	65.00	11.30	35.00	64.00
83	12.40	43.00	55.00	10.50	39.00	61.00
91	12.40	40.00	55.00	10.80	41.00	59.00
94	17.00	43.00	57.00	17.00	43.00	57.00
99	19.90	46.00	54.00	19.70	55.00	42.00
	DECEMBER			JANUARY		
82	11.80	45.00	52.00	13.10	34.00	65.00
83	11.50	48.00	52.00	16.60	45.00	55.00
91	11.30	41.00	50.00	10.80	47.00	51.00
94	15.10	47.00	52.00	14.80	43.00	51.00
99	18.20	44.00	51.00	18.60	38.00	53.00

(a) WBC = Total Leukocyte counts (10^3/cmm)
(b) TN = Total Neutrophils %
(c) L = Total Lymphocytes %

APPENDIX TABLES 7A

LEUKOCYTE VALUES

GROUP TWO

ANIMAL NUMBER	JULY WBC	JULY TN	JULY L	AUGUST WBC	AUGUST TN	AUGUST L
86	12.50	40.00	58.00	11.40	34.00	62.00
87	13.00	38.00	60.00	14.00	45.00	52.00
88	12.50	35.00	63.00	13.95	50.00	49.00
89	11.10	45.00	50.00	8.90	38.00	60.00
90	11.10	39.00	59.00	10.80	38.00	60.00
	SEPTEMBER					
86	15.20	48.00	50.00			
87	14.00	51.00	48.00			
88	12.80	49.00	50.00			
89	10.00	39.00	61.00			
90	12.10	42.00	61.00			
	OCTOBER			NOVEMBER		
86	8.60	72.00	28.00	14.80	56.00	43.00
87	14.00	71.00	29.00	12.60	58.00	41.00
88	13.90	44.00	52.00	17.50	45.00	53.00
89	8.10	58.00	40.00	8.10	61.00	38.00
90	9.80	56.00	42.00	-	-	-
	DECEMBER			JANUARY		
86	8.30	52.00	48.00	14.60	55.00	44.00
87	14.80	54.00	46.00	10.00	54.00	46.00
88	13.10	60.00	38.00	9.00	52.00	47.00
89	10.30	55.00	42.00	15.60	52.00	48.00
90	-	-	-	-	-	-

(a) WBC = Total White Blood Cell Counts (10^3/ cmm)
(b) TN = Total Neutrophils (%)
(c) L = Lymphocytes

APPENDIX TABLE 7A

GROUP THREE

ANIMAL NUMBER	JULY WBC(a)	JULY TN(b)	JULY L(c)	AUGUST WBC	AUGUST TN	AUGUST L
80	11.30	34.00	65.00	10.55	48.00	51.00
84	10.80	45.00	53.00	11.50	52.00	46.00
85	13.20	38.00	60.00	12.80	40.00	57.00
92	13.50	45.00	55.00	12.80	60.00	37.00
93	9.30	40.00	57.00	8.50	39.00	60.00

	SEPTEMBER		
80	10.20	48.00	52.00
84	11.20	55.00	42.00
85	12.50	60.00	39.00
92	12.80	48.00	52.00
93.	9.80	49.00	51.00

(a) WBC = Total White Blood Cell counts (10^3/cmm)
(b) TN = Total Neutrophils %
(c) L = Lymphocytes %

APPENDIX TABLE 7A

LEUKOCYTE VALUES

GROUP THREE

ANIMAL NUMBER	OCTOBER			NOVEMBER		
	WBC[a]	TN[b]	L[c]	WBC	TN	L
80	9.00	55.00	45.00	10.30	55.00	35.00
84	10.80	67.00	33.00	11.90	58.00	39.00
85.	12.80	64.00	35.00	17.00	72.00	28.00
92	13.90	58.00	42.00	13.10	62.00	38.00
93	8.60	48.00	52.00	9.30	47.00	51.00
	DECEMBER			JANUARY		
80	8.70	68.00	31.00	10.80	69.00	28.00
84	11.20	51.00	48.00	10.30	54.00	44.00
85	7.20	69.00	31.00	6.10	69.00	31.00
92	9.60	61.00	38.00	8.10	69.00	31.00
93	6.10	60.00	40;00	8.70	58.00	42.00

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(a) WBC = Total White Blood Cell counts (10^3/cmm)

(b) TN = Total Neutrophils %

(c) L = Lymphocytes %

APPENDIX TABLE 7A

LEUKOCYTE VALUES

ANIMAL NUMBER	JULY			AUGUST		
	WBC[a]	TN[b]	L[c]	WBC	TN	L
		GROUP FOUR				
95	13.90	38.00	60.00	14.20	35.00	65.00
96	14.90	35.00	64.00	13.20	36.00	63.00
97	15.90	50.00	49.00	14.90	43.00	57.00
98	13.90	47.00	53.00	14.20	45.00	55.00
100	12.80	42.00	57.00	11.20	39.00	60.00
		SEPTEMBER				
95	12.80	49.00	51.00			
96.	14.50	48.00	51.00			
97	15.80	60.00	40.00			
98	13.90	51.00	47.00			
100	10.80	45.00	45.00			

(a) WBC = Total white blood cell counts (10^3/cmm)

(b) TN = Total neutrophils %

(c) L = Lymphocytes %

APPENDIX TABLE 7A

LEUKOCYTE VALUES

GROUP FOUR

ANIMAL NUMBER	OCTOBER			NOVEMBER		
	WBC(a)	TN(b)	L(c)	WBC	TN	L
95	14.80	55.00	41.00	15.00	51.00	47.00
97	15.40	51.00	47.00	13.10	58.00	39.00
97	14.80	84.00	14.00	19.60	73.00	23.00
98	13.90	67.00	33.00	17.80	69.00	31.00
100	13.10	53.00	43.00	14.80	43.00	50.00
	DECEMBER			JANUARY		
95	9.00	33.00	67.00	6.00	43.00	55.00
96	13.10	52.00	38.00	8.40	52.00	46.00
97	17.30	65.00	32.00	14.30	65.00	35.00
98	7.50	65.00	34.00	11.10	58.00	42.00
100	6.80	48.00	52.00	8.80	45.00	54.00

(a) WBC = Total White Blood Cell Count (10^3/cmm)

(b) TN = Total Neutrophils %

(c) L = Lymphocytes %

APPENDIX TABLE 7B

TOTAL PROTEINS

ANIMAL NUMBER AND GROUP	DURATION OF DEFICIENCY IN MONTHS						
	JULY 1977	AUG.	SEPT.	OCT.	NOV.	DEC.	JANUARY 1978
Group 3							
80	8.0	8.2	8.4	8.0	8.0	7.8	7.4
84	7.8	8.1	6.7	6.7	7.0	7.4	7.4
85	7.2	7.4	6.8	6.5	7.4	7.4	6.8
92	6.5	8.2	7.2	6.5	7.2	7.2	7.2
93	7.0	7.0	7.4	7.0	7.4	7.2	7.4
Group 4							
95	7.3	6.8	7.3	6.7	7.0	7.0	7.4
96	7.2	6.9	7.3	7.2	7.0	7.2	7.4
97	7.4	8.2	7.5	8.3	7.6	7.6	7.4
98	7.4	7.4	7.4	7.4	7.4	7.4	7.4
100	7.2	7.0	7.4	7.4	7.0	7.0	6.8

APPENDIX TABLE 78

TOTAL PROTEINS IN PLASMA (mgs %)

