ZINC TOXICITY AND ITS PATHOLOGY IN RELATION TO FACIAL ECZEMA PROPHYLAXIS IN RUMINANTS

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ABSTRACT

"ZINC TOXICITY AND ITS PATHOLOGY IN RELATION TO FACIAL ECZEMA PROPHYLAXIS IN RUMINANTS."

Because of the possible administration of high dose rates of zinc compounds to ruminants as a prophylactic measure against facial eczema, the toxicities and pathological effects of certain zinc compounds have been examined in sheep and cattle.

At daily dose rates of zinc as low as 15 mg Zn/kg b.wt. lesions of zinc toxicity were observed but these were not always accompanied by clinical signs. After long term administration, zinc oxide treated sheep were heavier and fatter than controls. At higher dose rates of zinc oxide, body weight losses were recorded. Zinc sulphate caused greater weight loss, more deaths, more severe lesions and higher serum and organ zinc concentrations than zinc oxide which in turn was more toxic than zinc EDTA. Single zinc EDTA administrations resulted in more rapid rises and falls in serum zinc concentration and higher urinary zinc concentrations than the other two forms of zinc. Although most toxicity trials were conducted using sheep, some cattle were also dosed and these findings suggested that cattle react to high dose rates of zinc similarly to sheep.

The administration of zinc sulphate solutions to sheep and cattle by drenching gun caused abomasitis and other lesions of zinc toxicity were more severe and elevations of organ zinc higher than when the zinc was given by intraruminal intubation. Zinc solutions were shown to stimulate the reticular groove reflex causing the bolus to be deposited in the abomasum where it caused severe mucosal damage. When, for the same drenched dose rate of zinc sulphate, alternative combinations of volume and concentration were tried, it was shown that the greater volume alternative was more important than greater concentration in the production of zinc toxicity.

Single intraruminal doses of zinc sulphate (100 and 200 mg Zn/kg b.wt) caused reductions in food intake, elevations of duodenal pH and the secretion of acid from isolated abomasal pouches was doubled.

All forms of zinc administered caused lesions of the exocrine pancreas comprising duct necrosis and inflammation, acinar cell degeneration and inter- and intralobular fibrosis. The characteristics and chronology of these lesions suggested that the primary site of zinc-induced pancreopathy is in the pancreatic ductule. Severe pancreatic lesions caused by zinc oxide resulted in marked reductions of flow rates of pancreatic juice and in the concentration of protein and amylase in pancreatic juice. Although pancreatic bicarbonate output decreased, the concentration of bicarbonate in pancreatic juice was unaffected.

In subacute or chronic zinc sulphate toxicity of sheep and cattle there were often lesions indicative of one or more haemolytic episode. These consisted of a nephrosis and focal hepatic necrosis. Additional lesions seen in zinc toxicity were a periacinar fatty change, atrophy of ruminal papillae with epithelial metaplasia and a thymic atrophy.

When zinc was added to the milk of sucking lambs (0.2 g Zn ℓ) the typical pancreopathy of zinc toxicity occurred and, in addition, severe nephrosis with extremely high concentrations of kidney zinc were found.

Zinc concentrations were increased considerably in liver, kidney and pancreas, but not muscle, in all experiments. After cessation of zinc dosing zinc concentrations in these organs rapidly returned to normal.

Zinc was shown to interfere with selenium metabolism but this did not appear to be responsible for any of the lesions recognised in zinc toxicity.

The addition of zinc sulphate to the sole drinking water (0.25, 0.5 and 1.0 g Zn/ℓ water) of yearling cattle for nine weeks reduced water consumption. This effect was greatest soon after the introduction of zinc to the drinking water. At the highest zinc concentration, there were pancreatic lesions in all cattle and less body weight gains than in controls.

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PREFACE

Pithomycotoxicosis ('facial eczema'), a mycotoxic hepatopathy of ruminants, is caused by the ingestion of sporidesmin, a toxin of the saprophytic pasture fungus *Pithomyces chartarum*. The disease is characterised clinically by photosensitisation and causes considerable loss of production in New Zealand in certain years.

It has been shown that high doses of zinc can prevent or alleviate the liver damage of facial eczema when administered to ruminants at the time of the sporidesmin ingestion. Because the dose rates of zinc required to effect protection are very high and because news of the protective effect of zinc has been received with considerable enthusiasm by New Zealand farmers, concern has been expressed that the safety factor for zinc prophylaxis may not be large enough.

In order to define this safety factor, the toxicity of zinc salts to ruminants must be known and the effects of zinc toxicity studied and recognised. While some research has been conducted on zinc toxicity in ruminants this has been carried out under conditions foreign to New Zealand agricultural systems.

This thesis records a study of the toxicity of different zinc compounds to ruminants under conditions and methods of administration likely to be used by the New Zealand farmer. In addition the lesions of zinc toxicity are described, their pathogenesis examined and the effect of some lesions on organ function determined.

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The assistance of Mr D. Karl in performing the total volatile fatty acid determinations (Chapter 4.3) is gratefully acknowledged.

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INTRODUCTION

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INTRODUCTION

1.1 PITHOMYCOTOXICOSIS (FACIAL ECZEMA) IN NEW ZEALAND

Pithomycotoxicosis (facial eczema) is a hepatotoxicosis of sheep and cattle caused by the ingestion of sporidesmin, a toxin in the spores of the saprophytic fungus *Pithomyces chartarum*. This fungus multiplies and produces characteristic spores in pasture litter, especially under conditions of warmth and humidity as occurs in the autumn. Such conditions and consequently outbreaks of the disease, which is characterised clinically by photosensitisation, most frequently occur in the northern half of the North Island of New Zealand but can occur as far south as latitude 44 ^{o}S in the South Island.

The primary liver lesion is an obliterative pericholangitis causing bile stasis and leading to the retention of several compounds normally excreted in the bile. In the ruminant one of the most important of these is phylloerythrin, a breakdown product of chlorophyll. The bile stasis causes an increase in phylloerythrin in peripheral blood and some deposition in skin tissue. As phylloerythrin is a photodynamic compound its presence in the integument leads to photosensitivity. In the presence of sunlight, exposed areas (ears, face and plantar surfaces of limbs in sheep and pale or white skin areas, udder and escutcheon of cattle) develop the severe photodermatitis of photosensitisation.

The first record of the disease in New Zealand was in 1900 (Gilruth, 1900) and it has since been recognised in Australia (Hore, 1960), South Africa (Marasas *et al.*, 1972) and possibly South America (Camargo *et al.*, 1976). A comprehensive review of this disease has recently been made (di Menna *et al.*, 1977; White *et al.*, 1977; Mortimer *et al.*, 1977a,b,c).

The disease does not often cause death. However substantial economic losses are caused by lesser body weight gains, lowered milk production, hide damage, carcass and liver losses due to condemnation, the losses

incurred by disruption of farm management practices and the direct costs of controlling the disease. Estimates of the cost to New Zealand of pithomycotoxicosis vary between $4,000,000 and $27,000,000 per year (1978 estimate; J.D. Scott and S. Hudson, Liaison Section, Ruakura Agricultural Research Centre, personal communication) depending on the severity of the seasonal outbreaks. It is regarded as one of the more serious livestock diseases in New Zealand.

Very little of practical value can be done to treat animals already affected, apart from providing shelter from sunlight and adequate nutrition for liver regeneration. Effective control of the disease is carried out almost entirely by prevention. This can be achieved by recognition of dangerous periods and paddocks. Recognition is attained by spore counting (di Menna and Bailey, 1973) and a knowledge of conditions which favour spore production as well as some prior experience of areas known to produce high spore counts. Stock are then moved to safe pastures or crops in order to avoid the toxic pasture. The spraying of pasture with suitable fungicides (substituted benzimidazole compounds) has been shown to prevent the further production of *P. chartarum* spores. Usually control is effected by continuing spore counting and pasture spraying with the judicious placement of stock during danger periods.

This method of control, while reasonably effective, is an inconvenience and increases farm costs. Thus research is currently aimed at providing new ways of effective control. These methods include breeding for resistance (Campbell *et al.*, 1975) immunological research aimed at providing an effective vaccine (Jonas and Erasmason, 1977) stimulation of liver detoxification mechanisms (Mortimer *et al.*, 1978) and the administration of zinc to protect animals at risk during danger periods.

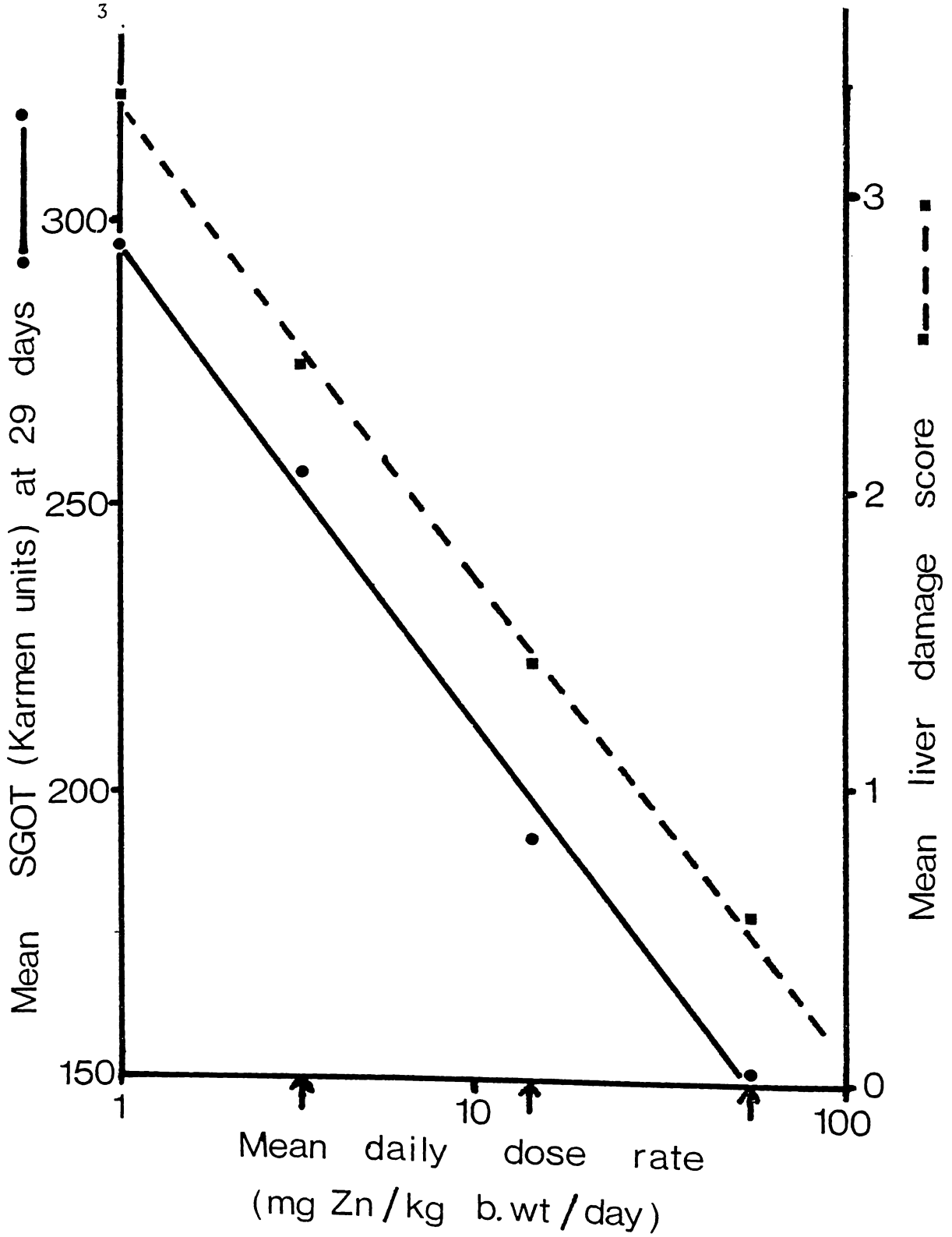

Figure 1.1 : Effect of concurrently drenched zinc sulphate on liver damage and serum glutamic oxaloacetate transaminase (SGOT) caused by administration of sporidesmin [4 mg prealumina sporidesmin (=0.56 mg sporidesmin/kg body-weight] to groups of 14 sheep. After Smith *et al.* (1977a).

1.2 ZINC AND PITHOMYCOTOXICOSIS (FACIAL ECZEMA)

The first scientific evidence that zinc may have a role to play in facial eczema came in 1973 when Dr N. Towers, Ruakura Animal Research Station, observed that rats maintained on a high zinc-content diet were more resistant to sporidesmin toxicity (Towers, 1974). This discovery led to collaborative efforts in which it was shown that zinc salts could prevent experimental sporidesmin toxicity in sheep (Towers *et al.*, 1975; Towers *et al.*, 1976; Smith *et al.*, 1977) and cattle (Towers *et al.*, 1975; Towers and Smith, 1978) and likewise prevent field outbreaks of pithomycotoxicosis in sheep (Towers *et al.*, 1975) and cattle (Smith *et al.*, 1978). Zinc sulphate, carbonate, oxide and EDTA have all been shown to be capable of preventing the disease. The zinc has been successfully administered by both drenching gun and by spraying pasture with zinc oxide.

In order to obtain this prophylactic effect zinc must be administered at or close to the time of sporidesmin intake (Towers, 1977; Smith *et al.*, 1977; B.L. Smith, unpublished observations). The protective effect of zinc increases, but at a diminishing rate, as zinc dose rate increases (Fig 1) with a maximum response being attained at very near the toxic zinc dose rate (Smith *et al.*, 1977).

The amount of zinc needed to be administered to attain worthwhile protection is large, 10-25 mg Zn/kg body weight/day over five days being regarded as optimal for sheep. Daily administration rates of 20 and 30 mg Zn (as sulphate)/kg have been shown to cause significant protection in dairy cows (Smith *et al.*, 1978; Towers and Smith, 1978).

These dose rates are very high in relation to normal intakes, being 20 to 30 times normal daily zinc intake. Because such large amounts of zinc are required to provide worthwhile protection against pithomycotoxicosis it was felt that if the farming community were to attempt to prevent the disease by zinc administration then there would be a considerable risk of zinc intoxication.

1.3 ZINC TOXICITY IN RELATION TO FACIAL ECZEMA

Early experiments indicated that the safety margin for the use of zinc to prevent facial eczema may not be large. Indeed in the early trials of the protective effect of zinc (Smith *et al.*, 1977; Smith *et al.*, 1978) there was evidence that the dose rate used to protect from facial eczema may have caused marginal zinc toxicity, indicated by a small weight loss about the time of zinc dosing. While early experimental work defined with some accuracy the amount of zinc needed to ensure worthwhile protection, there was no reliable data enabling the definition of toxic dose rate of zinc and hence to calculate a safety margin for the prophylactic use of zinc.

This has been made more apparent by the realisation that the zinc toxicity data would have to be applicable to the New Zealand situation. To be of any practical value the experimental methods and materials would need to be related to New Zealand classes of livestock, in the grazing situation and under systems of administration likely to be used for the administration of zinc for the prevention of facial eczema. Ideally, cheap, easy to obtain forms of zinc would be investigated provided it had been shown that the particular form of zinc was capable of eliciting the protective effect. Where possible, all deleterious effects of zinc should be measured e.g. weight changes, clinical changes, deaths, post mortem lesions, body composition changes and zinc tissue concentrations.

In addition to toxicology studies, a study of the pathology and pathogenesis of zinc toxicity will be necessary in order to determine which organs are affected by zinc and from their nature and the chronology of the changes to gain an insight into the stepwise progression of toxic lesions.

Before recommendations can be given favouring the use of zinc, factors which complicate zinc toxicity must be recognised. Interactions between zinc and other elements together with information on the extent to which zinc residues accumulate in organs and disappear after the cessation of zinc administration need to be studied. Not all these areas can be explored thoroughly in this limited study but

where possible experiments should be designed to provide some of the answers. In addition to the ongoing experiments on the protective function of zinc, some collaboration will be made with the purpose of monitoring the experimental groups of animals for evidence of zinc toxicity at the dose rates used for protection and at higher dose rates.

CHAPTER 2

REVIEW OF THE LITERATURE

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2.1 THE REQUIREMENT FOR ZINC IN RUMINANT NUTRITION

It is now well established that zinc is an essential element required for the growth and maintenance of farm and other animals (Underwood, 1977; Miller, 1970). Much early research concentrated on laboratory animals (particularly the rat) and chickens but many of the findings have been confirmed and extended in sheep and cattle.

The most well recognised lesions of zinc deficiency in ruminants are those of the integument. These have been well described for both sheep (Ott *et al.*, 1965; Mills *et al.*, 1967; Underwood and Summers, 1969) and cattle (Mills *et al.*, 1967; Kirchgessner *et al.*, 1978). In cattle the lesions of zinc deficiency appeared within three weeks of the commencement of zinc depletion as small areas of 'eczema' developing on the hind legs about the dew-claws and later on the fetlock and pastern. These areas developed into a scaly lesion with splits which oozed. Eventually the entire metatarsal area was affected. There was a drying and cracking of the lesion with a network of fissures occurring especially about the flexures of the joints. At four weeks this had spread over the hock and shank with some haemorrhaging also being apparent. These lesions later occurred on the udder, and, after six weeks, areas of both healing and fresh severe lesions occurred on the extremities and udder. When zinc supplementation was instigated udder and hind leg lesions healed within one and four weeks respectively (Kirchgessner *et al.*, 1978).

In the case of sheep similar changes occur but the effect of zinc deficiency on keratogenesis is more obvious. There is a loss of crimp definition, a thinning of fibres and sometimes a shedding of wool or lack of wool growth. Changes in hoof structure and horn growth of horned breeds also occur (Underwood and Somers, 1969).

Zinc deficiency also has been shown to have effects on growth of ruminants. Growth of lambs and calves ceased two weeks after the introduction of a very low zinc diet (Mills *et al.*, 1967). In ruminants the apparent digestibility of food is unaffected by zinc deficiency as was faecal excretion of nitrogen and sulphur. However urinary excretion of nitrogen and sulphur was greatly elevated in zinc deficient lambs (Somers and Underwood, 1969).

Hypogonadism appears to be a constant clinical sign of chronic zinc deficiency. It is a feature of zinc deficiency in bull calves (Miller *et al.*, 1966; Pitts *et al.*, 1966) and ram lambs (Underwood and Somers, 1969). This also occurs in laboratory animals (Follis *et al.*, 1941) and man (Halstead, 1977). Affected male ruminants have impaired testicular growth, histological changes and lowered or non-existent spermatogenesis. These lesions of zinc deficiency are completely reversible on repletion of the zinc status of the animal to normal.

There is evidence from other species that zinc deficiency has deleterious effects on the female reproductive cycle and fertility. Foetal malformations, stillbirths and dystokias have all been recorded in zinc deficient laboratory animals. However no such studies have yet been carried out on female ruminants although Egan (1972) has reported that more lambs were produced from Dorset Horn ewes which had been dosed with 140 mg Zn weekly.

Skeletal abnormalities have been recorded as a feature of zinc deficiency in birds, rats and pigs (Underwood, 1977) and Miller and Miller (1960) have observed reversible limb abnormalities in zinc deficient calves.

Many dietary components such as phytate (O'Dell and Savage, 1960), fibre (Becker and Hoekstra, 1971), or calcium (Cabell and Earle, 1965; Tucker and Salmon, 1955), have been shown to interfere with the availability or absorption of zinc in the alimentary tract and for this and other reasons it is not possible to accurately compare the levels of zinc in diets which have been used to produce zinc deficiency status in different experiments.

Zinc levels as low as 1.2 ppm Zn in diet have caused growth arrest and 2.4 ppm Zn caused impaired testicular growth and spermatogenesis (Underwood and Somers, 1969). They found that 17 ppm Zn was adequate for growth but inadequate for optimal testicular growth and function. However zinc concentrations of 18 ppm Zn in the diet have been found inadequate for maximal growth (Ott *et al.*, 1965) whereas 7 ppm Zn was found to be adequate by others (Mills *et al.*, 1967). Underwood and Somers (1969) found that while 17 ppm Zn was adequate for growth, it was inadequate for optimal testicular growth and function. Responses to zinc have been observed in cattle showing signs of zinc deficiency while grazing on pastures containing zinc concentrations as high as 83 ppm Zn (Dynna and Havre, 1963) an indication perhaps of the influence of other dietary components.

2.2 MINERAL INTERACTIONS WITH ZINC

There is considerable evidence in the scientific literature related to both ruminants and other mammalian species that there is considerable interaction between the many trace elements. While a comprehensive coverage of this field is outside the scope of this review, it is pertinent to comment that zinc is known to interact with copper (Petering *et al.*, 1971; Bremner and Davis, 1975), lead (Cerklewski and Forbes, 1976), selenium (Jensen, 1975), cadmium (Doyle and Pfander, 1975), and iron (Magee and Matrone, 1960).

These intereactions are pertinent for several reasons. Firstly, zinc's interaction with other minerals raises the possibility that its protective effects may be due to the depression of the concentration or activity of other trace elements within the animals concerned. Secondly, and more pertinent to this thesis, is that when a high zinc intake is maintained for a considerable time it presents a danger that such induced deficiencies may either be confused with zinc toxicity *per se* or may exacerbate lesions or changes which arise from zinc toxicity *per se*. Such interacting elements may also be important in preventing the occurrence of zinc toxicity or alleviating its manifestations.

2.3 PHARMACOLOGICAL OR THERAPEUTIC EFFECTS OF ZINC

There are a number of apparently diverse conditions which are now known to respond favourably to zinc administration. These effects of zinc are obtained at administration rates which are well in excess of those necessary for correcting deficiency states and these have been described as pharmacological or therapeutic effects of zinc. Zinc has been shown to promote healing or remission of wounds (Pories *et al.*, 1967; Miller *et al.*, 1967; Lavy, 1972), gastric ulcers (Frommer, 1975), venous leg ulcers (Hallbook and Lanner, 1972), bed sores (Cohen, 1968), acne (Michaelsson *et al.*, 1977), and rheumatoid arthritis (Simkin, 1976). In addition there is evidence that zinc has beneficial effects on pododermatitis of ruminants (Demertzis and Mills, 1973), atherosclerosis in man (Pories *et al.*, 1968) and sickle cell anaemia in man (Prasad *et al.*, 1975; Brewer *et al.*, 1977). Some of these pharmacological effects have been questioned on occasions and there has been controversy especially in the case of wound healing (Norman *et al.*, 1975; Wacker, 1976; Sandstead, 1970). There is also some evidence that some causes of deranged zinc metabolism e.g. steroid treatment or chronic illness (Beisel *et al.*, 1976; Sandstead *et al.*, 1976) predispose animals or man to some of these zinc responsive conditions.

Finally there are two heritable conditions which appear to be deficiencies of zinc absorption. They are Adema disease of cattle (Flagstad, 1976) and acrodermatitis enteropathica of man (Moynahan and Barnes , 1973; Nelder and Hambidge, 1975; Hambidge *et al.*, 1977). These conditions express themselves with many of the clinical signs of natural occurring zinc deficiency and can be corrected by the administration of high dose rates of zinc. All these conditions have given rise to an increasing interest in the absorption and pharmacokinetics of high zinc administration especially in man (Oelshlegel and Brewer, 1977; McKenzie, 1979).

2.4 ZINC AND THE PROPHYLACTIC EFFECT

2.4.1 GENERAL BACKGROUND

Various zinc salts administered experimentally via various routes to a wide range of experimental animals have been shown to have a protective effect in a number of different toxicities. The first such example of these relates to the work of Saldeen and Voigt (1964) who showed that toxic liver damage induced by carbon tetrachloride and manganese in the rat and golden hamster respectively was inhibited by the parenteral administration of zinc sulphate. This work was expanded by Saldeen (1969) who showed that zinc chloride or citrate also has a protective effect on 'choline free diet' induced fatty liver as well as the carbon tetrachloride hepatopathy. He showed that the zinc effect was more favourable during the latter half of the 10 day carbon tetrachloride dosing period and that a single subcutaneous 2 mg Zn dose gave optimal protective effect, a greater or lesser zinc dose giving lesser protective effects. At the highest subcutaneous dose (16 mg Zn) deaths were caused by zinc toxicity. In a histochemical investigation (Saldeen and Brunk, 1967) the protective effect of zinc chloride on carbon tetrachloride toxicity was confirmed in mice but there was no evidence to support the contention that the protective zinc effect was due to changes in the activity of zinc dependent enzyme systems.

Since these early experiments with carbon tetrachloride there have been confirmatory reports. Srinivason and Balwani (1969) showed some evidence in the form of serum enzyme changes and changed bromsulphthalein (BSP) excretion rate that some protection arose when zinc sulphate was administered intraperitoneally shortly before carbon tetrachloride. However they failed to show either that zinc changed the carbon tetrachloride induced lengthening of hexabarbitone sleeping time or any histologically demonstrable zinc induced protection. Chvapil (1973) has shown that gavage with zinc acetate (50 mg Zn/kg) as well as 1000 ppm zinc carbonate in the diet protected against carbon tetrachloride poisoning in rats. More recently Aterman and Yüce (1975) showed that subcutaneously dosed zinc chloride reduced the BSP retention and serum glutamic oxaloacetic transaminase

(SGOT) and glutamate pyruvate transaminase (SGPT) elevations caused by carbon tetrachloride. They concluded that the zinc protective effect is "quite remarkable" and should be further defined. Rana (1977) repeating the dose and form of zinc used by Chvapil (1973), showed evidence of protection against carbon tetrachloride poisoning of squirrels. He, however, recorded the presence of pancreatic lesions and focal hepatic necrosis in the zinc treated squirrels.

Zinc has also been shown to exert a protective effect against many other toxicities. The oedema caused by hyperbaric oxygen is prevented by intraperitoneal dosing of rats with 32 mg Zn/kg body weight as a soluble zinc salt (Radomski and Wood, 1970). No protective effect was recorded at one fifth of the dose rate of zinc while higher dose rates caused zinc toxicity. Nitrogen dioxide toxicity can similarly be reduced by zinc administration (Chvapil and Zukoski, 1974). Yanice and Lindeman (1976) demonstrated that zinc sulphate administration had a protective effect in ethanol intoxicated rodents and enhanced the disappearance of ethanol from blood. In rats which were given reserpine 18 hours before sacrifice (Ogle and Cho, 1977; 1978), zinc sulphate injected intraperitoneally 72 and 48 hours before sacrifice reduced the gastric glandular ulceration and inhibited mast cell degranulation.

The hepatocellular damage caused by bacterial *(Salmonella typhimurium)* endotoxins can also be prevented by intraperitoneal zinc chloride dosing (Sobocinski *et al.*, 1977a). The effect increased with increasing magnitude of the zinc dose up to 20 mg Zn/kg body weight after which there appeared to be diminished protection possibly related to zinc toxicity. In contrast however rats challenged with *S. typhimurium* cultures after intraperitoneal administration of zinc chloride (up to 10 mg Zn/kg body weight) showed enhanced mortality (Sobocinski *et al.*, 1977b).

2.4.2 ZINC AND THE PROPHYLACTIC EFFECT: FACIAL ECZEMA

The earlier scientific literature concerning the protective effects of zinc salts in alleviating various toxic effects was partly responsible for the research leading to the discovery that zinc also protects against sporidesmin poisoning. In 1973 Dr N. Towers working with high and low concentrations of zinc in the prepared diets of rats showed that zinc has a protective effect against sporidesmin intoxication (Towers, 1974; Towers, 1977a). This was followed by the demonstration that zinc similarly protects both sheep (Towers *et al.*, 1975; Smith *et al.*, 1977) and dairy cattle (Towers *et al.*, 1975; Towers and Smith, 1978). Zinc was also shown to be effective in preventing natural outbreaks of pithomycotoxicosis of sheep (Towers *et al.*, 1975; Towers *et al.*, 1976) and cattle (Smith *et al.*, 1978). These papers also showed that the protective effect was obtained either using the sulphate, oxide, carbonate or more recently the ethylenediaminetetracetic acid (EDTA) salt (Towers N.R., pers. comm.). The effective methods of administration of zinc have been by drenching gun, by intraruminal administration, by paste gun and by pasture spraying of suspensions. The protective effect has been shown to increase with increasing dose rates of zinc but at a diminishing rate (Smith *et al.*, 1977) with a substantial reduction in liver injury occurring at approximately 30 mg Zn/kg body weight/day (Fig 1). From the results of several experiments it has been calculated that the optimal protective dose rate of zinc (that which will produce worthwhile but not maximal protection) in the farming situation is approximately 20-25 mg Zn/kg body weight/d for sheep. While this dose rate has been used successfully for protection against experimental and natural facial eczema in cattle no experiments have yet ascertained the optimal or maximal protective dose rate of zinc in the case of cattle.

From these experiments in which sporidesmin was dosed and those quoted in relation to other toxicities it is obvious that the amounts of zinc needed to produce significant protection are very large and are approximately 20 to 30 times those required by animals for growth and maintenance. In some experiments there has been evidence that the protective dose rate is close to the toxic dose rate for zinc (Smith *et al.*, 1977; Smith *et al.*, 1978) and farmers have been warned against the use of zinc for the control of pithomycotoxicosis in the field (Smith, 1977).

2.5 TOXICOLOGY OF ZINC SALTS

2.5.1 ZINC TOXICITY IN MONOGASTRIC ANIMALS

One of the earliest experiments which demonstrated the toxic effects of zinc was that of Drinker *et.al.*, (1927). Zinc oxide was added to the feed of cats and dogs to give zinc intakes ranging from 40 to 250 mg Zn/kg body weight/day. Pancreatic lesions were recorded in the cats at dose rates greater than 180 mg Zn/kg b.wt./day for 8 or 13 weeks. However no pancreatic changes were recorded at calculated intakes of 83 mg Zn/kg b.wt./d for 45 weeks.

Scott and Fisher (1937) fed two groups, each of 15 cats, for 12-16 weeks. One group had zinc oxide added to its feed and the daily zinc dose rate based on initial body weight would have been approximately 66 mg Zn/kg b.wt./d. The control (non-dosed) cats gained nearly 300 g mean body weight while the zinc supplemented cats lost on average about 900 g. The pancreatic weights of the zinc dosed cats were 59% of those of controls and all cats in the zinc fed group were shown to have developed pancreatic fibrosis. The pancreatic and liver zinc concentrations of the zinc group were respectively about 7 and 15 times those of controls. These results contrast with those of Drinker *et al.*, (1927) which indicate that much higher intakes than these for approximately three times as long are safe for cats.

Grimett *et al.*, (1937) described zinc intoxication produced in the unweaned pig by feeding 1000 ppm Zn as zinc lactate in a mainly milk diet. This was confirmed by Sampson *et al.*, (1942) but they were unable to produce zinc toxicity in the growing weaned pig at a later stage of development. Brink *et al.*, (1959) introduced zinc carbonate in various concentrations to the diet of weanling pigs for 35 and 42 days. The addition of 1000 ppm Zn to the diet was the highest concentration tolerated. This represented a daily intake of approximately 140 mg Zn/kg b.wt./d. At concentrations of zinc approximately double and up to eight times this, representing 240 mg Zn/kg b.wt./d and greater, there were signs of zinc toxicity. Depressed weight gains, feed intake and efficiency of gain together with arthritis, haemorrhage of axillary spaces, gastroenteritis and ventricular haemorrhage in the brain were seen in pigs fed the highest amounts

of zinc. No mention was made of pancreatic lesions. In another experiment (Lewis *et al.*, 1975), designed to alleviate parakeratosis due to zinc deficiency, no toxic effect of zinc was identified when 1000 ppm Zn as $ZnSO_4 7H_2O$ was added to the diet for 20 weeks.

The rat has been the subject of zinc toxicity studies, often with conflicting results. Much of the early work is difficult to evaluate because of failure to report zinc intake. Likewise negative results are often recorded in instances where other workers have found evidence of toxicity. Often the anomalous results can be explained on the basis of the type of ration or the manner in which the zinc has been incorporated e.g. loose ration (Heller and Burke, 1927).

Grant-Frost and Underwood (1958) incorporated zinc oxide in the feed of growing rats at 5000 ppm Zn for 16 weeks. Feed consumption records indicated that a high level of 430 mg Zn/kg b.wt./d was consumed. This was found to reduce growth and food consumption, haemoglobin levels, copper retention and body fat content of the rats. They concluded from comparisons with pair-fed controls that the zinc effect was due to reduced food consumption, probably, they thought, due to the unpalatable nature of the diet and that the anaemia was due to the effect that zinc-induced copper deficiency would have had on haemopoiesis.

Young rats were fed with diets to which zinc oxide had been added to give zinc concentrations of 5000 and 10 000 ppm for 15 days (Sadasivan, 1951a, b). Both concentrations caused reductions in weight gain and caused the liver fat content to be much lower than the apparently elevated fat levels of the control rats.

Several experiments were designed to elucidate the cause of the anaemia which had been observed to occur in rats on high zinc diets (Sutton and Nelson, 1937). The anaemia is partly overcome by copper additives to the diet (Smith and Larson, 1946; Duncan *et al.*, 1953; Grant-Frost and Underwood, 1958). This was confirmed by Magee and Matrone (1960) who also showed that the addition of iron as well as copper to the diet more completely overcame the anaemia than copper alone.

Sutton and Nelson (1937) had also shown that diets containing high concentrations (5000 and 10 000 ppm Zn) of zinc can produce still-births and reduce numbers of newborn rats. This was confirmed by Schlicker and Cox (1968) who showed that 4000 ppm Zn in the diet for 20-40 days caused death and resorption of foetuses. If the high zinc concentration was fed both before and after conception it caused total foetal loss.

Several papers have been concerned with the effects of high zinc concentration in the diets of poultry. There seems general agreement that at zinc concentrations at or above 1500 ppm Zn (usually in the form of carbonate or oxide) there is growth depression with mortality occurring at levels above 3000 ppm (Roberson and Schaible, 1960; Klussendorff and Pensack, 1958; Johnson *et al.*, 1962). However in one experiment a zinc concentration as low as 800 ppm was toxic when added to a diet of sucrose and fish meal (Berg and Martinson, 1972). This paper emphasises the importance of diet composition in the production of zinc toxicity.

In man there are three recognised forms of zinc toxicity. Metal fume fever has been observed often in industrial workers who have inhaled fresh zinc oxide or chloride fumes (Burch and Sullivan, 1976). It is characterised by fever, chills, gastroenteritis and pulmonary manifestations. In recent years another form of zinc toxicity has been observed and is associated with the parenteral route (Gallery *et al.*, 1972; Brocks *et al.*, 1977). In the case reported by Gallery *et al.*, (1972), the zinc contamination of the haemodialysis water was traced to rainwater from a galvanised tank. In the other case the zinc was administered intravenously as part of a parenteral nutrition regimen. A prescribing error resulted in a massive intravenous overdose of zinc over 60 hours. The clinical signs were pulmonary oedema, hypertension, diarrhoea, vomiting, jaundice and oliguria. At postmortem examination there was evidence of an acute renal tubular necrosis, pulmonary oedema, macrophage infiltration and hyaline membrane formation in the lung and mild liver lesions.

The third form of zinc poisoning in man is associated with the ingestion of zinc. Murphy (1970) records the ingestion of 12 g of metallic zinc in a 16 year old male who subsequently showed lethargy, drowsiness and had increased serum lipase and amylase levels indicating pancreatic lesions. The ingestion of 45 g of zinc sulphate is reported to have caused death (Osol *et al.*, 1955). Vomiting often occurs in monogastrics following the ingestion of soluble zinc salts indicating a possible irritation of the gastric mucosa and acting to some extent as a protective mechanism. It is possible that zinc sulphate, like copper sulphate may cause vomiting by also acting on the chemoreceptor trigger zone in the brain. Zinc sulphate has been used as an emetic (Merck Index, 1960; Greig and Boddie, 1947). Vomition is hardly a likely protective mechanism for ruminants which have ingested zinc sulphate.

There has been controversy about the safety to man of using zinc sulphate orally and gastric lesions have been recorded where zinc has been used as a therapeutic agent (Glover and White 1977; Moore, 1978). However, in one trial 660 mg of zinc sulphate was administered orally daily for nearly a year without any adverse effects being observed (Prasad, 1976) and for periods over two years 'with little problem' (Oelshlegel and Brewer, 1977). It appears that the form of the soluble zinc salt may be as important as the actual salt chosen (Moore, 1978) and in man the dosage is recommended to be taken with meals to avoid the irritant effect on the stomach.

2.5.2 ZINC TOXICITY IN RUMINANTS

The effects of addition of zinc to the diet of penned sheep has been the subject of two papers (Ott *et al.*, 1966a,c). Zinc oxide was incorporated in the pelleted ration of lambs. In a series of experiments it was shown that at 500 ppm Zn in the feed for 10 weeks there was no effect of zinc. At 1000 ppm weight gains were normal but in one experiment there was slight pancreatic damage present. Pancreatic damage was extensive at 1500 ppm Zn in feed, and at 2000 ppm (85 mg Zn/kg b.wt./d) and above, feed intake and weight gains were reduced. Other effects of zinc recorded were high serum and organ (liver, kidney and pancreas) zinc concentrations, anaemia, lower

liver copper but higher iron concentrations and decreases in rumen volatile fatty acids together with a change in acetate/propionate ratio. Zinc sulphate was dosed intraruminally for 11 days and severe toxic effects of zinc appeared at this time with a dose rate of 100 mg Zn/kg b.wt./d. Much higher organ zinc concentrations were recorded in this experiment. In contrast to the experiment where zinc oxide was added to the diet, the intraruminally administered zinc sulphate caused an increase in haemoglobin concentration and packed cell volume. This was probably due to haemoconcentration resulting from a diarrhoea which developed. Using smaller numbers of animals Ott also examined the effect of additions of zinc oxide to the diet of beef cattle calves (Ott *et al.*, 1966b,d). At 500 ppm zinc in the diet no effect was noticed. However at 900 ppm zinc in the diet, zinc caused decreased weight gain and feeding efficiency while at 1700 ppm, depressed appetite and increased mineral and salt consumption occurred in addition to the decrease in weight gain and feed consumption. High zinc concentrations in the liver were accompanied by lower copper and higher iron concentrations in the liver. In an investigation of zinc additives to cattle diets of natural feedstuffs (Zurcher, 1970; Beeson *et al.*, 1977), there was evidence that at the highest concentration of zinc (640 ppm) the cattle gained 15% less than the average of the other groups (controls and lesser zinc additions) suggesting a possible zinc toxicity.

Some studies appear to have been prompted by the possibility of zinc (and other metals) poisoning stock as a result of either emissions from industrial sources or from the use of zinc industry byproducts in stock feed. Rosenberger and Gruender (1975) fed zinc oxide to heifers and dairy cows over four to five months and found that 1000 ppm in the food (i.e. approx. 24-28 mg Zn/kg b.wt./d) was well tolerated. However 1500 to 2200 ppm (approx. 40-50 mg Zn/kg b.wt./d resulted in inhibited development in heifers and a loss in body weight in cows but no decrease in milk yields. Some chronic zinc poisoning was caused by feeding 4400 ppm (110 mg Zn/kg b.wt./d) for four weeks. As feeding progressed haemoglobinuria, liver damage and progressive anaemia occurred prior to death. In another study Wietfeldt (1975) fed combinations of lead and zinc in the diet of young cattle. Cattle fed either lead (1500 ppm) plus zinc (4400 ppm)

or the zinc alone died after nine weeks. This author suggested that zinc potentiated lead poisoning but he attributed the deaths to zinc poisoning. For six weeks Miller *et al.*, (1965) added zinc oxide to the concentrate ration of dairy cows to give a concentration of 1280 ppm Zn in the feed. They were unable to demonstrate effects of zinc on body weight, milk production or red cell parameters nor any obvious effect on animal health. There was an approximate doubling and quadrupling of zinc plasma concentration at 690 and 1280 ppm of zinc in the feed respectively. The content of zinc in the milk doubled at both concentrations of zinc in the feed.

Miller (1970) in a review on zinc nutrition of cattle stated "it is improbable with adequate amounts of other nutrients that problems would arise in cattle receiving dietary zinc below 400-500 ppm. For most conditions it is possible that the tolerance may be considerably higher". This statement probably correctly states the safe limits for zinc additions to the feed of adult cattle. Young ruminants however appear to be more at risk from zinc toxicity. Miller *et al.*, (1970) using up to 633 ppm zinc in the feed of calves showed that homeostatic control of zinc absorption and tissue zinc concentrations is much less effective against this dietary zinc concentration than previously shown. The greatest changes in organ zinc concentration occurred between 233 and 633 ppm zinc in the diet indicating that homeostasis was less effective somewhere between these concentrations. No body weights were reported and no post#mortem examinations were carried out so no indication of zinc toxicity could be given. The zinc supplemented diets were only fed for 21 days.

Similar large increases in tissue zinc occurred in calves fed high (600 ppm) zinc diets (Stake *et al.*, 1975). The increases in tissue zinc concentration were present at seven days after commencement of zinc supplementation and there were further substantial linear increases with time up to 21 days. Their data indicated that at least part of the large increases in zinc deposition in tissues accompanied breakdown in zinc homeostasis and occurred very quickly. Neathery *et al.*, (1977) summarises differences between species of domestic animals in the extent of homeostasis. In particular they point out the difference between calves and mature non-lactating cows.

They found that 600 ppm added zinc to the diet of calves causes 4 to 20 fold increases in zinc concentration in the liver, kidney and pancreas whereas in the cow this level of zinc in the diet did not cause elevation in the liver or kidney. They suggested that in calves homeostatic control may be at the intracellular level.

A further indication of the sensitivity of young ruminants to zinc toxicity was provided by Davies *et al.*, (1977) who showed that severe toxicosis resulted from the ingestion of zinc in a milk substituted diet. They confirmed that a commercial yeast contaminated with zinc was responsible for poor growth and low appetite in suckling lambs. The reconstituted diet contained zinc sulphate and zinc intake was calculated to be 134 mg Zn/kg b.wt./d.

While no toxic effect of zinc on reproduction has been demonstrated in the male ruminant it has been shown that when pregnant ewes were fed a diet containing 750 mg Zn/kg from six weeks after conception, 11 foetuses were aborted and only three lambs survived more than two days after birth out of the 20 conceived lambs (Anon, 1974).

2.6 PATHOLOGY OF ZINC TOXICITY

The most significant feature of this examination of the literature on zinc toxicity is the paucity of detail on the pathological findings in zinc toxicity. Pathological lesions are most often reported as a chance finding and when reported are usually summaries or quotes from the reports of pathologists who have made examinations of tissues presented to them. Where an occasional emphasis on pathology has been made it has usually been with reference to one particular system, e.g. Aughey *et al.*, (1977), where the examination appears to have been mainly confined to the endocrine system.

