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# THE INDUCTION OF CALVING USING BETAMETHASONE AND THE PHARMACOLOGICAL ACTIVITY OF SELECTED FORMULATIONS HAVING DIFFERENT RATES OF ABSORPTION

A thesis presented in partial fulfilment
of the requirements for the
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Abstract of a thesis presented in partial fulfilment of the requirements for the Degree of Doctor of Philosophy.

THE INDUCTION OF CALVING USING BETAMETHASONE AND THE PHARMACOLOGICAL ACTIVITY OF SELECTED FORMULATIONS HAVING DIFFERENT RATES OF ABSORPTION

by Stuart Campbell MacDiarmid

A series of experiments was undertaken in cows to study the disposition and duration of activity of selected formulations of the synthetic corticosteroid betamethasone (BM). The aim was to select a combination of formulations which would be suitable for use in a two-injection treatment regimen designed to induce premature parturition in cattle.

In an initial experiment, 10 cows each received a subcutaneous injection of 20 ml of 2 mg/ml aqueous suspension of BM, 10 received 2 ml of a 20 mg/ml suspension and a further 10 cows served as saline-treated controls. The BM formulations differed only in their solids:vehicle ratio. Plasma BM, cortisol and glucose concentrations, and differential blood cell counts were studied for 3 days before, and for 19 days after, treatment.

The 2 mg/ml suspension produced a markedly higher peak plasma BM concentration than the 20 mg/ml suspension. However, plasma BM levels tended to be maintained for longer by the suspension having the higher solids:vehicle ratio.

The administration of BM resulted in depression of early-morning cortisol concentrations, elevation of plasma glucose levels, and elevation of circulating neutrophil numbers. The magnitude and duration of these changes was related to the solids:vehicle ratio of the injected suspensions, with the more concentrated formulation producing effects of greater duration.

A second experiment involved 9 cows which were divided into 3 groups, each of which was treated at a dose rate of

0.1 mg/Kg with sodium phosphate solution or a 2 mg/ml BM suspension or a 20 mg/ml BM suspension. Over a period of several weeks each cow received its allocated formulation by each of 3 routes; intravenous, intramusuclar and subcutaneous.

The bioavailability of the BM suspensions was low and the solids:vehicle ratio exerted a profound effect on the rate at which the steroid was absorbed. The disposition curves of the BM solution were similar regardless of the route of administration and the plasma half-life values of BM sodium phosphate, estimated from 3 experiments in each of 3 cows, were 5.64, 6.06 and 6.43 hours.

Ten cows were included in a third experiment. They were treated by subcutaneous injection with 2 ml of a 10 mg/ml BM suspension; a preparation intended for use in the induction of calving. Mean plasma concentrations of BM and glucose were elevated above pre-treatment values for 4 days and 8 days respectively. Mean plasma cortisol levels were profoundly depressed for 2 weeks and in some individuals showed no signs of returning to normal 4 weeks after treatment.

Two field trials, involving 619 and 553 cows respectively, were conducted to assess the suitability of BM formulations for the induction of premature calving in commercial dairy herds.

In the first trial, the mean stage of pregnancy at which cows were treated was approximately 250 days. Cows received an initial injection of either 2 ml of a 10 mg/ml suspension of BM, 2 ml of a 15 mg/ml BM suspension or 4 ml of a 5 mg/ml suspension of dexamethasone trimethylacetate (DTMA). All cows which had not calved within 10 days of this initial treatment received a 12.5 ml dose of a 2 mg/ml suspension of BM.

In comparison with those cows treated with DTMA, significantly fewer cows treated with the concentrated BM suspensions required a second corticostercid injection. In all other respects, such as calf mortality, incidence of retained foetal membranes and maternal illnesses, the results

of the treatments were not significantly different. The 10 mg/ml BM suspension was therefore deemed to be suitable for use in the induction of calving.

The second field trial confirmed the suitability of the 10 mg/ml suspension as an initial treatment to induce calving. Cows which had not calved within 7 days of the initial treatment were injected with 20 mg of BM, either as a 2 mg/ml suspension or as a 2 mg/ml solution of the sodium phosphate ester.

After the second steroid injection, those cows which had received the more rapidly absorbed BM solution calved sooner than those which had been treated with the 2 mg/ml suspension.

The results of these studies clearly showed that the duration of activity of BM suspensions could be prolonged by increasing their solids:vehicle ratio. It was also shown that a treatment regimen consisting of an initial injection of a 10 mg/ml BM suspension, followed 7 to 10 days later by an injection of a more rapidly absorbed BM formulation, was suitable for the induction of calving as currently practised in New Zealand.

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# CHAPTER I

## INTRODUCTION

Because of the seasonal nature of New Zealand dairying, dairy farmers in this country are always interested in new husbandry techniques which might lead to a reduction in the number of cows which calve inconveniently late in the season. Many were therefore receptive when, in 1970, the concept of treating cows with corticosteroids was put forward by researchers at Ruakura Agricultural Research Centre as a means by which late-calving cows could be 'brought into line' with their herdmates. There can be few management techniques which have found such widespread acceptance in such a short time.

By the time this present study was initiated in 1977, it was estimated that approximately 200,000 cows annually were being treated to cause premature parturition. Only one 'long-acting' corticosteroid product had been licensed for the induction of calving, and so the development of another product, capable of competing for a share of the market, was viewed as a commercially attractive proposition.

The most widely used method by which the pharmaceutical industry obtains 'long-acting' steroid formulations is by the preparation of derivatives of low solubility. While the number of possible derivatives of any one corticosteroid is enormous, the costs of developing and licensing a previously-untested derivative are similarly very great. A company, therefore, is more likely to favour the modification of an existing derivative for which basic toxicology and clinical testing criteria have already been satisfied.

The manufacturers of betamethasone alcohol had accumulated a substantial amount of data relating to its safety, as it has been marketed for many years in the form of an injectable 2 mg/ml suspension. Before the present study was begun, it was already known that the 2 mg/ml suspension was capable of inducing calving when administered close to term, or to cows already 'primed' by treatment with a 'long-acting' corticosteroid. Means were therefore sought by which the tried-and-tested betamethasone

alcohol could be formulated to produce effects of greater duration.

Pilot studies, conducted in England by Philip Box and his colleagues at Glaxo Group Research Limited, had indicated that the duration of activity of a given dose of betamethasone alcohol could be increased by administering that dose in a much-reduced volume. That is, by increasing the concentration, or more accurately the solids:vehicle ratio, formulations having prolonged activity could be prepared.

It was at this stage that the work presented in this thesis was begun.

Much of the information relating to the absorption of drugs from intramuscular and subcutaneous injection sites has been published in the form of incidental observations associated with a wide variety of other studies. It thus tends to be scattered throughout numerous, often unrelated journals. Because of the current veterinary interest in drug disposition, it was considered that a need existed for a comprehensive review of this subject. Accordingly, the preparation of such a review, with a veterinary emphasis wherever possible, was undertaken as an important supportive component of the major investigation. Similarly, a number of years has passed since the subject of corticosteroid-induced calving has been reviewed, and as there have been major advances in recent years, it was considered that an updated review of this subject should be included in the thesis.

The experimental investigations found necessary for this project were designed to provide background information on the duration of activity of certain betamethasone formulations after administration to cattle. In addition it was envisaged that once a 'suitable' formulation was found, its efficacy as an agent for the induction of premature parturition would have to be evaluated in large-scale field trials.

The investigations carried out did lead to the development of a suitable betamethasone product, and much general information relating to its use in cattle, and to the benefits and disadvantages of induced calving, was obtained.

# CHAPTER II

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# CHAPTER II

## THE ABSORPTION OF DRUGS FROM SUBCUTANEOUS AND INTRAMUSCULAR INJECTION SITES: A REVIEW

### 1. INTRODUCTION

A drug is administered to a patient with the aim of producing and maintaining at the site of drug action a concentration of free drug sufficient to produce an appropriate clinical response (Saunders, 1974; Baggot, 1977). The concentration of drug at its site of action is dependent on, among other things, its concentration in the blood (Baggot, 1977). The amount of administered drug which enters the blood stream depends on the size of the dose, the route of administration and the bioavailability of the dosage form (Baggot, 1977).

When a drug is administered other than by the intravenous route, absorption processes must occur for the drug to pass from its site of administration into the blood stream. Whenever an absorptive phase is involved in drug distribution, possible problems of bioavailability must be considered (Baggot, 1977). Bioavailability is a measure of the rate and extent to which a drug enters the systemic circulation in an active form, and is governed by the route of administration, the dosage form and certain physicochemical properties of the drug molecule (Baggot, 1977). The most important of these properties, in relation to absorption and distribution within the body, is the solubility of the drug in the body fluids, which comprise aqueous salt solutions of various pH values, as well as lipids (Feldman, 1974; Saunders,1974; Baggot, 1977). This is because to reach its site of action following administration, a drug usually has to pass from an aqueous medium, through a lipid-containing membrane and back into an aqueous medium (Saunders, 1974; Baggot, 1977). Therefore, drugs which are soluble in both water and lipid solvents are most likely to distribute readily throughout the body (Saunders, 1974). Weak organic acids and bases are particularly suitable for distribution because their ionised forms are soluble in water but are almost insoluble in lipids, while their non-ionised forms are soluble in lipids but are only poorly soluble in aqueous solutions (Feldman, 1974; Saunders, 1974; Baggot, 1977).

The main barriers to drug movement within the body are lipid in nature. These are the epithelial membranes of the gastro-intestinal tract, the endothelial membranes of the capillary walls and the various cell membranes (Saunders, 1974). These biological membranes consist predominantly of a lipoid-protein mosaic with some aqueous pores (Harvey, 1970; Saunders, 1974; Baggot, 1977). Drug molecules may cross these membranes either by passive diffusion or by specialized, energy-dependent active transport processes. When drug molecules are sufficiently lipid-soluble they are able to cross the predominantly-lipid membrane by passive diffusion, the rate of which is dependent on the concentration gradient across the membrane and the degree of lipid solubility of the drug. Drug molecules may also pass through biological membranes passively by filtration through the aqueous pores (Harvey, 1970; Feldman, 1974; Baggot, 1977). Drugs normally cross endothelial membranes (i.e. capillary walls) far more rapidly than they cross epithelial barriers (Baggot, 1977).

At physiological pH values most drugs are present in both ionised and non-ionised forms. The extent to which a drug is ionised depends on the dissociation constant (pKa) of the drug and on the hydrogen ion concentration (pH) of the surrounding medium. At a pH equal to the pKa of a drug, 50% of the drug molecules will be present in the ionised form. A partially ionised drug may be distributed in unequal concentrations on either side of a biological membrane, especially where pH differences exist across that membrane (Harvey, 1970; Saunders, 1974; Baggot, 1977). While the majority of drugs are weak electrolytes whose lipid-water solubility properties are readily altered by changes in pH, many drugs are non-ionised. These drugs are generally lipid soluble with only a very low degree of solubility in water. Their effects are usually due to their solubilization in the body lipids. Two groups of such drugs are the general anaesthetics and the steroids, both of which tend to become widely distributed in the lipids of the body (Saunders, 1974).

## 2. DRUG ABSORPTION

Drug absorption is defined as the passage of drug from its site of administration into the blood stream. The route by which a drug is administered will strongly influence the rate and extent to which it is absorbed. There are two major classes of route by which a drug may enter the body: the enteral and parenteral. Drugs administered by the enteral route are given orally and pass into the gastrointestinal (g.i.) tract from where absorption across g.i. epithelium takes place. The g.i. tract is by-passed when drugs are administered by a variety of parenteral routes which include topical application, inhalation and injection (Baggot, 1977).

Important parenteral routes of administration are intravenous, intramuscular (i.m.) and subcutaneous (s.c.) injection. When a drug is administered intravenously absorption is instantaneous and bioavailability complete. However, following i.m. or s.c. injection (i.e. extravascular injection), where diffusion to, and passage across, endothelial tissue barriers must take place, the possibility of delayed or incomplete absorption arises (Baggot, 1977). Drugs may differ in the rate and extent to which they are absorbed following extravascular injection (Baggot, 1977; Nouws & Ziv, 1977) and even different formulations of the same drug may show marked differences in their bioavailability (Nouws & Ziv, 1979; Ziv, 1980; Dowrick, 1980).

### 2.1 ABSORPTION FROM INTRAMUSCULAR AND SUBCUTANEOUS INJECTION SITES

Absorption of drugs from aqueous solutions injected by the i.m. or s.c. routes is usually relatively rapid (Ballard & Nelson, 1970; Bederka, Takemori & Miller, 1971; Baggot, 1977). Although water is the solvent of choice for parenteral drug products, not all drugs can be formulated as simple aqueous solutions, either because of poor solubility or instability. In such cases alternative biologically acceptable solvents, which may or may not be miscible with body fluids, may be used (Ballard & Nelson, 1970; Dowrick, 1980). Under certain circumstances it may be either impossible or undesirable to prepare a particular drug as a true solution, and it may be necessary to formulate a parenteral dispersion in which the drug is present as an

undissolved phase (Ballard & Nelson, 1970; Dowrick, 1980). A wide variety of such dispersions can be prepared in which the physical properties of the dispersed phase and the vehicle can be varied to produce solubilized systems, colloidal dispersions, emulsions and true suspensions. Vehicles may be miscible with water and plasma, or they may be oily and non-miscible (Dowrick, 1980).

It is usually assumed that following i.m. or s.c. administration, drugs are absorbed from the injection site according to first-order kinetics. That is, the rate of absorption is assumed to be proportional to the amount of drug remaining at the injection site (Wagner, 1961). While in many cases drugs do appear to be absorbed from parenteral sites according to first-order kinetics, absorption rate-constants may in some instances decrease with time (Franke *et al,* 1950; Sund & Schou, 1964; Munck *et al,* 1967; Binder, 1969; Kakemi *et al,*1969) or even increase (Binder, 1967; Brotherton, 1976). Hence absorption rates may be variable (Mulligan & Cassee, 1965; Brotherton, 1976; Greenblatt & Koch-Weser, 1976) and in some cases absorption may be better described by biexponential or even polyexponential kinetics (Munck *et al*, 1967; Ballard, 1968; Binder, 1969).

## 2.2 EVENTS OCCURRING AT INJECTION SITE

A factor of major importance in governing the rate at which an injected formulation is absorbed is the area of absorptive surfaces in contact with the injected volume (Wagner, 1961; Ballard, 1968; Harvey, 1970). However, the surface area of the injected depot changes continuously during the absorption process as the injected formulation diffuses or spreads within the tissue, and as the solute and solvent are removed at different rates (Ballard, 1968; Schou, 1971; Greenblatt & Koch-Weser, 1976).

When aqueous solutions of drugs are injected extravascularly they become rapidly distributed solely in the ground substance of either the subcutaneous connective tissue or of the connective tissue between muscle fibres (Ballard, 1968; Schou, 1971). Water-immiscible formulations, on the other hand, may take several days to spread and are distributed in spherical or ellipsoidal sacs in subcutaneous

connective tissue, or along planes of fascia or connective tissue surrounding muscles and groups of muscles (Ballard, 1968). Aqueous suspensions spread rapidly (Ballard, 1968).

In veterinary clinical practice, so-called intramuscular injections are very frequently made not into muscle tissue as such, but rather into fascial planes and connective tissue (Baxter & Evans, 1973; Marshall & Palmer, 1980) and might be better described as *intermuscular* injections.

Subcutaneous tissue contains a rich supply of both capillaries and lymphatic vessels, and while muscle tissue lacks lymphatics it too is well endowed with capillaries. The connective tissue sheaths and fascial planes of muscle groups are abundantly supplied with lymphatics, and tissue fluid moves through spaces along fascial planes between muscle fibres (Ballard, 1968). In the walls of lymphatics and capillaries there are small openings at the junctions of some of the endothelial cells which make up the vessels. Even mild trauma causes an increase in the number of such open junctions and leads to a marked increase in the permeability of the vessels (Casley-Smith, 1964; Ballard, 1968).

Some parenteral formulations intended for s.c. or i.m. injection may be irritant and cause marked tissue damage at the site of injection (Nouws & Ziv, 1977; Immelman, Botha & Grib, 1978; Rasmussen, 1980; Ziv, 1980). However, all i.m. and s.c. injections cause some degree of local trauma and aseptic inflammation. This tissue damage is due to the direct effect of the needle, to the distension and disruption caused by the injected volume and the composition of the injected substance (Sund & Schou, 1964a; Mulligan & Cassee, 1965; Secher-Hansen, Langgard & Schou, 1967c; Schou, 1971). For a given formulation the extent of the inflammation at the injection site is proportional to the volume injected (Secher-Hansen, Langgard & Schou, 1967c).

Pain at the site of injection can be caused by excessively acidic or basic solutions, anisotonic solutions, or by formulations which cause the liberation of vasoactive substances such as histamine and 5-hydroxytryptamine (5-OHT) (Schou, 1961; 1971).

The extent to which these amines are liberated depends on the composition, volume and concentration of the injected formulation (Schou, 1961; Sund & Schou, 1964a). Following extravascular injection many substances produce what is known as 'self-depression' of their own absorption (Schou, 1958a; 1958b; Milthers & Schou, 1958): a delay caused by the release of endogenous vasoactive amines at the injection site. The phenomenon may be blocked by the concurrent local or systemic administration of antihistamines (Schou, 1958a; 1958b) or 5-OHT antagonists (Milthers, 1959), and repeated injections into the same site will eventually fail to provoke self-depression as tissue amines become depleted. For a given substance, a greater self-depression occurs when the injection volume is increased (Sund & Schou, 1967; Schou, 1971) and when the concentration of the injected solution is increased (Schou, 1961; Binder, 1969). The biexponential absorption rates seen following the injection of some drug formulations (Ballard, 1968; Binder, 1969) may, in some cases, be explained by the fact that the initial absorption phase is inhibited by a self-depression phenomenon which gradually wears off (Binder, 1969).

Some drugs, such as the steroid anaesthetic alphaxalone, are absorbed more slowly from s.c. sites than from i.m. ones (Evans, Aspinall & Hendy, 1972). The vehicle for this steroid contains the solubilizing agent Cremophor EL which is known to release histamine (Lorenz *et al*, 1971). Histamine-containing mast cells are particularly abundant in the connective tissue of the skin (Barrett, 1978) and so it may well be that the delay in absorption, seen when such formulations are injected subcutaneously, is due to a greater self-depression at this site.

The absorption of drugs from injected solutions involves two major processes. The first process is diffusion within the injection depot ('stirring') and through the connective tissue ground substance, and the second is the passage of drug molecules from tissue fluid through capillary walls into blood plasma (Ballard, 1968; Schou, 1971).

Solvent and solute are absorbed from an injection site at different rates (Secher-Hansen, Langgard & Schou, 1967a; 1967b).

The hyaluronic acid gel and the structure of formed elements which constitute the tissue ground substance act as a macromolecular filter which permits solvent water to spread further than solute molecules. A concentration gradient is thus created (Ballard, 1968; Schou, 1971) and movement of drug molecules through the ground substance occurs partly by diffusion along this concentration gradient, and partly by filtration or 'solvent drag' due to a hydrostatic pressure difference (Secher-Hansen, Langgard & Schou, 1968; Secher-Hansen, 1968). The rate of filtration will influence the rate of absorption to some degree, especially with large, lipid-insoluble molecules (Schou, 1961; Sund & Schou, 1965).

While lipid-soluble molecules diffuse most readily through the capillary walls into blood plasma (Ballard, 1968; Schou, 1971) absorption of small molecules, regardless of lipid solubility, occurs almost entirely into capillaries. It is mainly in the absorption of molecules too large to cross endothelial barriers (M.W. > 10,000) that the lymphatics play an important role (Hollander, Reilly & Burrows, 1961; Schou, 1961; Ballard & Nelson, 1970; Harvey, 1970).

The rate at which injected water molecules disappear from an injection site is extremely rapid and yet is independent of the rate at which the injected *volume* disappears from the site (Secher-Hansen, Langgard & Schou, 1967a; 1967b). There is a very rapid exchange of water molecules between the injected solution and tissue fluids, with the result that the injected volume disappears at a much slower rate than the actual injected water molecules. It is probable that the surplus volume is removed from the injection site by way of the lymphatics (Secher-Hansen, 1968a; 1968b).

The absorption of drugs from non-aqueous solutions and from suspensions is a more complicated process and will be discussed later (Section 4.2), although it is appropriate at this point to mention that phagocytosis by leucocytes may play a role in the absorption of particulate and microcrystalline substances (Ballard, 1968; Greenblatt & Koch-Weser, 1976).

The rate at which drugs are absorbed from i.m. and s.c. injection sites, and hence their ultimate bioavailability, may be affected by a wide variety of factors which may be broadly

categorised as biological, physicochemical (pharmaceutical) or pharmacological.

## 3. BIOLOGICAL FACTORS AFFECTING ABSORPTION FOLLOWING EXTRAVASCULAR INJECTION

### 3.1 VASCULARITY, SITE AND REGION OF THE BODY

Absorption of injected drugs is usually more rapid from highly vascular tissues, as the distance through which drug molecules must diffuse before reaching capillary walls is very short (Harvey, 1970). It has traditionally been accepted that absorption of drug following i.m. injection is more rapid than following s.c. injection (Harvey, 1970; Brander & Pugh, 1977). However, this idea has been challenged in recent reviews (Reeves, 1975; Marshall & Palmer, 1980) in which it was suggested that in many instances differences in absorption rates from i.m and s.c. tissue sites at any given region of the body may be insignificant when compared with differences in absorption rates from the same tissue site in different anatomical regions. This has been confirmed in the use of aqueous solutions of insulin (Nora, Smith Cameron, 1964) and antipyrine (Munck *et al*, 1967) in humans and butorphanol in dogs (Pfeffer *et al*, 1980). Recently the studies of Marshall and Palmer (1980) have indicated that in calves the s.c. injection of aminopenicillins need not necessarily result in a lower bioavailability than from an i.m. injection.

Absorption from s.c. sites in one region of the body may be better than from i.m. sites in another (Marshall & Palmer, 1980; Palmer, 1980). In goats and dairy cows significant differences in bioavailability of ampicillin have been demonstrated between i.m. sites in the neck and gluteal region (Groothuis & Van Miert, 1979; Groothuis *et al*, 1980; Rutgers *et al*, 1980) and this is probably due to the fact that significant differences exist in blood flow to different muscles and hence in the rate at which substances are removed from them (Evans *et al*, 1975).

While s.c. sites have the advantage of avoiding valuable meat cuts, minimising problems of tissue residues and, in companion animals, minimising pain and inconvenience (Marshall & Palmer,

1980), usually with no reduction in clinical efficacy (for example, see English, 1965; Mercer *et al*, 1971), situations still arise in which absorption from an i.m. site appears significantly superior to absorption from a s.c. one. For instance, the steroid anaesthetic alphaxalone, which produces anaesthesia in cats following i.m injection, is ineffective by the s.c. route (Evans, Aspinall & Hendy, 1972).

Once a drug reaches the blood, the rate of blood flow through the capillaries (perfusion) determines the rate at which it is removed from the tissues surrounding the site of injection (Harvey, 1970; Schou, 1961; 1971). Mild inflammation, by inducing a hyperaemia, might be expected to result in enhanced absorption (Harvey, 1970); however, the presence of oedema may negate this effect by increasing the distance through which drug molecules must diffuse to reach capillaries (Schou, 1961; Harvey, 1970). For aqueous solutions of freely diffusable drugs of low molecular weight injected in relatively small volumes, tissue perfusion appears to be the rate limiting factor in absorption (Bederka, Takemori & Miller, 1971).

### 3.2. MOVEMENT AND EXERCISE

That movement at the site of extravascular injection, or exercise, may enhance absorption is well recognised (Ballard, 1968; Harvey, 1970; Reeves, 1975). Movement or massage tends to spread injected material through the tissues, thus increasing the surface area of the injection depot and decreasing the distance to capillaries (Harvey, 1970; Greenblatt & Koch-Weser, 1976). Muscular activity also increases the flow of blood and lymph through tissues (Harvey, 1970) and for large molecules such as toxins and proteins, which are absorbed primarily by the lymphatics, the resultant increase in absorption may be of major significance (Ballard, 1968). For example, immobilisation of a limb into which snake venom has been injected markedly increases the survival time of rabbits (Barnes & Trueta, 1941).

Hypothermia, which causes peripheral vasoconstriction and hence reduction in local blood flow, may lead to a marked

reduction in absorption. Harvey (1970) recounts how injured and chilled humans have been given multiple s.c. injections of morphine without apparent effect. However, when these patients have been hospitalised and warmed, morphine toxicity has occurred as peripheral circulation is restored.

The application of a tourniquet may dramatically reduce the absorption of injected drugs from a limb (Kety, 1949; McGirr, 1952).

### 3.3. DISEASE

The effects of local trauma have been briefly mentioned. Other local pathological conditions such as induration, abscessation or sclerosis may delay absorption (Ballard, 1968; Reeves, 1975).

Systemic disease states may also influence absorption of injected drugs. Conditions which decrease peripheral perfusion, such as cardiac failure, myxodema, shock, hypothermia or anaesthetic overdose, may all reduce absorption (Hollander, Reilly & Burrows, 1961; Ballard, 1968; Bederka, Takemori & Miller, 1971; Reeves, 1975). Fever has been shown to accelerate absorption of various antibiotics from some i.m. sites (Groothuis *et al*, 1978; 1980) and to depress it from others (Groothuis, Van Gogh & Van Miert, 1980; Groothuis *et al*, 1980). In ruminants it appears that absorption is enhanced from those muscle masses which shiver during fever (such as thigh muscles) and that the effect is thus one of exercise or muscle movement (Groothuis *et al*, 1980). A reduced absorption rate from non-shivering groups of muscles (such as occur in the neck) is probably a result of decreased perfusion (Groothuis, Van Gogh & Van Miert, 1980; Groothuis *et al*, 1980).

### 3.4 PHYSIOLOGICAL STATE

It has been demonstrated that the non-electrolyte anaesthetic urethane is absorbed much more rapidly from s.c. injection sites in starved or dehydrated mice than in normal mice (Hvidberg & Schou, 1959a). Starvation and dehydration both reduce the content of hyaluronic acid in connective tissue ground substance (Hvidberg, 1959) thus reducing the resistance to diffusion of the subcutaneously injected urethane.

## 3.5 AGE

Younger animals may absorb some drugs more rapidly from s.c. sites than older animals. Ballard (1968) has suggested that this may be due to an increase in thickness of s.c. tissue with age, as well as to changes in the composition of s.c. fat tissues.

Morphine administered by s.c. injection is more toxic to young animals than to adults (Milthers, 1959; 1960). Milthers demonstrated that in rats this difference in toxicity was due to differences in absorption rate which were related to a greater self-depression occurring in the adult. Morphine is a powerful liberator of histamine and 5-OHT, but in very young rats these amines, whether locally liberated or administered in test solutions, were without influence on absorption rates. Hence self-depression of absorption is not seen during the first few weeks of life.

Differences in absorption rate may be inferred to play a part in the reduced i.m. bioavailability of amoxycillin seen in calves of increasing age and weight (Marshall & Palmer, 1980).

## 3.6 SPECIES

In his discussion of sustained release formulations, Thompson (1960) indicated the need for caution when extrapolating to larger animal species (such as humans) the results of absorption studies conducted in small laboratory animals. Because there are 'certain relationships' between injection site, body size and injected volume which cannot be duplicated when a large difference in body size exists, Thompson suggested that the pattern of absorption of a small volume in a small animal may not closely resemble that seen following the injection of a large volume into a larger animal.

Marshall and Palmer (1980) have noted a tendency for individuals of physically smaller species (piglets, cats, dogs) to absorb amoxycillin from i.m. sites at a faster rate than larger species such as calves and horses.

# 4. PHYSICAL FACTORS AFFECTING ABSORPTION FOLLOWING EXTRAVASCULAR INJECTION

Absorption of drugs from i.m. or s.c. injection sites is frequently rate-limited by physical or biopharmaceutical factors. A list of these factors, based on reviews by Wagner (1961, 1971) and Chien (1981) includes:

1. Physicochemical properties of the drug itself.
2. Nature of the solvent or vehicle.
3. Surface area and geometry of the depot formed at the injection site.
4. Volume injected, and concentration of a solution, or the solids:vehicle ratio of a suspension.
5. Hydrogen ion concentration (pH) of the formulation.
6. Tonicity and viscosity of the formulation.
7. Particle size and size distribution of the drug in a suspension.
8. Presence or absence of pharmaceutical additives such as suspending agents.

## 4.1 PHYSICOCHEMICAL PROPERTIES OF THE DRUG MOLECULE

4.1.1 Molecular size or weight (M.W.) might be expected to have an effect on the rate at which a drug is absorbed. In diffusion limited processes, larger molecules can be expected to have slower penetration rates than smaller ones (Ballard, 1968). Several studies have shown this to be true for molecules small enough to be cleared by capillaries (Sund & Schou, 1964a; Secher-Hansen, Langgard & Schou, 1968; Secher-Hansen, 1968b). These studies have shown that carbohydrate molecules of greater M.W. are normally absorbed at a slower rate than smaller ones. That diffusion can be the rate-limiting factor in the absorption of these substances has been shown by experiments which alter the amount of ground substance in the connective tissue through which drug molecules must pass. Hyaluronidase, which depolymerises the ground substance, hastens the absorption of carbohydrate molecules and causes them to be absorbed at the same rate regardless of M.W. (Secher-Hansen, Langgard & Schou, 1968; Secher-Hansen, 1968b). Treatment with oestrogens, which increases the amount of hyaluronic acid in ground substance and the extent to which it is polymerised, delays the absorption of carbohydrate

molecules, and the effect is greater for molecules of greater M.W. (Secher-Hansen, 1968b).

The clinical relevance of these findings has been challenged by Bederka and co-workers (1971) who concluded that for a diverse group of compounds in aqueous solution absorption from i.m. sites was independent of M.W. or diffusion coefficients, and that local blood flow, rather than diffusion, was the factor limiting absorption rate.

The doses and volumes used in the study of Bederka, Takemori and Miller (1971) approximated those used in clinical medicine. However, a tenfold increase in volume led to a marked reduction in absorption rate, possibly because of the creation of a relatively large non-vascularised compartment from which the rate of absorption would be limited by diffusion. While in some of the experiments in which M.W. has been shown to influence absorption rate (Secher-Hansen, Langgard & Schou, 1968; Secher-Hansen, 1968b), the volume of test solution has been many times larger, relatively, than would be used in clinical practice, in others (Sund & Schou, 1964b) the volume was comparable to that used by Bederka and co-workers(1971). It appears, therefore, that the relationship between M.W. and the rate of absorption of common drugs from aqueous solutions still remains uncertain.

Drugs with a M.W. greater than about 10,000 are absorbed primarily by the lymphatics (Hollander, Reilly & Burrows, 1961; Ballard, 1968; Harvey, 1970) although it has been claimed that dextrans with M.W. ranging from 60,000 to 90,000 are absorbed across capillary walls following i.m. injection (Sund & Schou, 1964a; 1965). Dextrans are known to cause the local release of histamine, and this may enhance the permeability of the capillaries at the site of injection (Sund & Schou, 1964a; 1965).[1]

The absorption of large drug molecules which are cleared by the lymphatics is slower than that of those cleared by capillaries

[1] Schou (1971) states "... these large molecules (dextran, M.W. 60,000 to 90,000) are only cleared through the lymphatic system"; an apparent contradiction of his earlier claims (Sund & Schou, 1964a; 1965).

(Hollander, Reilly & Burrows, 1961; Secher-Hansen, Langgard & Schou, 1968) and tends to be limited by filtration rather than diffusion; it is thus little affected by hyaluronidase which only decreases the resistance to diffusion (Secher-Hansen, 1968a; 1968b).

The majority of drugs in common usage have M.W.'s of less than 10,000.

4.1.2 Solubility in tissue fluids. Following the injection of a suspension of a drug, an important factor governing its rate of absorption will be the rate at which the suspended drug particles dissolve in the tissue fluids at the site of injection. In large measure this will be governed by the intrinsic aqueous solubility of the particular drug (Wagner, 1971; Dowrick, 1980).

Injection of solutions by extravascular routes may lead to precipitation of the drug at the site of injection (Ballard & Nelson, 1970). This may be due to pH changes or to dilution of the vehicle by tissue fluids (Wagner, 1971; Wilensky & Lowden, 1973; Greenblatt & Koch-Weser, 1976). In such cases absorption is slower than one would expect for a solution.

Some drugs, for example procaine penicillin G (Buckwalter & Dickison, 1958), exist in two or more crystalline forms. They are said to exhibit polymorphism. The different crystalline forms of a drug may have different solubilities, and so suspensions of these drugs may be absorbed at different rates (Wagner, 1961; Ballard, 1968; Harvey, 1970).

4.1.3 Lipid solubility. Lipid-soluble molecules tend to be absorbed more rapidly from aqueous solutions than do lipid-insoluble substances of comparable M.W. (Schou, 1961). Whereas lipid-insoluble substances tend to be absorbed at a rate depending on their rate of diffusion in aqueous solution, the rate of absorption of lipid-soluble substances depends on their oil:water partition coefficient (Schou, 1961; Kakemi *et al*, 1969; Harvey, 1970) or, rather, the partition coefficient of the drug between the lipids of biological membranes and the aqueous tissue fluids (Keberle, 1971). This *in vivo* partition coefficient may be

strikingly different from *in vitro* oil:water partition coefficients because of the complex nature of biological lipids and tissue fluids (Keberle, 1971).

When a drug is injected in solution in an oil, or oil-like vehicle, the partition coefficient of the drug between the vehicle and the tissue fluid governs the rate at which it is absorbed (Wagner, 1971), as drug and vehicle are absorbed separately and at different rates (Honrath, Wolffe & Meli, 1963; Van der Vies, 1970). When the water solubility of a drug administered in this fashion is so low that a significant concentration of it in tissue fluid cannot be achieved, absorption may be negligible (Harvey, 1970).

4.1.4 Binding. Some drugs may bind with tissue constitue ts at the site of injection (Harvey, 1970; Secher-Hansen, 1970; Okumura, Sezaki & Kakemi, 1972). The strongly ionised mucopolysaccharides in connective tissue ground substance may retard the absorption of a number of drugs, especially large cationic molecules (Harvey, 1970). When succinylcholine is injected intramuscularly in saline solution its effects are weaker and slower in onset than when it is administered in water (Foldes, Brown & Lunn, 1962). This effect is probably due to reduced absorption, as saline enhances the spread of solute through ground substance (Secher-Hansen, Langgard & Schou, 1967b), thus allowing more of the cationic succinylcholine molecules to come into contact with, and bind to, tissue elements (Schou, 1971).

The fact that atropine is absorbed from i.m. injection sites more slowly than would be expected from its other physicochemical properties is probably because it binds to lipoproteins, proteins and amino acids at the injection site (Sund & Schou, 1964b).

## 4.2 NATURE OF SOLVENT OR VEHICLE

Drugs may be injected as aqueous solutions, solutions in non-aqueous solvents which may or may not be miscible with water, as aqueous or oily suspensions, or as emulsions.

Aqueous solutions are able to mix and diffuse directly into tissue fluid and the dissolved drug is able to be absorbed from there into capillaries or lymphatics (Dowrick, 1980). The

absorption of a diverse group of compounds from aqueous solutions injected intramuscularly was investigated by Bederka, Takemori & Miller (1971). The absorption rate constants for the six substances investigated were remarkably similar and the average absorption half-life was 4.4 minutes. The absorption rates appeared to be independent of the M.W.'s, diffusion coefficients, pka values and pharmacologic class of substances investigated. These workers used an injection volume comparable to that used in clinical medicine and they postulated that under such conditions the absorption of drugs from aqueous solution is limited only by the local blood flow in the injected muscle.

Drugs administered in water-miscible, non-aqueous solutions may behave very differently. Once the solvent becomes diluted by tissue fluid the water-insoluble drug may precipitate at the site of injection as a 'crystalline mush' (Deanesly & Parkes, 1937) from which absorption occurs as from an aqueous suspension (Eastland, 1951; Junkmann, 1957; Ballard & Nelson, 1970; Wagner, 1971).

To be cleared from an oily solution a drug molecule must diffuse to the oil:tissue fluid interface and dissolve into the aqueous tissue fluid before being absorbed into the capillaries. A particular drug may be absorbed at different rates from different oily vehicles depending on the oil:water partition coefficient of the drug in that vehicle and the rate at which the vehicle itself is absorbed (Eastland, 1951; Honrath, Wolff & Meli, 1963; Ballard & Nelson, 1970; Van der Vies, 1975).

The absorption of drugs from injected suspensions is an even more complex process. The stages in this process have been tabulated by Dowrick (1980) and are shown in Table 2:I. Each stage may be affected by a variety of biological, physical and physicochemical factors (Wagner, 1961; 1971).

Table 2:I Stages in the absorption of drugs from injected suspensions (Dowrick, 1980)

| Stage | Aqueous suspension | Oily suspension |
|---|---|---|
| 1 | Drug particle in aqueous depot | Drug particle in oil depot |
| 2 | Drug dissolves in aqueous depot | Drug particle diffuses to oil:water interface |
| 3 | | Drug particle becomes wetted |
| 4 | Drug dissolves in tissue fluid | |
| 5 | Drug diffuses through tissue fluid to capillaries | |
| 6 | Drug absorbed through capillaries into blood | |

## 4.3 EFFECT OF SURFACE AREA OF INJECTION DEPOT

It has already been mentioned that the rate at which an injected drug is absorbed is directly proportional to the area of absorbing membrane in contact with the depot of injected material.

Ballard (1968) has pointed out that with conventional injection techniques it is virtually impossible to control the area occupied by an injected solution, suspension or emulsion which is in contact with the tissues. Surface area may be increased by massage, which spreads the injected fluid, or by dividing the dose into multiple small injections (Harvey, 1970).

It has been demonstrated in cattle (Clark *et al*, 1974) that the absorption of oxytetracycline solution following i.m. and s.c. injection is more rapid when the dose is administered at multiple sites rather than at a single site. For the first five to six hours after injection the multiple-site technique resulted in significantly higher serum oxytetracycline levels.

## 4.4. VOLUME OF THE INJECTION AND DRUG CONCENTRATION

4.4.1 Aqueous solutions. Because of the large number of biological variables operating, a generally applicable mathematical equation to describe extravascular drug absorption has not been derived (Schou, 1971), although various approximations have been discussed by several authors (Schou, 1961; 1971; Ballard, 1968; Ballard & Nelson, 1970).

For aqueous solutions of non-electrolytes or very weak electrolytes which are pharmacodynamically inert at the injection site, one might predict that for a given dose the rate of absorption would be independent of the volume of injection (Schou,

1971), because either an increase in volume (and hence surface area) or an increase in concentration (and hence concentration gradient) could be expected to lead to an increase in absorption rate (Ballard, 1968; Ballard & Nelson, 1970; Schou, 1971).

However, in reality, changes in concentration are not balanced by changes in volume. Tissues do not readily accept large volumes and so the intimate contact between injected drug and absorptive surface is not maintained (Schou, 1971). Diffusion distance from the centre of the depot to the capillaries is increased (Ballard & Nelson, 1970; Bederka, Takemori & Miller, 1971) and when the volume of a given dose is increased the surface area of drug solution exposed to the absorbing capillaries does not increase to the same extent that drug concentration decre ses (Schou, 1971). The fact that it is virtually impossible to control the extent to which the injected solution spreads (Ballard, 1968) and that the geometry of the injection depot changes continuously (Schou, 1971) compound the difficulty of predicting the effect on absorption of changes in volume and concentration. Furthermore, increases in volume lead to greater self-depression of absorption (Schou, 1961; Secher-Hansen, Langgard & Schou, 1967) and marked increases in concentration may increase the osmotic pressure to the extent that the relative absorption rate is reduced (Schou, 1971).

The absorption rate of neutral, water-soluble substances without local pharmacodynamic effect administered in a constant volume is essentially identical over a wide range of concentrations (Sund & Schou, 1964a; Secher-Hansen, Langgard & Schou, 1967b; Kakemi *et al*, 1969). However, the absorption of a drug such as atropine, which has a local pharmacodynamic effect, decreases markedly as concentration increases (Schriftman & Kondritzer, 1957; Sund & Schou, 1964b).

Most studies have shown that the absorption rate of drugs from aqueous solutions injected into extravascular sites is significantly affected by alterations of the injection volume. For a given concentration, increases in volume of aqueous solutions of either inert substances (Sund & Schou, 1964a)

or atropine sulphate (Schriftman & Kondritzer, 1957) lead to decreases in absorption rates. It has been reported (Warner *et al*, 1953) that when a given dose of sodium chloride was administered intramuscularly in different volumes (and hence different concentrations) the absorption rate increased as the injected volume was decreased.

A four-fold increase in volume had no effect on the absorption rates of aqueous solutions of isonicotinic acid, isonicotinamide or thiamine (Kakemi *et al*, 1969; Okumura, Sezaki & Kakemi, 1972). Similarly, Bederka and co-workers (1971) noticed that the absorption of sodium penicillin from muscle was unaffected over a five-fold increase in volume, but above the upper limit of doses likely to be used clinically, a significant reduction in absorption rate occurred, probably because of the formation of a relatively large non-vascularized compartment from which the drug took longer to diffuse.

The rate at which insulin is absorbed has been shown (Binder, 1969) to be decreased both by increases in volume and increases in concentration of the injected solution. The greatest effect on absorption rate is that of concentration because insulin, like atropine, inhibits its own absorption by inducing a transient depression of blood flow in the capillaries around the injection site.

In summary, unless a drug has a local effect at the site of injection, it appears that alterations in concentration have very little effect on the absorption of aqueous solutions. On the other hand, alterations in volume may have an important impact on absorption rate, with an increase in volume leading to a decrease in the rate of absorption (Table 2:II).

4.4.2 Non-aqueous solutions. While alterations in concentration and volume also exert significant effects on the absorption rate of drugs from non-aqueous solutions, these effects have been less well defined than for aqueous solutions.

When oily solutions are injected, solvent and solute are absorbed separately (Van der Vies, 1970). The effect of a

change in volume or concentration will therefore depend not only on the extension of the absorptive surface area, but also on the partition coefficient of the drug between vehicle and tissue fluid (Honrath, Wolff & Meli, 1963; Wagner, 1971).

Table 2:II The effect of alterations of concentration and volume on the absorption of injected aqueous solutions

| Drug | Route of administration | Alteration | | Effect on absorption | Reference |
|---|---|---|---|---|---|
| Sodium chloride | i.m. | V+ | C- | - | Warner *et al*, 1953 |
| Sucrose | i.m. | Vk | C+ | k | Sund & Schou, 1964a |
| | s.c. | Vk | C+ | k | Secher-Hansen *et al*, 1967b |
| | i.m. | V+ | Ck | - | Sund & Schou, 1964a |
| Mannitol | i.m. | V+ | Ck | - | Sund & Schou, 1964a |
| Atropine | i.m. | Vk | C+ | - | Sund & Schou, 1964b |
| | i.m. | Vk | C+ | - | Schriftman & Kondritzer, 1957 |
| | i.m. | V+ | Ck | - | Schriftman & Kondritzer, 1957 |
| | i.m. | V+ | C- | - | Schriftman & Kondritzer, 1957 |
| Isonicotinic acid | i.m. | V+ | Ck | k | Kakemi *et al*, 1969 |
| Isonicotinamide | i.m. | V+ | Ck | k | Kakemi *et al*, 1969 |
| | i.m. | Vk | C+ | k | Kakemi *et al*, 1969 |
| Isoniazid | i.m. | Vk | C+ | k | Kakemi *et al*, 1969 |
| Thiamine | i.m. | V+ | Ck | k | Okumura *et al*, 1972 |
| Insulin | i.m. | V+ | Ck | - | Binder, 1969 |
| | i.m. | Vk | C+ | - | Binder, 1969 |
| Sodium penicillin | i.m. | V+ | Ck | k or-[a] | Bederka *et al*, 1971 |

a. Absorption not affected until volume administered was outside the usual clinical range.