Animal Number And Group	DURATION OF DEFICIENCY IN MONTHS						
	July 1977	Aug	Sept.	Oct.	Nov.	Dec.	January 1978
Group 1							
82	7.2	7.8	7.2	7.4	7.8	7.4	7.2
83	7.2	7.4	7.5	7.2	7.4	-	7.2
91	7.2	6.8	7.0	6.8	6.8	6.8	6.8
94	8.0	7.9	7.5	7.9	8.2	8.0	8.0
99	6.8	7.1	7.0	7.1	7.8	7.4	7.1
Group 2							
86	7.0	7.2	7.3	7.0	7.4	7.4	7.4
87	7.0	6.8	7.3	7.2	7.6	7.4	7.4
88	7.2	7.0	7.3	7.0	7.2	8.2	7.0
89	8.0	8.2	8.4	8.0	7.4	8.2	8.2
90	7.0	7.2	7.3	7.0	7.2	7.1	7.0

APPENDIX TABLE 8

CYANOCOBALMIN VALUES IN NORMAL AND COBALT DEFICIENT GOATS

Date of Blood Collection	ANIMAL NUMBER 82	83	91	GROUP ONE 94	99
Aug 8	340.0	500.00	460.00	550.00	460.00
Aug 16	415.0	530.00	310.00	400.00	330.00
Sept. 1	440.0	800.00	500.00	850.00	400.00
Sept. 16	315.0	620.00	300.00	600.00	700.00
Oct. 1	300.0	390.00	450.00	670.00	300.00
Oct. 16	300.0	410.00	450.00	420.00	400.00
Nov. 1	340.0	500.00	300.00	730.00	400.00
Nov. 16	460.0	650.00	460.00	410.00	320.00
Dec. 1	510.0	650.00	500.00	730.00	480.00
Dec. 16	430.0	580.00	330.00	540.00	300.00
Jan. 10	320.0	500.00	300.00	800.00	400.00

	ANIMAL NUMBER 86	87	88	GROUP TWO 89	90
Aug 8.	1030.00	700.00	580.00	535.00	530.00
Aug. 16	350.00	370.00	260.00	774.00	510.00
Sept. 1	925.00	500.00	285.00	232.00	515.00
Sept. 16	345.00	177.00	160.00	232.00	165.00

All values in pg/ml

Table 8 cont.

	ANIMAL NUMBER			GROUP TWO	
	86	87	88	89	90
Oct. 1	325.00	210.00	120.00	142.00	126.00
Oct. 16	167.00	110.00	155.00	477.00	-
Nov. 1	186.00	120.00	171.00	168.00	-
Nov. 16	165.00	170.00	125.00	180.00	-
Dec. 1	186.00	120.00	120.00	136.00	-
Jan. 10	235.00	55.00	170.00	171.00	-

	ANIMAL NUMBER		GROUP THREE		
	80	84	85	92	93
August. 8	390.00	105.00	400.00	670.00	730.00
August 16	375.00	620.00	495.00	620.00	400.00
Sept. 1	352.00	365.00	588.00	380.00	185.00
Sept. 16	150.00	200.00	177.00	100.00	150.00
Oct. 1	135.00	120.00	150.00	400.00	165.00
Oct16.	135.00	120.00	60.00	150.00	165.00
Nov. 1	145.00	120.00	100.00	150.00	168.00
Nov. 16	150.00	125.00	160.00	195.00	120.00
Dec.1	150.00	135.00	173.00	100.00	150.00
Dec. 16	120.00	140.00	136.00	155.00	140.00
Jan. 10	150.00	120.00	100.00	155.00	115.00

All values in pg/ml

Appendix Table 8 cont.

	ANIMAL NUMBER		GROUP FOUR		
	95	96	97	98	100
Aug. 8	516.00	350.00	1097.00	529.00	774.00
Aug. 16	335.00	220.00	335.00	387.00	129.00
Sept. 1	197.00	170.00	258.00	120.00	95.00
Sept. 16	158.00	150.00	171.00	206.00	90.00
Oct. 1	97.00	175.00	152.00	438.60	70.00
Oct. 16	152.00	220.00	165.00	165.00	130.00
Nov. 1	103.00	220.00	197.00	161.00	165.00
Nov. 16.	155.00	116.00	189.00	171.00	112.00
Dec. 1	130.00	170.00	195.00	171.00	95.00
Dec. 16	116.00	150.00	165.00	160.00	123.00
Jan. 10	64.50	177000	161.00	152.00	120.00

All values in pg/ml.

APPENDIX TABLE 9

CORTICOSTEROID CONCENTRATION IN NORMAL AND COBALT DEFICIENT GOATS

Duration of Deficiency in weeks	ANIMAL NUMBERS AND GROUP			
	82 Group One			
	82	83	94	99
0	25.75	20.80	19.32	20.32
2	20.75	15.85	27.74	21.22
4	20.55	16.93	19.13	16.84
6	19.15	15.85	22.92	21.44
8	20.50	23.19	21.81	20.89
11	20.44	20.70	23.87	20.90
13	83.20	14.00	18.04	20.90
16.	22.39	18.96	20.38	20.90
18	19.81	22.69	20.47	22.38
21	22.78	20.06	16.75	22.68
23.	19.22	11.64	20.09	20.25
	Group Two			
	87	88	89	
0	20.90	22.40	21.81	
2	26.44	55.96	23.12	
4	70.90	62.59	14.95	
6	91.63	60.40	81.28	
8	93.12	56.65	74.88	
11.	183.26	86.77	65.47	
13	126.35	86.77	60.50	
16	88.16	115.84	49.90	
18	82.59	133.68	66.93	
21	74.29	111.34	66.74	
23	50.52	66.32	36.87	

*Values in ng/10 ml

APPENDIX TABLE 9 cont.

Duration of Deficiency in weeks	ANIMAL NUMBERS AND GROUPS		
	GROUP THREE		
	80	84	85
0	10.82	22.75	20.89
2	50.52	47.83	30.89
4	66.86	47.83	66.86
6	78.82	121.79	104.01
8	92.62	64.39	51.29
11	94.10	63.79	54.48
13	59.62	49.53	106.49
16	57.15	62.10	68.23
18	54.46	68.33	53.40
21	67.86	53.86	53.77
23	64.46	58.67	68.42

* Values in ng/10 ml.

Table 9 cont.

Duration of Deficiency in weeks	Animal Numbers Group		
	GROUP FOUR		
	96	97	100
0	25.20	20.28	22.78
2	34.20	33.68	68.87
4	69.81	64.39	88.92
6	66.87	50.44	69.36
8	79.25	81.39	61.20
11	60.71	118.87	52.69
13	84.18	77.50	58.80
16	51.70	52.06	56.34
18	53.59	61.91	51.76
21	50.55	54.69	55.50
23	53.59	51.69	52.24

*Values in ng/10 ml

APPENDIX TABLE 10

THYROXINE (T_4) ug/100 ml) IN GOATS

Duration of Deficiency in weeks	GROUP ONE (CONTROL)			GROUP TWO		
	82	83	94	87	88	89
0	5.15	5.68	6.16	6.46	5.94	5.60
2	4.29	4.68	5.26	5.01	7.18	4.50
3	4.63	3.94	4.26	5.18	8.48	4.50
5	3.91	3.65	5.58	6.28	9.12	6.70
6	2.95	2.16	3.61	6.70	8.10	6.55
8	3.99	5.50	6.79	6.70	8.72	7.20
9	4.37	4.55	6.79	6.34	7.74	8.23
11	2.52	3.40	4.80	8.17	7.11	5.40
12	2.34	2.30	5.30	10.54	9.20	8.17
14	3.29	4.09	3.79	11.36	11.48	5.80
15	4.09	3.42	4.20	11.56	10.03	9.37
17	5.22	3.36	6.87	10.38	12.38	9.37
18	4.08	3.42	5.20	10.60	8.24	10.55
20	4.64	5.50	4.78	11.82	8.24	8.70
21	5.45	3.48	8.11	12.80	10.85	8.39

APPENDIX TABLE 10

THYROXINE (T_4) ug/100m)