Mention is made of the pancreatic lesions in several papers and it appears that this organ is a most sensitive indicator of zinc toxicity. Pancreatic lesions are recorded in zinc toxicity of dogs and cats (Drinker *et al.*, 1927; Scott and Fisher, 1937), rats (Veghelyi *et al.*, 1952), sheep (Ott *et al.*, 1966a,c), chickens (Soffietti and Bestetti,

1975) and man when pancreatic lesions were indicated by elevated serum amylase and lipase levels (Murphy, 1970). The gross lesion is variously described as an enlarged or small pancreas, firm or hard and often with nodules on the surface in chronic cases. Under the microscope the exocrine tissue is usually described as cystic with inflammatory cells occasionally present. Imperfect and disorganised acinar tissue is often described and the lesion is always described as being invaded by connective or fibrous tissue. In the case of Aughey *et al.*, (1977) only lesions of the islet cells were described in mice treated with zinc.

The caustic effect of zinc sulphate is well known and reference has occasionally been made to caustic erosions or haemorrhages of the gastric mucosa (Soffieti and Bestetti, 1975; Brink *et al.*, 1959; Moore, 1978). This irritant effect of zinc sulphate possibly is responsible for the emetic effect of zinc sulphate (Esplin, 1970). In the ruminant the direct effect of zinc on the rumen microflora has been suggested as a possible cause of gastrointestinal dysfunction and the changes in rumen pH, total volatile fatty acid concentration and acetic: propionic acid ratio (Ott *et al.*, 1966c). Zinc has been shown to have an effect on cellulose digestion by rumen microorganisms (Hubbert *et al.*, 1958; Martinez and Church 1970).

Liver lesions have been mentioned only once in cases of zinc toxicity (Wietfeldt, 1975). In this case cattle had been fed a diet containing 2200 or 4400 ppm of zinc for four months.

Kidney lesions appear to be mainly related to extremely high serum levels of zinc caused either by parenteral administration of zinc (Brocks *et al.*, 1977), or zinc toxicity of young animals (Davies *et al.*, 1977). An acute tubular necrosis of the lower nephron has been described in the former case and in the case of the suckling lambs enlarged pale kidneys were described and the microscopic changes described were glomerular atrophy, tubular necrosis and dilatation and interstitial fibrosis.

The effect of zinc on the haemopoietic system has occupied the attention of some authors (Grant-Frost and Underwood, 1958; Magee

and Matrone, 1960; Ott *et al.*, 1966c) but the investigations have been confined to describing the anaemia and the possible effect of zinc on copper and iron metabolism as a cause of the anaemia. In rats it appeared that the anaemia was due to the effect of zinc on copper and iron and these two substances when added to the zinc supplemented diet alleviated the anaemia (Magee and Matrone, 1960).

More recently, since the commencement of this study, a possible direct destructive effect on red blood cells has been recorded in zinc toxicity of cattle. Haemoglobinurea together with severe liver and kidney lesions were observed in the cattle (Wietfeldt, 1975).

2.7 SOURCES OF ZINC CONTRIBUTING TO ZINC TOXICITY

2.7.1 ZINC IN FOOD

Zinc occurs naturally in pasture plants but not at concentrations sufficiently high to cause toxicity to herbivores. Pasture zinc concentrations of 8-70 ppm throughout New Zealand grasses (Grace, 1972) and 7-50 ppm for Otago and Southland properties (Cornforth pers. comm.) have been found. At Ruakura the range over one year was 22-79 ppm (Towers, 1977b). These figures cause more concern over the possibility of zinc deficiency in herbivores rather than zinc toxicity. There is very little evidence for the accumulation of zinc by plants although there is one record of high levels (9000 ppm DMB) of zinc in *Arabidopsis thalianum* growing in association with zinc oxide, and *Viola calamineria* and *Philadelphus sp.* are known as indicator plants for zinc (Cannon, 1960).

Evidence for high zinc content of pasture comes from industrially contaminated areas as in Germany (Wagner and Siddiqui, 1973) or New Zealand where in a survey the only high concentrations of zinc in ruminant organs came from areas near a smelter (D.R. Harrison, pers. comm.). A potential source of zinc toxicity in herbivores associated with plant ingestion is seen in the case of zinc spraying of pastures for facial eczema control. Sufficient insoluble zinc oxide (which adheres to the plant surface) was sprayed onto pasture to cause

protection against pithomycotoxicosis and double this spray rate caused zinc damage to the pancreas (Smith, 1977). Average zinc content of the pastures that caused zinc toxicity was 1180 ppm (Towers *et al.*, 1976).

High concentrations of zinc are found in some natural foods of omnivores (Anon, 1954). Zinc concentrations greater than 1000 ppm are often found in oysters and oysters from zinc contaminated areas have zinc concentrations as high as 6000 ppm as were found in oysters from the Derwent Estuary in Tasmania (Eustace, 1974). Gastronomic eating records for oysters have been recorded as high as 558 oysters and at a zinc concentration of 1000 ppm and oyster weight of 6 g these could represent a zinc intake of >3 g. While no instances of zinc toxicity arising from such excesses have been recorded some risk from zinc toxicity is probably present.

2.7.2 INDUSTRIAL SOURCES OF ZINC

Fresh zinc oxide fumes from industrial situations are a well known source of inhalation toxicity in man. Zinc contaminated water from heavy metal processing and mining sources has been suspected of causing a problem in trout streams in Colorado (Nehring and Goettle, 1974). Their laboratory studies showed that the mean levels of zinc in the contaminated water were capable of causing death in trout. The zinc concentrations causing death (TD50) were between 0.41 and 0.96 mg Zn/ℓ depending on the species of trout tested.

Zinc contamination of milk which had lain in galvanised pipes is believed to have been responsible for an illness in piglets (Grimmett and McIntosh, 1936). Subsequent trial feeding with zinc lactate caused lameness and unthriftiness (Grimmett *et al.*, 1937).

The high zinc content of Toprina yeast associated with zinc toxicity of suckling lambs presumably arose from industrial contamination of this commercial yeast (Davies *et al.*, 1977).

2.7.3 ACCIDENTAL ADMINISTRATION OF ZINC

Two separate parenteral sources of zinc have been incriminated in zinc toxicity of man. In the first (Gallery *et al.*, 1972) rain-water from a galvanised tank was used in a home kidney dialysis unit. The high zinc concentration in the water is believed to be responsible for the illness which occurred in this patient. In another case where parenteral nutrition was being administered to an elderly woman a prescribing error resulted in a high zinc concentration occurring in the intravenously administered fluid. This caused severe zinc toxicosis and death.

Several cases of oral self administration of large amounts of zinc have been recorded in man. Murphy (1970) records a case of illness in a 16 year old boy who ingested 12 g of elemental zinc to hasten wound healing and death has been caused by the ingestion of 45 g of zinc sulphate (Osol *et al.*, 1955).

CHAPTER 3

TOXICITY OF ZINC SALTS TO RUMINANTS

CHAPTER 3

TOXICITY OF ZINC SALTS TO RUMINANTS

3.1 ZINC TOXICITY IN SHEEP

3.1.1 TOXICITY OF ZINC SULPHATE IN SHEEP

Introduction

Because the dose rates of zinc sulphate needed to effect protection against pithomycotoxicosis are so high (20 mg Zn/kg b.wt./day or approximately 20 times daily zinc requirements) it was predicted that the margin of safety for the use of zinc for protection would be narrow. To ensure accuracy of dosage, which in all likelihood would be essential in the field situation because of the predicted low safety margin, it was anticipated that zinc sulphate would have to be administered by hand to ruminants. At higher dose rates the solubility of zinc sulphate together with limitations imposed by maximum drenching gun volume precludes the use of a drenching gun and oral hand dosing must be by intraruminal intubation. However, at low dose rates these limitations do not apply and the drenching gun may be used.

In this experiment the toxic effects of orally administrated zinc sulphate were investigated at three separate daily dose rates.

Materials and Methods

Three pairs of 16 month old (50-60 kg) pasture grazed Romney wethers were given daily doses of zinc sulphate by intraruminal intubation for six weeks and, because the volume of the lowest dose rate allowed it, thereafter by drenching gun. The zinc sulphate was dissolved in 200 ml of water for intraruminal intubation and administered by drenching gun as a 59% w/v aqueous solution. The three dose rates were 180, 60 and 20 mg Zn/kg b.wt./day. Five control sheep were not dosed.

Body weights were recorded three times a week for the first six weeks of the experiment and less frequently (at least monthly) thereafter. Dosing continued until the sheep died or were killed *in extremis* for

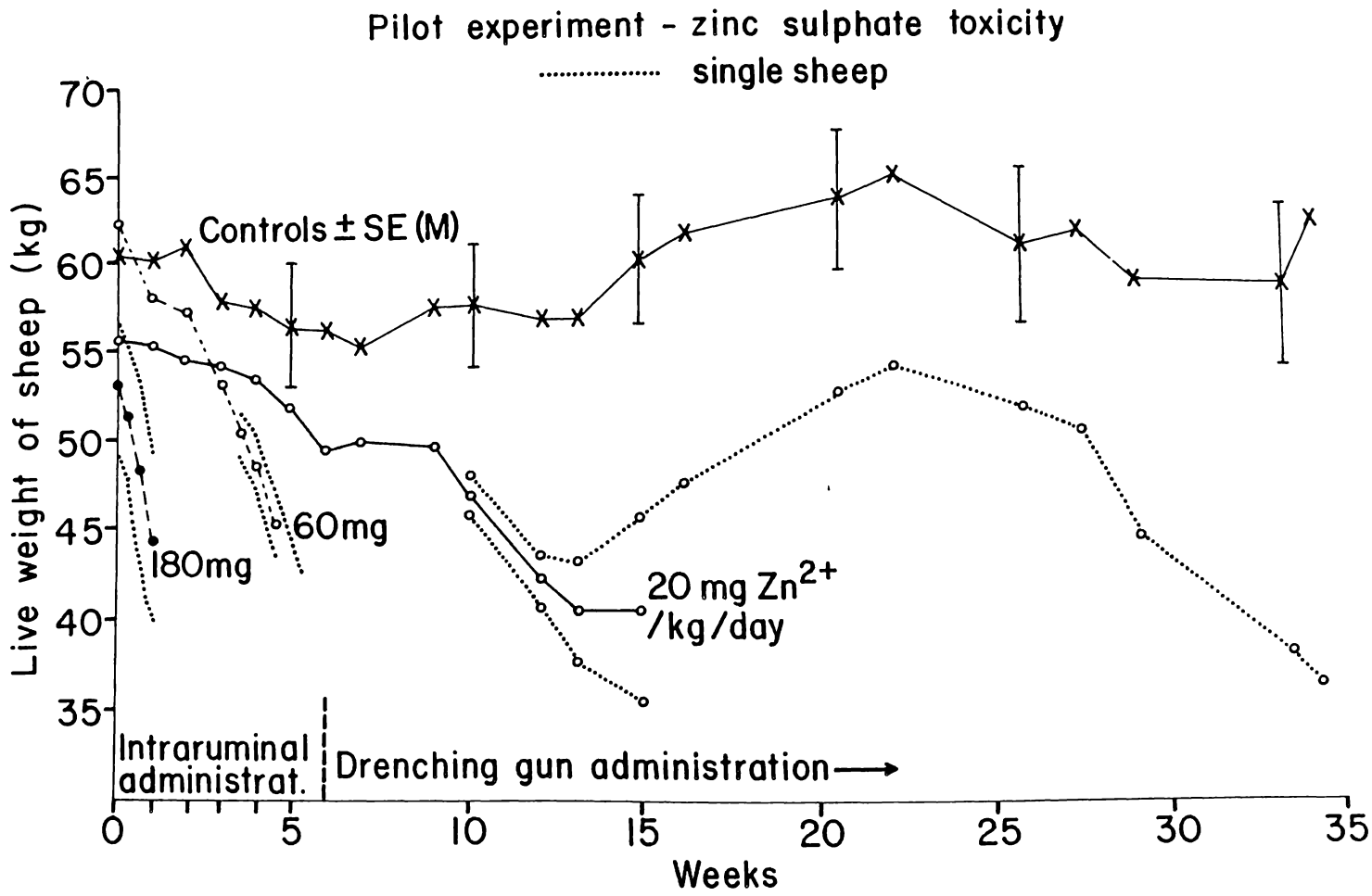


Figure 3.1 : Weight changes and survival times in pairs of sheep dosed daily with zinc sulphate solutions; x —— x mean bodyweight ±SEM for five non dosed control sheep; o —— o mean weights 20 mg Zn/kg/d; o ---- o means 60 mg Zn/kg/d; • ---- • means 180 mg Zn/kg/d; ..... weights of single sheep.

humane reasons. Postmortem examinations were carried out and samples of organs showing changes were taken for microscopic examination (Appendix B). Liver, kidneys and pancreas were weighed and samples taken for zinc analysis (Appendix A).

At 32 days after the start of dosing an intravenous glucose tolerance test (Kaneko, 1970) was performed on the two sheep dosed at 60 mg Zn/kg b.wt./d and two control sheep (Appendix E).

Results

Weight changes are recorded in Figure 3.1. The sheep receiving the highest daily dose (180 mg Zn/kg b.wt./d) developed severe diarrhoea and showed a rapid decline in body weight preceding death which occurred one week after the start of dosing. The two sheep dosed 60 mg Zn/kg b.wt./d) suffered a less severe diarrhoea and the faeces had a fish-like odour. These two sheep also showed a rapid decline in body weight and death occurred five weeks after the start of dosing. In the five days preceding death the animals showed lethargy, anorexia and finally coma. The remaining pair (20 mg Zn/kg b.wt./d) slowly lost weight especially over the terminal stages and deaths occurred at 16 and 35 weeks. Clinical signs were similar to those seen in the previous pair but more prolonged.

The two sheep given the highest dose rate showed gross pathological lesions, confined to the upper digestive tract. Necrosis of the mucosa of the lower oesophagus, rumen and abomasal mucosa and oedema of the abomasum was present. The sheep dying at one month or more after the start of dosing had severe pancreatic lesions. The pancreas was very small (pancreatic weight about a quarter of those recorded later in control animals), (Table 3.1) pale and firm. Severe pancreatic necrosis and fibrosis was seen at microscopic examination. This pancreopathy and a mild abomasitis, oesophageal and reticular groove necrosis (Plates 3.1 and 3.2) was also recorded in the pair of sheep receiving the lowest dose rate. In addition evidence of changes in fat metabolism were seen. A fatty liver change (Sheep No. 778 liver 52.3% fat DMB based on ether extract) (Plate 5.27) was present and abdominal fat reserves were good despite the emaciated condition of

Table 3.1 Effect of daily dosing of zinc sulphate on pancreas weight and organ zinc concentrations of sheep.

Treatment	Sheep	Pancreas wt (g)	Organ zinc concentrations µg Zn/g DMB Liver	Kidney	Pancreas
Controls		68.6 ±10.8	134.6 ±9.8	113.4 ±9.7	89.0 ±16.9
20 mg Zn/kg	778	12.5	242	3137	1719
	789	5.2	335	2184	1483
60 mg Zn/kg	707	9.0	1287	2057	1341
	790	8.0	1174	3236	499
180 mg Zn/kg	759	*	NA	NA	NA
	810	*	1000	1116	2489

Means ±SD are given for five control sheep; * weight not recorded but organ noted as normal size at post mortem examination; NA, not available; DMB, dry matter basis.

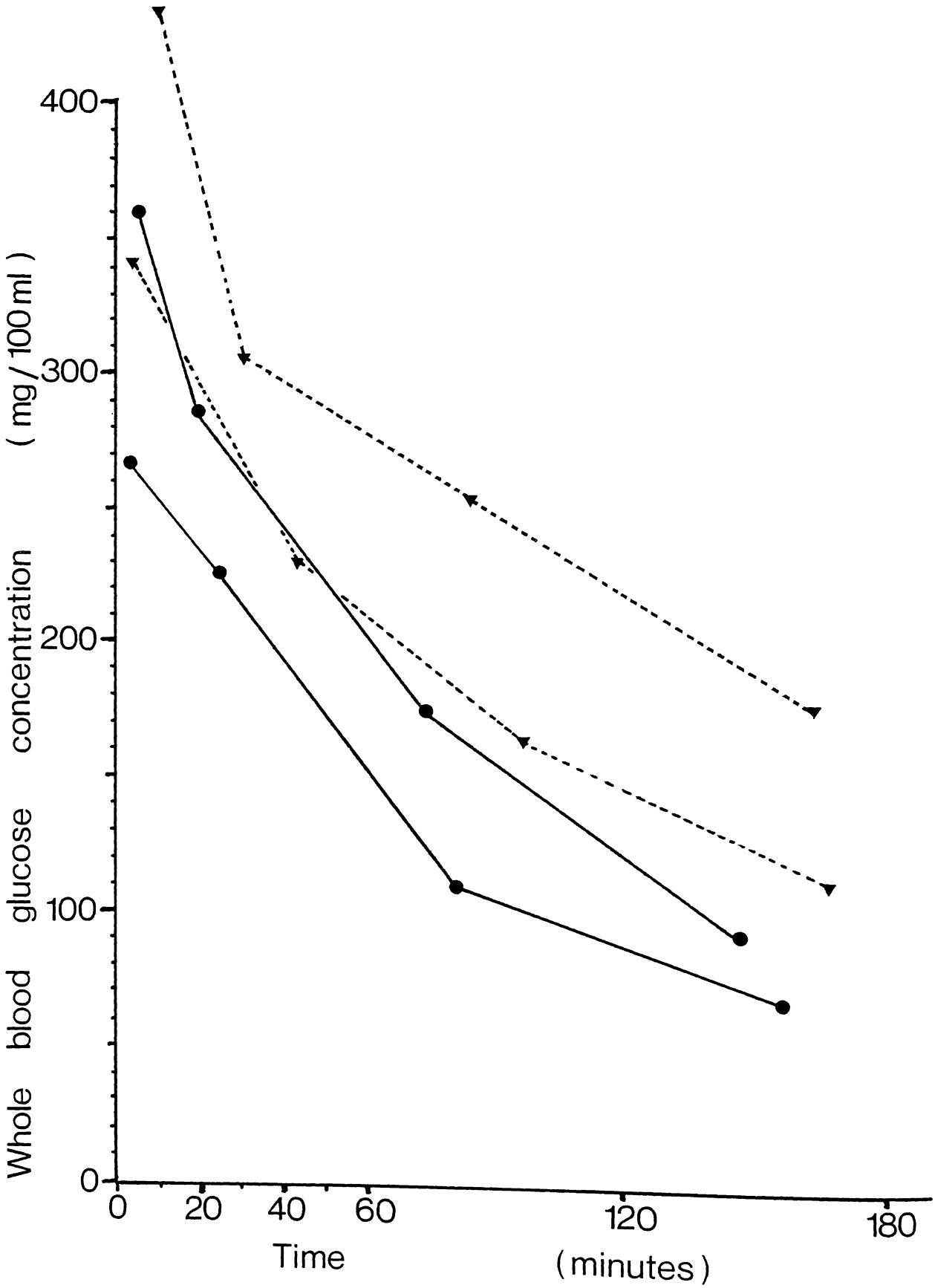


Figure 3.2 : Results of intravenous glucose tolerance tests performed on two control sheep (● —— ●) and two sheep which had been given 60 mg Zn/kg b.wt/d (as sulphate) (▼ ---- ▼) for 32 days.

the sheep. In the sheep which died at 35 weeks there was evidence of there having been one or more haemolytic episodes. This was seen as generalised icterus, yellow liver, dark kidneys and dark brown urine (Plate 5.24). The pathological changes in this and subsequent experiments are more fully recorded in the section on pathology of zinc toxicity (Chapter 5.1.3). The zinc concentrations for liver, kidney and pancreas are recorded in Table 3.1. In many cases these concentrations rose to over ten times those of the control sheep.

The glucose tolerance curves for two control and two zinc dosed (60 mg Zn/kg/d) are given in Figure 3.2. The zinc dosed sheep tended to have higher whole blood glucose concentrations at most times after the intravenous infusion of glucose.

Discussion

The weight losses, clinical signs, times of deaths and postmortem lesions show that daily dosing with zinc sulphate at all three dose rates caused zinc toxicity. Most significant was the effect of daily dosing at 20 mg Zn/kg b.wt./d, the dose rate found to give good protection against pithomycotoxicosis in sheep. Although prophylactic dosing of zinc would not be continued for the time that dosing continued (up to 35 weeks) in this experiment, these results did suggest that homeostasis was ineffective even at the lowest dose rate. Where dosing was prolonged as in this experiment the cumulative effect resulted in toxicity and elevated zinc concentrations in liver, kidney and pancreas. The sheep on the lowest dose rate had lower liver zinc concentrations than the other four sheep. This was probably at least partly due to the very high fat content seen at microscopic examination and in one sheep by the very high ether extract figure.

At the two highest dose rates the corrosive effect of the zinc sulphate was illustrated by the severe erosions of the upper digestive tract, even in areas covered by keratinised stratified squamous epithelium such as oesophagus, rumen, reticulum and reticular groove. These lesions were also present, but to a lesser extent in the sheep on the lowest dose rate and probably played some part in the breakdown in homeostasis and hence toxicity of zinc sulphate in this experiment.

In the other experiments with sheep (Ott *et al.*, 1966a) in which zinc oxide was added to the diet there were toxic lesions in the pancreas only, but at higher dose rates (greater than 53 mg Zn/kg b.wt./d) than the lowest recorded in this experiment. In one of Ott's experiments zinc sulphate was administered by intraruminal intubation and severe effects were recorded at 11 days at doses greater than 100 mg Zn/kg b.wt./d. These effects at this dose rate are comparable with those recorded in the present experiment.

In both sheep on the lowest dose rate of zinc sulphate, especially the last to die, there was evidence of one or more haemolytic episodes. The appearance of the organs resembled that seen in sheep with chronic copper poisoning. This is a new finding in the pathology of zinc toxicity and in addition to its significance in zinc toxicity it could be of considerable significance and value in elucidating the pathogenesis of chronic copper poisoning. Further discussion in the haemolytic crisis will be presented in later chapters (Chapters 3 and 5).

The significance of the intravenous glucose tolerance curves is uncertain. With only two pairs of sheep to compare it cannot be certain if the slower elimination of glucose in the zinc sulphate dosed sheep is significant or pathological. Even if so, it is difficult to interpret such sets in the absence of further tests. Derangements of fat and liver metabolism are known to affect the glucose tolerance test and in the absence of detectable (by light microscopy) islet cell lesions such an explanation must be considered.

The absence of lesions detectable by light microscopy of course does not exclude the possible existence of biochemical lesions or other lesions undetectable by light microscopy.

3.1.2 TOXIC EFFECTS OF THE LONG TERM ORAL ADMINISTRATION OF ZINC SULPHATE AND ZINC OXIDE TO SHEEP USING LOW DAILY DOSE RATES

Introduction

In the pilot experiment (Chapter 3.1.1) toxic effects of zinc sulphate were observed at daily dose rates as low as 20 mg Zn/kg b.wt./d which is about the daily dose found to be optimal for preventing pithomycotoxicosis in sheep (Smith *et al.*, 1977). It was therefore decided to examine the long term effects of dosing zinc sulphate and zinc oxide to sheep at dose rates near to those needed for preventing pithomycotoxicosis. Daily dosing by hand was chosen as the most accurate practical method of oral administration as it was realised that the safety margin in dosing zinc for pithomycotoxicosis control was likely to be small. The drenching gun and paste gun are two methods of stock medication commercially available to the New Zealand farmer.

This experiment examines the effects of long term daily dosing of sheep with zinc sulphate solution by drenching gun and zinc oxide paste by paste gun.

Materials and Methods

Forty Romney and Romney-cross pasture-grazed 15 month old (40-50 kg) wethers and ewes were randomly divided into five groups of eight. A control group was non-dosed and two groups were assigned to low (LS) (15 mg Zn/kg b.wt./d) and high (HS) (30 mg Zn/kg b.wt./d) zinc sulphate treatments delivered by drenching gun six days of each week as a 33% w/v $ZnSO_4.7H_2O$ aqueous solution. The two remaining groups were dosed by paste gun at the same time with 15 (LO) and 30 mg Zn/kg b.wt./d (HO) as a zinc oxide paste. The paste was made by mixing 140 g ZnO and 220 g H_2O with 2 g Tragacanth powder to which 25 mg of EtOH had been added.

Body weights were recorded at fortnightly intervals and doses of zinc adjusted fortnightly to the new mean body weight for each group. Serum and plasma (EDTA) samples were collected for zinc (Appendix A) and selenium analysis and haematology (Appendix D) at monthly intervals.

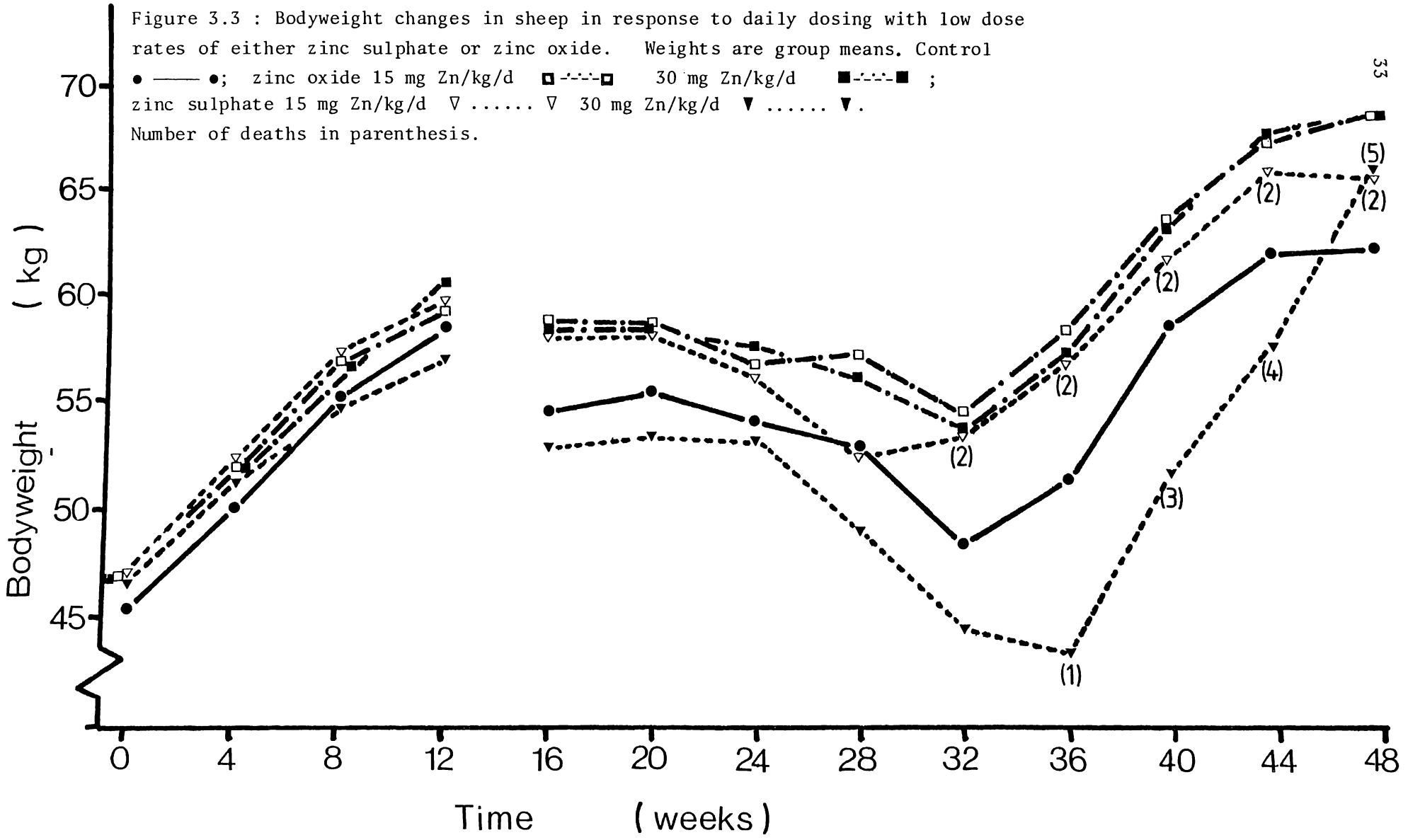


Figure 3.3 : Bodyweight changes in sheep in response to daily dosing with low dose rates of either zinc sulphate or zinc oxide. Weights are group means. Control ● —— ●; zinc oxide 15 mg Zn/kg/d ◘ -·-·- ◘ 30 mg Zn/kg/d ■ -·-·- ■ ; zinc sulphate 15 mg Zn/kg/d ∇ ..... ∇ 30 mg Zn/kg/d ▼ ..... ▼. Number of deaths in parenthesis.

An intravenous glucose tolerance test (Kaneko, 1970) was performed on clinically affected and three control sheep approximately seven months after the start of dosing (Appendix E). All sheep which died, or were slaughtered at the end of the experiment after 12 months of dosing, were subjected to a postmortem examination and tissues taken for zinc analysis (Appendix A) and microscopic examination of haematoxylin and eosin stained sections (Appendix B). Pancreatic lesions were scored on a 0-10 basis (Appendix C).

Results

One control sheep, a large Romney/Border Leicester cross wether, was excluded from the experiment because of its unpreventable habit of leaping or forcing its way into adjoining paddocks where the feed was more plentiful. This meant that the control group started the experiment approximately 0.8 kg (mean body weight) lighter than the other groups.

Weight changes The mean body weights for all groups are recorded in Figure 3.3. As the experiment progressed the two groups on zinc oxide administration gained weight at a faster rate than either the control or zinc sulphate groups. The effect was most noticeable after six months and by the time the experiment ended there was a mean gain over controls of +5.5 and +6.3 kg for the low and high zinc oxide groups respectively.

The HS group of sheep gained weight slower than the control group, the difference being apparent at six weeks. There was a further decline in weight gain in the HS group with mean differences from control weight of -2.3 and -4.9 kg at 14 and 32 weeks respectively. After this time deaths occurred in the HS group causing an upwards bias in mean weight of the survivors to the extent that by the end of the experiment the mean weight of the remaining three HS sheep was 3.5 kg more than controls. A similar effect of lesser magnitude was seen in the weight changes of the LS group.

All sheep were shorn after 16 weeks of dosing and shortly after this were rationed on winter feed (autumn saved pasture and hay), with all

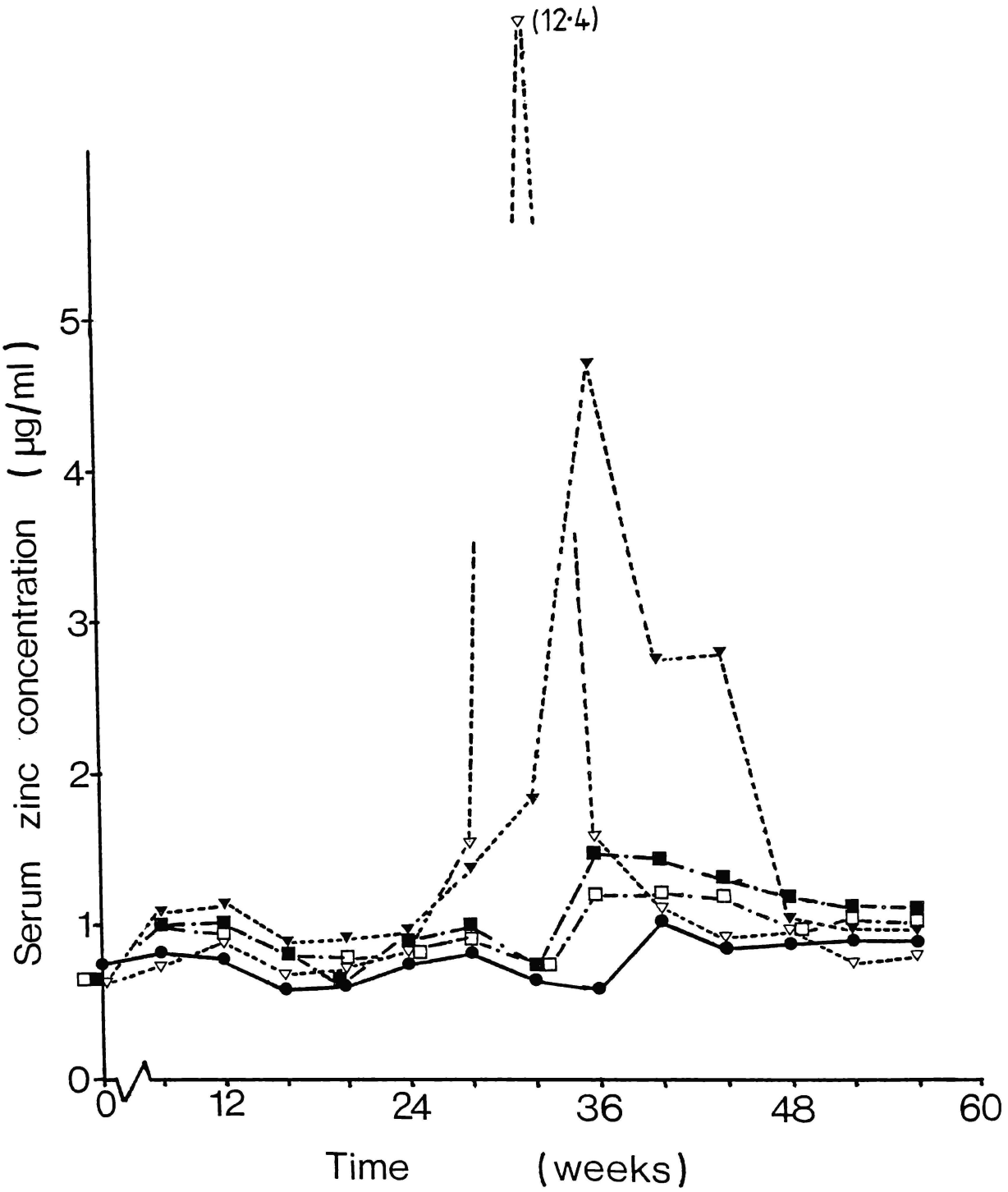


Figure 3.4 : Group mean serum zinc concentrations in sheep dosed daily with low doses of zinc oxide or zinc sulphate. Control ● —— ●; zinc oxide 15 mg Zn/kg/d □-·-·-·□ ; 30 mg Zn/kg/d ■-·-·-·■ ; zinc sulphate 15mg Zn/kg/d ▽ ..... ▽ , 30 mg Zn/kg/d ▼ ..... ▼.

groups losing weight over the winter. Body weights started to rise again after July 1977 (32 weeks) when a rising plane of nutrition became available.

Clinical signs and deaths Two sheep only from the LS group died after approximately 28 weeks. The first sheep of the HS group died after eight months of dosing and over the next six weeks a further four sheep died. For several weeks prior to each death there was a gradual decline in individual body weight of 15-25 kg accompanied by anorexia and lethargy. The affected sheep were observed to be just standing in the paddock and moved very slowly when disturbed. The weakness often resulted in sheep becoming cast. Occasionally head nodding, excessive salivation, lip licking, foetid diarrhoea and wool break were observed. Anaemia occurred in all sheep of the HS group that died and emaciation was present. All sheep that died weighed at death between 8 and 23 kg less than their starting weight.

Clinical Pathology Anaemia was present in some sheep of all groups, including controls, in the autumn of 1977, some three to four months after the start of dosing. The anaemia was due to *Haemonchus contortus* infection despite regular precautionary drenching of all sheep with a broad spectrum anthelmintic. The problem had resolved by the end of June and it was then possible to distinguish anaemic sheep in the zinc dosed groups. From this time onwards occasional sheep in the HS group became anaemic (defined as a PCV lower than the lowest value for the control group). The anaemia was occasionally only of short duration, the PCV being normal at the next blood sampling time, and four of the five sheep to die in the HS group were anaemic at the time of death with PCV values of less than 20%. Haemoglobin concentrations and total red cell counts were also low when PCV values were low.

Serum zinc concentrations are shown in Figure 3.4. Mean serum zinc concentration for the control group were always within the range 0.6 to 1.1 µg Zn/ml. Within the LO group the highest mean serum zinc concentration was 1.17 µg Zn/ml at 40 weeks with the highest individual level being 2.15 µg Zn/ml at 56 weeks. In the HO group the highest mean was 1.48 µg Zn/ml at 36 weeks with the highest individual concentration being 1.75 µg/ml also at 36 weeks.

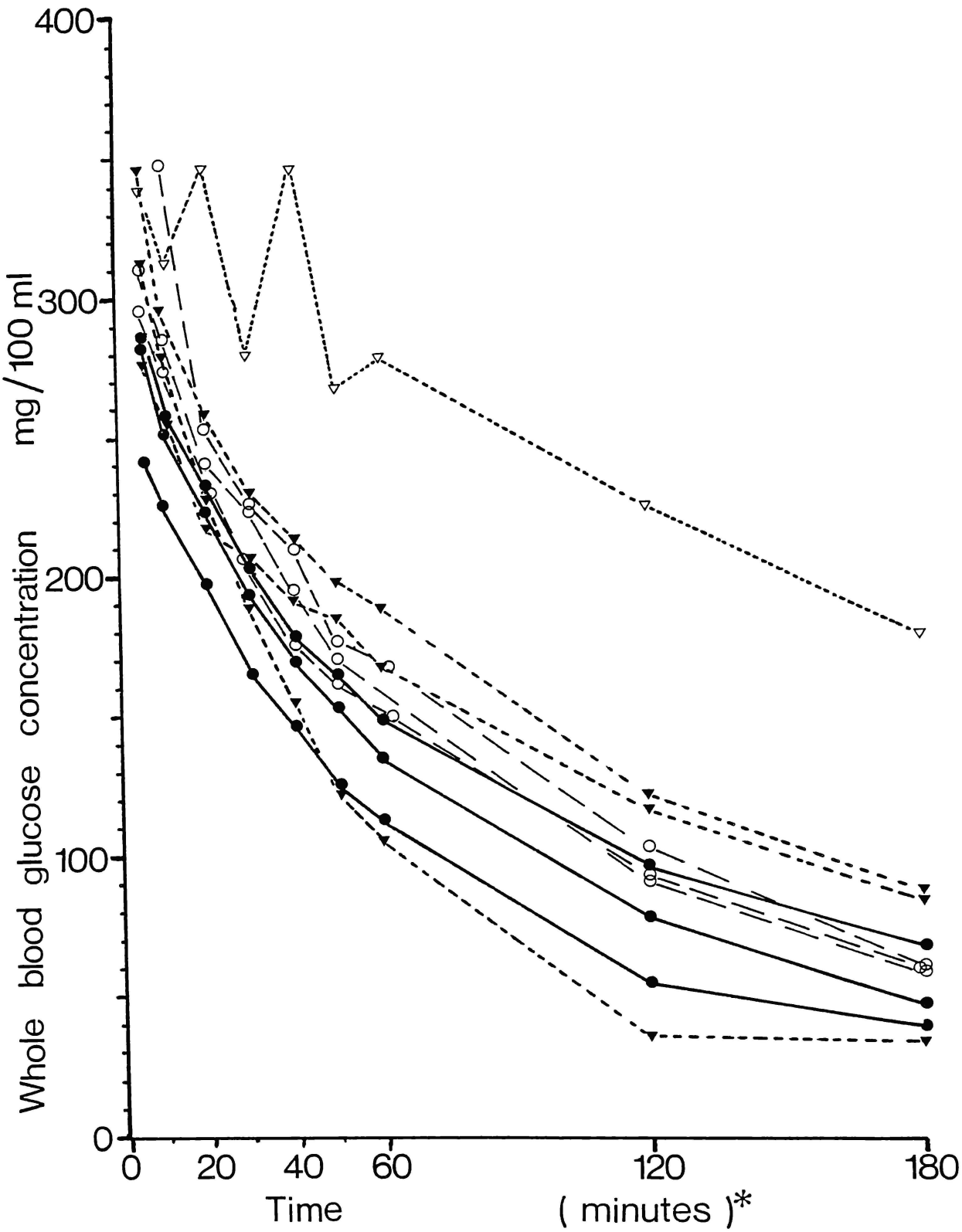


Figure 3.5 : Intravenous glucose tolerance curves for sheep that have been dosed with zinc for approximately 28 weeks. Control ● —— ●; zinc sulphate 30 mg Zn/kg/d ▼ ---- ▼; zinc sulphate 15 mg Zn/kg/d ▽ ----- ▽; zinc oxide 30 mg Zn/kg/d ● — — — ●; * minutes after i/v glucose injection.

Serum zinc concentrations were much higher in the zinc sulphate dosed sheep. The highest mean serum zinc concentrations in the LS group were 1.57 and 12.4 µg Zn/ml (includes one very high abnormal value) at 28 and 32 weeks respectively. At 28 weeks two sheep had serum zinc concentrations of 3.7 and 3.1 µg Zn/ml and at 32 weeks one sheep had a serum zinc value of 83.8 µg Zn/ml three days before its death. In the HS group all sheep showed high serum zinc values at 36 weeks (mean 4.62 µg Zn/ml) and all sheep had their highest values either at this time or at the blood sampling time immediately preceding their death. The highest individual value was 9.25 µg Zn/ml and six of the eight sheep in this group had values greater than 4 µg Zn/ml at some time during the experiment.

Intravenous glucose tolerance curves for three control and seven clinically affected zinc dosed sheep at 28 weeks after the start of dosing are given in Figure 3.5. With the exception of one sheep there did not appear to be a significant difference between the control curves and those of the zinc-dosed sheep.

Postmortem findings In all sheep that died during the experiment there were moderate to severe pancreatic lesions of zinc toxicity (Table 3.2) and six of the seven showed some evidence of haemolytic episodes or crises. This consisted of anaemia, jaundice, dark or black kidneys and haemoglobunuria. These are more fully described in the section on the pathology of zinc toxicity (Chapter 5). Fatty changes were seen in the livers of some of these sheep.

Pancreatic weights (Table 3.3) were lower in those animals which died during the experiment (10.7 g ±4.4 SD *cf* controls 75.8 g ±14.8 ($P<0.001$). Both sheep in the LS group had pancreatic damage scores of nine and the five from the HS group had moderate change (mean score 6.0; range 3 to 9; n=5). Only seven sheep other than these had pancreatic lesions, one from group LO (score 2) and five from the HO group (mean score 4.0; range 1 to 8; n=5). Only one sheep from the survivors of either the LS or HS group had a pancreatic lesion (score 1). The adrenal glands were enlarged in those sheep that died (mean 7.1 g ±2.6 SD of controls 5.0 g ±1.1 SD $P<0.05$) and livers were smaller (576 g ±110 SD *cf* controls 823±46 $P<0.001$).

Table 3.2 Extent of pancreatic injury and numbers of deaths occurring in sheep dosed daily with zinc sulphate or oxide for approximately 13 months. Low , 15 mg Zn/kg b.wt/d; high, 30 mg Zn/kg b.wt/d.

Treatment	Pancreas injury No/group	Mean	Range	Deaths
Control	0	0	0	0
Low ZnO	1	0.25	0-2	0
High ZnO	5	2.75	1-8	0
Low $ZnSO_4$	2	2.25	9	2
High $ZnSO_4$	6	2.9	1-9	5

Pancreas injury score 0-10; (0 no injury; 10 total area of section affected) based on microscopic examination.