V = volume, C = concentration, k = constant, + = increase, - = decrease.

Honrath and co-workers (1963) studied the absorption of testosterone and testosterone propionate following s.c. injection in sesame oil. A constant dose was administered in different volumes. They reported that testosterone was absorbed more rapidly from larger volumes of oil, whereas testosterone propionate was absorbed more slowly. These results indicate that the rate of absorption of testosterone from a particular oily vehicle is largely dependent upon the surface area available for absorption (Honrath, Wolff & Meli, 1963). Testosterone propionate, on the other hand, has a higher solubility in oil and a lower solubility in water than does testosterone (Blacow, 1972) and the vehicle: water partition coefficient (see Eastland, 1951; Wagner, 1971) is apparently so great that an increase in injection volume increases that proportion of the dose entrained in the oil depot and unavailable for absorption.

4.4.3 Suspensions. The injection of an aqueous suspension with a high solids:water ratio produces a depot from which there is usually slow absorption. For a given amount of drug, increasing the volume in which it is injected will usually increase the absorption rate (Wagner, 1971).

This effect has been reported for suspensions of procaine penicillin (Buckwalter & Dickison, 1958; Ober *et al*, 1958) and of amoxycillin trihydrate (Yeoman, 1977; Dowrick, 1980). Yeoman (1977) reported that when a given dose of amoxycillin was administered intramuscularly to calves or horses the blood level peaks were inversely related to the concentration of the suspension, with a 50 mg/ml suspension producing significantly higher peak levels than a 250 mg/ml suspension. The more concentrated suspension, because of its slower absorption, maintained blood levels of amoxycillin for a longer time.

There are three likely reasons for the effects of concentration on the absorption of suspensions:

1. The retarded absorption of concentrated suspensions is a result of the compact, poorly soluble deposit formed within the tissues (Ober *et al*, 1958). The surface area of the injected deposit is a critical determinant of its

rate of absorption (Heubner, 1971). Less concentrated suspensions will present a greater surface area to the absorptive capillary bed (Yeoman, 1977; Dowrick, 1980).

2. The greater the volume in which a given dose of a poorly soluble drug is suspended, the greater will be the proportion of the dose in *solution* in the vehicle and hence immediately available (Yeoman, 1977; Dowrick, 1980). In the case of amoxycillin trihydrate suspensions, 8.7% of a dose is in solution in a 50 mg/ml suspension, whereas in a 200 mg/ml suspension only 2% of the dose will be in solution (Dowrick, 1980).

3. The less concentrated suspension may have a lower viscosity (Dowrick, 1980).

## 4.5 THE EFFECT OF HYDROGEN ION CONCENTRATION ON ABSORPTION RATE

There is evidence that in some cases the pH of an injected formulation may influence the rate at which a drug is absorbed (Okumura, Sezaki & Kakemi, 1972). That some drugs may precipitate from solutions at physiological pH (7.2 - 7.4) has been mentioned. Extremes of pH may cause inflammation and destruction of tissue and may thus influence absorption by altering local perfusion (Schou, 1971; Feldman, 1974).

Absorption of sodium chloride solutions is not altered over a pH range of 2.5 to 10. However, above pH 10 absorption is reported to increase, while below pH 2 absorption decreases (Madison & Christian, 1950). A minor effect of pH on the absorption of aqueous sucrose solutions was noted by Secher-Hansen (1970): sucrose and water molecules were both absorbed slightly more rapidly at pH 8.9 than at pH 4.6.

Acid solutions of insulin (pH 2.9) are absorbed more slowly from extravascular sites than are neutral ones. The differences are apparent mainly during the initial phase of absorption and probably reflect a greater self-depression caused by the acid solutions exerting a more pronounced effect on tissues (Binder, 1969).

The work of White and Claflin (1963) and of Cutts and Walker (1966) has been interpreted (Ballard, 1968; Schou, 1971) as demonstrating a significant and important decrease in absorption rate (as assessed by acute toxic effects) of nitrogen mustard injected at pH 2 rather than at pH 8. However, these workers were not studying absorption *per se*, and some other explanation of their results is more likely because White and Claflin demonstrated the same reduced toxicity of the acidic solutions when they were administered intravenously.

In view of the rapid buffering activity of tissues (White & Claflin, 1963; Binder, 1971; Schou, 1971) it is likely that significant effects of pH on absorption rate will only occur as a result of local inflammation and the self-depression phenomenon.

## 4.6 THE EFFECT ON ABSORPTION OF VISCOSITY AND TONICITY

The viscosity and tonicity of a formulation may affect the rate at which a drug is absorbed from s.c. or i.m. injection sites (Wagner, 1961; 1971). The two properties are inter-related.

To investigate the effect of tonicity on absorption Secher-Hansen and associates (1967b) administered 6%, 10% and 14% (w/v) sucrose solutions by s.c. injection to mice. These solutions are hypotonic, isotonic and hypertonic, respectively. Following injection of the hypertonic solution the volume of the injection site increased significantly over several minutes as water was drawn in from the surrounding tissues to eliminate the osmotic imbalance. However, the volume of isotonic and hypotonic solutions were rendered osmotically inactive by the connective tissue ground substance.

Despite differences in the rate at which injected *volumes* disappeared from injection sites, over the range of concentrations studied, sucrose molecules disappeared at a rate independent of osmolarity. Tonicity thus had no effect on the absorption rate of this lipid-insoluble non-electrolyte. Osmotic equilibrium at the injection site was achieved within 5 minutes of injection.

The effect of tonicity on the absorption of the electrolyte sodium chloride was also studied by the same workers (Secher-

Hansen, Langgard & Schou, 1967b). Solutions containing 0.9% and 1.2% NaCl were injected, and it was noted that the hypertonic NaCl solution was absorbed at the same rate as the isotonic one because the tissue ground substance was able to bind the surplus ions of the 1.2% solution and the osmotic balance was maintained without an influx of water.

Munck and colleagues (1967) studied the absorption of the lipid-soluble drug antipyrine. They noted a highly significant difference in absorption rates of isotonic and hypotonic solutions. A hypotonic solution prepared in water had an absorption half-life of 7.7 minutes, whereas an isotonic solution had an absorption half-life of 1.4 minutes. However, the isotonic solution was prepared in saline rather than water, and it has been shown that saline enhances absorption in a similar fashion to hyaluronidase (Secher-Hansen, Langgard & Schou, 1967b; Section 5.2.1).

The absorption of drugs from aqueous solutions may be retarded by the addition of water-soluble or water-miscible agents which markedly increase both tonicity and viscosity (Eastland, 1951; Ballard & Nelson, 1970). The rate at which a drug is absorbed from a viscous solution may be determined by the slow rate at which drug molecules diffuse through such solutions (Harvey, 1970).

The rate at which radio-labelled sodium chloride is absorbed from s.c. sites has been shown to be reduced when it is administered in a 2 molar (78.5%) sucrose solution.

Reductions in acute toxicity of solutions of streptomycin, dihydrostreptomycin and isoniazid in mice have been achieved by the addition of a variety of non-electrolytes, amino acids or salts of amino acids (Presscott, Kauffman & James 1958a; 1958b). The effect is presumably due to a decrease in absorption rate (Ballard & Nelson, 1970). Non-electrolytes such as sucrose and glycerine increase the viscosity of aqueous solutions and decrease the rate at which ions such as streptomycin and sodium are able to diffuse through them. Electrolytes such as amino acids or calcium ions reduce the rate at which ionized drugs diffuse, partly by increasing viscosity and partly by the inter-ionic

forces which act between the drug and the added ions (Ballard & Nelson, 1970). In addition, the presence of a strongly hypertonic solution at the injection site could be expected to lead to a net flow of extracellular water into the site (Secher-Hansen, Langgard & Schou, 1967b) and so a solvent drag effect could also contribute to the reduction in absorption rate (Ballard & Nelson, 1970).

Brigham and Neilsen (1958) reported that the acute toxicity of solutions of streptomycin and dihydrostreptomycin was reduced when calcium pantothenate was added to the drug solution. Ballard & Nelson (1970) implied that this reduced toxicity was the result of reduced absorption caused by increased viscosity or tonicity. However, Brigham and Nielsen themselves suggested that the acute toxicity was reduced because of pharmacodynamic effects of the calcium ion, a view that is supported by recent studies (for example Adams, 1975).

Increases in viscosity will also retard the absorption of drugs from suspensions. Buckwalter and Dickison (1958) showed this for procaine penicillin suspensions injected intramuscularly into rabbits. Dowrick (1980) postulated that increased viscosity may be partly responsible for the retarded absorption seen with amoxycillin trihydrate suspensions having a high solids:vehicle ratio. The immune response in rabbits and guinea pigs to vaccines containing *Clostridium perfringens* type D toxoid was shown by Coles and co-workers (1965) to be positively correlated with the viscosity of the aqueous vehicle, presumably because the toxoid was more slowly absorbed from vaccines of greater viscosity.

Dowrick (1980) reported that an experimental antibiotic injected intramuscularly or subcutaneously was absorbed more rapidly from a less viscous formulation. The effect was more pronounced following i.m. injection.

## 4.7 RHEOLOGICAL BEHAVIOUR

Rheology is the study of the properties and behaviour of flowing substances such as suspensions. Although the physical properties of the individual suspended drug particles affect the bioavailability of the drug, the physical properties of the

suspension as a whole influence the bioavailability and *usefulness* of a formulation (Ballard & Nelson, 1970). For example, a suspension of procaine penicillin may have a desirably prolonged absorption characteristic, but may be too viscous to be readily injected (Buckwalter & Dickison, 1958).

Ober and his colleagues (1958) made a rheological study of procaine penicillin formulations and discovered that the best suspensions for prolonged action were those which exhibited thixotropy. Thixotropy is a property of certain gels which undergo liquefaction when shaken and return to the gel form upon standing. When pressure is exerted on a thixotropic paste it will flow easily through a hypodermic needle, but on deposition in the tissue it reverts to a gel, thus forming a compact depot which resists dissolution by presenting a minimum surface area to the tissue fluids. Such a depot has been dubbed a 'thixotropic pellet' (Thompson, 1960).

The degree of thixotropy of a formulation is expressed as a torque (T) value. Suspensions with T values of less than 100,000 dyne-cm do not have sufficient thixotropy to produce compact depots. Instead they form flat, more diffuse, fans which offer a larger area for absorption.

The rheological properties of a suspension are governed by the particle size and particle size distribution of the suspended drug, the solids:vehicle ratio of the formulation (Ober *et al*, 1958) and the nature of the vehicle (Ballard & Nelson, 1970).

## 4.8 PARTICLE SIZE AND PARTICLE SIZE DISTRIBUTION

In the case of aqueous suspensions, those with a small average particle size are absorbed at a faster rate than those containing larger particles (Miller & Fincher, 1971). This is because the effective surface area exposed to tissue fluids increases as particle size decreases. Buckwalter and Dickison (1958) clearly demonstrated this effect on the i.m. absorption of procaine penicillin.

In contrast, when drugs are administered as oily suspensions, the converse is the case (Buckwalter & Dickison, 1958; Dowrick,

1980; Chien, 1981). For a drug of low solubility in oil to be absorbed from an oily suspension the suspended drug particles must diffuse to the oil:tissue fluid interface (Dowrick, 1980). In oily suspensions the mobility of the smaller particles is reduced more than that of larger particles (Dowrick, 1980). As a result, when thick, oily suspensions of procaine penicillin were injected intramuscularly, those with a smaller average particle size maintained blood levels of penicillin for longer than those containing larger particles (Buckwalter & Dickison, 1958). Coles and co-workers (1958) reported that when a particulate toxoid was administered as an oily vaccine, a reduction in particle size led to an increased antibody response, presumably because of a more prolonged release of antigen from the vaccine.

### 4.9 EFFECT ON ABSORPTION OF PHARMACEUTICAL ADDITIVES

The presence of pharmaceutical additives such as suspending or wetting agents may alter the rate of absorption of injected drugs (Thompson, 1960; Blecher & Burnette, 1969; Ballard & Nelson, 1970; Wagner, 1971; Feldman, 1974). By increasing the viscosity of formulations, suspending agents such as carboxymethylcellulose, gelatin or polyvinylpyrrolidone (PVP) may delay drug absorption. Surfactants such as the tweens and spans may accelerate absorption by increasing the rate of wetting of suspended particles.

## 5. PHARMACOLOGICAL FACTORS AFFECTING ABSORPTION FOLLOWING EXTRAVASCULAR INJECTION

There are a number of drugs which may retard or enhance the rate at which other drugs are absorbed from extravascular injection sites. The influence that such drugs exert on absorption may result from their actions on connective tissue ground substance, on local peripheral circulation and tissue perfusion, or on the self-depression phenomenon.

### 5.1 DRUGS ACTING PRIMARILY ON LOCAL PERFUSION

5.1.1 Adrenaline and noradrenaline. Pharmacologically active substances such as adrenaline or noradrenaline may be added to injection solutions to retard absorption and thus either prolong local activity or reduce systemic toxicity.

At a concentration of 5 µg/ml or greater, adrenaline and noradrenaline delay absorption from both i.m. (Kety, 1949; McGirr, 1952; Gosselin, 1966; Schou, 1968; Bederka,Takemori & Miller, 1971) and s.c. sites (Secher-Hansen, 1968b; Gangarosa & Cheong, 1969). These drugs exert vasoconstrictor effects on the terminal vascular bed in the zone of absorption and, as a result of this vasoconstriction, local blood flow in the region of the injection site is reduced (Schou, 1961). The absorption of water molecules, and of solutes which are normally absorbed by capillaries, is reduced (Schou, 1968; Secher-Hansen, 1968b; Gangarosa & Cheong, 1969; Bederka, Takemori & Miller, 1971), but the absorption of surplus water and large molecules which are absorbed by the lymphatics is unaffected (Secher-Hansen, 1968b).

The absorption-retarding effect of adrenaline is used clinically when vasoconstrictors are added to solutions of local analgesics. As a result, the local effect of these solutions is prolonged and the systemic toxicity of the analgesic is reduced (Schou, 1971). In cases of acute allergic reactions following the injection of penicillin, infiltration of adrenaline around the injection site may be used to reduce the uptake of antigens. Similarly, the infiltration of adrenaline around certain snake bites may dramatically reduce systemic toxicity (Schou, 1971).

5.1.2 Atropine. The addition of atropine to injection solutions has been shown to delay the absorption of a number of compounds including organic and inorganic phosphates (Ramachandran & Agren, 1963), sulphacetamide and sucrose (Sund & Schou, 1964b). Atropine also inhibits its own absorption (Schriftman & Kondritzer, 1957; Sund & Schou, 1964b).

It is probable that atropine exerts its effect on absorption by a direct, local vasoconstrictor action which reduces tissue perfusion, although a self-depression phenomenon involving local release of histamine and 5-OHT may also be involved (Ramachandran & Agren, 1963; Sund & Schou, 1964b). The effect of atropine is entirely a local one, dependent on the atropine and test drug being administered at the same site. Even massive doses of atropine at other sites have no effect on the absorption of the test drug (Ramachandran & Agren, 1963; Sund & Schou, 1964b).

The effect of atropine on absorption is dose-dependent over the concentration range of 0.5 mg/ml (below which no effect is apparent) to 5 mg/ml (above which there is no further increase in response) (Ramachandran & Agren, 1963; Sund & Schou, 1964b).

Other anticholinergic drugs including methylscopolamine, homatropine, adephenine, propantheline and oxyphenone, also depress absorption (Sund & Schou, 1964b).

5.1.3 Cholinergic drugs. Acetylcholine, methacholine and carbachol, and the anticholinesterase agent paraoxon, have been shown to accelerate absorption from i.m. injection sites (Hyman *et al*, 1959; Jorgenson & Schou, 1965; Gosselin, Cameron & Audino, 1967; Gangarosa & Cheong, 1969).

The effect of cholinergic agents is probably due to an increased tissue perfusion caused by a dilation of precapillary sphincters in the region of the injection site (Jorgenson & Schou, 1965; Gosselin, Cameron & Audino, 1967). As is the case with atropine, the effect is a local one, dependent on the addition of the cholinergic agent to the injection solution. The effect is also dose-dependent (Jorgenson & Schou, 1965).

5.1.4 Other vasodilators. The addition of isoproterenol to injected drug solutions has been shown to enhance their absorption from s.c. (Gangarosa & Cheong, 1969) and i.m. sites (Gosselin, 1966; Gosselin, Cameron & Audino, 1967). Isoproterenol increases local blood flow by stimulating beta-adrenergic receptors, and its action is blocked by systemic treatment with beta-blocking agents such as propranolol (Gangarosa & Cheong, 1969).

The non-specific vasodilator papaverine has been shown to increase the rate at which subcutaneously administered substances are absorbed (Gangarosa & Cheong, 1969) and this effect is not blocked by propranolol. Nitroglycerin is another vasodilator which enhances the absorption of solutions to which it is added (Gosselin, Cameron & Audino, 1967), as do certain vasodilating prostaglandins (Bederka, Takemori & Miller, 1971).

## 5.2 DRUGS ACTING PRIMARILY ON GROUND SUBSTANCE

5.2.1 Hyaluronidase. The addition of the enzyme hyaluronidase enhances the absorption of a variety of compounds from i.m. (Schriftman & Kondritzer, 1957; Sund & Schou, 1965) and s.c. sites (Franke *et al*, 1950; Hvidberg & Schou, 1959a; 1959b; Secher-Hansen, Langgard & Schou, 1967b; Secher-Hansen, Langgard & Schou, 1968).

By depolymerizing hyaluronic acid this enzyme reduces the water binding capacity of the ground substance and decreases the resistance it offers to diffusion of solutes (Schou, 1961). The degree to which it influences absorption depends on the variable hyaluronic acid content of the ground substance (Hvidberg & Schou, 1959a; Secher-Hansen, 1968a). Hyaluronidase does not affect the permeability of capillaries (Sund & Schou, 1965).

As the mucopolysaccharide barrier to diffusion is broken down differences in the absorption rate of substances of different M.W. are abolished (Secher-Hansen, Langgard & Schou, 1968b), with the exception that those large molecules which are absorbed by the lymphatics are unaffected (Secher-Hansen, 1968a).

It has already been mentioned that a surplus volume of solvent water at the injection site is usually removed much less rapidly than the actual injected molecules of water (see Section 2.2., Secher-Hansen, Langgard & Schou, 1967a). While the addition of hyaluronidase to solutions has no effect on this already very rapid exchange of water molecules (Sund & Schou, 1965), because of effects on the water-binding capacity of ground substance it may hasten the rate at which surplus volume disappears (Secher-Hansen, Langgard & Schou, 1967b; Secher-Hansen, 1968a).

Sodium chloride in solution has a similar effect to hyaluronidase on the absorption of other solutes (Secher-Hansen, Langgard & Schou, 1967b).

5.2.2 Oestrogens. In mice which have been pretreated with oestradiol on two occasions during the week before injection, absorption from s.c. sites is significantly retarded (Hvidberg & Schou, 1958b; Secher-Hansen, 1968a; 1968b). Oestrogen treatment increases the amount of ground substance in the skin, alters its

composition and increases the extent to which the hyaluronic acid is polymerized (Hvideberg, Szporny & Langgard, 1963; Grosman, Hvidberg & Schou, 1971; Grosman, 1973). The extent of these changes is greater in sexually immature animals (Grosman, 1972).

Through such tissue changes, oestrogen treatment influences the rate at which the surplus water volume is removed from an injection site. Absorption is decreased because of the increased water-binding capacity of the ground substance, and the injected water molecules are cleared at a reduced rate because of increased diffusion distances (Secher-Hansen, 1968a). Solute molecules such as urethane and several carbohydrates are absorbed more slowly because the permeability of the ground substance is reduced (Hvidberg & Schou, 1959b; Secher-Hansen, 1968b). Substances of low M.W. are less affected than those of higher M.W. (Secher-Hansen, 1968b) and the effects of oestrogen treatment are more pronounced in mice with thicker skin (Secher-Hansen, 1968a).

It is possible, however, that this effect of oestrogens on the rate of absorption from s.c. sites may not be a consistent phenomenon. In rats and rabbits the absorption of sulphacetamide sodium was unaffected by prior treatment with oestrogens (Hvidberg & Schou, 1959b), and the composition of the ground substance remained unaltered (Schou, 1961).

5.2.3 Diuretics. The possibility that diuretics might influence the water content of connective tissue ground substance, and hence the absorption rate of drugs from injection sites, was investigated by Hvidberg and colleagues (1958). They found that the mersalyl-theophylline complex diuretic investigated was without effect on the absorption of saline or urethane from normal s.c. connective tissue.

## 5.3 DRUGS AFFECTING THE SELF-DEPRESSION PHENOMENON

5.3.1 Corticosteroids. The absorption of certain drugs from s.c. injection sites is enhanced by systemic treatment with glucocorticoids.

The absorption of the anaesthetic urethane in mice is accelerated by prior systemic treatment with cortisone acetate (Cooper, Schmidt & Schou, 1957) and treatment of rabbits with cortisone acetate, prednisone or hydrocortisone greatly enhances the rate at which sulphacetamide sodium is absorbed from s.c. sites (Schou, 1958c; 1959a; 1959c; 1960).

Glucocorticoids enhance the absorption without altering the ground substance of the s.c. connective tissue (Schmidt, 1958; Hvidberg & Schou, 1959a), and so their influence is probably attributable to their effects on the local circulation and on the histamine content of connective tissue (Schmidt, 1958). Corticosteroids not only inhibit the production of oedema fluid at the site of injection, but also accelerate its reabsorption (Cooper, Schmidt & Schou, 1957). Therefore, it is possible that under the influence of anti-inflammatory corticosteroids, there is a solvent drag effect which promotes the passage of injected drug from interstitial fluid into the circulation (Schou, 1961).

It is also likely that corticosteroids may act by reducing the self-depression mechanism. Schou (1961) suggests that while corticosteroid treatment reduces the rate at which histamine and 5-OHT are synthesised, the normal turnover of these amines in tissues is unaffected, so that over a period of days a depletion occurs. As a result of this depletion of histamine and 5-OHT, the self-depression phenomenon is reduced or abolished (Schou, 1958a; Milthers, 1959). This explains why the corticosteroid-induced enhancement of absorption is more pronounced in cross-over studies (Schou, 1958c) and why absorption is less affected by a single intravenous administration of a soluble hydrocortisone ester 10 minutes prior to injection than by 5 days of pretreatment with cortisone (Schou, 1959c). The same hypothesis explains why the addition of dexamethasone to an oxytetracycline solution may have no discernible effect on the rate at which that solution is absorbed after s.c. injection in cattle (Clark *et al*, 1974).

Finally, Schou (1961) suggests that glucocorticoid treatment reduces the sensitivity of the absorptive capillary bed to the *effects* of vasoactive amines liberated at injection sites.

Mineralocorticoids appear to be without effect on absorption (Schou, 1959b).

5.3.2 Vasoactive amines and their antagonists. The role of histamine and 5-OHT in the self-depression phenomenon (the phenomenon whereby drugs inhibit their own absorption) has already been mentioned.

Whether vasoactive amines are added to injection solutions or are liberated within the tissues, they have been demonstrated to depress the absorption of sulphacetamide sodium in rabbits and rats (Schou, 1958a; 1958b) and morphine in rats (Milthers & Schou, 1958; Milthers, 1959). It is likely that the liberation of endogenous amines slows the absorption of many drugs following extravascular injection (Schou, 1961; 1971) and this effect has been cited to explain observed absorption phenomena following s.c. injection of morphine in rats, rabbits and humans (Milthers, 1959; 1960) and i.m. injection of insulin in humans (Binder, 1969).

It is not surprising, therefore, that pretreatment with systemic antihistamines such as mepyramine maleate, or the addition of such agents to injection solutions, will reduce or abolish the self-depression phenomenon and enhance absorption. This acceleration of absorption by antihistamines has been demonstrated for sulphacetamide sodium in rats and rabbits (Schou, 1958a; 1958b) and for morphine in rats (Milthers & Schou, 1958; Milthers, 1959). Blood levels of morphine are more than doubled following concurrent local or systemic treatment with antihistamines (Milthers & Schou, 1958).

Similarly, the 5-OHT antagonist BOL 148 (2-bromo-2-lysergic acid diethylamide) greatly enhances the absorption of morphine (Milthers, 1959).

Depletion of endogenous histamine by pretreatment of rabbits and rats with Compound 48/80 has been shown to enhance the absorption of both sulphacetamide solutions (Schou, 1958a; 1958b) and morphine solutions (Milthers & Schou, 1958). Depletion of tissue 5-OHT, by pretreating adult rats with reserpine, reduces the self-depression of morphine absorption (Milthers, 1959).

While generally acting to retard absorption, there are some instances when histamine may act to enhance absorption. Normally, solutes with a M.W. greater than 10,000 are unable to enter capillaries and so are drained away relatively slowly by the lymphatic vessels (Ballard, 1968). The increase in capillary permeability caused by histamine may allow those large molecules to enter the capillaries, thus accelerating their absorption (Sund & Schou, 1964a; 1964b).

There are reports in the literature of two studies in which histamine enhanced, rather than retarded, the absorption of even small solute molecules. In the first study (McGirr, 1952) it was reported that histamine accelerated the rate at which radio-labelled sodium chloride was cleared following i.m. injection in humans. However, McGirr's technique differed from that of later workers in that the injection needle was left *in situ* during the experiment, and readings were taken on the rate at which NaCl solution, NaCl with adrenaline and finally NaCl with histamine were cleared. The three test solutions were injected sequentially within the space of a few minutes. Results of such a study are thus hardly comparable with those obtained following single injections into different injection sites.

In the second study, reported by Hyman and co-workers (1959), i.m. injections of radio-labelled iodine were made in cats. When the histamine-liberating agent Compound 48/80 was administered by intra-arterial injection into the vessel supplying that muscle, the clearance of iodine was accelerated. The results of this experiment are thus not comparable with studies in which histamine is included in the test solution or is liberated at the injection site. The effect of Compound 48/80 intra-arterially was to increase the blood flow in the affected muscle, thus hastening the clearance of injected solutes.

## 6. SUSTAINED-RELEASE FORMULATIONS

The previous sections have outlined how the absorption of drugs from extravascular injection sites may be affected by various biological, pharmaceutical and pharmacological factors. It is

obvious that there exists a large number of ways by which the pharmacist may prepare drug formulations having widely differing absorption rates.

For the optimum use of many drugs intended for injection, formulations giving slow absorption and hence sustained action are desirable. When low blood levels of prolonged duration are more important to the clinician than high levels of short duration, such sustained-release formulations have the advantage that, by maintaining therapeutic concentrations over a longer period of time, the frequency of injection can be reduced (Thompson, 1960; Ritschel, 1973; Vermeulen, 1975; Chien, 1981).

## 6.1 TYPES OF SUSTAINED-RELEASE FORMULATIONS

Apart from the pharmacological methods (for example, the inclusion of vasoconstrictors), which have not found widespread application, the important methods by which injectable sustained-release formulations may be prepared have been classified by Thompson (1960), Ballard and Nelson (1970), Ritschel (1973) and Chien (1981) as follows:

1. Chemical. The use of salts, esters and complexes of low aqueous solubility.

2. Physical
   - i) The selection of a vehicle from which absorption is slow (e.g. oily vehicles).
   - ii) The addition of macromolecules which increase viscosity (e.g. carboxymethylcellulose, PVP, tragacanth, etc.).
   - iii) The addition of metallic soaps (e.g. aluminium monostearate) which act as gelling agents to increase the viscosity of oily vehicles.
   - iv) The use of solutions from which, upon contact with tissue fluids, the drug is precipitated (e.g. propylene glycol).
   - v) The addition of adsorbants (e.g. aluminium hydroxide).
   - vi) The use of aqueous or oily suspensions.

Any single substained-release formulation may employ several of these.

6.1.1 Derivatives of low solubility. The most common and the best method of preparing sustained-action formulations for injection involves the use of a drug form of low aqueous solubility (Eastland, 1951; Ballard & Nelson, 1970). Such derivatives of the parent compound are usually formulated as aqueous or oily suspensions (Ballard & Nelson, 1970), but may be oily solutions (Van der Vies, 1975; Vermeulen, 1975; Brotherton, 1976), or dissolved in an organic solvent from which the drug precipitates on contact with tissue fluids (Eastland, 1951; Junkmann, 1957; Ritschel, 1973).

Derivatives having low aqueous solubility have been prepared from many steroids, including glucocorticoids, mineralocorticoids, androgens, anabolic steroids, oestrogens and progestagens (Vermeulen, 1975; Chein, 1981). The commonest means by which such derivatives are obtained is by esterification of the steroid with various carboxylic acids (Junkmann, 1957; Vermeulen, 1975; Brotherton, 1976). The duration of activity of these esters increases with length of the side chain (Van der Vies, 1975; Vermeulen, 1975; Brotherton, 1976) up to a certain point, beyond which activity is lost, possibly because of very poor solubility in tissue fluids (Woollet & Evans, 1971; Brotherton, 1976).

Once prepared, these steroid esters may be formulated for use as oily solutions or aqueous suspensions; the latter usually giving a more sustained, if less even, release pattern (Van der Vies, 1975; Brotherton, 1976).

Relatively insoluble complexes or salts of a number of acidic or basic drugs are employed in repository preparations. The procaine salt of penicillin is a common example, as are the zinc and protamine complexes of insulin (Eastland, 1951; Harvey, 1970).

6.1.2 The addition of macromolecules to formulations. The absorption-retarding effect of increased viscosity has been made use of in several formulations. High concentrations of gelatin have been used to prolong the absorption of penicillin solutions, adrenaline, ephedrine, morphine and heparin (Eastland, 1951). The addition of gelatin to solutions of ACTH enhances their effect not

so much by retarding their absorption, but by protecting the injected hormone from binding and destruction at the injection site (Thompson, 1960).

Viscous solutions of PVP have been used to prolong the absorption of insulin and other hormones, procaine, sulphathiazole, penicillin and many other drugs (Eastland, 1951; Blecher, & Burnette, 1969; Ritschel, 1973). More recently PVP has been incorporated into solutions of oxytetracycline and has been found to markedly reduce the local tissue damage caused by such formulations (Immelman, Botha & Gribb, 1978; Ziv, 1980), possibly through the formation of less irritant physicochemical complexes (Blecher & Burnette, 1969; Immelman, Botha & Gribb, 1978).

6.1.3 The addition of absorbents. Many of the clostridial vaccines which are so important in veterinary medicine consist of toxoids adsorbed onto aluminium hydroxide or aluminium phosphate to produce large, relatively insoluble complexes (Ritschel, 1973; Trinca, 1979). The prolonged release of antigen from such vaccines produces an enhanced immune response.

Similarly, steroid action may be prolonged by preparing complexes of the hormones with inorganic adsorbents (Junkmann, 1957).

## 6.2 RELATIVE RATES OF ABSORPTION OF DIFFERENT TYPES OF FORMULATION

Ritschel (1973) has ranked the different types of injectable formulation according to their relative rates of absorption (Table 2:III). In general, aqueous solutions are absorbed most rapidly and oily suspensions containing gelling agents, the least. There are exceptions to this classification, but it serves as a useful rule of thumb when considering the likely duration of activity of injectable drug formulations.

Table 2:III Classification of parenteral formulations according to rate of absorption (after Ritschel, 1973)

| Rate of absorption | Type of formulation |
|---|---|
| Most rapid absorption | Aqueous solution |
| | Aqueous solution + macromolecules |
| | Aqueous suspension |
| | Aqueous suspension + macromolecules |
| | Oily solution |
| | Oily solution + metallic soaps |
| | Oily suspension |
| Least rapid absorption | Oily suspension + metallic soaps |

## 7. CONCLUSIONS

The main barrier to the absorption of drugs injected into i.m. or s.c. sites is the capillary endothelial membrane. Absorption across this membrane is governed by the physicochemical properties of the injected drug and by conditions in the connective tissue on one side of that membrane and the blood flow on the other.

The physicochemical properties of the injected drug which are of major significance in absorption from extravascular sites are its solubility in the aqueous interstitial fluid, its solubility in the lipids of the endothelial membranes and its ability to diffuse from the injection depot through the connective tissue ground substance separating it from those membranes. Water solubility favours diffusion of the injected drug in the interstitial fluid while lipid solubility favours its passage across the endothelial cells.

Biological factors which influence the rate of absorption of injected drugs from i.m. or s.c. sites are the total area of the absorbing capillary membrane at the particular site, the rate of blood flow through the capillary bed and the amount and composition of the connective tissue ground substance. The density of the capillary bed differs between tissues and anatomical regions of the body, and these differences are reflected in differences in absorption rates. The permeability of the capillaries may be

affected by a variety of physiological, pathological and pharmacological factors, as may the rate of blood flow through tissues and the condition of the ground substance.

While some authors state that absorption of drugs from extravascular sites is dependent on the rate of diffusion through connective tissue ground substance (Schou, 1971), others believe that local tissue perfusion is the rate-limiting factor (Bederka, Takemori & Miller, 1971). It is likely that under certain circumstances each viewpoint could be correct, but that in the clinical situation it is probably the degree of local tissue perfusion which governs the rate of absorption, at least of aqueous solutions (Bederka, Takemori & Miller, 1971).

Absorption may be enhanced or retarded by the concurrent administration of a variety of drugs, in some cases to a clinically significant extent. Various biopharmaceutical techniques have been described and by the use of these the uptake of injected drugs may be significantly delayed.

# CHAPTER III

## A COMPARISON OF THE PHARMACOLOGICAL ACTIVITY IN COWS OF TWO SUSPENSIONS OF BETAMETHASONE ALCOHOL

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# CHAPTER III

## A COMPARISON OF THE PHARMACOLOGICAL ACTIVITY IN COWS OF TWO SUSPENSIONS OF BETAMETHASONE ALCOHOL

### 1. INTRODUCTION

In the preceding chapter (Chaper II, Section 4.4) the solids: vehicle ratio was discussed as one of the factors which govern the rate of absorption, and hence the pharmacological activity, of injected drug suspensions. Wagner (1971) pointed out that, in general, aqueous suspensions having a high solids:vehicle ratio produce compact depots from which absorption is slow.

While discussing the glucogenic activity of several betamethasone formulations, Woollett and Evans (1971) mentioned that the rate of absorption was influenced by the concentration of steroid in the suspending vehicle. They did not, however, elaborate on this point and it fell to P.G. Box (Glaxo Group Research Ltd., London, pers. com.) to demonstrate that when aqueous suspensions of betamethasone alcohol were administered subcutaneously to calves, for a given dose the rate at which betamethasone was absorbed was related to the volume in which that dose was administered. In other words, as would be expected (Wagner, 1961; 1971), the rate of absorption of betamethasone alcohol appeared to be inversely related to the concentration of the injected suspension.

Corticosteroids increase gluconeogenesis and inhibit glucose utilisation in peripheral tissues, thus causing hyperglycaemia (McDonald, 1977; Brander & Pugh, 1977). The magnitude and duration of this hyperglycaemia may be used as an index of the relative activities of different corticosteroid formulations (Pechet, 1964) because the rate at which an administered steroid is absorbed determines the intensity and duration of its action (Van der Vies, 1964; 1975). In cattle, Woollett and Evans (1971) showed that the extent to which blood glucose concentrations are elevated is influenced by the rate at which different betamethasone formulations are absorbed, and Box (pers. com.) noted that a more concentrated suspension of betamethasone alcohol produced a more prolonged,

although less intense, hyperglycaemia than a more rapidly absorbed dilute suspension.

Administration of exogenous corticosteroids may result in suppression of the secretory activity of the adrenal glands (Brander & Pugh, 1977) and Kancher and co-workers (1976) demonstrated that the administration of betamethasone to cattle results in lowered concentrations of endogenous cortisol. In humans it has been shown that slowly absorbed formulations of betamethasone produce a more prolonged suppression of cortisol concentrations than more rapidly absorbed ones (Mollman *et al*, 1977): it is believed that the duration of this suppressive effect correlates well with the duration of anti-inflammatory activity (Mikhail *et al*, 1969).

The depressant effect on circulating eosinophil numbers has long been recognised as an index of the activity of a corticosteroid formulation (Pechet, 1964; Silber & Arcese, 1964). In addition, corticosteroids usually cause a depression in numbers of circulating lymphocytes and an increase in numbers of neutrophils (Brander & Pugh, 1977). These effects are more pronounced in cattle than in other domestic species (Anon., 1977; Austin, 1979).

The aim of this first experiment was to confirm Box's preliminary observations of the influence of the solids:vehicle ratio on the rate of absorption of betamethasone alcohol suspensions. Two different suspensions of betamethasone were administered to cattle and the effects of these on glucose and cortisol concentrations and differential white blood cell counts were studied.

## 2. MATERIALS AND METHODS

### 2.1 ANIMALS

Thirty clinically normal, non-lactating, non-pregnant cows were divided into groups of ten. Ages were unrecorded, but all had experienced at least one lactation. Twenty-three Friesian and seven Jersey cows were used. Body weights were not available. Cows were grazed on rather poor pasture and were fed hay at the daily rate of one bale per five cows. Apart from ensuring that at least two Jerseys appeared in each group, allocation to groups was on a random basis.

## 2.2 TREATMENTS

Treatments were administered by subcutaneous injection into that part of the neck which was covered when the left ear was laid back flat, and were as follows;

Group I/Treatment I; 40 mg betamethasone alcohol as 20 ml of a 2 mg/ml suspension(1)

Group II/Treatment II: 40 mg betamethasone alcohol as 2 ml of a 20 mg/ml suspension(2)

Group III/ Control; 20 ml normal saline.

A dose of 40 mg per cow of betamethasone was estimated to be similar to a dose of 0.1 mg/kg body weight.

The two aqueous betamethasone alcohol suspensions differed only in their betamethasone content. The vehicle in each formulation was identical.

Animals were examined and blood samples were collected once daily on the three days directly preceding treatment and at the same time of day on 14 occasions over 19 days following treatment.

## 2.3 BLOOD COLLECTION

Blood samples were collected by jugular venepuncture into evacuated 10 ml glass tubes. Each tube contained 20 mg potassium oxalate and 25 mg sodium fluoride.

Pools of plasma were made up from blood collected in aliquots of 500 ml into plastic bottles containing 1 g potassium oxalate and 1.25 g sodium fluoride.

Blood was centrifuged within two hours of collection and plasma was removed, frozen and stored at -20°C until required for assay.

(1) Betsolan, Glaxo New Zealand Limited, Palmerston North

(2) EPHE/1/4, experimental formulation, Glaxo New Zealand Limited.

## 2.4 CORTISOL ASSAY

The cortisol radioimmunoassay technique used was that of Ruder *et al* (1972) as modified by Evans (1979).

2.4.1 Materials. Assay buffer was 0.01 M phosphate-buffered saline (PBS), pH 7.3, containing 0.1% (W/V) gelatin and 0.01% (W/V) sodium merthiolate.

Antiserum (AS) to cortisol - 2-BSA (courtesy of Dr R.J. Fairclough, Ruakura Agricultural Research Centre, Hamilton, New Zealand) was stored frozen as 1 ml aliquots at 1:200 dilution in assay buffer. Immediately before use, AS was further diluted in the same buffer to 1:2000.

(1,2,6,7(n) -$^{3}$H) cortisol ($^{3}$H-C), specific activity 285 mCi/mg, radioactive concentration 1 mCi/ml (Radiochemical Centre Ltd., Amersham, UK) was used to prepare a stock solution of 4 μCi/ml by diluting 0.1 ml in 25 ml of ethanol-water (1:24). After storage at 4°C, this stock solution was diluted 1:10 with assay buffer immediately before use.

Cortisol (Sigma Chemical Co., St Louis, USA) was dissolved in ethanol to prepare a range of standards from 0.1 to 32 ng/ml. Ethanol was used as the 'zero standard'.

Bovine gamma globulin, Cohn Fraction II (Sigma Chemical Co., St. Louis, USA) was dissolved in assay buffer to give a 1.5% (W/V) working solution of non-specific protein (PBS-BGG).

Polyethylene glycol 4000 (PEG) (BDH Chemicals Ltd., Poole, UK) was dissolved in distilled water to give a 16.2% (W/V) working solution which was used to separate bound from unbound $^{3}$H-C.

The scintillation fluid was toluene:triton x-100 (2:1) containing 3 g of 2,5 diphenyloxazole (PPO) (Sigma Chemical Co., St Louis, USA) and 100 mg of 1,4-bis (2-(5-phenyloxazolyl)) benzene phenyloxazolylphenyl-oxazolyl-phenyl)(POPOP) (Sigma Chemical Co., St Louis, USA) per litre.

Dichloromethane (DCM) and ethanol were distilled twice before use; the latter after reflux distillation over m-phenylenediamine.

2.4.2 Radioimmunoassay procedure. Plasma samples, in 0.5 ml aliquots, were extracted with 4 ml of DCM in screw-capped glass culture tubes (16 x 125 mm) by vortexing for 30 seconds. The solvent was transferred in 0.8 ml aliquots to labelled assay tubes and evaporated under a stream of air.

Assays were performed in 12 x 75 mm glass culture tubes, and unknown plasma samples, plasma pools and assay standards were assayed in triplicate.

The range of cortisol concentrations from which the standard curve was derived was prepared by adding 0.1 ml aliquots of standard solutions to assay tubes and evaporating the solvent under a stream of air.

To each tube was added 0.1 ml of AS and 0.1 ml of PBS-BGG. Tubes were vortexed briefly and left to stand at room temperature for 30 minutes. Labelled cortisol, 0.1 ml, was then added to all tubes which were again vortexed and incubated at 4°C overnight.

Separation of free from bound $^{3}$H-C was achieved by the addition of 1 ml of PEG at 4°C to each tube while vortexing. Tubes were allowed to stand for 10 minutes at 4°C, then were centrifuged at the same temperature for 20 minutes at 2000 rpm. The supernatant was aspirated and the precipitate redissolved in 1 ml of distilled water. Solutions were decanted into scintillation vials and 6 ml aliquots of scintillation fluid were added to each. Scintillation counting was done in either a Beckman LS7000 or a Beckman LS350 Liquid Scintillation Counter.

A Sord M222 computer was used to calculate plasma cortisol concentrations by the method of Burger *et al* (1972). A representative standard curve is shown in Figure 3:1.