DURATION OF DEFICIENCY IN WEEKS	GROUP THREE (ENERGY)			GROUP FOUR (PROTEIN)		
	80	84	85	95	96	97
0	5.58	4.90	7.06	5.95	3.07	1.80
2	5.78	4.40	3.50	6.03	8.60	2.90
3	5.39	7.90	8.50	6.13	6.10	5.80
5	7.57	5.90	5.20	11.65	5.08	6.30
6	9.15	6.90	6.20	9.31	5.58	7.85
8	5.59	5.60	7.30	10.15	5.20	8.85
9	9.19	6.50	8.70	7.62	8.58	5.80
11	21.30	7.30	9.20	10.27	6.36	6.99
12	9.79	6.10	8.70	13.33	7.45	8.50
14	10.46	9.40	11.50	7.32	6.68	9.58
14	9.79	8.50	14.20	16.52	8.77	7.36
17	11.59	16.50	7.51	13.58	9.21	8.66
18	12.51	14.19	9.38	14.32	7.92	6.77
20	7.38	10.20	9.38	11.80	9.28	11.92
21	9.38	9.50	11.39	13.03	7.21	7.21

APPENDIX TABLE 11A

THYROPAC 3 VALUES

Duration of Deficiency in weeks	Group One			Group Two		
	82	83	84	87	88	89
0	122.62	107.79	111.26	103.96	110.78	118.74
3	113.57	110.91	101.62	121.06	105.26	118.30
6	108.83	99.85	103.23	120.27	78.78	144.24
9	122.52	103.33	120.46	123.34	64.94	142.31
12	117.57	117.71	110.49	106.12	91.62	119.66
15	103.04	111.52	112.67	70.27	98.00	142.60
18	107.10	121.52	112.67.	97.24	86.62	100.08
21	105.91	100.42	151.10	96.48	87.53	95.25
Group mean	112.45			105.64		

	Group Three			Group Four		
	80	84	85	95	96	97
0	120.42	117.95	119.71	105.37	116.50	106.12
3	125.03	119.31	118.80	107.44	119.94	116.12
6	126.98	106.17	106.25	106.08	143.00	111.64
9	127.35	115.15	104.60	106.61	112.31	122.85
12	107.60	92.39	104.66	95.79	100.94	102.20
15	91.52	.	65.60	99.49	103.17	78.11
16	91.52	93.56	93.60	92.52	109.54	80.20
21	90.50	98.48	62.04	91.53	98.62	38.19
Group mean	103.79			103.01		

APPENDIX TABLE 110

FREE THYROXINE INDEX

Duration of deficiency in weeks	Group One			Group Two		
	82	83	94	87	88	89
0	4.19	5.27	5.54	6.21	5.36	4.72
3	4.08	3.55	4.19	4.28	8.06	3.80
6	2.71	2.16	3.50	5.57	11.44	4.54
9	3.57	4.40	5.64	5.14	11.92	5.78
12	1.99	1.95	4.80	9.93	10.04	6.83
15	3.94	3.07	3.73	16.45	10.23	6.57
18	3.81	2.81	4.62	10.90	9.51	10.54
21	5.15	3.37	15.37	12.20	12.40	8.81

	Group Three			Group Four		
	80	84	85	95	96	97
0	4.63	4.14	5.90	5.65	3.32	1.70
3	4.31	6.62	7.15	5.71	5.09	4.99
6	7.21	6.50	5.84	8.78	3.90	7.03
9	7.22	5.64	8.32	7.15	7.64	4.72
12	9.10	6.60	8.31	13.92	7.38	8.32
15	10.71	9.24	21.65	16.60	8.56	9.42
18	13.67	15.17	10.02	15.48	7.23	7.68
21	10.36	9.65	18.36	14.24	7.31	18.88

APPENDIX TABLE 12A

OESTROGENS, PROGESTERONE AND LUTEINIZING HORMONE VALUES IN CONTROL GROUP GOATS

ANIMAL NUMBER 82: GROUP ONE

Date of Blood Collection	Oestr. pg/ml	Prog. ng/ml	LH ng/ml
9/8	21.80	0.99	3.00
10/8	41.83	0.90	12.00
10/8	66.03	0.90	11.50
15/8	7.56	2.03	2.50
15/8	10.74	2.15	3.20
18/8	10.75	6.55	1.50
22/8	13.43	7.05	0.50
23/8	18.47	8.95	1.50
27/8	20.15	5.15	2.50
30/8	11.76	4.00	3.75
31/8	69.39	0.42	11.00
31/8	77.39	0.42	10.50
3/9	20.15	2.50	2.50
7/9	20.15	3.61	0.50
12/9	11.76	6.50	1.50
16/9	20.15	3.35	2.45
18/9	71.90	0.87	10.50
20/9	23.20	1.60	2.00
24/9	11.08	7.15	1.50
28/9	23.51	8.95	1.75
2/10	21.83	8.50	2.75
5/10	69.40	0.77	8.50
10/10	7.40	3.18	2.00
14/10	20.99	6.99	0.50
18/10	29.39	8.90	2.00
22/10	56.77	0.98	3.50
25/10	58.77	0.98	11.00
25/10	94.46	0.55	11.50
29/10	9.07	2.58	2.50
2/11	18.47	3.50	1.00
5/11	16.37	6.18	2.50
6/11	18.20	7.70	1.75
9/11	32.79	8.99	2.50
13/11	73.39	0.89	9.50
17/11	16.79	2.79	2.00

Appendix Table 12 a Cont.

Animal Number 82: Group One

Date of Blood Collection	Oestr. pg/ml	Prog. ng/ml	LH ng/ml
21/11	11.76	5.35	2.00
25/11	40.38	5.00	1.00
29/11	20.15	6.90	1.50
3/12	66.94	0.99	9.50
6/12	11.08	2.21	3.50
6/12	6.55	-	0.50
11/12	16.79	5.20	2.00
15/12	23.51	7.45	2.50
17/12	41.98	7.10	1.50
17/12	-	6.25	2.50
19/12	50.38	7.10	3.50
23/12	73.47	0.82	9.50
28/12	18.47	2.70	1.60
3/1	16.37	3.70	0.50
7/1	41.98	4.20	1.50
7/1	-	6.70	3.00
8/1	65.07	0.32	11.50
9/1	14.03	0.32	5.30
14/1	-	1.37	1.50

TABLE 12 A cont.

ANIMAL NUMBER 83: GROUP ONE

Date of Blood Collection	Oestr. pg/ml	Prog. ng/ml	LH ng/ml
8/8	7.39	8.50	2.10
11/8	20.99	8.00	2.30
14/8	10.08	8.50	1.90
17/8	9.24	8.40	2.00
20/8	49.24	0.68	8.50
20/8	66.23	0.66	11.50
21/8	9.23	0.68	1.90
23/8	9.24	1.85	2.50
26/8	20.99	5.85	1.60
29/8	8.40	5.85	2.50
1/9	20.15	4.35	2.50
6/9	79.51	0.58	9.00
6/9	-	1.85	6.00
10/9	12.67	3.73	1.50
14/9	11.76	2.47	2.00
19/9	57.10	0.70	8.50
23/9	15.39	3.15	2.50
27/9	18.47	6.70	1.50
1/10	30.15	6.65	1.90
5/10	72.50	0.65	8.50
9/10	7.74	-	1.45
13/10	10.06	6.50	1.75
17/10	35.75	3.50	3.50
21/10	77.80	0.43	9.75
25.10	9.74	3.10	2.20
28/10	7.39	5.35	2.50
1/11	-	3.75	2.80
4/11	19.02	0.95	2.80
8/11	51.02	0.80	17.00
14/11	8.40	2.42	2.50
16/11	7.29	3.50	2.30
20/11	8.22	7.50	-
24/11	20.56	3.46	2.70
27/11	65.12	0.97	11.50
1/12	6.82	1.95	1.75
3/12	7.45	3.85	1.75

TABLE 12 A cont.