Table 3.3 Effect of daily dosing of low doses of zinc oxide and zinc sulphate on mean body and organ weights and carcass fat parameters (±SD); low dose 15 mg Zn/kg/d; high dose 30 mg Zn/kg/d.

	Weight change kg	Carcass Wt. kg	Pancreas g	Liver g	Adrenal g	Thymus g	Heart g	Kidney g	Fat depth (mm) Back(C)	Fat depth (mm) Rib(J)
Control	17.1 ±3.7	33.1 ±3.0	75.9 ±14.8	823 ±46	5.0 ±1.1	17.5 ±8.9	263 ±23	137 ±12	10.7 ±2.3	22.7 ±3.1
Low ZnO	21.8 ±5.3	37.4 ±3.2	71.9 ±7.6	812 ±82	4.8 ±1.6	14.1 ±6.3	251 ±24	135 ±8.5	17.6 ±4.4	32.9 ±7.1
High ZnO	22.9 ±4.6	37.8 ±4.9	67.4 ±18.7	834 ±109	4.8 ±1.0	15.6 ±7.1	251 ±25	135 ±8.7	17.9 ±7.9	33.4 ±10.0
Low $ZnSO_4$	13.3 ±14.6	36.1 ±2.8	55.5 ±29.2	760 ±133	5.0 ±1.2	11.8 ±5.7	256 ±38	138 ±18	NA	NA
High $ZnSO_4$	-3.6 ±19.8	36.2 ±4.3	34.6 ±33.3	662 ±176	6.2 ±3.0	13.3 ±6.1	190 ±41	130 ±14	NA	NA

Table 3.4 Effect of daily dosing of low doses of zinc oxide and zinc sulphate on mean organ zinc concentrations. Low dose 15 mg Zn/kg/d; high dose 30 mg Zn/kg/d; ±SD.

	Mean organ zinc concentration ±SD (µg Zn/g DMB)					
	Liver	Kidney	Pancreas	Adrenal	Heart	Vastus medialis
Control	111 ±27	103 ±14	98 ±21	62 ±2.7	68 ±16	134 ±23
Low ZnO	189 ±73	231 ±83	179 ±40	60 19.1	71 ±26	137 ±34
High ZnO	290 ±162	528 ±424	347 ±117	64 ±6.6	76 ±10	140 ±20
Low $ZnSO_4$	417 ±469	274 ±159	1246 ±2632	85 ±47	90 ±24	169 ±42
High $ZnSO_4$	843 ±920	1458 ±1207	2193 ±2645	80 ±26	132 ±70	208 ±66

A noticeable feature at postmortem examination was the tendency of zinc-dosed sheep to have greater reserves of depot fat compared with controls. This was especially noticeable in those sheep which died in an emaciated state. At slaughter of the survivors at the end of dosing the fat depth over the ribs (J) and loin (C) was measured in the LO and HO groups and compared to controls. The mean measurements (Table 3.3) were significantly greater than controls for both groups and both measurements (all $P<0.01$).

Organ zinc concentrations (Table 3.4) were highest in the liver and pancreas of the zinc dosed sheep. The zinc sulphate dosed sheep that died had zinc concentrations in the pancreas ranging from 496 to 7670 µg Zn/g. The zinc concentrations in heart and vastus medialis muscle were significantly higher ($P<0.01$ and <0.001 respectively) than controls in those sheep that died during the dosing period.

Discussion

These results show that long term hand dosing of the zinc salts, zinc oxide and especially zinc sulphate at daily dose rates similar to those used for facial eczema protection can result in zinc toxicity of sheep. In the case of the zinc sulphate dosed sheep deaths occurred between seven and eleven months after the start of dosing. Although two deaths occurred earlier in the LS group than in the HS group the HS sheep were more severely affected than the LS sheep. Deaths occurred in the sulphate groups only after seven months of dosing but zinc toxicity was probably present as early as four months judging from the lower weight gains in both sulphate groups. The only sign of toxicity in the oxide groups was the presence of pancreatic lesions and these were more extensive in the HO group.

The variation in response to zinc dosing was great in all groups. Even in the HS group, where five out of eight sheep showed severe loss of weight and died with severe lesions of zinc toxicity, the remaining three animals remained clinically normal throughout the experiment and two of the three showed no lesions, even of the pancreas, at postmortem examination. The overall findings indicate a greater susceptibility of adult sheep to zinc salts than is indicated by previous published

work (Ott *et al.*, 1966a), conducted under different conditions. Their sheep were penned and fed a concentrate diet in which zinc oxide was incorporated for only 12 weeks.

The greater weight gains in the zinc oxide groups compared to controls was an unexpected result of the experiment. The difference was apparent in carcass weights at the conclusion of the experiment suggesting that visceral weight alone was not responsible for the difference from controls. In fact mean carcass weight was 52% of mean body weight at slaughter for controls and 55% for both zinc oxide groups. Approximately 75% of the difference in live weight between the zinc oxide dosed groups and controls was due to the difference in carcass weights. The measurements of carcass fat showed that the zinc oxide groups had greater fat depots than controls, suggesting that at least some of the difference from controls in body weight was due to lipid content.

This increase in fattiness is possibly a result of greater growth rate in the zinc oxide dosed groups. This has been shown to occur in sheep (Barton and Kirton, 1958). The reason for the greater growth rates can only be speculated on. Feed intakes could not be measured in this experiment. In previous experiments with lambs (Ott *et al.*, 1966a), weight gains greater than those for the control groups were recorded as the amount of zinc added to feed increased up to 0.5 or 1.0 g Zn (as zinc oxide)/kg feed. This addition of zinc was equivalent to zinc intakes of 40-50 mg Zn/kg b.wt./d. An interesting finding in Ott's paper (1966a) was that when the weights of the zinc-supplemented lambs gained faster than the controls the ratio of feed consumed to weight gain decreased indicating a greater efficiency of food conversion. The present results support those of Ott *et al.* (1966a) and their feed to gain ratios suggest a possible reason for the weight gains in this experiment although it is still not known what causes the greater efficiency.

The possibility that the fattiness was a consequence of the pathological changes (e.g. pancreatic lesions) was considered. However there was no correlation between pancreatic lesions and the fat measurements of the zinc oxide dosed groups. Had fattiness resulted from the pancreatic change a correlation might have been expected.

The mean serum zinc concentration of *all groups* showed an upward movement (significance $P<0.001$ even in controls) at the late winter - early spring period. The significance of this movement is unknown although it does coincide with the onset of spring growth and the start of the increase in growth rates in all groups. There is no evidence for a strong seasonal variation in pasture concentrations of zinc in New Zealand (Towers, 1977b). It is possible however that other constituents of pasture, which may influence zinc absorption or metabolism, may vary on a seasonal basis and cause changes in the serum zinc concentration.

The pancreatic lesions were present in all sheep which died and in several of the zinc oxide sheep which survived. As expected, the pancreas appeared to be the organ most sensitive to zinc toxicity but its importance in producing the final weight loss and death in the zinc sulphate dosed sheep is doubtful. In some zinc oxide-dosed sheep pancreatic lesions were judged by microscopic examination to be moderately severe but atrophy as judged by loss of pancreatic mass was not severe and certainly not significantly related to lack of weight gain. On the other hand anaemia and the postmortem signs of haemolytic episodes or crises were present in most sheep which died and there was a correlation between the presence of these signs and deaths. Pancreatic mass was also reduced in those sheep which died. A more full discussion of the significance of the lesion of zinc toxicity is included in the section on the pathology of zinc toxicity (Chapter 5).

3.2 ZINC TOXICITY IN CATTLE

3.2.1 DAILY DRENCHING OF DAIRY COWS (LACTATING AND NON-LACTATING) WITH ZINC SULPHATE

Introduction

The protective effect of zinc in preventing pithomycotoxicosis has been shown to occur in cattle (Towers and Smith, 1978; Smith *et al.*, 1978) as well as sheep. The protective effect has so far only been demonstrated for cattle with zinc sulphate, having been administered by drenching gun at very high dose rates of zinc. Judging by the results

of experiments with sheep, the margin of safety for zinc sulphate was not expected to be great. For this reason zinc sulphate was dosed to cattle by drenching gun because more accurate administration of the dose rate could be obtained by this method.

The following experiments were designed to more closely define the margin of safety for the use of the drenching gun administration of zinc sulphate for facial eczema control in cattle.

Materials and Methods

Two trials were designed and executed to investigate the toxicity of zinc sulphate to dairy cows.

Experiment A In a pilot experiment, four pasture grazed Jersey/Friesian-cross cows were dosed at two dose rates of zinc sulphate for six days of each week for four weeks. Approximately 60-70% w/v aqueous zinc sulphate solutions were administered by drenching gun so that two cows received 50 and two cows 100 mg Zn/kg b.wt./d. A further four cows were not dosed and used throughout the experiment as controls for body weight changes. They were not slaughtered at the end of the dosing period. Body weights were recorded twice weekly and blood samples for serum zinc analysis (Appendix A) were collected at least twice weekly. At the end of the dosing period a postmortem examination was conducted on the drenched cows and samples of tissue taken for zinc analysis (Appendix A) and for microscopic examination (Appendix B).

Experiment B In the second experiment (B) 12 lactating and 12 non-lactating pasture grazed dairy cows of mixed breeds (mainly Friesians, Jerseys and Friesian/Jersey cross) were randomly divided into three groups of four. One group of four from the lactating group, and one group of four from the non-lactating group were not dosed and were used as a control group. Each of the remaining groups of lactating and dry cows were assigned to either a high (80 mg Zn/kg b.wt./d or low (40 mg Zn/kg b.wt./d) treatment group for drenching of zinc sulphate for a period of five weeks. An approximately 50% w/v aqueous zinc sulphate was used, the low dose rate group receiving approximately

Table 3.5 Pancreatic injury and organ zinc concentrations in dairy cows drenched daily with zinc sulphate for four weeks.

Animal No.	Treatment mg Zn/kg/d	Pancreas injury score	Liver	Kidney	Pancreas	Heart
			µg Zn/g DMB			
9110	100	8	2228	1710	1347	153
9130	100	9	1130	2076	763*	106
9111	50	1	801	545	602	116
9142	50	1	1046	699	1399*	189

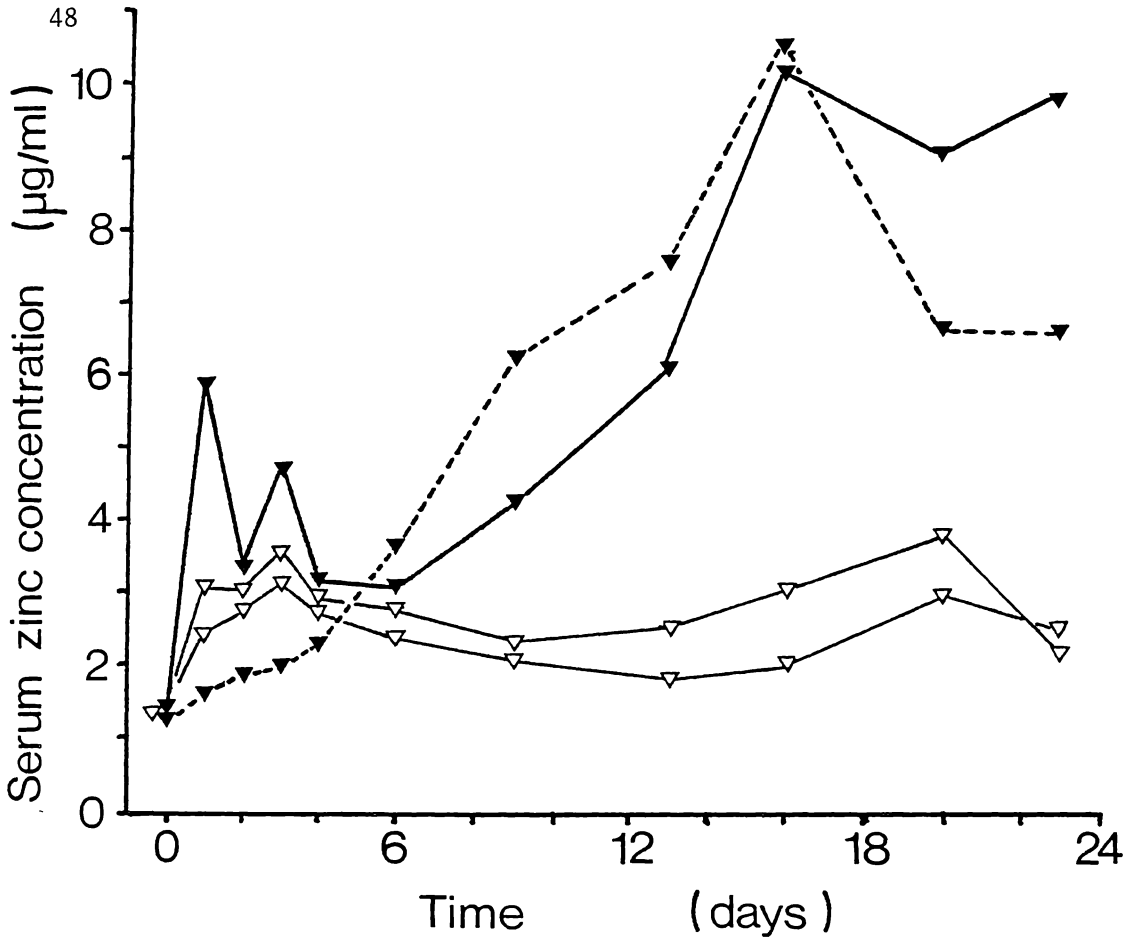
*Results reverse of expected;
suspect samples or labels inadvertently transposed.

120 ml of drench at one operation of the drenching gun and the high group receiving approximately 240 ml as two operations of the drenching gun. The dose rate was adjusted weekly by varying the drenching gun volume according to the mean weekly body weight of each group.

Body weights were recorded weekly on Fridays. Milk weights were recorded twice daily and a 15 ml sample of milk collected weekly on Mondays in acid washed vials for zinc analysis (Appendix A). Blood samples for serum analysis (Appendix A) were collected on Monday mornings before drenching. At the completion of the drenching programme all surviving cows were slaughtered. Postmortem examinations were carried out on these and on cows which died during the experiment. Liver, kidney, pancreas and spleen were weighed and samples taken for zinc analysis (Appendix A) and microscopic examination (Appendix B). A sample of skeletal muscle (*Longissimus dorsi*) was also taken for zinc analysis.

Results

Experiment A One cow (9130) on the highest dose rate (100 mg Zn/kg b.wt./d) of zinc sulphate developed a severe diarrhoea over the last 10 days of the dosing period and both cows on the high dose rate lost weight over the experimental period (Fig 3.6). No other clinical signs were observed in the other animals. At postmortem examination cow 9130 had necrotic lesions and ulceration of the abomasal mucosa especially about the margins of the fundic folds. The pancreas was greatly enlarged and oedematous with an irregular pale hard surface. The other cow (9110) (on the high dose rate) had a small hard pancreas. On microscopic examination each cow was seen to have a severely damaged pancreas whereas the two cows on the lower dose rate (50 mg Zn/kg b.wt./d) had only minor pancreatic lesions (Table 3.5). Serum zinc concentrations (Fig 3.7) rose to high levels in the two cows on the high dose rate (up to 10 µg Zn/ml) and to 3 µg Zn/ml in the serum of the cows on the lower dose rate of zinc sulphate. There were high concentrations of zinc in the liver, kidney and pancreas in all cows which had received zinc especially those which had received the higher dose rate (Table 3.5).


Serum zinc concentration (µg/ml)
0
2
4
6
8
10
0
6
12
18
24
Time (days)

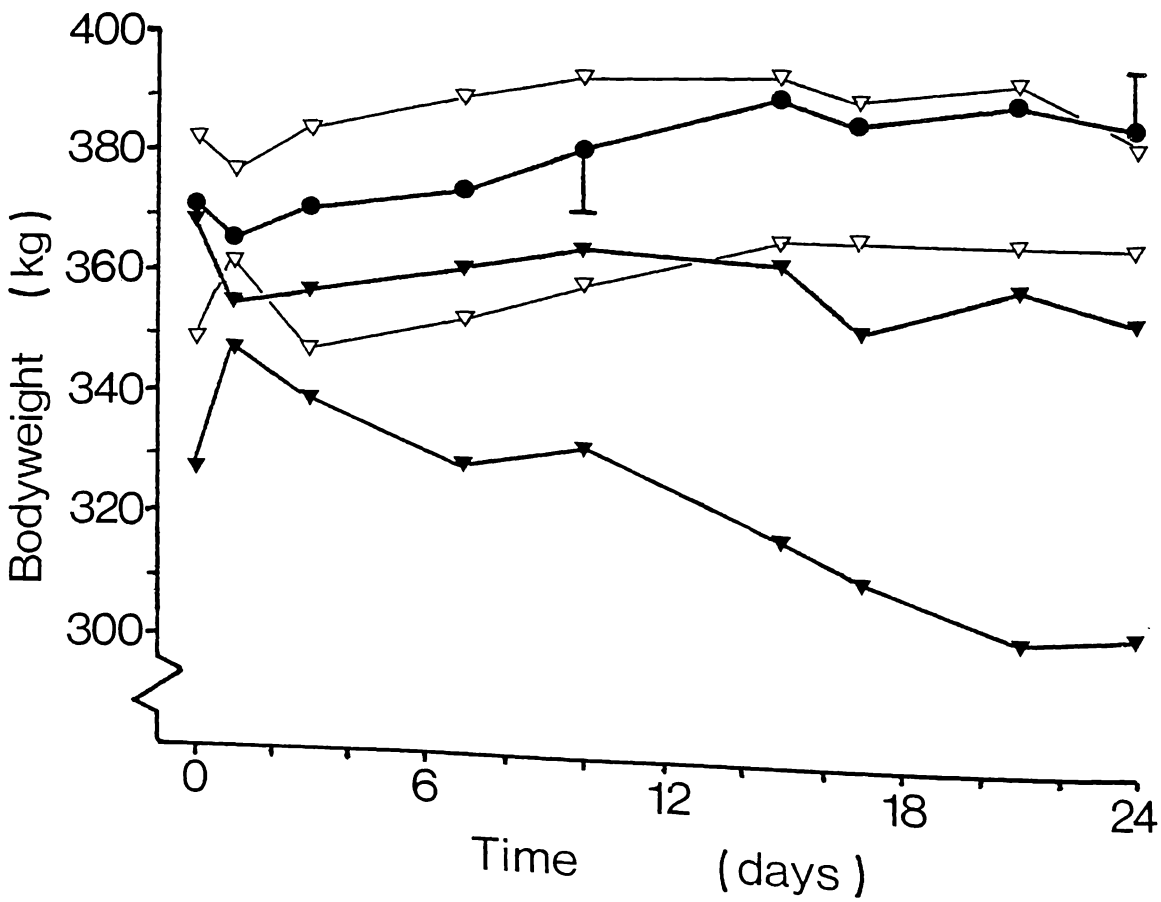

Bodyweight (kg)
300
320
340
360
380
400
0
6
12
18
24
Time (days)

Figure 3.7 : Experiment A: Serum zinc concentrations in individual cows drenched daily with zinc sulphate for four weeks. 50 mg Zn/kg/d ∇ ——— ∇; 100 mg Zn/kg/d ▼ ═══ ▼.

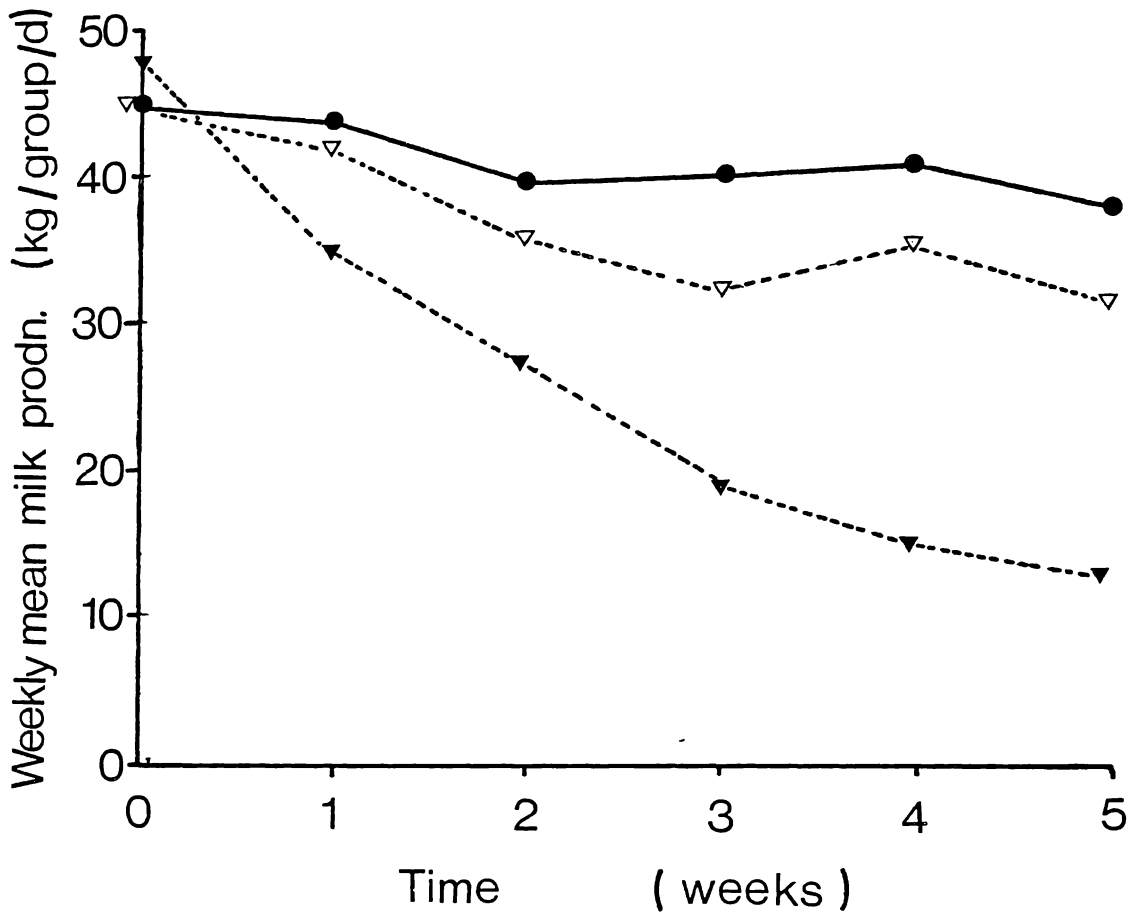
Figure 3.6 : Experiment A: Bodyweight changes in individual non-lactating cows dosed daily with zinc sulphate for four weeks. Control ±SEM ● —— ●; 50 mg Zn/kg/d ▽——▽; 100 mg Zn/kg/d ▼—— ▼.

Experiment B Three of the four cows in each of the high zinc dose rate groups (lactating and dry) developed diarrhoea, anorexia and lethargy resulting in emaciation and death in five of these six cows. In at least four of these there was some evidence of one or more haemolytic episodes. These animals showed while alive anaemia, haemoglobinurea and after death lesions indicative of haemolytic crisis at postmortem examination (Plate 5.26) or microscopic examination (Plates 5.31 and 5.34). Cows in all groups which received zinc, either lost weight or gained less weight than the cows in the control groups.

Moderate to severe pancreatic damage was present in all eight cows on the highest dose rate of zinc sulphate and moderate pancreatic damage was present in three of the four non-lactating cows and one of the four lactating cows in the low dose rate. Abomasal lesions were present in seven of eight cows in the highest dose rate group and four of the eight in the lower dose rate group. These lesions ranged in severity from very severe necrosis of the entire abomasal mucosa (Plate 5.5) to oedema only of the fundic folds.

Milk yields in the lactating cows which received zinc decreased more rapidly than the controls over the dosing period (Fig. 3.8) and this was especially dramatic in those cows on the highest dose rate where three of the four ceased lactation during the dosing period. One cow in the low dose rate group and none in the control group ceased lactation. Two cows in the high dose rate group ceased lactation 3 and 5 days before death and the other ceased lactation 14 days before slaughter at the end of the experiment. In the low-dose-rate group the one cow ceased lactation seven days before slaughter.

Whole-milk zinc concentrations were elevated in animals which had received zinc sulphate. In the low-dose-group the zinc concentrations in milk rose to approximately double or treble those of controls, while those which were on the high dose rate had greater elevations reaching an individual maximum of 27 µg Zn/g (mean maximum 13.7) or approximately six to seven times the concentration in controls (Fig 3.9). There was wide variation in milk zinc concentrations in the high dose group. Lactose, protein and fat analyses were carried out on milk samples taken from the lactating cows 17 days after the start of zinc dosing


Weekly mean milk prodn. (kg/group/d)
50
40
30
20
10
0
0
1
2
3
4
5
Time (weeks)

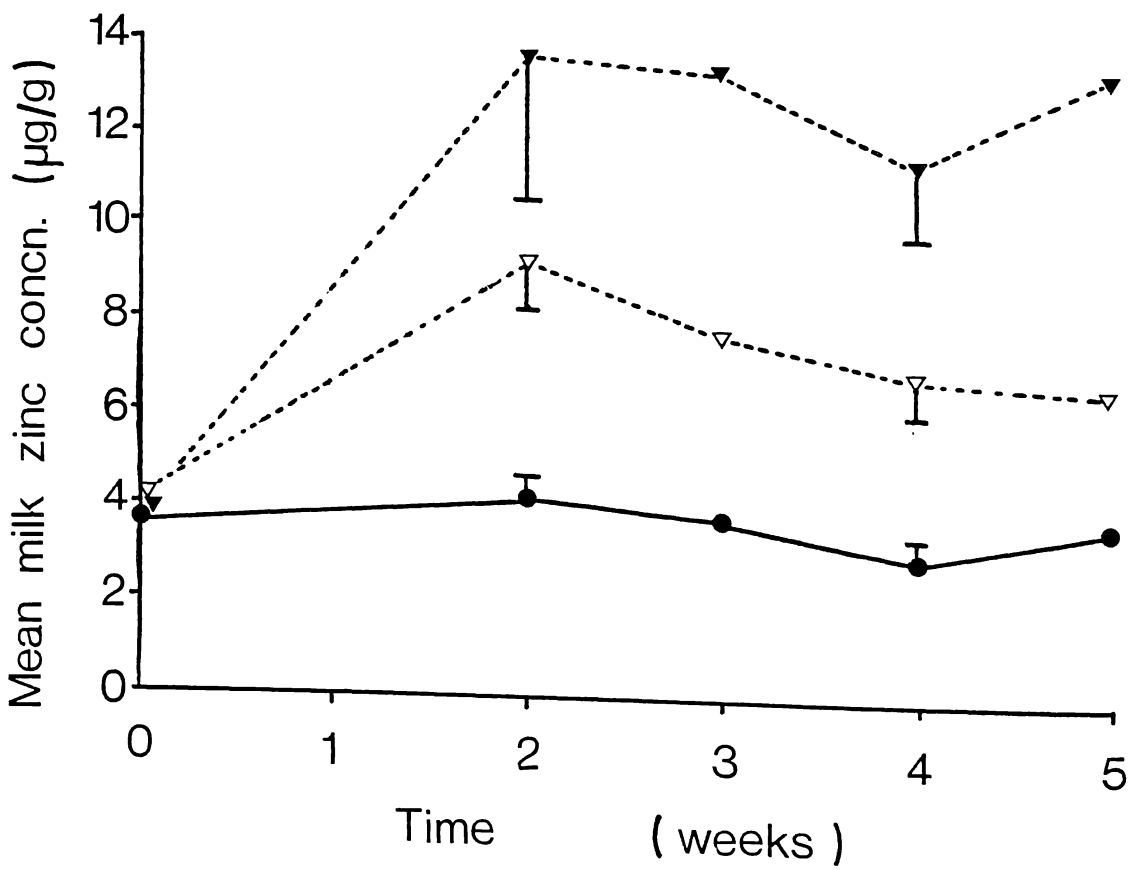

Mean milk zinc concn. (µg/g)
14
12
10
8
6
4
2
0
0
1
2
3
4
5
Time (weeks)

Figure 3.8 : Effect of daily drenching of lactating dairy cows with zinc sulphate on milk production. Weekly mean milk production per day is plotted for each group. Control group ● —— ●; 40 mg Zn/kg/d ∇ .... ∇; 80 mg Zn/kg b.wt/d ▼ .... ▼.

Figure 3.9 : Mean group milk zinc concentration (±SEM) in dairy cows drenched daily with zinc sulphate for five weeks. Control group ● —— ●; 40 mg Zn/kg b.wt/d ∇ .... ∇; 80 mg Zn/kg b.wt/d ▼.... ▼.

Table 3.6 Fat, protein and lactose content of milk collected from zinc sulphate dosed lactating cows 17 days after start of dosing.

Treatment	Cow	Fat %	Protein %	Lactose %
Control No zinc	6097	7.23	4.02	3.92
	7101	4.39	3.08	4.55
	8129	5.29	3.62	3.86
	7060	5.13	3.31	4.24
Low zinc 40 mg Zn /kg/d	7080	5.48	3.48	4.44
	5111	7.60	3.54	1.78*
	7059	5.00	3.13	3.90
	1128	6.77	3.81	4.05
High zinc 80 mg Zn /kg/d	5123	7.82	4.74	2.35*
	2100	4.39	3.34	4.45
	7105	2.41*	2.15*	1.07*
	9102	6.28	3.51	1.18*

* Values lower than lowest control value; all of these cows were the most severely affected in their groups and all four had the most severely damaged pancreata irrespective of group of origin.

(Table 3.6). The very low lactose concentrations for the cows which had pathological lesions suggest a primary or secondary toxic effect of zinc or an indirect effect through lowered pasture consumption.

Serum zinc concentrations also varied considerably within groups. The highest individual level recorded was 34.3 µg Zn/ml which was the last preceding death as were levels of 26.5 and 14.6 µg Zn/ml in two other cows. Weekly mean serum zinc concentrations are recorded in Figures 3.10 and 3.11.

The zinc concentrations in the liver, kidney and pancreas were elevated in all groups which had received zinc but there was considerable variation within each group (Table 3.7). Zinc concentrations in the longissimus dorsi muscle from the zinc dosed cows were not significantly higher than those of the control cows.

Discussion

The occurrence of early deaths and severe lesions of zinc toxicity at the high dose rate in the second experiment and the presence of less acute zinc toxicity (decline in body weight lower milk yield and pancreatic lesions) at the lowest dose rate (40 mg Zn/kg b.wt./d) indicated that cattle have a susceptibility to zinc sulphate similar to that found for sheep in previous experiments. The dose rate of zinc found to protect cows against pithomycotoxicosis was 23 mg Zn/kg b.wt./d (Towers and Smith, 1978). However it is not known, at present, to what extent doses of zinc above or below this dose rate will effect protection. Presuming that this figure is considered optimal as is the case in sheep, then the margin of safety for drenched zinc sulphate to prevent facial eczema is, as in sheep, very low.

Figure 3.10
Lactating cows

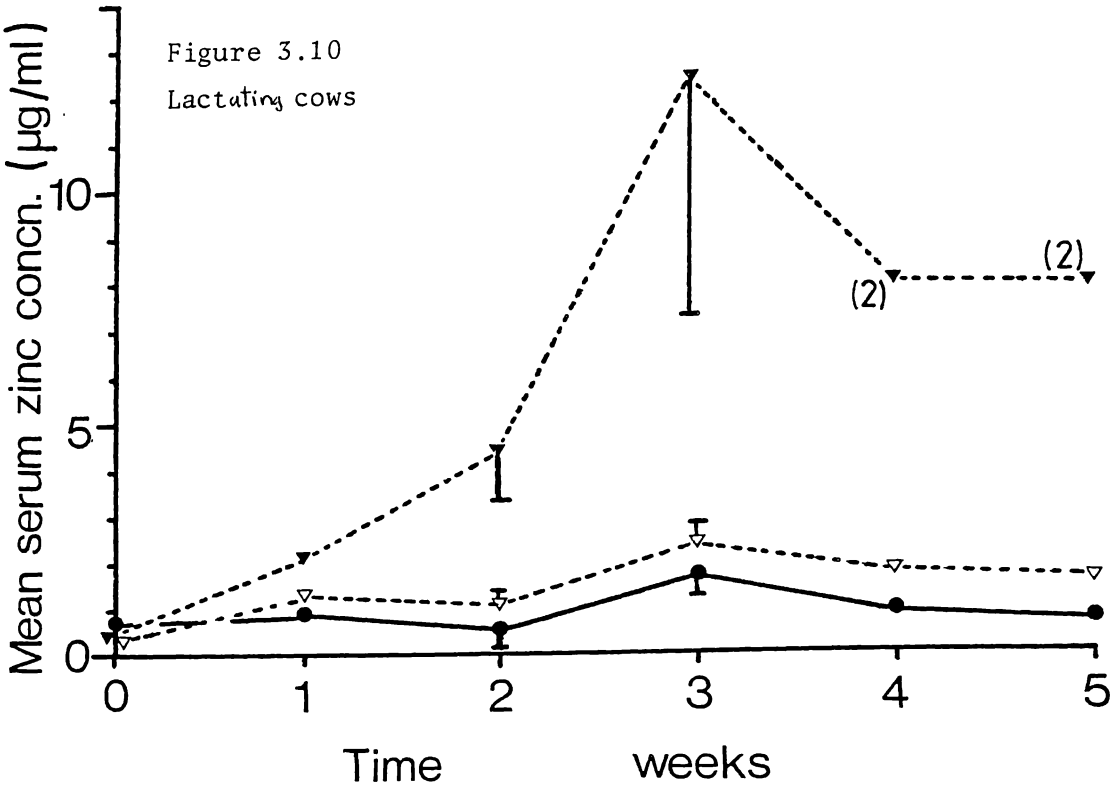
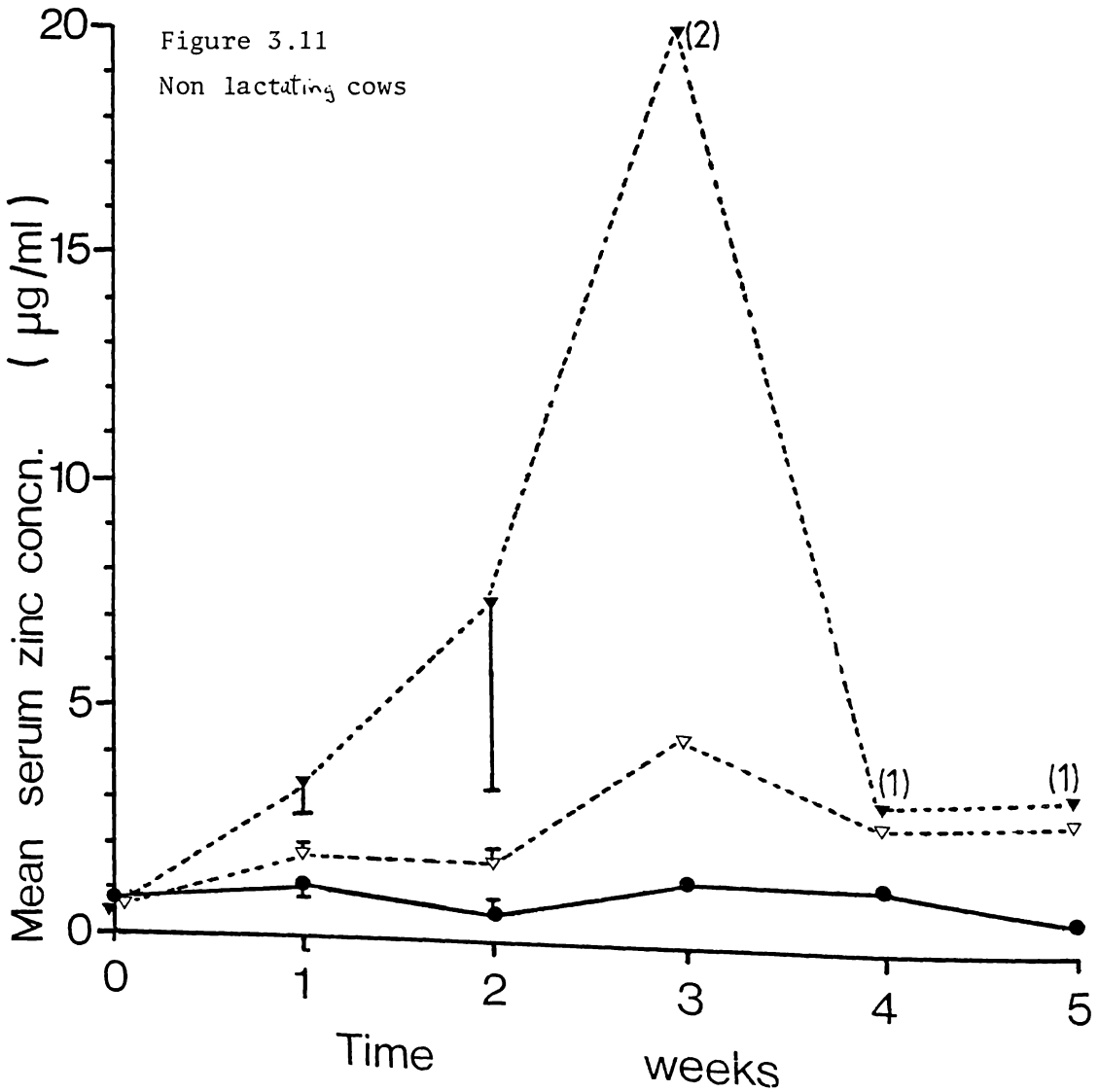


Figure 3.11
Non lactating cows



Figures 3.10 and 3.11 : Weekly mean group serum zinc concentrations in lactating and non-lactating dairy cows dosed daily with zinc sulphate for five weeks. Controls ● —— ●; 40 mg Zn/kg b.wt./d ∇..... ∇; 80 mg Zn/kg b.wt./d ▼..... ▼. No. of surviving cows in parenthesis.

Table 3.7 Zinc concentrations in organs and summary of pathological findings in lactating and dry cows after five weeks of daily drenching with zinc sulphate.

Group	Cow No.	Zinc concentration µg/ Zn/g DMB Liver	Kidney	Pancreas	Muscle	Post-mortem findings Death	Haem.	Abom.	Pan-creas
Control Lactating		121 ±25	78 ±15	146 ±48.0	164 ±41	-	-	0	0
40 mg Zn /kg/d	7080	239	145	691	120	-	-	0	0
	5111	1155	1329	4298	148	-	+	3	6
	7059	514	246	848	149	-	-	2	0
	1128	503	206	893	110	-	-	0	0
80 mg Zn /kg/d	5123	1218	1606	1413	162	-	+	2	8
	2100	1313	1057	3877	164	-	-	0	4
	7105	1707	1037	3520	ND	+	+	4	8
	9102	1567	1973	1486	ND	+	+	4	8
Control Non-lactating		108 ±11	70 ±4	106 ±29	151 ±13	-	-	0	0
40 mg Zn /kg/d	6	1358	1353	3805	187	-	-	2	2
	614	891	997	2973	120	-	-	1	0
	618	1365	2290	2632	225	-	-	2	6
	628	551	637	1958	109	-	-	0	4
80 mg Zn /kg/d	619	1450	725	2388	128	+	-	4	6
	644	1362	842	623	ND	+	+	1	6
	613	ND	ND	ND	ND	+	+	5	8
	663	1121	1284	4688	104	-	-	1	6

Means ± Standard Deviations given for control groups. Death (+) indicates died before end of dosing. Haem. (+) indicates presence of evidence of haemolytic episodes or crisis at post mortem examination or microscopic examination.

Abomasum 0-5; 0, no lesion; 5, very severe lesion. Pancreas 0-10; 0, no lesion; score is percentage ÷10 of tissue showing changes on microscopic examination. ND, not determined.

The lesions of zinc toxicity were also similar to those recorded in sheep. Of particular interest was the presence of severe abomasal lesions in the cows, an indication perhaps that the drenched solutions were being channelled direct to the abomasum by the reticular groove mechanism.

The haemolytic episodes (in some cases crises) occurred earlier and in a more dramatic fashion than in the case of sheep. In all the instances recorded in sheep the haemolytic episodes occurred after several months of zinc sulphate dosing at low dose rates (30 mg Zn/kg b.wt./d or lower). In the present experiment six cows showed signs of a haemolytic episode or became moribund with a haemolytic crisis between 18 and 39 (at slaughter) days after the start of dosing. Four of the six cows which died showed signs of a haemolytic crisis and in at least two of these the postmortem lesions of the haemolytic crisis (icterus, orange liver, black kidneys, very dark urine) were quite dramatic. These occurred 18 and 25 days after the start of dosing. A more extensive discussion of the significance of the haemolytic crisis will be presented in the section on the pathology of zinc toxicity (Chapter 5).

Serum zinc concentrations were at their highest at three weeks after the start of dosing, the time at which the most dramatic haemolytic crises occurred. The highest mean zinc serum concentrations were due mainly to the exceptionally high concentrations in one or two animals within the groups. Often but not always, these very high serum zinc concentrations preceded death (and haemolytic crisis) by only a day or two. Serum zinc concentrations were recorded only weekly and they may have risen sharply before death in those cows having relatively low serum zinc concentrations at their last bleeding before death.

The zinc dosing regimes had a considerable effect on milk yield and in the highest dose rate group there was an obvious decline in group milk yield from the start of dosing. Individual milk yields tended to decline rapidly and even cease when cows were showing clinical signs of zinc toxicity and on occasions when very high serum or milk zinc concentrations were recorded. These were followed or accompanied by very low milk yields.

A feature of the results was that the highest mean whole milk zinc concentrations were recorded at two weeks, one week prior to the maximum serum zinc concentrations and were higher than the serum concentrations at the same time. No satisfactory explanation for these results can be given but they may relate to the times the samples for milk and serum zinc analysis were taken relative to the drenching time. The exceptionally low lactose levels in the milk in cows at 17 days after the start of dosing indicates that zinc dosing may have interfered with lactose metabolism. The low lactose levels were recorded in the milk of cows in which signs of zinc toxicity were evident.

The effect of the low dose rate of zinc on milk yield, while minor, could be of considerable significance to the milk industry if this method and level of zinc administration was used for the prevention of pithomycotoxicosis.

3.2.2 THE EFFECT OF HIGH CONCENTRATIONS OF ZINC SULPHATE IN THE DRINKING WATER OF GRAZING YEARLING DAIRY CATTLE

Introduction

The addition of medicines to the drinking water of animals is commonly employed for disease control e.g. antibiotics to poultry, antifoaming agents for cattle; and has been considered for the administration of zinc salts for facial eczema control. The inconvenience of drenching ruminants together with the danger of causing corrosive abomasitis when using concentrated solutions has made drinking water medication with zinc sulphate an attractive proposition. In the case of sheep, drinking frequency is notoriously sporadic and often they simply do not drink. With cattle however, drinking water medication was considered to be a reasonable proposition for the administration of zinc salts.