Mean assay sensitivity was 0.28 ± 0.03 ng/ml ($\bar{x}$ ± SEM) with a range of 0.13 - 0.51 ng/ml. The specificity of the antiserum is shown in Table 3:I (Evans, 1979). Between-assay coefficients of variation were calculated for four plasma pools, both undiluted and diluted 1:1 with assay buffer. Data are shown in Table 3:II and it is considered that these figures provide satisfactory tests of

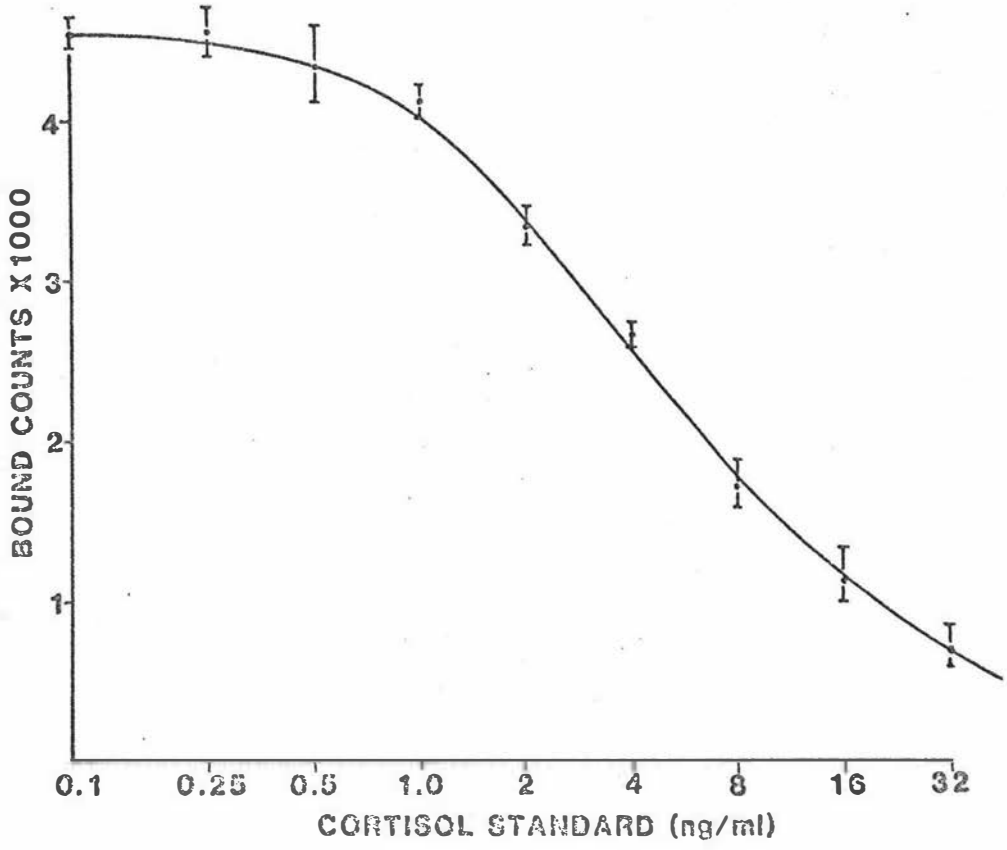


Figure 3:1 Representative standard curve for the cortisol assay, computed by the method of Burger *et al* (1972).

parallelism and assay repeatability. Within-assay coefficients of variation were calculated from three plasma pools (Table 3:III).

## 2.5 BETAMETHASONE ASSAY

The assay technique was based on methods used by Johnson *et al* (1976) and Bradley, C.E. *et al* (Glaxo Research Ltd., London, pers. com.) but was modified as suggested by Dr R.J. Fairclough (pers. com.).

2.5.1 Materials. The assay buffer was tricine-buffered saline (TBS) with 0.1% (W/V) gelatin. It was prepared by dissolving 9 g sodium chloride, 1 g sodium azide, 1 g gelatin and 17.92 g of tricine (N-tris(hydroxymethyl)methylglycine) (Sigma Chemical Co., St Louis, USA) in 1 L of distilled water. The pH was adjusted to 7.4 with 5 N sodium hydroxide and the solution was stored at 4°C.

Antiserum (AS) to betamethasone-CMO-thyroglobulin (courtesy of Dr K.J. Child, Glaxo Research Ltd., London) was stored frozen in 1 ml aliquots at 1:100 dilution in assay buffer. Immediately before use AS was further diluted to 1:3000.

(1, 2, 4(n)-$^{3}$H) betamethasone ($^{3}$H-B), specific activity 81 mCi/mg and radioactive concentration 1.0 mCi/ml (Radiochemical Centre Ltd., Amersham, UK) was used to prepare a stock solution of 0.8μ Ci/ml in assay buffer. This stock solution was stored at 4°C and diluted 1:5 with the same buffer immediately before use.

Betamethasone (Sigma Chemical Co.) was dissolved in ethanol to prepare a range of standard solutions from 0.125 to 128 ng/ml. Ethanol was used as the 'zero standard'.

Bovine gamma globulin, Cohn Fraction II (Sigma Chemical Co.) was dissolved in tricine buffer to give a 1.5% (W/V) working solution of non-specific protein (TBS-BGG).

Polyethylene glycol solution and the scintillation fluid were the same as used for the cortisol assay.

2.5.2 Radioimmunoassay procedure. The assay was performed on plasma without prior extraction and all samples and standards were assayed in triplicate in 12 x 75 mm glass culture tubes.

Table 3:I Specificity of antiserum to cortisol-3 BSA: cross reaction with other steroids (Evans, 1979)

| Steroid | Cross reaction (%) |
|---|---|
| Cortisone | 9.6 |
| Deoxycorticosterone | 0.9 |
| Tetrahydrocortisol | < 0.5 |
| Betamethasone | < 0.1 |
| 17-hydroxyprogesterone | < 0.1 |
| Progesterone | < 0.1 |
| Aldosterone | < 0.1 |
| Testosterone | < 0.1 |
| Androstenedione | < 0.1 |
| Oestradiol-17β | < 0.1 |

Table 3:II Cortisol assay : between assay coefficients of variation

| Plasma pool | No. assays | Mean cortisol (ng/ml) | C.V.(%) |
|---|---|---|---|
| 83 | 11 | 1.10 | 33.1 |
| 83 diluted 1:1 | 4 | 0.78 | 9.2 |
| 100 | 12 | 1.35 | 22.6 |
| 100 diluted 1:1 | 4 | 0.67 | 19.6 |
| 145 | 4 | 29.35 | 6.3 |
| 145 diluted 1:1 | 4 | 21.82 | 5.1 |
| 150 | 12 | 17.42 | 14.8 |
| 150 diluted 1:1 | 9 | 8.95 | 11.0 |

Table 3:III Cortisol assay : within assay coefficients of variation

| Plasma pool | No. replicates | Mean cortisol (ng/ml) | C.V.(%) |
|---|---|---|---|
| 83 diluted 1:1 | 7 | 0.62 | 10.3 |
| 150 | 10 | 20.91 | 7.1 |
| 150 diluted 1:1 | 5 | 11.68 | 1.8 |

To prepare a standard curve, 0.1 ml aliquots of standard solutions were added to assay tubes and solvent was evaporated under a stream of air. To each tube was added 0.1 ml of bovine plasma (No. 150) known to be free of betamethasone.

To the standards and to 0.1 ml aliquots of unknown plasma samples was added 0.1 ml of AS and 0.1 ml of TBS-BGG. The tubes were vortexed briefly and left to stand for 30 minutes at room temperature. Labelled betamethasone, 0.1 ml, was added to all tubes which were vortexed and then incubated overnight at 4°C. Separation of free from bound $^{3}$H-B, scintillation counting and assay computation were performed according to methods already described for cortisol. A representative standard curve is shown in Figure 3:2. The mean assay sensitivity was 0.32 ± 0.02 ng/ml ($\overline{x}$ ± SEM) with a range of 0.18 - 0.61 ng/ml. The specificity of the antiserum is shown in Table 3:IV (Bradley *et al*, pers. com.). Between-assay and within-assay coefficients of variation were calculated for plasma pools and are shown in Tables 3:V and 3:VI. Twelve different plasma pools were assayed undiluted and diluted 1:1 and 1:3 with the same betamethasone-free plasma (No. 150) that was used in the preparation of the standard curve. The estimated steroid concentrations at each dilution were transformed to the logarithms $\log_{10}x$, $(\log_{10}x)+ 0.301$ and $(\log_{10}x)+ 0.602$, respectively. An analysis of variance was performed on the transformed data and showed no significant deviation from parallelism.

## 2.6 GLUCOSE ANALYSIS

Plasma glucose estimations were made by the glucose oxidase-peroxidase method of Rosevear *et al* (1969). The technique is a colourimetric one utilising an AutoAnalyzer (Technicon Corporation, USA).

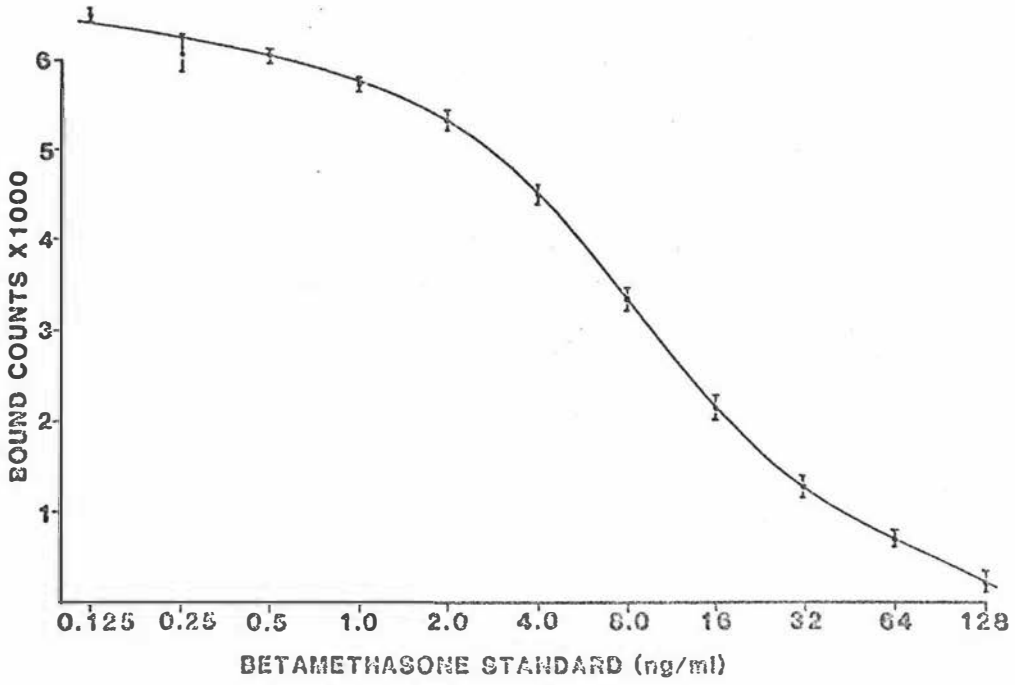


Figure 3:2 Representative standard curve for the betamethasone assay, computed by the method of Burger *et al* (1972).

Table 3:IV Specificity of antiserum to betamethasone-CMO-thyroglobulin: cross reaction with other steroids (Bradley *et al*, pers. com.)

| Steroid | Cross reaction (%) |
|---|---|
| Cortisol | < 1% |
| Corticosterone | < 1% |
| Testosterone | < 1% |
| Dexamethasone | 4.4% |
| 11-Dehydrobetamethasone | 12.0% |
| 6β-hydroxybetamethasone | 3.9% |
| Betamethasone sodium phosphate[a] | 0.05% |

[a] The sodium salt of betamethasone 21-phosphate does not hydrolyse in tricine buffer. Thus the 21-ester does not cross react with the betamethasone antiserum.

Table 3:V Betamethasone assay: between assay coefficients of variation

| Plasma pool | No. assays | Mean betamethasone (ng/ml) | C.V.(%) |
|---|---|---|---|
| 83 | 27 | 5.68 | 16.4 |
| 100 | 27 | 14.07 | 9.3 |

Table 3:VI Betamethasone assay: within assay coefficients of variation

| Plasma pool | No. replicates | Mean betamethasone (ng/ml) | C.V.(%) |
|---|---|---|---|
| 83 | 10 | 6.26 | 6.3 |
| 100 | 10 | 14.37 | 9.2 |
| 2 | 5 | 1.82 | 17.4 |
| 3 | 5 | 4.60 | 10.3 |

## 2.7 HAEMATOLOGY

Total white cell counts were made within one hour of collection. A haemocytometer counting chamber was used. Differential white cell counts were made by counting and classifying 200 leucocytes on air-dried smears stained with May-Grunwald-Giesma stain (Benjamin, 1961).

## 2.8 STATISTICAL ANALYSIS

The concept of statistical significance is based on estimates of the probability, P, of a particular occurrence being due to the operation of chance factors. By convention, a probability of 5 percent ($P = 0.05$) is described as 'significant'. Throughout this study levels of significance are denoted thus;

| | | |
|---|---|---|
| * | $P < 0.05$ | 'significant' |
| ** | $P < 0.01$ | 'highly significant' |
| *** | $P < 0.001$ | 'very highly significant' |

Analyses of variance were applied to steroid and glucose concentrations. In these analyses the 'main effects' of treatment and sampling day, and their interactions, were partitioned using orthogonal coefficients (Cochran & Cox, 1960) taken from the tables of Fisher and Yates (1963). For plasma glucose concentrations a non-orthogonal comparison was also made between Group I and Group II over the sampling period from day 9 to 19.

In three instances where samples were not available the missing data were substituted by the mean of the preceding and succeeding measurements.

All estimates of steroid concentration were transformed to logarithms prior to statistical analyses (Bliss, 1967). The following relationship was used:

$$\text{transformed steroid concentration} = \log_{10}(x + 1.1)$$

where x is the steroid concentration in ng/ml. This relationship had been established previously on empirical grounds (Barrell, 1976; Wilson, 1977) by the finding of linear relationships between the estimated mean and its standard error for subgroups of non-transformed steroid data.

For each group the mean concentration of each steroid and its ninety-five percent confidence interval ($CL_{95}$) (Bliss, 1967; Green, 1972) was calculated from log-transformed data. For cortisol, the $CL_{95}$ was based on a pooled variance (Bliss, 1967) estimated over all sampling days in all groups. The $CL_{95}$ for each betamethasone mean concentration was based on a within-group pooled variance as one group did not receive betamethasone.

The pooled variance (S2) from which $CL_{95}$s were calculated was derived as follows:

$$S^2 = \frac{\sum_{d.n} \left( \sum_{N} X^2 - \frac{\left( \sum_{N} X \right)^2}{N} \right)}{[d.n.(N-1)] - m}$$

where X = individual log-transformed data point,
N = number of individuals per group,
n = number of treatment groups,
d = number of sampling days,
m = number of missing data points.

Mean variances ($S^2_{\bar{x}}$) were then derived:

$$S^2_{\bar{x}} = \frac{S^2}{N}$$

or $$S^2_{\bar{x}} = \frac{S^2}{N-m}$$ for those means based on data which include a missing data point.

From this mean variance $CL_{95}$s were calculated:

$$CL_{95} = \bar{X} \pm t.\ S_{\bar{x}}$$

using $t$ for [d.n.(N-1)] -m degrees of freedom.

Mean plasma glucose concentrations were calculated from non-transformed data and a pooled variance was used to estimate the $CL_{95}$s of each mean.

The $CL_{95}$s, calculated for each mean, provided estimates of the probability of a repeat experiment resulting in means of different magnitude. The probability that repetitions of the

experiment would produce mean values outside the range delineated by the $CL_{95}$s is thus 1 in 20. Where the $CL_{95}$s of two treatment groups did not overlap the probability that the mean values for those two groups were different is therefore greater than 95 percent ($P < 0.05$) and the difference can be described as significant.

White blood cell counts were compared on days 0,1,2,9 and 19 using Students' *t* test (Bliss, 1967). A comparison Group I + II *versus* Group III was made, followed by a comparison between Group I and Group II.

## 3. RESULTS

### 3.1 BETAMETHASONE CONCENTRATIONS

Marked differences in mean peak plasma levels of betamethasone produced by suspensions of different concentration were recorded; 4.70 ng/ml and 1.78 ng/ml in Groups I and II respectively. However, differences in duration of detectable plasma levels were not so apparent (Fig. 3:3). In both treatment groups the mean peak concentration occurred on the day after injection. By day 4 the mean betamethasone concentration in plasma for each group had fallen to levels not significantly different from that of the control group. However, on day 5 the mean plasma concentration in Group II was again significantly different from the control (Fig. 3:3). The analysis of variance of betamethasone concentrations in Group I and II indicated highly significant differences in peak levels and in their rate of decline (Table 3:VII).

The rate of absorption of betamethasone alcohol suspensions was variable; particularly that of the more concentrated formulation. While two cows in Group I did not experience peak levels until day 2, 4 cows in Group II did not experience peak concentrations until day 3. Within each treatment group, peak plasma concentrations of betamethasone varied widely. In Group I (mean peak concentration, 4.70 ng/ml) individual concentrations ranged from 2.25 to 11.92 ng/ml and in Group II (mean, 1.78 ng/ml) the range was 0.52 to 5.87 ng/ml.

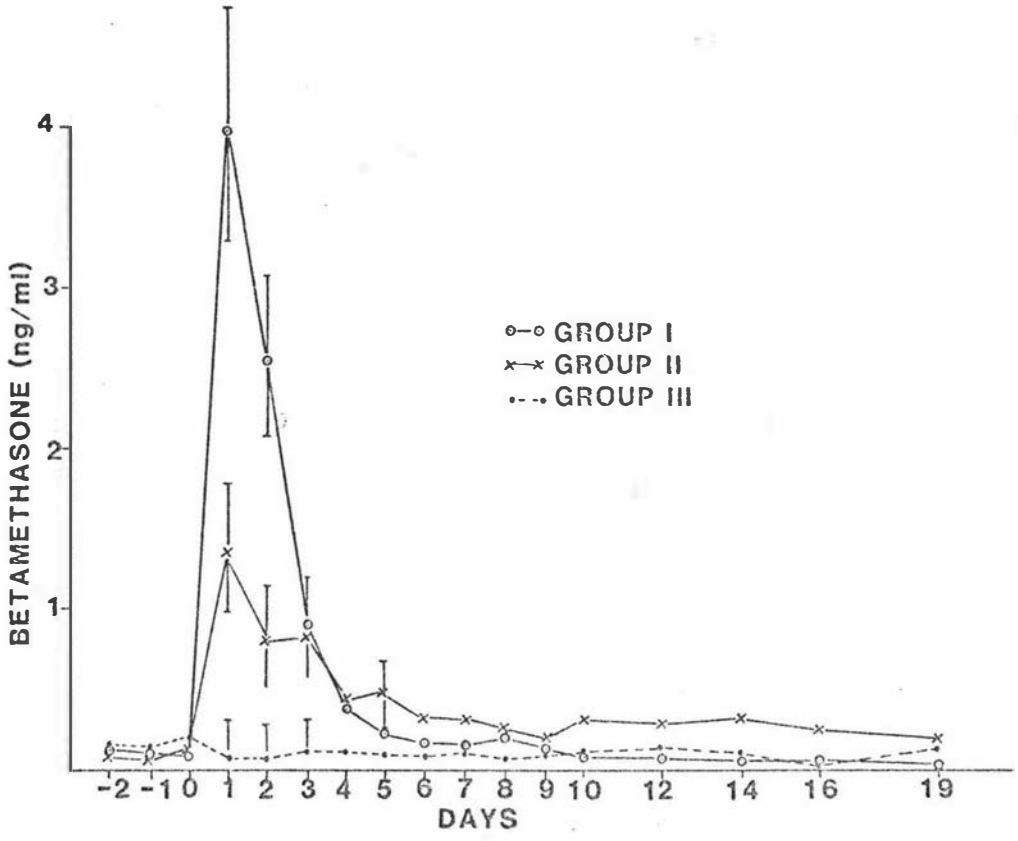


Figure 3:3 Plasma betamethasone concentrations (means and $CL_{95}$s) in cows following administration of two different formulations at a dose rate of 40 mg/cow.
Group I; 2 mg/ml suspension. Group II: 20 mg/ml suspension.
Group III; saline-treated control group.

Table 3:VII Summary of the analysis of variance of betamethasone concentrations in the plasma of cows treated with two different formulations.

| | Source of variation | Degrees of freedom | 'F' |
|---|---|---|---|
| A. | Treatment I *vs* Treatment II | 1 | $9.99 \times 10^{-4}$ |
| B. | Sampling days | 13 | |
| | post-treatment - linear | 1 | 263.27*** |
| | post-treatment - quadratic | 1 | 112.67*** |
| | post-treatment - cubic | 1 | 34.31*** |
| | remainder | 10 | 0.38 |
| C. | Interaction A x B | 13 | |
| | Treatment I *vs* Treatment II x post-linear | 1 | 44.30*** |
| | Treatment I *vs* Treatment II x post-quadratic | 1 | 28.36*** |
| | Treatment I *vs* Treatment II x post-cubic | 1 | 8.56** |
| | remainder | 10 | 1.04 |
| D. | Residual mean square | 252 | 0.01199313 |

In some of the pre-treatment and control group samples a 'blank' effect was apparent. That is, in some instances animals not treated with betamethasone apparently had low concentrations of this steroid present in their plasma. In most cases the estimated concentration of this betamethasone 'blank' was below the sensitivity limit of the assay. However, one cow in the control group had a 'betamethasone' concentration of between 0.44 and 0.73 ng/ml on 14 of the 22 sampling days.

## 3.2. CORTISOL CONCENTRATIONS

There was a wide range of cortisol concentrations recorded in individual cows prior to treatment with betamethasone, as well as in cows in the untreated control group (Table 3:VIII). Immediately before treatment, individual cortisol concentrations ranging from 0.19 to 44.10 ng/ml were recorded. An examination of the $CL_{95}$s for the mean plasma concentrations of the untreated cows showed signficant differences between individuals over the 22 day period of the experiment. One cow, No. 56, had a particularly low concentration of cortisol throughout.

Table 3:VIII Early-morning[a] plasma cortisol levels in untreated cows over a twenty-two day period.

| Cow no. | Mean cortisol (ng/ml) | Upper $CL_{95}$[b] - Lower $CL_{95}$ |
|---|---|---|
| 27 | 15.85 | 21.62 - 11.55 |
| 52 | 5.41 | 6.84 - 4.23 |
| 54 | 8.28 | 11.34 - 5.97 |
| 56 | 0.27 | 0.30 - 0.23 |
| 67 | 7.67 | 10.58 - 5.49 |
| 70 | 9.78 | 13.68 - 6.92 |
| 75 | 1.75 | 2.92 - 0.91 |
| 88 | 7.85 | 11.46 - 5.29 |
| 93 | 1.86 | 2.54 - 1.31 |
| 98 | 10.98 | 13.86 - 8.66 |
| Group mean | 5.34 | 9.10 - 2.96 |

a. Samples collected between 6.00 - 7.30 am.

b. Based on *individual* variances.

Administration of either betamethasone formulation caused a profound and long-lasting depression of early-morning cortisol levels. Twenty-four hours after treatment mean cortisol concentrations of 0.76 and 0.78 ng/ml were recorded in Groups I and II respectively. Individual cows had cortisol concentrations ranging from zero to 1.23 ng/ml at that time. Those cows which had received the 2 mg/ml suspension showed some recovery after 12 days (mean concentration, 2.04 ng/ml), whilst the cortisol levels in those cows treated with the more slowly absorbed 20 mg/ml suspension were still maximally depressed after 19 days (mean concentration, 0.54 ng/ml) (Fig. 3:4).

After treatment there was a highly significant difference in mean cortisol profiles, as indicated in the summary of the analysis of variance (Table 3:IX) by the interaction between treatments and sampling days. Graphically, this is shown (Fig. 3:4) as an elevation of mean cortisol concentration recorded for Group I after day 10. On days 12, 16 and 19 the mean plasma cortisol concentrations of Group I and the control group (Group III) were not significantly different. Mean cortisol concentrations in Group II showed no signs of returning toward their pre-treatment level within the experimental period. One cow in Group II had detectable cortisol recorded on only one day post-treatment.

## 3.3 GLUCOSE CONCENTRATIONS

Betamethasone treatment caused a highly significant elevation in mean plasma glucose concentration (Fig. 3:5, Table 3:X). The greatest elevation was recorded in those cows which were treated with the low concentration suspension, while the high concentration formulation exerted a more prolonged effect. In Groups I and II respectively, the maximum mean glucose concentrations were 84.1 percent and 47.6 percent above mean pre-treatment values. Differences in peak glucose concentrations were highly significant, as were the rates at which plasma glucose concentration declined (Table 3:X).

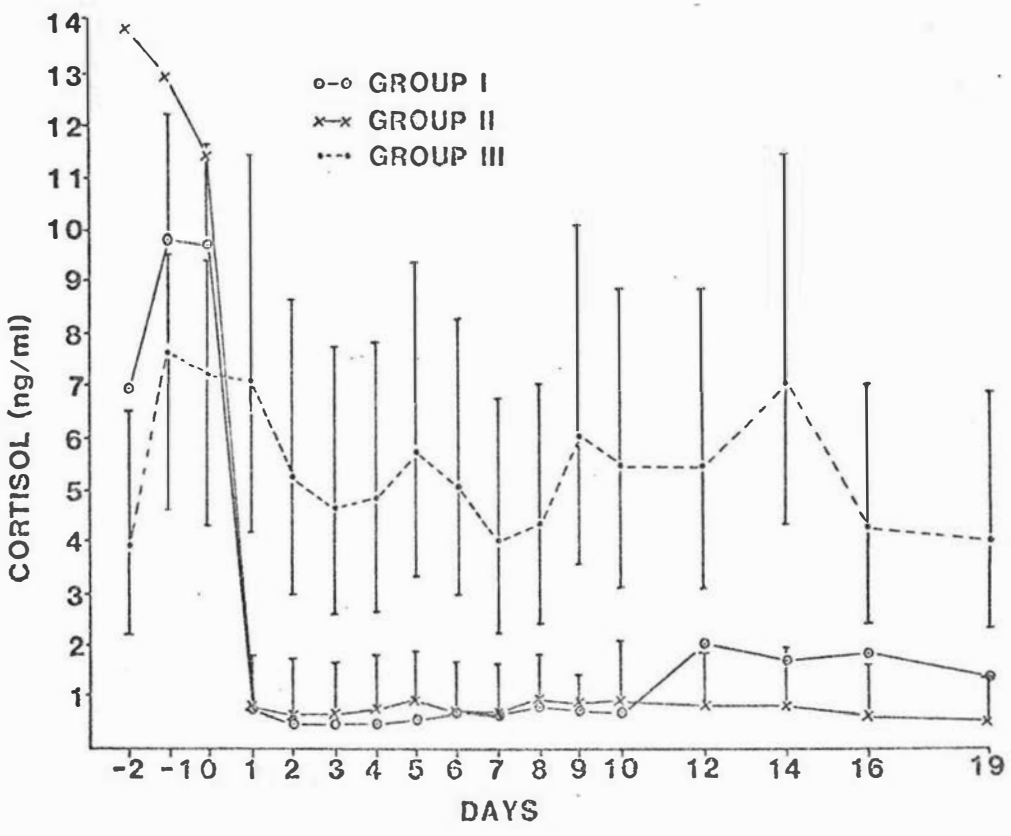


Figure 3:4 Early morning plasma cortisol concentrations (means and $CL_{95}$s) in cows before and after administration of two betamethasone formulations at a dose rate of 40 mg/cow. Group I; 2 mg/ml suspension. Group II; 20 mg/ml suspension. Group III; saline-treated control group.

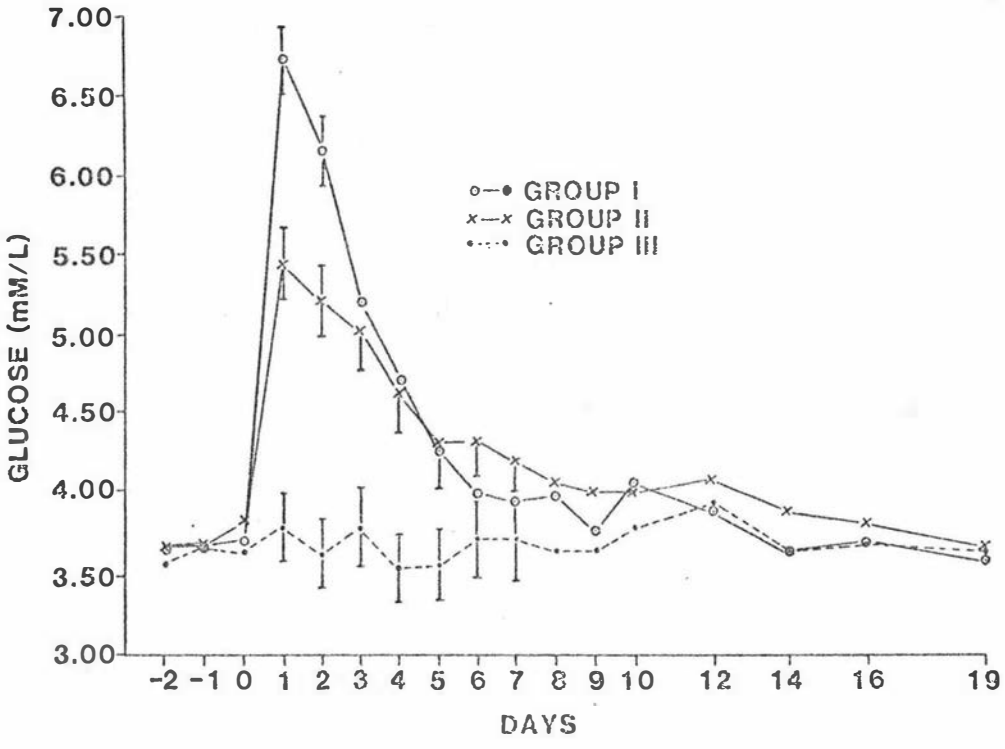


Figure 3:5 Plasma glucose concentrations (means and $CL_{95}$s) in cows treated with two betamethasone formulations at a dose rate of 40 mg/cow. Group I; 2 mg/ml suspension. Group II; 20 mg/ml suspension. Group III; saline-treated control group.

Table 3:IX Summary of the analysis of variance of cortisol concentrations in the plasma of cows treated with two betamethasone formulations.

| | Source of variation | Degrees of freedom | 'F' |
|---|---|---|---|
| A. | Treatments | 2 | |
| | control *vs* treated | 1 | 192.70*** |
| | Treatment I *vs* Treatment II | 1 | 0.04 |
| B. | Sampling days | 16 | |
| | pre - *vs* post-treatment | 1 | 244.84*** |
| | remainder | 15 | 0.71 |
| C. | Interaction A x B | 32 | |
| | control *vs* treated x pre - *vs* post - | 1 | 98.63*** |
| | control *vs* treated x post-linear | 1 | 3.86[a] |
| | Treatment I *vs* Treatment II x pre - *vs* post- | 1 | 4.83* |
| | Treatment I *vs* Treatment II x post-linear | 1 | 8.32** |
| | remainder | 28 | 0.60 |
| D. | Residual mean square | 459 | 0.08750 |

[a]. Just fails to reach significance (P = 0.05, F = 3.92).

Table 3:X Summary of the analysis of variance of plasma glucose concentrations in cows treated with two betamethasone formulations.

| | Source of variation | Degrees of freedom | 'F' |
|---|---|---|---|
| A. | Treatments | 2 | |
| | control *vs* treated | 1 | 260.29*** |
| | Treatment I *vs* Treatment II | 1 | 1.79 |
| B. | Sampling days | 16 | |
| | pre- *vs* post-treatment | 1 | 126.01*** |
| | post-linear | 1 | 536.81*** |
| | post-quadratic | 1 | 124.13*** |
| | post-cubic | 1 | 42.26*** |
| | remainder | 12 | 1.31 |
| C. | Interaction A x B | 32 | |
| | control *vs* treated x pre- *vs* post- | 1 | 45.74*** |
| | control *vs* treated x post-linear | 1 | 280.04*** |
| | control *vs* treated x post-cubic | 1 | 54.06*** |
| | control *vs* treated x post-quadratic | 1 | 5.92* |
| | Treatment I *vs* Treatment II x post-linear | 1 | 44.05*** |
| | Treatment I *vs* Treatment II x post-cubic | 1 | 40.36*** |
| | Treatment I *vs* Treatment II x post-quadratic | 1 | 1.34** |
| | remainder | 25 | 0.62 |
| D. | Non-orthogonal comparison | | |
| | Treatment I *vs* Treatment II x Days 9 - 19 | 1 | 7.83** |
| E. | Residual mean square | 459 | 0.13899 |

### 3.4 HAEMATOLOGY

Betamethasone treatment exerted significant effects on differential white blood cell counts (Fig. 3:5). On days 1 and 2 there was a very highly significant difference in neutrophil numbers between treated and untreated cows, and a significant difference ($P < 0.02$) in neutrophil numbers between Groups I and II. Highly significant differences in numbers of circulating eosinophils were recorded between treated and control cows on days 1, 2 and 9, but significant differences were not recorded between Groups I and II. Lymphocyte counts were not significantly different, although on day 1 the difference between treated and untreated animals approached significance ($0.05 < P < 0.1$).

While neutrophil numbers were elevated to a greater degree in the group receiving the 2 mg/ml suspension, they remained elevated for a longer period of time (approximately three days longer) in the group treated with the 20 mg/ml suspension.

## 4. DISCUSSION

This first experiment confirmed the observation of P.G. Box that the rate at which an injected suspension of betamethasone alcohol is absorbed is dependent on the volume in which the dose is administered.

When 40 mg of betamethasone alcohol was administered subcutaneously to cows, the greater peak plasma concentration was clearly produced by the suspension with the lower concentration (2 mg/ml). The results also demonstrated that the length of time for which betamethasone was detectable in plasma was influenced by the concentration of the injected suspension, with the more concentrated formulation (20 mg/ml) producing detectable plasma concentrations for at least two days longer than the dilute suspension. The absorption of the more concentrated suspension was, however, more variable, as reflected in the range of times taken for peak plasma concentrations of betamethasone to be reached in individual cows. The mean peak plasma concentration

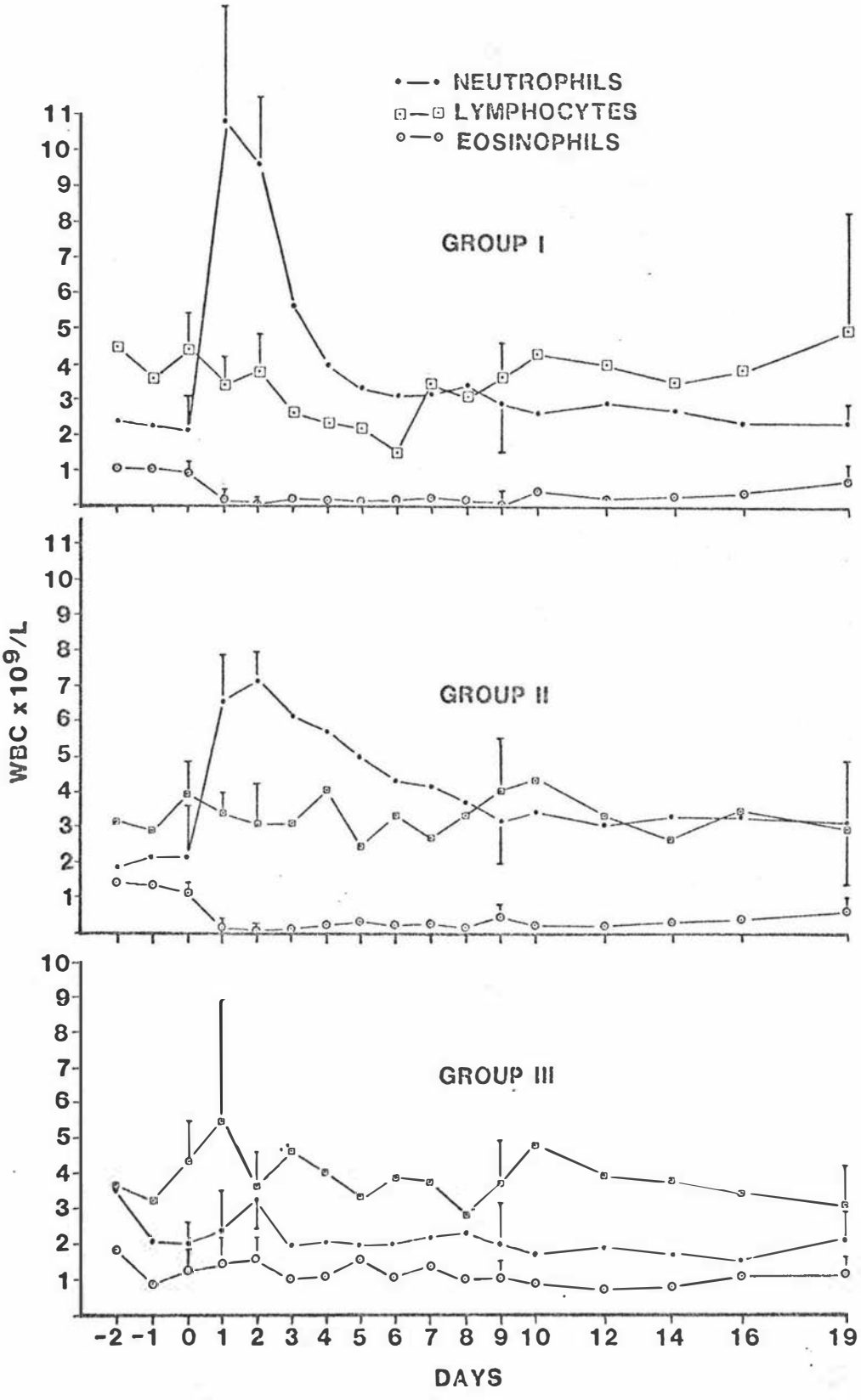


Figure 3:6 Differential white blood cell counts (means ± SD) in cows treated with two betamethasone formulations at a dose rate of 40 mg/cow. Group I; 2 mg/ml suspension. Group II; 20 mg/ml suspension. Group III; saline-treated control group.

of betamethasone in Group II would have been considerably higher had all cows within that group experienced their peak plasma concentration on the same day.

This effect of concentration on the rate of absorption of an injected suspension, an effect of a high solids:vehicle ratio, has been demonstrated for other poorly-soluble drugs (Chapter II; Section 4.4).

That the differences in the plasma betamethasone profiles produced difference in *effects* is demonstrated by the results of the cortisol assays, the plasma glucose analyses and the haematology results.

While the magnitude of the cortisol depression produced by each formulation was the same, that recorded in the group treated with the more rapidly absorbed 2 mg/ml suspension persisted for a much shorter time. Other workers (Kanchev *et al*, 1976) have reported that heifers treated with the same formulation had their endogenous cortisol levels depressed for 'at least 16 days'; the duration of their study. Their results are not comparable to those reported here, however, as in their trial betamethasone was administered daily for nine days.

The prolonged depression of cortisol recorded in the group treated with the slowly absorbed 20 mg/ml suspension is comparable to that seen by Fairclough, Hunter and Welch (1981) in a cow treated with a long-acting dexamethasone formulation. Following the injection of 20 mg of dexamethasone trimethylacetate suspension these workers noted that endogenous cortisol secretion was still depressed 22 days after treatment. Similarly Mollman and co-workers (1977) demonstrated that in humans betamethasone formulations which are slowly absorbed depress endogenous cortisol concentrations for a longer period than do more rapidly absorbed formulations.

In cattle, cortisol concentrations are usually highest in the early morning (Wagner & Oxenreider, 1972; MacAdam & Eberhart, 1972; Fulkerson *et al*, 1980) although in some individuals cortisol levels may peak at other times of the day (Welsh *et al*,

1979). Fulkerson and co-workers (1980) noted that within an individual cow, cortisol levels may alter by as much as 30 ng/ml several times in the course of a single day. For these reasons, Fulkerson and colleagues (1980) cautioned against establishing a basal mean cortisol concentration from samples drawn once daily and against comparing mean concentrations established by such methods in different studies. Even so, the early morning mean plasma cortisol concentrations recorded in the present experiment (Table 3:VIII) are similar to those reported by other workers using comparable assay methods (Wagner & Oxenreider, 1972; MacAdam & Eberhart, 1972; Welsh *et al*, 1979; Fulkerson *et al*, 1980, Breves *et al*, 1980). Breves and co-workers (1980) studied early morning cortisol concentrations in a group of 20 cows for several weeks before and after calving: mean cortisol concentrations for individual cows ranged from 0 to 13 ng/ml.

It is well recognised that the intensity and duration of action of steroids is influenced by their rate of absorption (Junkmann, 1957; Van der Vies, 1965; 1975; Woollett & Evans, 1971; Brotherton, 1976) and this point is illustrated by the plasma glucose concentrations recorded in the present study. The elevation of glucose levels reflects, both in magnitude and duration, the mean plasma levels of betamethasone in the two treatment groups.

Several workers have shown that in cattle rapidly absorbed solutions of corticosteroids produce a more rapid and more pronounced elevation of plasma glucose than do suspensions of the same, or comparable, corticosteroids (Burns, 1963; Stockl *et al*, 1969; Woollett & Evans, 1971; Schillinger & Klee, 1979). However, the duration for which plasma glucose is elevated is longer following administration of suspensions, which are absorbed over a longer period of time. These findings are complemented by those of the present study in which the more slowly absorbed suspension of betamethasone produced a more prolonged, if less intense, elevation of plasma glucose than the more rapidly absorbed suspension.

The 84% elevation in plasma glucose concentration produced in the present study by the 2 mg/ml suspension is almost identical

to that reported by Woollett and Evans (1971) following administration of a similar dose of the same formulation.

The intensity and duration of effect of each betamethasone suspension on circulating neutrophil numbers again reflected their differences in absorption rate. The magnitude and duration of this elevation in the group receiving the 2 mg/ml suspension is very similar to that reported by others using this suspension at the lower dose rate of 0.05 mg/kg (Anon., 1977). No clear effect on lymphocyte numbers was noted in the present experiment, although in those cows treated with the dilute suspension, numbers slowly declined over several days. This observation is different from that of other workers (Anon., 1977) who have noted that betamethasone caused a marked depression in numbers of lymphocytes within 24 hours. Mean pretreatment lymphocyte numbers in that earlier study though were about twice as high as those seen here, and this may have contributed to the differences in observed effects. In both studies, pretreatment lymphocyte numbers were within the normal range (Anon., 1975).

Goetsch and colleagues (1959) reported that while another synthetic corticosteroid, 9 α-fluoroprednisolone, caused an elevation in numbers of circulating neutrophils in cattle, it was without effect on lymphocyte numbers. Schalm (1972) on the other hand, reported that 9 α - fluoroprednisolone caused lymphocyte numbers to decline over a period of 30 to 60 hours.

In this experiment an attempt has been made to relate plasma profiles of betamethasone to the intensity and duration of effect of two different suspensions of betamethasone alcohol. Depression of endogenous cortisol levels does not appear to be a good indicator of duration of action, as with the concentrated suspension especially, cortisol levels were still maximally depressed long after plasma betamethasone fell below detectable levels. Plasma glucose levels and circulating neutrophil numbers seemed to be the parameters which best reflected the differences in betamethasone profiles produced by the two different suspensions.

# CHAPTER IV

## THE DISPOSITION OF CERTAIN BETAMETHASONE FORMULATIONS ADMINISTERED TO CATTLE BY DIFFERENT PARENTERAL ROUTES

1. INTRODUCTION

2. MATERIALS AND METHODS
   - 2.1 Animals
   - 2.2 Treatments
   - 2.3 Calculation of diposition kinetics

3. RESULTS

4. DISCUSSION

5. CONCLUSIONS

# CHAPTER IV

## THE DISPOSITION OF CERTAIN BETAMETHASONE FORMULATIONS ADMINISTERED TO CATTLE BY DIFFERENT PARENTERAL ROUTES

## 1. INTRODUCTION

The experiment described in the previous chapter clearly demonstrated that following subcutaneous injection, the rate of absorption of a given dose of betamethasone alcohol is governed by the concentration, or solids:vehicle ratio, of the administered suspension. It is recognised that the route by which a parenteral formulation is administered may influence the rate of absorption (Chapter II, Section 3.1), so an experiment was planned in which two suspensions of betamethasone alcohol would be administered to cattle by three different parenteral routes and the resulting plasma profiles compared with those produced following the administration of a water-soluble ester of betamethasone. The data derived from these studies would enable disposition kinetics of betamethasone in cattle to be defined.