ANIMAL NUMBER 83: Group One

Date of Blood Collection	Oestr. pg/ml	Prog. ng/ml	LH ng/ml
3/12	7.45	3.85	1.75
6/12	20.67	7.25	1.60
9/12	20.67	-	-
18/12	95.60	0.80	7.75
22/12	22.47	1.80	0.50
26/12	23.09	5.50	2.[illegible]
30/12	54.00	8.65	2.00
2/1	21.86	5.30	2.00
6/1	20.58	5.30	2.15
7/1	68.30	0.45	10.15
15/1	-	3.00	-

ANIMAL NUMBER 91: GROUP ONE

10/8	10.80	5.55	1.75
13/8	7.10	4.85	2.85
16/8	35.54	5.15	3.50
19/8	73.98	0.35	11.50
22/8	15.53	1.80	2.50
25/8	9.24	2.25	0.50
27/8	12.42	5.75	1.50
30/8	9.24	7.50	1.00
3/9	34.45	3.65	2.00
7/9	76.94	0.85	9.00
12/9	14.26	1.00	2.50
16/9	12.10	2.75	0.75
20/9	5.88	4.65	1.50
24/9	35.76	8.25	2.50
27/9	65.53	0.95	7.00
28/9	18.55	1.20	2.00
1/10	5.23	1.95	1.15
2/10	8.92	5.10	1.00

Table 12 A cont.

ANIMAL NUMBER: 91 Group One

Date of Blood Collection	Oestr. pg/ml	Prog. ng/ml	LH ng/ml
5/10	5.85	5.10	0.50
6/10	5.85	6.55	2.00
9/10	7.72	6.05	1.50
10/10	18.20	-	2.50
13/10	59.32	3.10	1.50
14/10	65.86	0.80	8.25
17/10	6.22	2.50	2.75
18/10	23.09	3.00	1.50
22/10	18.47	4.65	1.00
25/10	18.50	4.35	2.00
29/10	60.50	6.70	1.50
30/10	-	4.05	3.75
1/11	78.55	0.95	9.50
5/11	6.25	1.05	2.00
9/11	15.45	3.65	1.50
13/11	68.54	7.27	1.75
17/11	108.54	0.58	8.50
22/11	11.59	2.25	0.75
26/11	28.99	3.25	2.50
3/12	36.14	8.25	1.50
5/12	55.42	0.52	5.50
11/12	35.74	1.16	0.56
15/12	18.54	5.80	1.55
19/12	15.09	5.75	2.50
23/12	52.48	0.69	11.00
23/12	-	0.59	1.60
27/12	16.84	3.65	1.50
31/12	14.24	5.75	0.50
4/1	26.86	8.25	-
8/1	15.80	8.00	1.75
11/1	59.56	0.95	8.50

TABLE 12A cont.

Animal Number 94: Group One

Date of Blood Collection	Oestr. pg/ml	Prog. ng/ml	LH ng/ml
10/8	-	4.55	-
12/8	30.95	3.05	3.00
15/8	69.15	0.40	11.00
18/8	10.63	2.05	2.50
21/8	7.30	5.80	3.50
23/8	27.59	7.80	1.50
27/8	12.56	8.00	1.50
27/8	-	8.90	1.85
30/8	37.85	2.95	3.00
3/9	66.70	0.95	10.50
7/9	16.22	2.65	2.00
12/9	3.42	2.95	1.50
16/9	37.02	5.40	1.50
21/9	69.15	0.90	9.00
24/9	27.30	2.80	2.50
28/9	21.74	3.40	2.50
2/10	11.42	6.10	2.50
6/10	66.37	0.95	9.85
10/10	7.32	4.85	1.50
14/0	10.75	8.40	0.50
18/10	23.55	5.40	2.15
22/10	27.51	3.35	7.50
25/10	31.70	2.60	3.50
29/10	61.70	0.35	13.50

Table 12A cont.

Date of Blood Collection	Oestr. pg/ml	Prog. ng/ml	LH ng/ml
Animal Number 94: Group One			
2/11	9.24	2.65	2.00
5/11	31.70	1.70	1.00
9/11	69.00	1.05	2.50
13/11	9.80	4.20	1.50
17/11	4.60	7.20	3.50
22/11	9.80	5.60	1.50
26/11	50.44	3.65	0.80
29/11	65.66	0.25	2.00
4/12	18.57	1.60	0.75
7/12	9.05	1.60	4.00
11/12	11.07	5.60	2.00
15/12	26.27	5.95	2.00
20/12	82.25	0.70	12.50
21/12	16.55	0.70	1.00
24/12	16.55	5.60	0.50
27/12	18.77	8.15	2.50
28/12	28.55	3.20	2.65
3/1	77.56	0.65	27.50
7/1	76.55	3.15	2.50
9/1	27.50	0.95	2.50
12/1	20.55	-	2.65
Animal Number 99: Group One			
9/8	5.37	4.35	2.50
12/8	12.88	2.20	1.50
15/8	18.47	1.95	2.50
18/8	-	1.85	-
20/8	70.53	0.85	10.50
23/8	3.36	1.80	3.00
26/8	10.08	2.65	1.15
29/8	16.79	3.65	2.00
3/9	8.90	4.25	2.50
6/9	90.38	3.35	2.00
11/9	78.77	0.75	8.00
12/9	8.90	1.85	0.75
14/9	8.90	2.40	0.50
19/9	7.90	4.85	2.50
23/9	-	6.80	2.85
28/9	26.87	0.85	7.25
1/10	2.60	3.30	0.50
5/10	8.40	5.55	0.50
9/10	6.05	9.00	2.50
15/10	100.36	0.50	9.50

Table 12A cont.

Animal Number 39: Group One

Date of Blood Collection	Oestr. pg/ml	Prog. ng/ml	LH ng/ml
19/10	16.87	1.75	1.50
22/10	4.62	5.35	2.50
25/10	23.51	6.80	1.00
28/10	30.88	6.35	2.50
31/10	90.68	0.80	16.50
4/11	16.79	1.38	0.60
8/11	-	3.65	2.00
1/11	41.98	6.55	2.00
15/11	40.38	0.85	8.00
18/11	70.38	0.55	9.00
20/11	4.20	0.55	1.00
24/11	8.40	2.35	2.00
27/11	38.62	3.50	3.00
2/12	28.65	6.55	2.50
4/12	52.16	4.10	2.50
6/12	102.46	0.55	8.50
10/12	13.43	2.40	0.50
14/12	18.72	2.40	2.00
18/12	-	3.95	0.80
24/12	88.16	0.70	10.50
26/12	8.40	0.50	3.50
30/12	6.51	1.00	2.50
2/1	20.99	3.00	1.50
6/1	15.11	6.55	2.50
10/1	54.58	8.56	2.50
12/1	62.06	0.75	10.50
17/1	-	-	1.85

APPENDIX TABLE 128

OESTROGENS, PROGESTERONE AND LUTEINIZING HORMONE VALUES IN COBALT DEFICIENT GROUP (TWO)