Relatively little is known of the effect of high zinc concentrations in the sole drinking water of ruminants. Mehren and Church (1977) offered calves an alternative of either tap water or various concentrations of zinc sulphate up to 0.06 g Zn/ℓ. Neither discrimination nor rejection were evident. Wright *et al.* (1978) using zinc sulphate in the sole water supply for beef cattle showed that there was no lasting effect on water consumption at levels of zinc up to 0.25 g Zn/ℓ. There was however a temporary setback in water consumption for one day after the zinc solutions were introduced. The mean group zinc consumption of the cattle was slightly lower than that found to be optimal for facial eczema control in sheep (Smith *et al.*, 1977).

This study of the effect of high zinc concentrations in the drinking water of cattle has been made because higher concentrations than those used by Wright *et al.* (1978) may be required to ensure facial eczema control especially during wet periods when water consumption is lowered. The concentration of zinc in water which is necessary to cause toxicity is unknown and the situation will be complicated by the large variations of water intake known to exist between animals and the various environmental influences on drinking frequency and quantity.

Materials and Methods

Twenty yearling dairy cross steers and heifers were divided into four groups and each group allocated to similar and adjacent 0.54 hectare rectangular paddocks. At one end of each paddock was a raised 160 litre plastic water drum reservoir supplying a drinking trough with the water treatments via a level regulating ballcock valve. An electric fence at right angles to the adjoining long boundaries of each paddock was progressively shifted away from the water troughs to make more pasture available. Hay was available in racks in each paddock. Rainfall was recorded throughout the experiment which lasted from early September to the end of November 1977. Evaporation losses were not recorded.

The treatments comprised (a) control - tap water supply (0.11 mg Zn/ℓ); (b) low zinc (0.25); (c) intermediate (0.5); and (d) high 1.0 g Zn as $ZnSO_4$ $7H_2O$/ℓ of water.

Water consumption for each group was recorded at 9 am daily by reading a sight glass on the 160 ℓ drum. For the first two weeks water only was supplied, after which the different water treatments were applied for a period of nine weeks. Treatment water was prepared by measuring the amount of water needed to top up the reservoir drum and adding enough concentrated stock solution to give the correct drinking water concentration. Throughout the entire trial each group of yearlings with its treatment was rotated one paddock weekly in order to eliminate possible differences between paddocks.

Body weights were recorded weekly and blood samples taken for serum zinc analysis. At the conclusion of the experiment the cattle were slaughtered and their organs examined. Samples were removed and prepared for zinc analysis (McLeay and Smith, 1977; Appendix A) by atomic absorption spectrometry and for histological examination after routine paraffin blocking, sectioning and staining with haematoxylin and eosin. Pancreatic damage was scored on a 0-10 basis depending on the estimated percentage of exocrine tissue area showing degenerative changes on microscopic examination (Appendix C).

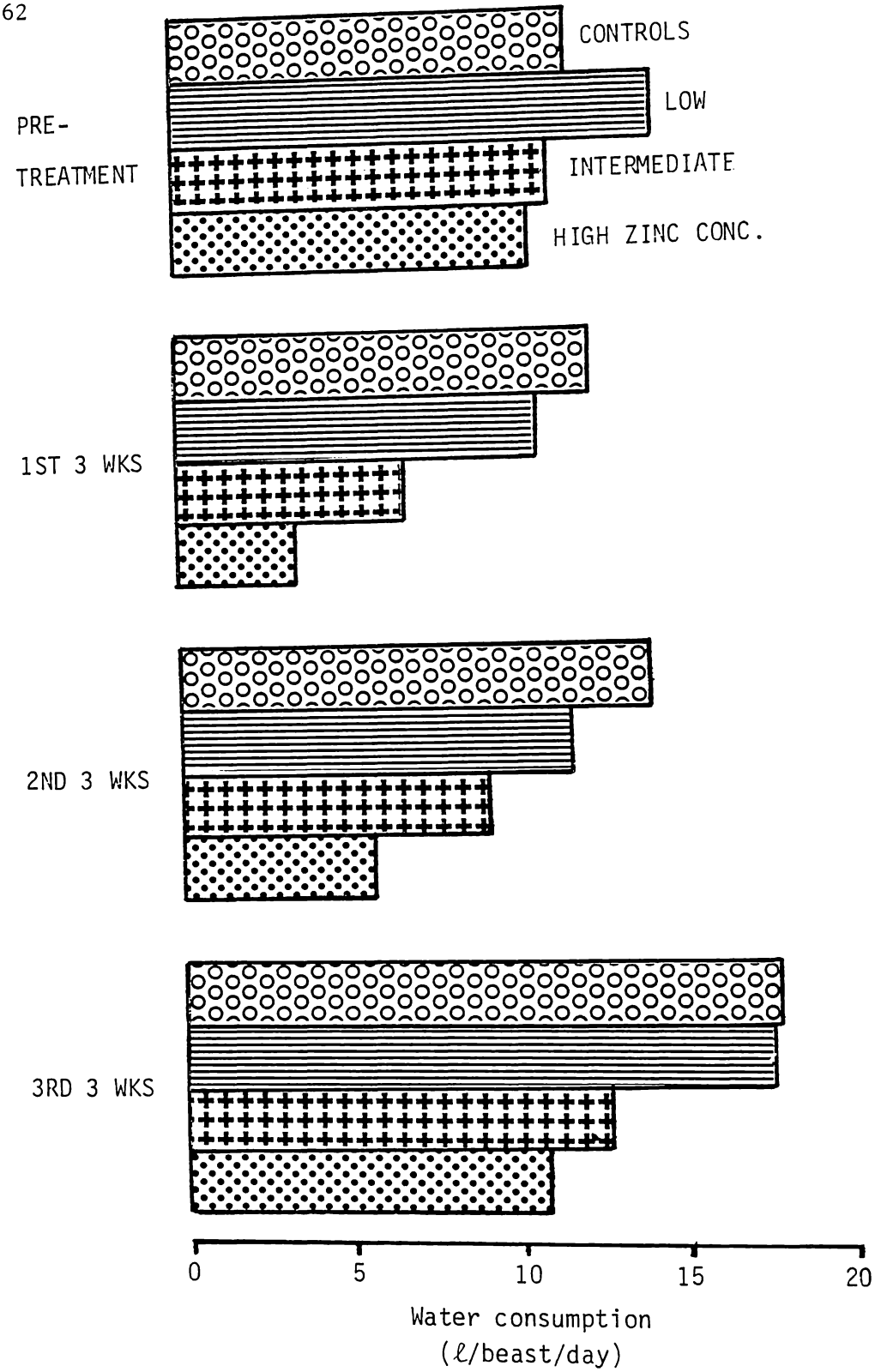


Figure 3.12 : Effect of zinc concentration in sole drinking water of yearling cattle on consumption of trough water. After the initial two week pretreatment period on water alone zinc sulphate was added to the source of trough water to give the following concentrations of zinc for each group. Control (none added); low (0.25); low (0.5); high (1.0 g Zn/ℓ).

Results

Consumption of trough water was markedly reduced by the presence of zinc in the drinking water (Fig 3.12, Table 3.8).

In a total of 61 treatment days, recorded intakes for the high, intermediate and low zinc groups were lower than control intakes on 61, 55 and 47 days respectively when 30-31 days would be the expected number of days if there was no effect of zinc. Over the nine week treatment period the high, intermediate and low zinc treated groups drank respectively 54, 35 and 8% less than controls over the same period.

The effect was greatest early in the trial where for the first three weeks the high, intermediate and low groups drank, respectively 71, 44 and 12% less than controls. The effect was particularly marked over the first week with reduction of 74, 66 and 34% - respectively from the pretreatment levels. As the experiment progressed the differences in water consumption between treatment and control groups lessened. The high intermediate and low groups consumed respectively 59, 35 and 15% less than controls for the middle three weeks and 38, 29 and 0% less for the final third of the treatment period. Throughout the experiment the mean water intake of controls also increased, being 12.2, 14.1 and 17.4 litres for the first middle and final thirds of the treatment period.

A new break of grass or a fall of rain appeared to result in a drop in water consumption in all groups, the effect being most noticeable and lasting in the high zinc group (Fig 3.13). Three new allocations of fresh pasture were made available during the trial. The total water consumption for all groups for the three days following the new breaks was 47% of that recorded for the three days prior to the new break. On 10 of 11 occasions when rain (>1 mm) followed a dry day (<1 mm), excluding four days following each new break of grass, all four groups decreased their water intakes from the previous day.

A highly significant ($P<0.001$) decrease in weight gain (11 kg) was evident in the high zinc group after the first week on zinc treatment (Table 3.9). A significant ($P<0.01$) difference was again evident at

Table 3.8 Zinc added to the drinking water of yearling steers. Effect on drinking water and zinc intake.

(a) Water intake litres/beast/day					(b) Estimated group mean supplemented zinc intake mg Zn/kg bodywt/d.			
Period of experiment (weeks)	Control 0.0	Low 0.25	Intermediate 0.5	High 1.0*	Control	Low	Intermediate	High
1-3	12.1	10.7 (88.4)	6.8 (56.2)	3.5 (28.9)†	0	13.3	16.7	18.1
4-6	14.1	11.9 (84.4)	9.2 (65.2)	5.6 (39.7)	0	13.4	20.4	25.5
7-9	17.4	17.3 (99.4)	12.3 (70.7)	10.7 (61.5)	0	17.9	25.6	44.8

* g Zn/ℓ

† figure in parenthesis represents percentage of control value for same period of time.

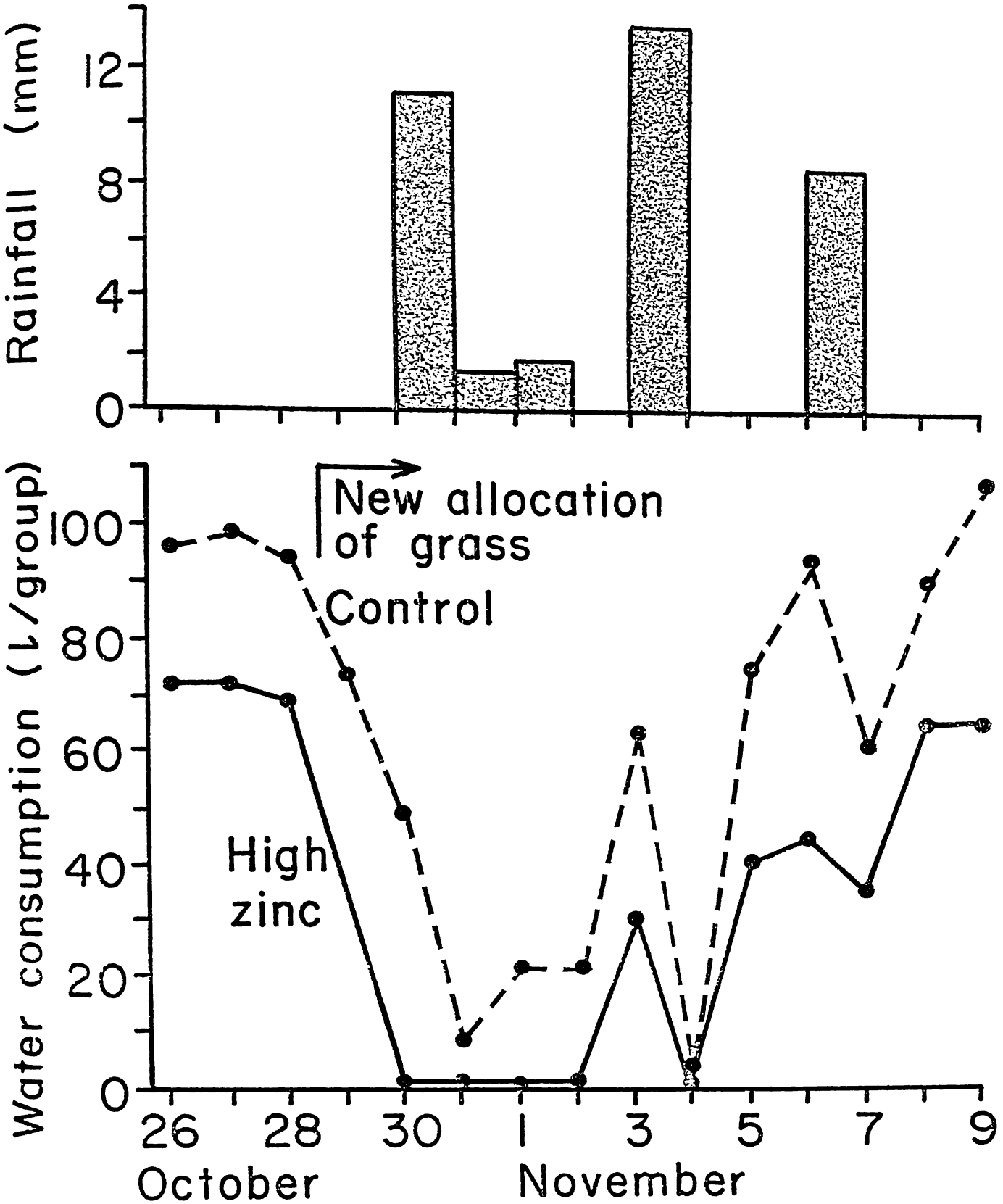


Figure 3.13 : Example of the effect of new pasture allocation and rainfall on the water consumption of yearling cattle. Only values for control and high zinc water concentration groups are plotted. The water consumptions for the low and intermediate groups occupied positions between the two plotted values.

Table 3.9 Effect of zinc in the drinking water of yearling dairy cattle on bodyweight and serum zinc concentrations.

Group treatment	Mean bodyweight (kg) Initial	Cumulative changes			Serum zinc concentration µg/ml[+]		Organ zinc µg/g DMB[+]		
	0 d	7 d	49 d	58 d	14 d	28 d	Pancreas	Liver	Kidney
Control	194	+4.0	+39.2	+48.6	0.94 ±0.05	1.01 ±0.06	139 ±22	175 ±28	124 ±5
Low (0.25 g Zn/ℓ)	194	+5.2	+41.8	+50.4	0.53 ±0.05	1.74 ±0.06	356 ±40	258 ±36	171 ±18
Intermediate (0.5 g Zn/ℓ)	199	+1.0	+36.8	+41.6	2.00 ±0.07	2.18 ±0.12	549 ±38	464 ±29	318 ±51
High (1.0 g Zn/ℓ)	194	-7.8	+32.0	+38.6	1.43 ±0.22	2.22 ±0.18	1834 ±608	1004 ±109	790 ±122
		(5.2)	(6.8)	(8.4)					

[+]Values are group mean ±SE: least significant differences between groups (5%) are given in parenthesis.
d Days after start of treatments.

49 and 58 days after the start of treatment. Between these times the weights of the zinc dosed animals approached those of controls but only temporarily and after rain or a fresh break of grass.

From body weights and water intakes, mean group zinc intakes were calculated for each third of the treatment period (Table 3.8). For the first middle and final thirds of the treatment the low zinc group received 13, 13 and 18 mg Zn/kg/d. The corresponding figures were, for the intermediate group 17, 20 and 26 and for the high group 18, 26 and 45 (range 0 to 75 mg/kg/d).

On histological examination of the pancreas it was seen that all five animals in the high group had some evidence of pancreatic damage (mean score 5.6 range 4-8) and damage was present in two animals each in the intermediate (scores 2 and 2) and low zinc (scores 4 and 2) groups. There was no pancreatic damage in the controls.

Zinc concentrations in serum and organs are recorded in Table 3.9. Serum zinc concentrations rose in all groups reaching over 2 µg/ml in the intermediate and high groups. A maximum group mean of 2.22 (range 1.9-2.9) µg Zn/ml was reached at six weeks by the high group. Analysis of organ zinc concentrations (Table 3.9) showed that pancreas levels rose the most with increasing concentration of zinc in drinking water. Liver, followed by kidney, also had high zinc concentrations. Muscle (vastus medialis), heart and adrenal tissue showed no significant rise in zinc concentration.

Discussion

The results of this experiment show that the presence of high concentrations of zinc in drinking water cause reductions in the water intakes of yearling dairy cattle. This effect on drinking water consumption was greatest early in the trial in all treatment groups and the reductions in intake lessened as the trial progressed.

The marked initial reduction in consumption of water by those cattle offered water containing high zinc concentrations suggests that the avoidance or rejection of the zinc solutions was due to its unpalatable

nature rather than an indirect effect of zinc toxicity. The zinc solutions were tasted by four laboratory staff who all agreed that the high and intermediate treatments were very unpalatable and that the low sinc solution was just detectably unpalatable. The unpalatable nature of zinc sulphate solutions appears to be due to the zinc ion. Digesti and Weeth (1976), using weanling heifers showed that the discrimination and rejection thresholds for sulphate were 2.0 and 3.3 g So_4 per litre suggesting that zinc rather than sulphate was responsible for the probable unpalatable nature of the water.

As the experiment progressed it appeared that an adaptation to the unpalatable taste occurred leading to the relatively greater increase in volume of zinc treated water being consumed compared with controls. The increase in consumption of water by the control group probably reflected increased body weight and a greater need for water as ambient temperatures rose and the paddocks dried as summer approached. These factors would cause a greater physiological need and reinforce any adaptation which may have been occurring.

Body weight difference between the high zinc group and controls was at its greatest one week after commencement of treatments. The greatest effect in terms of water consumption was also occurring at this time and it seems possible (no diarrhoea was observed) that the weight differences which were recorded were due to a continuing water deficit.

Body weight and water consumption data were used to calculate mean group zinc intakes, ignoring the zinc in the natural tap water (0.11 mg Zn/ℓ). These calculated zinc intakes indicate that the high group was receiving enough daily zinc to cause some toxicity for at least the last three weeks of the trial. The postmortem and histological examinations of pancreata showed that this was the case with all animals of the high group having pancreatic lesions. Although not examinable in the present data, Wright *et al.* (1978) have shown that up to a four fold variation in water intake can occur between animals. Such a variation together with the natural between-animal variation in susceptibility to zinc is thought sufficient to explain the finding of mild pancreatic lesions in two animals each of the low and intermediate group.

The elevated serum zinc concentrations in the high and intermediate groups, more than double those of controls, were not very high compared with the levels (5-50 µg/ml) attainable when concentrated zinc solutions are administered to sheep by drenching guns (Smith *et al.*, 1979) when severe abomasal lesions were obtained. Pancreas, liver and kidney, in descending order, all showed significant rises in zinc concentration which is in agreement with the findings of others (Miller *et al.*, 1970).

The effect of both rain and herbage water content on the intake of trough water by cattle has been reported before (Phillips, 1968; Young, 1975; Wright *et al.*, 1978; Castle and Watson, 1973). Wright *et al.* (1978) noted this effect and drew attention to the problems of using zinc solutions in drinking water for the control of facial eczema. They pointed out that as *Pithomyces chartarum* spore numbers rise rapidly after rain the need for zinc for facial eczema control would be greatest at the time when zinc consumption was least. They also suggested that a greater concentration of zinc temporarily in the drinking water may overcome this problem. This may not be without its dangers. The most probable effect would be to cause a more sudden decline or even cessation of water consumption with possible effects on lactation in the case of dairy cows. Provided the cattle are not kept on the high zinc level for more than a few days at a time zinc toxicity *per se* is not likely to be a serious problem. The possible effects of water denial on lactating cows in the North Island of New Zealand could have serious consequences and these problems need to be specifically investigated using lactating cows. It is certain from these and from other experiments using sheep (B.L. Smith, unpublished) that the unpalatable nature of zinc solutions is not sufficient to prevent zinc toxicity where the zinc solution is the sole source of drinking water.

CHAPTER 4

SPECIAL FEATURES OF ZINC TOXICITY

CHAPTER 4

SPECIAL FEATURES OF ZINC TOXICITY

4.1 ZINC SOLUTIONS AND CLOSURE OF THE RETICULAR GROOVE IN SHEEP

Introduction

In preliminary experiments concentrated zinc solutions, when given by drenching gun, almost invariably produced severe lesions of the abomasum but the same solutions when given by intraruminal intubation did not produce these lesions. This led to the hypothesis that zinc solutions in drenches caused reflex closure of the reticular groove and consequent direct passage of the solutions to the abomasum.

In ruminants reticular groove action is important in determining the route by which ingested or administered substances reach the abomasum. Certain substances are known to evoke the reticular groove reflex in adult ruminants (Titchen and Newhook, 1975), particularly solutions of copper salts in sheep (Watson and Jarrett, 1944). The action of zinc sulphate solutions on the reticular groove reflex has been investigated by both Clunies Ross (1931) and Monnig and Quin (1935) with inconclusive results.

These experiments investigated the route taken by zinc solutions when administered by drenching gun to sheep.

Materials and Methods

Romney cross sheep aged seven to twelve months (24-36 kg) were starved for 18 h prior to slaughter. They were dosed by drenching gun with 20 ml of a solution of 0.4% (w/v) crystal violet containing either zinc sulphate, zinc acetate, sodium acetate or cupric sulphate (laboratory grades) at the concentrations indicated in Table 4.1 and Figure 4.1. Control groups received only the crystal violet solution. Slaughter, which was effected by cutting the throat and spinal cord, was carried out within 30 secs of drenching. The sheep were hung by the hind legs

Table 4.1 Crystal violet staining scores as indicators of the effect of zinc and other ions on the passage of fluids administered by drenching gun to sheep.

Experiment	Group	No. of sheep	Mean staining scores ± SEM		
			Omasal mucosa	Abomasal mucosa	Abomasal contents
1	H_2O	15	1.60±0.45 bB*	1.33±0.44 bA	2.00±0.58 bA
	Zn sulphate	15	3.07±0.18 aA	2.73±0.37 aA	3.73±0.42 aA
	Cu sulphate	15	3.00±0.37 aAB	2.60±0.47 aA	3.47±0.47 aA
2	H_2O	13	0.54±0.18 bB	0.46±0.27 bB	1.15±0.52 bB
	Zn sulphate	13	2.31±0.29 aA	2.54±0.49 aA	3.46±0.50 aA
	Zn acetate	13	2.23±0.26 aA	2.00±0.47 aAB	2.69±0.51 aAB
	Na acetate	13	1.00±0.23 bB	0.69±0.38 bB	1.00±0.45 bB

Ionic concentrations for both experiments were: zinc 45 mg/ml; copper 44 mg/ml; acetate 82 mg/ml.

*Values within each experiment not followed in the same vertical column by the same letter are significantly (ab, $p<0.05$; AB, $p<0.01$) different.

and, immediately following evisceration, ligatures were tied around the omasal-abomasal junction and the pylorus.

The abomasal contents were mixed within the abomasum and a 25 ml sample placed in a universal bottle. The omasum and abomasum were then opened and the mucosa rinsed. Grading for the degree of staining of omasal and abomasal mucosal tissue was carried out at the end of each experiment without knowledge of the treatment origin of each sample. Similarly, abomasal contents were ranked in order of dye intensity and scored for degree of staining. Scores ranged from 0 (no staining) to 5 (intense staining). It was determined that, in comparison with crystal violet stain alone, the addition of zinc sulphate to the stain at concentrations used in these experiments did not influence the fixation of stain or the intensity of *in vitro* staining of mucosa of freshly obtained strips of abomasal wall.

Results

The addition of zinc salts to the dye-containing drench resulted in an increase in the mean staining scores of omasal and abomasal mucosa contents. An example of the stained abomasum (Score 5) of a sheep after the administration of crystal violet drench plus zinc sulphate (20%) by drenching gun is given in Plate 4.1. The mean staining scores obtained with zinc were similar to those obtained with copper (Table 4.1).

Dye solutions containing zinc sulphate or zinc acetate produced comparable mean staining scores for tissues and abomasal contents. Solutions containing sodium acetate produced a mean staining score similar to that obtained in controls (Table 4.1). These findings indicate that the effect was due to the zinc ion.

Mean staining scores were also influenced by the concentration of zinc sulphate. With the exception of the staining score for omasal mucosa obtained using 20% zinc sulphate (Fig 4.1), staining scores increased with increasing zinc sulphate concentration ($p<0.01$ for abomasal mucosa).

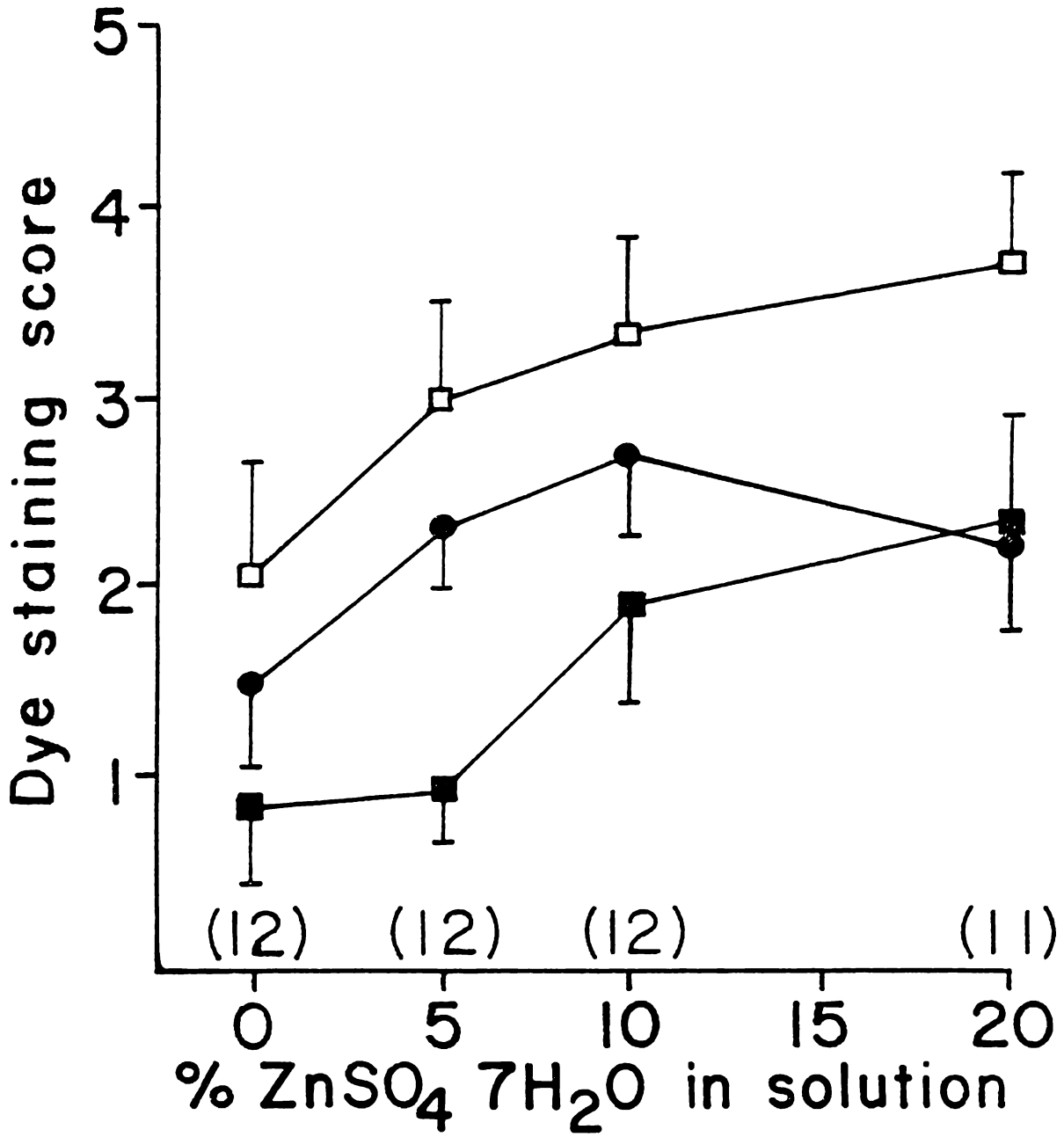


Figure 4.1 : Effect of concentration of zinc sulphate on passage of zinc sulphate/crystal violet solutions administered by drenching gun to sheep. Abomasal mucosa ■——■; Omasal mucosa ●——●; Abomasal contents □——□ . Values are expressed as mean ±SEM. Figure in parenthesis refers to number of sheep at each concentration.

Discussion

The results indicate that zinc stimulates the reticular groove reflex in adult sheep and that this effect is dependent on the concentration of the zinc solution. Clunies Ross (1931) used low concentrations of zinc sulphate and smaller numbers of sheep either of which may account for the inconclusive nature of his results.

There is a variable latent period of several seconds duration in the action of the reticular groove reflex (Watson and Jarrett, 1944; Comline and Titchen, 1951). The duration of this latent period relative to the time taken for the drench to reach the cardia is critical in determining the destination of the drenched zinc solution. This could explain the high variability recorded in the staining scores within each group. Had the zinc solutions been administered several seconds prior to the dye marker, as practised by Watson and Jarrett (1944), the variability in scores may have been lower. However in the present experiments it was necessary to incorporate zinc in the dye solution in order to identify the immediate destination of zinc solutions when given by drenching gun.

There are criticisms of this method of determining the effect of zinc sulphate. The first is that mixing of the contents of the different ruminant stomach compartments is possible after death and during evisceration. An attempt was made to avoid mixing by tying off the omasal-abomasal junction as soon as possible after the abdomen was opened.

A further criticism is that crystal violet itself may stimulate the reticular groove. However no such evidence for this exists in the scientific literature and any such action of the crystal violet would once again mask the effect of zinc sulphate. An objection that zinc and the dye together but not separately may cause the groove to contract is not easily refuted. However the pathological findings (abomasal lesions, Chapter 4.2) support the contention that zinc salts cause the effective contraction of the reticular groove.

It should be noted that these results probably do not necessarily indicate any subtle effect of zinc ions on the reticular groove reflex. The concentrations of zinc used in these experiments are corrosive to membranes as seen by the effects of zinc drenching on the abomasum (Chapters 4.2, 5; Plates 5.3, 5.5, 5.6) and may stimulate the reflex by nature of an irritant or perhaps by some special property of zinc itself. The concentration of zinc salts used here were chosen because they would be those necessary for the drenching of ruminants with soluble zinc for the prevention of pithomycotoxicosis.

4.2 THE EFFECT OF METHOD OF ORAL ADMINISTRATION OF ZINC SULPHATE ON ACUTE ZINC TOXICITY IN THE SHEEP

Introduction

In preliminary experiments with sheep conflicting data on toxic dose rates of zinc occurred and it appeared that exacerbation of zinc's toxic effect was related to the method of administration and in particular to the use of drenching guns. It was subsequently shown that zinc solutions were capable of stimulating the reticular groove reflex (Chapter 4.1; Smith *et al.*, 1977b). These experiments demonstrate the extent to which the method of oral administration influences the toxicity of zinc sulphate when administered to sheep.

Materials and Methods

Romney cross sheep (25-35 kg), approximately 11 months old, were grazed on pasture throughout each experiment.

Experiment I In experiment I two groups, each of four sheep, were dosed at alternate three and four day intervals with zinc (100 mg Zn/kg) given as a 20% (w/v) $ZnSO_4.7H_2O$ solution. One group was dosed by drenching gun, the other by intraruminal intubation. After five initial doses over 14 days the methods of administration were transposed for a further three doses over seven days. Serum samples for zinc analysis were obtained at 0, 2, 3, 4, 6, 8, 12, 24 and 48 h after each dose.

Experiment II In experiment II, two groups, each of seven sheep, were dosed daily for six days of each week for three weeks with zinc (50 mg Zn/kg/d) as a 20% (w/v) $ZnSO_4.7H_2O$ solution. One group received the zinc solution by drenching gun and the other by intraruminal intubation. Live weights were recorded weekly. Serum samples for zinc analysis were obtained on days one and three of each week immediately before dosing and six hours after dosing.

At the completion of the dosing periods all sheep were slaughtered and postmortem examinations were made. The pancreas, liver and kidney were weighed and samples taken for histological examination and for zinc analysis. Zinc analyses were carried out by atomic absorption analysis on serum and organ specimens (McLeay and Smith, 1977; Appendix A).

The extent of damage seen in the abomasum was scored (0-5) according to the estimated percentage of abomasal mucosa showing obvious necrotic changes; 0 indicated no detectable damage, 1 less than 10% affected, 3 indicated 20-50% affected and 5 more than 80% affected (Appendix C). Pancreatic tissue damage was scored according to the estimated area of exocrine tissue showing severe degenerative changes on histological examination of haematoxylin and eosin stained sections (Appendix C).

Ruminal papillae were measured on formalin fixed samples taken from the same area of the anterior wall of the ventral sac of each sheep. Measurements were made using a taper gauge and recorded as the average length x width product of 12 measured papillae (Appendix C).

Results

Experiment I Drenching gun dosing of zinc sulphate proved much more toxic than intraruminal intubation. Five of the eight sheep in the trial died within 48 h of drenching gun administration of the zinc sulphate solution, four after receiving their first dose in this manner, and the fifth after the second dose. Deaths were preceded in each case by a marked increase in serum zinc concentrations which rose from less than three to 30-50 µg Zn/ml within four to eight hours of dosing

Table 4.2 Experiment I. Effect of method of administration of zinc on peak serum concentrations of zinc (µg/ml) and time of peak after dosing (h) in parenthesis. Two groups of four sheep were given 100 mg Zn/kg bodyweight as a single dose of 20% zinc sulphate aqueous solution at three and four day intervals. After five doses the methods of administration were transposed.

Peak serum concentration of zinc (µg/ml)**

Sheep No.	Method of Administration	DAY 0	3	7	10	14	Method of Administration	DAY 17	21	24
1	Drenching gun	42.5* (4)					Intraruminal intubation			
2		10.0 (6)	36.3* (6)							
3		2.5 (4)	6.0 (4)	17.0 (6)	9.4 (6)	3.4 (8)		4.0 (12)	3.6 (8)	3.8 (6)
4		1.5 (4)	2.4 (2)	22.0 (8)	4.7 (3)	2.5 (4)		2.4 (12)	2.0 (8)	2.8 (3)
5	Intraruminal intubation	3.0 (8)	3.2 (4)	3.1 (8)	3.5 (4)	3.1 (6)	Drenching gun	41.5 (4)	7.3 (3)	23.8 (4)
6		3.5 (12)	38.3 (4)	2.1 (8)	3.1 (8)	2.1 (6)		34.3* (6)		
7		3.5 (12)	3.5 (8)	4.6 (6)	4.9 (8)	4.4 (12)		51.5* (8)		
8		2.0 (4)	2.3 (8)	2.7 (6)	2.8 (8)	2.9 (6)		45.0* (6)		

**pre experimental mean serum zinc concentration (± standard deviation) 0.63±0.23 µg Zn/ml;
*sheep died within 48 h of zinc administration

(Table 4.2). On a further eight of fourteen occasions when zinc was administered by drenching gun, peak serum zinc concentration exceeded 5 µg Zn/ml. Pre-experimental mean serum zinc concentration for all sheep was 0.63±0.23 SD µg Zn/ml.

In contrast, on only one occasion of 26 intraruminal administrations of zinc sulphate, did peak serum zinc concentration exceed 5 µg Zn/ml and the mean peak (4-12 h) serum zinc concentrations on these 25 occasions was 3.16±0.80 SD µg Zn/ml. Intraruminal administration of the zinc was not associated with the death of any sheep.

Severe lesions of the abomasum and occasionally of the upper duodenum were seen in all sheep that died immediately after administration of zinc by drenching gun and those slaughtered immediately after their third dose of zinc by drenching gun.

Experiment II Chronic drenching gun dosing of zinc sulphate was found to be more toxic than intraruminal administration of equal amounts of zinc.

Table 4.3 summarises the differences in effect seen when two groups, each of seven sheep, received zinc sulphate solution by either of the two oral methods of administration. The sheep which received zinc (50 mg/kg/d) by intraruminal intubation showed no abomasal damage on postmortem examination or pancreatic damage on histological examination. All these sheep had live weight gains of 6 kg or more over the three weeks of the experiment. Increases in serum and organ concentrations of zinc occurred (mean serum zinc pretrial 0.61; day 15 predosing 1.4, 6 h postdosing 2.9 µg Zn/ml) but the increases were small in comparison with those found in sheep which received zinc sulphate by drenching gun (pretrial 0.54; day 15 predosing 3.3, 6 h postdosing 11.7 µg Zn/ml). Six of the seven sheep dosed by drenching gun had lower weight gains than those dosed by intubation, five of the seven actually recording weight losses over the three weeks of the experiment. The sheep with markedly lower weight gains or weight losses all showed varying degrees of abomasal and pancreatic damage as well as greater organ zinc concentrations than did those dosed by intraruminal intubation. The abomasal

Table 4.3 Experiment II. Effect of method of oral administration on zinc toxicity in sheep. Two groups of seven sheep were given zinc (50 mg Zn as $ZnSO_4.7H_2O$/kg bodyweight/d) for three weeks either by drenching gun or by intraruminal intubation.

Method of administration	Sheep	Weight change at 3 weeks kg	Abomasal damage 0-5	Pancreatic damage	Rumen papillae size LxB (mm) n=12	Serum zinc** at 2 weeks µg/ml # 0 hr	6 hr	Organ zinc µg/g DMB Liver	Kidney	Pancreas
Drenching gun	1*	-4.0	4	3	0.91	7.2	26.5	1306	1145	7093
	2	-1.5	2	4	1.36	2.6	4.9	1635	2977	6268
	3	+7.5	0	0	6.40	1.5	3.8	585	699	304
	4	-1.5	2	9	1.75	3.0	12.0	1376	3579	3584
	5	+4.0	1	9	5.76	2.1	5.6	459	1047	1335
	6	-1.0	2	8	2.20	3.6	15.1	1135	3253	2867
	7	-1.5	2	8	1.82	2.9	14.1	2520	2452	4884
Group mean		+0.29			2.89	3.27	11.71	1288	2160	3762
Intraruminal intubation	8	+7.0	0	0	12.7	1.5	3.4	402	658	368
	9	+6.0	0	0	10.5	1.7	3.2	632	838	189
	10	+7.5	0	0	5.4	1.4	2.1	510	595	198
	11	+7.0	0	0	7.6	1.5	2.9	485	525	136
	12	+8.5	0	0	14.0	1.3	3.2	425	522	216
	13	+7.0	0	0	10.0	1.2	3.0	309	406	295
	14	+6.5	0	0	12.9	1.2	2.8	286	360	149
Group mean		+7.07			10.44	1.4	2.94	436	578	222
t test significance P<		0.01	ND	ND	0.01	0.05	0.05	0.05	0.05	0.01

**Pre experimental mean serum zinc concentrations; drenching gun group 0.54, intraruminal group 0.61 µg Zn/ml;
*Sheep died on last day of drenching; #sheep bled immediately prior to drenching (0 h) and 6 h after drenching with zinc; ND not done.

and pancreatic damage appeared to be associated with the higher serum and organ concentrations of zinc. One of the drenched sheep (#1) died on the last day of the experiment while another (#3) showed no indication of zinc toxicity.

In those sheep in which abomasal lesions were most apparent ruminal papillae were smaller in size (Plate 5.42) than in those not showing abomasal lesions (Table 4.3). Where smaller ruminal papillae were found, microscopic examination showed less vacuolation of the ruminal epithelium and a more complete keratinisation of a narrower squamous epithelium (Plate 5.43).

Discussion

These experiments show that the method of oral administration of zinc sulphate solution can markedly influence its toxicity. The use of a drenching gun for the oral administration of zinc sulphate causes an exacerbation of its toxicity. In addition, the use of the drenching gun caused considerable variation in response to zinc sulphate administration.

It has been shown (Chapter 4.1; Smith *et al.*, 1977b) that solutions of zinc, when administered by drenching gun, may stimulate the reticular groove reflex causing such drenches to travel directly to the abomasum. In these experiments the presence of severe abomasal lesions in many of the sheep dosed by drenching gun provides further evidence that zinc solutions stimulate the reticular groove reflex. The evidence of McLeay and Smith (1977) indicates that zinc secretion via the abomasum is not likely to be responsible for the observed abomasal lesions. In experiment I most administrations by drenching gun resulted in substantial increases in serum zinc concentrations. However in six out of nineteen occasions this did not occur. Smith *et al.* (1977b) have suggested that variations in the reticular groove reflex latent period, which may be of several seconds duration, may result in some drenches reaching the cardia before the groove musculature has contracted. This variable period could result in the occasional ineffectual closure of the groove and be responsible for the variation in

response to drenching gun administration of zinc sulphate solutions. The fact that one sheep drenched by drenching gun showed no signs of zinc toxicity (Table 4.3) suggests that none or few of the drenches were channelled into the abomasum. In this connection an inability to stimulate the reflex in certain animals has been recorded (Watson and Jarrett, 1944).

It is suggested that drenching gun administration of zinc solutions, which involves contact of zinc with the pharyngeal mucosa results in stimulation of the reticular groove reflex. If the reticular groove closure has occurred before the bolus arrives at the cardia then the drench or part of it is channelled directly into the abomasum. Here, and sometimes also in the upper small intestine, high concentrations of zinc solutions cause severe mucosal damage resulting in breakdown in homeostatic mechanisms. The resulting high serum levels of zinc appear to result in the high levels of zinc in certain organs (liver, kidney, pancreas) and the pancreatic damage typical of zinc toxicity.

These results may be compared with those of acute copper poisoning of sheep in which the presence of abomasal lesions is presumed to be due to stimulation of the reticular groove reflex and the consequent deposition of the administered solution in the abomasum (Sharman, 1969).

The reduction in size of ruminal papillae recorded in experiment II appeared to be most pronounced in sheep with more severe abomasal damage. Reductions in the size of ruminal papillae have been reported in ruminants on low energy rations (Nockels *et al.*, 1966) and in cases of starvation (R.G. Clarke, pers. comm.). While food intake was not recorded in these experiments the drenching gun group of sheep were observed not to graze, appeared to have reduced rumen contents, and had reduced weight gains or even weight losses. It is possible that these changes in papillation are related more to abomasal damage and inappetance than to zinc toxicity *per se*. It has been shown that sheep on high zinc intakes suffer reduced food intake (Ott *et al.*, 1966a; McLeay and Smith, 1977). In addition, damage to the abomasum causes reduction in food intake (McLeay *et al.*, 1973).

There are differences of opinion regarding the relative safety of ingested zinc sulphate. Burch and Sullivan (1976), referring to man, state "zinc sulphate and zinc oxide are relatively innocuous compounds when ingested". However Moore (1978) draws attention to a bleeding gastric erosion resulting from zinc sulphate medication.

In ruminants, the work of Ott (1966a,b,c,d) is often quoted as evidence for the low toxicity of zinc. Most of his work involved the incorporation of zinc oxide into the diet. He also administered zinc sulphate to sheep for 11 days by intraruminal intubation at similar and higher dose rates (up to 160 mg Zn/kg/d) than used here. The survival of his sheep for this time was probably due to the method of administration. Unless the importance of method of administration is recognised such results may give a false impression of the safety of zinc. It seems clear from these results and those of others that zinc sulphate is capable of causing gastric irritation and that special care should be taken in the administration of this substance not only to ruminants but also to other species.