## 2. MATERIALS AND METHODS

### 2.1 ANIMALS

Nine clinically normal, non-lactating, non-pregnant, Friesian cows were used. All were 3 or 4 years old and in good condition. They were grazed as a single mob on good quality pasture and hay was offered *ad libitum* whenever the cows were brought into the yards.

Body weights were recorded prior to the first intravenous injection. As it was not possible to weigh animals subsequently, a weigh-band was used prior to subsequent treatments to check for any marked variations in body weights.

### 2.2. TREATMENTS

Three formulations of betamethasone were used;

I. Betamethasone sodium phosphate solution, 4 mg/ml(1)

II. Betamethasone alcohol suspension, 2 mg/ml(2)

III. Betamethasone alcohol suspension, 20 mg/ml(3)

All treatments were administered at the rate of 0.1 mg/kg body weight. Three cows were allocated to each treatment group, and during the course of the experiment each group received its allocated formulation by the intravenous, the intramuscular and the subcutaneous route. The timing of these treatments is shown in Table 4:I.

Subsequently, the second treatment group (II), received an intravenous injection of betamethasone sodium phosphate solution.

Table 4:I Days from commencement of experiment on which different betamethasone formulations were administered to cows.

| | Treatment | | |
|---|---|---|---|
| Route of administration | I | II | III |
| I V | 3 | 2 | 1 |
| I M | 15 | 13 | 9 |
| S C | 29 | 28 | 52 |
| | | 48[a] | |

I. betamethasone solution

II. 2 mg/ml suspension

III. 20 mg/ml suspension

a Supplementary treatment, intravenous solution

Intravenous injections were made into the jugular vein, subcutaneous injections were made in that part of the neck which was covered when the left ear was laid back flat and intramuscular injections were made with a 2.5 cm needle inserted to its full length into the cranial portion of the biceps femoris muscle.

Following injection, blood samples were collected at various intervals, the frequency of which differed for each treatment.

(1) Betnesol Injection, Glaxo Laboratories Ltd., Greenford, England.
(2) Betsolan Suspension, Glaxo New Zealand Ltd., Palmerston North.
(3) EPHE/1/4, experimental suspension, Glaxo New Zealand Ltd., Palmerston North.

Cows treated with betamethasone sodium phosphate solution (Group I) had samples drawn at 0.25, 0.5, 0.75, 1 and 1.5 hours after injection, then at hourly intervals until 10 hours. After the administration of a supplementary intravenous injection of the solution to cows in Group II, samples were also drawn at 16 hours, 24 hours, and then hourly until 31 hours post-injection. In most cases following extravascular injection, sampling commenced at 0.5 hours post-injection and continued, in some cases, for up to 80 hours.

Plasma was separated and stored prior to betamethasone assay as described previously (Chapter III, Sections 2.3 and 2.5).

## 2.3 CALCULATION OF DISPOSITION KINETICS

Following intravenous injection, disposition curve data from individual cows were analysed according to a single compartment open model (Ritschel, 1976; Baggot, 1977) described by the equation;

$$Cp = Be^{-\beta t} \qquad \text{(Equation 4:1)}$$

where Cp = the plasma concentration at time t.

B = the zero time plasma drug concentration intercept of the terminal phase of the exponential disposition curve.

e = the base of the natural logarithm (ln).

$\beta$ = the apparent first-order disappearance rate constant obtained from the terminal slope of a semilogarithmic plot of plasma drug concentration *versus* time (Fig. 4:1).

Following transformation of plasma concentration values to logarithms, iterative least-squares linear regression lines were used to derive the values for B and $\beta$ which best fitted the terminal exponential phase of the disposition curve. The overall elimination rate constant, $\beta$, was calculated from;

$$\beta = -b \times 2.303 \qquad \text{(Equation 4:2)}$$

where b is the slope of the least-squares linear regression line describing the disappearance of drug from the plasma (Baggot, 1977). From the rate constant $\beta$ was calculated the plasma half-life ($t_{\frac{1}{2}}$ or $t_{\frac{1}{2}\beta}$) of betamethasone according to the formula (Baggot, 1977);

$$t_{\frac{1}{2}} = \frac{\ln 2}{\beta} \qquad \text{(Equation 4:3)}$$

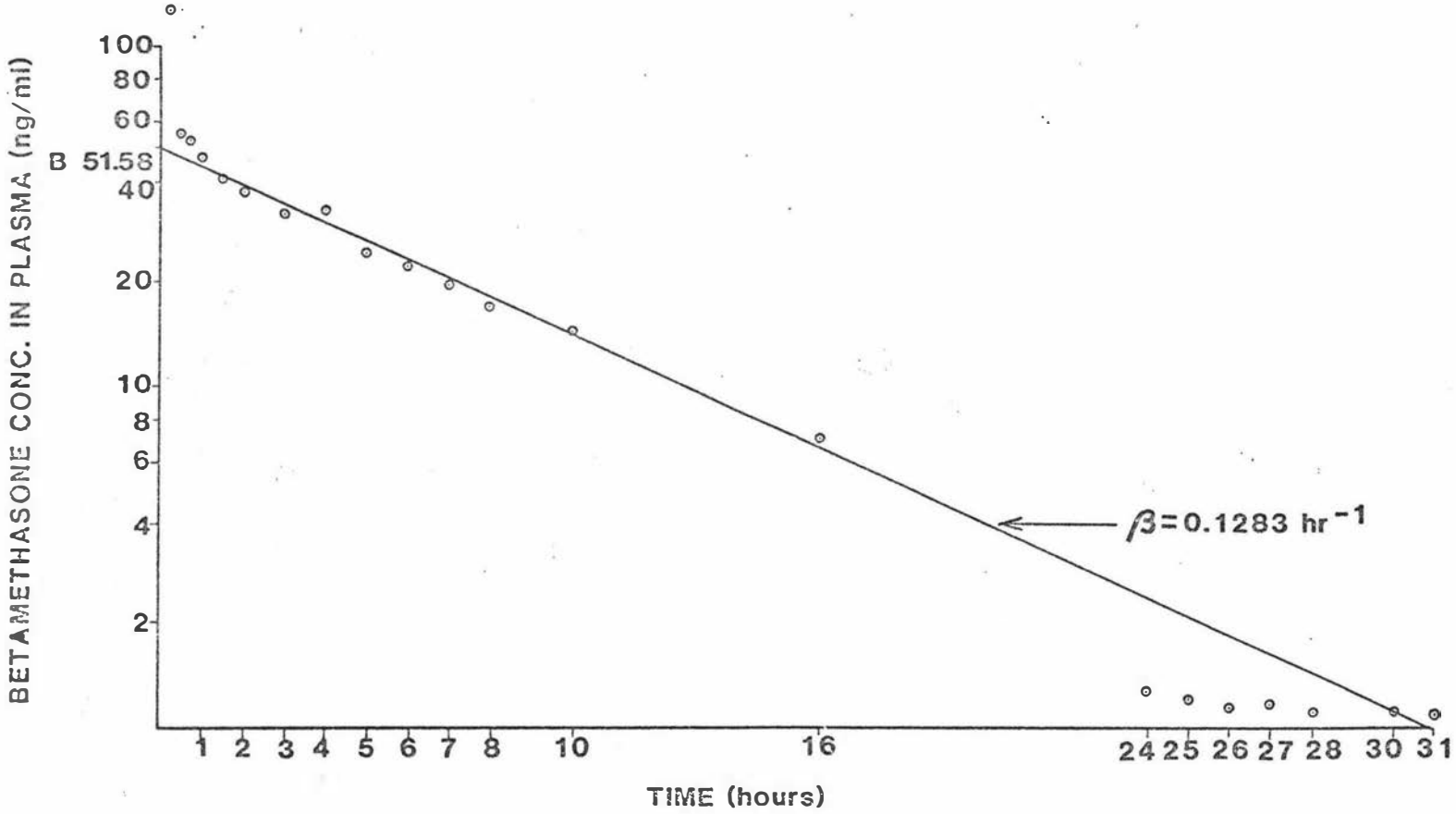


Figure 4:1 Semilogarithmic graph depicting plasma concentrations of betamethasone after intravenous injection of the sodium phosphate ester at a dose rate of 0.1 mg/kg to a single cow (no. 128).

The zero-time concentration intercept, B, was used to calculate the apparent specific volume of distribution (V'd) (Baggot, 1977);

$$V'd = \frac{Dose}{B} \qquad \text{(Equation 4:4)}$$

The body clearance of betamethasone, defined as the volume of blood cleared of the drug by the various elimination processes per unit time (Baggot, 1977), was calculated from the formula

$$Cl_B = \beta.Vd(area) \qquad \text{(Equation 4:5)}$$

where $$Vd(area) = \frac{Dose}{(Area).\beta} \qquad \text{(Equation 4:6)}$$

and 'Area' is area under the disposition curve (Equation 4:11).

Body clearance is expressed in units $ml.kg^{-1}.hr^{-1}$.

Because of the instantaneous dilution in circulating blood, the differences in concentration between the two suspensions of bethamethasone alcohol could not be expected to exert any influence on disposition following intravenous injection. Therefore, the data obtained following administration of the suspensions by this route were pooled.

Where appropriate, the disposition curve data obtained following extravascular injection were analysed according to the single compartment open model described by the equation (Ritschel, 1976);

$$Cp = B'e^{-\beta't} - B''e^{-K_{ab}t} \qquad \text{(Equation 4:7)}$$

where B' = the zero time plasma drug concentration intercept of the terminal phase of the exponential disposition curve.

$\beta'$ = the apparent first-order disappearance rate constant obtained from the slope of the terminal phase of the disposition curve.

B'' = the zero time concentration intercept of the exponential absorption curve (equivalent to 'A' in Ritschel's nomenclature).

$K_{ab}$ = an apparent first-order absorption rate constant obtained from the slope of the absorption curve.

Following extravascular administration the regression line describing the absorption phase was obtained by the method of

residuals (Ritschel, 1976). Actual plasma concentration values were subtracted from points lying on the extrapolated exponential line which describes the elimination phase. A least-squares linear regression line was then placed through the 'residual' points (Fig. 4:2)(5)

From the absorption phase regression line was calculated the absorption rate constant, $K_{ab}$;

$$K_{ab} = -b'' \times 2.303 \qquad \text{(Equation 4:8)}$$

where b'' is the slope of that line. The absorption half-life, that is the time taken for half of the injected dose to be absorbed, is obtained from the equation;

$$t^{\frac{1}{2}}_{ab} = \frac{\ln 2}{K_{ab}} \qquad \text{(Equation 4:9)}$$

To determine the extent to which a formulation was systemically available following extravascular administration, the area under the plasma concentration curve (AUC) was compared with the AUC obtained after intravenous injection. The AUC was calculated by the trapezoidal method (Ritschel, 1976; Baggot, 1977) according to the formula;

$$AUC_{o \to tn} = (C_1 \times t_1) + \left[\frac{C_1 + C_2}{2} \times (t_2 - t_1)\right] + \left[\frac{C_2 + C_3}{2} \times (t_3 - t_2)\right] + \ldots$$

$$\left[\frac{C_{n-1} + C_n}{2} \times (t_n - t_{n-1})\right] \text{ ng/ml.hr} \qquad \text{(Equation 4:10)}$$

where $C_n$ = the plasma concentration after n time intervals t.

For this relative bioavailability study, AUC was calculated by this method from 0 to 24 hours only.

The area under the curve from 0 hours to infinity was calculated thus;

$$AUC_{o \to \infty} = AUC_{0 \to tn} + \frac{Cp(t^*)}{\beta'} \qquad \text{(Equation 4:11)}$$

where Cp(t*) is the last measured concentration of drug in the

(5) The intercept of this regression line with the ordinate should be equal to the intercept of the elimination phase (Baggot, 1977; Curry, 1977) but is not always so (Ritschel, 1976). (See discussion, page 96).

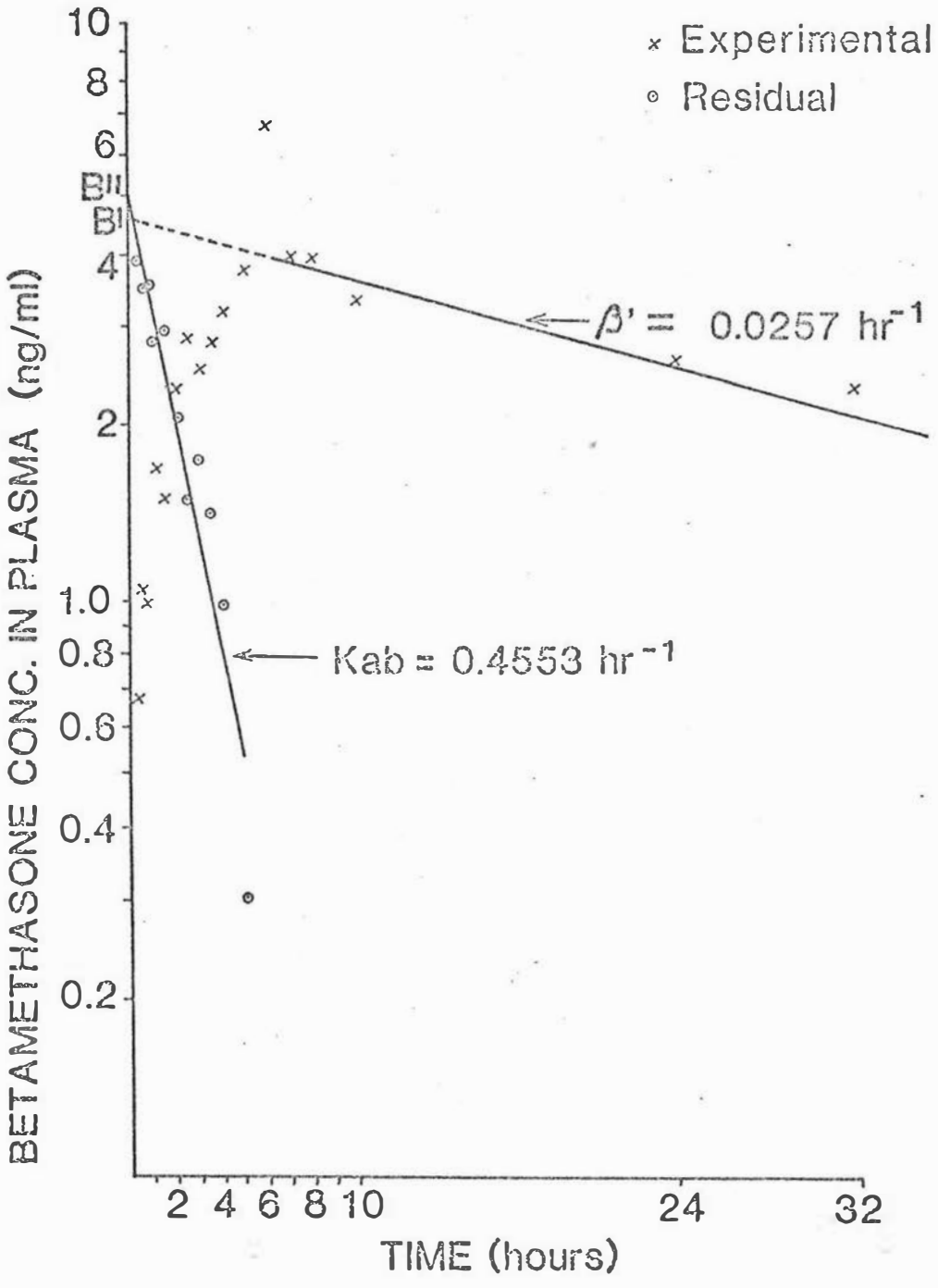


Figure 4:2 Semilogarithmic graph depicting the plasma concentration of betamethasone after intramuscular injection of the 2 mg/ml suspension at a dose rate of 0.1 mg/kg to a single cow (no. 128).

plasma and β' is the apparent overall elimination rate constant (Baggot, 1977).

In those instances where absorption following extravascular injection was extremely rapid, the rate of absorption could not be estimated from the data collected. In such instances an estimate of absorption half-life was made using the method of Swintosky, Dittert and Doluisio (1969). This method is only applicable to drugs in which the absorption rate is more than five times greater than the elimination rate, and assumes that when plasma concentration reaches its peak, absorption is 95% to 99% complete. The percentage of a drug remaining unabsorbed (that is 5% and 1%) is plotted against the time at which the plasma concentration reaches its maximum. The slope of lines joining these 5% and 1% points with the 100% unabsorbed point (that is, time zero) give estimates of the absorption half-life (Fig. 4:3).

## 3. RESULTS

For most of the duration of the experiment body weight, as estimated by weigh-band, remained remarkably constant. However, in those cows treated on day 48 and day 52, an average weight loss of about 7.5% was apparent. No adjustment was made in the pre-calculated dose administered to these cows.

Betamethasone sodium phosphate solution injections produced very similar plasma concentration curves regardless of the route of administration. Following intravenous, intramuscular or subcutaneous injection, the highest mean plasma concentration was recorded at the first sampling time of 0.25 hours post-injection (Fig. 4:4). Individual peak plasma concentrations were also recorded at this time except in two cows (No.s 41 and 62) in which the peak was recorded at 0.5 hours after subcutaneous injection.

After intravenous injection of betamethasone sodium phosphate solution, peak plasma levels at 0.25 hours ranged from 36.51 ng/ml to 128.77 ng/ml (mean, 64.28 ng/ml) (Table 4:II). By 10 hours concentrations ranged from 9.41 ng/ml to 20.27 ng/ml (Mean, 12.95 ng/ml), and in those 3 cows which were sampled up until 31 hours, concentrations between 1.04 and 2.17 ng/ml were recorded at that time.

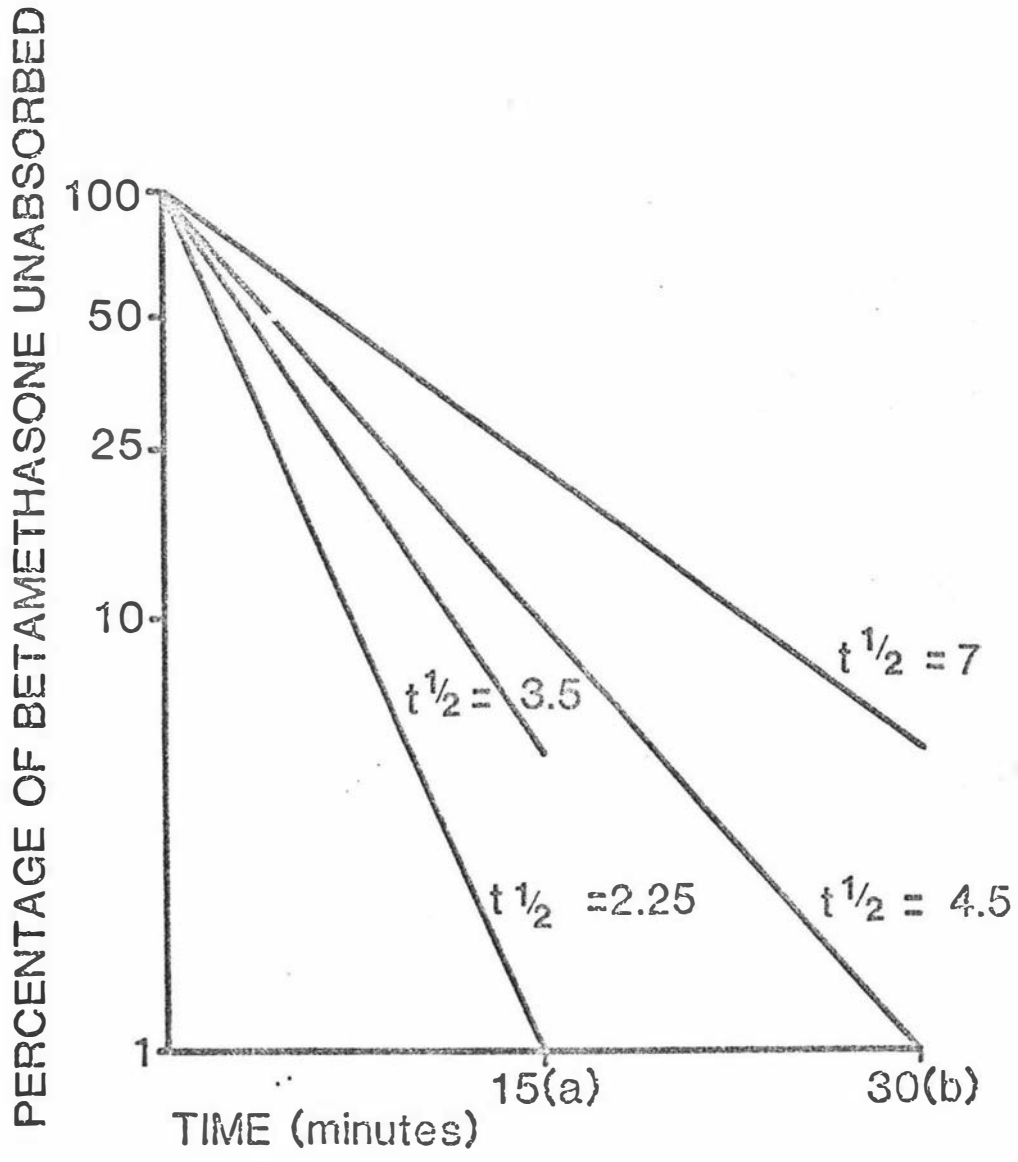


Figure 4:3 The absorption half-life of betamethasone sodium phosphate administered by extravascular injection to cattle. (Calculated by the method of Swintosky, Dittert & Doluisio, 1969).

a. The $t_{\frac{1}{2}ab}$ calculated at this point applied to all the intramuscular injections as well as to one subcutaneous injection (cow no. 34).

b. The $t_{\frac{1}{2}ab}$ calculated at this point applied to the subcutaneous injection in cows no. 41 and 62.

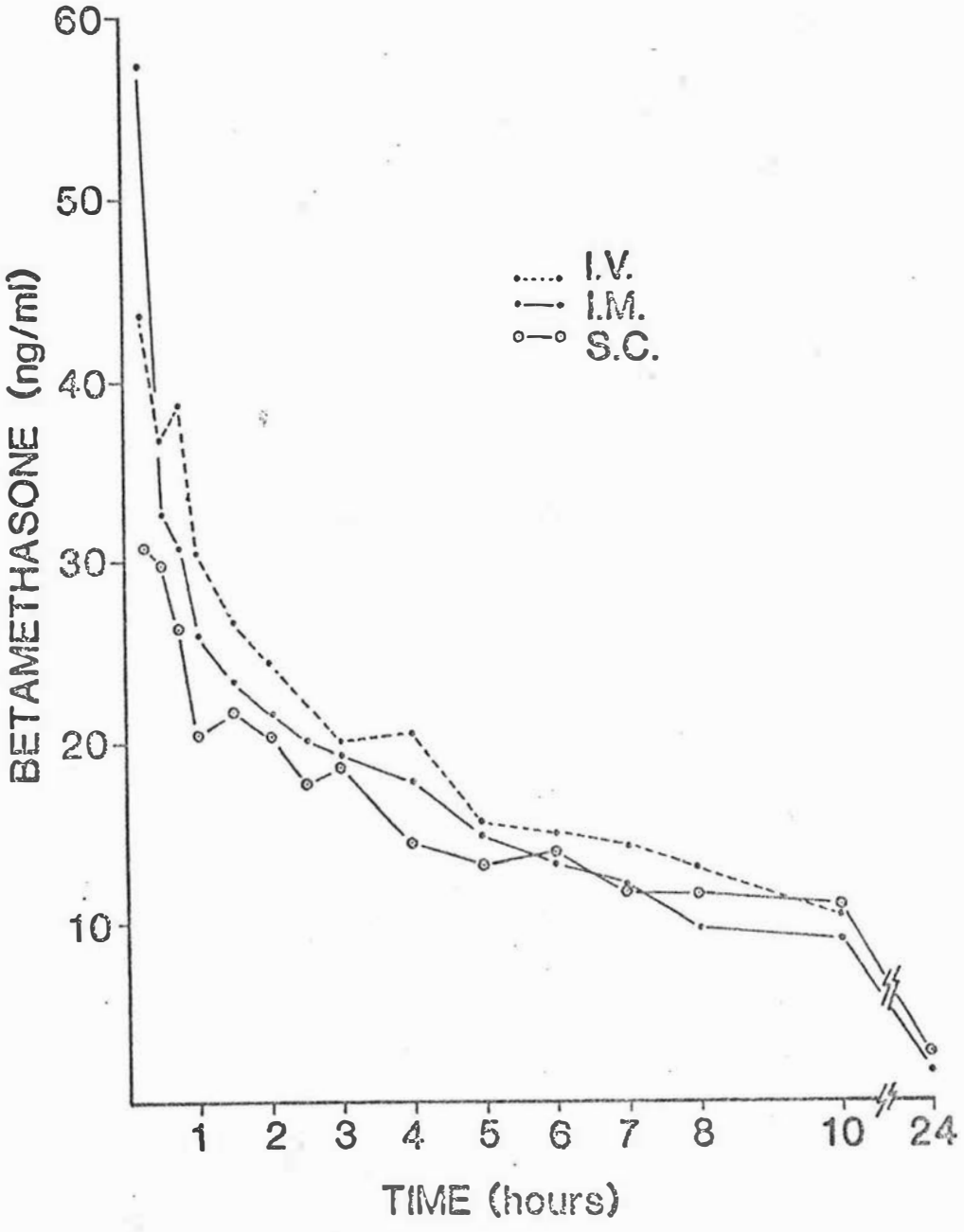


Figure 4:4 Mean plasma concentrations of betamethasone in adult cattle (Group I) following parenteral administration of the sodium phosphate ester at a dose rate of 0.1 mg/kg.

Table 4:11 Plasma betamethasone concentrations (ng/ml) in cows following the administration of betamethasone sodium phosphate solution, 4 mg/ml, at a dose rate of 0.1 mg/kg

| | Peak level | | | 10 hours | | | 32 hours | | |
|---|---|---|---|---|---|---|---|---|---|
| Cow No. | IV | IM | SC | IV | IM | SC | IV | IM | SC |
| 34 | 36.51 | 60.78 | 42.96 | 9.41 | 8.59 | 11.83 | NA | 0.38 | 1.36 |
| 41 | 61.33 | 70.69 | 29.17 | 12.43 | 10.57 | 10.64 | NA | 1.24 | 0.72 |
| 62 | 45.82 | 43.74 | 29.51 | 10.16 | 7.83 | 9.81 | NA | 0.19 | 0.53 |
| 113 | 83.81 | NA | NA | 20.27 | NA | NA | 2.11[a] | NA | NA |
| 128 | 128.77 | NA | NA | 14.43 | NA | NA | 1.04[a] | NA | NA |
| 153 | 63.18 | NA | NA | 13.40 | NA | NA | 2.17[a] | NA | NA |

NA Not assayed

[a] 31 hours, rather than 32

Peak values recorded following extravascular injection of the betamethasone solution were not remarkably different from those obtained following intravenous administration (Table 4:II).

The best fitting least-squares linear regression lines for the disposition curves produced following intravenous injection of betamethasone sodium phosphate indicated that, in 4 out of 6 cows, distribution was completed by 0.5 hours. In the remaining 2 cows distribution was completed by 0.75 and 1.0 hour respectively. The other disposition kinetics calculated from these regression lines (Table 4:III), indicated that betamethasone had a large volume of distribution in cows (mean, 2665.0 ml/kg), an apparent first-order disappearance rate constant ranging from 0.0947 to 0.1283 per hour, and a half-life which ranged from 4.63 to 7.32 hours.

The range of values calculated for body clearance ($Cl_B$) of betamethasone showed considerable variation between individuals, with a two-fold difference between the fastest and the slowest rates (Table 4:IV).

Following extravascular injection of betamethasone solution, absorption occurred too rapidly for a rigorous pharmacokinetic analysis to be applied to the absorption phase (Fig. 4:5). However, estimates of the absorption half-life ($t_{\frac{1}{2}ab}$) were made using the method of Swintosky, Dittert and Doluisio (1969). Absorption half-lives were estimated by this method to be between 2.25 and 3.5 minutes after intramuscular injection of the solution, and between 2.25 and 7.0 minutes following subcutaneous administration (Fig. 4:3).

The elimination phase of each disposition curve was analysed conventionally and values for the y-axis intercept (B or B'), the apparent first-order elimination rate constant ($\beta$ or $\beta'$) and the plasma half-life ($t_{\frac{1}{2}\beta'}$) are shown in Table 4:V. The terms B' and $\beta'$ obtained from the analysis of the disposition curve following extravascular injection are not normally considered to be the same as B and $\beta$ obtained following intravenous injection because of the influence absorptive processes have on the disposition curve. However, in this experiment the absorptive

Table 4:III Disposition kinetics of betamethasone in cattle following intravenous injection of the sodium phosphate solution (0.1 mg/kg). Single compartment open model.

| Cow No. | Weight(kg ) | B(ng/ml) | $\beta(hr^{-1})$ | $t_{\frac{1}{2}}(hr)$ | V'd(ml/kg) |
|---|---|---|---|---|---|
| 34 | 293.4 | 29.17 | 0.1214 | 5.71 | 3365.1 |
| 41 | 283.5 | 42.06 | 0.1497 | 4.63 | 2549.3 |
| 62 | 308.2 | 32.44 | 0.1114 | 6.22 | 3039.6 |
| 113 | 266.4 | 49.34 | 0.1015 | 6.83 | 2054.2 |
| 128 | 252.0 | 51.58 | 0.1283 | 5.40 | 1923.3 |
| 153 | 231.7 | 32.45 | 0.0947 | 7.32 | 3058.6 |
| mean | 272.55 | 39.51 | 0.1178 | 6.02 | 2665.0 |
| ± SD | 28.12 | 9.54 | 0.0199 | 0.98 | 586.8 |

Table 4:IV Body clearance of betamethasone in cows following the intravenous injection of betamethasone sodium phosphate, 0.1 mg/kg.

| Cow | AUC $o \rightarrow \infty$(ng/ml.hr) | Vd(area)(ml/kg) | $\beta(hr^{-1})$ | $Cl_B(ml.kg^{-1}.hr^{-1})$ |
|---|---|---|---|---|
| 34 | 244.94 | 3362.9 | 0.1214 | 408.3 |
| 41 | 287.95 | 2319.8 | 0.1497 | 347.3 |
| 62 | 288.32 | 3113.4 | 0.1114 | 346.8 |
| 113 | 491.26 | 2005.5 | 0.1015 | 203.5 |
| 128 | 442.47 | 1761.5 | 0.1283 | 226.0 |
| 153 | 355.36 | 2971.5 | 0.0947 | 281.4 |
| mean | 351.72 | 2589.1 | 0.1178 | 302.2 |
| ± SD | 97.17 | 650.8 | 0.0199 | 79.1 |

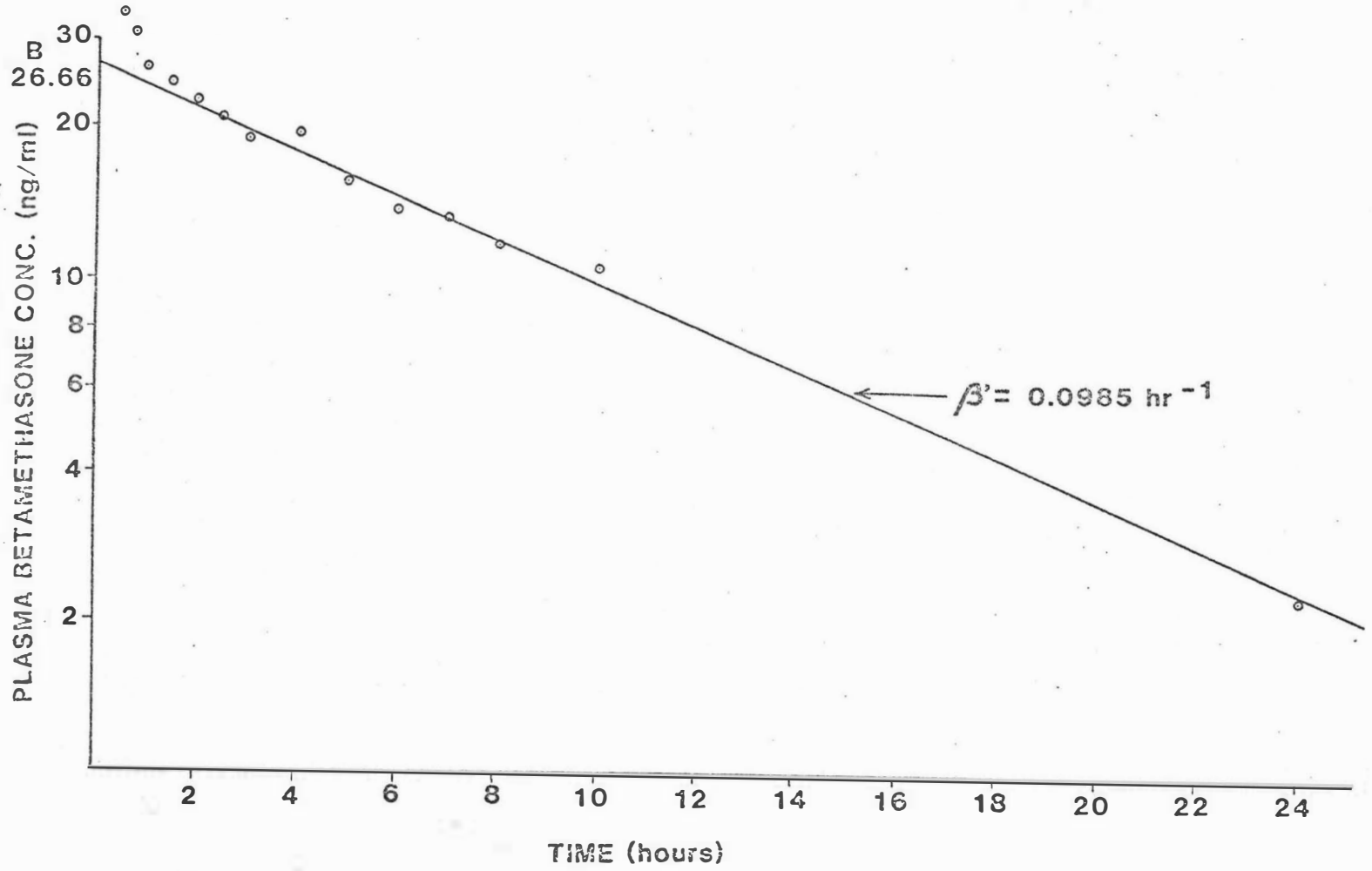


Figure 4:5 Plasma concentrations of betamethasone following intramuscular injection of the sodium phosphate ester (4 mg/ml) at a dose rate of 0.1 mg/kg to single cow (no. 41).

Table 4:V Elimination kinetics of betamethasone following the administration of the sodium phosphate ester by different routes. Dose, 0.1 mg/kg.

| Route | Cow no. | B or B'(ng/ml) | β or β'($hr^{-1}$) | $t_{\frac{1}{2}}$(hr) |
|---|---|---|---|---|
| IV | 34 | 29.17 | 0.1214 | 5.71 |
| | 41 | 42.06 | 0.1497 | 4.63 |
| | 62 | 32.44 | 0.1114 | 6.22 |
| | $\bar{x}$ ± SD | 34.56 ± 6.70 | 0.1275 ± 0.0199 | 5.52 ± 0.81 |
| IM | 34 | 27.64 | 0.1317 | 5.26 |
| | 41 | 26.66 | 0.0985 | 7.03 |
| | 62 | 30.08 | 0.1464 | 4.73 |
| | $\bar{x}$ ± SD | 28.13 ± 1.76 | 0.1255 ± 0.0245 | 5.67 ± 1.20 |
| SC | 34 | 23.29 | 0.0832 | 8.33 |
| | 41 | 25.76 | 0.1060 | 6.53 |
| | 62 | 24.62 | 0.1160 | 5.97 |
| | $\bar{x}$ ± SD | 24.59 ± 1.18 | 0.1017 ± 0.0168 | 6.94 ± 1.23 |

phase was so rapid, in relation to sampling times, that B' and β' can be considered equivalent to B and β respectively. Because of this, and because there were no significant differences between routes in estimates of mean values for elimination kinetics, for each cow an overall mean value for the elimination $t_{\frac{1}{2}}$ was calculated. Thus half-life estimations based on 3 experiments in each of the 3 cows gave values (mean ± SD) of 6.43 ± 1.66, 6.06 ± 1.26 and 5.64 ± 0.80 hours for cows 34, 41 and 62 respectively. These estimates of $t_{\frac{1}{2}}$ were not significantly different.

Intravenous administration of suspensions of betamethasone alcohol produced no clear peak in plasma concentrations (Fig. 4:6) and while the mean plasma concentration over the first four hours was lower than that recorded following the injection of the soluble ester of betamethasone, this difference was significant over the first hour only. The disposition curves produced were unsuitable for pharmacokinetic analysis; they were too flat and the sampling period was too short. Plasma concentrations at 0.25 hours ranged from 6.67 ng/ml to 19.5 ng/ml (mean, 14.86 ng/ml) and at 10 hours ranged from 6.26 ng/ml to 23.91 ng/ml (mean, 12.63 ng/ml).

Up to 32 hours after extravascular injection, mean plasma concentrations produced by the two suspensions were significantly different (Fig. 4:7, Fig. 4:8). The 2 mg/ml suspension produced peak concentrations within 6 to 10 hours in all individuals. Peak concentrations ranged from 3.31 ng/ml to 6.69 ng/ml, and declined over the sampling period to between 0.14 ng/ml and 1.57 ng/ml at 56 hours. On the other hand, plasma concentrations produced by the 20 mg/ml suspension never exceeded 1.61 ng/ml, and maximum levels were not produced until 10 to 25 hours after injection (Table 4:VI)

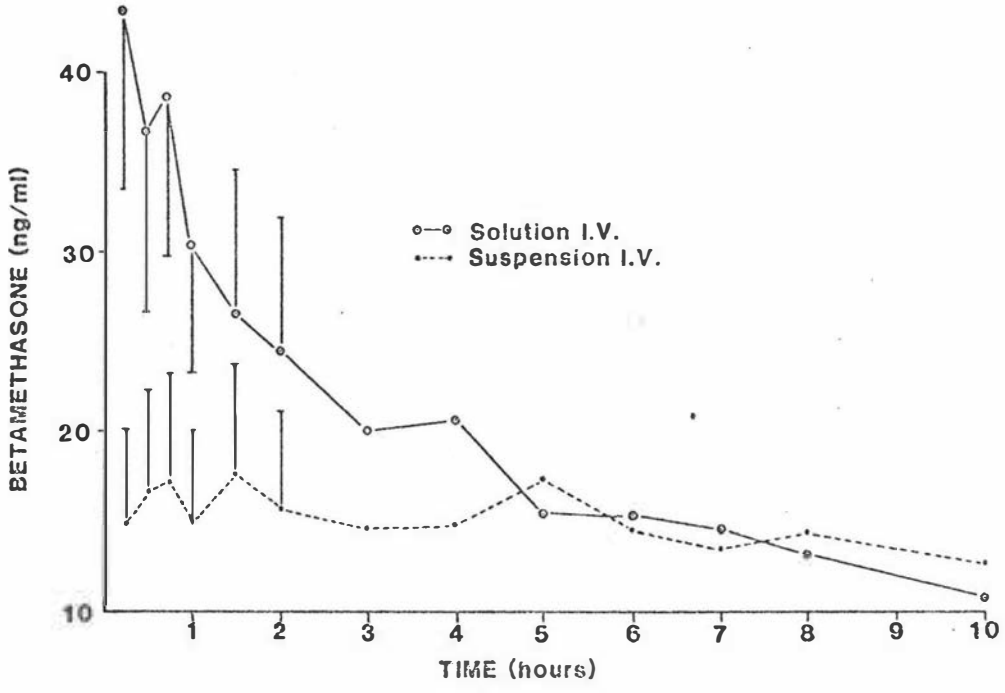


Figure 4:6 Plasma concentrations (means and $CL_{95}$s) of betamethasone following intravenous injection of different formulations to cows at a dose rate of 0.1 mg/kg.

Table 4:VI Plasma concentration of betamethasone (ng/ml) following extravascular injection of suspensions of different concentration. Dose, 0.1 mg/kg.

| Route | Conc. of suspension | Cow no. | Plasma concentrations at periods after administration: Peak at time (hours) | | 32 hours | 56 hours | 80 hours |
|---|---|---|---|---|---|---|---|
| IM | 2 mg/ml | 113 | 6.49 | 8 | 2.70 | 1.18 | NA |
| | | 128 | 6.69 | 6 | 2.29 | 0.94 | NA |
| | | 153 | 4.97 | 8 | 2.14 | 0.14 | NA |
| | 20 mg/ml | 141 | 0.58 | 10 | 0.41 | 0.29 | 0.74 |
| | | 170 | 0.77 | 25 | 0.57 | 0.53 | 0.46 |
| | | 171 | 0.64 | 10 | 0.26 | 0.37 | 0.0 |
| SC | 2 mg/ml | 113 | 4.68 | 10 | 2.98 | 1.57 | NA |
| | | 128 | 3.31 | 10 | 2.15 | 1.04 | NA |
| | | 153 | 3.95 | 8 | 3.00 | 0.72 | NA |
| | 20 mg/ml | 141 | 1.53 | 10 | 1.42 | 0.78 | 0.53 |
| | | 170 | 1.61 | 14 | 0.82 | 0.98 | 0.30 |
| | | 171 | 0.39 | 10 | 0.16 | 0.08 | 0.03 |

NA Not assayed.

While the mean peak plasma concentration of betamethasone produced by the 2 mg/ml suspension was higher after intramuscular injection than after subcutaneous injection, the difference was not significant. Neither was the difference in mean peak concentrations produced by the injection of the 20 mg/ml suspension into different sites. After intramuscular injection, the more dilute suspension produced peak levels sooner than the 20 mg/ml suspension. However, such time differences were not seen after subcutaneous administration, nor were they discernible when the more concentrated suspension was injected into different sites.

Elimination rate constants and absorption rate constants following extravascular injection of the 2 mg/ml suspension differed markedly between individuals (Table 4:VII). Elimination half-lives ranged from 11.63 hours up to 29.70 hours, and in 2 out of 3 animals, betamethasone was eliminated more slowly following subcutaneous, as opposed to intramuscular, injection. No clear difference in the rate of absorption from different sites was noted, and absorption half-lives ranged from 1.52 hours to 4.05 hours.

As disposition curves produced for the 20 mg/ml suspension (Fig. 4:7, Fig. 4:8) were too flat for analysis, absorption and elimination rate constants were not calculated for this formulation.

The relative bioavailability of betamethasone alcohol suspensions was low following extravascular injection. Over the first 24 hours after injection of the 2 mg/ml suspension, relative availability ranged from 15.04 % to 27.99%; the lowest values being obtained after subcutaneous administration (Table 4:VIII). The relative availability of the 20 mg/ml suspension could not be calculated from data obtained in these experiments, but it was obviously very low (Fig. 4:7, Fig. 4:8).