ANIMAL NUMBER 86: GROUP TWO

Date of Blood Collection	Oestr. pg/ml	Prog. ng/ml	LH ng/ml
10/8	78.28	0.70	13.50
13/8	16.07	2.37	1.25
14/8	-	4.37	0.50
16/8	15.04	5.32	2.50
22/8	17.21	8.51	1.25
24/8	60.87	2.05	3.50
25/8	84.34	0.55	18.75
28/8	25.70	1.95	3.00
31/8	28.13	3.30	3.00
4/9	34.84	7.46	3.50
8/9	6.72	9.20	2.50
13/9	41.98	7.49	2.60
17/9	47.77	1.58	2.53
21/9	79.81	0.58	8.75
25/9	12.25	0.09	0.80
3/10	20.78	0.89	1.00
7/10	18.19	2.20	2.50
11/10	48.38	0.15	9.50
15/10	14.20	0.11	1.25
19/10	8.59	0.53	1.25
23/10	10.92	3.20	2.25
26/10	5.57	5.40	2.50
30/10	14.67	4.45	3.00
2/11	18.47	4.45	2.00
6/11	38.05	2.98	2.75
10/11	44.98	0.70	11.50
14/11	10.25	0.73	1.50
18/11	6.75	0.98	2.00
22/11	22.25	1.70	1.00
26/11	14.00	0.53	1.00
28/11	18.05	0.41	1.00
30/11	11.75	1.00	2.00
4/12	2.85	0.65	1.00
8/12	9.15	0.81	1.00
12/12	19.52	0.97	1.75
16/12	-	0.32	0.75
24/12	10.55	0.44	0.75
28/12	13.43	0.53	0.85
31/12	6.77	0.12	1.15
3/1	13.55	0.82	1.25
7/1	18.05	0.31	2.00
11/1	9.2	0.05	1.00
15/1	6.72	0.20	1.50

Table 128 cont.

Animal Number 87: Group Two

Date of Blood Collection	Oestr. pg/ml	Prog. ng/ml	LH ng/ml
8/8	30..65	8.50	3.75
11/8	22.25	1.85	3.00
14/8	42.12	2.55	5.25
17/8	79.76	0.25	16.75
20/8	19.73	2.25	1.50
24/8	9.80	3.50	3.55
26/8	12.59	4.50	2.00
29/8	20.15	8.30	3.00
1/9	23.09	6.45	4.50
5/9	39.04	4.40	2.95
10/9	60.52	0.98	18.75
10/9	59.30	0.85	12.75
14/9	19.04	1.78	3.56
19/9	34.51	3.23	3.57
23/9	34.34	3.90	6.75
27/9	61.97	6.83	2.25
1/10	20.56	8.20	2.50
5/10	39.04	8.25	1.25
9/10	56.11	3.75	6.25
13/10	56.88	0.12	26.75
18/10	-	0,55	0.50
21/10	18.13	0.55	0.50
25/10	19.33	-	1.50
28/10	11.04	0.78	2.00
31/10	21.04	0.75	2.25
4/11	46.41	0.30	9.50
8/11	21.33	2.50	3.75
12/11	11.33	3.40	1.75
16/11	4.75	3.34	2.00
18/11	10.40	6.10	1.50
20/11	14.69	5.27	1.50
24/11	10.40	4.05	1.50
26/11	11.55	0.78	0.50
27/11	-	0.78	2.50
2/12	38.50	0.02	5.50
2/12	38.50	0.02	9.50
6/12	20.30	1.20	1.00
10/12	8.37	5.95	1.15
14/12	8.38	1.55	5.50

Table 128 cont.

Date of Blood Collection	Oestr. pg/ml	Prog. ng/ml	LH ng/ml
Animal Number 87: Group Two			
18/12	-	0.37	1.50
22/12	4.20	0.45	1.75
26/12	4.20	0.79	1.00
30/12	17.63	0.55	0.75
1/1	14.27	0.27	0.80
6/1	8.82	1.11	0.60
10/1	10.76	1.21	1.00
13/1	-	0.95	-
Animal Number 88: Group Two			
10/8	4.62	5.35	2.00
13/8	16.67	2.35	0.50
16/8	11.08	3.25	1.50
19/8	54.42	0.25	11.50
22/8	30.23	2.25	2.00
24/8	11.33	8.30	2.00
25/8	4.20	8.00	1.00
28/8	28.58	8.46	1.50
31/8	8.30	8.20	2.15
4/9	19.31	3.28	3.00
6/9	26.03	2.50	-
13/9	57.14	0.45	17.00
13/9	26.03	0.58	6.25
18/9	11.75	1.57	1.50
21/9	8.60	2.44	2.50
22/9	8.59	4.81	8.55
23/9	4.21	15.20	11.11
27/9	4.21	11.50	2.50
30/9	-	8.50	2.50
1/10	18.19	5.40	3.20
5/10	43.96	0.80	27.50
9/10	18.89	0.80	0.50
13/10	8.89	1.80	1.40
17/10	4.21	1.70	0.50
21/10	4.20	3.02	0.75
25/10	36.67	-	1.50
28/10	28.50	4.40	2.00
31/10	-	0.34	17.50
4/11	38.28	0.02	8.50
7/11	4.20	0.73	2.25

Table 128 cont.

Date of Blood Collection	Oestr. pg/ml	Prog. ng/ml	LH ng/ml
ANIMAL NUMBER 88: GROUP TWO			
16/11	22.48	0.78	0.50
18/11	40.56	0.02	13.50
20/11	13.02	1.21	2.50
24/11	6.62	1.55	0.50
27/11	6.89	0.37	1.50
28/11	13.01	0.50	0.65
2/12	11.75	0.45	0.60
6/12	-	0.79	2.00
10/12	54.58	0.21	1.00
14/12	8.62	0.27	2.25
18/12	26.18	1.02	0.50
22/12	26.18	0.70	2.50
26/12	10/18	0.81	4.00
30/12	13.58	1.21	1.50
2/1	17.85	1.25	1.50
6/1	10.50	1.23	0.50
10/1	13.01	1.30	0.50
17/1	37.78	0.85	0.68
Animal Number 89: Group Two			
8/8	30.50	0.95	2.00
11/8	82.40	0.88	11.50
14/8	9.53	1.25	3.25
17/8	18.40	3.50	2.50
20/8	5.20	4.50	1.00
23/8	-	5.50	0.85
26/8	42.80	8.20	3.50
29/8	56.82	0.99	12.55
1/9	25.05	1.50	3.56
5/9	12.80	5.80	3.50
10/9	28.78	7.50	2.50
14/9	32.40	8.55	1.25
20/9	18.50	5.35	2.25
23/9	42.40	0.68	2.25
27/9	52.55	0.22	13.50

TABLE 128 cont.

Animal Number 89: Group Two

Date of Blood	Oestr. pg/ml	Prog. ng/ml	LH ng/ml
1/10	18.89	1.00	0.50
5/10	9.50	1.22	2.50
9/10	16.50	3.50	0.80
13/10	18.99	6.50	1.20
17/10	22.80	6.50	3.00
20/10	38.40	4.50	2.00
24/10	45.54	0.22	8.00
28/10	18.29	0.50	1.00
31/10	29.19	1.50	3.00
4/11	8.89	0.50	1.50
8/11	5.84	0.80	1.75
12/11	6.87	1.50	1.25
16/11	6.87	0.50	1.75
24/11	5.84	0.95	-
27/11	6.70	1.50	1.50
2/12	19.50	1.20	1.95
6/12	18.50	0.86	1.00
10/12	10.50	0.09	2.00
14/12	10.50	0.09	1.95
22/12	8.89	0.02	1.75
22/12	3.50	0.08	1.55
26/12	4.20	0.58	1.65
29/12	-	0.23	2.25
30/12	8.20	0.68	1.00
2/1	11.87	1.11	0.95
6/1	22.87	0.95	1.25
10/1	-	0.95	1.00
17/1	-	1.05	0.98

Table 128 cont.