4.3 EFFECT ON ZINC TOXICITY OF VOLUME AND CONCENTRATION OF ZINC SULPHATE SOLUTION WHEN ADMINISTERED BY DRENCHING GUN TO SHEEP

Introduction

The manner of oral administration of zinc sulphate is critical in determining its toxic effect in ruminants (Smith *et al.*, 1979; Chapter 4.2). The exacerbation of zinc toxicity when zinc sulphate is delivered by drenching gun appears to be due to the routing, by the reticular groove, of the drench to the abomasum where corrosive gastritis occurs. The severity of reaction of other organs to zinc sulphate when delivered by drenching gun also seems to be related to the extent of the corrosive lesion in the abomasum. Because of this, it was decided that various possible alternatives of drench concentration and volume should be investigated in order to determine their relative importance in producing the exacerbation of zinc toxicity.

Increasing the concentration of the zinc sulphate solution up to 20% appeared to increase the effective operation of the reticular groove (Smith *et al.*, 1977b; Chapter 4.1). However when large volumes are used which may involve more than one swallow by the sheep, or multiple operation of the drenching gun for each dose, there may be an increased chance of the reticular groove operating effectively as has been suggested (Smith *et al.*, 1977b; Chapter 4.1). Alternatively, lower concentrations, even if effectively channelled to the abomasum, may cause less damage in the abomasum.

In this experiment three dose rates of zinc sulphate were given to sheep by drenching gun over three weeks each in three different combinations of volume and concentration and the effect of each combination on toxicity compared.

Materials and Methods

Fifty 12 month old, 25-35 kg body weight Romney cross wethers were randomly grouped into 10 groups of five of approximately equal mean weight. One control group was not dosed. The remaining nine groups of five were drenched daily by drenching gun with zinc sulphate solutions for three weeks at three separate dose rates of zinc each given as three different combinations of concentration and volume as seen below.

Design of experiment to investigate the difference in toxic effect of zinc sulphate in sheep caused by variations in volume and concentration of the drenching gun solutions.

Concentration of zinc sulphate % w/v	Volume of drench		
	10 ml	20 ml	40 ml
5			A 5/40
10		A 10/20	B 10/40
20	A 20/10	B 20/20	C 20/40
40	B 40/10	C 40/20	
80	C 80/10		

Dose rates
A 15 mg Zn/kg b.wt./d
B 30 mg Zn/kg b.wt./d
C 60 mg Zn/kg b.wt./d

The three dose rates based on mean initial weights were A 15, B 30 and C 60 mg Zn/kg b.wt./d. The effects of zinc at the various combinations of volume and concentration were measured by weight changes, abomasal and pancreatic damage (Appendix C) histological changes and size of rumen papillae (Appendix C).

Results

The measured toxic effects of the different concentration and volume combinations are presented in Tables 4.4, 4.5, 4.6 and 4.7. As expected the greatest toxic effect was seen in those animals receiving the highest dose rate (groups C). Within each dose rate subgroup (A, B or C) the greatest effect was seen in the highest volume/lowest concentration combination at each dose rate, with the greatest effect seen in this combination of the highest dose rate (C 20/40). At the two lower dose rates the effects due to changes in volume/concentration were not large and were outweighed by the differences in dose rate. However this could not be said for the differences in volume/concentration effects at the highest dose rate (subgroup C) where quite large differences were due to changes in concentration and volume.

Table 4.4 Effect of volume and concentration of zinc sulphate drenching on numbers of sheep* with abomasal lesions.

Concentration % w/v	Volume of drench (ml) 0	10	20	40
0	0			
5				0
10			0	1
20		0	0	4
40		1	1	
80		1		

Dose rates 15, 30 and 60 mg Zn/kg b.wt/d

*Five sheep per group

Table 4.5 Effect of volume and concentration of zinc sulphate drenching fluid on size of rumen papillae (length x breadth mm) ± SEM.*

Concentration % w/v	Volume of drench (ml) 0	10	20	40
0	6.9±0.8			
5				11.0±1.9
10			7.9±0.7	7.4±0.9
20		12.2±1.1	8.1±0.3	4.2±0.8
40		8.8±0.7	7.1±1.7	
80		6.7±1.1		

Dose rates 15, 30 and 60 mg Zn/kg b.wt/d

*Five sheep per group

Table 4.6 Effect of volume and concentration of zinc sulphate drenching fluids on body weight changes (kg) at 3 weeks*.

Concentration % w/v	Volume of drench (ml) 0	10	20	40
0	+3.4±0.4			
5				+2.6±0.5
10			+4.9±0.4	+3.2±1.1
20		+4.7±0.6	+3.2±1.1	-4.9±1.0
40		+4.5±1.1	+0.8±1.5	
80		+1.5±1.8		

Dose rates 15, 30 and 60 mg Zn/kg b.wt/d
*Values are group means ± SEM.

Table 4,7 Effect of volume and concentration of zinc sulphate drenching on numbers of sheep with pancreatic lesions*.

Concentration % w/v	Volume of drench (ml) 0	10	20	40
0	0			
5				0
10			0	0
20		0	0	4 (3.0)
40		0	4 (2.0)	
80		1 (1.8)		

Dose rates 15, 30 and 60 mg Zn/kg b.wt/d
*Mean pancreatic injury score for groups in parenthesis.

Rumen total volatile fatty acid concentrations were calculated for the two extremes of volume and concentration for the lowest and highest dose rates (A and C). The only significant difference ($p>0.01$) was seen in the high volume low concentration combination of the high dose rate group (C 20/40) where the concentration was lower than that in the other groups for which it was calculated (Control mean 25.6; Group C 20/40 16.0 mM/ℓ).

At the lowest dose rate there seemed to be a positive response to zinc especially at the lower volume higher concentration combinations. This was seen particularly in the measurements of weight change (Table 4.6) and size of rumen papillae (Table 4.5).

Discussion

The results indicate that, at least for the highest dose rate, the most exacerbating influence of volume and concentration was at that combination giving the highest volume and lowest concentration. This volume (40 ml) required four squeezes of the drenching gun to administer each dose and although carried out rapidly without any spillage would surely have allowed time for any latent period in the operation of the reticular groove reflex to be overcome. Thus the chances of the drenched bolus arriving at the groove before it had contracted would be much less than in the other combinations of this dose rate and is one possibile explanation for this exacerbated effect. The lowest concentration (20%) at this dose rate has certainly been shown to be sufficient to cause stimulation of the reticular groove reflex (Smith *et al.*, 1977b; Chapter 4.1).

However there are other possible explanations for this effect. At low volumes (e.g. 10 ml) the drenched bolus has a greater chance of being spread out and dissipated along the squamous epithelium of the oesophagus and reticular groove. In addition, assuming that all of the various drenches were channelled to the abomasum, the high volume drench may have covered and damaged a greater surface area in the abomasum allowing a greater absorption of subsequent drenches and hence greater effect in terms of pancreatic lesions and weight change.

Table 4.8 Number of deaths caused by various volumes and concentrations of zinc sulphate solution when administered twice to mice by intragastric intubation. Results are expressed as number of deaths over numbers of mice in group.

Concentration of zinc sulphate % w/v	Volume of drench (ml)			
	0.125	0.25	0.5	1.0
0.5				$^{0}/_{3}$
1.0			$^{0}/_{4}$	$^{0}/_{4}$
2		$^{0}/_{4}$	$^{0}/_{4}$	$^{2}/_{4}$
4	$^{0}/_{4}$	$^{0}/_{4}$	$^{1}/_{3}$	$^{4}/_{4}$
8	$^{0}/_{4}$	$^{0}/_{4}$	$^{2}/_{3}$	
16	$^{0}/_{4}$	$^{4}/_{4}$		
32	$^{1}/_{4}$			

In view of this a small trial was conducted with 16 groups each of three or four mice of approximately 26 grams weight. The mice were dosed, by intragastric intubation, twice at a one day interval with zinc sulphate at four dose rates, each dose rate being administered at four different combinations of volume and concentrations. The trial design, treatments and results in terms of number of mice dying within four days of commencement of dosing is shown in Table 4.8. These results clearly show that once again that greater volume has a greater exacerbating effect than greater concentration at the same dose rate. In this case no reticular groove mechanism is involved and it suggests that in the sheep even if all the drench travels to the abomasum then greater volume still exerts an exacerbating effect.

At the outset of this experiment it was hoped that at the same dose rate of zinc greater drench volume might have prevented the exacerbating effect of drenching gun administration. This did not occur and in fact the opposite result was obtained i.e. greater volume caused more exacerbation than greater concentration at the same dose rate.

Sufficient evidence from this and previous experiments (Smith *et al.*, 1979; Smith *et al.*, 1977b; Chapter 4.1 and 4.2) had been obtained to show that zinc sulphate dosed by drenching gun produced adverse effects and that the margin of safety for zinc sulphate above dose rates required for protection against pithomycotoxicosis was not great when the drenching gun was used. Therefore, this line of investigation was discontinued in favour of investigations of the toxicity of different forms of zinc and other methods of administration.

4.4 THE INFLUENCE OF CHEMICAL FORM OF ZINC ON THE EFFECTS OF INTRARUMINAL DOSES OF ZINC TO SHEEP

Introduction

Since high oral doses of zinc have been shown to prevent several toxicoses (Saldeen, 1969; Voigt and Saldeen, 1965; Chvapil *et al.*, 1974) including sporidesmin poisoning (Towers, 1977a; Smith *et al.*, 1977a; Towers and Smith, 1978) there has been some interest in zinc toxicity

in ruminants and the serum zinc levels produced by different forms of zinc at high dose rates. Certain aspects of the toxicity of zinc to ruminants have been studied by Ott *et al.* (1966a,b,c,d) who mainly administered the zinc by incorporation into food or drinking water. Because of the pastoral situation in New Zealand some research emphasis has been placed on oral hand administration of zinc salts. The sulphate, oxide and EDTA forms of zinc have all been shown to prevent pithomycotoxicosis. These forms of zinc all have different physical and chemical properties and are of potential value for the field control of pithomycotoxicosis. The effect of oral administration of these compounds to ruminants is therefore of considerable interest.

The availability of zinc to animals may be either impaired or improved by chelating agents in the diet (Davies and Nightingale, 1975; O'Dell and Savage, 1960). The degree to which synthetic chelating agents influence the absorption of zinc (as judged by growth promotion in zinc deficient turkey poults) was related to the stability constant of the particular chelating agent (Vohra and Kratzer, 1964). Too low or too high a stability constant resulted in lowered effectiveness of the chelate. Dietary (Powell *et al.*, 1967) or intravenous (Vagg, 1971) EDTA when administered to ruminants also influenced the excretory route of zinc, there being a marked increase in the urinary output of zinc.

The availability of other salts of zinc appears to be dependent at least in part on their solubility. In this experiment the effect of intraruminal administration of three different forms of zinc (oxide, sulphate and EDTA) as either a single or multiple dose to sheep is examined.

Materials and Methods

Single dose experiment (SDE) The sulphate, oxide or EDTA forms of zinc were administered as a single dose by intraruminal intubation to pasture grazed, 12 month old 30-40 kg Romney sheep at three dose rates (480, 240 and 120 mg Zn/kg). All sheep were wethers except for one ewe in each of the groups receiving the highest and intermediate dose.

There were eight non-dosed control sheep. Three groups of eight sheep each received the highest dose rate of one each of the three salts. Six groups each of four sheep received the three forms at the two lower dose rates. The zinc sulphate and zinc EDTA aqueous solutions were administered as 29% w/v $ZnSO_4$ and 47% w/v Zn EDTA and the zinc oxide as a 200 ml suspension. All doses were washed down with approximately 200 ml of water.

Multiple dose experiment (MDE) Twenty eight pasture grazed 15 month old Romney cross wethers were divided into four groups of seven. One group of controls was not dosed. Each of the other three groups was dosed by intraruminal intubation with one of the three forms (oxide, sulphate or EDTA) of zinc at 240 mg Zn/kg/dose on Mondays, Wednesdays and Fridays of each week for 26 days to give approximately 100 mg Zn/kg mean daily dose.

The zinc sulphate and zinc EDTA were administered as a 26% w/v zinc sulphate and a 43% w/v zinc EDTA aqueous solution, each being washed down the dosing tube with approximately 200 ml of water. The zinc oxide was administered as a 200 ml suspension in water, also washed down with 200 ml of water.

In both experiments all sheep were penned shortly before dosing and remained penned for 12 h after initial dosing after which they were returned to pasture and mustered for subsequent bleedings and dosings as required. In the SDE a mid-stream urine sample for zinc analysis was collected from each ewe at 8, 30 and 72 h after zinc administration.

Blood samples for zinc analysis of serum were taken by jugular puncture from a pair of sheep (SDE) and three sheep (MDE) from each group at 0, 2, 4, 8, 12, 18, 24, 36 and 48 h for both experiments and at 3, 5, 7, 10 and 15 days after dosing in the SDE. In the MDE all sheep were bled prior to dosing on the Friday of each week of the experiment and at the above times after dosing on days 1, 13 and 26 after the start of dosing.

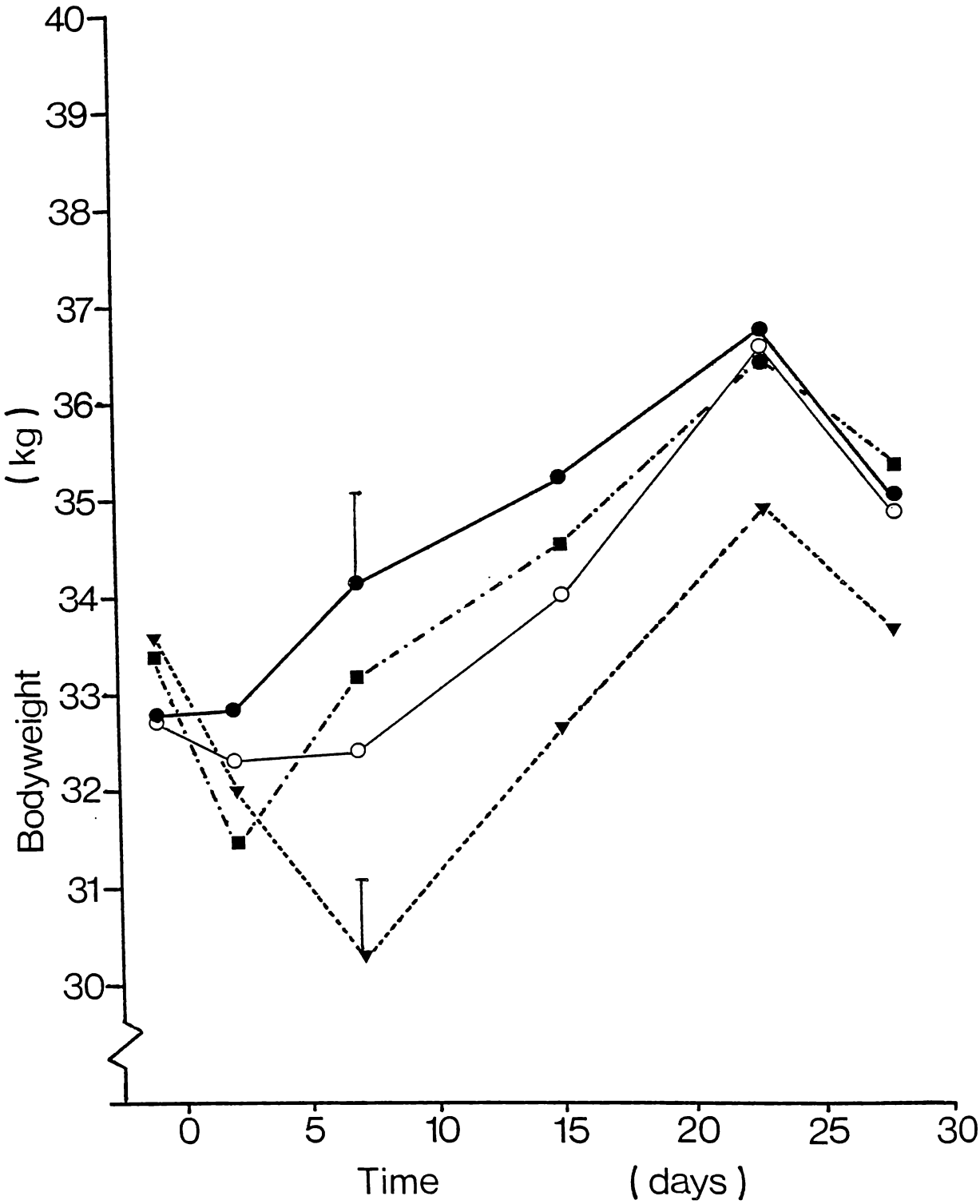


Figure 4.2 : Effect of high single intraruminal dose (480 mg Zn/kg) of different zinc salts on bodyweight of sheep. Control ● —— ●; zinc sulphate ▼ ..... ▼; zinc oxide o —— o; zinc EDTA ■-.-.-.■ . Plotted values are group means ±SEM's.

Chemical changes and body weights were recorded throughout each experiment. At the end of each experiment, four weeks after initial dosing, the sheep were slaughtered and a postmortem examination conducted. Organ weights were recorded and samples collected for zinc analysis (Appendix A) and histological examination (Appendix B). Serum and urine samples were diluted (McLeay and Smith, 1977) and subjected to atomic absorption analysis for zinc content.

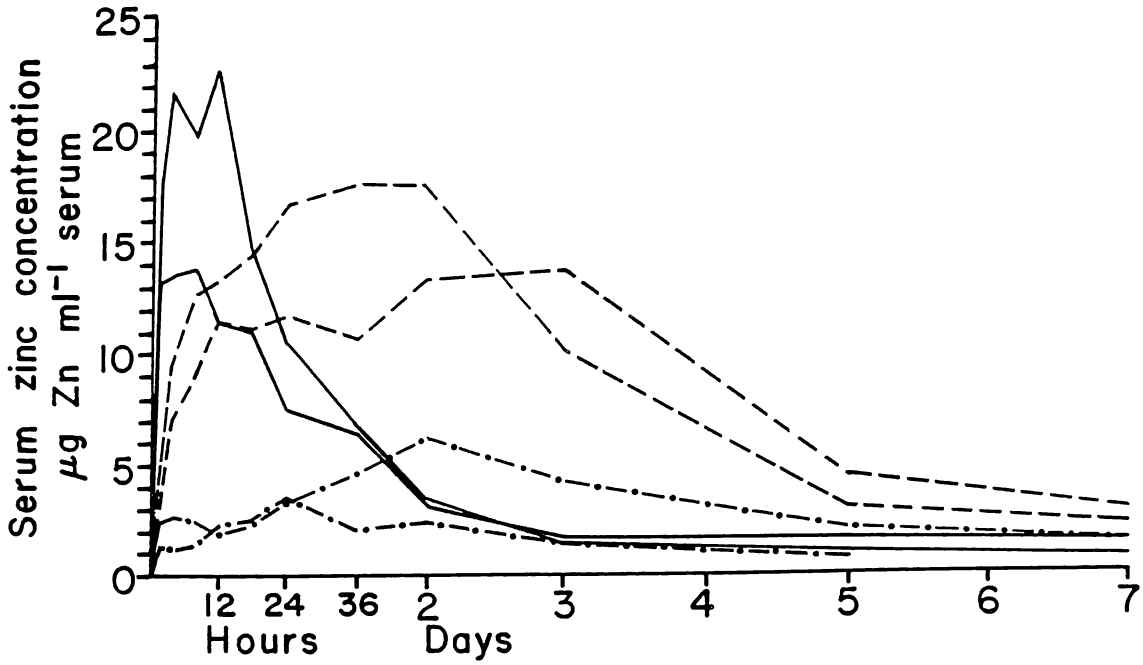
Pancreatic damage was scored on histological examination of sections on a 0-10 basis (where 0, no change; 10, severe extensive damage of pancreatic exocrine tissue; Appendix C).

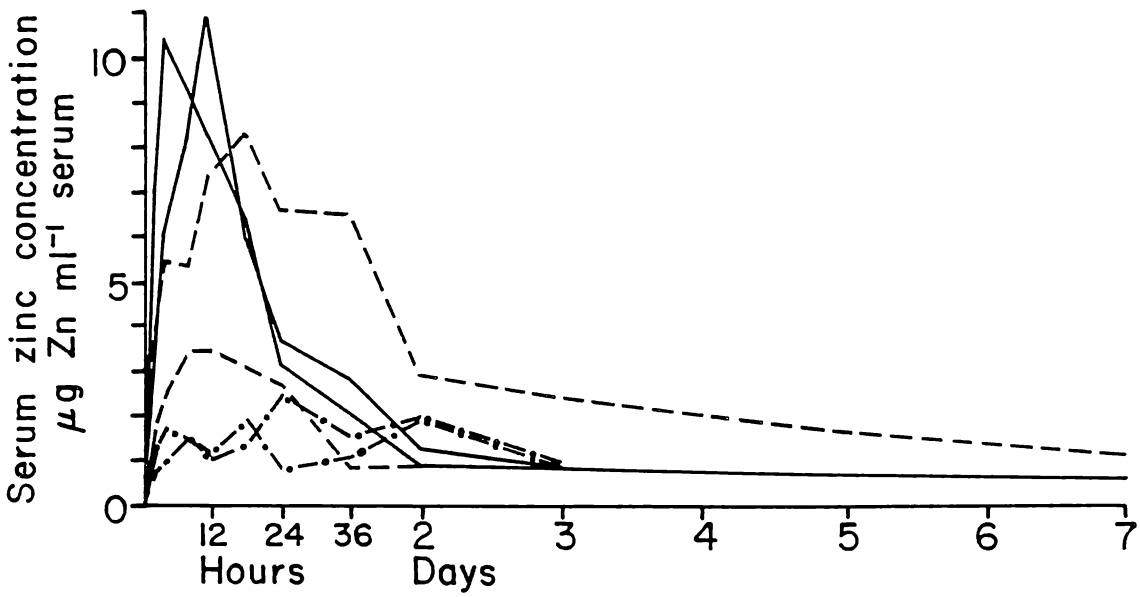
Results

Single dose experiment

Clinical and postmortem findings The only clinical sign noted was an acute diarrhoea. This started at 8 h in the EDTA group. By 18 h all the EDTA sheep and one sheep in the high sulphate group had diarrhoea. Over the next two to four days most of the high and medium sulphate group animals developed diarrhoea. All animals recovered from their diarrhoea within a few days. The diarrhoea was reflected at least in part by significant reductions in body weight which were recorded early in the experiment (Fig 4.2). Weight losses were also recorded in the medium sulphate group at two and seven days after dosing. At slaughter there was a significantly lower mean carcass weight in the high sulphate group.

The only lesions detected at postmortem were in the pancreas. Here a mild to moderate acinar necrosis with atrophy and ductular hyperplasia of lobular distribution with occasional periductular inflammation was seen. There were pancreatic lesions only in those sheep receiving zinc at the highest dose rate. Seven of eight sheep in the high sulphate group had pancreatic lesions (mean score 2.25 range 0-5) compared with one of eight in the oxide (score 4) and EDTA groups (score 1).


Serum zinc concentration
μg Zn ml⁻¹ serum
25
20
15
10
5
0
12 24 36 2 3 4 5 6 7
Hours
Days


Serum zinc concentration
μg Zn ml⁻¹ serum
10
5
0
12 24 36 2 3 4 5 6 7
Hours
Days

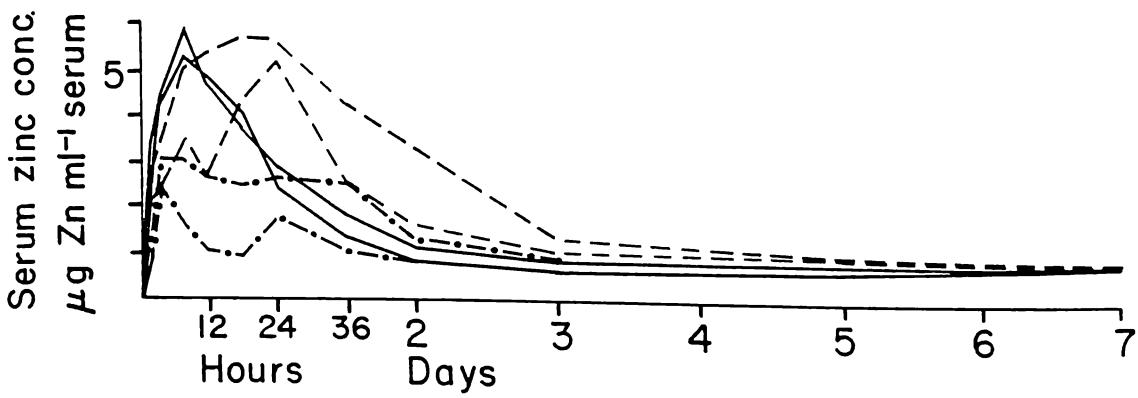

Serum zinc conc.
μg Zn ml⁻¹ serum
5
12 24 36 2 3 4 5 6 7
Hours
Days

Figure 4.3 a, b and c : Serum zinc concentrations in sheep after a single intraruminal dose of zinc salts at three different dose rates. Zinc sulphate ---; zinc EDTA ——; zinc oxide -·-·-·.

Figure a: Dose rate 480 mg Zn/kg bwt.

Figure b: Dose rate 240 mg Zn/kg b.wt.

Figure c: Dose rate 120 mg Zn/kg b.wt.

Table 4.9 Effect of a single intraruminal dose of different forms of zinc (480 or 240 mg Zn/kg) on urinary zinc concentration.

Form of zinc	Dose rate mg Zn/kg b.wt	Urinary zinc concentration (µg/ml) at time* after single dose		
		8 h	30 h	72 h
EDTA	480	1127	451	NA
Sulphate	480	0	1.5	3.5
Oxide	480	0	0	0.5
EDTA	240	1002	301	5.0
Sulphate	240	0	1.0	4.5
Oxide	240	0	0.5	NA
Control	0	0	0	0

NA not available *h hours

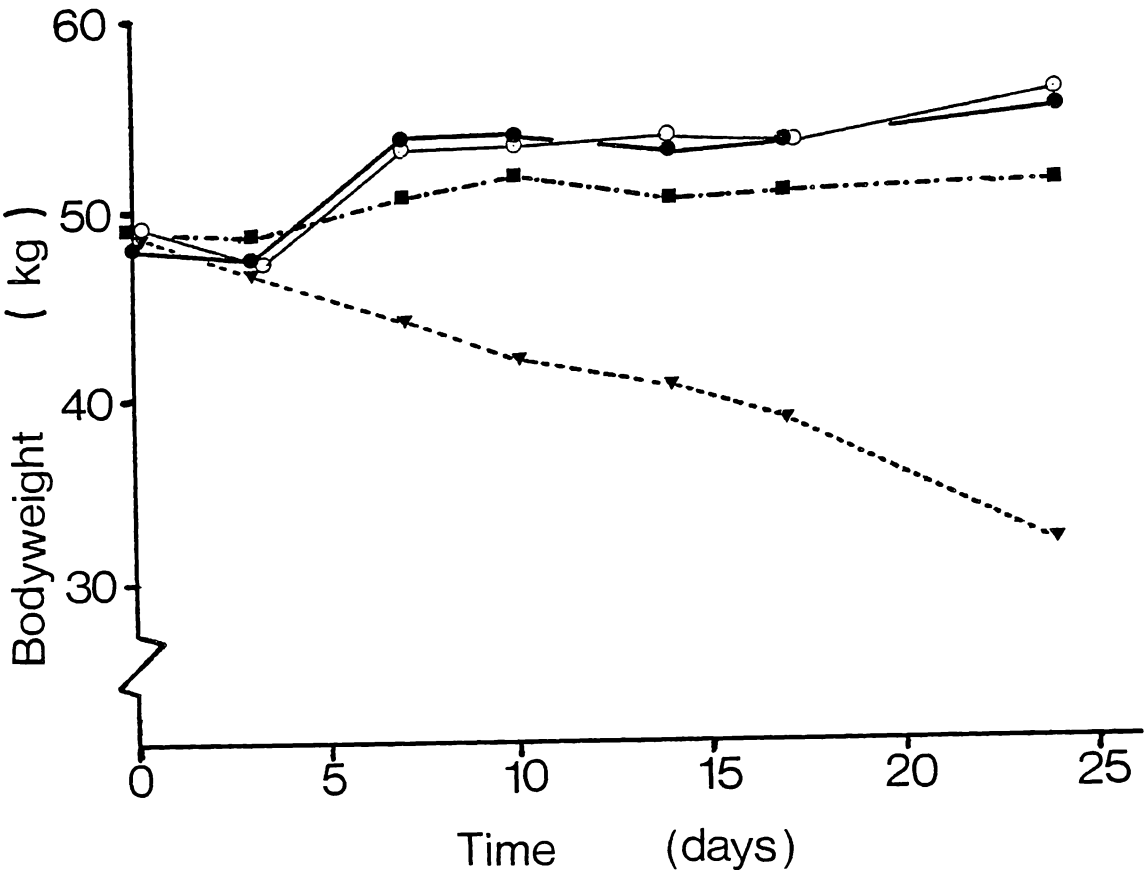
Laboratory findings Serum zinc concentrations are shown in Figures 4.3a,b,c. In those sheep receiving zinc EDTA serum zinc concentrations rose more rapidly, peaked earlier (6-18 h) and at a higher concentration and subsequently declined more rapidly than the sheep receiving the equivalent dose as either of the other two salts. In the case of zinc sulphate serum zinc levels were also high but were slower to rise, peaking later (12-48 h) but maintained higher concentrations for longer than was the case with EDTA. In the case of zinc oxide, serum concentrations rose slowly and to a lesser extent than for the sheep receiving the other two salts but maintained their elevations for two or three days.

Urinary zinc concentration was only measured from one ewe in each group of the high and medium zinc treatments. These results (Table 4.9) indicate that urinary zinc excretion was high in those animals receiving zinc as zinc EDTA.

There were no significant differences between controls and dosed animals for zinc concentrations in liver, pancreas, muscle (*Vastus medialis*) or spleen collected at slaughter. There was however a significant ($p<0.05$) elevation of kidney zinc in the high sulphate dosed animals. The mean kidney zinc concentrations were: control 107.2, high sulphate 129.0, high oxide 122.6, high EDTA 108.4 µg Zn/g DMB.

Multiple dose experiment

Clinical and postmortem findings A bright dark green mucoid severe diarrhoea commenced in the zinc sulphate dosed animals after a week of dosing and persisted throughout the trial. This diarrhoea was accompanied by a drop in body weight in the sulphate group (Fig 4.4) with all animals in this group dying between 13 and 26 days after the start of dosing. Apart from a mild diarrhoea starting after a week of dosing and persisting for a further week in the zinc EDTA group there were no clinical signs in any animals of the three groups other than those receiving zinc sulphate. Six out of the seven zinc sulphate dosed sheep died before the last dose.


60
50
40
30
Bodyweight (kg)
0
5
10
15
20
25
Time (days)

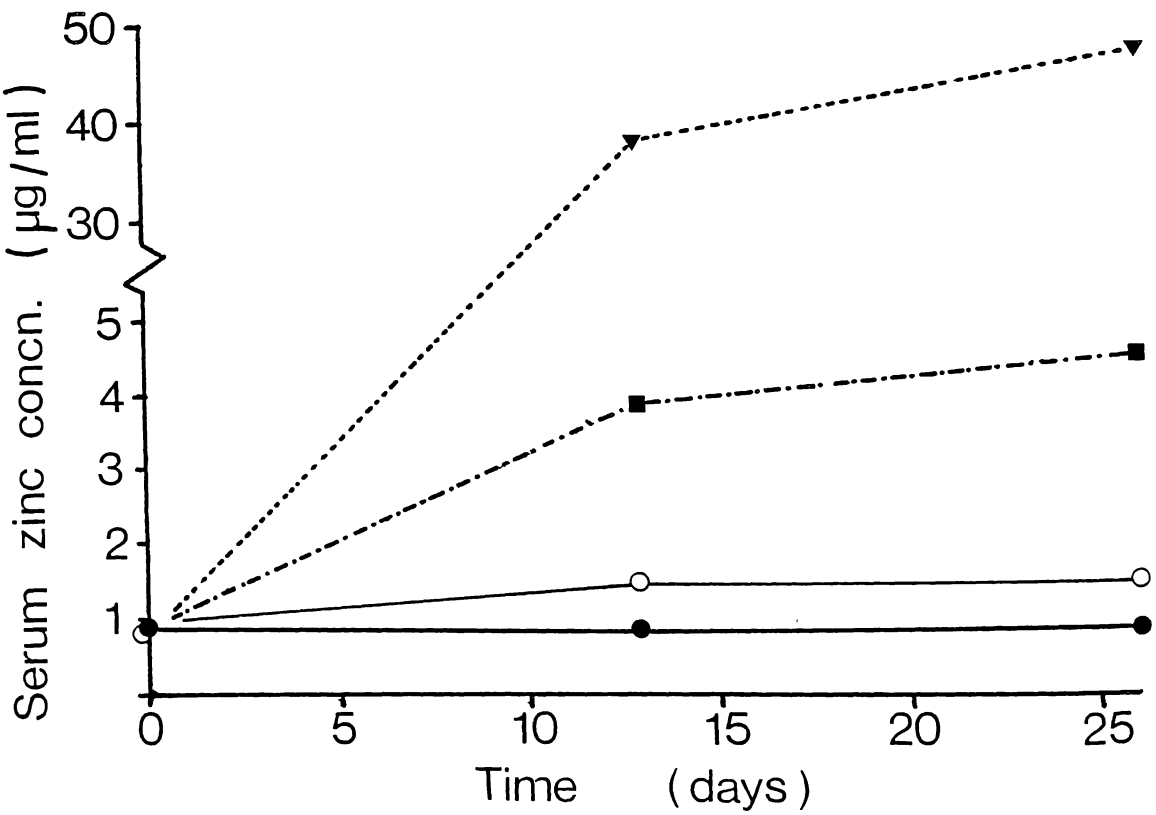

50
40
30
5
4
3
2
1
Serum zinc concn. (µg/ml)
0
5
10
15
20
25
Time (days)

Figure 4.4 : Bodyweight changes in sheep dosed with three forms of zinc thrice weekly for 26 days. Controls ● —— ●; zinc sulphate ▼---- ▼; zinc oxide ■-.-.-.■; zinc EDTA o —— o.

Figure 4.5 : Mean serum zinc concentrations in sheep dosed with three forms of zinc three times a week. Serum samples collected 48 h after previous dose and immediately before each 240 mg Zn/kg dose. Control ● —— ●; zinc sulphate ▼ ---- ▼; zinc oxide ■-.-.-■; zinc EDTA o —— o.

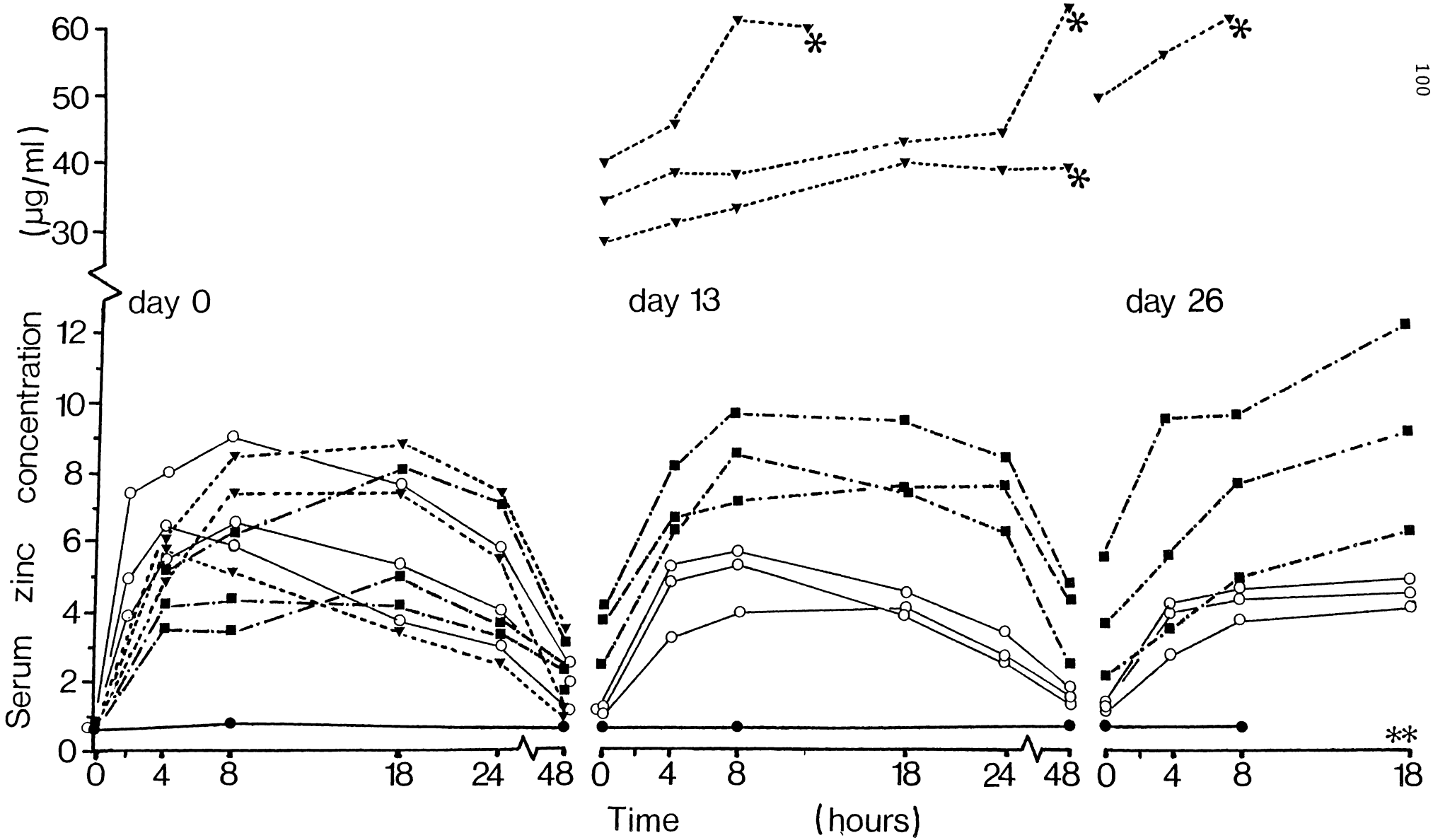

Serum zinc concentration (μg/ml)
60
50
40
30
12
10
8
6
4
2
0
day 0
day 13
day 26
*
*
*
*
**
0
4
8
18
24
48
Time (hours)

Figure 4.6 : Serum zinc concentrations over 48 hour post dosing periods at 0, 13 and 26 days during a 26 day thrice weekly dosing period with three different forms of zinc at 240 mg Zn/kg b.wt/dose. Values are plotted for the same individual sheep at each 48 h bleeding period. Control (mean of 3 sheep) ● —— ●; zinc sulphate ▼ ---- ▼; zinc oxide ■-·-·-■ ; zinc EDTA o —— o; *last value before death of sheep; **24 h sample inadvertently destroyed.

Animals in the zinc sulphate group showed extensive lesions on post-mortem examination. The rumen contents were small and dehydrated and the papillation of the rumen wall was much reduced. The fundic folds of the abomasum were oedementous and the mucosal surface often covered with a white curd like material or showing evidence of sloughing. The abomasal contents were usually dark green and slimy. Small intestinal contents were usually bright green. The liver was often finely mottled with an orange peel-like surface and a nutmeg brown colour. The pancreas was always small, pale and firm often with an irregular serosal surface. Pancreatic damage was evident in all animals (damage score range 5-8; mean 6.9). The kidneys were enlarged with red or brown speckled capsular surface and moist cut surface. The urinary bladder often contained dark brown urine.

In the zinc oxide group pancreatic damage was observed in all animals (range 3-10; mean 7.3). In the zinc EDTA group only mild pancreatic damage was observed in four of the seven animals (range 1-3; mean 1.0).

Serum zinc concentrations following the first dose tended to follow the trends noted in the single dose experiment (Fig 4.6). EDTA dosed sheep tended to show serum zinc concentrations peaking earlier and declining earlier than did the other groups. However with repeat dosing the resting serum levels tended to become progressively higher (Fig 4.5) except in the EDTA group where predosing zinc concentrations tended to be only slightly more than control and pretreatment concentrations. Serum zinc concentrations in the sulphate group rose to very high levels, 30-60 µg/ml, the highest levels preceding the death of these sheep. In the case of zinc EDTA dosed animals the peak serum zinc concentrations at bleeding times late in the dosing period tended to become progressively lower whereas those in the zinc oxide group starting from a higher resting level tended to become slightly higher. The more rapid decline in serum zinc concentrations in the case of zinc EDTA dosed animals was not as pronounced as in the SDE.

Organ zinc concentrations (Table 4.10) were highest in pancreas, kidney and liver (in descending order) and were mugh higher in the sulphate and oxide groups than in the EDTA group which in turn also had organ

Table 4.10 Mean organ concentration of zinc in groups of sheep at post mortem following twenty six days of dosing with zinc as either sulphate, oxide or EDTA three times a week at 240 mg Zn/kg/dose.

Treatment	Mean organ zinc concentration (µg Zn/ml) ±SD			
	Liver	Kidney	Pancreas	Muscle
Control	154± 18	112± 15	163± 49	166±20
EDTA	424± 65	534± 107	654± 259	163±14
Sulphate	1773±630	2023± 674	2673±1286	NA
Oxide	1299±263	2348±1078	2956±1796	164±25

NA,Not available

zinc concentrations significantly higher ($p<0.01$) than control group sheep. Mean organ zinc concentrations reached as high as 3000 µg/g DMB in the pancreas in the oxide group. There was no significant rise in zinc concentration in any muscle samples taken from the oxide or EDTA group.

Discussion

The rapid and high rises in serum zinc concentration when zinc was administered as the sulphate and EDTA (SDE) suggests either that little control of absorption exists or that some change to absorptive surfaces occurred under these dosing regimes. The equally rapid decline in serum zinc concentration in the case of zinc EDTA indicates either a more rapid excretion or the more rapid removal into body tissues. The much higher concentrations of zinc in the urine of EDTA animals indicates that excretion by this route is at least in part responsible for the rapid decline in serum zinc. Whatever the reason it appears that in the case of the EDTA it exists in serum in a different form to that resulting from sulphate dosage and is recognised and excreted differently as such.

In the MDE the more rapid decline of serum zinc in the EDTA group was not as pronounced but sufficient to ensure that serum zinc concentrations had almost returned to predosing levels before each new dose of zinc EDTA was administered. The more rapid rise of serum zinc in the EDTA group was present after the first dose, but not apparent at subsequent bleedings in the middle and at the end of the experiment. The mean peak to which the serum zinc rose in the EDTA group was smaller at these subsequent bleeding times whereas the peaks for sulphate and oxide became progressively higher. It appeared that in the case of the EDTA group either zinc absorption declined, or removal from serum became more rapid as the experiment progressed. As organ zinc in the EDTA group was much less than the other two groups it appears that excretion rather than removal from serum to an organ is the more likely adjustment.