Table 4:VII Disposition kinetics of betamethasone in cattle following extravascular injection of a 2 mg/ml suspension. Dose, 0.1 mg/kg.

| Cow no. | Route | B'(ng/ml) | B"(ng/ml) | $\beta'(hr^{-1})$ | $t_{½}\beta'(hr)$ | $Kab(hr^{-1})$ | $t_{½}ab(hr)$ |
|---|---|---|---|---|---|---|---|
| 113 | IM | 9.57 | 8.22 | 0.0373 | 18.56 | 0.1957 | 3.54 |
| | SC | 5.37 | 4.35 | 0.0233 | 29.70 | 0.1442 | 4.80 |
| 128 | IM | 4.60 | 4.99 | 0.0257 | 26.99 | 0.4553 | 1.52 |
| | SC | 4.16 | 4.27 | 0.0268 | 25.82 | 0.2041 | 3.39 |
| 153 | IM | 7.35 | 6.81 | 0.0596 | 11.63 | 0.1711 | 4.05 |
| | SC | 5.16 | 4.64 | 0.0286 | 24.26 | 0.2338 | 2.96 |

Table 4:VIII Area under disposition curve (ng/ml.hr) and relative bioavailability (%) of betamethasone alcohol following extravascular administration to cows. Dose, 0.1 mg/kg.

| Cow no. | IV[a] | IM[b] | SC[b] |
|---|---|---|---|
| 113 | 450.38<br>(100) | 126.08<br>(27.99) | 80.36<br>(17.84) |
| 128 | 413.49<br>(100) | 73.97<br>(17.89) | 62.18<br>(15.04) |
| 153 | 315.70<br>(100) | 73.03<br>(23.13) | 52.29<br>(16.56) |

a. Betamethasone sodium phosphate solution, 4 mg/ml.

b. Betamethasone alcohol suspension, 2 mg/ml.

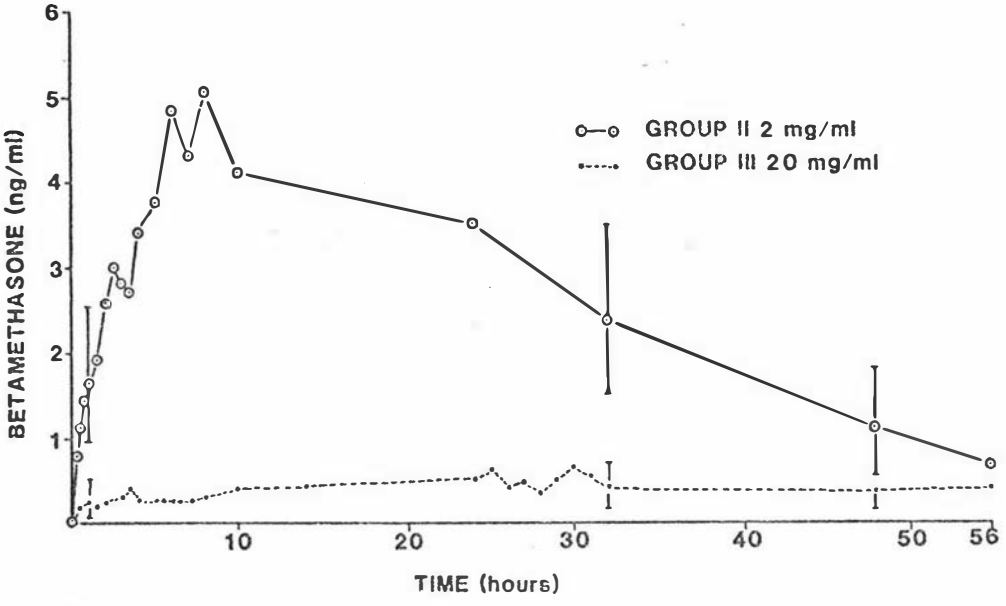


Figure 4:7 Plasma concentrations of betamethasone (means and $CL_{95}$s) following intramuscular injection of different suspensions to cows at a dose rate of 0.1 mg/kg.

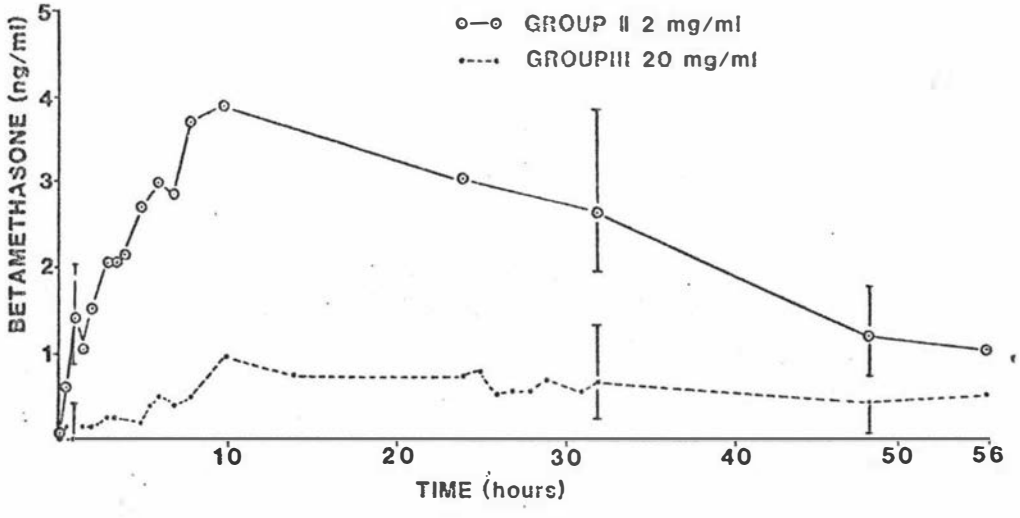


Figure 4:8 Plasma concentrations of betamethasone (means and $CL_{95}$s) following subcutaneous injection of different suspensions to cows at a dose rate of 0.1 mg/kg.

# 4. DISCUSSION

In this second series of experiments the sodium phosphate ester of betamethasone was used as a highly soluble reference compound, and its absorption and elimination characteristics were compared with those of suspensions of the relatively insoluble betamethasone alcohol. Such sodium phosphate esters are rapidly hydrolysed *in vivo* to free corticosteroid (Melby & Dale, 1969; Hare *et al*, 1975; Panaretto & Wallace, 1978).

There are very few published reports which deal with those aspects of betamethasone which have been examined in the present study. There are, however, some reports pertaining to dexamethasone, an epimer of betamethasone. The two steroids are so similar in structure that in the absence of information to the contrary it is reasonable to assume that the pharmacokinetics and pharmacodynamics of the two steroids should be very similar. There is, however, some suggestion that betamethasone may be slightly more potent than dexamethasone in some species (Pechet, 1964; Peets *et al*, 1969; Brander & Pugh, 1977) and in several species the plasma protein binding of the two steroids is different (Peets *et al*, 1969; Mollman, 1977). For instance, in the cow 32% more dexamethasone than betamethasone is bound to plasma proteins (Peets *et al*, 1969) and as only unbound steroid is capable of biological activity, the difference in binding may partially explain the marginally greater activity of betamethasone (Peets *et al*, 1969; Silber & Arcese, 1964). Although plasma betamethasone concentrations attained in cattle have not previously been published, the very rapid absorption of the soluble ester after administration by extravascular injection is as expected (Silber & Arcese, 1964). The sodium phosphate ester of dexamethasone produced peak plasma concentrations within 30 minutes of intramuscular injection in 4 out of 5 human subjects (Melby & Dale, 1969) and within 2 to 20 minutes of intramuscular injection in cattle (Fairclough, Hunter & Welch, 1981). After subcutaneous injection in sheep this soluble ester of dexamethasone produced peak plasma levels within 30 minutes (Panaretto, 1979).

The peak plasma concentrations recorded in the present study following the extravascular injection of betamethasone sodium phosphate (29.17 to 70.69 ng/ml) are not markedly dissimilar from those recorded by Fairclough, Hunter & Welch (1981) after the intramuscular injection of dexamethasone sodium phosphate. Those workers recorded peak concentrations of 22 ng/ml to 70 ng/ml after the administration of a smaller dose than that used here; they used 20 mg for a Jersey cow.

The half-life of absorption of betamethasone sodium phosphate following extravascular injection, estimated to be between 2.25 and 7.0 minutes (Page 78) is of a similar magnitude to the mean absorption half-life of 4.4 minutes calculated for a wide variety of drugs in solution (Bederka, Takemori & Miller, 1971). The rate at which aqueous solutions disappear from a given intramuscular injection site has been found to be similar for all drugs tested (Bederka, Takemori & Miller, 1971; Chapter II, Section 4.2).

Published references to the plasma half-life of betamethasone following intravenous injection are rare. Several publications (Melby, 1977 and Swartz & Dluhy, 1978, are examples) dealing with the human subject described 'dexamethasone' and 'betamethasone' as having a half-life of around 5 hours, but do not give details of administration. Following intravenous administration of dexamethasone sodium phosphate to humans, plasma half-lives ranging from 1.6 hours to 8.3 hours have been recorded (Araki *et al*, 1965; Hare *et al*, 1975).

Data reported by Johnson and co-workers (1976) suggest a plasma half-life of 2.4 hours for betamethasone sodium phosphate in the human.

The half-life of dexamethasone in the dog has been reported as 1.0 to 1.5 hours (Silber, 1959; Silber & Arcese, 1964).

An inspection of data presented by Fairclough, Hunter and Welch (1981) suggests that dexamethasone may have a half-life in cattle of about 7.5 to 9.0 hours following administration of the sodium phosphate ester by the intramuscular route.

It would appear, therefore, that in a given species betamethasone and dexamethasone have a similar plasma half-life, that the plasma half-life can vary markedly between individuals of the same species, and that the plasma half-life values reported here for betamethasone sodium phosphate (4.63 hours to 8.33 hours; Table 4:III and Table 4:V) are of similar magnitude to those estimated from the disposition curves of dexamethasone sodium phosphate in cows (Fairclough, pers. com.).

The similarity of the disposition curves of betamethasone sodium phosphate following administration by different routes (Fig. 4:4) suggests that in the cow at least, the intravenous route offers no advantage over intramuscular or subcutaneous injection in terms of plasma steroid concentrations attained or maintained.

The large values obtained for the apparent specific volume of distribution of betamethasone (Table 4:III), all of which exceed the actual volume of the body (that is, are greater than 1 L/kg ) imply that betamethasone is widely distributed (Baggot, 1977). It has been demonstrated that *in vivo*, dexamethasone rapidly diffuses into cells (Hare *et al*, 1975) and presumably the same occurs with betamethasone.

The term 'body clearance' describes rates of drug removal from the body independently of the distribution processes (Baggot, 1977). In 15 human subjects, the body clearance for dexamethasone following the intravenous administration of the sodium phosphate ester, ranged from 91.2 to 379.8 $ml.hr^{-1}.kg^{-1}$ (1.52 to 6.33 $ml.min^{-1}.kg^{-1}$) (Hare *et al*, 1975). The values for betamethasone in cows, presented in Table 4:IV, are comparable both in magnitude and individual variation.

While it was recognised that betamethasone alcohol is 'practically insoluble' in water (Anon., 1971) it was anticipated that following injection into a large volume of circulating blood the injected crystals would rapidly dissolve and that a first-order elimination curve would result. That this did not occur can be seen from Figure 4:6. It appears that

even after intravenous administration the particles of betamethasone alcohol take several hours to dissolve.

In the betamethasone suspensions used, 98% of the particles were smaller than 5μ (Holmes, M.B., Glaxo New Zealand Ltd., pers. com.). Studies in dogs have shown that when insoluble particles of this size are injected intravenously they become lodged in the liver and spleen (Kanke *et al*, 1980). It is therefore likely that the intravenously administered suspensions of betamethasone alcohol found temporary residence in those tissues before finally dissolving in plasma.

The kinetics of the extravascular injection of betamethasone alcohol suspensions again clearly demonstrated that absorption, and hence the disposition curve, is strongly influenced by the solids:vehicle ratio of the injected suspension; the more concentrated suspension producing much lower plasma levels than the more dilute one.

As outlined under materials and methods (2.3), the regression line which describes the absorption phase of a single compartment open model following extravascular administration is obtained by the method of residuals. The intercept of this line with the ordinate should equal that of the elimination phase. That this may not always be the case is implicit in the biexponential equation used by Ritschel to describe this model (Equation 4:7). There are three possible reasons why B' and B'' may not coincide (Reuning, R.H., The Ohio State University, pers. com.). In the first case, experimental inaccuracies arising from the difficulties inherent in measuring such small quantities may result in a relatively poor fit of the regression line to the data points. Another possibility is that the disposition of the drug is, in fact, best described by a two-compartment open model but there are too few early data points to determine this. A third possibility is that the absorption phase itself may not be monoexponential (Ballard, 1968); the pattern of absorption of microcrystalline suspensions is likely to be complex (Van der Vies, 1975; Brotherton, 1976).

In each instance when betamethasone alcohol, 2 mg/ml, was administered by extravascular injection B' and B'' failed to coincide (Table 4:VII). In situations such as this, where the intercepts of the two regression lines are not the same, the rate constant derived from the regression line describing the absorption phase should not, strictly speaking, be called $K_{ab}$, as it may represent a mixture of absorption and distribution rate constants (Reuning, pers. com.).

A comparison of the rates of absorption (Fig. 4:3, Table 4:VII) and elimination (Table 4:V; Table 4:VII) shows marked differences between the soluble and relatively insoluble formulations of betamethasone; the soluble formulation being absorbed many times more rapidly than either of the suspensions. Prolonged absorption influenced the rate at which betamethasone was eliminated from the plasma, as can be seen by comparing the apparent elimination rate constants and plasma half-lives (Table 4:V; Table 4:VII). Following the extravascular injection of the 2 mg/ml suspension, half-life values were two to five times longer than following administration of the solution.

The bioavailability of the 2 mg/ml suspension (low in all cases) was influenced by the route of injection, with the smallest area under the curve (AUC) resulting from subcutaneous injection. While in two cows at least, absorption occurred more slowly following subcutaneous injection, and less completely in all three, it is possible that this result is more a reflection of a difference due to the region of the body rather than a difference due to the route of injection. Recent studies in cattle (see Chapter II, Section 3.1) have shown that the region of the body in which the injection is made is at least as important as the route of administration, that injection by the same route may show different availabilities between body regions and, in some instances, subcutaneous injection in one region may give a better availability than intramuscular injection in another. The differences described in these experiments cannot, therefore, be ascribed solely to the route of injection, as regional differences may well have been more important.

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# 5. CONCLUSIONS

The experiments described in this chapter have provided further confirmation that the rate at which injected suspensions of betamethasone alcohol are absorbed is governed by the volume in which the dose is given. The concentration of a suspension exerts a profound effect on the rate and extent to which the injected steroid is absorbed.

Some data suggested that the site and the route of injection may influence the bioavailability of the suspended betamethasone, and that the availability of the soluble form of betamethasone is relatively unaffected by the route of administration.

Where absorption and elimination rate constants could be determined for betamethasone, and where comparable data were available for dexamethasone, the disposition kinetics of betamethasone appeared to be very similar to those reported for its epimer.

# CHAPTER V

## THE PHARMACOLOGICAL ACTIVITY IN COWS OF A 10 MG/ML SUSPENSION OF BETAMETHASONE

1. INTRODUCTION

2. MATERIALS AND METHODS

2.1 Animals

2.2 Treatments

2.3 Samples

2.4 Experimental design

3. RESULTS

3.1 Betamethasone concentrations

3.2 Cortisol concentrations

3.3 Glucose concentrations

4. DISCUSSION

5. CONCLUSION

# CHAPTER V

## THE PHARMACOLOGICAL ACTIVITY IN COWS OF A 10 MG/ML SUSPENSION OF BETAMETHASONE

### 1. INTRODUCTION

The studies reported in Chapters III and IV of this thesis, and those of P.G. Box (pers. com.) confirmed that a betamethasone formulation having a prolonged effect could be prepared by increasing the solids:vehicle ratio of an aqueous suspension of the alcohol. Thus, for a given dose, a less intense effect, but one of greater duration, could be achieved by merely decreasing the volume in which that dose was administered.

It was noticed, however, that when the manufacturer's[1] recommended dose of betamethasone (0.05 mg/Kg) was administered as a 20 mg/ml suspension, the volume of the appropriate dose was inconveniently small. For example, a Jersey cow would receive a dose of approximately 1 ml.

It was for this reason that a decision was made to investigate the suitability of a 10 mg/ml suspension of betamethasone. A suspension of this concentration had already been shown in pilot studies (P.G. Box and colleagues, pers. com.) to produce effects of promising intensity and duration.

It was envisaged that any commercial product available at this concentration would initially be recommended for use in the induction of parturition in dairy cattle, and that a dose of 2 ml would be administered by subcutaneous injection.

The aim of this experiment, therefore, was to examine a 10 mg/ml suspension for its effects on plasma concentrations of glucose and cortisol, and to relate changes in these to the plasma betamethasone concentrations produced.

(1) Glaxo New Zealand Ltd., Palmerston North.

## 2. MATERIALS AND METHODS

### 2.1 ANIMALS

Ten healthy, non-pregnant, non-lactating cows of mixed ages were used. Four were Jerseys and 6 were Friesians. Four had been spayed some months previously for reasons not connected with this study.

During the course of the experiment, the cows were grazed as a single mob on good quality pasture and were fed hay at the rate of 2 bales per day.

Before treatment the cows' weights ranged from 370 to 515 Kg (mean ± S.D. = 453 ± 47.6 Kg).

### 2.2 TREATMENTS

All cows received 2 ml of a 10 mg/ml aqueous suspension of betamethasone alcohol(2), administered by subcutaneous injection on the left side of the neck in an area over the fourth and fifth cervical vertebrae. This site more closely approximates that commonly used in clinical practice than does the site behind the ear used in the studies described in Chapters III and IV.

Individual dose rates ranged from 39 to 54 µg/Kg (mean ± S.D. = 44.4 ± 0.5 µg/Kg).

### 2.3 SAMPLES

Blood samples were collected from all cows once daily between 7.30 and 8.00 a.m. on 6 occasions over a 7 day period before treatment and on 21 occasions over a 29 day period after treatment. Plasma was separated and stored at -20°C. Estimations of betamethasone, cortisol and glucose concentrations were carried out according to methods described in Chapter III, Sections 2.4, 2.5 and 2.6 respectively.

For a supplementary study involving betamethasone estimations only, additional blood samples were collected from 3 cows at more frequent intervals throughout the 48 hours after injection.

(2) Betsopart, Glaxo New Zealand Ltd.

### 2.4 EXPERIMENTAL DESIGN

No control group was included in this study and, in keeping with several published studies of this nature (Goetsch, McDonald & Odell, 1959; Maplesden, McSherry & Stone, 1970; Neff, Connor & Bryan, 1960; Heidrich *et al*, 1963; Woollett & Evans, 1971; Schillinger & Klee, 1979), the pretreatment values for each parameter examined were used to establish a reference baseline.

For each parameter studied a mean value and its $CL_{95}$ were calculated (Chapter III, Section 2.8) for;

i) each day
ii) the overall pretreatment period.

After treatment with betamethasone, each mean observation for a parameter was considered to be significantly different from its mean pretreatment value only for as long as the $CL_{95}$s of each did not overlap.

## 3. RESULTS

### 3.1 BETAMETHASONE CONCENTRATIONS

A betamethasone 'blank' effect greater than the sensitivity of the assay was recorded in 7 pretreatment samples from 2 cows. This led to an erroneously high mean pretreatment 'betamethasone' level and made interpretation of subsequent betamethasone profiles difficult.

A mean peak plasma level of betamethasone of 0.6 ng/ml was recorded 24 hours after injection (Fig. 5:1). Individual peak plasma levels ranged from 0.32 to 1.83 ng/ml and all but 3 occurred at that time.

The additional samples, from the 3 cows included as a supplementary study, showed slowly rising betamethasone levels reaching a peak between 25 and 31 hours (Fig. 5:2).

The mean plasma concentration of betamethasone was elevated above the pretreatment 'blank' concentration for 7 days, but was significantly different from it for 4 days only (Fig. 5:1).

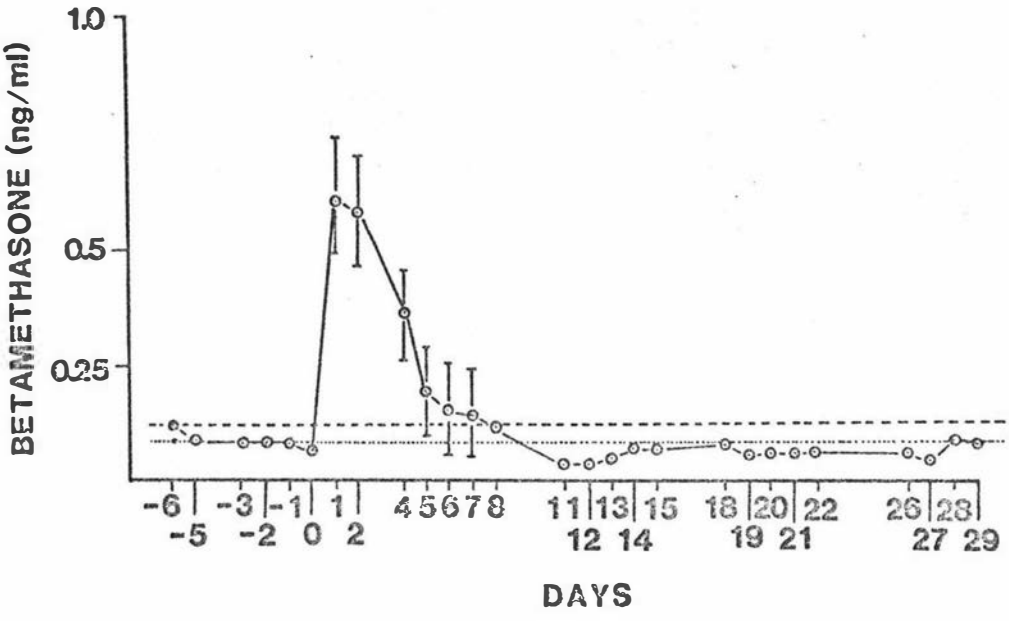


Figure 5:1 Plasma concentrations of betamethasone (means and $CL_{95}$s) in cows following subcutaneous injection of 2 ml of a 10 mg/ml suspension. Horizontal lines indicate the pretreatment mean and its upper $CL_{95}$.

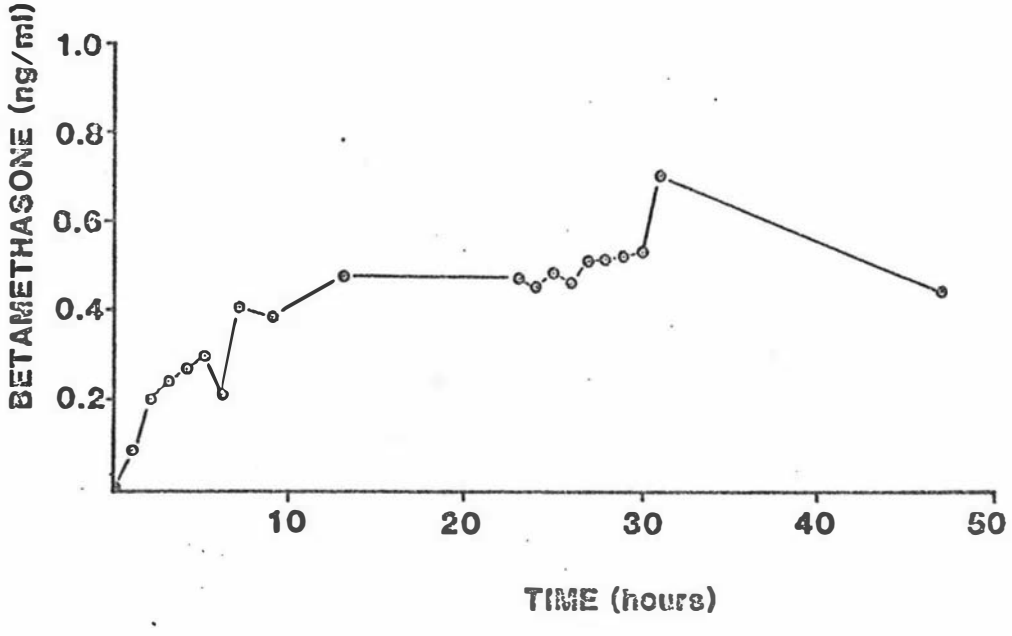


Figure 5:2 Mean plasma betamethasone concentrations in 3 cows over a 48 hour period following the subcutaneous injection of 2 ml of a 10 mg/ml suspension.

## 3.2 CORTISOL CONCENTRATIONS

In all cows, betamethasone treatment profoundly depressed endogenous cortisol levels (Fig. 5:3). Cortisol was depressed to a mean concentration which was not significantly different from zero and levels did not start to recover until the end of the second week. While cortisol levels in some individuals (for example, No. 117, Fig. 5:4) recovered rapidly after this time, in others (for example, No. 67, Fig. 5:4) there was still no sign of recovery by the end of the experimental period; that is, 29 days after treatment.

Individual mean pretreatment cortisol concentrations ranged from 10.50 ng/ml to 21.04 ng/ml (Table 5:I), but differences between cows were not significant. On the morning of treatment individual cows had cortisol levels ranging from 9.74 ng/ml to 22.75 ng/ml.

## 3.3 GLUCOSE CONCENTRATIONS

Increases in plasma glucose ranging from 31.8% to 106.9% above pretreatment concentrations were recorded (Table 5:II). The mean peak glucose concentration was seen on the second day after betamethasone treatment and was 41.3% greater than the pretreatment mean for the group. The plasma glucose concentration remained significantly elevated for 8 days (Fig. 5:5).

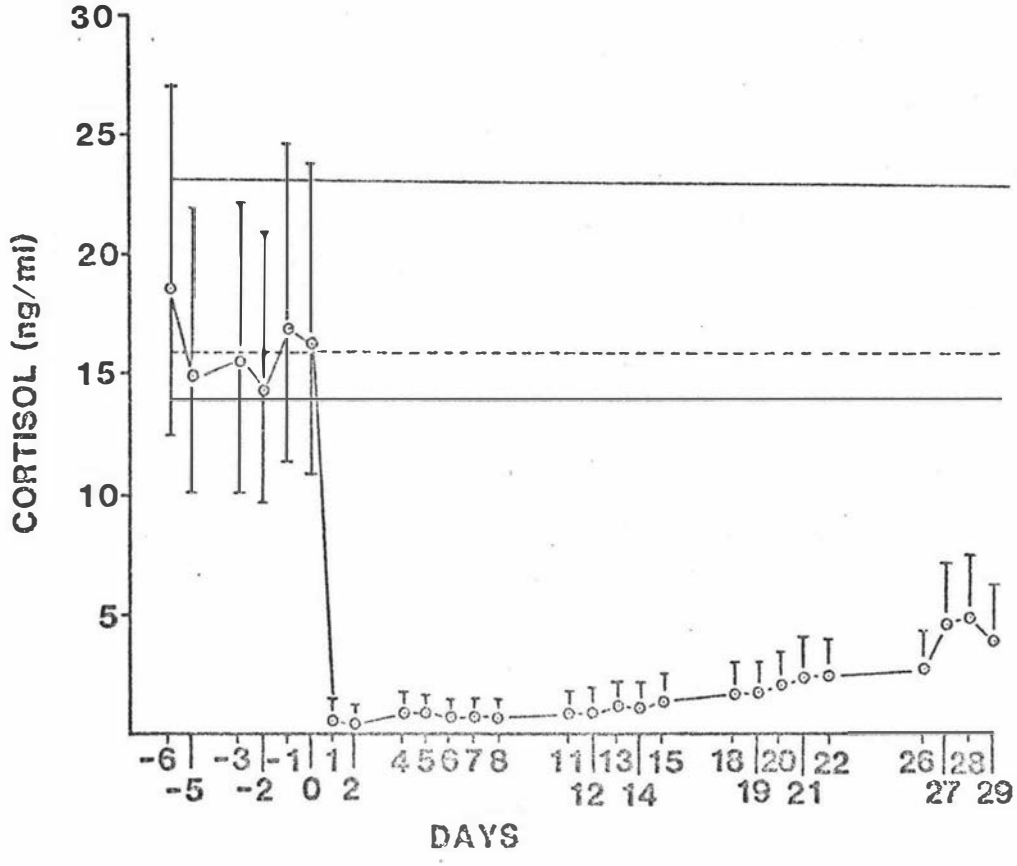


Figure 5:3 Early-morning plasma cortisol concentrations (means and $CL_{95}$s) in cows following the subcutaneous injection of 2 ml of a 10 mg/ml suspension of betamethasone. Horizontal lines indicate the pretreatment mean and its upper and lower $CL_{95}$s.

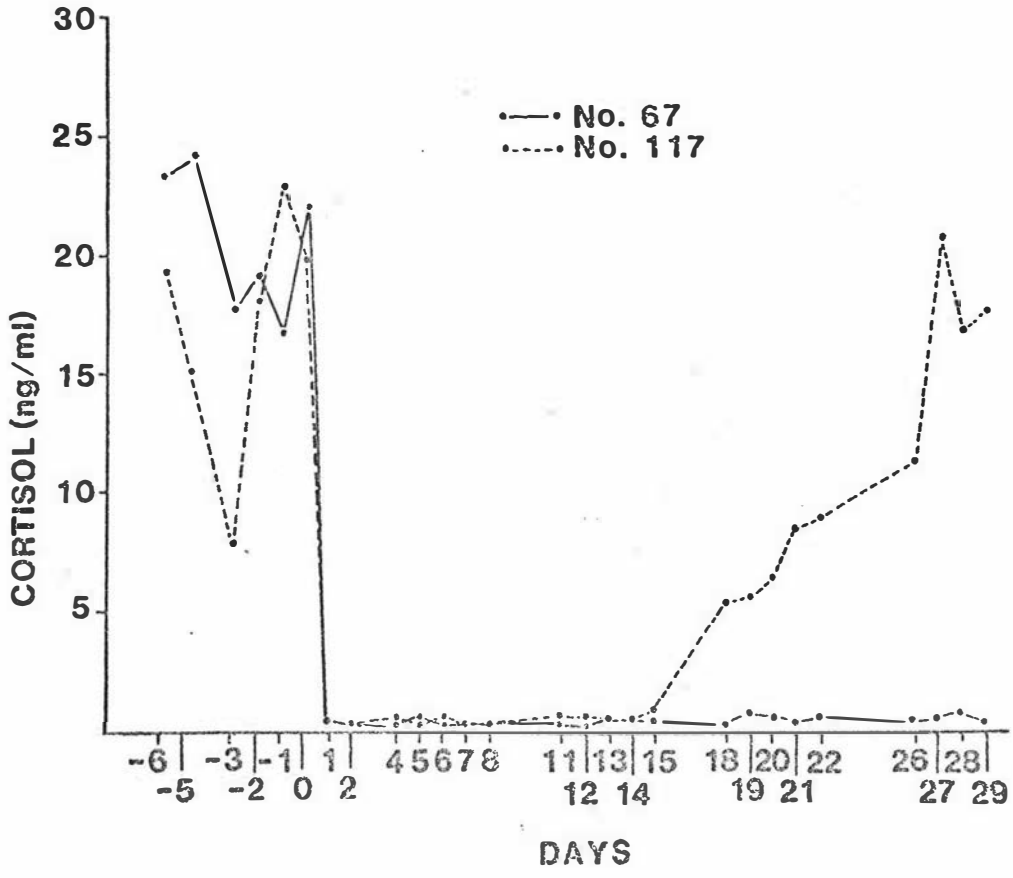


Figure 5:4 Early-morning plasma cortisol concentrations in 2 cows following subcutaneous injection of 2 ml of a 10 mg/ml suspension of betamethasone.

Table 5:I Early-morning[a] plasma cortisol concentrations in cows over the six sampling days before treatment

| Cow no. | Mean cortisol (ng/ml) | Upper $CL_{95}$[b] - Lower $CL_{95}$ (ng/ml) |
|---|---|---|
| 0[d] | 16.70 | 18.12 - 15.38 |
| 39 | 15.42 | 21.74 - 10.86 |
| 41[d] | 10.50 | 19.42 - 5.46 |
| 67 | 20.28 | 23.86 - 17.21 |
| 82[d] | 21.04 | 25.32 - 17.45 |
| 117 | 16.67 | 24.37 - 11.29 |
| 128 | 14.26 | 20.64 - 9.76 |
| 170 | 15.42 | 26.43 - 8.82 |
| 350[d] | 18.32 | 23.04 - 14.52 |
| 777 | 12.95 | 21.70 - 7.56 |
| Group mean | 15.88 | 23.00[c] - 13.95 |

a. collected between 7.30 and 8.00 a.m.

b. based on individual cow variance

c. based on pooled pretreatment variance

d. spayed cows.

Table 5:II Plasma glucose concentrations in cows: pretreatment concentrations and peak concentrations following subcutaneous injection of 2 ml of betamethasone alcohol suspension, 10 mg/ml[a]

| Cow no. | Pretreatment mean (± S.D. ; mM/L) | Post-treatment peak (mM/L) | Increase (%) | Day of peak level |
|---|---|---|---|---|
| 0 | 3.37 ± 0.19 | 4.60 | 36.5 | 1 |
| 39 | 3.18 ± 0.26 | 4.85 | 52.5 | 2 |
| 41 | 3.42 ± 0.17 | 5.30 | 55.0 | 1 |
| 67 | 3.58 ± 0.17 | 5.40 | 51.8 | 7 |
| 82 | 3.02 ± 0.14 | 6.25 | 106.9 | 2 |
| 117 | 3.23 ± 0.15 | 4.75 | 47.0 | 2 |
| 128 | 3.40 ± 0.20 | 4.60 | 35.3 | 4 |
| 170 | 3.02 ± 0.17 | 4.40 | 45.7 | 1 |
| 350 | 3.30 ± 0.16 | 4.35 | 31.8 | 1 |
| 777 | 3.38 ± 0.20 | 4.65 | 37.6 | 1 |
| Group mean | 3.29 ± 0.24 | 4.65 ± 0.63[b] | 41.3 | 2 |

a. Dose, mean ± S.D. = 44.4 ± 0.5 µg/Kg

b. Based on daily means

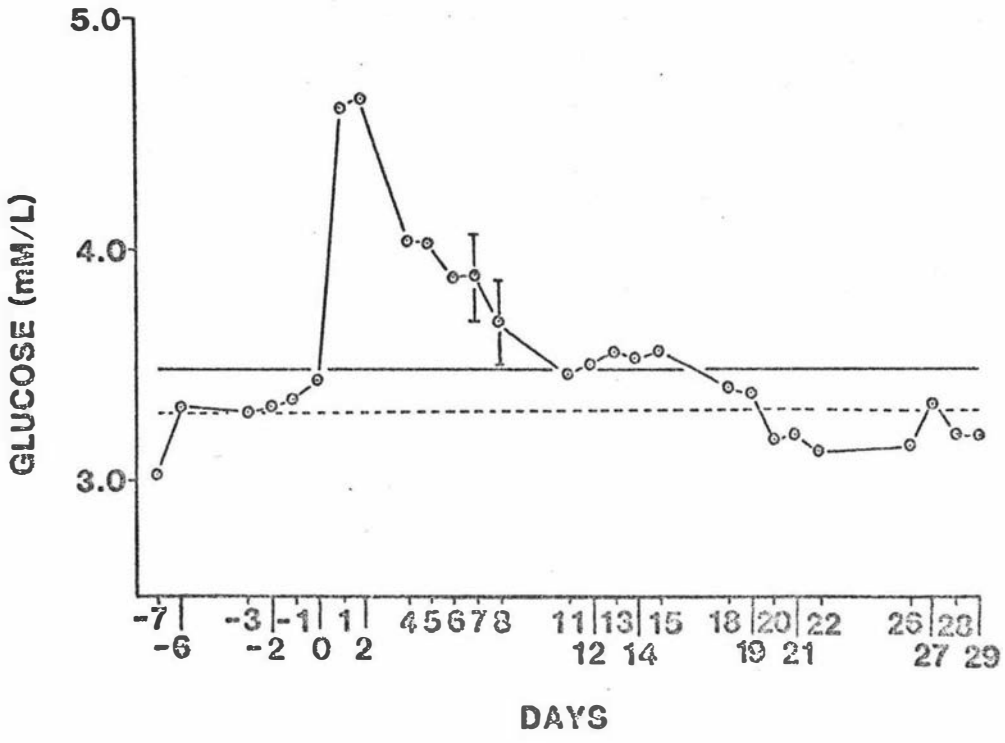


Figure 5:5 Plasma glucose concentrations (means and $CL_{95}$s) in cows following subcutaneous injection of 2 ml of a 10 mg/ml suspension of betamethasone. Horizontal lines indicate pretreatment mean and its upper $CL_{95}$.

## 4. DISCUSSION

A 2 ml dose of a 10 mg/ml suspension of betamethasone, when administered to cattle by subcutaneous injection, produced a lower plasma concentration than did the same volume of a 20 mg/ml suspension (Chapter III, Section 3.1). However, the duration for which betamethasone was detectable was similar and absorption of the 10 mg/ml suspension was less variable between animals than that of the 20 mg/ml suspension.

The 10 mg/ml betamethasone suspension is intended for use in the induction of premature parturition in cattle and so it is of interest to compare its effects with those of other corticosteroid formulations intended for the same purpose.

Fairclough, Hunter and Welch (1981) studied the concentrations of dexamethasone in the plasma of 2 cows treated with 20 mg of dexamethasone trimethylacetate (TMA) suspension(3), a formulation widely used in New Zealand to induce calving (Chapter VI, Section 3.14). The peak dexamethasone concentration of 0.5 ng/ml was similar to the betamethasone concentration seen in the present study. Dexamethasone was detectable by their assay for 8 to 14 days; that is appreciably longer than the period reported here for betamethasone. However, had pretreatment 'blank' values not been encountered in the present study, it is possible that post-treatment concentrations of betamethasone would have remained significantly different from pretreatment values for longer.

The profound depression of endogenous cortisol concentrations that was seen following injection of other betamethasone formulations (Chapter III, Section 3.2) was also recorded in this experiment following a smaller total dose of betamethasone.

While the cortisol levels in some of the cows in the present study had returned to the normal range within the observation period, others were still maximally depressed after 29 days. A similar prolonged and profound depression of endogenous cortisol

(3) Opticortenol, Ciba Geigy, Basel, Switzerland.

has been demonstrated following administration of a 20 mg dose of dexamethasone TMA (Fairclough, Hunter & Welch, 1981) or triamcinolone acetonide (Stellflug, Louis & Hafs, 1978), another long-acting corticosteroid used to induce calving (Chapter VI, Section 3.23).

Pretreatment cortisol concentrations were significantly higher than those seen in untreated cows in the experiment reported in Chapter III (Section 3.2). The mean pretreatment cortisol levels reported for the group of cows in this study are also higher than those reported by other workers using comparable assay methods, although individual animal means are similar in many cases (MacAdam & Eberhart, 1972; Wagner & Oxenreider, 1972; Breves *et al*, 1980). However, because of the pulsatile nature of cortisol secretion, mean concentrations reported in different studies should be compared with caution (Fulkerson *et al*, 1980; Chapter III, Section 4).

The peak elevation of 41.3% in plasma glucose concentrations, recorded in the present study, is less than that reported by other workers using comparable doses of more rapidly absorbed formulations of betamethasone (Burns, 1963), its epimer dexamethasone (Maplesden, McSherry & Stone, 1960; Stockl, Onderscheka & Zacherl, 1969; Schillinger & Klee, 1979), and 9 α-fluoroprednisolone (Neff, Connor & Bryan, 1960). However, plasma glucose was maintained at elevated levels for only 56 to 96 hours by these rapidly-absorbed formulations. While elevated levels could be maintained for longer by increasing the dose (Goetsch, McDonald & Odell, 1959; Neff, Connor & Bryan, 1960; Burns 1963; Woollet & Evans, 1971), in no case did the duration of elevation approach the 8 days seen in the present study after using the slowly-absorbed betamethasone formulation.

Comparable doses of the slowly absorbed dexamethasone TMA formulation have been shown to elevate plasma glucose by between 33% and 48%, but elevated glucose concentrations were maintained for 3 to 4 days only (Heidrich *et al*, 1963; Stockl, Onderscheka & Zacherl, 1969).

Results from different studies should be compared with caution, as undefined variations in experimental conditions may mask or accentuate differences in response. In different studies, comparable doses of the same formulation of corticosteroid may produce effects on blood glucose of either different magnitude (for example, Stockl, Onderscheka & Zacherl, 1969 and Schillinger & Klee, 1979) or different duration (for example, Goetsch, McDonald & Odell, 1959 and Neff, Connor & Bryan, 1960). While it has been shown that increasing the dose of a corticosteroid will increase the intensity and duration of its effect on plasma glucose (Burns, 1963; Woollett & Evans, 1971), in some studies a greater dose has actually produced a *smaller* response than that seen in other studies where the same formulation has been used (for example, Woollett & Evans, 1971 and Schillinger & Klee, 1979).

## 5. CONCLUSION

The results reported here indicate that a commercially produced 10 mg/ml aqueous suspension of betamethasone, when administered to cattle as a 2 ml dose by the subcutaneous route, can be expected to produce effects of moderate intensity and extended duration. The effects of this betamethasone formulation compare favourably, in terms of magnitude and duration, with those produced by the 'long-acting' dexamethasone formulation most commonly used in New Zealand for the induction of premature calving.

# CHAPTER VI

## INDUCTION OF PARTURITION IN CATTLE USING CORTICOSTEROIDS; A REVIEW


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# CHAPTER VI

## INDUCTION OF PARTURITION IN CATTLE USING CORTICOSTEROIDS; A REVIEW

### 1. INTRODUCTION

The first report on the use of corticosteroids to induce premature parturition in cattle was published by Adams in 1969 (see also, Adams & Wagner, 1969; Adams, 1970). However, the suggestion that corticosteroids might induce calving, if administered in late gestation, had been raised several years earlier (Tucker & Meites, 1965), and had been demonstrated for dexamethasone in 1965 (Frerking & Grunert, 1971) and for flumethasone in 1967 (Brown *et al*, 1970).

Following these initial reports, the use of corticosteroids to induce calving received much attention from researchers in Europe and North America (Jochle, 1971; 1973; Carroll, 1974) but it was only in New Zealand that this procedure gained widespread acceptance (Welch, 1971; 1972; Welch, Newling & Anderson, 1973). For example, it was estimated that in 1972 approximately 120,000 New Zealand dairy cows, or 5% of the national herd, were induced to calve prematurely (Welch, Newling & Anderson, 1973). By 1975 the figure had risen to 150,000 cows (McGowan, Welch & Hunter, 1975) and by 1978 it is likely that up to 75% of New Zealand dairy farmers were using the technique on at least some of their cows (Welch & Scott, 1979).

Induction of calving was readily accepted in New Zealand because of the largely seasonal pattern of dairying in which the peak of lactation should coincide with the maximum pasture growth. Cows must maintain a 12 month calving cycle for lactation to coincide with pasture availability, and induction of calving gives the otherwise late-calving cow the opportunity to become pregnant in time to calve at the optimal time the following year (Welch, Newling & Anderson, 1973; Widdows, 1974; Tervit , 1976). These same seasonal pressures also apply in parts of Australia (Allen & Herring, 1976) and to a lesser extent in Ireland (O'Farrell, 1979).

New Zealand dairy farmers were also attracted to the technique because it offered them a means of terminating the calving season, thus allowing the milking herd to be managed as a single unit (Welch, Newling & Anderson, 1973).

The technique has also been strongly promoted as a means by which the number of non-pregnant cows in a herd may be reduced. This aim can be achieved by extending the mating period by a further eight weeks. Then to avoid the inconvenience of a correspondingly extended calving period, parturition can be induced in selected cows about half-way through the following calving season (Anon., 1977; Welch, 1977; Welch & Scott, 1979).