ANIMAL NUMBER 90 : GROUP TWO

Date of Blood Collection	Oestr. pg/ml	Prog. ng/ml	LH ng/ml
8/8	60.50	0.41	8.05
11/8	19.24	1.79	0.75
14/8	17.35	2.52	1.50
17/8	14.69	7.36	0.50
20/8	7.35	5.37	2.00
24/8	46.82	3.77	3.00
26/8	95.80	0.88	15.50
29/8	39.05	1.28	3.50
1/9	14.21	3.92	2.50
5/9	8.25	3.85	0.50
10/9	15.25	2.61	2.75
14/9	50.38	0.34	11.50
19/9	8.82	3.00	1.75
23/9	5.88	1.94	0.75
25/9	10.50	1.94	1.20
28/9	9.24	1.41	1.25
2/10	7.35	1.79	0.50
5/10	14.69	2.52	1.25
8/10	0.75	0.92	0.75
12/10	2.00	0.92	2.00
15/10	1.50	0.85	1.50
19/10	1.50	0.61	1.50
21/10	0.73	1.94	0.73
24/10	-	1.21	0.50
28/10	0.60	0.50	0.60

APPENDIX TABLE 12C

OESTROGENS, PROGESTERONE AND LUITEINIZING HORMONE VALUES IN GROUP THREE GOATS.

ANIMAL NUMBER 80: GROUP THREE

Date of Blood Collection	Oestr. pg/ml	Prog. ng/ml	LH ng/ml
8/8	23.93	1.20	1.50
11/8	20.99	3.65	0.50
14/8	35.68	1.20	2.00
17/8	82.00	0.95	15.50
20/8	22.40	2.05	2.50
23/8	-	4.05	1.50
26/8	28.55	7.75	10.00
29/8	21.56	9.75	2.75
3/9	18.89	11.85	1.00
5/9	41.98	2.55	2.00
6/9	71.56	0.75	19.50
10/9	29.20	2.10	4.00
12/9	14.27	5.45	3.75
17/9	39.04	6.50	3.50
20/9	42.82	5.70	3.75
22/9	61.56	0.50	14.75
28/9	4.98	1.20	2.00
2/10	18.89	1.25	1.50
3/10	3.25	3.00	3.75
7/10	7.95	3.25	2.75
10/10	11.87	4.70	2.00
15/10	19.25	4.10	2.38
19/10	42.27	0.25	16.75
23/10	5.20	1.35	1.00
26/10	5.05	3.70	3.00
29/10	8.40	4.55	1.50
30/10	8.40	2.50	4.50
2/11	15.68	3.50	2.00
6/11	12.59	3.75	1.50
10/11	22.48	4.15	5.00
14/11	34.69	2.00	3.00
18/11	44.69	0.90	15.20
22/11	11.75	0.70	2.25
24/11	8.90	1.80	2.50
26/11	11.33	0.99	5.00
30/11	11.33	2.90	0.50
4/12	4.20	1.05	2.00
8/12	14.69	1.60	1.00
12/12	14.69	0.66	1.00
16/12	4.69	1.60	3.00

Table 12C cont.

Date of Blood Collection	Oestr. pg/ml	Prog. ng/ml	LH ng/ml
Animal Number 80: Group Three			
20/12	8.55	0.70	10.50
25/12	9.07	0.55	3.00
31/12	9.39	1.10	2.00
4/1	10.50	0.58	1.00
8/1	10.50	1.65	2.00
14/1	-	1.65	0.85
Animal Number 84: Group Three			
9/8	34.31	1.43	3.50
12/8	69.55	0.63	14.54
15/8	25.77	1.79	2.00
18/8	18.50	6.43	2.50
21/8	16.77	5.07	2.50
23/8	12.05	10.18	6.00
27/8	8.55	5.77	4.50
30/8	26.55	6.79	5.00
3/9	89.55	0.87	12.50
7/9	18.40	2.03	3.25
10/9	15.45	5.12	2.25
12/9	13.97	5.66	6.55
16/9	8.40	4.23	1.50
20/9	50.38	7.91	3.25
24/9	4.20	3.78	3.75
28/9	16.79	6.05	1.75
30/9	-	3.73	5.50
2/10	55.26	0.59	18.00
2/10	60.50	0.93	6.00
6/10	7.80	0.93	0.98
10/10	16.53	2.36	2.00
14/10	11.90	3.60	4.50
18/10	8.82	3.60	2.00
20/10	15.68	4.73	3.50
25/10	8.40	4.82	3.75
26/10	11.40	3.82	3.00
29/10	18.40	2.13	2.50
1/11	32.79	0.65	11.50
5/11	16.26	0.16	2.00
9/11	11.15	0.58	1.00
13/11	10.58	2.13	2.50
17/11	18.33	3.04	2.00
18/11	20.55	4.80	1.65

Table 12C cont.

Animal Number 84: Group Three			
Date of Blood Collection	Oestr. pg/ml	Prog. ng/ml	LH ng/ml
21/11	-	1.60	-
27/11	22.68	1.82	3.50
29/11	46.66	0.20	25.00
3/12	5.68	2.03	1.00
7/12	8.40	1.07	1.00
10/12	10.08	1.70	2.75
11/12	5.87	2.37	3.50
15/12	34.20	1.99	0.50
19/12	10.20	1.07	1.60
23/12	12.50	1.07	-
27/12	-	1.07	1.50
3/1	8.17	2.52	1.50
7/1	15.50	0.81	2.00
11/1	4.20	0.93	0.50
17/1	6.40	0.33	0.95
Animal Number 85: Group Three			
10/8	12.59	3.50	2.00
12A/8	71.37	0.23	8.50
12B/8	54.58	0.22	5.50
13/8	4.20	1.50	0.75
15/8	8.20	0.85	1.00
18/8	15.88	2.50	0.50
21/8	14.20	4.50	1.50
23/8	14.20	6.50	1.75
27/8	20.50	6.78	1.25
30/8	50.50	5.78	2.08
1/9	-	3.20	2.75
3/9	94.95	0.89	13.75
7/9	32.49	1.89	0.98
12/9	4.20	3.85	2.00
16/9	12.49	8.20	2.85
20/9	-	8.30	1.25
24/9	44.20	4.43	2.25
28/9	51.83	0.92	11.00
2/10	11.33	1.92	2.00
6/10	10.50	2.30	1.00
8/10	-	5.80	0.75
10/10	4.20	4.60	2.00
14/10	19.60	5.60	-
18/10	58.00	3.60	3.00

Table 12C cont.

Animal Number 85: Group Three			
Date of Blood Collection	Oestr. pg/ml	Prog. ng/ml	LH ng/ml
20/10	62.83	0.45	8.25
22/10	11.45	0.85	0.75
25/10	10.68	2.30	1.00
29/10	8.50	3.30	1.25
1/11	20.55	3.50	0.50
5/11	22.15	4.50	0.50
9/11	15.35	3.50	2.00
12/11	-	1.50	2.00
13/11	48.28	0.50	11.00
17/11	2.89	0.89	1.00
21/11	7.89	1.23	1.00
25/11	45.25	3.25	1.00
29/11	20.30	4.25	1.50
3/12	30.40	3.39	2.50
5/12	48.75	0.29	11.00
5/12	-	1.05	0.75
11/12	10.15	0.29	0.75
15/12	22.10	0.35	1.00
18/12	11.55	0.65	1.00
23/12	8.50	0.88	-
27/12	11.50	0.78	1.00
3/1	12.55	0.75	0.75
7/1	11.55	1.11	1.50
11/1	4.20	0.95	0.75
13/1	8.20	0.96	1.00
17/1	9.95	0.85	2.50
Animal Number 92 Group Three			
9/8	28.50	1.89	3.00
11A/8	66.55	0.50	7.50
11B/8	41.50	0.09	3.00
12A/8	11.50	0.89	1.55
12B/8	12.75	0.92	0.58
15/8	11.50	3.50	2.00
18/8	13.50	5.20	-
21/8	46.50	1.00	3.20
23/8	58.50	0.90	11.55
27/8	5.58	3.50	2.00
30/8	6.75	6.50	1.75
3/9	15.70	8.80	2.25
5/9	27.00	7.50	3.50
7/9	-	7.50	4.20
9/9	15.50	6.50	2.50

Table 12C cont.