These findings support those of Vagg (1971) who showed in sheep that intravenously administered zinc EDTA is excreted to a large extent via the kidneys and urine. His results indicated that the overall retention of zinc was not affected by the form of zinc but rather the route by which it was excreted was changed. The retention of metals *in vivo* appears not to be entirely dependent on the stability constants of the chelate, the competitive affinity of the tissue systems for the metal being equally important. The urinary excretion of zinc in the case of chelate may be the result of a gross excess of absorbed zinc, probably in the EDTA form and its subsequent removal in this form. The urinary excretion of chelates and their value in the therapeutic removal of metals is well known (Seven and Johnson, 1960). A large urinary output of zinc in ruminants dosed with EDTA alone indicates that even free EDTA has a high affinity for zinc (Powell *et al.*, 1967).

The acute diarrhoea which developed in both experiments in the case of the EDTA and sulphate forms of zinc suggests that zinc interferes with gut function either directly as in the case of local gut lesions (Smith *et al.*, 1979; Smith, 1977) or indirectly via a systemic route (Winek and Buehler, 1966) on either motility or mucosal integrity or through an effect on gut micro-organisms (Hubbert *et al.*, 1958). Diarrhoea in the EDTA sheep was of shorter duration and caused a temporary weight loss probably due to loss of gut fill. In the case of the sulphate-dosed sheep the intensity and duration of diarrhoea was greater and may have been responsible for keeping body weights depressed for the duration of the trial and even affecting carcass weights at slaughter in the SDE. In the MDE there were substantial body weight losses in the sulphate dosed group of sheep. While the persistent diarrhoea would have contributed to this body weight loss other factors such as the abomasal and pancreatic damage, together with the observed reluctance to feed, probably contributed to this loss.

A greater degree of pancreatic damage existed in the high sulphate group in the SDE. The greater exposure of the organ to zinc (time x serum zinc concentration) possibly accounts for this difference.

When zinc is administered as EDTA the sequestered nature of zinc in the chelate may account for the lesser pancreatic damage in this group.

Zinc concentrations were measured in organs collected at slaughter. Despite the fact that organ zinc concentrations are known to return to normal soon after zinc dosing ceases (Towers *et al.*, 1976) there still was a small but significant elevation of kidney zinc in the sulphate and EDTA-dosed groups in the SDE. In the MDE in all zinc-dosed groups large increases in organ zinc were recorded in the liver, kidney and pancreas, but not muscle. The elevations of organ zinc were much greater in the sulphate and oxide groups than the EDTA-dosed group once again pointing to the fact that the metabolism of Zn EDTA appears to be different in several ways.

The survival of all sheep which received the single zinc sulphate dose by intraruminal intubation is in marked contrast to what would happen if the zinc was given by drenching gun. The channelling of zinc into the abomasum by the reticular groove results in the death of sheep at doses even lower than the lowest used in these experiments (Smith *et al.*, 1979). In the MDE abomasal lesions were present in the sulphate-dosed group but this was not unexpected with such high dose rates of zinc sulphate being administered for four weeks. The group receiving zinc oxide were found to have slightly more pancreatic damage than the sulphate-dosed group. This may be because animals in the sulphate-dosed group died before the pancreatic lesions could develop properly. However in the case of zinc sulphate it does illustrate the lesser importance of the pancreatic lesion as a factor contributing to ill health and emphasises the importance of other lesions, e.g. abomasitis. Nevertheless there was a lesser but significant reduction in body weight gain in the zinc oxide-dosed group which may more fairly reflect the effect of the pancreatic damage as this was the only pathological change in this group of animals.

4.5 THE EFFECT OF ADDING ZINC TO THE MILK FED TO SUCKLING-LAMBS

Introduction

It is known that young animals absorb greater amounts of zinc than adults (Miller and Cragle, 1965) but the ability to absorb zinc has not been investigated in experiments designed to test the toxicity of zinc salts in very young animals.

In a pilot experiment kidney lesions were observed in a young lamb bottle fed with milk to which zinc sulphate had been added to give a zinc concentration of 200 mg Zn/ℓ milk. Similar lesions have been observed by Davies *et al.* (1977) where lambs consumed a similar amount of zinc.

Because this lesion is different to that observed in adult animals and occurred at calculated dose rates of zinc expected to be relatively safe in adult sheep when dosed over a similar length of time it was decided that a more extensive investigation of zinc toxicity in suckling lambs should be carried out. This experiment examines the toxic effect of two different salts of zinc and the sulphate ion in young lambs.

Materials and Methods

Thirty 2 to 6 day old Romney male and female lambs were obtained from a synchronised lambing trial at Ruakura, after they had received at least two days of colostrum. The lambs were identified, weighed and allotted to one of five groups for treatment. All lambs were fed reconstituted 'Anlamb' for three days followed by 'Ancalf' (both balanced milk diets; N.Z. Co-op Dairy Co. Ltd) for the remainder of the 20 week experimental period. 'Ancalf' milk powder contained 72.6 µg Zn/g. To the milk of each group of six lambs was added a stock solution containing a zinc and/or sulphate source to give the different treatments. The salts were added to water so that one ml of stock added to 99 ml milk gave the correct concentration of zinc or sulphate. The daily milk intake of all lambs was restricted to that

of the lambs drinking the lowest amount of milk. The experimental design and final concentrations of zinc and sulphate in milk are shown below.

Experimental design and concentrations of zinc and sulphate in the milk treatments of groups.

Group (n=6)	Stock g/ℓ	Final conc. milk Zn mg/ℓ	SO_4
1. $ZnSO_4.7H_2O$	88	200	293
2. $Zn(OAC)_2 2H_2O$	67.2	200	0
3. Na_2SO_4 (anhyd)	43.4	0	293
4. $ZnSO_4$ (+Se*)	88	200	293
5. Control	Nil	0	0

*Selenium was injected into the muscle of all lambs of group 4 on day 0 (start of treatments) and at four weeks after the start of treatments. A sterilised aqueous solution of sodium selenite was injected so that each lamb received 1 mg Se per dose.

The additions of stock solutions to the milk continued for 10 weeks at which time the lambs were slaughtered and a postmortem examination performed. Visceral organs were weighed and samples taken for zinc analysis (Appendix A) and light microscopic examination of H and E stained sections (Appendix B).

During the experiment the lambs were weighed weekly and blood samples taken for serum zinc and selenium analysis every two weeks. At the end of the experiment all lambs were bled and blood samples examined for haematocrit, haemoblobin concentration and total red cell count (Appendix D).

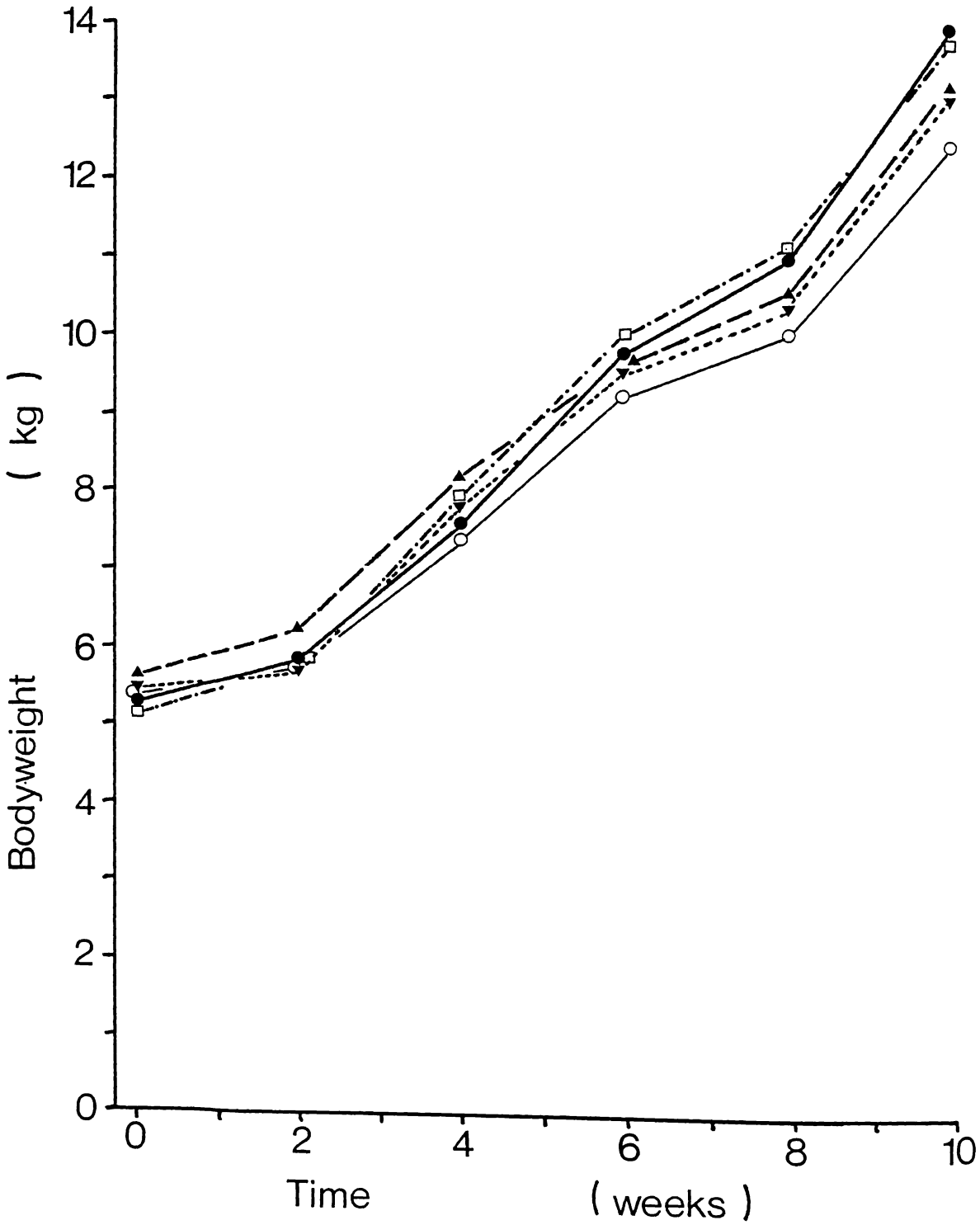


Figure 4.7 : Bodyweight changes in suckling-lambs receiving added zinc or sulphate in their bottled milk for 10 weeks. Control ● —— ●; zinc sulphate ▼ ---- ▼; zinc acetate o —— o; sodium sulphate □- - -□; zinc sulphate and parenteral selenium ▲— — ▲.

Results

One early death not related to treatment occurred in the zinc plus selenium group and this lamb and its related data were removed from the experiment.

There was a slight effect of treatment on body weight (Fig 4.7) with all lambs which received zinc having a smaller growth rate ($p<0.01$ at 10 weeks; no Zn vs Zn, excluding Se group) than the control or sodium sulphate group lambs. The zinc dosed lambs gained at the rate of 102 g/d and the non-zinc lambs at 119 g/d over the experimental period.

Serum zinc concentrations (Fig 4.8) rose from predosing levels of 1.71±0.41 SD µg Zn/ml for all groups to between 6 and 10 µg Zn/ml for the three zinc dosed groups at 14 and 28 days after the start of zinc supplementation. Thereafter the concentration in all zinc dosed groups declined to approximately 2.5 µg/ml at 10 weeks after the start of the experiment.

At the conclusion of the experiment haematology parameters were slightly lower in the zinc groups than the non-zinc groups. Excluding the zinc plus selenium group the statistical significance of the zinc versus no zinc data was $p<0.05$; <0.05 and <0.01 for haematocrit, haemoglobin concentration and total red cell values respectively. The mean values for non-zinc groups were 31.5%, 10.3 g/100 ml and 8.0×10^6/µl and for the zinc groups (excluding the zinc plus selenium group) were 29.4%, 9.6 g/100 ml and 7.2×10^6/µl for haematocrit, haemoglobin concentration and red cell counts respectively.

Kidney weights at postmortem examination are recorded in Table 4.11. The most significant change was in kidney weights. All lambs of the zinc groups had much greater kidney weights (101 g ± 16.0 SD) than non-dosed lambs (62 g ± 6.4; $p<0.001$). Thymus weights were low in those lambs with the greatest pancreatic damage but although the pancreatic weight in these lambs was also low the group means for either thymus or pancreas weight were not significantly lower than those of the control lambs. Lesions were present in the pancreata and kidneys

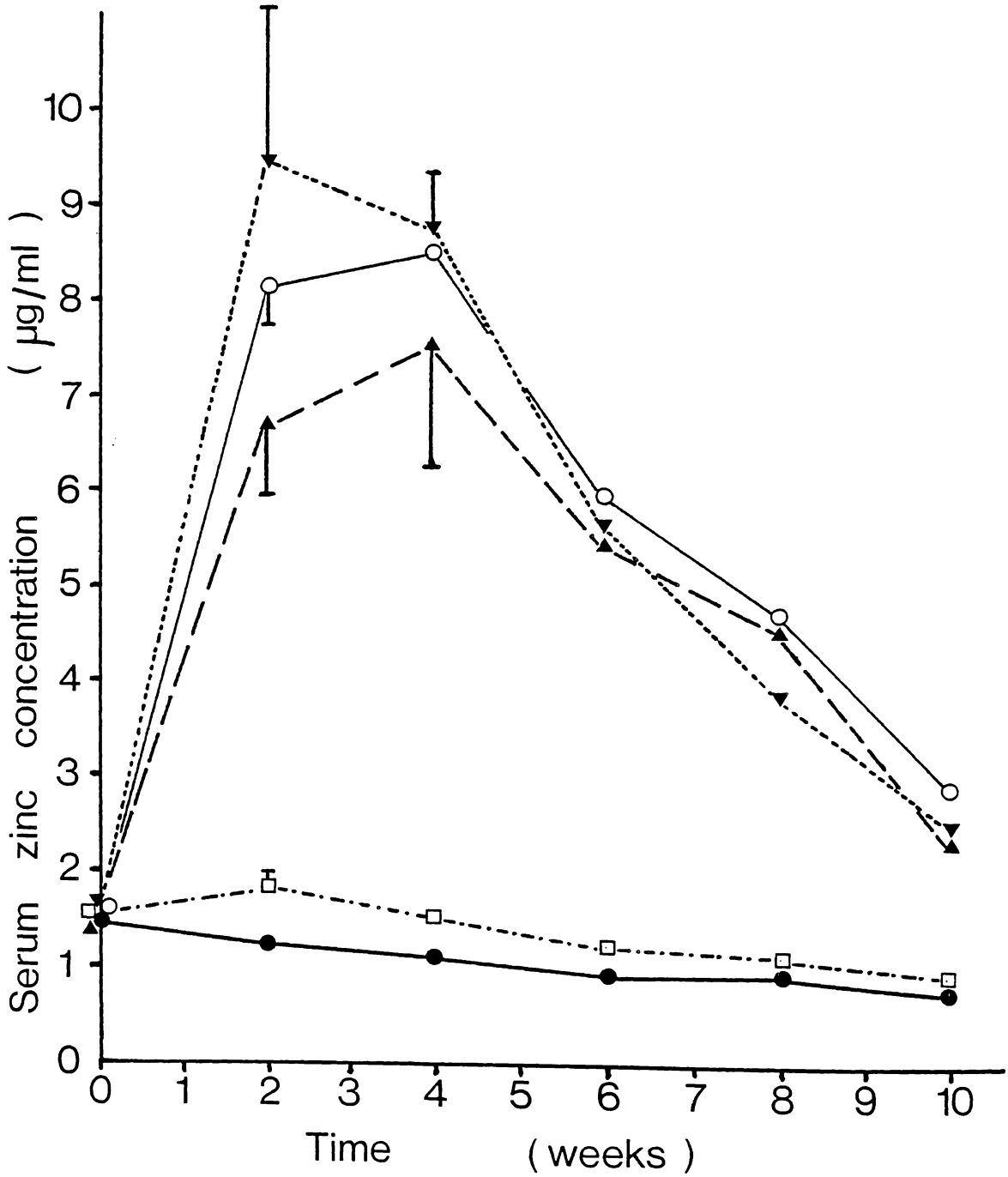


Figure 4.8 : Serum zinc concentrations (group means ±SEM) in suckling lambs receiving added zinc or sulphate in their bottled milk for 10 weeks. Control ● —— ●; zinc sulphate ▼---- ▼; zinc acetate o —— o; sodium sulphate □-.-.-.□; zinc sulphate and parenteral selenium ▲ — — ▲

Table 4.11 Effect of zinc and sulphate in the milk* of bottle fed lambs on kidney weight and organ lesions.

Treatment	Kidney wt (g)/ kg b.wt ±SD	Kidney lesions	Pancreas damage score mean	range
Zinc sulphate	7.1±0.49	$^{6}/_{6}$	2.8	7-0
Zinc acetate	7.0±0.74	$^{5}/_{6}$	3.8	8-0
Zinc sulphate plus parenteral Se	7.8±1.8	$^{4}/_{5}$	3.6	6-1
Sodium sulphate	4.4±0.39	$^{0}/_{6}$	0	0
Control	4.2±0.27	$^{0}/_{6}$	0	0

* Zinc treatments contained 200 mg added Zn/ℓ;
sulphate treatments contained 293 mg added SO_4/ℓ.

Table 4.12 Effect of zinc and sulphate in the milk* of bottle fed lambs on group mean organ zinc concentrations.

	Organ zinc concentration (µg/g DMB) ±SD				
Treatments	Liver	Pancreas	Kidney	Spleen	Vastus medialis
Zinc sulphate	1180 ±376	1399 ±806	6454 ±2512	116 ±10.0	144 ±23.4
Zinc acetate	1132 ±491	1652 ±985	8387 ±1647	107 ±7.2	149 ±83
Zinc sulphate plus parenteral Se	1280 ±519	1700 ±595	6252 ±2032	120 ±2.4	167 ±19.3
Sodium sulphate	143 ±20.4	99 ±15.5	104 ±9.8	105 ±9.4	143 ±41.6
Control	146 ±17.9	99 ±11.2	105 ±11.0	106 ±7.9	189 ±58

*Zinc treatments contained 200 mg added Zn/ℓ; sulphate treatments contained 293 mg added SO_4/ℓ.

of the zinc dosed lambs (Table 4.11) and a complete description of these changes is included in the section on the pathology of zinc toxicity (Chapter 5.1.2 and 5.1.4).

Organ zinc concentrations (Table 4.12) were elevated in all groups which received additional zinc. The most significant rise in organ zinc was in the kidneys of these lambs. The mean kidney zinc concentrations were approximately four to five times those of pancreas in the zinc supplemented groups. Concentrations of up to 10 530 µg Zn/g were recorded from the kidneys of the zinc supplemented lambs.

Discussion

From the zinc concentrations in milk, the intake of milk and the body weights at the conclusion of the experiment the mean daily zinc dose rate was calculated to be about 20 mg Zn/kg b.wt. This was a high dose rate, but not higher than that found from previous experiments (Smith *et al.*, 1977a) to prevent pithomycotoxicosis in adult sheep and certainly lower than dose rates of zinc which would cause zinc toxicity in mature sheep after 10 weeks of dosing.

Predosing serum zinc concentrations were high (1.71±0.41 SD µg Zn/ml) compared with those experienced in adult sheep in previous experiments (Chapters 3.1.2, 4.2 and 4.4). These concentrations declined in the controls throughout the 10 weeks of the experiment to concentrations (0.83±0.13 µg Zn/ml) (Fig 4.8) similar to those expected from previous experiments using adult sheep. In the zinc supplemented lambs the serum zinc concentrations rose to very high levels (>6 µg Zn/ml) at two and four weeks after the start of supplementation and then steadily declined over the remaining weeks of the experiment despite the continuing administration of the zinc supplemented milk. This and the decline observed in the control lambs suggests that some homeostatic mechanism was developing as the lambs matured. This early lack of homeostasis appears to be a feature of zinc metabolism in young animals (Strain *et al.*, 1978; Miller *et al.*, 1968). Whether or not this decline in serum zinc concentrations after four weeks in this experiment was due to a change in intestinal absorption or some other mechanism such as

kidney secretion is not known. It is known that zinc absorption from the intestine is greater in young animals (Miller and Cragle, 1965). In this experiment there was a much greater accumulation of zinc in the kidney than previously described in adult sheep despite the presence of higher serum zinc concentrations in the older sheep (Chapters 3.1.1, 3.1.2 and 4.2), a difference which could be due either to nutrition (grass versus milk) or to an age effect. The high kidney concentrations of zinc have also been recorded in lambs fed high zinc supplemented milk for only 33 days (Davies *et al.*, 1977). They also showed that the high kidney zinc levels were also present two weeks after the zinc supplemented diet had been suspended at 33 days and that the zinc concentration was much higher in the cortex than medulla of the kidney.

In the present experiment both severe pancreatic and kidney lesions were observed at postmortem and subsequent microscopic examination of tissues. The kidney lesions and high zinc concentrations have not been observed before in this series of experiments. Davies *et al.* (1977) observed the kidney lesions but not those in the pancreas. It seems likely that both were related to the high serum zinc concentrations but that the kidney lesions are also related to the immaturity of the lambs as these have not been recorded before in older animals with zinc toxicity. Judging from the results of Davies *et al.* (1977) and our serum levels it seems likely that the high zinc concentrations and lesions in the kidney may have been well developed in the first half of the experiment.

Despite the lesions in both kidney and pancreas in these lambs the body weight gains in the zinc supplemented groups were not so different from controls as might have been expected from the results of Davies *et al.* (1977). They recorded daily weight gains in the zinc supplemented lambs much less (approximately 40-45%) than those of controls. This may have been because of differences between the two experiments in the milk substitute used which was reconstituted dried milk in their experiment and commercial milk replacers in our experiment. The 'Ancalf' contained added zinc.

These results are of little relevance to the use of zinc for the control of pithomycotoxicosis as the disease does not occur in lambs of this age. However milk substitutes containing excessive zinc may present a danger to lambs fed such diets as was experienced by Davies *et al.* (1977). The highest zinc concentration in bovine milk recorded in these experiments was 27 µg Zn/ml (Chapter 3.2.1 Exp. B) from a cow suffering from zinc sulphate toxicity. This was much less than the zinc concentration of milk in this experiment (200 µg Zn/ml). It would seem unlikely that lambs or calves would suffer secondary zinc toxicity from their dams milk.

The enthusiastic use of zinc as a 'cure all' by farmers has been extended to young ruminants (J. Graham personal communication) and the greater danger of zinc toxicity in young animals should be appreciated. In addition it has been shown that the inherited disorders of zinc absorption (in man, acrodermatitis enteropathica; in cattle, Adema disease) can be treated successfully with luxus amounts of zinc salts. The susceptibility of calves or suckling-infants to zinc toxicity may be of importance in this context.

4.6 THE EFFECT OF ZINC DOSING ON SELENIUM METABOLISM IN SHEEP AND AN INVESTIGATION ON THE POSSIBLE ROLE OF ZINC INDUCED SELENIUM DEFICIENCY IN THE PATHOGENESIS OF ZINC TOXICITY IN THE SUCKING LAMB

Introduction

The main pathological manifestation of zinc toxicity is a degeneration, fibrosis and atrophy of the pancreas. This has been recorded in the rat (Veghelgi *et al.*, 1952) cats and dogs (Drinker *et al.*, 1927; Scott and Fisher, 1938) and sheep (Ott *et al.*, 1966a; Smith, 1977). It has also been shown that atrophy of the pancreas has been caused by diets low in selenium in the chick (Thompson and Scott, 1970; Cantor *et al.*, 1975) and mouse (De Witt and Schwarz, 1958). There is also evidence of some interdependence or association of zinc and selenium in the biological situation (Schicka *et al.*, 1972; Masson and Young, 1967). In addition, muscular dystrophy and exudative

diathesis, both manifestations of selenium deficiency have been produced in chickens by feeding diets containing high concentrations of zinc (Jensen, 1975). However in that experiment both zinc and copper were used as the sulphate and the effects described could have been due to the sulphate rather than or in addition to either zinc or copper. Sulphate is well known to affect selenium metabolism in animals (Ganther and Baumann, 1962; Hintz and Hogue, 1964; Whanger *et al.*, 1969). Therefore in the case of zinc sulphate the effects of both zinc and sulphate need to be differentiated.

Because of the above evidence it seemed possible that a zinc induced selenium deficiency could occur in sheep and even contribute to the lesions of zinc toxicity. If so, the possible prevention of some aspects of zinc toxicity (e.g. pancreatic lesions) by selenium supplementation would be of considerable interest and value.

Because selenium deficiency and its manifestations (Underwood, 1977) and lack of zinc homeostasis (Miller and Cragle, 1965; Strain *et al.*, 1978; Chapter 4.5) occur most readily in young animals the suckling-lamb was chosen as the experimental animal in the experiments described here.

This section records investigations on the effect of zinc dosing on blood, serum and tissue selenium concentration and also on the role of selenium in the pathogenesis of zinc toxicity in young lambs.

Materials and Methods

Whole blood and organ selenium concentration in adult sheep subjected to long term zinc administration Liver and pancreas tissue samples were obtained from groups of eight 2 year old sheep that had been subjected to approximately 13 months dosing with zinc sulphate or zinc oxide (30 mg Zn/kg/d for six days of each week). The zinc oxide dosed sheep and control sheep were slaughtered at the end of dosing. Organs from the zinc sulphate-dosed sheep were either collected at that time (three sheep) or at postmortem examination after death. The details of this experiment may be seen in Materials and Methods of Chapter 3.1.2.

Tissue samples were cut and weighed, heated to 103 ^{o}C for 16 h re-weighed and selenium analyses performed (Watkinson, 1979).

For whole blood selenium analyses 0.5 ml of whole blood was lysed in 2.0 ml of distilled water and subjected to selenium analysis (Watkinson, 1979). All selenium analyses were performed in the laboratory of Dr J. Watkinson, Ruakura Agricultural Research Centre using a semi-automated fluorimetric method.

Pilot investigation in sucking lambs A pair of six day old twin lambs (one male and one female) which had been born prematurely (approximately 10 days) were bottle fed *ad libitum* with fresh cows milk (ex Ruakura No. 4 dairy). Zinc sulphate stock solution (1 ml in 99 ml of milk) was added to the milk fed to the male lamb to give a final concentration of 200 mg of added zinc per litre of milk. Both lambs were fed four to five times per day for four weeks after which time both were slaughtered and a postmortem examination conducted on each lamb and organ samples taken for histological examination and zinc and selenium analyses. A serum sample was also taken from each lamb at the end of the experiment for selenium analysis (Watkinson, 1979).

The effect of zinc and sulphate in the milk fed to sucking lambs on selenium metabolism, and the possible effect of parenteral selenium in alleviating the effects of zinc toxicity Thirty 2 to 6 day old male and female Romney lambs were bottle fed with reconstituted milk replacer ('Anlamb' for three days followed by 'Ancalf', both N.Z. Co-op Dairy Co. Ltd) to which zinc or sulphate salts had been added to give the concentrations indicated as follows:

Group	Salt	Concentration of Zn or SO_4 mg/ℓ milk	
$ZnSO_4$	Zinc sulphate	Zn 200;	SO_4 293
$Zn(OAc)_2$	Zinc acetate	Zn 200	-
Na_2SO_4	Sodium sulphate	-	SO_4 293
Zn+Se	Zinc sulphate*	Zn 200;	SO_4 293
Control	Control (no additives)	-	-

*Parenteral selenium injected 1 mg Se/lamb at days 0 and 28 as a sterile sodium selenite solution. Ancalf contained added zinc and the powder contained 72.6 µg Zn/g.

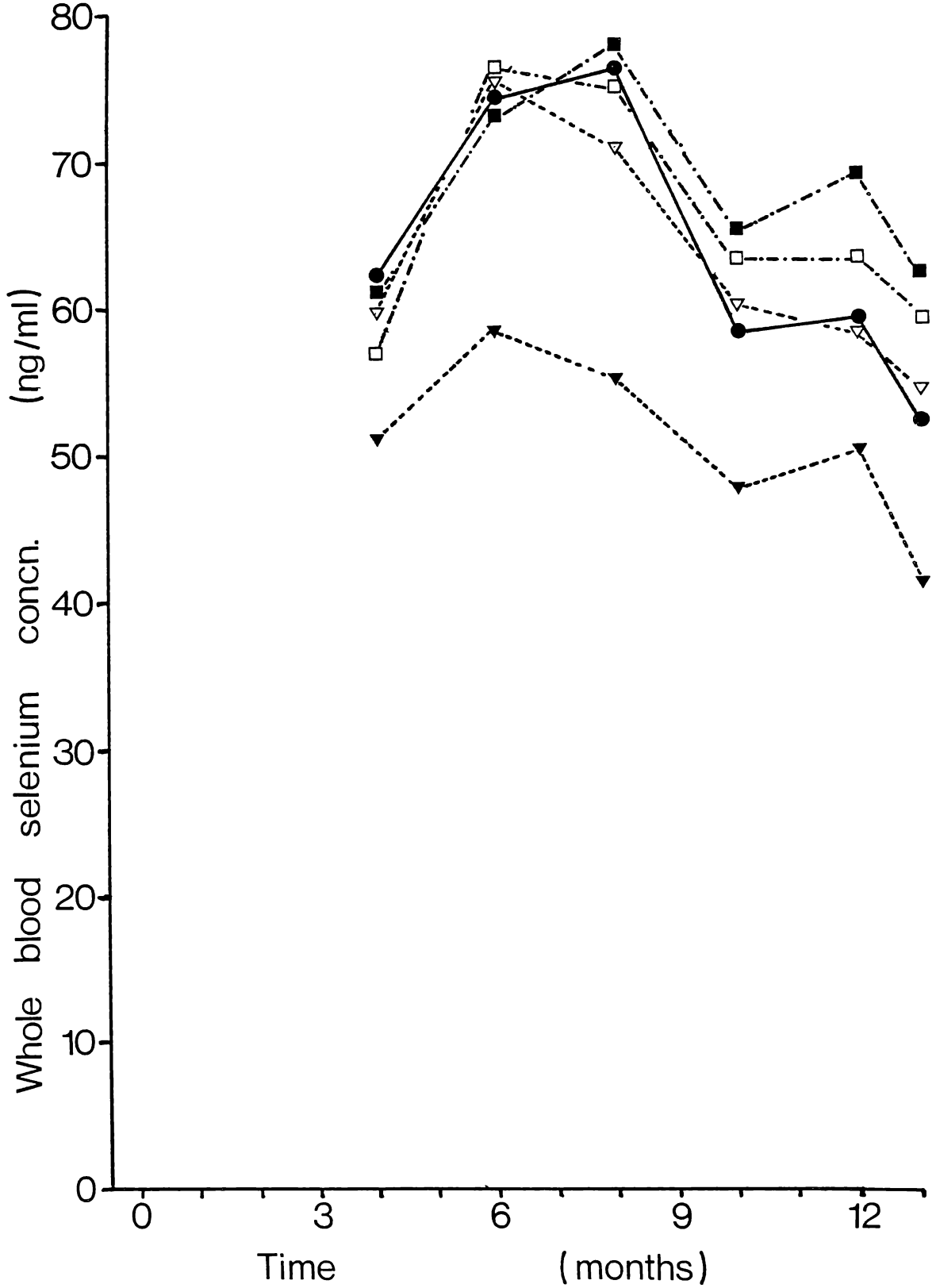


Figure 4.9 : Effect of long term dosing of adult sheep with zinc salts on whole blood selenium concentrations. Control ● —— ●; zinc sulphate, 15 mg Zn/kg/d ▽ .... ▽; zinc sulphate, 30 mg Zn/kg/d ▼ ---- ▼; zinc oxide 15 mg Zn/kg/d □-.-.-.□; zinc oxide, 30 mg Zn/kg/d ■-.-.-■ .

The treatments continued for 10 weeks at which time the lambs were slaughtered. Postmortem examinations were conducted and tissues were taken for zinc and selenium analyses. Serum selenium concentrations were measured in samples collected at two weekly intervals throughout the experiment. A complete description of Materials and Methods is given in the Materials and Methods section of Chapter 4.5.

Results

Adult sheep experiment Whole blood selenium concentrations for all groups are given in Figure 4.9. As the experiment progressed, the high sulphate group developed a lower whole blood selenium concentration than did controls. The difference from controls was apparent at four months and significantly different ($p<0.05$) at six months after the start of dosing. After six months the mean selenium concentrations in whole blood for the zinc oxide groups became greater than those for controls. The difference between the means for high and low zinc oxide groups combined was significantly greater ($p<0.05$) than the control mean at the end of the experiment.

The selenium concentration (WMB)* for liver and pancreas of the control, high oxide and high sulphate groups are given in Table 4.13. The mean selenium levels for both liver and pancreas of the high sulphate group were lower than those of controls, the mean pancreatic selenium concentration of the sulphate group being significantly ($p<0.01$) lower than that of controls.

A comparison of the selenium values for pancreas and liver of the high sulphate group together with the pancreatic damage and occurrence of death in the sheep (Table 4.14) shows that the animals which died with considerable pancreatic damage had the lowest selenium values in pancreas. The liver selenium concentrations when compared with the occurrence of pancreatic damage and death did not appear to be correlated.

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*All Se tissue values expressed on wet matter basis (WMB) as method involves wet ashing procedure

Table 4.13 Effect of daily dosing of sheep with zinc salts on organ selenium concentration.

	Mean selenium concentration ng/g WMB ±SD	
Treatment	Liver	Pancreas
Control	81.3±11.1	131±29.5
Oxide*	88.0± 6.4	131±27.8
Sulphate*	68.3±17.8	74.9**±33.4

*Both treatments 30 mg Zn/kg body weight/d for approx. 13 months or less in case of sulphate-dosed sheep which died before end of experiment;

**statistically significant difference from controls $P<0.01$.

WMB, wet matter basis.

Table 4.14 Comparison of incidence of deaths, pancreatic injury scores and pancreas and liver selenium concentrations (means ±SD) in eight sheep dosed daily with 30 mg Zn (as $ZnSO_4.7H_2O$)/kg b.wt/d with seven undosed control sheep.

Sheep No.	Death	Pancreatic Injury Score	Selenium Concentration (ng/g) Pancreas	Liver
1	-	0	111	82
2	-	0	120	58
3	-	1	94	64
4	+	3	45	81
5	+	4	68	91
6	+	6	47	42
7	+	8	39	52
8	+	9	NA	68
Controls (n=7)	-	0	81±11.1	131±29.6

NA; not available WMB; wet matter basis

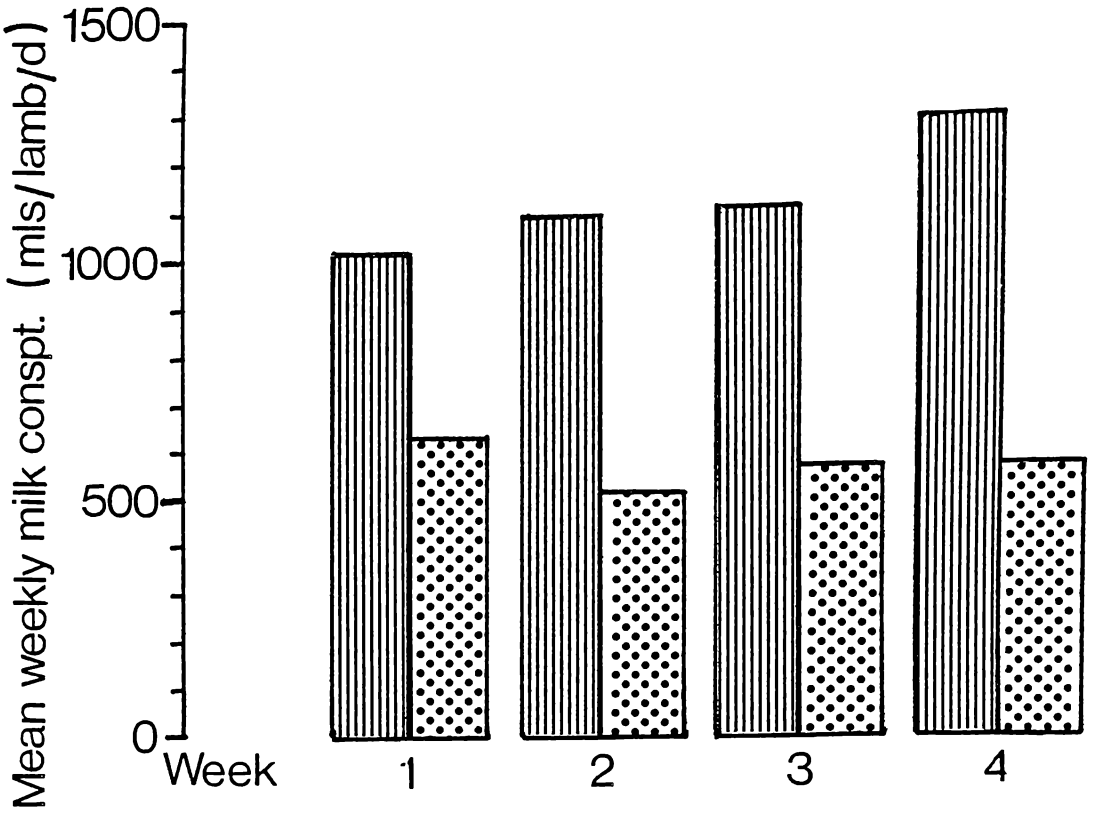


Figure 4.10 : Weekly mean consumption of milk (ml/lamb/day) for twin pair of lambs, one of which received 200 mg of added zinc (as sulphate) per litre of cows milk. Control ▥, zinc sulphate ⁘.

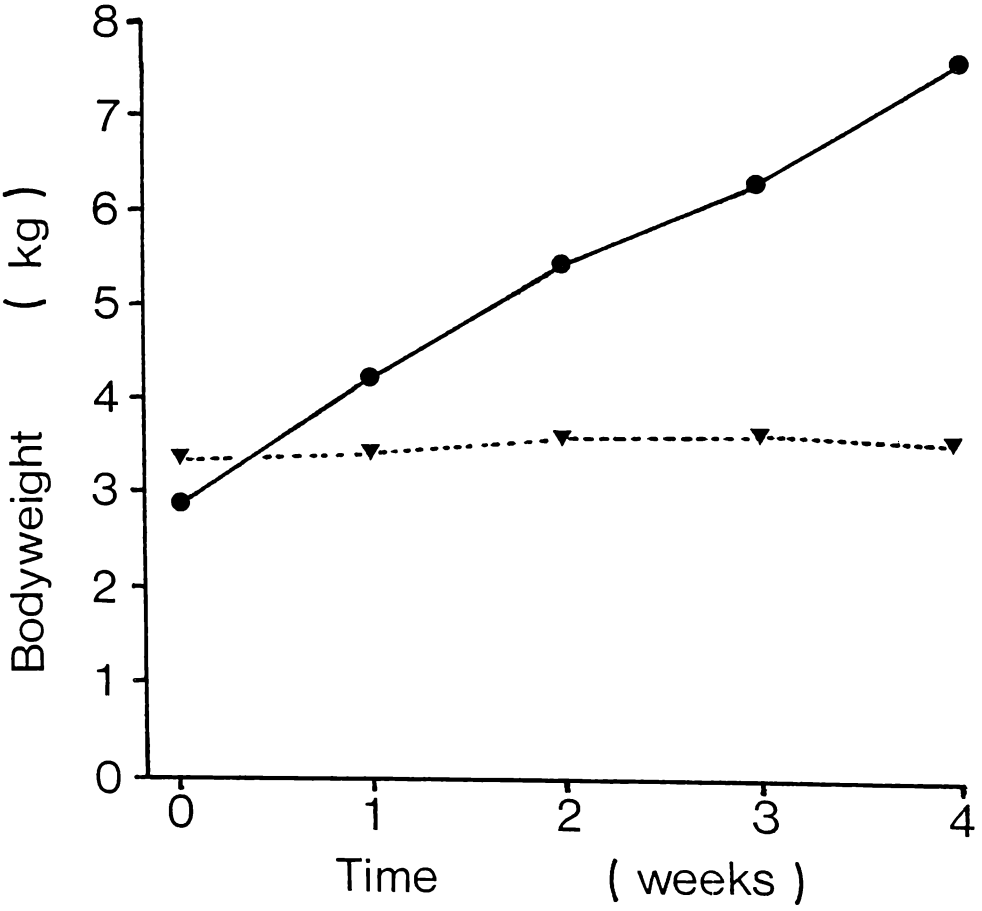


Figure 4.11 : Bodyweight changes in twin pair of lambs, one of which received 200 mg of added zinc (as sulphate) per litre of cows milk. Control ● —— ●; zinc sulphate added ▼ ---- ▼.

Pilot investigation in sucking lambs The zinc-fed lamb consumed less milk (Fig 4.10), failed to thrive (Fig 4.11) and was much weaker than its twin, at the conclusion of the experiment. Serum selenium concentrations at the end of the experiment were 12 and 7 ppb for the control and zinc supplemented lambs respectively. For the zinc dosed lamb the selenium concentrations (ng/g WMB) were for liver 94, kidney 110, pancreas 59, longissimus dorsi 43, adrenal 267, and spleen 105 at the conclusion of the experiment.

At postmortem examination there were no lesions present in the control lamb. The zinc supplemented lamb was found to have lesions characteristic of zinc toxicity in the pancreas (Chapter 5.1.2) However it also had grossly visible severe lesions of the skeletal muscle [longissimus dorsi, biceps femoris and quadriceps] and cardiac muscle [chalky white subendocardial plaques of both left and to a lesser extent right ventricles (Plate 5.45)]. The microscopic lesions of the skeletal muscle and heart were typical of those described for white muscle disease. In addition the kidneys were enlarged (73 g), pale and distorted (Plate 5.35). A description of the lesions is given in the section on the pathology of zinc toxicity (Chapter 5).

Zinc, sulphate and selenium in sucking lambs The effects of the different treatments on the health and organ pathology of the lambs, organ and serum zinc concentrations and aspects not related to selenium are given in the section on pathology (Chapter 5.1.4) and the section on zinc toxicity in sucking lambs (Chapter 4.5). No lesions of white muscle disease were present.

The injection of selenium into the lambs of one group which received zinc sulphate had no effect in alleviating either the kidney or pancreatic lesions which were present in approximately equal prevalence and severity in all three groups which received zinc (Table 4.11).