There are, however, features of the technique which in some cases may be considered unacceptable, or at least undesirable. For instance, the mortality rate among calves born to induced parturition is very high under New Zealand conditions (Welch, 1972; Welch, Newling & Anderson, 1973; Welch & Kaltenbach, 1977; Welch, Crawford & Duganzich, 1977; Welch *et al*, 1979), but this loss is acceptable because of the low value of dairy calves in relation to what farmers see as the benefits of the technique (Welch, Newling & Anderson, 1973; Tervit, 1976). In other countries, in which calves are more valuable, the high calf mortality rate has meant that induction of calving, as practised in this country, has not found a similar widespread application (O'Farrell & Crowley, 1974; Carrol, 1974; Kudlac, 1978; Plenderleith, 1979).

In his 1973 review of the subject of induced parturition, Jochle made a distinction between 'precocious' and 'premature' parturition. Precocious parturition, he stated, is the delivery one to two weeks early of a fully developed foetus which does not need special care and which has an excellent chance of survival. Premature parturition, on the other hand, he defined as the delivery sometime during the last timester of gestation of a live foetus which needs special care if it is to survive. Thus, according to Jochle's definition, it is the induction of premature parturition which is often practised in New Zealand, while overseas it is the induction of precocious parturition which has been more readily accepted.

The ability of synthetic corticosteroids to induce premature or precocious parturition in cattle has found applications in five main areas. These are:

i) to synchronize the calving period with seasonal requirements;
ii) to synchronize the calving period with the availability of labour, to facilitate observation and management of calving, and to overcome the inconvenience caused by late-calving cows;
iii) to avoid or minimise dystocia problems and to terminate unwanted pregnancies;
iv) for the therapeutic termination of pregnancy for various clinical reasons;
v) and in conjunction with a milk fever control programme using vitamin D analogues.

The use of induced calving to suit the seasonal nature of New Zealand farming has been mentioned in the preceding paragraphs.

## 1.1 SYNCHRONIZATION OF THE CALVING PERIOD WITH AVAILABILITY OF LABOUR

Wagner, Willham and Evans (1974) showed that induction of calving using corticosteroids could be an effective management procedure for reducing the amount of supervision required and allowing more efficient use of available labour. Instead of constant daily supervision of beef cows during the calving season, observation could be limited to attending small groups of treated cows during a two-day period each week.

Each Saturday, they selected from their herd of 304 cows all those which were within 7 to 14 days of expected term. These cows were treated with short-acting formulations of either flumethasone or dexamethasone, with the result that they calved within 36 to 60 hours of injection. This system ensured a reduction in calf losses because adequate assistance was always available during the restricted calving period each week. It was estimated that substantial financial savings could be achieved with this technique.

For successful application of induced precocious parturition Wagner, Willham and Evans (1974) recommended that cattle should be in 'adequate to good body condition' at the time of treatment, that treated cows should be closely supervised during the two days after treatment, that newborn calves be assisted in their initial attempts to suckle, that all cases of retained foetal membranes be adequately treated and that the level of disease problems should be minimal in any herd in which this procedure is applied.

Winter and colleagues (1974) also recommended the induction of precocious parturition to compress the calving period of beef cattle into a predictable time span which could be synchronized with available labour, while similar recommendations were made for dairy herds by Beardsley and co-workers (1974).

The use of induced calving to cause expected late-calvers to calve about the same time as the majority of the herd was advocated for beef cattle by Poncelet and Moody (1975) and for dairy cattle by O'Farrell and Crowley (1973), Thomas (1975) and O'Farrell and Langley (1975).

## 1.2 INDUCTION OF CALVING TO MINIMISE DYSTOCIA PROBLEMS

From the time that the technique of inducing parturition with corticosteroids was introduced (Adams, 1969), it has been suggested that it could provide a useful means whereby potential dystocia problems due to foetal oversize could be minimised (Jochle, 1971). During the last week or so of gestation the reported weight gains of calves *in utero* range from about 0.3 to 0.6 kg/day (Beardsley, *et al*, 1974; Wagner, Willham & Evans, 1974; Muller *et al*, 1975; O'Farrell & Langley, 1975) and so it is reasonable to expect that calves delivered several days premature would be significantly smaller than if they had been carried to term, and hence less likely to have a difficult birth.

However, the results of field trials, in which the influence of induction on calving difficulty has been investigated, often appear contradictory.

Kelly, McLennan and Bell (1973) used a corticosteroid to induce calving in two groups of heifers bred to a Charolais bull: circumstances in which herd mates had experienced serious dystocia problems. They found that by reducing gestation by even 7 to 10 days, birth weights were reduced and the incidence of dystocia minimised. Calf vigour and survival were good and the authors commented that because the time of calving was predictable following treatment, close supervision could be provided to minimise or avoid any potential problems. Plenderleith (1974; 1979) found that induction of parturition with corticosteroids was a useful means of reducing dystocia problems in early-bred

dairy heifers and in heifers cross-bred to bulls of the larger beef breeds. These results are confirmed by those of Terblanche, Kritzinger and Van Heerden (1976), Gravetz and Kordts (1979), Holness and Sprowson (1979), Holden and Reader (1979), Herak, Makek and Tomaskovic (1979) and Diskin and Box (1981).

Several workers, however, found that while birth weights of calves born to induced calvings were reduced, there was either no reduction in calving difficulty, or the calving difficulty was actually greater amongst induced cows (O'Mary & Coonrad, 1973; Winter *et al*, 1974; Beardsley *et al*, 1974; La Voie *et al*, 1975; Beardsley *et al*, 1976).

In a 1974 study by Winter and his colleagues, it was shown that whereas beef cows induced to calve had significantly higher calving difficulty scores than naturally calving cows, calf vigour was not affected. Wagner (1975) stated that by reducing birth weight the chance of survival of crossbred calves could be improved, but Carroll (1974) pointed out that most controlled experiments clearly indicated no reduction in calving difficulty resulted even when a significant decrease in birth weight was achieved.

A probable reason for this apparent failure of the technique to reduce calving difficulty is that induced cows often calve without adequate preparation. Osinga, Stegenga and Jochle (1971) reported that relaxation and preparation of the birth canal was often incomplete, and Mutiga and Kiptoon (1978) encountered dystocia as a result. Furthermore, Welch, Newling and Anderson (1973) reported that uterine inertia was sometimes encountered during induced parturition. It was noted by Wollrab and Dittmer (1979) that the further from term parturition was induced, the greater were the calving problems, despite the reduced birth weight of the calves.

Diskin and Box (1981) suggested that when calving is induced using those corticosteroids which produce a quick response (that is, the so-called 'short-acting' formulations), higher rates of dystocia may be encountered. This suggestion,

in conjunction with a variety of biological differences including the stage of gestation at the time of treatment, may explain the differences in results between some published studies.

It also appears that where a herd history indicates that severe dystocia problems necessitating embryotomy or caesarean operation are likely to be encountered, then the induction of parturition with corticosteroids, by reducing calf birth weight, may reduce the severity of the problem. However, available evidence also suggests that where the incidence of dystocia due to maternal/foetal disproportion is not great, then calving difficulty may be increased as a result of incomplete preparation of the birth canal.

Corticosteroids have been shown to be reasonably safe and efficient abortifacients for use in mismated cows and heifers in the middle and last trimester of pregnancy (Sloan, 1976), although in some cases of unwanted pregnancy in young heifers, severe dystocia problems may still be encountered (Jackson, 1979).

## 1.3 INDUCTION OF PARTURITION FOR THERAPEUTIC REASONS

There are instances when the induction of premature or precocious parturition may be desirable either as a means of alleviating the clinical condition of a cow suffering a severe illness in late pregnancy, or as a means of salvaging a live calf from her (Hansen & Christiansen, 1971).

In 1971 Ballarini reported on the use of the technique to save either the cow or the calf in severe cases of traumatic reticuloperitonitis, cardiovascular disease or bronchopneumonia. Other clinical conditions which have benefitted from induction include preparturient prolapse of the vagina (Carroll, 1974; Herak, Makek & Tomaskovic, 1979; Herak & Kunst, 1979), 'downer cows' (Vujosevic *et al*, 1972; Mutiga & Kiptoon, 1978) and severe preparturient udder oedema (Mutiga & Kiptoon, 1978; Herak & Kunst, 1979). Prolonged gestations have been successfully terminated with corticosteroids (Karg *et al*, 1971; Mutiga & Kiptoon, 1978; Herak & Kunst, 1979).

Spence (1978) and Plenderleith (1979) tried unsuccessfully to treat cows with pregnancy toxaemia by inducing premature parturition.

Carter, Butler and Valli (1971) treated a cow with *hydrops allantois* by inducing parturition using dexamethasone on day 211 of pregnancy. Because the cow developed severe diarrhoea, metritis and pneumonia, and died 4 days after calving, they concluded that the technique was too risky to be useful. However, other workers (Christiansen & Hansen, 1974; Vandeplassche *et al*, 1974; Herak, Makek & Tomaskovic, 1979; Plenderleith, 1979) have subsequently found that induction of parturition using corticosteroids between the 7th and 9th months of pregnancy is generally a very successful means of treating *hydrops allantois*.

Corticosteroid treatment will not induce parturition if the calf is dead (Ballarini, 1971; Vanderplassche *et al*, 1974).

### 1.4 INDUCTION OF CALVING AS PART OF A MILK FEVER CONTROL PROGRAMME

Parenteral administration of vitamin $D_3$ or its derivatives has been used for a number of years to prevent milk fever in periparturient dairy cows. However, the efficacy of these drugs depends on their being given within the correct time interval before parturition. When given too soon and repeated there is a danger of toxicity. Researchers in Israel and in Northern Ireland have recently used corticosteroids to ensure that cows treated with vitamin $D_3$ analogues calve within the optimum period of time (Sachs & Hurwitz, 1978; Bar, Sachs & Hurwitz, 1980; McMurray, Rice & McBride, 1980).

## 2. THE MECHANISM BY WHICH CORTICOSTEROIDS INDUCE PARTURITION

In recent years there has developed an appreciation of the central role played by foetal cortisol in the initiation of normal calving. It is this understanding of the role of endogenous corticosteroids that has led to an explanation of the probable mechanism by which certain synthetic corticosteroids induce premature parturition. However, while the hormonal

changes which occur in the maternal and foetal circulations are now fairly well documented, there remain some areas of uncertainty about how such changes come about, and in separating cause and effect.

## 2.1 CHANGES IN MATERNAL PERIPHERAL PLASMA CONCENTRATION OF HORMONES ABOUT THE TIME OF CALVING

2.1.1 Cortisol. Adams and Wagner (1970) reported that there is a rise in maternal corticosteroid concentrations during the last 4 days before calving and that these concentrations then decline rapidly after delivery. While most other studies have indicated that maternal cortisol concentrations increase sometime during the last few days of pregnancy to peak at or shortly before delivery (Eberhart & Patt, 1971; Hoffman, Schams & Karg, 1972; Hoffman *et al*, 1973; Smith *et al*, 1973; Hudson *et al*, 1976; Seren *et al*, 1977; Bolte *et al*, 1977; Hoffman *et al*, 1977) such rises may be small (Welch *et al*, 1975; McGowan, Welch & Hunter, 1975; Hoffman *et al*, 1977) and, in some instances, because of individual variation, may not represent a statistically significant difference from the normal (Comline *et al*, 1974; Garverick *et al*, 1974; Fairclough *et al*, 1975; Hunter *et al*, 1977).

Prepartum cortisol concentrations in the dam are reported to range from between 4 to 6 ng/ml (Comline *et al*, 1974; Hudson *et al*, 1976; Hunter *et al*, 1977; Hoffman *et al*, 1977) up to 10 or 20 ng/ml (Comline *et al*, 1974; Fairclough *et al*, 1975; Welch *et al*, 1975; McGowan, Welch & Hunter, 1975; Hunter *et al*, 1977). The peak concentrations reported at or shortly before calving range from 8 to about 20 ng/ml (Hudson *et al*, 1976; Hoffman *et al*, 1977).

2.1.2 Oestrogens. A ten-fold increase in peripheral plasma oestrogen levels during the last month of pregnancy is the first major change in maternal steroid hormones as cows approach parturition (Smith *et al*, 1973). Over the last 4 or 5 weeks before calving oestrogen concentrations increase in a gradual fashion until the last week of gestation (Edqvist *et al*, 1973; Smith *et al*, 1973; Welch *et al*, 1975)

when concentrations rise sharply to a peak at, or even a day or two before, parturition (Hoffman *et al*, 1973; Smith *et al*, 1973; Mather *et al*, 1974; Comline *et al*, 1974; Peterson *et al*, 1975; Welch *et al*, 1975; Drinan, Wong & Cox, 1976; Seren *et al*, 1977; Stellflug *et al*, 1978).

A very rapid decline in plasma oestrogens begins at, or shortly before, delivery and minimum levels are reached within 24 to 36 hours (Hoffman, Schams & Karg, 1972; Edqvist *et al*, 1973; Hoffman *et al*, 1973; Smith *et al*, 1973; Mather *et al*, 1974; Comline *et al*, 1974; Tsang, Hackett & Turner, 1975; Peterson *et al*, 1975; Stellflug *et al*, 1978).

During late pregnancy, oestrone is the major oestrogen, being present at concentrations 5 to 10 times higher than oestradiol (Edqvist *et al*, 1973; Smith *et al*, 1973; Tsang, Hackett & Turner, 1975). Mather and co-workers (1974) reported differences in prepartum oestrogen concentrations between breeds, while Tsang, Hackett and Turner (1975) reported considerable variation between individuals.

2.1.3 Progesterone. Concentrations of progesterone in maternal peripheral plasma decline gradually over the last month or so of pregnancy (Fairclough, Hunter & Welch, 1975; Welch *et al*, 1975; Drinan, Wong & Cox, 1976; Hunter *et al*, 1977; Hoffman *et al*, 1977) until one or two days prior to parturition, at which time there is a sudden sharp decline (Edqvist *et al*, 1973; Hoffman *et al*, 1973; Comline *et al*, 1974; Seren *et al*, 1977). This sharp decline occurs either slightly before (Smith *et al*, 1973) or at the same time as (Hoffman, Schams & Karg, 1972) the rise in maternal cortisol.

2.1.4 Prostaglandins. Hunter and co-workers (1977) found little change in prostaglandin F concentrations in maternal peripheral plasma until between 48 and 36 hours prepartum. From that time on there was a gradual increase until 24 hours before delivery when there was a dramatic rise to peak levels during labour. In one cow they measured short, sharp peaks of prostaglandin F on days 14 and 9 prepartum, and suggested that

these surges of prostaglandin activity may have occurred in other cows but been missed because of the infrequent nature of the sampling.

2.1.5 Prolactin. Concentrations have been reported to show a sharp increase, lasting for about 24 hours, just before calving (Hoffman, Schams & Karg, 1972; Hoffman *et al*, 1973).

## 2.2 PLASMA HORMONE CONCENTRATIONS IN THE UTERINE AND OVARIAN VEINS

Oestrogen concentrations in the uterine vein or the ovarian vein rise slowly over the last few weeks of gestation before increasing sharply to a peak about 3 days before calving (Peterson *et al*, 1975; Hunter *et al*, 1977). However, oestrogen concentrations are 2 to 5 times higher in plasma flowing in the uterine vein than in the peripheral circulation (Comline *et al*, 1974; Evans, 1974; Peterson *et al*, 1975; Hoffman *et al*, 1977). Concentrations in the uterine vein reflect more accurately short term changes in oestrogen metabolism than do concentrations in the peripheral circulation (Hoffman, Schmidt & Schallenberger, 1979).

Changes in concentration of progesterone in the uterine and ovarian veins reflect those seen in the peripheral circulation. However, in contrast to the oestrogens, progesterone levels are lowest in the uterine vein and highest in the ovarian vein (Comline *et al*, 1974; Evans & Wagner, 1976; Hoffman *et al*, 1977). Progesterone concentrations in plasma from the ovarian vein may be 50 to 150 times higher than in the uterine vein or peripheral circulation (Fairclough, Hunter & Welch, 1975).

The concentration of prostaglandin F in uterine venous plasma does not show any consistent changes until two or three days before term, at which time levels rise sharply to peak during labour (Fairclough, Hunter & Welch, 1975).

## 2.3 HORMONAL CHANGES IN THE FOETUS

In 1970 Adams and Wagner suggested that the rise in maternal plasma cortisol concentrations seen just prior to calving might be the result of an increased cortisol secretion by the foetus and the transport of this hormone across the placenta into the maternal circulation. While it is now clear that cortisol does

not cross the placenta from the foetus in significant amounts (Hudson *et al*, 1976; Hoffman, Schmidt & Schallenberger, 1979) there is universal agreement that cortisol levels in the foetal calf rise as parturition approaches.

Between 20 days and about one week prepartum cortisol concentrations in the foetus increase slowly from 5 to 15 ng/ml to 10 to 20 ng/ml (Comline *et al*, 1974; Welch *et al*, 1975; Hunter *et al*, 1977). In the last 10 to 7 days of gestation foetal cortisol concentrations rise more rapidly, with a sharp increase in the last few hours before delivery (Comline *et al*, 1974; Fairclough *et al*, 1975; Welch *et al*, 1975; Hunter *et al*, 1977). Cortisol concentrations in the foetus at, or shortly before, delivery range from 50 to 100 ng/ml (Comline *et al*, 1974; Fairclough *et al*, 1977) in contrast to maternal concentrations which remain below 20 ng/ml (Fairclough *et al*, 1975).

Cortisol concentrations in the foetal calf 1 hour prepartum are about half those measured in the newborn calf 15 minutes after birth (Comline *et al*, 1974) and these levels reflect an abrupt increase in cortisol secretion by the calf in the last 5 minutes before delivery. Comline and his colleagues (1974) warn that cortisol levels measured in the newborn are thus unrepresentative of cortisol concentrations in the foetus *in utero*. Cortisol levels in the calf fall sharply after delivery (Eberhart & Patt, 1971; Comline *et al*, 1974).

In contrast to maternal oestrogen concentrations those in the foetal calf show little or no change as parturition approaches (Peterson *et al*, 1975; Hunter *et al*, 1975) although there may be a slight increase up to about 3 days before delivery (Hunter *et al*, 1974; Hoffman, Schmidt & Schallenberger, 1979). It is unlikely that oestrogens cross the bovine placenta in significant amounts (Hoffman *et al*, 1977; Hoffman, Schmidt & Schallenberger, 1979).

## 2.4 HORMONAL CHANGES ASSOCIATED WITH INDUCED CALVING

There is general agreement that apart from a profound decline in maternal endogenous cortisol concentrations following

induction of calving using synthetic glucocorticoids, the changes seen prior to delivery are essentially similar to those preceding natural birth.

Following the injection of 'short-acting' formulations of flumethasone (Hoffman, Schams & Karg, 1972; Hoffman *et al*, 1973; Drinan, Wong & Cox, 1976; Mostl *et al*, 1980) or dexamethasone (Adams & Wagner, 1970; Evans, 1974; Comline *et al*, 1974; Garverick *et al*, 1974; Evans & Wagner, 1976; Beardsley *et al*, 1976; Bolte *et al*, 1977) to induce calving, maternal cortisol levels decrease profoundly within a few hours and remain depressed for up to 5 days. 'Long-acting' formulations can be expected to depress endogenous cortisol levels for longer periods (Stellflug, Louis & Hafs, 1978; Fairclough, Hunter & Welch, 1981).

Under the same regimens cortisol concentrations in the calf remain unaffected prior to parturition, but exhibit the typical sharp rise during the birth process (Comline *et al*, 1974) so that concentrations equivalent to those recorded following natural birth are found (Drinan, Wong & Cox, 1976).

The injection of dexamethasone or flumethasone in late pregnancy causes a pronounced rise in maternal plasma oestrogen concentrations (Edqvist *et al*, 1971; Evans, 1974; Chew *et al*, 1978): an increase which begins about 24 hours after administration of the synthetic corticosteroid (Evans & Wagner, 1976; Terblanche & Labuschagne, 1980). While some workers report that total oestrogen concentrations are not significantly different from those seen in association with natural birth (Hoffman *et al*, 1973; Barth *et al*, 1978) others report that the *rate* at which oestrogen levels increase in the few days prior to calving is greater in induced cows (Comline *et al*, 1974; Beardsley *et al*, 1976; Bolte *et al*, 1977). Maternal levels at birth may be higher than (Drinan, Wong & Cox 1976; Kesler *et al*, 1976), lower than (Mostl *et al*, 1980), or essentially similar to (Hoffman, Schams & Karg, 1972; Comline *et al*, 1974; Bolte *et al*, 1977; Barth *et al*, 1978) those recorded in association with natural parturition.

Following delivery, oestrogen concentrations are reported to decline sharply (Evans, 1974; Lindell, Kindahl & Edqvist, 1977; Chew *et al*, 1978; Terblanche & Labuschagne, 1980) in a manner not different from that seen after natural calving (Bolte *et al*, 1977; Chew *et al*, 1978). In one report, however, it was reported that following induced calving, oestrogens took longer than normal to decline to basal levels (Drinan, Wong & Cox, 1976).

Progesterone concentrations are reported to decline in essentially the same pattern whether calving is natural or induced (Edqvist *et al*, 1971; Hoffman, Schams & Karg, 1972; Comline *et al*, 1974; Evans & Wagner, 1976; Drinan, Wong & Cox, 1976; Beardsley *et al*, 1976; Kesler *et al*, 1976; Bolte *et al*, 1977; Chew *et al*, 1978; Terblanche & Labuschagne, 1980). Evans and Wagner (1976) reported that the decline in progesterone levels seen prior to induced parturition preceded the increase in oestrogen levels.

Following administration of the 'long-acting' corticosteroid dexamethasone trimethylacetate, prostaglandin metabolites in maternal plasma increase gradually until 24 hours before birth, after which time they increase abruptly until delivery. An increase in oestrogen concentration precedes the increase in prostaglandin. The final precipitous decline in progesterone concentrations before birth is synchronous with the final rise in prostaglandin metabolites (Lindell, Kindahl & Edqvist, 1977; Fairclough *et al*, 1981).

Comline and co-workers (1974) reported that cows which failed to calve following administration of dexamethasone still exhibited a transient rise in plasma oestrogen concentrations, and Evans and Wagner (1974) noted that such cows had significantly lower oestrogen concentrations prior to treatment than those which did calve. Cows which failed to calve after dexamethasone treatment were also recorded as having higher pre-treatment concentrations of progesterone, and after treatment, progesterone levels in these non-responding cows did not decline as much as in cows in which induction was successful (Chew *et al*, 1978).

When parturition is induced by the administration of dexamethasone to the *calf*, maternal hormone changes are essentially identical to those recorded prior to natural calving, but changes may occur more rapidly as the dose of dexamethasone is increased (Hunter *et al*, 1974; Fairclough *et al*, 1975; Fairclough *et al*, 1981).

Minor differences in hormonal changes reported by different researchers may be due to differences in both the intensity and duration of action of different corticosteroid formulations used, the different stages of pregnancy at which calving was induced, the different sampling methods used and, in addition, to individual biological variation.

## 2.5 INTERPRETATION OF THE ENDOCRINE EVENTS PRECEDING PARTURITION IN THE COW

It is universally accepted that the signal to initiate parturition comes from the foetal hypothalamic-pituitary-adrenal axis (Kennedy, Kendrick & Stormont, 1957; Drost, 1969; Eberhart & Patt, 1971; Welch, Frost & Bergman, 1973; McGowan, Welch & Hunter, 1975; Hunter *et al*, 1977; Thorburn, Challis & Currie, 1977; Fairclough *et al*, 1981; and many others). Rising foetal cortisol levels provide the signal which initiates the parturition process and the transmission of this signal to the maternal compartment is mediated not by passage of hormones across the placenta from calf to mother (Hudson *et al*, 1976; Hoffman, Schmidt & Schallenberger, 1979), but through a change in the activities of steroidogenic enzymes in the placenta (Flint, Ricketts & Craig, 1979).

The increases in maternal cortisol levels at term arise more as a result of the process of parturition than as part of the mechanism involved in the termination of pregnancy (Smith *et al*, 1973; Hudson *et al*, 1976; Hunter *et al*, 1977; Hoffman *et al*, 1977). Cortisol concentrations in the dam probably rise in response to stress and, in fact, are highest in those cows experiencing calving difficulties (Hudson *et al*, 1976). The increase in prolactin concentrations is also probably a response rather than an initiating mechanism (Hoffman, Schmidt & Schallenberger, 1979).

That the corpus luteum in the cow is the only significant source of progesterone during late pregnancy is indicated by the high levels of progesterone in the ovarian venous plasma (Edqvist *et al*, 1973; Fairclough, Hunter & Welch, 1975; Hoffman *et al*, 1977). The high levels of oestrogen in the uterine venous plasma indicate that the foetoplacental unit is the main source of oestrogens (Edqvist *et al*, 1973; Peterson *et al*, 1975; Welch *et al*, 1975; Evans & Wagner, 1976). The lack of change in oestrogen concentrations in the calf rule out the possibility that the foetus itself is the source of the increased oestrogen production (Peterson *et al*, 1975; Hoffman, Schmidt & Schallenberger, 1979).

It is probable that in late pregnancy the rising foetal cortisol either induces the production of new enzymes or activates pre-existing enzymes in the placenta (Flint, Ricketts & Craig, 1979). The placenta then increases its production of oestrogens (Edqvist *et al*, 1973; Fairclough *et al*, 1975; Hunter *et al*, 1977; Fairclough *et al*, 1981), possibly by an enhanced uptake and conversion of progesterone (Comline *et al*, 1974; Lindell, Kindahl & Edqvist, 1977; Flint, Ricketts & Craig, 1979). Such a hypothesis would explain the gradual changes seen in these hormones over the last weeks of pregnancy. However, it is not proven that an enhanced conversion of progesterone to oestrogen by the bovine placenta is responsible for these hormonal changes (Fairclough *et al*, 1975; Fairclough *et al*, 1981) and this hypothesis has been disputed by some workers (Hoffman, Schmidt & Schallenberger, 1979) despite the fact that it has been confirmed in the sheep and goat (Flint & Ricketts, 1979; Flint, Ricketts & Craig, 1979).

The rising oestrogen concentrations may themselves cause regression of the corpus luteum (Flint, Ricketts & Craig, 1979) or they may induce the production by the cotyledons of luteolytic prostaglandin (Edqvist *et al*, 1973; Fairclough, Hunter & Welch, 1975; Hunter *et al*, 1977; Fairclough *et al*, 1981). Some workers, however, have suggested that luteolysis has already occurred before this final major surge of prostaglandin secretion and that the high levels of progesterone present up until that time are sufficient

to prevent the luteolytic action of prostaglandins (Thorburn, Challis & Currie, 1977; Hoffman, Schmidt & Schallenberger, 1979).

The uterine musculature and birth canal are prepared for parturition by the rising oestrogen concentrations which increase uterine contractility and enhance responsiveness to oxytocin and to prostaglandins (Edqvist *et al*, 1973; Evans & Wagner, 1976; Henricks, Rawlings & Ellicot, 1977; Thorburn, Challis & Currie, 1977; Hoffman, Schmidt & Schallenberger, 1979).

In summary then, while there remains some uncertainty over which hormonal changes are 'cause' and which are 'effect', there is little doubt that the initiating signal for the hormonal cascade which terminates pregnancy comes from the foetal calf through an increased secretion of cortisol. When premature parturition is induced by administration of synthetic corticosteroids it is this signal which is being crudely mimicked (Jochle, 1971; McGowan, Welch & Hunter, 1975). It is known that some synthetic steroids such as dexamethasone readily cross the placenta from the maternal circulation (see Nathanielsz, in the discussion section of Hoffman *et al*, 1977), and such synthetic corticosteroids presumably activate or induce the placental enzymes which are normally the target of endogenous foetal cortisol. Such a hypothesis is supported by the close similarity of the hormonal events preceding natural and induced calving.

## 3. CORTICOSTEROID FORMULATIONS WHICH HAVE BEEN USED IN AN ATTEMPT TO INDUCE CALVING

### 3.1 CORTISOL

Even when doses as high as 1 g of the naturally occurring corticosteroid cortisol (hydrocortisone) are used close to term, such treatment will not induce premature calving (Lauderdale, 1972; Hagg & Schlitz, 1973; Comline *et al*, 1974). Similarly, the use of adrenocorticotrophic hormone (ACTH), which stimulates the adrenal cortices to produce and release cortisol, is not effective in bringing about calving (Welch, Frost & Bergman, 1973).

### 3.2 PREDNISOLONE

Brown and colleagues (1970) reported that when 4 cows were injected intramuscularly with 200 mg of prednisolone on two occasions 24 hours apart during the last month of gestation, all animals developed signs of impending parturition, but none actually calved prematurely.

### 3.3 METHYLPREDNISOLONE

Hagg and Schlitz (1973) reported that 40 mg of methylprednisolone administered by intramammary infusion failed to induce premature calving in 10 cows between days 213 and 257 of gestation.

### 3.4 9α-FLUOROPREDNISOLONE ACETATE

Tucker and Meites (1965) noted that administration of 9α-fluoroprednisolone acetate caused udder development in pregnant heifers, and warned that 15 mg/day for several days in late gestation may induce premature parturition.

Lauderdale (1972) reported that 9α-fluoroprednisolone acetate at a dose of 20 mg/day for 3 days "....does not cause abortion, but does tend to shorten gestation length when administered within two weeks of expected parturition."

### 3.5 6α-9α - DIFLUOROPREDNISOLONE SODIUM PHOSPHATE

When 6α-9α-difluoroprednisolone sodium phosphate solution was injected intramuscularly in 1 to 3 doses of 10 to 20 mg at intervals of 2 to 3 days to 100 cows that were 4 to 9 months pregnant, none aborted or calved prematurely. Of another 45 cows with systemic disease which were also treated with this steroid, only 3 aborted, possible because of the severity of their disease symptoms (Ballarini & Bonomini, 1975).

### 3.6 EFFICACY OF CORTICOSTEROIDS LACKING A $C_{16}$ SUBSTITUTION

The results reported above for cortisol, prednisolone, methylprednisolone, 9α-fluoroprednisolone acetate and 6α-9α-difluoroprednisolone sodium phosphate, have led some workers to suggest that corticosteroids which lack a substitution in the $C_{16}$ position are ineffective as inducers of parturition

(Jochle, 1973; Anon., 1974; Ballarini & Bonomini, 1975). However, while in practice these steroids are relatively unreliable for this purpose, studies conducted by the Syntex Corporation (J.C. Siegrist, pers. com.) have demonstrated that both prednisolone and 9α-fluoroprednisolone acetate are, in fact, capable of causing premature calving.

It may be that corticosteroids lacking a substitution of the $C_{16}$ position do not cross the placenta as readily as those which do, and so may be less able to activate the steroidogenic enzymes which initiate the calving process. It is known that the placenta seems to be an effective barrier to cortisol (Hoffman *et al*, 1977) while dexamethasone, which is very effective for inducing calving, readily enters the calf following administration to the cow (Husband, Brandon & Lascelles, 1973; Nathanielsz, in Hoffman *et al*, 1977). In this same context it has been established that dexamethasone and triamcinolone, both of which are capable of inducing calving (Welch, Newling & Anderson, 1973) have a greater volume of distribution and apparently enter cells more readily than do cortisol or prednisolone (Araki *et al*, 1965).

### 3.7 DEXAMETHASONE SODIUM PHOSPHATE SOLUTION

Bosc (1971) treated 43 cows intramuscularly with either 8 mg or 16 mg of dexamethasone as the sodium phosphate ester. Cows were treated on either day 265 or day 274 of pregnancy. Response time varied with the size of the dose and stage of pregnancy. The average interval from treatment to calving for cows treated on day 265 was 127.9 hours following the 8 mg dose, and 51.2 hours following the 16 mg dose. Cows treated with these same doses on day 274 responded in 58.7 and 34.9 hours respectively. Some cows failed to respond to the 8 mg dose, especially when treated at the earlier stage of pregnancy.

Frerking and Grunert (1971) reported that a 10 mg dose of dexamethasone sodium phosphate would reliably induce calving when administered close to term, but at any time earlier than the last month of gestation a dose of even 40 mg was unreliable.

They reported also that heifers responded to treatment less consistently than cows.

In the last month of gestation, doses of dexamethasone sodium phosphate in the order of 16 to 20 mg have produced average response times of between 32.6 hours and 69 hours (Vujosevic *et al*, 1972; 1973; Bosc, Fevre & Vaslet de Fontaubert, 1975; Wollrab & Dittmer, 1979): the shorter response times being recorded for cows closer to term.

A few failures, even close to term, have been reported (Bosc, Fevre & Vaslet de Fontaubert, 1975; Wollrab & Dittmer, 1979).

## 3.8 'AZIUM' FORMULATIONS OF DEXAMETHASONE

Dexamethasone, in various formulations, is the most widely used and reported glucocorticoid suitable for the induction of calving. Many reports fail to specify the formulation of dexamethasone used, but most of the North American studies probably refer to a 2 mg/ml solution of dexamethasone in the organic solvent polyethylene glycol(1) or to a 2 mg/ml aqueous suspension of dexamethasone(2). Steroids injected in organic solvents frequently precipitate at injection sites (Ballard & Nelson, 1970), and it is not unreasonable to expect similar behaviour from dexamethasone in polyethylene glycol (W.B. Young, Schering Corporation, pers. com.). Absorption of such precipitated steroid occurs as from an aqueous suspension. For this reason, no great differences in effect between the two 'Azium' formulations need be anticipated, and so no attempt to distinguish between them is made in the following discussion.

Dexamethasone, specified only as 'Azium', was the formulation used in the first reported study of corticosteroid-induced calving (Adams, 1969). Adams treated 22 cows between days 235 and 280 of gestation with an intramuscular injection of 20 mg of dexamethasone. Nineteen of the 22 calved within 22 to 56 hours, with the mean response time being 45 hours.

(1) 'Azium Solution', Schering Corporation, Kenilworth, USA

(2) 'Azium Aqueous Suspension', Schering Corporation, Kenilworth, USA.

In a study aimed at reducing dystocia associated with foetal oversize and attempting to synchronise the calving period with availability of labour, Beardsley and co-workers (1974) induced dairy cows pregnant for 273 days using doses of 20 mg or greater. The average response time was 45 hours and the average gestation length was shortened by 5 days as compared to untreated cows. Calving difficulty was greater in the induced cows, however, because of inadequate preparation of the birth canal.

Wagner, Willham and Evans (1974) used an 'Azium' formulation of dexamethasone in a trial designed to assess the usefulness of inducing parturition in beef cattle as a management technique under conditions similar to those found in the field. The dose used ranged from 20 to 60 mg and cows were injected 7 to 14 days before their expected calving date. There were some failures (30 of 189) among the cows receiving a lower dose rate, but most cows calved between 36 and 60 hours after injection, 48 hours being the mean response time.

Similar results are reported from other major studies where 'Azium' formulations have been used (Muller *et al*, 1975; Beardsley *et al*, 1976; Kesler *et al*, 1976). The response time is shorter in cows treated closer to term (Adams & Wagner, 1970).

## 3.9 'DEXAMETHASONE', FORMULATION NOT SPECIFIED

Several studies have been reported in which neither the formulations of dexamethasone used, nor their source, are specified. Following administration of 20 mg doses of these formulations during the last three weeks of pregnancy mean response times were reported as 31.3, 57.4 and 52.5, 44.5, 47.7 52.8 and 44.5 hours (Levis, Slyter & Cotton, 1974; LaVoie, Winter & Moody, 1977; Piper, Combs & Peterson, 1978; Barth *et al*, 1978 and Davis *et al*, 1979, respectively).

For the dexamethasone formulations so far discussed, 'failure' rates, that is, cows not calving within 72 hours of injection, may range as high as 10 to 20%, even amongst cows treated during

the last three weeks of gestation (Adams & Wagner, 1970; Levis, Slyter & Cotton, 1974; Beardsley *et al*, 1974; Evans, 1974; Bosc, Fevre & Vaslet de Fontaubert, 1975; Garverick *et al*, 1974; Kesler *et al*, 1976; Barth *et al*, 1978; Davis *et al*, 1979).

## 3.10 DEXAMETHASONE IN INTRAMAMMARY FORMULATIONS

Fifteen of 103 cows treated with an intramammary mastitis preparation containing dexamethasone aborted or calved prematurely within 1 to 13 days. The total dose administered ranged from 10 to 40 mg, (Gindelle, Buchegger & Meyer, 1971). In another study, 5 out of 10 cows treated with 10 mg/quarter between day 213 and day 256 of gestation calved within 3 days (Hagg & Schlitz, 1973). Ballarini (1973) reported that parturition was induced more frequently by intramammary dexamethasone as gestation advanced and milk production declined. He also reported that the effect was dose related.

## 3.11 DEXAMETHASONE METASULPHOBENZOATE SOLUTION

Holness and Sprowson (1979) induced parturition in 11 cows and heifers using an intramuscular injection of dexamethasone metasulphobenzoate solution. A dose of 50 mg injected between days 262 and 271 of gestation induced calving within 42 to 73 hours; 13 days earlier, on average, than untreated control cows.

## 3.12 DEXAMETHASONE ISONICOTINATE

A 20 mg dose of dexamethasone isonicotinate suspension(3) has proven suitable as a means of inducing premature parturition in cows with a variety of disease conditions (Hansen & Christiansen, 1971; Christiansen & Hansen, 1974); most animals calving within 4 days of injection.

A group of 20 heifers treated with this preparation between days 269 and 280 of gestation had a mean response time of 42.2 hours, and calved, on average, 5 days earlier than untreated control animals (Grunert, Ahlers & Jochle, 1975).

(3) 'Voren', Boehringer Ingelheim, German Federal Republic

## 3.13 'SHORT-ACTING' AND 'LONG-ACTING' FORMULATIONS OF CORTICOSTEROIDS

The corticosteroid formulations discussed up to this point are usually described as 'short-acting' (S.A.). They are injected as solutions or as dilute aqueous suspensions of fine particles which dissolve relatively rapidly in body fluids. Such S.A. formulations are rapidly absorbed, producing peak blood concentrations within a few hours of injection and are eliminated from the body within 3 days (Anon., 1977).

So called 'long-acting' (L.A.) or depot formulations of corticosteroids may also be used to induce parturition. Such formulations may be prepared by a variety of methods (Vermeulen, 1975) which result in a preparation which is absorbed only slowly following injection. Depot corticosteroids take 2 to 4 days to achieve relatively low peak blood concentrations which may, however, take two or more weeks to decline to undetectable levels (Anon., 1977).

When used to induce calving, these two different classes of corticosteroid formulation differ in their effects.

## 3.14 DEXAMETHASONE TRIMETHYLACETATE

Of the L.A. corticosteroid formulations, dexamethasone trimethylacetate (TMA) suspension (5 mg/ml)[4] has been the most widely used for the induction of calving.

Following intramuscular injection into cattle, this ester, which has very low aqueous solubility, takes 2 to 3 days to produce peak blood concentrations of dexamethasone (McGowan, Welch & Hunter, 1975; Fairclough, Hunter & Welch, 1981).

Welch, Newling and Anderson (1973) first reported on the use of dexamethasone TMA to induce calving. Several hundred cows were injected intramuscularly with 20 mg at an average of 33 days before the expected calving date. The average response

(4) 'Opticortenol', Ciba-Geigy, Basel, Switzerland.

time was 16.3 days; much longer than the response time expected for the S.A. formulations so far discussed. However, the cows in this trial calved on average 16.7 days prematurely; earlier than obtainable with the S.A. corticosteroids. The earlier in pregnancy a cow was treated, the longer the response time (0.16 days longer per day of gestation).

Less than six months after the report by Welch, Newling and Anderson, Bailey and co-workers (1973) reported on a trial in which 15 Friesian cows were given 30 mg of dexamethasone TMA between days 240 and 252 of gestation. All calved between 4 and 20 days after treatment (mean 12.1), with no apparent relationship (probably because of the small number of animals involved) between response time and stage of gestation. Calves were born 14 to 32 (mean 23.7) days prematurely.

Several other workers have used doses of between 20 and 30 mg of dexamethasone TMA, in cows between 219 and 270 days pregnant, to induce calving within 7 to 45 days of treatment (O'Farrell & Crowley, 1973; 1974; Bachmann *et al*, 1975; Thomas, 1975; Sloan, 1976). Mean response times are reported to be between 11.5 and 14 days (O'Farrell & Crowley, 1974; McGowan, Welch & Hunter, 1975; Sloan, 1976; Welch, Crawford & Duganzich, 1977) and calves have been born an average of 14.6 days prematurely (McGowan, Welch & Hunter, 1975; Welch, Crawford & Duganzich, 1977).

The response to dexamethasone TMA is more variable than the response to S.A. corticosteroids, but cows may be induced to calve considerably earlier in pregnancy. The variability in response to L.A. corticosteroids may be no greater than the spread of natural calvings on either side of a mean 'normal' gestation length. For instance, cows calving in response to a single injection of 20 to 25 mg of dexamethasone TMA do so over a period ranging from 17 to 18 days (Bailey *et al*, 1973; O'Farrell & Crowley, 1974) to 22 or 23 days (Welch, Newling & Anderson, 1973; Welch, Crawford & Duganzich, 1977).

In the trial reported by Bailey and colleagues (1973) untreated control cows calved over a period of 18 days about

their mean calving date. MacMillan and Curnow (1976) from a study involving well over 25,000 untreated New Zealand dairy cows in a single season, reported a 21 day spread about the mean calving date. When data were corrected for possible recording errors, MacMillan and Curnow reported a standard deviation of 4.5 days about the mean gestation period of 282 days. However, when uncorrected data were used, data which included probable errors in recording the date of insemination, as may occur on the farm, a standard deviation of 13.9 days was calculated. This latter figure is greater than the standard deviation of 9.9 days reported by Welch, Crawford and Duganzich (1977) for cows induced in their field trial. Welch and Kaltenbach (1977) reported a standard deviation of 9.8 days for the calving date of their untreated control cows.

Dexamethasone TMA is widely used as the first injection in a two-injection regimen to induce premature calving (McGowan, Welch & Hunter, 1975; Welch, Crawford & Duganzich, 1977; Welch *et al*, 1979) and its use in this context is discussed in a later section (3.25).

## 3.15 DEXAMETHASONE TMA WITH PREDNISOLONE

Several workers have induced premature parturition with a formulation of dexamethasone TMA (2.5 mg/ml) in combination with prednisolone (7.5 mg/ml)(5) (Kelly, Mclennan & Bell, 1973; O'Farrell & Crowley, 1973; 1974; Bachmann *et al*, 1975; Lindell, Kindahl & Edqvist, 1977). When a particular dose of dexamethasone TMA is administered either alone or as the combination product, the response time is essentially similar (Bachmann *et al*, 1975) which is to be expected in light of the low efficacy of prednisolone as an agent for inducing parturition (Brown *et al*, 1970). Any response to this combination product is thus likely to be due to the dexamethasone component alone.

(5) 'Opticortenol-S', Ciba-Geigy, Basel, Switzerland.

## 3.16 DEXAMETHASONE ISONICOTINATE IN LONG-ACTING FORMULATION

A L.A. formulation of dexamethasone isonicotinate(6) has been used as the first injection in a two-injection regimen to induce calving (Welch *et al*, 1979). On average, cows that were 3 weeks or less from term at the time of treatment calved within 7 days of receiving 21 mg of this formulation and before receiving a second injection.