Animal Number 92 : Group Three			
Date of Blood Collection	Oestr. pg/ml	Prog. ng/ml	LH ng/ml
12/9	35.65	2.50.	0.89
16/9	20.80	3.50	1.50
20/9	25.50	3.65	3.10
24/9	45.50	0.65	8.00
24/9	45.50	0.70	5.00
28/9	7.40	2.70	0.50
30/9	8.20	1.98	0.75
2/10	10.50	3.00	0.50
6/10	8.20	5.00	1.75
10/10	-	8.55	1.50
14/10	10.20	6.55	2.00
18/10	12.24	2.55	9.00
22/10	42.24	0.45	3.00
25/10	11.28	0.65	3.00
29/10	5.50	2.70	0.75
1/11	8.50	2.50	2.50
5/11	9.78	3.66	3.50
9/11	11.59	3.50	0.75
13/11	11.10	4.50	0.75
17/11	21.50	1.55	3.50
20/11	48.77	0.22	10.50
24/11	12.17	1.11	0.50
29/11	4.62	2.10	0.50
3/12	17.21	0.50	0.75
7/12	8.77	1.20	0.50
11/12	10.99	0.95	0.65
15/12	25.94	1.30	1.65
19/12	41.98	0.55	9.25
23/12	4.80	0.09	2.00
27/12	4.80	0.05	2.10
31/12	8.40	0.11	1.00
3/1	4.62	0.80	-
7/1	19.39	0.50	3.50
11/1	9.36	0.65	2.50
15/1	5.38	-	2.25
Animal Number 93: Group Three			
9/8	65.64	0.12	15.30
12/8	19.20	2.90	1.25
15/8	15.30	3.50	4.25

Table 12C cont.

Animal Number 93: Group Three

Date of Blood	Oestr. pg/ml	Prog. ng/ml	LH ng/ml
17A/8	16.30	0.85	4.50
17B/8	10.95	1.20	12.00
20/8	17.30	2.35	1.55
23/8	21.30	4.35	8.50
26/8	25.86	5.55	3.55
29/8	28.90	10.65	-
1/9	52.10	6.05	3.50
5/9	65.64	0.95	15.50
10/9	11.72	1.75	0.50
14/9	12.92	4.50	2.00
19/9	17.00	5.05	3.20
20/9	8.06	5.25	2.00
24/9	8.10	4.24	5.00
28/9	12.30	3.10	0.85
2/10	30.66	2.55	2.40
4/10	32.80	-	5.60
6/10	59.26	0.85	20.00
8/10	7.85	1.85	1.40
10/10	11.75	1.97	2.00
14/10	15.20	3.15	1.40
18/10	16.70	-	1.00
22/10	18.70	3.19	0.50
25/10	-	5.41	2.00
29/10	47.50	0.95	9.00
1/11	4.50	0.19	3.50
5/11	8.50	0.26	3.50
9/11	15.20	1.30	2.31
13/11	8.50	4.80	7.00
17/11	20.67	3.05	0.50
21/11	10.52	3.17	3.60
25/11	15.60	0.95	4.50
29/11	42.47	0.40	15.00
29/11	-	0.03	14.00
3/12	13.09	0.70	4.00
5/12	21.88	0.70	2.00
7/12	10.75	0.10	1.40
11/12	20.75	0.53	1.20
15/12	11.75	0.27	0.80
19/12	8.75	0.20	2.00
23/12	8.20	0.41	1.30
27/12	5.25	0.19	1.10
1/1	-	1.11	3.00
7/1	4.55	0.89	3.00
11/1	6.65	0.58	0.50
12/1	9.55	0.38	2.60
13/1	8.55	0.12	-
17/1	15.65	1.20	2.85

Table 120

OESTROGENS, PROGESTERONE AND LUTENIZING HERMONE VALUES IN GOATS

ANIMAL NUMBER 95; GROUP FOUR

Date of blood collection	Oestr. pg/ml	Prog. ng/ml	LH ng/ml
8/8	67.98	1.46	1.50
11/8	63.38	0.97	11.50
14/8	4.62	0.44	1.25
17/8	21.51	0.78	2.00
20/8	7.90	5.35	1.50
22/8	5.88	11.85	0.85
26/8	18.93	3.15	2.50
29/8	65.57	0.78	8.00
1/9	7.35	2.50	2.50
5/9	25.19	8.35	1.00
10/9	15.42	6.05	2.50
14/9	5.10	7.05	-
19/9	45.45	13.80	0.50
23/9	45.40	3.05	2.50
24/9	43.45	0.85	11.55
28/9	7.14	2.12	2.50
1/10	11.50	3.89	2.75
5/10	16.37	4.25	3.00
9/10	66.94	0.85	10.25
9/10	60.94	0.80	6.50
13/10	7.35	0.85	0.55
17/10	6.75	3.75	0.55
21/10	16.18	4.75	1.25
26/10	8.76	3.15	1.25
30/10	8.75	2.35	4.25
4/11	37.56	0.85	21.50
8/11	34.58	1.87	0.50
10/11	13.09	-	0.50
12/11	35.64	1.16	8.00
15/11	9.10	0.92	1.55
20/11	18.16	1.00	2.00
24/11	19.76	1.60	0.51
27/11	39.76	1.80	0.85
2/12	11.75	2.45	1.25
6/12	7.35	-	0.85
10/12	12.07	1.26	1.50
14/12	10.07	1.02	1.25
18/12	12.43	0.55	1.25

Table 120 cont.

Animal Number 95: Group Four

Date of Blood Collection	Oestr. pg/ml	Prog. ng/ml	LH ng/ml
22/12	13.49	1.05	0.75
26/12	14.85	0.73	3.50
30/12	7.35	0.31	1.80
2/1	18.59	1.26	1.80
6/1	25.07	0.53	2.50
10/1	13.96	0.07	4.50
15/1	11.98	0.38	2.50

ANIMAL NUMBER 96: GROUP FOUR

9/8	31.45	1.60	4.00
15/8	71.42	0.99	13.00
15/8	-	0.80	17.50
23/8	11.[illegible]	2.66	0.63
19/8	17.85	0.80	3.25
24/8	33.58	8.50	0.55
25/8	-	5.70	5.00
28/8	67.17	5.00	2.00
30/8	13.51	3.89	2.86
3/9	67.17	1.89	3.00
7/9	68.08	0.97	15.00
12/9	20.15	2.21	3.50
16/9	2.86	14.05	3.11
20/9	23.55	9.35	6.50
24/9	45.52	5.70	5.20
27/9	43.42	0.49	25.00
29/9	38.77	1.94	2.20
2/10	15.40	3.70	1.20
5/10	12.77	4.25	1.75
6/10	-	5.94	1.50
10/10	7.35	5.50	6.50
14/10	6.72	4.50	1.50
17/10	22.88	5.70	2.40
23/10	12.18	5.70	3.50
25/10	33.38	2.80	2.50
27/10	-	2.57	6.50
30/10	40.88	0.42	11.40

Table 120 cont.