An increase in serum selenium concentrations occurred in all lambs over the first 28 days of the dosing period and declined thereafter. As this occurred a difference between the serum selenium concentrations of the zinc supplemented lambs and the non-zinc lambs emerged (Fig 4.12). This difference in serum selenium between zinc and non-zinc

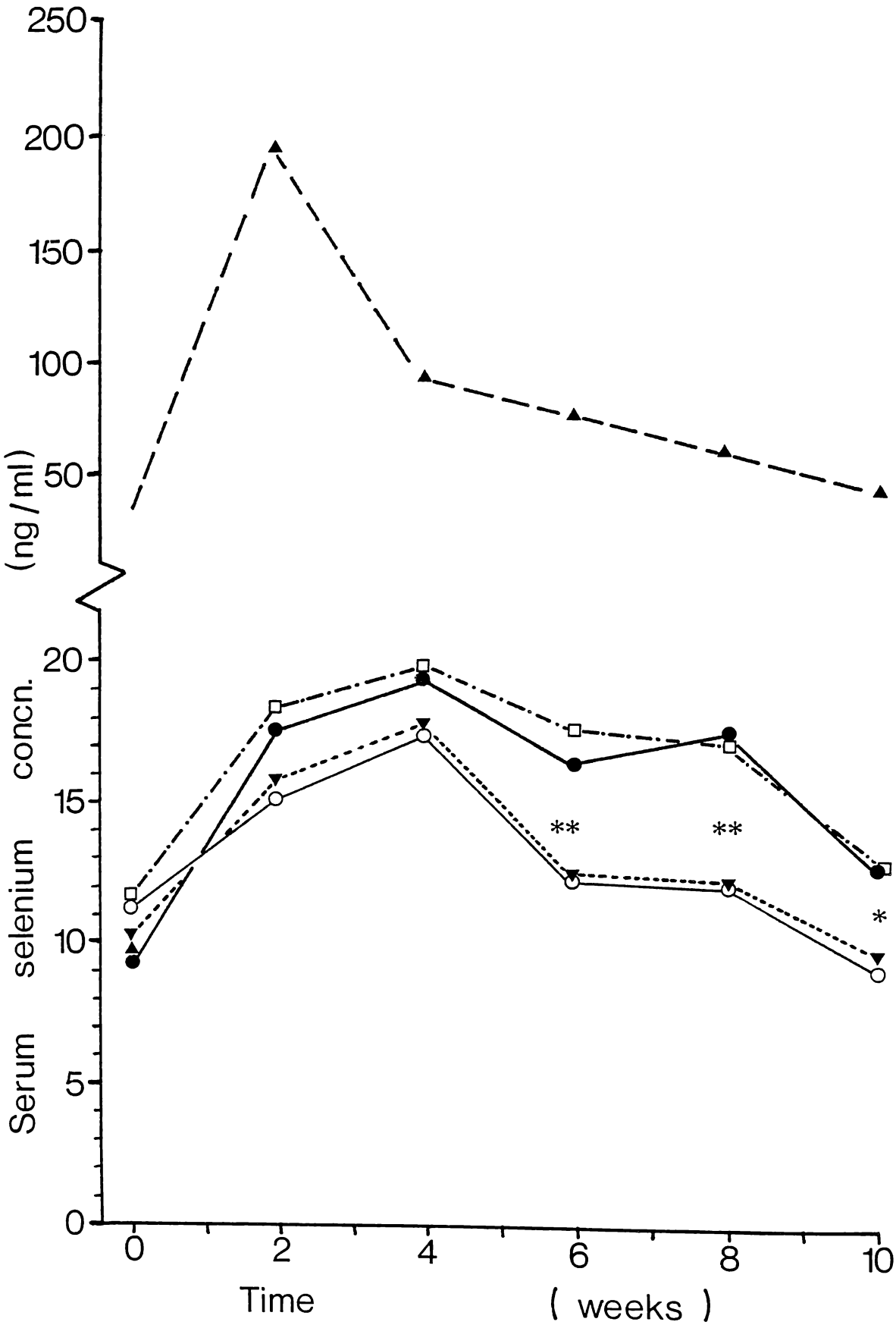


Figure 4.12 : Effect of zinc and sulphate in the milk of bottle fed lambs on serum selenium concentrations. Control ● —— ●; zinc sulphate ▼—— ▼; zinc acetate o —— o; sodium sulphate □-.-.-□; zinc sulphate and parenteral Se ▲ ----▲; ** $p<0.01$; * $p<0.05$.

Table 4.15 Mean selenium concentrations in organs of lambs receiving zinc and sulphate in bottle fed milk for ten weeks.

Treatment	Selenium concentration ng/g WMB				
	Liver	Kidney	Pancreas	Spleen	Muscle
Zinc sulphate	65.3	467	61	70	17.3
Zinc acetate	53.0	379	54	81	16.3
Zinc sulphate plus parenteral selenium	282	943	189	210	66.6
Sodium sulphate	62.2	601	79	86	14.5
Control	72.8	642	76	87	15.1
Pooled SD	16.5	108	12.9	11.7	2.1
Significance (excluding +Se group)					
Zn vs No Zn	NS	<0.001	<0.01	<0.05	<0.05
SO_4 vs No SO_4	NS	NS	NS	NS	NS

SD; standard deviation. NS; not significant
WMB; wet matter basis

lambs (excluding selenium injected lambs) was highly significant ($p<0.01$) at 42 days and stayed significant for the remainder of the experiment.

Organ selenium concentrations are given in Table 4.15. Zinc supplementation appeared to have significantly lowered the selenium concentration of kidney ($p<0.001$) and pancreas ($p<0.01$) compared with those of controls and sodium sulphate-dosed groups. The highest concentration of selenium in all groups was in kidney and the lowest was in muscle.

Discussion

The results of these three separate experiments all indicate that zinc interferes in the selenium metabolism of both young and adult sheep.

In the long-term zinc-dosing experiment, the high zinc sulphate dose rate caused a significant major depression of whole blood selenium whereas the zinc oxide group had a slight but significant elevation of whole blood selenium. The reason for the depression of whole blood selenium in the case of zinc sulphate is unknown but could be due to either zinc or sulphate. The depression caused by zinc sulphate was also present in the selenium concentrations of liver and pancreas. However in the case of the pancreas in which the difference is greatest and statistically highly significant a possible reason for the difference is provided by an examination of the pathological findings. Those animals with particularly lower pancreatic selenium concentrations have also pancreatic lesions. One of the most significant features of the pathological change in the pancreas is a change in the predominant tissue type from exocrine parenchyma to fibrous tissue infiltration of the organ. This is an alternative explanation for the very low pancreatic selenium concentration rather than it being due to the direct effect of zinc in selenium metabolism. The slight elevation of serum selenium concentrations in the zinc oxide group is of unknown significance. It possibly signifies a shift of selenium from an organ to serum.

In the experiments using sucking lambs there was further evidence that zinc interferes with selenium metabolism. In the pilot experiment the lesions of white muscle disease (a selenium responsive disease due to low selenium in the diet) and low serum selenium concentrations occurred in the lamb which had zinc sulphate added to its milk.

In the main experiment with suckling lambs there was a definite effect of zinc on selenium content of serum and certain organs and the effect of the different treatments showed clearly that the effect on selenium was due to zinc and not sulphate. Likewise it was shown that the pathological lesions of the kidney and pancreas were clearly due to zinc and not sulphate. Furthermore these effects of zinc on the different organs were not alleviated in any way by parenteral selenium administration suggesting that these lesions were not caused by a zinc induced selenium deficiency. The findings of the main sucking lamb experiment also suggest that the selenium depressing effect of zinc sulphate (cf. lack of effect with zinc oxide) in the long term zinc dosing experiment was due to some characteristic of *zinc* sulphate (e.g. solubility, astringency, effect on reticular groove) rather than a sulphate effect. However a definitive experiment in adult sheep is needed to resolve the role of zinc sulphate in causing this effect.

It must be recognised that the concentrations of zinc in the tissues of all these zinc dosed sheep were very high. In the case of lambs the greatest effect on selenium concentration was in the kidney, the organ which contained the highest concentration of zinc recorded in this series of experiments.

No lesions of white muscle disease occurred in the main experiment with suckling-lambs despite the effect of zinc on selenium metabolism. Possible reasons for this include the difference in the maturity of lambs (premature lambs were used in the pilot experiment) and the different sources of milk for the two experiments. In the pilot experiment it is recognised, of course, that the zinc treatment and results applied to only a single lamb and therefore no firm conclusions can be drawn.

4.7 EFFECTS OF INTRARUMINAL ADMINISTRATION OF ZINC ON GASTRIC ACID SECRETION IN SHEEP

Introduction

Preliminary studies have shown that zinc sulphate solutions administered by drenching gun or in large doses by intraruminal intubation caused gross lesions of the abomasal mucosa, and sometimes resulted in death (Chapter 4.2 and 5.1.1). Abomasal pouch preparations were used in these experiments to investigate the effects of zinc on abomasal secretion and to see whether the lesions resulted from direct contact of zinc with the abomasal mucosa or were a secondary effect following absorption of zinc and its subsequent secretion by the abomasum. The pouches were isolated from the abomasum proper and did not come into direct contact with administered zinc which was given intraruminally to avoid the gross damage often incurred when given by mouth.

Materials and Methods

Two Dorset Horn ewes (Sheep 1 and 2) and one Romney x Merino wether (Sheep 3) weighing 30-45 kg and fed *ad libitum* on chopped meadow hay of 85% dry matter were prepared with an isolated pouch of the body of the abomasum and a duodenal cannula situated 5 cm caudal to the pylorus. The management of the sheep, their surgical preparation and the 24 h collections of pouch secretion were as described previously (McLeay and Titchen, 1970; 1974). At 9 am daily the previous 24 h collection of secretion was measured and samples were taken of this and of duodenal contents. Estimates of titratable acidity of secretion by electrometric titration to pH 7 and measurement of duodenal pH were made by pH meter (Radiometer, Copenhagen).

Zinc was administered by intraruminal intubation as a 22% aqueous solution of $ZnSO_4$ $7H_2O$ at pH 5.3 in doses of 100 or 200 mg Zn/kg body weight on three occasions in sheep 1 and 2 and on four occasions in sheep 3 over a period of 24-30 days. Successive doses were given when the response to the previous dose was judged to have ceased. The 200 mg Zn/kg was given only as the last dose in each animal.

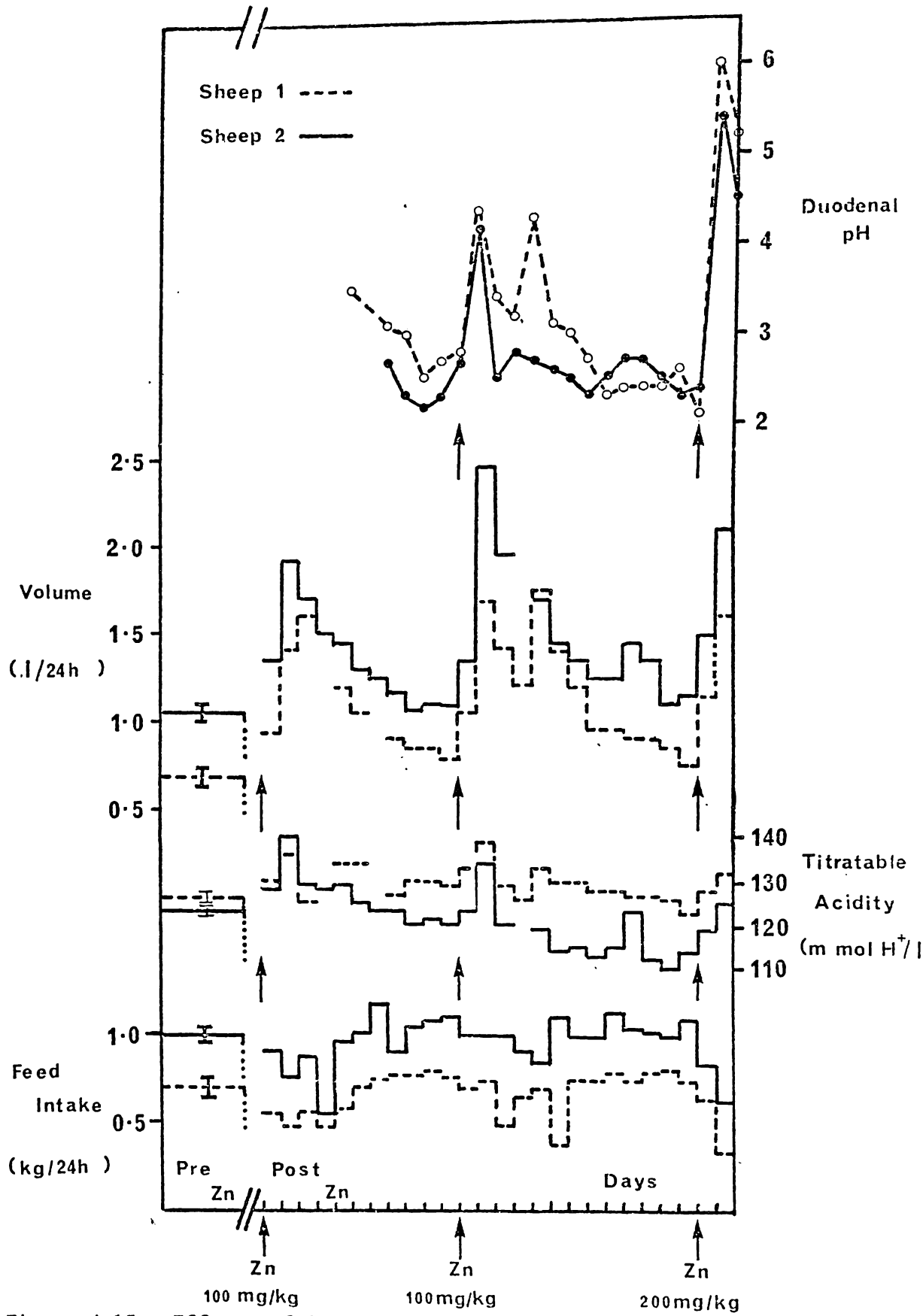


Figure 4.13 : Effects of intraruminal administration of zinc sulphate solution (100 and 200 mg Zn/kg) on duodenal pH, volume of abomasal pouch secretion and feed intake (wet matter) of sheep 1 (----) and 2 (——). Daily control measurements obtained before the administration of zinc (indicated by ↑) are given as the mean ±SEM (n=9 for sheep 1 and n=8 for sheep 2).

In sheep 1 and 2 blood samples were obtained by puncture of the jugular vein at 0, 7, 10, 24 and 48 h following dosing. The concentration of zinc in serum and gastric pouch secretion were determined directly by atomic absorption spectrophotometry (AAS) (Varian Techtron 1200) on diluted serum and undiluted gastric secretion samples. Duodenal contents were prepared as for tissue samples (Appendix A).

Before the administration of zinc to the sheep, control 24 h observations on food intake, volume of pouch secretion and its H^+ and Zn^{2+} concentrations were made for seven to nine days. In sheep 3, control observations were made on duodenal pH before, but in sheep 1 and 2 duodenal contents were sampled after the first dose of zinc. On some days collections of pouch secretion were incomplete and data concerning these have been omitted. Sheep 1 and 2 were killed on the second day and sheep 3 on the ninth day following dosing with 200 mg Zn/kg.

Results

Abomasal pouch secretion and duodenal pH Intraruminal administration of zinc at 100 mg/kg increased the daily volume of pouch secretion and its acidity so that the maximal acid output (volume x titratable acidity) was more than twice the average control level. Increases were observed in the first 24 h and reached their maxima after two to five days. Thereafter secretion gradually declined and by eight to ten days was near control levels. The increases in acid output were accompanied by reductions in food intake (Fig 4.13).

The pH of the duodenal contents increased after zinc administration reaching a maximum in all but one case within 24 h of dosing. Predosing levels were 2.6-3.5 and the maximal increase was to 6.0. The pH then declined rapidly towards the predosing levels within two or three days, but in sheep 1 and 3 a second increase occurred four days after dosing.

Doses of 200 mg Zn/kg produced similar increases in pouch secretion, greater increases in duodenal pH and greater reductions in food intake than 100 mg Zn/kg. The responses to 100 and 200 mg Zn/kg in sheep 1

and 2 are shown in Figure 4.13. Sheep 3 showed responses similar in nature but lesser in degree; in one case there was no increase in pouch secretion or duodenal pH following a third dose of 100 mg Zn/kg.

Zinc in abomasal pouch secretion Attempts to monitor the concentration of zinc in the pouch secretions were marred in sheep 2 and 3 by zinc contamination. Initial higher levels (1-2.5 µg/ml) gradually declined over the experimental period to levels of 0.10 µg/ml and it was discovered that zinc was being leached from newly introduced indwelling urethral catheters used to facilitate drainage of the pouches. Zinc levels in two unused catheters were 6396 and 6062 µg/g; the zinc levels in catheters taken from sheep 1 and 2 at postmortem were 143 and 86.2 µg/g respectively. The catheter in sheep 1 had been in the pouch for 10 months and zinc levels before dosing were 0.16±0.02 µg/ml (mean ± SEM n=10) and after dosing 0.08±0.01 (n=36). In all animals dosing with zinc did not increase its concentration in pouch secretion and there were similar or lesser concentrations in greater volumes of secretion. The output of zinc increased above predosing levels on only four occasions and this simply reflected the increased volume of secretion.

Zinc in serum and duodenal contents The concentration of zinc in serum increased from less than 1.15 µg/ml to 4.05 µg/ml maximum at 10 h after dosing with 100 mg Zn/kg. Zinc concentrations in duodenal contents increased from 1 to 120 µg/g (wet matter) for 100 mg Zn/kg doses and to 240 µg/g for 200 mg Zn/kg. Maximal levels were achieved within 24 h of dosing and then declined over the next four days to reach predosing levels.

Postmortem examinations Sheep 1 and 2 were killed near the height of the response to 200 mg Zn/kg and sheep 3 five days after the height of the response. No lesions attributable to zinc administration were observed at postmortem or on histological examination of the mucosa of either the abomasal pouch or the abomasum proper in all animals.

Discussion

Intraruminal administration of zinc increased abomasal pouch secretion and duodenal pH. Both were closely associated and secretion increased despite reductions in food intake. The siting of the duodenal cannulae was such that the pH of the contents sampled would be expected to be similar to that of abomasal antral contents (Harrison and Hill, 1962) and in sheep 1 and 2 at postmortem, the pH of both antral and duodenal contents was greater than 4.0. Increases in pouch secretion and abomasal pH and reductions in food intake have been demonstrated following abomasal parasitic infection (McLeay *et al.*, 1973) in which hypergastrinaemia has been shown to occur (Anderson *et al.*, 1976). Increased gastrin release resulting from raised antral (Becker *et al.*, 1973) and duodenal pH could have been responsible for the increased pouch secretion observed in the present experiments. This, however, may not be the only explanation as the increases in secretion endured longer than those in pH.

It is not known what caused the elevation in pH of the duodenal contents but it is probable that reduced abomasal secretion of HCl was the major factor. It would seem that an inhibitory effect on HCl secretion, possibly initiated by the greatly increased concentration of zinc in digesta, was local as the secretory function of the isolated pouches was certainly not impaired. Although histological examination showed little damage to the abomasal mucosa (proper) following dosing with 200 mg Zn/kg, gross lesions and elevated pH occur if this dose is administered directly to the abomasum (C. Ramberg personal communication) or given by drenching gun which appears to result in chanelling of the drench direct to the abomasum. In the present experiments effects on the acid secreting cells may have only been detected by ultrastructural examination (McLeay *et al.*, 1973).

The failure of intraruminal administration of zinc to result in increased concentrations and outputs of zinc in pouch secretion despite markedly higher concentrations in serum indicated that little of the administered zinc was secreted by the body of the abomasum. Thus abomasal lesions produced by oral dosing of zinc do not appear to be due to absorption and subsequent abomasal secretion of zinc.

4.8 EFFECT OF ZINC OXIDE ADMINISTRATION TO SHEEP ON PANCREATIC EXOCRINE SECRETION

Introduction

Although there is ample evidence that toxic doses of zinc result in pancreatic lesions, weight losses and higher feed gain ratios, the effect of the pancreatic fibrosis and atrophy alone on the health of these animals and their production efficiency parameters is unknown. Severe abomasal and upper intestinal lesions are known to cause inappetance and considerable weight loss. Zinc sulphate administration causes such lesions and the weight loss associated with zinc sulphate toxicity is most likely due to either these lesions or direct effects of soluble zinc on the gut microflora. In zinc oxide toxicity weight losses are less dramatic than in zinc sulphate toxicity and this could be due to the absence of the more severe gut lesions.

The secretory function of the zinc damaged exocrine pancreas is most likely affected. Pancreatic mass and the proportion of normal exocrine tissue remaining are both reduced to less than 25% of original in severe chronic zinc toxicity. A correlation between pancreatic mass and maximum bicarbonate output is recognised in normal dogs (Hansky *et al.*, 1964). However Tietz (1976) has stated that it is estimated that pancreatic insufficiency cannot be clearly demonstrated until at least 50% of the acinar cells have been destroyed. In man the secretin test of pancreatic exocrine secretory function under stimulated conditions is one of the main diagnostic tests for chronic pancreatitis.

It appears therefore that, in zinc toxicity, when either pancreatic lesions are extensive or pancreatic mass is reduced then pancreatic secretion is likely to be adversely effected.

The main aims of this experiment were to determine the effect of pancreatic lesions caused by zinc toxicity on the exocrine secretion of the pancreas and to determine the zinc concentration in bile and pancreatic juice over the period of the experiment.

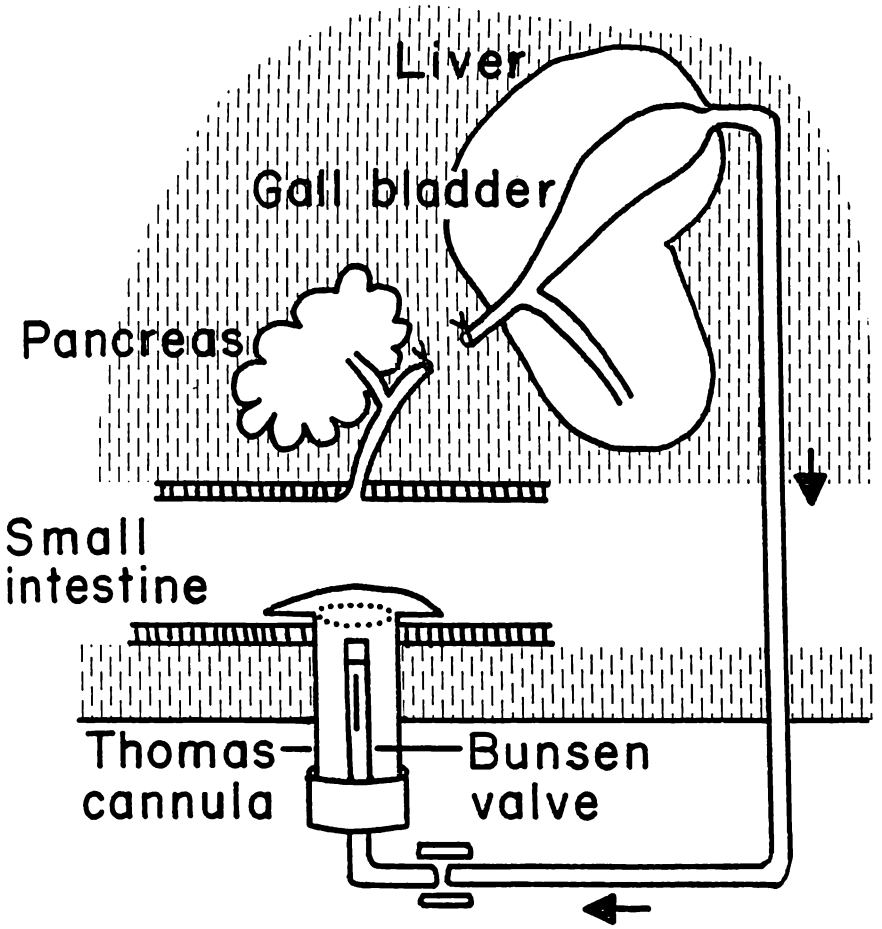
Materials and Methods

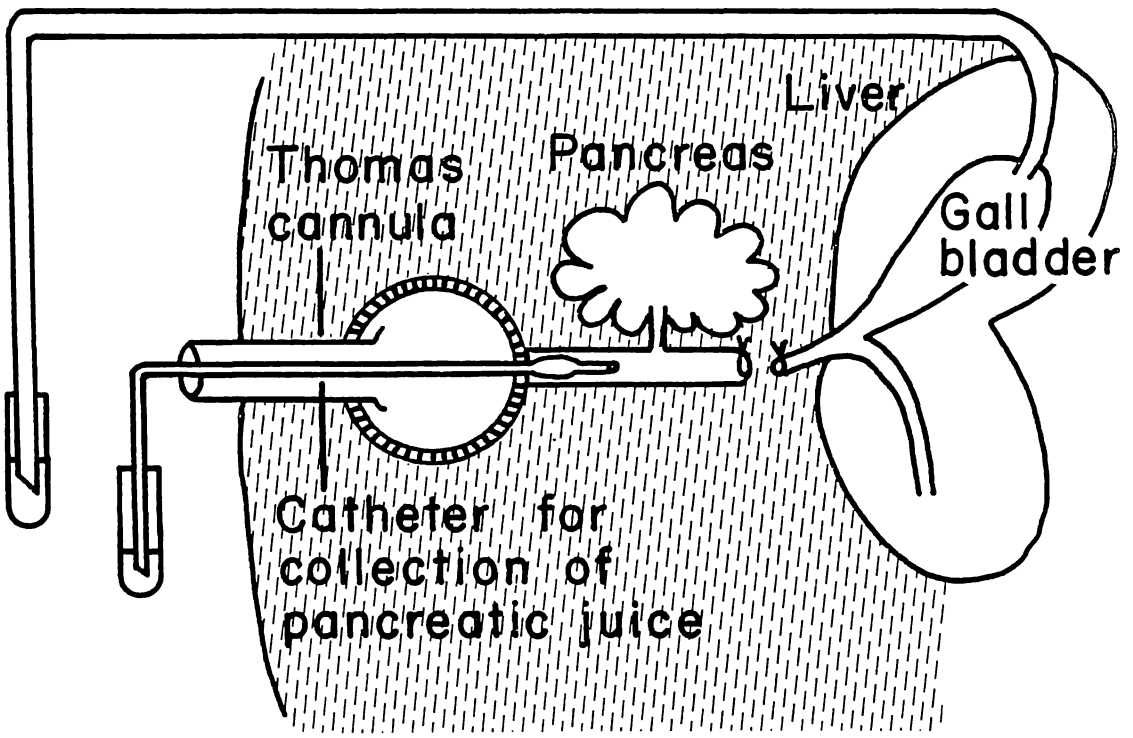
Five Romney cross 18 month old wethers (approx. 50 kg) were surgically prepared so as to exteriorise the bile flow and make accessible the sphincter of Oddi so that intermittent collections of pancreatic juice could be made. Each sheep was maintained before and after surgery in an individual pen with water and *ad libitum* feeding of freshly cut green grass allocated twice daily except on weekends when grass was fed only once a day.

Surgical technique The method of collection of bile and pancreatic juice was a modification of the technique of Taylor (1960) and was arrived at after considerable experimentation with different methods of pancreatic duct cannulation and collection of the pancreatic secretion. Nearly all attempts to collect pancreatic juice by directly cannulating the common bile duct from which the bile flow had been diverted were not successful because of inflammation of the pancreatic duct which developed in long standing (>1 month) preparations.

Anaesthesia was induced by intravenous injection of pentobarbitone (Nembutal, Abbott) and maintained by closed circuit administration of fluothane.

Access to the surgical field was gained by a long paracostal incision on the right flank. The gall bladder was drained, a cannula placed in the free end of the gall bladder and secured by purse string sutures. The common bile duct was occluded by a ligature just below the cystic and hepatic duct junction. The free end of the gall bladder cannula was then exteriorised via a stab puncture in the lumbar fossa. The Thomas cannula was inserted in the duodenum slightly cranial to and opposite the sphincter of Oddi, held by a purse string suture and exteriorised by a further stab puncture in the lumbar fossa but on the other side of the incision. Modifications to the method of Taylor (1960) were (a) the use of larger Thomas cannula (OD 20 mm), (b) the lack of Roux-Y side arm and oblique to side anastomosis, (c) exteriorisation of the bile flow, and (d) return of the exteriorised bile via a bunsen valve in the cap of the Thomas cannula (Fig 4.14a).





Figures 4.14 a and b. : Surgical preparation for the exteriorisation of bile flow and separation from pancreatic juice (a) and method for collection of bile and pancreatic juice (b).

After surgery the abdominal suture line and point of emergence of the catheter and cannula were dusted with aureomycin powder and the skin sutures removed at 10-14 days. The bile flow and appearance was checked daily and the bunsen valve removed from the Thomas cannula and checked regularly.

Collection of samples Samples of pancreatic juice and bile were collected on either five or six occasions from each sheep starting 14 days after surgery and within a period ranging from 4 to 34 days prior to the start of zinc dosing and thereafter at weekly intervals after the start of dosing on Tuesdays between 0830 and 1000 hrs. During collection the sheep was restrained in an elevated metal crate and allowed access to its usual feed.

Pancreatic juice was collected by opening the Thomas cannula and clearing the inside with a suction tube. The sphincter of Oddi was visualised and a PVC catheter with a bevelled end (Taylor, 1960) was carefully inserted into the pancreatic duct so that the bulbous swelling on the catheter disappeared beyond the sphincter. The catheter was kept in place by packing cottonwool about it in the outer Thomas cannula (Fig 4.14b). When clear pancreatic juice flowed out of the end of the catheter the end was inserted into the bottom of a graduated centrifuge tube containing a layer of liquid paraffin (approx. 1 ml). Pancreatic juice was collected for approximately 20-30 min, or longer if the flow rate was small.

Bile was also collected under paraffin but from the disconnected exteriorised PVC bile cannula into a 25 ml measuring cylinder, usually for a shorter collection time than for pancreatic juice. Collection of bile and pancreatic juice was of either such short duration or small volume respectively that return of the sample or substitute secretion was considered unnecessary. Both samples were taken immediately to the laboratory for bicarbonate determinations. After this the remaining sample was stored in acid washed, well rinsed glass containers and deep frozen (-10 ^{o}C) to await zinc, protein and amylase determinations.

Bicarbonate determinations Bicarbonate determinations were performed by a titrimetric method (Peters and van Slyke, 1956) using a PHM62 pH meter and TTT60 titrator coupled with a TTA31 titration assembly and ABU11 autoburette (all Radiometer, Copenhagen). Pancreatic juice or bile (0.5 ml) was withdrawn from below the liquid paraffin layer into a 'Hamilton' microsyringe, added to 2.5 ml of 0.01 N HCl in a plastic centrifuge tube, swirled vigorously on a bench vortex mixer and poured and washed into a plastic mixing cup with a magnetic stirrer. An electrometric titration was performed back to the pH of the original sample using approximately 0.01 N NaOH which was standardised by a blank titration against 0.01 N HCl immediately prior to the sample titration.

Bicarbonate concentration (μ mole/ml) was calculated from the following formula.

$$HCO_3^- \ (\mu\text{mole/ml}) = \frac{1000}{Vs} \ (Na \times Va) \ (1 - \frac{Vb_2}{Vb_1})$$

where Vs sample volume (0.5 ml)
Va volume of acid (2.5 ml)
Vb_1 volume of alkali in blank titration
Vb_2 volume of alkali to back titrate sample
Na normality of acid (0.010 N)

Total protein Pancreatic juice protein was determined by a modification of the biuret method of Reinhold, described by Varley (1960), for plasma proteins. Duplicate 50 μl pancreatic juice samples were each mixed with 2 ml 0.9% saline, 1 ml aliquots mixed with 2.5 ml working Biuret reagent (test) or 2.5 ml alkaline tartarate iodide reagent (blank) and incubated for 10 min at 37 ^{o}C. A series of standards of equivalent protein concentrations to the diluted pancreatic juice (range 12-96 mg protein/ml sample) were prepared by suitable dilution of stock solution containing 0.293 g bovine albumen (Sigma)/100 ml and treated similarly to the pancreatic juice to enable a calibration curve of corrected standard absorbance against protein concentration to be plotted.

Absorbance of the incubated pancreatic juice and standard solutions were read in a Shimadzu QV-50 spectrophotometer at 555 nm against a reagent blank. The pancreatic juice blank and standard blanks were read against alkaline tartarate-iodide reagent absorbances subtracted from their respective test absorbances.

Pancreatic juice protein concentration was obtained by reference to the calibration curve. Pancreatic juice having a protein concentration of less than 5 mg/ml was determined by using lesser dilutions and appropriate correction factors.

Amylase in pancreatic juice Amylolytic activity of suitably diluted pancreatic juice was estimated by the chromogenic method of Klein *et al.* (1969) using 'Amylochrome' (Roche Diagnostics) serum amylase kits.

Measured enzyme activity was converted to enzyme concentration by the system suggested by Taylor (1962). Serial dilutions of pancreatic juice in ice cold NaCl containing 0.002 M phosphate buffer (pH 7) and 0.001 M $CaCl_2$ were used to plot a curve of amylase activity vs pancreatic juice concentration for each pancreatic juice sample taken prior to zinc dosing. The lower limit of detectable activity was 50 U/ℓ designated as three standard deviations above the mean of 29 blank determinations.

Values for amylase concentration were obtained from each dilution series curve by dividing each pancreatic juice concentration in the linear dilution range by the pancreatic juice concentration value corresponding to 1000 U/ℓ of amylase activity and a composite plot of amylase activity against amylase concentration prepared. A regression formula relating activity to concentration of enzyme was calculated.

Samples of pancreatic juice collected after the start of zinc dosing were diluted sufficiently for the amylase activity to fall within the observed range of validity. The measured activity of the diluted samples was converted to amylase concentration (U/ml sample) by application of the regression equation and multiplication by the appropriate dilution factor. In one sheep (15) the amylase concentration fell below the detection threshold (=2.5 U/ml sample).

Bile and pancreatic juice zinc Both bile and pancreatic juice zinc concentrations were determined by atomic absorption spectrophotometry after simple dilution of the samples in distilled water (Appendix A). A minimum dilution of one in five was employed with all bile samples. With most pancreatic juice samples the minimum dilution was one in 25 with the minimum sample dilution being one in six on samples in which zinc concentration was very low.

Postmortem examination At the conclusion of the experiments each sheep was slaughtered and a postmortem examination performed. In particular the pancreas was carefully examined and sections taken for microscopic examination and for scoring for injury (Appendix C).

Treatments Two surgically prepared sheep were maintained for three months without any zinc treatment, with only occasional catheterisation of the pancreatic duct. After an initial recovery period between surgery and dosing of six weeks, the other three successfully prepared sheep were given intraruminal zinc oxide on three days (Monday, Wednesday, Friday) of each week. Each dose was 240 mg Zn/kg b.wt, equivalent to approximately 100 mg Zn/kg/d, which was maintained for the remainder of the experiment, a period of 43 days.

Results

Both sheep which were kept for three months with implanted bile duct catheters and intestinal cannulae and not dosed with zinc salts were found to have pancreata of normal size (54 and 48 g) and appearance. There were no lesions in the pancreas and one had minor thickening of the major bile ducts.

The bile ducts of two of the three dosed sheep (15 and 898) also showed minor thickening probably due to the implanted catheters. The pancreata of the three zinc dosed sheep varied in the degree to which they reacted to the zinc oxide. The pancreata of sheep 15, 880 and 898 weighed 5, 54 and 56 g respectively. Sheep 15 had a very pale firm small pancreas which on microscopic examination showed lesions consistently seen in zinc toxicity cases and gave an injury score (Appendix C) of 9. Sheep

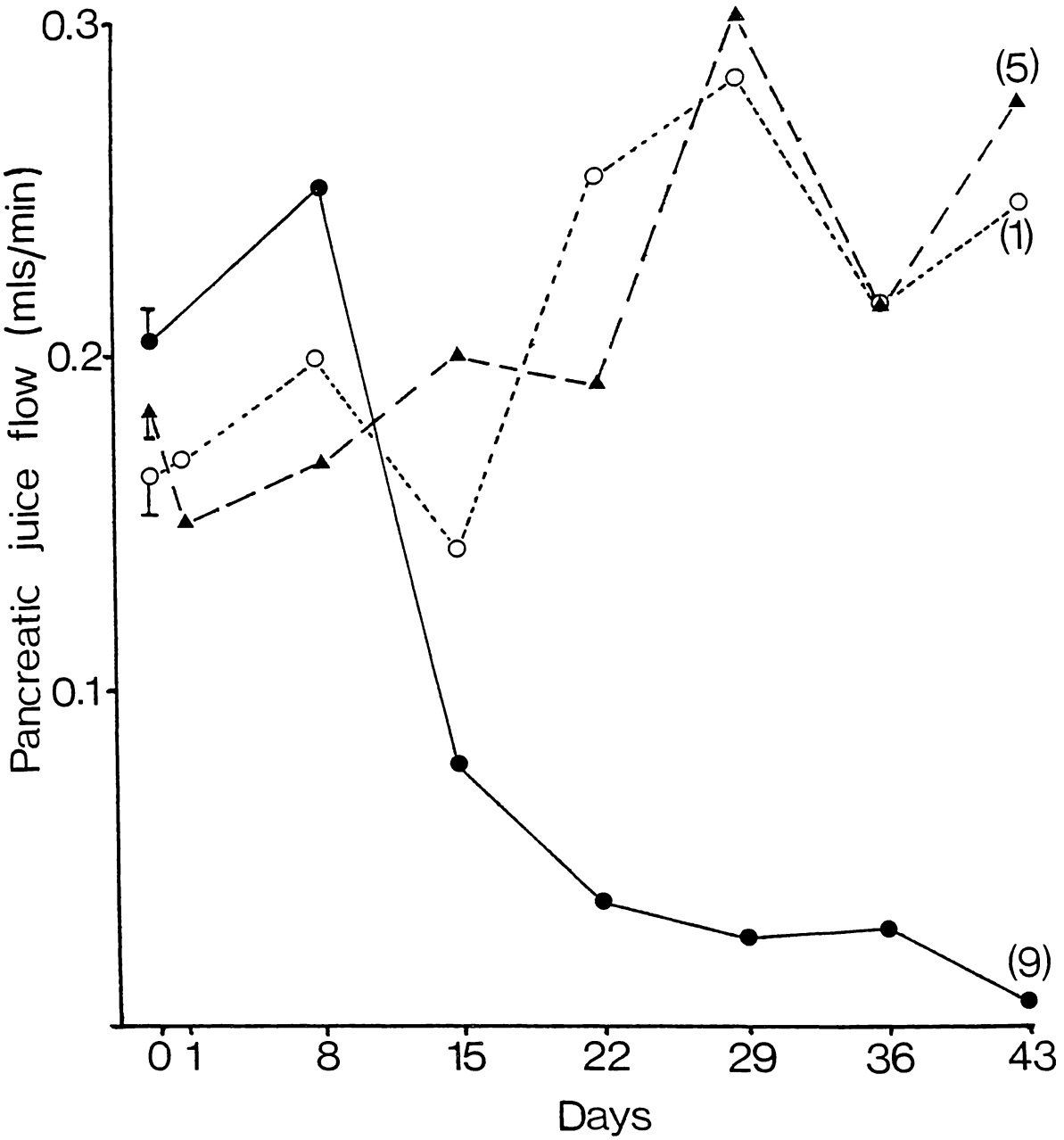


Figure 4.15 : Effect of zinc oxide dosing (240 mg Zn/kg/dose) thrice weekly to three separate sheep on flow of pancreatic juice. Flow at day 0 is the mean ±SEM of 5 or 6 predosing flow measurements. Pancreatic injury scores in parenthesis. Sheep 15 ● —— ●; Sheep 898 ▲ __ __ ▲; Sheep 880 o ---- o.

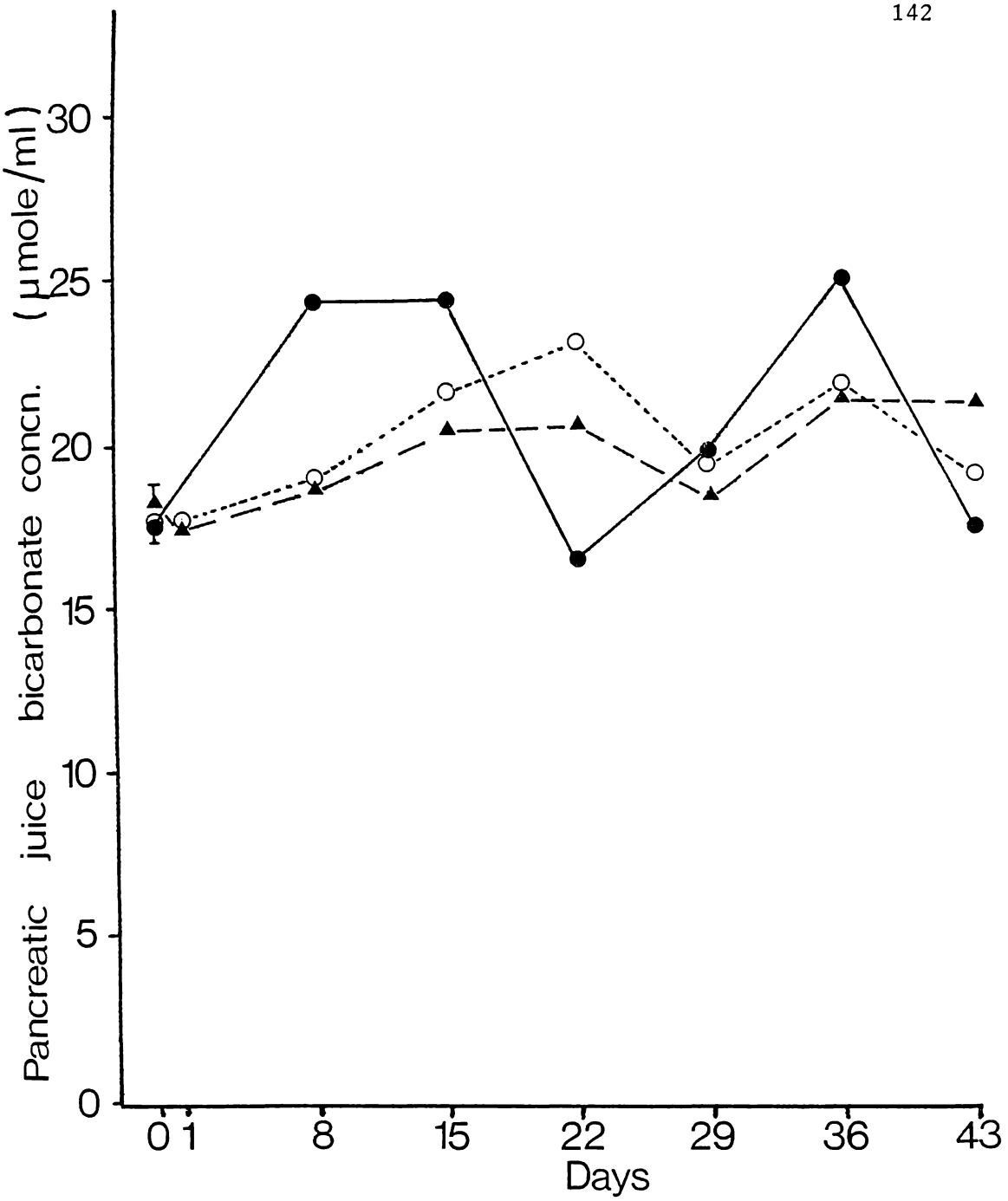


Figure 4.16 : Effect of zinc oxide dosing (240 mg Zn/kg/dose) thrice weekly to three separate sheep on the bicarbonate concentration in pancreatic juice. Concentration at day 0 is the mean ±SEM of 5 or 6 measurements of bicarbonate concentration prior to commencement of zinc oxide treatments.