## 3.17 DEXAMETHASONE PHENYLPROPIONATE WITH DEXAMETHASONE SODIUM PHOSPHATE

Some workers have induced parturition in cows with a formulation consisting of 2 mg/ml of dexamethasone as the L.A. phenylpropionate ester suspended in a 1 mg/ml solution of dexamethasone sodium phosphate(7) (Allen & Herring, 1976; Terblanche, Kritzinger & Van Heerden, 1976; Mutiga & Kiptoon, 1978).

In a large field trial Allen and Herring (1976) administered 10 ml of this formulation to 716 dairy cows, 64.5% of which calved within 11 days. Treatment had been administered between 5.5 and 8 months of gestation.

Terblanche, Kritzinger and Van Heerden (1976) administered 13 ml of the same formulation to a line of 17 heifers in an attempt to minimise an anticipated dystocia problem. Treatment was given on about day 267 of pregnancy and 15 of the heifers calved between 36 and 60 hours afterwards (mean 52 hours). Similar results were reported by Mutiga and Kiptoon (1978).

While the results reported by Allen and Herring (1976) are apparently similar to those expected following administration of a L.A. dexamethasone formulation prior to the last month of gestation (3.14), the rather short response times reported by Terblanche, Kritzinger and Van Heerden (1976) and Mutiga and Kiptoon (1978) indicate that nearer to term it is probably the dexamethasone sodium phosphate component of this formulation

(6) 'Voren AP', Boehringer Ingelheim, German Federal Republic
(7) 'Dexafort', Intervet, Boxmeer, The Netherlands

which achieves the desired result.

3.18 BETAMETHASONE ALCOHOL

A short-acting 2 mg/ml suspension of betamethasone alcohol[8] was employed by Plenderleith (1974; 1979) to minimise dystocia problems. Doses of 20 to 30 mg administered approximately 10 to 14 days before term caused most cows to calve within 72 hours, although the author warned that a failure rate of 5 to 10% can be expected.

In two trials, reported by O'Farrell and Langley (1975) and O'Farrell (1979), a total of 95 late-calving dairy cows were treated with 40 mg of betamethasone alcohol. Cows were injected intramuscularly between days 255 and 288 of pregnancy and, in the two trials, 75% and 72% calved within 4 or 5 days of receiving a single injection.

The mean response times of 60, 62 and 69 hours reported by Plenderleith (1974) and 62 and 51.3 hours reported by O'Farrell and Langley (1975) and O'Farrell (1979), are similar to those reported for S.A. dexamethasone formulations administered at a similar stage of pregnancy (3.7 to 3.12 inclusive).

3.19 BETAMETHASONE ALCOHOL, LONG-ACTING FORMULATION

Diskin and Box (1981) reported on a small trial in which a L.A. formulation of betamethasone was used as the first injection in a two-injection regimen. Two-injection regimens are discussed below (3.25).

3.20 FLUMETHASONE SOLUTION

After dexamethasone, flumethasone is the steroid most widely investigated for use in the induction of calving. It is the most potent of the corticosteroids studied, having up to 50 times the glucogenic activity of dexamethasone in rats (McDonald, 1977) and 4 times the activity in calves (Woollett & Evans, 1971).

(8) 'Betsolan Injection', Glaxovet Ltd., Greenford, U.K.

The ability of flumethasone to induce premature calving was first reported by Brown and colleagues (1970) who, in 1965, administered doses of 2.5 mg twice in the space of 24 hours to 3 cows in late gestation. The cows calved within 24 to 36 hours to produce calves which were 20 to 33 days premature.

Osinga, Stegenga and Jochle (1971) administered single intramuscular doses of 2.5 mg, 5 mg or 10 mg of flumethasone to 26 cows and heifers between days 268 and 270 of gestation. Twenty-three calved within 72 hours, but 3 of the 10 given the smaller dose failed to calve for 7 to 9 days, despite the fact that signs of impending parturition did develop in response to the corticosteroid. The mean response time was 47.4 hours.

Ballarini (1971) also studied the effects of single doses of flumethasone ranging from 2.5 mg to 10 mg and administered at different stages of pregnancy. No dose was effective at less than 230 days gestation. After 260 days, 12 of 13 animals calved between 12 and 48 hours after receiving either a 5 mg or 10 mg dose.

While doses of flumethasone as low as 3.5 mg are reported to produce reliable results when administered in the last 12 days of gestation (Poncelet & Moody, 1975) most workers have chosen to use single 10 mg doses to induce calving (Karg *et al*, 1971; Jochle *et al*, 1972; Vujosevic *et al*, 1973; Winters *et al*, 1974; La Voie *et al*, 1975; Drinan, Wong & Cox, 1976; Hoerlein & Jones, 1977). Doses as high as 15 mg (Poncelet & Moody, 1976) and 20 mg (Kudlac, 1978) have also been used. Treatment administered in the last three weeks of pregnancy has produced mean response times ranging from 32.7 to 58 hours (Vujosevic *et al*, 1973; Winters *et al*, 1974; La Voie *et al*, 1975; Drinan, Wong & Cox, 1976; Poncelet & Moody, 1976; Hoerlein & Jones, 1977). The percentages of cows failing to calve within 72 hours range from 2.5% to 29% (Karg *et al*, 1971; Jochle *et al*, 1977). The closer cows are to term, the more quickly they respond to flumethasone (Vujosevic *et al*, 1973; Drinan, Wong & Cox, 1976; Kudlac, 1978).

As is the case with dexamethasone (Bosc, 1972), at a given stage of pregnancy larger doses produce shorter response times (Osinga, Stegenga & Jochle, 1971; Winters *et al*, 1974).

Induction regimens using 2 or 3 injections of flumethasone solution have been described (Lauderdale, 1972; Kordts & Jochle, 1975; Gravert & Kordts, 1979). Lauderdale (1972) was able to induce parturition in all of 5 cows treated at days 215, 216 and 217 of gestation with 3 successive 5 mg doses of flumethasone.

In terms of response-time, 'failure' rate and the stage of gestation at which parturition may reliably be induced, flumethasone solution is thus very similar in effect to S.A. formulations of dexamethasone and betamethasone. It may, however, be relied on to induce calving at doses one quarter to one half of those of the other two steroids.

### 3.21 FLUMETHASONE SUSPENSION

Welch, Newling and Anderson (1973) reported a trial in which flumethasone, formulated as a L.A. suspension, was compared with two other L.A. corticosteroids. A single 10 mg dose of the flumethasone suspension was administered to 165 cows which were, on average, 30.5 days from their expected calving date. The mean response time was 10.2 days, shorter than the response time for the other L.A. steroids tested and shorter than the response times reported for dexamethasone TMA in other trials (3.14).

While the response time to flumethasone suspension is much longer than the response time recorded for flumethasone solutions (3.20), cows can be induced to calve much earlier in pregnancy when the L.A. suspension is used.

### 3.22 FLUMETHASONE GRANULATE FOR ORAL ADMINISTRATION

When flumethasone solution is administered to cows by intramuscular injection there is a sharp increase in blood glucose concentrations. Peak levels occur within 18 to 22 hours and thereafter decline rapidly (Mulling, 1970; Woollett & Evans, 1971).

However, oral administration of a suspension of flumethasone produces a less marked elevation of plasma glucose which takes about 36 hours to reach its maximum but which is then maintained for a further 24 to 36 hours (Mulling, 1970). The oral formulation of flumethasone can thus be considered 'long-acting' (Jochle, 1971).

Osinga, Stegenga and Jochle (1971) treated 11 cows and heifers orally with either 5 mg or 20 mg of flumethasone granulate suspended in water. Treatment was administered on day 270 of gestation and 7 of the treated animals calved within 3 days. Another showed signs of impending parturition which regressed, with a calf being eventually born 9 days later. The remaining 3 showed no response to treatment.

### 3.23 TRIAMCINOLONE ACETONIDE

The L.A. steroid triamcinolone acetonide was compared with dexamethasone TMA or flumethasone suspension by Welch, Newling and Anderson (1973). A single 30 mg dose of triamcinolone induced calving in 166 cows which were, on average, treated 35.3 days before their expected calving date. The mean response time was 15.8 days, similar to that observed for dexamethasone TMA. However, significantly more dead calves were born to cows treated with triamcinolone.

The recommended 'therapeutic' dose of triamcinolone acetonide for cattle is 12 to 30 mg (McDonald, 1977). Davis and co-workers (1979) used low doses of 4 to 8 mg, administered 8 to 12 days before term, as the first of a two-injection regimen. Two out of 15 cows thus treated calved before a second corticosteroid injection could be administered 6 days after the triamcinolone.

### 3.24 INTRAVENOUS INJECTION OF CORTICOSTEROIDS

The response time following an intravenous injection of flumethasone (Carroll, 1974) or dexamethasone (La Voie, Winter & Moody, 1977) is no shorter than when the intramuscular route of administration is used. There may be a significantly

greater incidence of dystocia, however, following intravenous injection (La Voie, Winter & Moody, 1977).

### 3.25 TWO-INJECTION TREATMENT REGIMENS

In an attempt to improve the precision and reliability of the technique of induction of calving and to reduce side effects, various two-injection treatment schedules have been examined.

In situations where cows are known to be close to term, treatment schedules involving two injections of S.A. corticosteroids administered 12 to 24 hours apart have been reported (La Voie *et al*, 1975; Kordts & Jochle, 1975; La Voie, Winter & Moody, 1977; Gravert & Kordts, 1979). In some schedules one of the injections has been administered intravenously, but administration by this route has no advantage over intramuscular injection (La Voie, Winter & Moody, 1977). Only one of these investigations (La Voie *et al*, 1975) compared a single injection with a two-injection regimen in the same trial. Two 5 mg injections of flumethasone administered 12 hours apart were compared with a single 10 mg injection. Treatment was administered 8 to 13 days before term and no significant differences in effects were demonstrated.

When induction of calving is attempted relatively early in pregnancy and/or the expected calving dates are unknown, two corticosteroid injections administered several days apart have produced good results. The corticosteroid formulations used may be short-acting (Poncelet & Moody, 1975), long-acting (McGowan, Welch & Hunter, 1975; Sloan, 1976; Allen & Herring, 1976; Welch, Crawford & Duganzich, 1977) or the first injection may be a L.A. steroid and the second a short-acting one (McGowan, Welch & Hunter, 1975; Welch, Crawford & Duganzich, 1977; Welch *et al*, 1979; Davis *et al*, 1979; Holden & Reader, 1979; Diskin & Box, 1981).

While an initial injection of S.A. corticosteroid has a 'priming' effect leading to a better response to the second

injection 7 days later (Poncelet & Moody, 1975), the best results are obtained when an initial injection of L.A. steroid is followed 6 to 12 days later by an injection of a S.A. formulation (McGowan, Welch & Hunter, 1975; Welch, 1977; Welch, Crawford & Duganzich, 1977). The blood profile of glucocorticoid produced by such a regimen more closely mimics the pattern of foetal cortisol secretion which occurs prior to natural parturition (McGowan, Welch & Hunter, 1975).

Injection schedules in which an initial 'priming' dose of corticosteroid is followed some days later by an injection of a prostaglandin analogue have been reported too (Bosc, Fevre & Vaslet de Fontaubert, 1975; Beal *et al*, 1976; Day, 1977; Day, 1979; Welch *et al*, 1979), but will not be further discussed here.

Single injections of L.A. glucocorticoids are able to induce abortion or parturition as early as the middle trimester of pregnancy (Sloan, 1976), but the response time is very variable, ranging from 6 to 29 days (Welch, Newling & Anderson, 1973; 3.13). The administration of a S.A. formulation 6 to 12 days after the long-acting one will result in most calves being born within 3 days after that second injection (McGowan, Welch & Hunter, 1975; Welch, & Kaltenbach, 1977; Welch *et al*, 1979; Davis *et al*, 1979). The response to this second injection is thus very similar to the response seen following a single S.A. corticosteroid injection during the last two or three weeks of pregnancy and the undesirable variability in response to L.A. corticosteroids alone is eliminated.

Initial doses of corticosteroid that are by themselves too small to induce calving may, through their 'priming' action, provide for a more predictable response to a second injection administered some days later and may reduce the incidence of undesirable side effects (Poncelet & Moody, 1975; Davies *et al*, 1979).

# 4. EFFECTS OF INDUCED CALVING ON THE CALF

## 4.1 CALF MORTALITY FOLLOWING INDUCED CALVING

Mortality amongst calves born as a result of induced parturition is mainly, but not solely, a function of their prematurity (Adams, 1969; Welch, Newling & Anderson, 1973).

Most workers report that when calving is induced by S.A. glucocorticoid formulations administered within about 2 weeks of term, little or no difference is seen in the number of stillbirths, in calf 'vigour' or in calf viability (Osinga, Stegenga & Jochle, 1971; Hansen & Christiansen, 1971; Vujosevic *et al*, 1973; La Voie & Moody, 1973; O'Farrell & Crowley, 1974; Plenderleith, 1974; Poncelet & Moody, 1975). Despite these observations, in some trials greater calving difficulty has been recorded in the induced cows (Winter *et al*, 1974; La Voie *et al*, 1975; Muller *et al*, 1975).

Researchers are not unanimous, however, on this subject. Christiansen and Hansen (1974) induced cows in the last 3 weeks of gestation and recorded 15% stillbirths from cows calving within 4 days of treatment and 23% from cows taking longer. They noted more stillbirths from cows treated before day 277 of gestation, but reported that there was no increase in mortality during the first 8 days of life among calves born alive. This latter finding is in contrast to that of Wollrab and Dittmer (1979) who treated cows prior to day 275 of gestation and reported that calf losses in the first 4 weeks of life were twice as great as occurred in calves born to untreated cows.

Carroll (1974) reported that when cows from a Charolais herd (mean gestation length 290 days) were treated on day 270, calf losses were 'distressingly high' during the first 60 days after parturition.

There are also reports of *improved* viability amongst calves born to calvings induced late in gestation. Winter

(1974) reported that calf viability in beef cattle decreases from an optimum as gestation length is either decreased or increased from 275 days, thus when parturition was induced to produce calving at or near day 275, calf mortality was reduced (Winter, 1974) and calf vigour was improved (Poncelet & Moody, 1975; 1976).

Whenever cows are induced to calve more than about 2 weeks prematurely the incidence of stillbirths rises (O'Farrell & Crowley, 1974; Thomas 1975). O'Farrell and Langley (1975) advanced the mean calving date by 2 weeks and recorded a 6.6% incidence of stillbirths and a total of 13.3% of calves dead within 7 days. These losses are still not markedly greater than would be expected in Great Britain after natural calving; 3.9% to 5.2% of calves born dead (Wijeratne & Steward, 1970; Roy, 1980) and a further 6% dying 'before sale' (Roy, 1980).

However, when Allen and Herring (1976) induced 709 cows to calve between 5.5 and 8 months of gestation, 14.2% of calves were born dead. By 4 weeks the loss had risen to 24.3% as compared with a 2.7% loss amongst 4,330 calves born naturally on the same farms.

In other trials in which calving has been induced relatively early in gestation using single injections of L.A. corticosteroids, the reported incidences of stillbirths range from 11.2% to 44.6% (Welch, Newling & Anderson, 1973; O'Farrell & Crowley, 1973; 1974; McGowan, Welch & Hunter, 1975). The incidence of stillbirths is reported to increase by 2.4% for each week earlier in pregnancy that calving is induced (Welch, Crawford & Duganzich, 1977).

The mortality among calves is claimed to be higher than would be predicted solely on the basis of prematurity (Welch, Newling & Anderson, 1973). Both uterine inertia and 'premature separation of the placenta' have been postulated as factors contributing to the high incidence of stillbirths.

The particular L.A. corticosteroid used may influence the number of calves born dead. This feature is indicated in

the report of Welch, Newling and Anderson (1973) in which the 44.6% mortality among calves following the treatment of cows with triamcinolone acetonide was significantly greater than the 31.3% and 31.5% recorded following dexamethasone TMA or flumethasone suspension treatments respectively.

Both calf mortality (Adams, 1969; Welch, Crawford & Duganzich, 1977) and response time (Adams & Wagner, 1970; Welch, Newling & Anderson, 1973) increase with prematurity. However, the response time *per se* also influences the proportion of calves born dead following induction of calving (Welch, Newling & Anderson, 1973; Christiansen & Hansen, 1974). Welch, Newling and Anderson (1973) noted that for a given stage of pregnancy, a cow gave birth to a live calf an average of 3.2 days sooner than to a dead one. It was thus partially with the aim of reducing calf mortality that McGowan, Welch and Hunter (1975) introduced their two-injection regimen to shorten response time.

In one trial, in which calves were born on average 2 weeks premature, the lowest incidence of stillbirths was recorded amongst calves born to a two-injection treatment schedule involving a L.A. corticosteroid followed 6 days later by a short-acting one. Six percent of calves were born dead following this treatment, a percentage significantly lower than the 11.2% recorded in the same trial for calves born to a single injection of L.A. steroid. The response time following the two-injection schedule was also shorter, but the difference was not statistically significant (Welch, Crawford & Duganzich, 1977).

When calving is induced with a two-injection regimen, mortality is understandably lower amongst calves born to the first injection than amongst those born following the second injection (3 to 8% as against 23 to 34%) (Welch *et al*, 1979), as cows calving to the first injection are those closer to term at the time treatment is initiated.

Welch and his co-workers (Welch & Kaltenbach, 1977; Welch *et al*, 1979) have related calf mortality to calf size as well as to prematurity. In a trial where calves were born

up to 1 month premature, the mortality amongst calves weighing 15 kg or more at birth was the same (5 to 6%) regardless of whether they were born naturally or following induction. No calves from the untreated control group of cows weighed less than 15 kg at birth, but amongst those calves which weighed less than 15 kg and which were born as a result of corticosteroid treatment, mortality rates ranged from 24% to 53% (Welch & Kaltenbach, 1977). In another trial (Welch *et al*, 1979) a 13% calf mortality was recorded for induced calvings in which calves weighed more than 15 kg, while a 32% mortality was recorded for calves of less than that weight. Overall, 16.6% of calves were born dead and the figure increased to 35% by 4 days after birth. This latter figure may be compared to the 5.7% of naturally born calves either stillborn or dying within the first week of life (Anon.,1977-78).

## 4.2 IMMUNE STATUS OF CALVES

When S.A. corticosteroids are used to induce calving close to term, the immunoglobulin concentration of colostrum is no different from that in naturally calving cows (Beardsley *et al*, 1976; Hoerlein & Jones, 1977). Even so, the total immunoglobulin content may be reduced because the total volume of colostrum may be reduced (Hoerlein & Jones, 1977). The ability of these calves from induced births to absorb immunoglobulins from colostrum is not impaired, as serum gamma globulin concentrations after suckling are similar to those of calves born naturally (Muller *et al*, 1975; Langley & O'Farrell, 1976; Hoerlein & Jones, 1977; Vukotovic *et al*, 1978).

The situation is different when calves are born earlier in pregnancy after using L.A. corticosteroids. Such premature calves are lethargic, slow to stand and suck properly (Adams, 1969; Welch, Newling & Anderson, 1973) and, in the critical time available for immunoglobulin absorption, they may not receive an adequate intake of colostrum.

The colostrum available to such calves has a reduced content of immunoglobulins (Bailey *et al*, 1973) and the

composition of the secretion may be further altered by the pre-calving milking which is often found to be necessary to avoid udder damage (Welch, 1972; O'Farrell & Crowley 1973; Bachmann *et al*, 1975; Allen & Herring, 1976).

Calves born to cows treated with L.A. corticosteroids also have an impaired ability to absorb immunoglobulins (Bailey *et al*, 1973; Husband, Brandon & Lascelles, 1973). This is because the steroid is available to act upon the foetus for a sufficient length of time to promote premature 'closure' of the immunoglobulin absorption processes of the gut (Husband, Brandon & Lascelles, 1973). It is not surprising, therefore, that some 60% of calves born following induction with L.A. corticosteroids may be hypogammaglobulinaemic (Bailey *et al*, 1973).

### 4.3 CALF GROWTH RATES SUBSEQUENT TO INDUCTION

When cows are induced to calve close to expected term, calves grow at the same rate as calves after natural births: little or no difference in body weight is apparent at weaning (Carroll, 1974; Levis, Slyter & Cotton, 1974; Muller *et al*, 1975; Winter & Moody, 1977; Chew *et al*, 1978).

In general though, the smaller a calf is at birth, the slower will be its absolute growth rate for at least the first 6 months of life (Roy, 1980) and when calves are delivered more than a few days prematurely, a significant depression in growth rate will become apparent. When Bailey and co-workers (1973) caused calves to be born 14 to 32 days early, the calves grew at a significantly slower rate (0.43 kg/day) than calves born to untreated cows (0.57 kg/day).

## 5. EFFECTS OF INDUCED CALVING ON THE COW

### 5.1 RETAINED FOETAL MEMBRANES AND UTERINE INVOLUTION

Virtually all workers have reported that cows induced to calve prematurely have a greater than normal incidence of retained foetal membranes (RFMs). Results reported in different trials, and hence following different treatments, are not strictly comparable unless control groups have been included in

each trial. This is because the incidence of RFMs differs widely from country to country (a range of 1.96% to 11.7% has been cited by Arthur, 1979) and between herds, seasons, years (Sandals *et al*, 1979) and breeds (Wagner, Willham & Evans, 1974). A further complication to any comparison is the time after calving at which membranes are said to be 'retained'. Most workers assess foetal membrane retention at 24 hours post-partum although some assess them at 12 hours and a few at 72 hours. Also, although most workers rely on a visual appraisal, this method tends to under-estimate the incidence (Carroll, 1974; Day, 1979) as substantial portions of the membranes may be retained *in utero* without being visible at the vulva. Accordingly a few workers assess retention by palpation.

In trials using S.A. corticosteroids within about 2 weeks of term, and in which untreated control cows have been included, the reported incidence of RFMs in treated cows ranges from 8.3% to 93%, while 0% to 20% of naturally calving cows had RFMs (Table 6:I). Levis, Slyter and Cotton (1974) reported no significant difference between untreated cows and induced cows in the incidence of RFMs.

Table 6:I The incidence of RFMs in different trials using short-acting corticosteroids within 3 weeks of term.

| Incidence (%) of RFMs | | Reference |
|---|---|---|
| Control cows | Induced cows | |
| 0.6 | 53.6 to 62.5 | Winter *et al*, 1974 |
| 10 | 76 | Beardsley *et al*, 1974 |
| 0 to 2 | 70 to 78 | La Voie *et al*, 1975 |
| 15 | 61.9 | Grunert, Ahlers & Jochle, 1975 |
| 1 | 20 to 39 | Poncelet & Moody, 1976 |
| 0 to 6 | 23 to 78 | La Voie, Winter & Moody, 1977 |
| 16.6 | 66.6 | Bolte *et al*, 1977 |
| 0 | 8.3 | Barth *et al*, 1977 |
| 14 to 20 | 52 to 93 | Davis *et al*, 1979 |

The impression gained from the literature is that the incidence of RFMs tends to be lower when calving is induced with L.A., rather than S.A., corticosteroids. However, as mentioned earlier, comparisons between trials must be made with caution. Bachmann and co-workers (1975) reported a 7.7% incidence of RFMs in cows delivering single calves after treatment with dexamethasone TMA. Untreated control cows in the same trial had no RFMs. In the trial reported by Bailey and others (1973), 2 out of 15 (13.3%) induced cows had RFMs, whereas 3 out of 15 (20%) untreated cows had them.

In other trials using L.A. formulations, in which no untreated control cows have been included, the incidence of RFMs has been zero, (Thomas, 1975), 8.6% (O'Farrell & Crowley, 1974), 9% (Welch, Newling & Anderson, 1973) and 11.5% and 22% (Welch, Crawford & Duganzich, 1977).

Allen and Herring (1976) induced parturition in 709 cows, the majority of which were between 5.5 and 8 months pregnant, using a product containing a combination of L.A. and S.A. dexamethasone esters (see 3.17). They reported a 50% incidence of RFMs. Terblanche, Kritzinger and Van Heerden (1976), using the same product on heifers within 2 weeks of term, a stage of pregnancy at which the response is most likely to be due to the S.A. component, reported that 68.8% had RFMs.

Davis and colleagues (1979) induced calving using either a single injection of a S.A. steroid or an injection of a S.A. steroid administered some days after a priming injection of a L.A. steroid. The cows in the latter group had significantly fewer RFMs than the cows treated with the S.A. corticosteroid alone. However, they still had more RFMs than untreated cows. Uterine involution also occurred more quickly when calving was induced with two injections rather than one (Davis *et al*, 1979).

The incidence of RFMs following induction using other two-injection regimens has ranged from 15.1% to 40% (Welch & Kaltenbach, 1977; Welch Crawford & Duganzich, 1977; Welch

*et al*, 1979; Davis *et al*, 1979).

For any treatment regimen, the incidence of RFMs increases with prematurity (Vujosevic *et al*, 1973; Christiansen & Hansen, 1974; Wagner, Willham & Evans, 1974; Grunert, Schultz & Ahlers, 1975; Welch *et al*, 1979): under New Zealand conditions the incidence increases, on average, by 2.9% per week (Welch, Crawford & Duganzich, 1977). The incidence of RFMs is highest amongst induced cows experiencing difficulty in calving (Beardsley *et al*, 1974).

Most, if not all, of the cows which retain the placenta following induced calving will experience some degree of metritis (Adams, 1969; Adams & Wagner, 1970; Evans, 1974) and uterine involution is delayed. Retention of the foetal membranes delays re-epithelialization of caruncular surfaces as well as inhibiting resolution of the caruncular mass and delaying the decline to normal in the numbers of neutrophils in the endometrium (Evans, 1974). Uterine tone is reduced in cows induced to calve using corticosteroids and this delays the early stages of involution (Piper, Combs & Peterson, 1978).

In large-scale field trials, between 22.4% and 25% of all cows with RFMs have required antibacterial therapy for infections subsequent to retention (Welch, Newling & Anderson, 1973; Welch & Kaltenbach, 1977; Welch *et al*, 1979).

## 5.2 EFFECTS OF RETAINED FOETAL MEMBRANES ON SUBSEQUENT REPRODUCTIVE PERFORMANCE

Following natural calving, significantly more non-pregnant cows are found 2 to 3 months later amongst those which retained their foetal membranes than amongst those which did not (Moller *et al*, 1965; Kay, 1978). While retention does not *in itself* have a marked effect (Arthur, 1979; Sandals *et al*, 1979) cows which retain their foetal membranes are much more likely to develop metritis than those which do not and metritis does have a detrimental effect on subsequent reproductive performance (Sandals *et al*, 1979). It might be expected,

therefore, that because of the high incidence of RFMs associated with the technique, induction of calving would result in reduced reproductive efficiency.

While the high incidence of metritis amongst induced cows with RFMs has already been mentioned (5.1) surprisingly few reports mention any reduction in fertility following induction. The reproductive performance of cows induced to calve by Adams (1969) and Adams and Wagner (1969) was suboptimal, but numbers were small and no untreated control animals were available for comparison.

Poncelet and Moody (1976) reported that although there was no significant difference in subsequent fertility between their treated group and the control group, those cows which retained foetal membranes had a significantly lower pregnancy rate and required more services per conception than cows which did not have RFMs.

Welch and Kaltenbach (1977) induced cows to calve about a month prematurely. They reported that the calving to conception interval increased by 0.54 days for each day of prematurity and concluded that while the fertility of the induced cows was reduced in comparison with the controls, their mean conception date was earlier than if they had been left to calve naturally. After a later trial, Welch and his colleagues (1979) reported that while RFMs had no effect on the interval from either calving to first oestrus, or calving to conception, more cows were non-pregnant 2 to 3 months later amongst those which had been induced to calve. Holness and Sprowson (1979), in a rather small trial, reported that the conception rate was markedly lowered by induction, as did Kudlac (1978). O'Farrell (1979) reported that induced cows with RFMs had significantly longer calving to conception intervals.

The overwhelming majority of workers, however, claim that induction of calving, together with its attendant high

incidence of retained afterbirth, has no adverse effect on subsequent reproductive performance (Osinga, Stegenga & Jochle, 1971; Lauderdale, 1972; Poncelet *et al*, 1974; Levis, Slyter & Cotton, 1974; Beardsley *et al*, 1974; Wagner, Willham & Evans, 1974; Carroll, 1974; La Voie *et al*, 1975; Schmitt *et al*, 1975; Beardsley *et al*, 1976; Bolte *et al*, 1976; La Voie, Winter & Moody, 1977; Barth *et al*, 1978) although in many trials untreated control groups, against which valid comparison could be made, were lacking (Welch, Newling & Anderson, 1973; O'Farrell & Crowley, 1973; Christiansen & Hansen, 1974; O'Farrell & Langley, 1975; Allen & Herring, 1976; Terblanche, Kritzinger & Van Heerden, 1976).

In several of the trials referred to above, all cows recorded as retaining their foetal membranes received local and/or systemic antibiotic therapy (Osinga, Stegenga & Jochle, 1971; Beardsley *et al*, 1974; 1976; Wagner, Willham & Evans, 1974; Levis, Slyter & Cotton, 1974), a practice shown to improve reproductive performance (Bannerjee, 1965).

The effects of induction of calving on subsequent fertility must remain open to debate. It is, however, reasonable to assume that in the New Zealand situation at least, in which a high proportion of cows require veterinary treatment for uterine infections resulting from retention of the foetal membranes, the practice of inducing premature calving may lead to some increase in infertility. Any such detrimental effect on fertility must be balanced against the advantage accruing from the significant advance in calving date and the lengthened mating period (Anon., 1977).

## 5.3 ATTEMPTS TO REDUCE THE INCIDENCE OF RETAINED FOETAL MEMBRANES

On the basis of a small trial involving a total of 18 cows, Garverick and co-workers (1974) claimed that administration of oestradiol benzoate, in conjunction with dexamethasone, significantly reduced the incidence of retained foetal membranes. Other studies have failed to confirm these results (Adams, 1969;

Karg *et al*, 1971; La Voie & Moody, 1973; Grunert, Ahlers & Jochle, 1975; Grunert, Schultz & Ahlers, 1975; Barth *et al*, 1978; Davis *et al*, 1979; Wollrab & Dittmer, 1979). Oestrogen treatment may shorten response time (Garverick, *et al*, 1974; Muller *et al*, 1975; Grunert, Ahlers & Jochle, 1975; Barth *et al*, 1978; Piper, Combs & Peterson, 1978), but may also increase the incidence of dystocia (Karg *et al*, 1971; La Voie & Moody, 1973; Grunert, Ahlers & Jochle, 1975). Massive doses (120 mg) of diethylstilboestrol, administered to cows which are induced to calve within a week of term, may reduce the incidence of RFMs (Grunert, Ahlers & Jochle, 1975; Grunert, Schultz & Ahlers, 1975).

Adams and Wagner (1970) suggested that administration of progestagens might reduce the severity of the RFM problem. However, other workers (Osinga, Stegenga & Jochle, 1971; Jochle *et al*, 1972) demonstrated that progestagens have no influence on the incidence of RFMs and interfere with the parturition process, resulting in an increase in the incidence of dystocia, calf mortality and cow mortality.

Ecbolic drugs appear to be without influence on the incidence of RFMs (Adams, 1969; Piper, Combs & Peterson, 1978).

There are, therefore, no means yet available for reducing the number of induced cows which subsequently retain their foetal membranes. The severity of the effects of the problem may be reduced, however, by routinely administering oral, parenteral or local antibiotic therapy (Carroll, 1974; Wagner, Willham & Evans, 1974; Poncelet & Moody, 1976).

## 5.4 THE RISK OF MASTITIS IN COWS TREATED WITH CORTICOSTEROIDS

Because corticosteroids are known to decrease resistance to infection under certain circumstances, and may exacerbate pre-existing infections (McDonald, 1977), it has been suggested that induction of calving is contraindicated in cows in which mastitis is present (Widdows, 1974). Buddle, Midgley and Ashby (1976) even suggested that the use of corticosteroids might predispose cows to mastitis.

The latter workers reported that during the first 14 days after treatment with dexamethasone TMA, 9 quarters in 4 cows shed staphylococci whereas only 2 quarters in 3 untreated cows did so. The validity of their conclusions may be questioned, however, because of the high incidence of subclinical mastitis recorded prior to dexamethasone treatment. Welch and Kaltenbach (1977) suggested that unsatisfactory milking shed hygiene could have been responsible for the apparent increase in mastitis reported by Buddle and his colleagues.

Certainly, Welch and Clayton (1977) found no increased incidence of mastitis in a retrospective survey of induced cows, nor was any increase recorded in a trial carried out to study production after induced parturition (Welch & Kaltenbach, 1977). Measurements of leucocyte concentrations in the milk of treated cows have not indicated any increased incidence of clinical or subclinical mastitis (Bailey *et al*, 1973; Beardsley *et al*, 1974; Welch & Kaltenbach, 1977).

Several workers have warned of the need to commence milking some corticosteroid-treated cows before they calve, otherwise their engorged udders become subject to trauma which may predispose to mastitis (Welch, 1972; O'Farrell & Crowley, 1973; Bachmann *et al*, 1975; Allen & Herring, 1976).

## 5.5 METABOLIC DISEASE IN COWS FOLLOWING INDUCTION OF PARTURITION

Welch (1971) stated, without giving specific reasons, that S.A. corticosteroids used to induce calving, but not L.A. ones, may increase the incidence of metabolic disease.

Widdows (1974) claimed that when the response time following treatment with dexamethasone TMA is prolonged the onset of metabolic disease may be 'more rapid than usual'. Beardsley and co-workers (1974) recorded a small but non-significant increase in milk fever cases in a group of cows induced to calve using a S.A. dexamethasone formulation.

It is, however, unlikely that corticosteroids used to induce parturition have any significant effect on the incidence

of periparturient metabolic disease. Serum calcium and magnesium levels are unaffected by fluorinated corticosteroids (Neff, Connor & Bryan, 1960; Carter, Butler & Valli, 1971; Hartman & Kronfeld, 1973; Kelly, Mclennan & Bell, 1973; Beardsley *et al*, 1976; Sali *et al*, 1976; Kudlac, 1978) and most studies have shown serum phosphorus levels to be essentially unaffected. Nevertheless, Neff, Connor and Bryan (1960) recorded a depression of serum phosphorus after administration of 9α-fluoroprednisolone acetate, a steroid relatively ineffective for inducing calving (see 3.4 and 3.6).

Beardsley and co-workers (1976) reported that serum concentrations of calcium, magnesium and phosphorous did not differ between induced and untreated control cows. Sachs and Hurwitz (1978), Bar, Sachs and Hurwitz (1980) and McMurray, Rice and McBride (1980) have all used corticosteroids to induce calving in cows previously treated with vitamin D analogues to prevent milk fever. McMurray and colleagues concluded that the corticosteroids used had no significant detrimental effects on calcium metabolism, an opinion shared by Sachs (pers. com.).

It has been suggested (Widdows, 1974) that when metabolic disease does occur in cows which have been induced to calve, some individuals are less responsive to treatment than usual. Such cows may be less able to counteract the stress of metabolic disease because the corticosteroids used to induce parturition have the effect of depressing endogenous cortisol secretion (2.4).

## 5.6 MISCELLANEOUS ILLNESSES ASSOCIATED WITH INDUCTION OF CALVING

In two large New Zealand trials mortality amongst induced cows in the month following calving was 0.25% and 2% (Welch, Newling & Anderson, 1973). While no control groups were available for comparison, these figures may indicate that the technique is responsible for some increase in cow deaths, because Moller (1978), in a survey of 15 New Zealand dairy herds over a 4 year period, reported an *annual* loss of between 1.1% and 1.7%.

Welch and colleagues (Welch, Newling & Anderson, 1973) suggested that at least some of the cows which died following induction ".... may have had a pre-existing condition exacerbated by the treatment". For example, facial eczema (Anon., 1977) has been incriminated, and as a reversible, dose-dependent hepatopathy has been seen in a variety of glucocorticoid-treated animals (Rogers & Ruebner, 1977), it is reasonable to assume that induction of calving with these drugs could cause deterioration in an animal suffering from the effects of a pre-existing liver condition.

## 5.7 MILK PRODUCTION SUBSEQUENT TO INDUCTION OF CALVING

Most workers have reported that the milk production of cows which have been induced to calve is not different from that of cows calving naturally (Osinga, Stegenga & Jochle, 1971; Welch, 1971; Bailey *et al*, 1973; Thomas, 1975; Schmitt *et al*, 1975; Bolte *et al*, 1976; Welch & Kaltenbach, 1977; Bolte *et al*, 1977; Chew *et al*, 1978; O'Farrell, 1979) although lactation may be slow in onset (Osinga, Stegenga & Jochle, 1971; Welch, 1972; Kudlac, 1978).

However, reports on lactation following induced calving are not unanimous. Beardsley and co-workers (1976) reported that cows induced to calve barely a week prematurely had a significant (12.6%) reduction in average daily milk yield over a 9 week period, in comparison with untreated control cows. In an earlier trial (Beardsley *et al*, 1974) a 10.8% decrease in average daily milk yield had been recorded, but this reduction was not statistically significant. Reduced production was also reported by Holness and Sprowson (1979).

Welch (1972) reported on a trial involving 14 identical twins. In each pair, the cow with the later expected calving date was induced to calve at the same time as the earlier calving twin. Over the whole season, production from the induced cows was about 20% below that of their naturally calving twins. However, had the induced cows been left to calve naturally (on average about 23 days later), their

production over the season would have been even less. Subsequently, Welch and co-workers (1979) have reported on a trial in which a significant 4% reduction in daily milk yield in induced cows was more than compensated for by a 17 day (9%) longer period of lactation.

## 6. CONCLUSIONS

While there are a variety of farming situations in which induction of precocious or premature calving could be considered a useful management tool, it is only in New Zealand, and to a lesser extent other countries where intensely seasonal dairying is practised, that the technique has found widespread application and acceptance.

A variety of corticosteroid formulations and treatment schedules have been assessed for their suitability for different management requirements. When it is desirable that calving should be induced in cows within the last two or three weeks of gestation, single injections of a number of short-acting corticosteroid formulations have been shown to produce reliable and predictable results. In most cases, calving has resulted within 3 days of treatment.

Earlier in pregnancy, the short-acting corticosteroids are less effective, and long-acting formulations have been shown to be more reliable. The response time to such formulations tends to be variable and unpredictable, and so, in an attempt to improve the precision of the technique, two-injection schedules have been developed. An initial priming dose of a long-acting steroid followed some days later by an injection of a short-acting formulation has been shown to produce a reliable and predictable response. For this reason, two-injection schedules are now most widely used in New Zealand, where cows are often induced to calve up to six weeks or more before term.

It is believed that the blood glucocorticoid profile produced in the cow by such schedules more closely mimics that

of cortisol, which is normally secreted by the foetal calf to initiate parturition through the medium of enzyme changes in the placenta.

Calf mortality can be very high following induced parturition and this loss is largely a function of prematurity, although other factors, such as prolonged response time following treatment, may contribute. The health of calves which survive is generally good, provided they receive adequate colostrum.

Induction of calving markedly increased the incidence of retained foetal membranes. While there is debate on whether or not subsequent reproductive efficiency is impaired by RFMs, it is certain that retention predisposes cows to metritis, and metritis definitely leads to reduced fertility. Because of the relatively high proportion of cows in this country which require veterinary attention for uterine infections associated with RFMs, it is reasonable to assume that the technique of induced calving may well lead to an increase in the number of barren cows.

There is little evidence to suggest that induction of calving increases the incidence of other periparturient disease. However, because cows treated with synthetic glucocorticoids have a marked and often long-lasting depression of adrenal function, induced cows may be less able to respond to the stress of disease in the immediate post-calving period. There is also some suggestion that pre-existing liver disease may be exacerbated by administration of corticosteroids, and for this reason induction of parturition may be contra-indicated in herds with a recent history of facial eczema.

In the decade since induction of calving first found application in New Zealand, a substantial amount of information has been accumulated on the effects and side-effects of the technique. It is surprising, therefore, that no-one has assessed the economic value of the technique and examined

whether or not it truly achieves what it is purported to achieve. While it may be true, as Welch and colleagues (1979) claim, that any reduction in milk production is more than offset by a lengthening of lactation, it has yet to be demonstrated that such a lengthening of lactation compensates for costs associated with calf losses, retained foetal membranes and possible infertility.

The extent to which the technique of inducing parturition actually helps to bring late-calving cows 'into line' with their herd mates may be judged from the fact that in one large field trial (Welch *et al*, 1979), 19% of cows which became pregnant after induced calving had to be induced to calve again the following year.

# CHAPTER VII

## A COMPARISON BETWEEN SUSPENSIONS OF BETAMETHASONE, AND DEXAMETHASONE TRIMETHYLACETATE, USED FOR THE INDUCTION OF PARTURITION IN DAIRY COWS

1. INTRODUCTION

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# CHAPTER VII

## A COMPARISON BETWEEN SUSPENSIONS OF BETAMETHASONE, AND DEXAMETHASONE TRIMETHYLACETATE, USED FOR THE INDUCTION OF PARTURITION IN DAIRY COWS(1)

### 1. INTRODUCTION

Induction of premature calving by the administration of corticosteroid formulations is now a widely accepted practice in New Zealand (Chapter VI, Section 1). The treatment regimen most commonly used is that of McGowan, Welch and Hunter (1975) in which an injection of a slowly-absorbed corticosteroid formulation is followed 7 to 14 days later by an injection of a rapidly-absorbed corticosteroid.

It was demonstrated in Chapters III, IV and V of this thesis that aqueous suspensions of betamethasone which have a high solids:vehicle ratio are slowly-absorbed and thus have a prolonged effect. In Chapter V it was concluded that a 10 mg/ml suspension of betamethasone alcohol, administered to cattle as a 2 ml dose, can be expected to produce effects which compare favourably in terms of intensity and duration with those produced by the formulation of dexamethasone trimethylacetate (TMA) which is widely used for induction of calving.

The clinical trial reported in this chapter was designed to assess whether or not a concentrated suspension of betamethasone alcohol was suitable for use as the first injection in a two-injection treatment regimen for induction of premature calving. Two different betamethasone concentrations were used and compared for a number of factors with a suspension of dexamethasone TMA.

(1) A paper based on the study described in this chapter has been previously published (MacDiarmid, 1979; Appendix I)

## 2. MATERIALS AND METHODS

The trial was carried out during August and September 1978 on 41 dairy farms in the central Waikato area. Farmers taking part in the trial would normally have had cows induced at this time and had been notified of the experimental requirements beforehand. Within each herd, individual cows were allocated to one of three treatment groups on a random basis.

### 2.1 ANIMALS

Most of the 619 cows in the trial were Jerseys, Friesians or Jersey x Friesian crosses. Ages ranged from 2 to 12 years. An estimate of the stage of pregnancy at the time of treatment was based on artificial insemination records, when these were available. The expected calving date, and thus the prematurity of the calf, was based on a gestation length of 282 days which is reported to be the mean for New Zealand dairy cattle (Moller *et al*, 1967; MacMillan & Curnow, 1976). Before treatment all cows were examined *per rectum* to confirm pregnancy and to substantiate the estimate of the stage of pregnancy.

In two herds, cows had been inseminated with semen from *Bos indicus* bulls, so that extended gestation periods were expected (Plasse *et al*, 1968). Farmers reported that herd mates had carried similar calves for 10 to 19 days beyond the estimated calving dates for Jerseys and Friesians.

### 2.2. TREATMENTS

The first injection of the two-injection schedule consisted of;

1) 20 mg of betamethasone as a 10 mg/ml suspension(2) (Group I).
2) 30 mg of betamethasone as a 15 mg/ml suspension(3) (Group II).

(2) Betsopart, Glaxo New Zealand Ltd.
(3) Experimental suspension, Glaxo New Zealand Ltd.