Date of Blood	Oestr. pg/ml	Prog. ng/ml	LH ng/ml
Animal Number 96: Group Four			
1/11	10.26	0.42	1.00
5/11	32.35	1.84	2.50
9/11	9.38	0.61	1.00
13/11	7.35	0.92	0.50
17/11	18.30	0.65	0.50
24/11	8.89	-	1.50
29/11	8.89	0.53	0.50
1/12	12.77	1.43	1.50
3/12	12.44	2.20	0.50
6/12	31.78	1.41	1.25
7/12	41.98	1.81	1.50
12/12	19.38	0.14	3.50
16/12	7.35	-	-
19/12	19.76	0.53	0.50
23/12	18.77	0.26	1.00
27/12	7.75	0.30	1.75
3/1	15.66	0.30	0.50
7/1	5.88	0.36	1.25
11/1	22.11	0.60	0.50
16/1	22.88	0.75	0.50
Animal Number 97: Group Four			
10/8	18.47	1.70	3.00
13/8	23.56	2.30	1.89
15/8	-	8.50	0.50
16/8	30.39	9.70	1.90
20/8	29.39	3.50	3.35
23/8	83.49	1.45	20.00
27/8	26.22	2.45	2.50
30A/8	7.56	8.35	1.50
30B/8	18.89	5.40	4.00
3/9	18.89	3.20	3.00
4A/9	86.55	0.38	-
4B/9	49.49	0.06	11.00
4C/9	33.58	0.20	17.50
8/9	15.00	1.50	3.50
12/9	-	3.20	3.50
17/9	4.20	5.50	0.89
20/9	20.76	8.50	1.22
23/9	33.30	5.24	7.00
24/9	-	3.98	3.50
26/9	55.66	0.24	16.50
30/9	14.47	-	2.25
3/10	10.58	3.10	4.00

Table 12D cont.

Animal Number 97: Group Four

Date of Blood Collection	Oestr. pg/ml	Prog. ng/ml	LH ng/ml
11/10	8.23	4.00	1.00
15/10	5.20	5.00	0.50
19/10	26.03	3.05	2.60
20/10	39.02	0.30	15.50
20/10	36.00	0.05	10.50
23/10	19.76	0.30	2.00
26/10	9.76	0.77	8.50
28/10	-	1.55	2.50
30/10	19.76	3.75	0.85
2/11	15.07	3.90	2.50
6/11	-	4.35	5.00
10/11	36.94	3.40	3.20
14/11	40.14	0.20	9.50
18/11	24.20	0.34	1.50
22/11	14.20	0.50	1.00
30/11	17.63	1.70	1.00
4/12	13.63	3.40	1.00
8/12	11.75	0.80	0.50
12/12	9.95	0.11	0.50
16/12	16.87	0.10	3.50
20/12	12.36	0.18	1.50
24/12	13.93	0.20	2.00
28/12	7.55	0.20	1.50
30/12	9.55	0.24	0.50
2/1	-	1.00	0.50
8/1	20.38	0.42	1.50
11/1	12.30	-	2.50
15/1	20.90	-	2.00

Table 12D cont.

Animal Number 98:	Group Four		
Date Blood Collection	Oestr. pg/ml	Prog. ng/ml	LH ng/ml
8/8	4.20	2.00	1.85
11/8	71.37	2.60	0.75
14/8	6.30	3.15	2.00
17/8	20.50	7.05	1.20
18/8	92.36	2.20	3.20
19/8	104.15	0.88	5.00
19/8	58.77	0.73	18.00
20/8	6.30	1.07	2.65
26/8	23.51	2.08	6.00
29/8	30.14	8.95	3.15
1/9	5.98	9.45	2.00
5/9	55.42	4.35	7.00
10/9	110.83	0.87	18.50
11/9	16.09	1.50	3.25
14/9	25.50	1.30	4.15
17/9	5.98	3.30	0.40
19/9	16.62	2.80	-
23/9	27.17	1.50	4.00
27/9	41.51	0.75	11.50
28/9	26.87	0.28	2.00
29/9	-	0.55	1.80
1/10	8.62	3.50	0.50
5/10	20.36	4.75	3.25
9/10	30.40	3.79	2.50
13/10	42.01	0.79	15.50
17/10	13.35	1.89	1885
21/10	13.35	4.40	1.85
26/10	-	5.40	-
28/10	29.02	5.10	2.00
31/10	47.50	0.28	7.50
4/11	14.62	3.80	.75
8/11	6.25	2.65	4.00
12/11	29.39	4.00	6.00
16/11	40.30	0.24	16.25
20/11	12.60	3.50	0.60
24/11	15.68	6.95	0.75
27/11	9.76	0.73	2.00
2/12	4.20	0.73	1.50
10/12	4.20	0.15	0.95
14/12	18.47	1.74	2.00
18/12	10.92	0.85	1.50
22/12	13.93	0.92	0.95
26/12	13.93	1.11	0.90
30/12	4.20	0.37	0.50
2/1	4.20	0.78	1.50
4/1	16.94	0.46	0.75

Table 120 cont.

Animal Number 98: Group Four			
Date of Blood Collection	Oestr. pg/ml	Prog. ng/ml	LH ng/ml
6/1	8.79	0.61	0.60
10/1	8.00	1.31	0.80
16/1	17.55	-	1.00
ANIMAL NUMBER 100: GROUP FOUR			
8/8	16.80	0.97	1.50
11/8	16.80	2.05	-
14/8	16.79	0.90	1.25
17/8	20.36	2.06	2.85
19/8	61.15	0.53	11.00
19/8	24.57	0.90	7.00
19/8	19.30	1.78	4.00
20/8	4.20	5.95	2.50
26/8	55.25	0.92	11.00
28/8	14.95	1.46	2.75
1/9	-	3.68	1.50
5/9	5.65	8.20	1.50
10/9	20.15	9.37	3.50
14/9	19.73	7.75	4.00
19/9	52.36	0.21	21.00
23/9	9.90	1,80	5.20
27/9	21.83	3.12	2.25
1/10	4.20	4.35	2.15
5/10	15.50	3.67	4.00
9/10	-	2.67	3.40
13/10	37.45	0.25	11.00
17/10	12.60	1.80	2.00
21/10	4.20	3.00	0.75
25/10	15.60	0.53	0.50
28/10	12.63	2.50	1.45
31/10	4.20	2.10	2.76
4/11	12.74	1.10	2.00
8/11	34.20	0.29	2.00

TABLE 12 D Cont.

ANIMAL NUMBER 100:

Date of Blood Collection	Oestr. pg/ml	Prog. ng/ml	LH ng/ml
12/11	40.28	0.78	2.00
13/11	53.13	0.25	9.50
16/11	17.20	0.56	0.50
20/11	25.33	0.65	2.10
24/11	35.33	0.55	0.50
27/11	46.18	1.16	1.50
29/11	18.47	0.61	0.75
2/12	8.40	0.96	0.75
6/12	10.90	1.16	2.15
14/12	-	-	0.85
18/12	4.20	2.66	1.45
22/12	4.20	0.44	1.50
26/12	7.50	0.05	0.80
30/12	27.49	0.16	0.95
2/1	12.97	0.50	1.50
6/1	8.88	0.27	0.60
10/1	22.36	0.78	0.80
15/1	10.40	0.77	-
17/1	-	0.58	1.85

Appendix Table 13

PITUITARY LH VALUES AND WEIGHTS IN NORMAL AND COBALT DEFICIENT GOATS

Group number	Animal number	Extracted protein(mg)	Total LH(Ug)	ng/LH/ug protein	Pituitary weights (mgs)
I	82	25700	85.17	3.34	242.00
	83	25600	87.62	3.42	272.00
	94	18700	134.62	7.20	310.00
	99	10600	96.77	9.13	358.00
II	86	35100	27.40	0.78	282.00
	87	18700	25.00	1.34	520.00
	88	15386	31.10	2.02	452.00
	89	22600	36.51	1.62	346.00
III	80	31974	40.55	1.27	441.00
	84	34496	45.11	1.31	304.00
	85	18500	41.97	2.27	496.00
	92	17004	42.59	2.50	331.00
	93	14600	63.33	4.34	486.00
IV	95	19500	56.92	2.92	368.00
	96	15575	45.00	2.89	530.00
	97	30160	60.91	1.60	400.00
	100	30860	71.43	2.31	346.00