3) 20 mg of dexamethasone as a 5 mg/ml suspension of the trimethylacetate(4) (Group III).

These initial treatments were all administered subcutaneously.

Cows which had not calved by the tenth (or in a few cases, the eleventh) day after this initial injection received by intramuscular injection 25 mg of betamethasone as a 2 mg/ml suspension(5).

2.3 STATISTICAL DESIGN AND EVALUATION

The primary comparison was made between the combined betamethasone treatments (Groups I and II) and Group III. A secondary comparison was made between Groups I and II. Accordingly, cows were allocated to Groups I, II or III in the ratio of 1: 1: 2 respectively.

Results were subjected to an orthogonal (independent) analysis I + II *versus* III, and then I *versus* II. This method gave a more powerful comparison between the experimental treatments and the standard treatment (Bliss, 1967). Supplementary direct comparisons, I *versus* II and II *versus* III were made whenever these differences appeared to be interesting.

2.3 OBSERVATIONS RECORDED

The following were recorded;

1) Response time; *i.e.* the number of days between receiving the *first* injection and calving.
2) Number of cows calving within 10 days of the first injection.
3) Number of cows calving within 7 days of receiving a second injection of corticosteroid. For the purposes of this trial it was decided that if any cow did not

(4) Opticortenol, Ciba-Geigy New Zealand Ltd.
(5) Betsolan, Glaxo New Zealand Ltd.

calve within this period, the treatment would be considered to have failed.

4) Prematurity of calves.

5) Calf mortality rates. These were recorded at birth and at 4 days after calving.

6) Incidence of metabolic disease.

7) Incidence of retained foetal membranes; if visible at the vulva at 24 hours after calving, membranes were recorded as 'retained'.

8) Incidence of dystocia.

9) Incidence of mastitis occurring between the time of initial injection and time the cows entered the milking herd.

10) Incidence of other maternal illnesses.

11) Cow mortality.

## 3. RESULTS

Results are presented in two categories:

1) Data obtained from all cows treated.

2) Data obtained from a restricted category consisting only of those cows for which mating records and the stage of pregnancy as estimated by rectal examination were in agreement.

Cows treated within 10 days before their expected calving date, and cows carrying *Bos indicus* calves were also excluded from the restricted category.

### 3.1 STAGE OF PREGNANCY AT THE TIME OF TREATMENT

The day of treatment relative to the expected day of calving was calculated for those cows in the restricted category. The figures (Table 7:I) indicated no significant differences between treatment groups.

A highly significant difference in the stage of pregnancy existed, however, between cows which calved to the first injection alone and those which calved after the second injection. There was also a highly significant difference in the

stage of pregnancy between cows which calved within the experimental period and those which failed to calve.

Table 7:I Stage of pregnancy and days before expected calving (mean ± SD) on which the first injection was administered (restricted category).

| Group | Number | Stage of pregnancy (days) | Days before expected calving |
|---|---|---|---|
| I | 70 | 249.1 | 32.9 ± 17.0[a] |
| II | 93 | 251.7 | 30.3 ± 11.9[a] |
| I + II | 163 | 250.5 | 31.5 ± 14.4[a] |
| III | 178 | 250.9 | 31.1 ± 14.9[a] |
| Cows calving to first injection | 136 | 256.3 | 25.7 ± 10.1[b] |
| Cows calving to second injection | 180 | 248.7 | 33.3 ± 14.8[b,c] |
| Cows which failed to calve within 7 days of second injection | 25 | 234.9 | 47.1 ± 18.6[c] |

Comparison in all tables is between means with the same superscript.

a Not significant

b ***

c ***

3.2 RESPONSE TIME

There were no differences between groups (Table 7:II).

Table 7:II Response time in days (mean ± SD) between first injection and calving

| Treatment group | Cows calving to 1st injection | Cows calving to 2nd injection |
|---|---|---|
| I | 7.0 ± 2.1 | 12.6 ± 1.3 |
| II | 7.2 ± 2.3 | 12.0 ± 0.8 |
| III | 7.6 ± 2.4 | 12.4 ± 1.6 |
| I + II + III | 7.5 ± 4.9 | 12.4 ± 1.4 |

3.3 NUMBER OF COWS CALVING AFTER EACH TREATMENT

In the groups treated with concentrated betamethasone suspensions (I and II), a greater proportion of cows calved to a single injection than in the group treated with dexamethasone TMA (III). The difference was highly significant (Table 7:III).

In all treatment groups, the peak of calving occurred on the second day after administration of the second injection (Fig. 7:1). Of the cows in Groups I, II and III which received a second injection, 52%, 75% and 62% respectively calved within 2 days of that injection. The only significant difference is that between Groups I and II ($P < 0.01$) although that between Groups II and III approaches significance ($\lambda^2 = 3.718$; $0.05 < P < 0.10$).

In comparison with those in Group III, significantly fewer cows in Group II failed to calve within the allocated 7 days after the second injection (Table 7:III).

Table 7: III Cows calving following first injection and cows calving within 7 days of second injection.

| Group | No. receiving first injection | No. (%) calving to first injection only | No. calving within 7 days of second injection | No. (%) failing to calve |
|---|---|---|---|---|
| I | 131 | 72[a] (55.0) | 50 | 9 ( 6.9) |
| II | 166 | 102[b] (61.4) | 58 | 6[c] ( 3.6) |
| III | 322 | 85[ab] (26.4) | 204 | 33[c] (10.2) |
| I + II + III | 619 | 259 (41.8) | 312 | 48 ( 7.7) |

a. ***
b. ***
c. *

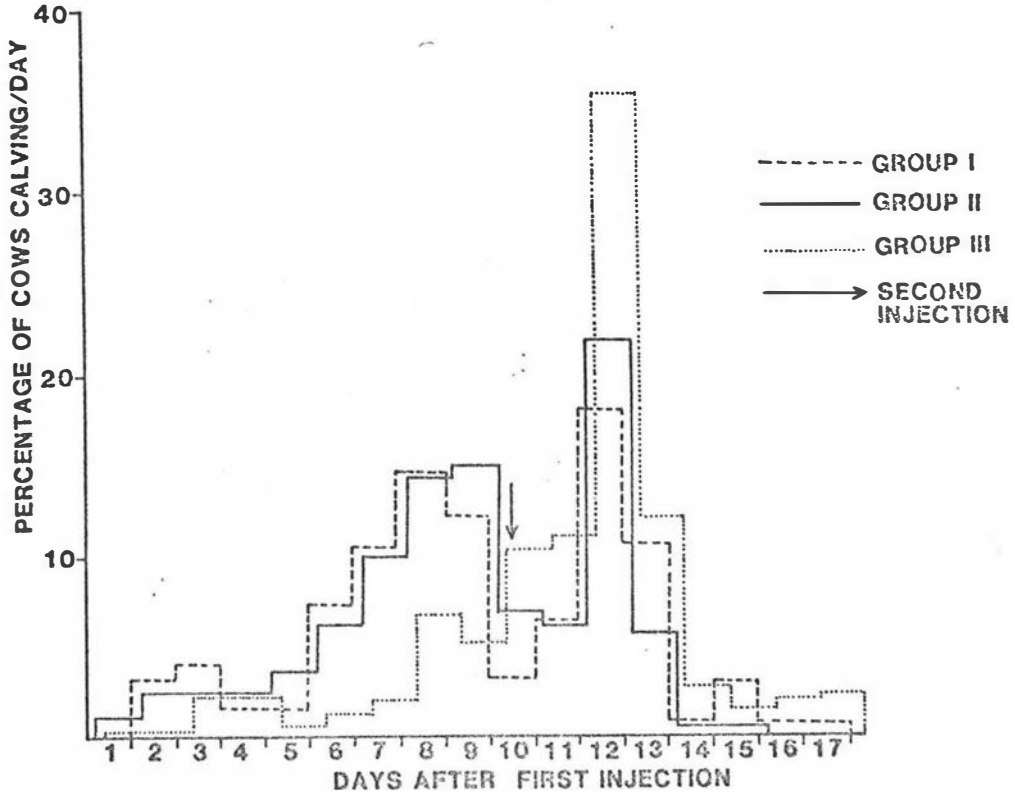


Figure 7:1 Calving pattern after induction of parturition using corticosteroids.
Group I; 2 ml of a 10 mg/ml suspension of betamethasone.
Group II; 2 ml of a 15 mg/ml suspension of betamethasone.
Group III; 4 ml of a 5 mg/ml suspension of dexamethasone TMA.
Second injection; 12.5 ml of a 2 mg/ml suspension of betamethasone.

## 3.4 CALF PREMATURITY

In the restricted category, within-group variability was high, but the calves born to cows in Group I + II were significantly more premature than those born to cows in Group III (Table 7:IV).

Overall, the calves born following the second injection were more premature than those born to the first injection.

Table 7:IV Prematurity of calves (Mean ± SD) born to cows in the restricted category.

| | Calves born to 1st injection | | Calves born to 2nd injection | | All calves born | |
|---|---|---|---|---|---|---|
| Group | Number | Days prem. | Number | Days prem. | Number | Days prem. |
| I | 39 | 16.9±6.5 | 27 | 30.2±20.1 | 66 | 22.4±15.1 |
| II | 59 | 18.8±9.8 | 30 | 23.5±10.4 | 89 | 20.4±10.2 |
| I + II | 98 | 18.1±8.7 | 57 | 26.7±15.9[a] | 155 | 21.1±12.5[b] |
| III | 38 | 18.1±12.9 | 123 | 18.3±13.4[a] | 161 | 18.2±13.1[b] |
| I + II + III | 136 | 18.1±10.0[c] | 180 | 21.0±14.7[c] | 316 | 19.7±12.9 |

a. ***
b. *
c. *

## 3.5 CALF MORTALITY

In the first 4 days after parturition many calves either died or were destroyed by farmers who felt that the calves were 'too small'. It was not possible in this study to differentiate between those which were destroyed and those which died from other causes.

There were no significant differences between groups in the number of calves stillborn, nor in the number dead by 4 days of age (Table 7:V). There was a highly significant difference in mortality between those calves born following a single injection and those born following a second injection.

Table 7:V Number (%) of calves stillborn and dying within 4 days of birth following induction of parturition.

| Group | Born to: / Dead at: | 1st Injection Birth | 1st Injection 4 days | 2nd Injection Birth | 2nd Injection 4 days | Total Birth | Total 4 days |
|---|---|---|---|---|---|---|---|
| I | | 5 (6.9) | 10 (13.9) | 19 (38) | 26 (52) | 24 (19.7) | 36 (29.5) |
| II | | 8 (7.8) | 15 (14.7) | 12 (20.7) | 23 (39.6) | 20 (12.5) | 38 (23.7) |
| III | | 5 (5.9) | 8 (9.4) | 38 (18.6) | 70 (34.3) | 43 (14.9) | 78 (27) |
| I + II + III | | 18 (6.9) | 33[a] (12.7) | 69 (22.1) | 119[a] (38.1) | 87 (15.2) | 152 (26.6) |

a. ***

3.6 METABOLIC DISEASE

Metabolic disease was seen in all treatment groups and occurred both before and after calving. Differences between groups were not significant (Table 7: VI).

Table 7:VI Incidence of metabolic disease amongst cows induced to calve by the administration of corticosteroids.

| Group | No.calving | Percentage incidence |
|---|---|---|
| I | 122 | 8.2 |
| II | 160 | 5.0 |
| III | 289 | 5.5 |
| I + II + III | 571 | 5.9 |

3.7 RETAINED FOETAL MEMBRANES

No significant differences were detected between groups in the incidence of RFMs (Table 7: VII).

Twenty-five of the 116 cows that were recorded as having RFMs required veterinary attention for RFM-related problems and there was no significant difference in the distribution between groups.

Table 7:VII Incidence of retained foetal membranes amongst cows induced to calve by the administration of corticosteroids

| Group | No. calving to single injection | Percentage incidence | Total No. calving | Percentage incidence |
|---|---|---|---|---|
| I | 72 | 26.4 | 122 | 22.1 |
| II | 102 | 24.5 | 160 | 22.0 |
| III | 85 | 17.6 | 289 | 18.6 |
| I + II + III | 259 | 22.8 | 571 | 20.3 |

3.8 DYSTOCIA

Thirty-nine cows required assistance at calving. These were distributed among Groups, I, II and III as 13, 9 and 17 respectively. The difference in incidence was not significant.

3.9 MASTITIS

In the immediate post-calving period there were 16 cases of mastitis; 4 in Group I and 12 in Group III. There was no significant difference between these two groups in the incidence of mastitis.

There were no cases of mastitis recorded in Group II, and the difference between Group II and Group III is significant ($P < 0.01$). When Groups I and II are combined, however, the difference in incidence between the combined group and Group III is not significant ($P > 0.2$).

3.10 OTHER ILLNESSES

There were a few cases of severe diarrhoea and a few cases of undiagnosed illness: 12 cows were affected in all. Differences between groups were not significant.

3.11 COW MORTALITY

Four cows died in Group I, one in Group II and 6 in Group III. There was no significant difference between groups.

## 4. DISCUSSION

The concentrated betamethasone suspensions used in this trial had one important economic advantage over the dexamethasone TMA formulation; a significantly smaller percentage of cows which had been treated with the betamethasone suspensions (Groups I and II) required a second injection of corticosteroid.

While the reasons for this difference in pattern of response are not clear, undefined and perhaps rather subtle differences in absorption rate could be responsible.

The peak of calving occurred two days after the second injection (Fig. 7:1) and this is in accord with the results of other trials (Welch, Crawford & Duganzich, 1977; Welch *et al*, 1979). In the group treated with the 15 mg/ml betamethasone formulation (Group II) there was a tendency for more cows to calve within 2 days of receiving their second corticosteroid injection. As cows in this group received 50% more total glucocorticoid in the initial injection, this response is possibly a reflection of a dose effect (Osinga, Stegenga & Jochle, 1970; Brown *et al*, 1970; Bosc, 1971) which also carried over to result in a greater proportion of Group II calving within the experimental period.

Calf mortality at birth was high; about 15%. This is comparable, however, to that reported by others for a double injection regimen of this nature (Welch & Kaltenbach, 1977; Welch *et al*, 1979). The mortality rate for all groups had risen to 26% by the fourth day after birth, a figure which is inflated by the number of viable calves which were destroyed because the farmer considered them too small. On some farms these very small calves were kept until heavy enough to go to the freezing works as bobby calves. Understandably (Welch *et al*, 1979), those calves born as a result of the first injection experienced a lower mortality rate (6.9% against 22.1%) as they were nearer to term at the time of injection.

Significant differences were not detected between treatments in respect to maternal illness. The incidence of foetal membranes still retained at 24 hours was relatively high in all treatment groups, but was similar to that reported elsewhere for a two-injection regimen of this nature (21%, Welch & Kaltenbach, 1977; 21% - 26%, Welch *et al*, 1979).

While 21.5% of cows with RFM's required veterinary attention for this problem, most expelled them without intervention within a few days.

Before a possible association between corticosteroid-induced parturition and mastitis can be ruled out (Chapter VI, section 5.4), more experimental data need to be obtained and evaluated. However, the nil-incidence of mastitis in the group which received the highest dose of steroid (Group II) would seem paradoxical if any direct association between the use of corticosteroids and a predisposition to mastitis is postulated (Buddle, Midgley & Ashby, 1976).

## 5. CONCLUSIONS

In a previous chapter (Chapter III), results of laboratory studies were reported which indicated that a formulation of betamethasone alcohol having a high solids:vehicle ratio could be expected to produce clinical effects which compared favourably with other long-acting corticosteroid formulations used for the induction of premature calving. The trial reported in this chapter confirmed that such effects are also demonstrable under field conditions. In addition, concentrated betamethasone suspensions had certain advantages over dexamethasone TMA in terms of the number of cows calving to a single injection.

# CHAPTER VIII

## A COMPARISON OF DIFFERENT FORMULATIONS OF BETAMETHASONE USED FOR THE INDUCTION OF PARTURITION IN DAIRY COWS

1. INTRODUCTION

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3. RESULTS
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   - 3.8 Retained foetal membranes
   - 3.9 Other illnesses
   - 3.10 Cow mortality

4. DISCUSSION

5. CONCLUSION

# CHAPTER VIII

## A COMPARISON OF DIFFERENT FORMULATIONS OF BETAMETHASONE USED FOR THE INDUCTION OF PARTURITION IN DAIRY COWS(1)

### 1. INTRODUCTION

The trial reported in Chapter VII established that a 10 mg/ml suspension of betamethasone alcohol may be satisfactorily substituted for dexamethasone trimethylacetate (TMA) in the two-injection treatment regimen commonly used in New Zealand to induce calving.

In this treatment regimen (McGowan, Welch & Hunter, 1975), an initial injection of a slowly-absorbed corticosteroid is followed 7 to 14 days later, if necessary, by an injection of one which is more rapidly absorbed. For several years a 2 mg/ml suspension of betamethasone has been widely used as the second injection (McGowan, Welch & Hunter, 1975; Welch, Crawford & Duganzich, 1977; Welch & Kaltenbach, 1977). It was postulated that, when used as a second injection, a corticosteroid formulation which is more rapidly absorbed than the 2 mg/ml suspension might produce a shorter response time and hence a contraction of the calving period.

The trial reported in this chapter was therefore designed to compare the effect on calving pattern of the commonly-used 2 mg/ml betamethasone suspensions with that of a 2 mg/ml solution of the highly soluble betamethasone sodium phosphate.

### 2. MATERIALS AND METHODS

The trial was carried out in September 1979 on 27 dairy farms in the central Waikato area. Farmers taking part in the trial would normally have had cows induced to calve at this time and had been notified beforehand of the experimental requirements.

(1) A paper based on the study described in this chapter has been published (MacDiarmid, 1980; Appendix II).

## 2.1 ANIMALS

The cows used were Jerseys, Friesians or Jersey x Friesian crosses. Ages ranged from 2 to 13 years and data were collected from 553 cows.

An estimate of the stage of pregnancy at the time of treatment was based on artificial insemination records where these were available. The expected calving date, and thus the prematurity of the calf, was based on a gestation length of 282 days (Moller *et al*, 1967; MacMillan & Curnow, 1976). Before treatment all cows were examined *per rectum* to confirm pregnancy and to substantiate the estimate of the stage of gestation.

## 2.2 TREATMENTS

All animals received an initial injection of 2 ml of a 10 mg/ml suspension of betamethasone(2). Cows calving to this single injection were classified as Group I.

Animals which had not calved by the seventh day after injection received either:

1) 20 mg of betamethasone alcohol as a 2 mg/ml suspension(3) (Group II), or
2) 20 mg of betamethasone as a 2 mg/ml solution of the sodium phosphate ester(4) (Group III).

All treatments were administered by subcutaneous injection. As cows became available they were randomly allocated to Groups II and III.

## 2.3 EVALUATION OF TREATMENTS

The effects of treatments were compared using the appropriate statistical tests (Chi square or Student's *t* test). The observations recorded and compared were:

1) Response time: the number of days between receiving the *first* injection and calving.

(2) Betsopart, Glaxo New Zealand Ltd.
(3) Betsolan, Glaxo New Zealand Ltd.
(4) Betsolan Soluble Injection, Glaxovet Australia Pty. Ltd Melbourne.

2) Number of cows calving within 7 days of receiving the first injection.
3) Number of cows calving within 10 days of receiving a second injection of corticosteroid. For the purposes of this trial it was decided that if any cow did not calve within this period the treatment would be considered to have failed.
4) Prematurity of calves.
5) Calf mortality rates. These were recorded at birth and at 4 days after calving.
6) Birthweights of calves.
7) Incidence of metabolic disease amongst the cows.
8) Incidence of retained foetal membranes; if visible at the vulva 24 hours after calving membranes were recorded as 'retained'.
9) Incidence of dystocia.
10) Incidence of mastitis occurring between the time of initial injection and the time cows entered the milking herd.
11) Incidence of other maternal illnesses.
12) Cow mortality.

## 3. RESULTS

Results are considered in two categories:

1) From all cows treated.
2) From a restricted category consisting only of those cows for which mating records and the stage of pregnancy as estimated by rectal examination were in agreement. Cows treated within the 10 days before their expected calving date were also excluded from this category.

### 3.1 STAGE OF PREGNANCY AT THE TIME OF TREATMENT

The day of treatment relative to the expected day of calving was calculated for those cows in the restricted category. The figures (Table 8:I) demonstrate significant differences in stage of pregnancy between Group I and Group II + III, and between Group II + III and the 'failures'. No difference was demonstrated between Groups II and III.

Table 8:I Stage of pregnancy and number of days before expected calving (mean ± S.D.) on which first injection administered (restricted category)

| Group | Number | Stage of pregnancy (days) | Days before expected calving |
|---|---|---|---|
| I | 30 | 265.4 | 16.6 ± 7.2[a] |
| II | 90 | 251.8 | 30.2 ± 14.0[b] |
| III | 104 | 252.4 | 29.6 ± 13.0[b] |
| II + III | 194 | 252.1 | 29.9 ± 13.4[a,c] |
| 'Failures' | 14 | 231.5 | 50.5 ± 16.6[c] |

Comparison in all tables is between means with same superscript.

a. ***

b. Not significant

c. ***

3.2 RESPONSE TIME

Group III had a significantly shorter response time than Group II (Table 8:II).

Table 8:II Response time (mean ± S.D.) between first injection and calving

| Treatment group | Number calving | Days |
|---|---|---|
| I | 103 | 4.9 ± 2.0 |
| II | 207 | 9.6 ± 2.0[a] |
| III | 210 | 9.1 ± 1.6[a] |
| II + III | 417 | 9.3 ± 1.8 |

a. **

3.3 NUMBER OF COWS CALVING AFTER EACH TREATMENT

On hundred and three (18.6%) of the 553 cows which received the first injection calved within 7 days (Group I). Three of the 553 received a repeat of the initial injection because udder development at 7 days was considered unsatisfactory. These 3 cows were dropped from the trial. Two cows died before calving and before receiving a second injection.

In both groups which received a second injection the peak of calving occurred on the second day after the administration of that injection (Fig. 8:I). In Group III a significantly greater percentage of cows had calved within 2 days of the second injection (Table 8:III). There was no significant difference between Groups II and III in the number of cows failing to calve within the time allocated.

Table 8:III Number (%) of cows calving within a specified time after receiving a second injection of betamethasone

| Group | Treated | Calving within 2 days | Calving within 10 days | Failing to calve within 10 days |
|---|---|---|---|---|
| II | 222 | 151 (68.0)[a] | 207 (83.2) | 15 (6.8) |
| III | 223 | 171 (76.7)[a] | 210 (94.2) | 13 (5.8) |
| II + III | 445 | 322 (72.4) | 417 (93.7) | 28 (6.3) |

a. *

3.4 CALF PREMATURITY, MORTALITY AND BIRTH WEIGHT

Although there was no difference in prematurity between calves born in Groups II and III, the difference between calves in Group II + III and Group I was highly significant (Table 8:IV).

In comparison with Groups II and III, fewer calves were born dead to those cows which calved to a single injection only (Group I) and more of these calves were still alive 4 days later (Table 8:V). There was no significant difference in calf mortalities between Groups II and III.

A total of 142 calves (27.3%) were dead by the fourth day after birth. Fifty-four (38%) of these had been destroyed by farmers because of small size. Calves dead 4 days after birth were calculated to have been more premature than those surviving (Table 8:IV).

Birth weights were obtained for 348 calves (Table 8:VI). For those calves born following a single injection only, the difference in mean birth weights between calves alive 4 days after calving and those dead at this time was not significant.

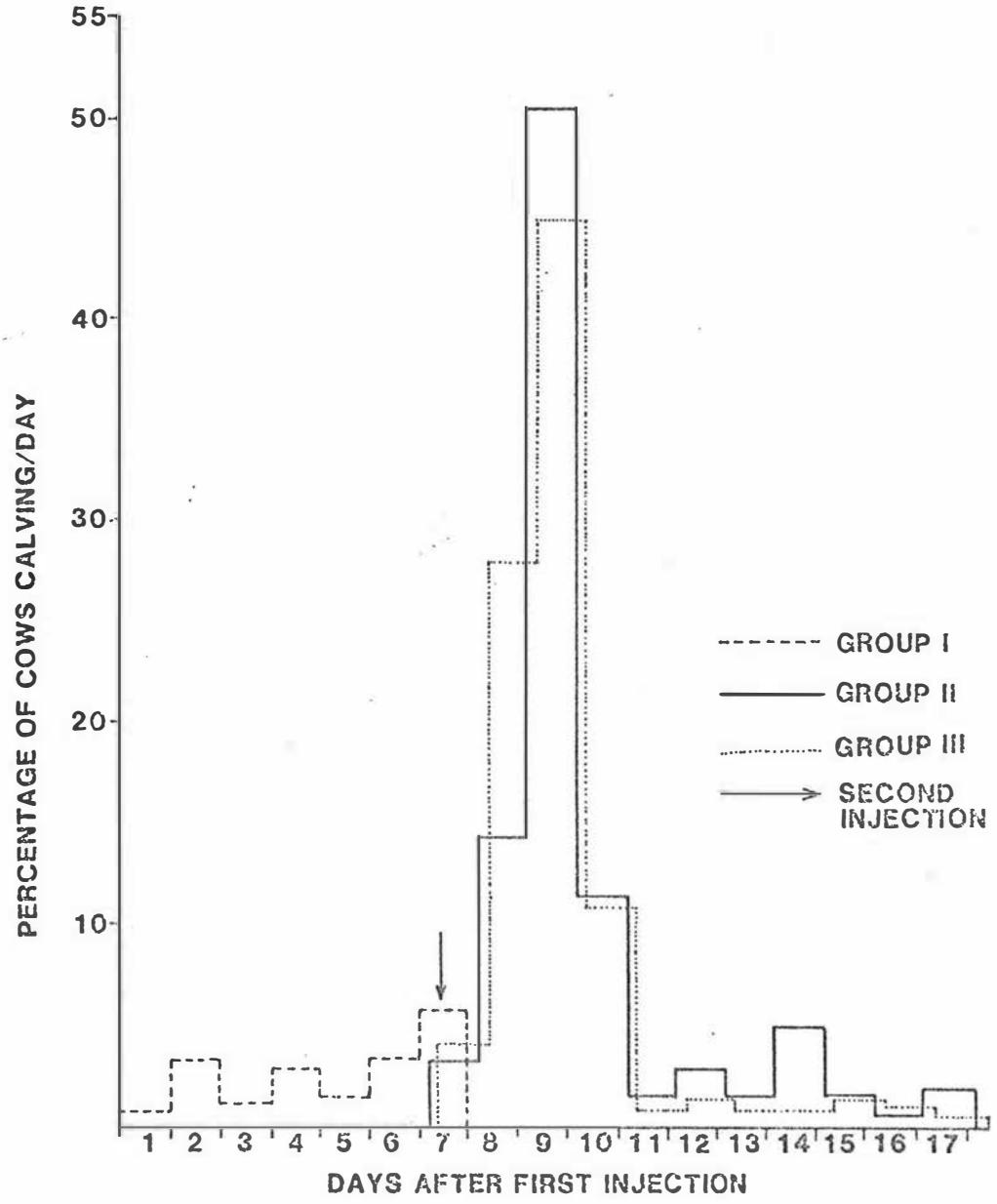


Figure 8:1 Calving pattern after induction of parturition using betamethasone formulations.
Group I; those cows calving within 7 days of receiving 2 ml of a 10 mg/ml suspension.
Group II; 2 ml of a 10 mg/ml suspension followed by 10 ml of 2 mg/ml suspension (second injection).
Group III; 2 ml of a 10 mg/ml suspension followed by 10 ml of a 2 mg/ml solution (second injection).

However, for calves born after a second injection, the mean birth weight of those surviving for 4 days was significantly greater than that of calves dead by that time.

Table 8:IV Prematurity of calves born to cows in the restricted category

| | | Days premature (mean ± S.D.) | | |
|---|---|---|---|---|
| Group | No.calves | Calves alive at 4 days | Calves dead at 4 days | Total calves |
| I | 30 | 10.8 + 7.6 | 11.3 ± 6.0 | 10.9 ± 7.4[a] |
| II | 90 | 16.1 ± 10.8 | 26.7 ± 14.5 | 20.6 ± 13.5 |
| III | 105[c] | 18.5 ± 10.1 | 24.4 ± 16.7 | 20.4 ± 13.1 |
| II + III | 195 | 17.4 ± 10.4[b] | 25.5 ± 15.6[b] | 20.6 ± 13.2[a] |

a. ***

b. ***

c. includes one set of twins

Table 8:V Number (%) of calves stillborn and dying within 4 days of birth following induction of parturition

| Group | Calves born | Dead at birth | Dead at 4 days |
|---|---|---|---|
| I | 103 | 5 ( 4.9)[a] | 8 (7.8)[b] |
| II | 207 | 24 (11.6) | 70 (33.8) |
| III | 211[c] | 26 (12.3) | 64 (30.3) |
| II + III | 418 | 50 (12.0)[a] | 134 (32.1)[b] |
| I + II + III | 521 | 55 (10.6) | 142[d] (27.3) |

a. *

b. ***

c. includes one set of twins

d. 54 calves destroyed: remainder died from natural causes.

Table 8:VI Birth weights (mean ± S.D.) of calves born following induction of parturition.

| Group | Calves alive at 4 days | | Calves dead at 4 days | |
|---|---|---|---|---|
| | Kg. | (no.) | Kg. | (no.) |
| I | 31.5 ± 6.6[a] | ( 69) | 25.7 ± 16.7[a] | ( 4) |
| II | 24.4 ± 5.2 | ( 89) | 16.3 ± 5.1 | (46) |
| III | 24.5 ± 5.2 | (100) | 16.7 ± 6.2 | (40) |
| II + III | 24.6 ± 5.0[b] | (189) | 16.5 ± 5.6[b] | (86) |

a. N.S.

b. ***

## 3.5 METABOLIC DISEASES

Metabolic disease was seen in all treatment groups and occurred both before and after calving: the total incidence was 5.2% (Table 8:VII). The difference in incidence between Groups II and III was significant.

## 3.6 DYSTOCIA

A total of 11 cows (2.1%) required assistance at calving. Differences between groups were not significant (Table 8:VII).

## 3.7 MASTITIS

Thirty-two cows (6.1%) developed clinical mastitis. The difference in incidence between groups was not significant (Table 8:VII).

## 3.8 RETAINED FOETAL MEMBRANES

The incidence of retained foetal membranes (RFMs) was significantly higher in those cows calving to a single injection (Table 8:VII). The difference in incidence between groups which calved to the second injection was not significant.

Of the 97 cows (18.6%) recorded as having RFMs, 30 required veterinary attention for RFM-related problems. Significantly more of those cows in Group II + III which retained their foetal membranes required veterinary attention.

Table 8:VII Incidence of periparturient illness in cows induced to calve using betamethasone formulations

| Treatment group | Number | Metabolic disease | | Dystocia | | Mastitis | | RFMs | | | Other illnesses | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No. | (%) | No. | (%) | No. | (%) | No. | (%) | No.treated | No. | (%) |
| I | 103 | 4 | (3.9) | 2 | (1.9) | 5 | (4.8) | 35 | (34.0)[b] | 5[c] | 2 | (1.9) |
| II | 207 | 6 | (2.9)[a] | 2 | (1.0) | 10 | (4.8) | 26 | (12.6) | 10 | 7 | (3.4) |
| III | 210 | 17 | (8.1)[a] | 7 | (3.3) | 17 | (8.1) | 36 | (17.1) | 15 | 6 | (2.9) |
| II + III | 417 | 23 | (5.5) | 9 | (2.3) | 27 | (6.5) | 62 | (14.9)[b] | 25[c] | 13 | (3.1) |
| I + II + III | 520 | 27 | (5.2) | 11 | (2.1) | 32 | (6.1) | 97 | (18.6) | 30 | 15 | (2.9) |

(Some cows experienced more than one illness)

a. *

b. ***

c. Difference N.S. when related to number calving, but highly significant ($P < 0.01$) when related to RFMs present at 24 hours.

### 3.9 OTHER ILLNESSES

Eleven cows showed erythema and dermatitis of the udder consistent with a diagnosis of photosensitisation. Four had infectious diseases other than mastitis or RFM-related problems. There was no significant difference between groups in the incidence of other illnesses (Table 8:VII).

### 3.10 COW MORTALITY

Five cows died during the trial. There was no significant difference between groups in the cow mortality rate.

## 4. DISCUSSION

The cows in the trial reported here were treated at a significantly later stage of pregnancy than the cows of the previous trial reported in Chapter VII (Table 8:VIII), and it is this difference which is responsible for the lower incidence of stillbirths (10.6% *versus* 15.2%, $P < 0.05$) in the present trial. Calves in that earlier trial were significantly more premature (Table 8:IX).

Table 8:VIII Comparison of the stage of gestation in days (mean ± S.D.) at which the first injection was administered in the 1978 trial and the 1979 trial

| | 1978 | 1979 | P |
|---|---|---|---|
| Cows calving to 1st injection | 256.3 ± 10.1 (n = 136) | 265.4 ± 7.2 (30) | *** |
| Cows calving to 2nd injection | 248.7 ± 14.8 (180) | 252.1 ± 13.4 (194) | * |

Table 8:IX Comparison of the prematurity at birth in days (mean ± S.D.) of calves born in the 1978 trial and in the 1979 trial.

| | 1978[a] | 1979 | P |
|---|---|---|---|
| Calves born to 1st injection | 16.9 ± 6.5 (n=39) | 10.9 ± 7.4 (30) | *** |
| Calves born to 2nd injection | 30.2 ± 20.1 (27) | 20.6 ± 13.2 (195) | * |

a. Only calves from cows treated with 10 mg/ml suspension of betamethasone alcohol.

Despite the lower incidence of stillbirths, by the fourth day after birth the calf mortality rate had risen to 27.3%, a figure not significantly different from the 26.6% reported in the earlier trial. Of the calves which died between birth and 4 days of age, 38% were destroyed by the farmer (Table 8:V), usually because he considered them too small to rear economically to the 25 Kg minimum weight required for bobby calves. These percentages, therefore, may not be a realistic estimate of actual calf viability.

Mortality rates were understandably lower in calves born as a result of the first injection alone (4.9% *versus* 12.0%), as these calves were nearer to term at the time of injection (Table 8:I). The differences in birth weights of calves further illustrate the point that mortality is a reflection of prematurity. In Group II + III, the mean birth weight for calves dying was 16.5 Kg and these calves were, on average, 25.6 days premature. The survivors had a mean birth weight of 24.6 Kg and were, on average, 17.4 days premature.

The peak of calving occurred 2 days after the second injection (Fig. 8:I) and this is in accord with the results of the earlier trial. A significantly higher proportion of those cows which received betamethasone sodium phosphate solution (Group III) had calved by this time. The more rapid response to the soluble betamethasone is illustrated by the significantly shorter response time of Group III (Table 8:II).

Following administration of the highly-soluble sodium phosphate ester, peak concentrations of betamethasone in the plasma are always greater than those produced by the less soluble alcohol; up to 10 times greater in some cases (Chapter IV; Section 3; Also Box, pers. com.). It is thus likely that once a cow has been sufficiently 'primed' by her exposure to a slowly-absorbed corticosteroid, the speed with which she responds to a second injection may be related, within limits, to the plasma corticosteroid concentration produced by that second injection.

The differences in plasma betamethasone concentrations produced by the different formulations may also be responsible for the significant difference between Groups II and III in the incidence of metabolic disease. However, a direct association is difficult to establish because, as explained in Chapter VI (Section 5.5) betamethasone is unlikely to have any significant adverse effect on calcium, magnesium or phosphorus metabolism. Further experimental clarification is needed in this area, as any significant increase in the incidence of metabolic disease must be regarded as a serious side-effect.

The incidence of foetal membranes retained at 24 hours was relatively high and was close to the 20.3% reported for the earlier trial described in Chapter VII (Section 3.7). In most cases RFMs were expelled without assistance within a few days.

One gains an impression from a review of the literature that cows induced to calve by the use of 'long-acting' corticosteroids are less likely to retain their foetal membranes than cows treated with 'short-acting' formulations (Chapter VI, Section 5:1). Presumably such a difference could be explained by the fact that a longer exposure to the glucocorticoid would encourage the maturation processes responsible for separation and expulsion of the placenta. It is interesting, therefore, that those cows which calved following a single injection of betamethasone (Group I) had

a greater incidence of RFMs than those cows which calved after receiving two injections (and were thus exposed to the steroid action for longer). However, when RFMs did occur in cows which had been treated with two injections, they were more likely to require veterinary attention (Table 8:VII), possibly because the affected cows were so far from term that their exposure to betamethasone was insufficient to cause maturation of the placental separation process before the foetus was expelled.

The incidence of other maternal illnesses did not differ significantly between groups. Dystocia was recorded less frequently than in the earlier trial whereas mastitis was recorded more frequently.

Because in most instances the signs of erythema and dermatitis of the udder, considered to be due to photo-sensitisation, were very mild, it is likely that some farmers failed to report cases. The incidence of this possible side-effect may be greater than the results reported here would indicate. Veterinarians working in the practice where the trial was carried out reported that photosensitisation occurs occasionally following the use of long-acting dexamethasone esters. The reasons for this phenomenon are not known, but the skin lesions may be a result of corticosteroid-induced liver damage (Chaper VI, Section 5.6). It was suggested (H.C. MacDiarmid, Cambridge Veterinary Services Inc., pers. com.) that similar signs are occasionally seen in untreated, naturally-calving cows.

## 5. CONCLUSION

The trial results reported in this chapter further confirm the suitability of a 10 mg/ml suspension of betamethasone as the initial 'priming' injection of a two-injection schedule to induce calving.

Cows in this second trial were induced to calve at a significantly later stage of pregnancy than those in the

earlier trial (Chapter VII) and this fact explains certain differences in results.

It is apparent from the data that corticosteroid formulations having different rates of absorption may produce differences in the pattern of induced calving. The possibility is also raised that the soluble betamethasone formulation, while having a minor advantage over the 2 mg/ml suspension in terms of a more compact calving period, may achieve this at the cost of an increased incidence of metabolic diseases.

# CHAPTER IX

## GENERAL DISCUSSION

The results of the studies presented in this thesis are significant in two respects. Firstly, by a simple pharmaceutical technique designed to prolong its activity, the usefulness of a tried and tested drug has been extended. Secondly, by prolonging the activity of this drug, another management tool has been provided which is able, partially at least, to correct some of the effects that poor animal husbandry or other factors may have had on the calving pattern of seasonal dairy herds.

The pharmacological investigations reported in Chapters III, IV and V were undertaken to confirm that by the pharmaceutical manipulation of increasing the solids:vehicle ratio of a suspension of betamethasone alcohol, a 'long-acting' formulation could be produced. Studies in cattle confirmed the practicality of this modification and demonstrated that a 10 mg/ml suspension of betamethasone had an intensity and duration of activity comparable to that of commercially available 'long-acting' corticosteroid products.

More recently, the 'long-acting' nature of the 10 mg/ml suspension of betamethasone has also been demonstrated in dogs (P.G. Box, pers. com.) and horses (Lea Stogdale, University of Saskatchewan, pers. com.).

Another investigation into the relationship between the solids:vehicle ratio and the intensity and duration of gluco-corticoid activity of betamethasone suspensions in cattle has also been recently completed (Maw, 1981). As expected, a 10 mg/ml suspension produced an elevation of plasma glucose which persisted for a longer period than that produced by the same dose of a 2 mg/ml suspension. Contrary to expectations though, the glucogenic effect of a 5 mg/ml suspension was not intermediate between that of the 2 mg/ml and 10 mg/ml suspensions. Rather, its intensity of effect was similar to that of the 2 mg/ml

suspension while its duration of effect matched that of the 10 mg/ml suspension. It is the opinion of the present writer that the absorption rate was probably intermediate, but the apparently anomalous effects on glucose levels were due to the economy of steroid utilisation from the different formulations. Such a hypothesis is tenable provided one accepts that the rate of glucogenesis is a saturable response. That is, when plasma steroid concentrations exceed some optimum, no further increase in glucogenesis can be expected.

The greatest effect on plasma glucose levels will be produced by the formulation which maintains betamethasone concentrations close to the optimum for the maximum time. The more rapidly absorbed suspensions will produce very high plasma steroid concentrations, well in excess of the optimum, but such concentrations will be maintained for a short period only. In terms of the glucogenic response, such formulations are 'wasteful' of betamethasone. On the other hand, very slowly absorbed formulations may never produce optimum concentrations of steroid and so will never generate the maximum glucogenic response. It thus becomes apparent that a formulation having an 'intermediate' absorption rate may be the one capable of causing the greatest glucogenic response. Further study of this possibility is desirable.

Once it had been established that the 10 mg/ml suspension of betamethasone was a suitable 'long-acting' product, field trials were carried out to demonstrate its effectiveness as an agent for the induction of calving. The first trial (Chapter VII) was based on the hypothesis that the betamethasone suspension would be no different in its effect from a commonly used dexamethasone formulation. In terms of response time, however, the betamethasone product turned out to have certain practical advantages. Because more cows calved after a single injection, an economic advantage could be claimed. However, the possible dangers of an over-rapid response were indicated by the results of the second trial (Chapter VIII) in which a further compression

of the calving period (brought about by the use of a very rapidly absorbed second injection) was obtained, but at the expense of a small yet significant increase in the incidence of metabolic disease.

On the basis of the results of these two trials, Diskin and Box (1981) in Ireland investigated the use of the 10 mg/ml product as part of a two-injection regimen designed to induce precocious parturition in ovum-transplanted heifers. The treatment schedule was designed to avoid an anticipated dystocia problem and in all respects the results were considered very satisfactory. Further trials are now underway in Europe (P.G. Box, pers. com.).

It was mentioned in the review of the induction of parturition (Chapter VI, Section 6) that no investigations had been made into the economic benefits of the technique as it is applied in New Zealand. In an attempt to rectify this situation, Moller and MacDiarmid (1981a; Appendix III) carried out a cost-benefit analysis of the technique, and investigated the subsequent reproductive performance of cows after induction of calving (Moller & MacDiarmid, 1981b; Appendix IV).

Their cost-benefit analysis (Moller & MacDiarmid, 1981a) was based on data in relevant New Zealand publications, together with a survey which they carried out as a follow-up to the induction trial described in Chapter VIII. They examined the 'extra costs', the 'costs saved', the 'income lost' and the 'extra returns' following the use of the technique. It was their contention that there is no economic benefit from the induction of calving unless long-term advantages are obtained in subsequent seasons. About two-thirds of the cows in their survey provided such benefits, but one third caused further economic losses.

In a subsequent paper (Moller & MacDiarmid, 1981b) the same authors showed that in terms of reproductive performance after induction of calving, cows could be classified into one

of two populations. Whereas the reproductive performance of the majority was satisfactory, a minority of cows failed to become pregnant again despite their ability to come into oestrus at the expected time after calving and despite being mated on several occasions. It was suggested that the decrease in reproductive efficiency was a result of the high incidence of retained foetal membranes.

The present study has provided much new data relating to the technique of induction of calving using corticosteroids. Although this management tool is widely used, the convenience associated with the compressed calving period must be measured against the costs of impaired reproductive performance. The present indication is that the economics of the technique, as it is currently utilised in New Zealand, are precariously balanced. Such an indication should provide the stimulus for further research, directed towards the development of more flexible schedules designed to meet the needs of individual cows. Eventually, a better understanding of the cascade of changes occurring toward the end of pregnancy may lead to new combinations of drugs which would be capable of initiating parturition prematurely as well as ensuring that the subsequent process proceeded normally in all other respects.

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