Sheep 15 ● —— ●; Sheep 898 ▲ — — ▲; Sheep 880 o ---- o.

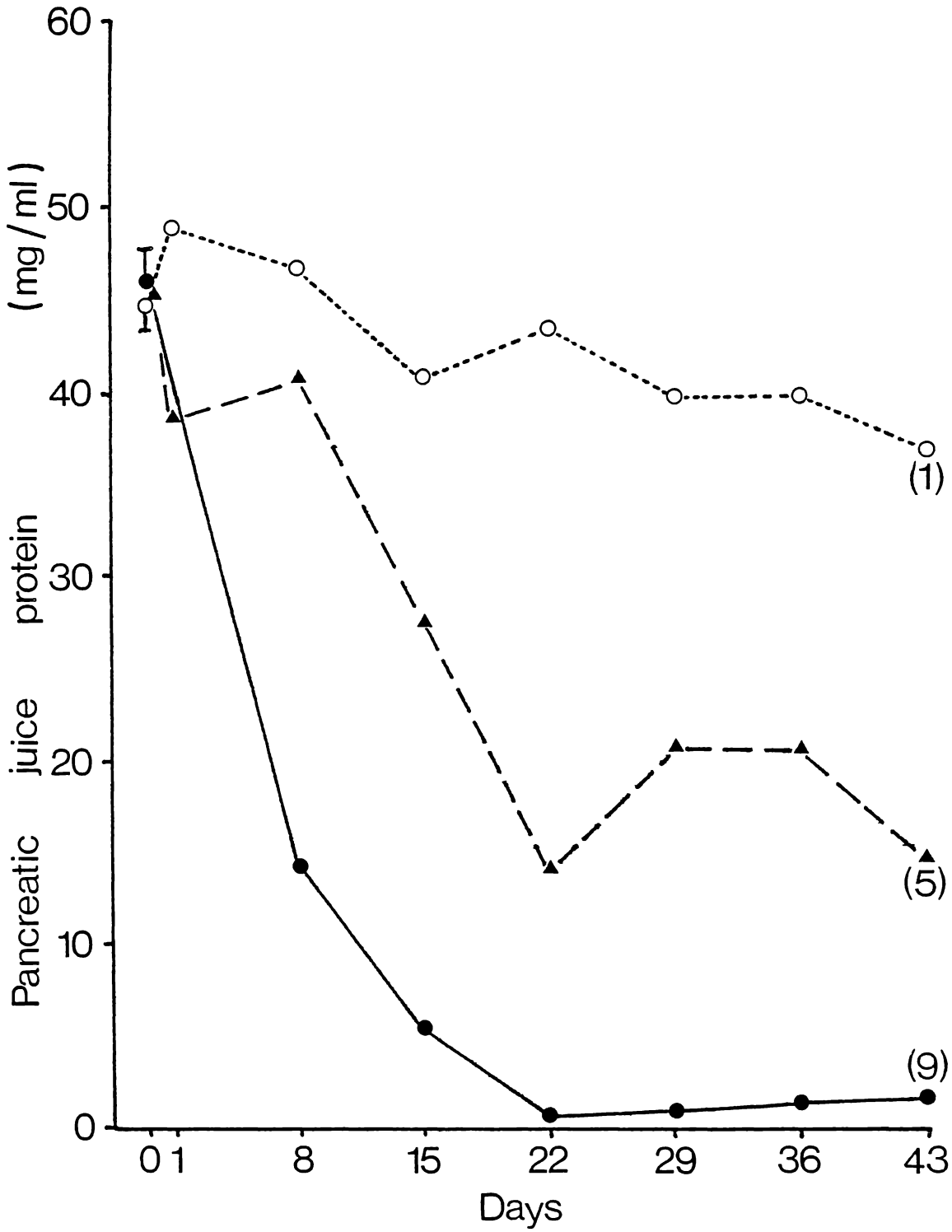


Figure 4.17 : Effect of zinc oxide dosing (240 mg/kg/dose) thrice weekly to three separate sheep on protein concentration in pancreatic juice. Protein concentration at day 0 is the mean ±SEM of 5 or 6 measurements of protein concentration prior to the start of zinc oxide treatment. Pancreatic damage scores in parenthesis.

Sheep 15 ● —— ●; Sheep 898 ▲ — — ▲; Sheep 880 o ---- o.

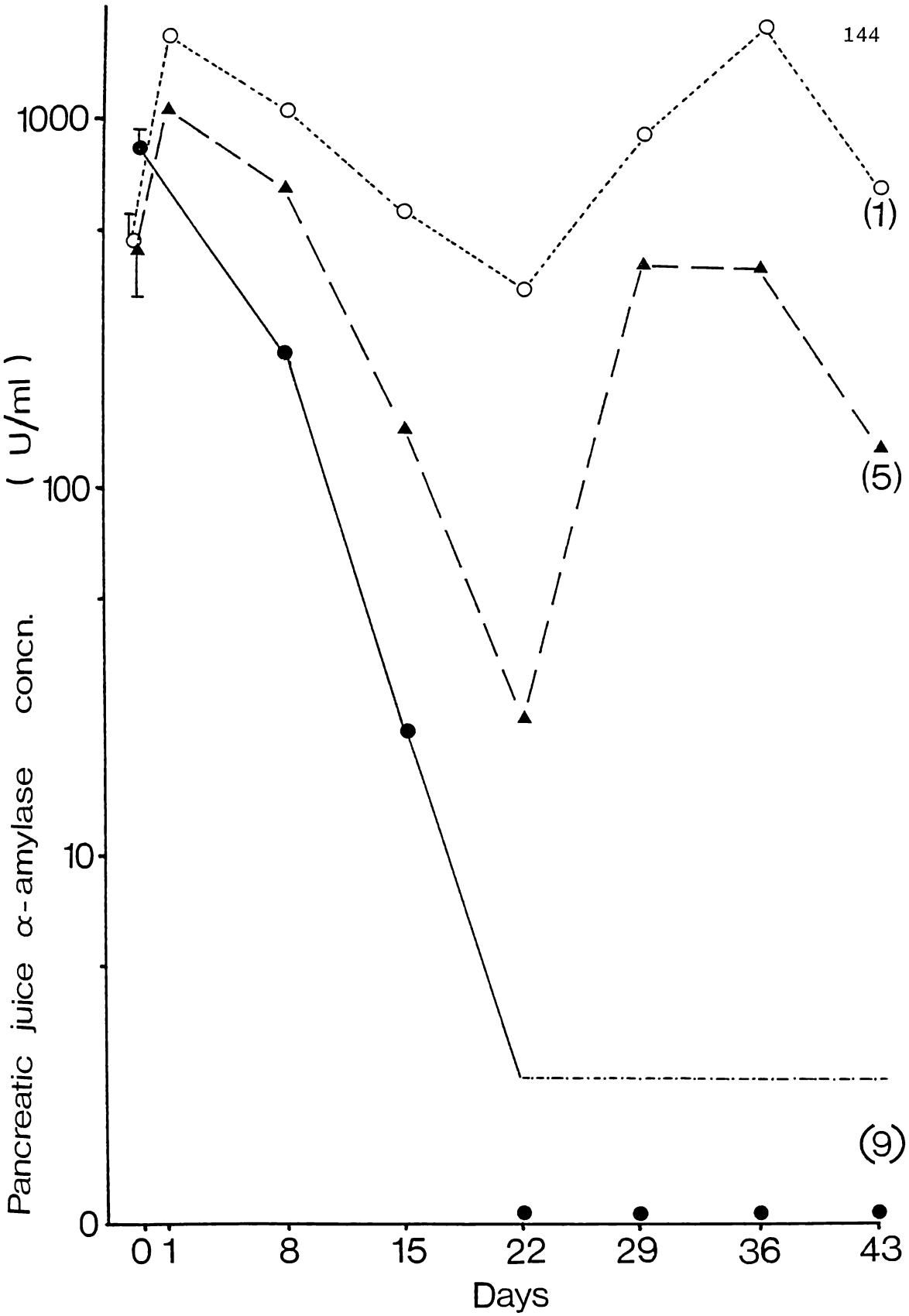


Figure 4.18 : Effect of zinc oxide dosing (240 mg Zn/kg/dose) thrice weekly to three separate sheep on α-amylase concentration in pancreatic juice. Concentration at day 0 is mean ±SEM of 5 or 6 measurements of α-amylase activity prior to zinc oxide treatment. Pancreatic damage scores in parenthesis. Sheep 15 ● —— ●; Sheep 898 ▲ — — ▲; Sheep 880 o ---- o: Activity of α-amylase undetectable below 2.5 u/ml. -·-·-.

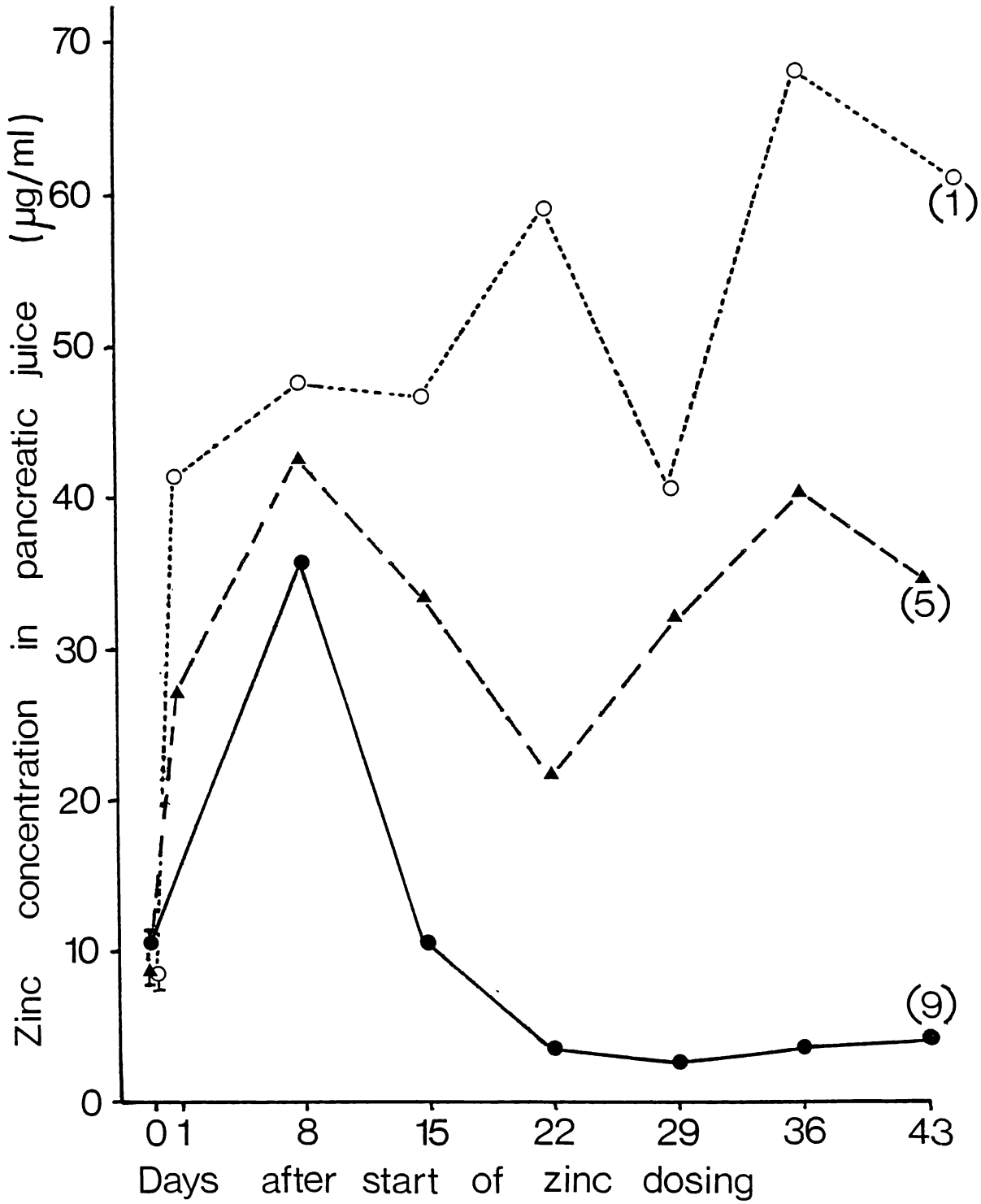


Figure 4.19 : Zinc concentration in the pancreatic juice of three separate sheep dosed thrice weekly with zinc oxide (240 mg Zn/kg/dose). Concentration at day 0 is mean ±SEM of 5 or 6 measurements of zinc concentration before the start of the zinc oxide dosing. Pancreatic injury scores in parenthesis. Sheep 15 ● —— ●; Sheep 898 ▲ — —▲; Sheep 880 o ---- o.

880 had minimal pancreatic change with fibrosis affecting less than 10% of the grossly observed pancreas and which gave a microscopic examination injury score of 1. Sheep 898 had half of the pancreas firm and pale while the remaining half was also firm but less severely affected. Its pancreas had a microscopic examination injury score of 5.

The variability in response of the pancreas to zinc oxide administration was also seen in the response of pancreatic secretion. The effect of zinc dosing on the various parameters of pancreatic secretion is seen in Figure 4.15 (flow rates), Figure 4.16 (bicarbonate concentrations), Figure 4.17 (protein concentration), Figure 4.18 (amylase activity) and Figure 4.19 (zinc concentration). The cumulative effects of both volume and concentration changes can be seen in Tables 4.16 and 4.17. It appears that where severe pathological lesions of zinc toxicity were present in the pancreas then the effect on pancreatic output of bicarbonate and amylase could be clearly demonstrated. In the case of bicarbonate output the effect was mostly through the effect of zinc on flow rate but in the case of amylase output both flow rate and to a greater extent, enzyme activity contributed to the decline in output.

The effect of zinc toxicity on enzyme and protein output from the pancreas was much more dramatic than on bicarbonate. In the case of sheep 898 (damage score 5) protein concentration appears to be a more sensitive indicator of pancreatic injury than bicarbonate in the unstimulated pancreas (Figs 14.16 and 14.17).

Zinc concentration in the pancreatic secretion is much higher than that in bile both before and after zinc dosing. There was an approximately three to six fold maximum increase in pancreatic juice zinc concentration after dosing of zinc oxide commenced and although serum levels of zinc were not determined, the concentrations seen here in pancreatic juice (>60 µg/ml) were much greater than would be expected to occur in serum from such dosing. The zinc concentrations in bile both before and after zinc dosing were very low and the output of zinc in both the non-dosed and zinc-dosed situations is much less in bile

Table 4.16 Effect of dosing zinc oxide (240 mg Zn/kg b.weight / dose) to three sheep for three days per week output of bicarbonate, protein, amylase and zinc from the pancreatic duct.

Sheep No.	Days after start of dosing	Output via pancreatic juice (per min)			
		Bicarbonate µ mole	Protein mg	Amylase u	Zinc µg
15	Predosing*	3.7 ±0.29	8.31±0.10	168±14	2.19±0.10
	8	6.1	3.5	56	8.9
	15	1.6	0.34	1.5	0.7
	22	0.6	0.03	0.1	0.13
	29	0.5	0.03	0.1	0.07
	36	0.7	0.04	0.1	0.09
	43	0.1	0.01	0.1	0.02
898	Predosing*	3.38±0.21	9.64±0.74	85±22	1.59±0.09
	8	3.3	7.0	110	7.2
	15	4.1	5.5	29	6.7
	22	3.9	2.7	5	4.1
	29	5.6	6.5	122	9.5
	36	4.5	4.3	85	8.4
	43	5.8	4.0	36	9.2
880	Predosing*	2.93±0.32	7.53±0.96	79±20	1.45±0.22
	8	3.8	9.4	220	9.5
	15	3.1	5.8	80	6.5
	22	5.8	10.7	85	14.7
	29	5.5	11.1	269	11.2
	36	4.5	8.3	415	14.2
	43	4.4	8.5	160	13.8

*Predosing mean ±SEM; n=5 or 6

Table 4.17 Effect of dosing zinc oxide (240 mg Zn/kg b.weight/dose) to three sheep for three days per week on bile flow and the concentration and output of bicarbonate and zinc in bile.

Sheep No.	Days after start of dosing	Bile flow ml/min	Concentration in bile		Output in bile	
			Bicarbonate µ mole/ml	Zinc µg/ml	Bicarbonate µ mole/min	Zinc µg/min
15	Predosing*	0·85±0.11	14.1±1.1	0.06±0.05	12.9±2.2	0.05±0.04
	8	0.50	14.1	0.1	7.8	0.05
	15	0.43	22.6	0.4	9.7	0.17
	22	0.40	31.3	0.3	12.6	0.12
	29	0.32	27.9	0.2	8.9	0.06
	36	0.23	45.4	0.3	10.3	0.07
	43	0.34	27.6	0.6	9.4	0.20
898	Predosing	0.87±0.06	16.2±1.5	0.05±0.04	13.9±1.5	5.05±0.03
	8	0.60	17.7	0.25	10.6	0.15
	15	0.55	15.5	0.30	8.5	0.17
	22	0.33	13.8	0.60	4.6	0.20
	29	0.39	12.7	1.05	5.0	0.41
	36	0.38	16.6	1.05	6.3	0.40
	43	0.25	14.4	4.60	3.6	1.15
880	Predosing*	0.66±0.12	14.1±1.00	0.07±0.03	9.7±2.1	0.04±0.02
	8	0.61	19.6	0.15	12.0	0.09
	15	0.34	17.0	0.60	5.7	0.20
	22	0.57	17.7	0.25	10.1	0.14
	29	1.03	15.7	0.15	16.2	0.15
	36	0.60	19.0	0.45	11.5	0.27
	43	0.88	19.4	0.50	17.1	0.44

*Predosing mean ±SEM; n=5 or 6

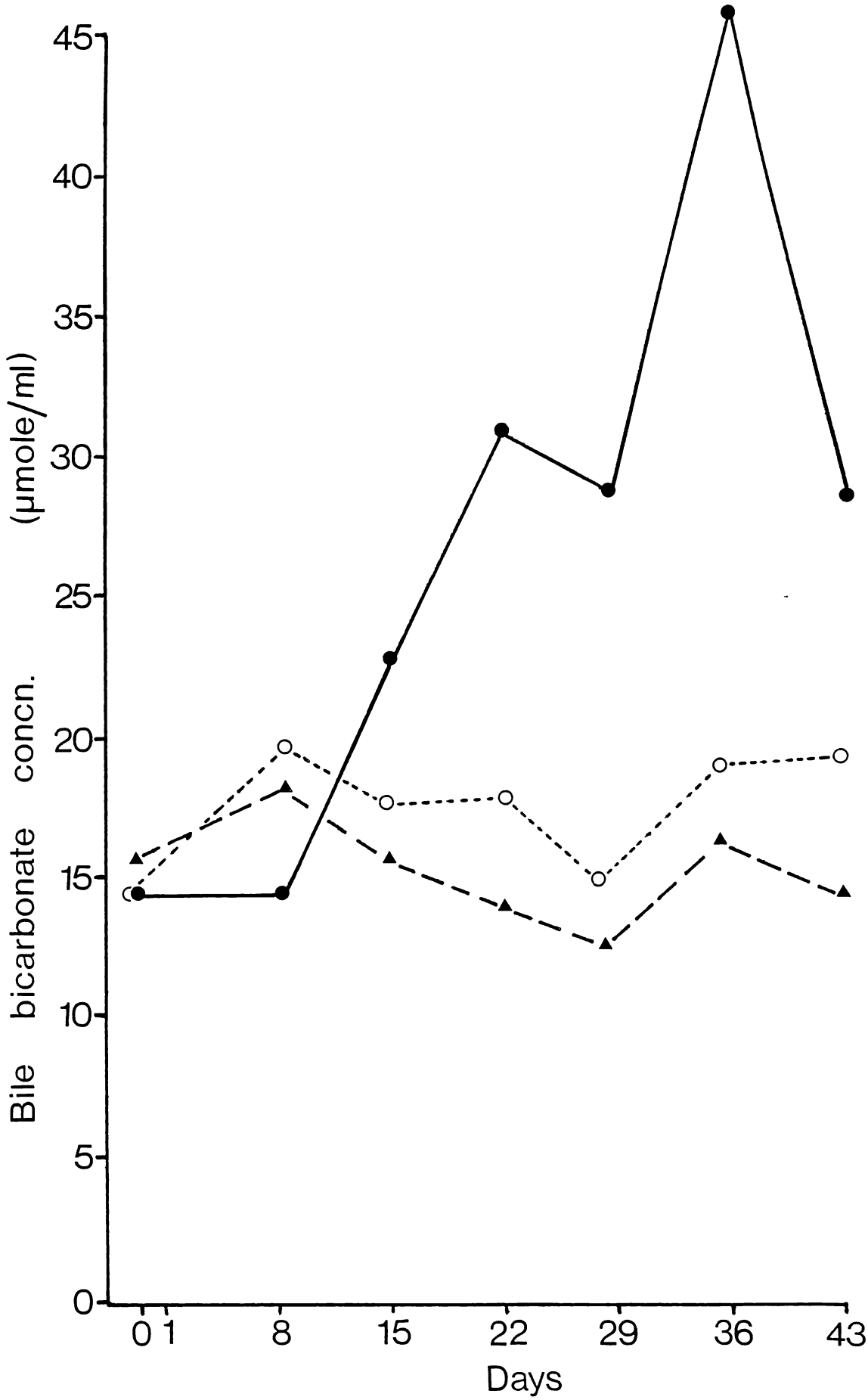


Figure 4.20 : Effect of zinc oxide dosing (240 mg Zn/kg dose) thrice weekly to three separate sheep on the bicarbonate concentration in bile. Concentration at day 0 is the mean ±SEM of 5 or 6 measurements of bicarbonate concentration prior to commencement of treatment.

Sheep 15 ● —— ●; Sheep 898 ▲ — — ▲; Sheep 880 o ---- o.

than in pancreatic juice. This is in contrast to bicarbonate output which is much greater from liver than from pancreas and is not diminished in bile by zinc dosing.

In the sheep (15) with the most severe pancreatic injury, where bicarbonate output from the pancreas was drastically reduced, in contrast to the other two sheep there was an increase (at least two fold) of bicarbonate concentration in bile (Fig 4.20; Table 4.17). However the lowered bile flow in this sheep after commencement of zinc dosing meant that total bile bicarbonate output was not increased.

It appeared also that the concentration of zinc in pancreatic juice was inversely proportional to the amount of pancreatic injury observed at the end of the experiment.

Discussion

These results suggest that zinc oxide dosing of sheep results in diminished pancreatic juice flow and protein and amylase concentration when pancreatic damage is severe. Although these results only demonstrated a spectacular drop in amylase activity it seems likely that the lipid and protein specific enzyme concentrations in pancreatic juice were likewise affected. This is also supported by the observed decline in protein concentrations. These observed or inferred changes are also magnified by the decline in pancreatic juice flow rate.

The relative importance to ruminants of pancreatic exocrine secretion or of losses of individual components is not fully understood. Jarrett and Filsell (1972) showed that in sheep in which the pancreatic duct had been ligated, there was no difference from controls in faecal lipid content although faecal nitrogen was increased. There was considerable decline in wool production and body weight but these changes could have resulted from causes other than loss of pancreatic juice. Heath and Morris showed that ewes and lambs deprived of pancreatic juice had a much lower lipid content in intestinal lymph than in normal sheep. Likewise Gooden and Lascelles (1973) showed in calves that deprivation of pancreatic juice resulted

in substantial reduction in lipid absorption. They also demonstrated that this was due to lipase loss rather than a loss of buffering activity due to depletion of bicarbonate. This is supported by the work of Caple and Heath (1972) who showed that bile contributes the majority of bicarbonate to the duodenal contents. The present results support this finding.

In the present experiment the loss of bicarbonate output occurred only in the sheep with the most severely damaged pancreas and it was due mainly to the reduction in pancreatic juice flow as bicarbonate concentration stays within normal range. In this sheep the bicarbonate concentration in bile appeared to increase; possibly a compensatory reaction for the loss of pancreatic output of bicarbonate. The increase in bicarbonate concentration in bile occurs in this sheep in spite of a decrease in bile flow rate to less than half that recorded prior to the treatment period. The normal physiological reationship in sheep, where secretion of bicarbonate in bile is more important, is for increases in bicarbonate concentration in bile to occur with increases in bile-flow-rate (Caple and Heath, 1972) and the results here indicate that the opposite has occurred.

Although the output of zinc from the pancreas to the intestine is a small percentage of the total zinc administered (<0.5%) it may be a significant proportion of the absorbed amount of zinc. The percentage absorbed (after zinc sulphate dosing) may be as low as 1% (C. Ramberg personal communication). If such a low absorption figure was the case in the present experiment then the percentage of absorbed zinc which is secreted via the pancreas could be as high as 50%. These speculative calculations, however, are based on several extrapolations and no account is taken of a possible entero-pancreatic circulation of zinc.

The role of zinc in the production of the pancreatic lesion remains unknown. It appears that the primary lesion in the pancreas is in the pancreatic duct (Chapter 5.2) and because the pancreas accumulates and excretes zinc it might be hypothesised that zinc causes local damage to the pancreatic duct. However in this experiment the highest

and lowest concentrations of zinc in pancreatic juice were found in those sheep with the least and greatest pancreatic damage respectively. Early changes in the zinc concentration of pancreatic juice were not frequently determined in this experiment and it could be argued that the lower zinc concentration in the pancreatic juice from the most damaged pancreas may have resulted from pancreatic injury.

No flow or concentration measurements were carried out on pancreatic juice from non-zinc dosed sheep for as long as these sheep were maintained (approx. two months). Therefore it might be suggested that these results may arrive from or be influenced by the long standing nature of the surgical preparations rather than the zinc treatment. However it should be noted that (a) no significant changes were recorded in pancreatic output or concentration during the time prior to zinc administration, (b) the changes recorded after zinc administration were related to the degree of pancreatic injury and the one sheep with minimal pancreatic damage showed no significant changes in its pancreatic secretion, and (c) two sheep were maintained for two months after surgery and there were no histological or macroscopic changes seen in their pancreata at postmortem examination. In addition it should be noted that this particular surgical preparation involves virtually no interference with the pancreas or its ducts except for the brief weekly catheterisation of the common bile duct. Finally Taylor (1960) stated that such preparations lasted for up to 15 months during which time many collections of pancreatic juice were made.

CHAPTER 5

THE PATHOLOGY OF ZINC TOXICITY IN SHEEP AND CATTLE

CHAPTER 5

THE PATHOLOGY OF ZINC TOXICITY IN SHEEP AND CATTLE

5.1 GENERAL PATHOLOGY

Introduction

Various authors have briefly described lesions of zinc toxicity but none have either summarised the general lesions of different forms of zinc toxicity in different species or described a particular lesion in detail (see Chapter 2.6). Throughout this series of experiments many different lesions of zinc toxicity of varying age, severity and extent were found at postmortem examination of animals. The circumstances which lead to the different lesions were often distinct and were sometimes predictable. The following paragraph summarises the different lesions and the circumstances leading to their appearance in this series of experiments.

Abomasal lesions were present when zinc sulphate was administered by hand to sheep or cattle and were especially prominent when the zinc was administered by drenching gun. The pancreatic lesions were present in most animals showing signs of zinc toxicity no matter what form of zinc was used or how it was administered. These lesions were also often present in animals dosed with zinc but showing no clinical signs of toxicity. However the lesion took two to four weeks to develop and in those animals which suffered from acute abomasitis and early death from zinc sulphate administration, the pancreatic lesion was often not apparent at postmortem examination. Two different types of kidney lesion were experienced. The first, an interstitial nephritis and 'mineralisation' of the tubules was associated with zinc toxicity in lambs. The second, a tubular necrosis with the presence of intratubular haemoglobin casts, was present in both sheep and cattle which had shown signs of having undergone one or more haemolytic crisis. The evidence of haemolytic crises was only seen in animals which had been dosed with zinc sulphate. In cattle the haemolytic crisis was present in acute zinc toxicity whereas in sheep it occurred more often

after several months of zinc administration. Liver lesions were present in those animals in which haemolytic crises had occurred. In those animals in which severe abomasal lesions had occurred there were ruminal papillary changes and in all animals with more than mild lesions of zinc toxicity thymus regression was marked. In those animals with severe lesions, resulting usually in death, enlarged adrenals were present. These latter three lesions occur in other severe disease entities and are regarded as non-specific lesions of zinc toxicity.

Materials and Methods

The methods of postmortem examination, collection and preparation of histological material are described in Appendix B. In addition to routine haematoxylin and eosin staining the following stains were used when required.

1. Oil Red O (American Registry of Pathology, 1968d).
2. Masson's Trichrome (American Registry of Pathology, 1968c).
3. van Gieson Collegen Fibre Staining (American Registry of Pathology, 1968a).
4. Perl's Prussian Blue Reaction (American Registry of Pathology, 1968e).
5. Gomori's Reticulin Stain (American Registry of Pathology, 1968b).
6. Alcian Blue/PAS (Pearse, 1968).

Results

5.1.1 ABOMASAL LESIONS

A common feature of zinc sulphate toxicity in sheep and cattle was the presence in the abomasum of lesions of varying extent and severity. When very high dose rates of zinc sulphate ($\geq$60 mg Zn/kg/d) were given daily for several days by intraruminal intubation extensive oedema and necrosis of the abomasal submucosa and sloughing of the mucosa itself could be expected (Chapter 3.1.1). When lesser dose rates ($\geq$20 mg Zn/kg/d) were given by drenching gun similar lesions were observed or an exacerbation of the lesions produced by intraruminal intubation at the same dose rate of zinc occurred.

Macroscopic changes The mildest lesion observed was an oedematous change of the mucosa most easily discerned in the fundic folds of the abomasum varying from just discernible to very thick swelling of the folds to the extent that the edges of the folds rounded considerably and the folds of the opened abomasum stood thick and turgid above the flattened abomasum (Plate 5.1). Incised edges of the folds rapidly lost fluid.

Necrotic changes of the abomasum were more often observed. Oedema sometimes accompanied this change, especially if the necrosis was extensive. In its mildest form the necrosis was confined to the dependent part (greater curvature) of the abomasum (Plate 5.3) and the tips of the fundic folds (Plate 5.2). The normally glistening mucosal surface was roughened, discoloured, friable and had a dry appearance. Haemorrhage sometimes accompanied these changes (Plates 5.6 and 5.7) and the depth of this mucosal erosion was such that in one animal it penetrated the submucosa and muscular sheaths to form a deep ulcer visible on the serosal surface (Plate 5.4). In the most extensive cases the whole of the fundic region and sometimes the pyloric antrum was affected. In such severe cases the whole surface was covered with a caseous white pseudomembrane (Plate 5.5) or showed a fixed grey/white appearance (Plate 5.6).

In the most severe cases the necrotic changes also extended up to two metres into the small intestine. Such severe lesions were invariably associated with acute zinc sulphate poisoning resulting in death of the animal.

On several occasions chronic or healed lesions of the fundic folds were observed. These were detected as abomasa with shrunken areas in the fundus which included grossly distorted fundic folds. These folds were shortened both in length and depth and the edges were considerably thickened and distorted (Plates 5.8 and 5.9).

Microscopic changes On microscopic examination oedematous abomasa had minor sloughing and necrosis of the outer epithelial cells (Plate 5.10). The lamina propria and especially the submucosa was distended

and contained a faint to strongly pink staining homogeneous substance and increased numbers of leucocytes, the main cell type being neutrophils. These were occasionally seen in focal aggregations and occasionally packed thin walled vessels believed, because they contained no red blood cells, to be lymph vessels.

Where necrosis of the mucosa was present this was accompanied by hyperaemia and greater numbers of leucocytes in the areas beneath the sharply demarcated necrotic area which extended on occasions to beneath the muscularis mucosa (Plate 5.11).

5.1.2 PANCREATIC LESIONS

Throughout the several experiments in which zinc salts were administered to sheep and cattle at various dose rates and lengths of time a wide range of severity of pancreatic changes was observed. In addition, one experiment (Chapter 5.2) was designed with the objective of observing the sequential changes which occur when zinc oxide was administered over four weeks and sheep were slaughtered in groups at specific times during and after this four week dosing period.

Macroscopic changes The earliest change recorded was oedema in the interlobular pancreatic tissue. This was seen as a separation of lobules by opalescent whitish fluid in bands of up to 3 mm width. Often at this stage the fat surrounding the pancreas, and occasionally the other abdominal organs, developed plaques of the flat white chalky appearance usually associated with fat necrosis (Plate 5.13) contrasting with the normal appearance (Plate 5.12) of the pancreas. Mild to moderate pancreatic lesions were seen as scattered, well-defined, firm white patches 2-10 mm across of irregular shape but appearing to conform to a lobular pattern on the surface of the pancreas. These varied from just a few discrete areas to coalescing areas covering most of the uncut surface. When the lesion was so extensive,the pancreatic mass was noticeably reduced (Plates 5.14 and 5.25) and the pancreas as a whole was pale and very firm, often 'crunchy' when cut by knife. In severely affected pancreata the organ was reduced to less than 25% of its original mass.

Microscopic lesions Examination of haemotoxylin and eosin stained sections of pancreas by light microscope was carried out on all pancreata, of animals from zinc toxicity experiments. In sheep the earliest lesion after the commencement of zinc administration was a necrotic change of the epithelium of pancreatic ducts. This change occurred in both large and small ducts and was seen to be a coagulative necrosis of the epithelial cells (Plates 5.15 and 5.47) with apparent leakage of exudate fluid into the duct lumen (Plate 5.46). The exudate was an homogeneous strongly eosinophilic material which almost always appeared to be adherent to the necrotic side of the duct. Most often the change was confined to one side or segment of the duct wall. Associated with this change was usually a local infiltration of mononuclear inflammatory cells of varying intensity (Plates 5.15, 5.16 and 5.47). In other cases the whole duct wall appeared necrotic and the entire circumference of the duct was surrounded by infiltrated leucocytes. Occasionally a necrotic centre surrounded by infiltrated leucocytes was the only evidence of the prior existence of a pancreatic duct (Plate 5.18). At this stage changes to the exocrine pancreatic parenchyma were obvious. The most significant feature of the lesion found in sheep was its lobular distribution. In some pancreata, different lobules were seen in the one section showing a range of changes from not affected to severely affected (Plates 5.19, 5.49 and 5.51). The earliest lobular changes consist of a separation of pancreatic acini and an intralobular infiltration of fibrocytes and collagen. Pancreatic acinar cells lost their cuboidal shape, their nucleii shrank and became fragmented, the glandular end pieces became dilated and the whole lobule was infiltrated lightly by polymorphonuclear leucocytes (Plate 5.20). Occasional aggregations of mononuclear leucocytes were seen. At this stage the architecture of the lobule appeared quite different and a ductular hyperplasia appeared evident with dilated duct lumina giving the lobule a slightly 'lacy' or cystic appearance (Plate 5.21). At this stage fibrosis was evident and as the lobular degeneration proceeded this became the prominent feature both of the intra and interlobular areas (Plates 5.22 and 5.23).

In the early stages when oedema and duct necrosis was evident fat necrosis was seen in occasional intrapancreatic areas of fat. Instead of the usual clear vaculolar area of the lipocyte the lipocyte vacuole was filled with a pale blue, finely granular substance and accompanied by surrounding macrophages and foreign body giant cells (Plate 5.50).

In comparison with the zinc damaged sheep pancreas, the bovine lesion displayed a few differences. The lobular pattern of the lesion in cattle was less obvious, the early necrotic changes in the exocrine parenchyma were more pronounced and the invasion of perivascular areas by lymphocytes was a more prominent feature. The more diffuse intralobular change was accompanied by an infiltration of both neutrophils and eosinophils.

5.1.3 PATHOLOGY OF THE HAEMOLYTIC CRISIS

Clinical In several sheep and cattle an anaemia, usually detected as a lowered packed cell volume, developed over the course of zinc administration.

Occasionally the packed-cell volume would rise again only to become depressed again as the animal underwent consecutive haemolytic episodes. In severe cases the urine was observed to be dark and in several cows in one experiment (Chapter 3.2.1) the urine developed a very dark 'soy sauce' characteristic. These animals were icteric, lethargic and anorectic and in severe acute cases this rapidly progressed to death over a period of one to three days.

Macroscopic changes In all of the cases in which there was evidence of one or more haemolytic episodes the usual pancreatic lesions were present and in addition there were several other organ changes related to the haemolytic crisis (Plate 5.24).

The liver was usually discoloured but normal in shape. Its consistency was slightly friable and usually in the case of sheep there was a distinct 'greasy' feel to the cut surface. The discolouration

varied from the usual red/brown colouration through a 'nutmeg' brown colour to an orange colour (Plates 5.24 and 5.25). Accompanying these changes the fat depots had an icteric appearance. The kidneys also varied from their usual red/brown colour to a nutmeg brown, often with a speckled appearance, to, in some of the cattle, a very dark almost black colouration on both the cut and uncut surfaces (Plate 5.26). Where urine was present in the urinary bladder this varied from a light brown to black colour.

Microscopic changes In the case of sheep which had shown haemolytic episodes the liver invariably showed some degree of fatty change (Plates 5.27 and 5.28). Empty clear vacuoles of varying size from those just detectable to those occupying almost the whole hepatocyte were shown by Oil Red O staining of frozen sections to be lipid in nature. They varied from a mainly periacinar distribution to those occupying all regions of the hepatic lobule but the density and size of the vacuoles tended to be greater towards the centre of the lobules (Plate 5.29). Occasional yellow to brown pigment-containing epitheloid cells could be found throughout the lobule but tended to be concentrated near the portal triad. Occasional small clusters of neutrophils were seen throughout the parenchyma (Plates 5.27 and 5.28) but there was no regular pattern of distribution for these. In severely affected sheep a periacinar area of hepatocyte necrosis was apparent throughout most lobules (Plates 5.28 and 5.30) and this change sometimes occupied up to half the lobule area.

In cattle the predominant liver lesion consisted of prominent areas of focal hepatic necrosis. These large areas usually contained degenerating hepatocytes on the periphery and in the centre masses of neutrophils and lymphocytes (Plate 5.31). In severe cases these areas of necrosis contained a large mass of yellow staining (H & E) pigment. The fatty change was also present in cattle, its distribution within the lobule being similar to that in sheep.

The kidneys of sheep had dilated tubules (Plate 5.32) occasionally containing casts of pink to red/brown homogeneous material interpreted from special stains (Prussian blue + ve) to be haemoglobin casts.

The epithelial cells of the tubular epithelium contained a dark brown granular pigment, shown to contain iron (positive Prussian blue reaction ,(Plate 5.33). In the cattle showing the signs of haemolytic crisis the kidney tissue contained similar but more pronounced changes in the lumen of the tubules (Plate 5.34). However the proximal convoluted tubular epithelium in severe acute cases was completely necrotic and the pigment observed in the tubular epithelium of sheep was not as prominent. In cattle in which zinc dosing was prolonged and evidence of minor haemolytic crises was present there was considerable pigmentation of the tubular epithelium. In these cases the brown granular colouration was present but was dominated by an intense dark blue accumulation of substance in the cytoplasm adjacent to the nucleus. This change appeared similar to that referred to as 'mineralisation'.

In both sheep and cattle all animals which had lesions indicative of one or more haemolytic episodes also had the pancreatic lesions typical of zinc toxicity.

5.1.4 PATHOLOGY OF ZINC TOXICITY IN YOUNG RUMINANTS

In a pilot experiment and one major experiment the effect of zinc added to the milk of suckling-lambs was examined (Chapter 4.5). In these experiments no abomasal lesions were recorded but the expected pancreatic lesions were present along with kidney lesions not experienced in previous experiments with adult ruminants.

Macroscopic changes Pancreatic lesions similar to those recorded in adult sheep were seen. Pale firm pancreata with occasionally an irregular 'knobbly' serosal surface were present but the reduction in pancreatic mass was not conspicuous. The most prominent lesion in these lambs was seen in the kidneys which were swollen, pale and often distorted (Plates 5.35 and 5.36). The distortion appeared to be due to planes of tension which had restricted the outward movement of the swollen cortex. A fine white mottling was present on the subcapsular surface of some kidneys.

Microscopic changes The pancreatic lesions of zinc toxicity in lambs were similar to those observed in adult sheep. The necrotic and accompanying inflammatory changes of the pancreatic ducts were not seen and the interlobular fibrosis was much more prominent (Plate 5.37) than in adult sheep.

A range of pathological lesions was present in the kidneys of lambs suffering from zinc toxicity. Some degree of tubular dilatation was always present. The most prominent dilatation usually occurred locally in discrete segmental areas and extended right through the collecting tubules in the medulla to the outer cortex. In many cases a severe tubular degeneration occurred with the tubules showing varying degrees of deposition of a basophilic material, this material often showing an onion-like concentric ring structure (Plate 5.38). This change appeared to start as an intracellular accumulation of basophilic pigment (near the nucleus). As the concretion increased in size, the epithelial cells fragmented and the tubule and its lumen often appeared to be replaced by a mass of this material. Interstitial areas of mononuclear cell infiltration and radial zones of fibrosis were also present as well as occasional periglomerular fibrosis and thickening of Bowmans capsule (Plate 5.39).

5.1.5 OTHER LESIONS

RUMEN EPITHELIUM

Macroscopic changes In sheep drenched with zinc by drenching gun, in which severe abomasal lesions occurred, a change in appearance of ruminal epithelium was also observed. The epithelial surface appeared much smoother (Plate 5.40) than in the unaffected sheep and the rumen papillae and reticular folds were much reduced in size compared with controls. This was especially noticeable in the rumen papillae which in some sheep were very small (Plates 5.41 and 5.42).

Microscopic changes The main changes in the ruminal papillae which were reduced in size were seen in the stratified epithelium. The epithelium was thinner and less cellular. The stratum germinativum appeared normal as did the succession of layers through the stratum

granulosum to the stratum corneum which, although present showed keratinisation (Plate 5.43). In contrast, those sheep which received zinc by intraruminal intubation had a more cellular basal layer and all of the various layers of the epithelium were thicker. The stratum corneum had a more cellular appearance, the cells being less flattened than the small papillae and the keratinisation appeared less complete. There was a tendency for the centre of these cells to exhibit basophilia and the outer layer had a scalloped appearance due possibly to the loss of basophilic centre (Plate 5.44).

THYMUS CHANGES

Macroscopic changes All sheep which had severe abomasal lesions, or long-standing pancreatic lesions and had lost considerable weight during the course of experiments showed evidence of thymic regression. The thymus was small and reduced in weight and had a more grey colouration than seen in control sheep.

Microscopic changes When thymus regression was present the microscopic change was immediately detected by the presence of cleavages throughout the organ. Under higher powered magnification these areas were seen to be of a fatty nature and followed areas of regression of the cortex which in severe cases had all but disappeared leaving only the medulla containing Hassels corpuscles.

WHITE MUSCLE DISEASE IN LAMBS DOSED ZINC SULPHATE

Macroscopic changes In the pilot experiment, the zinc dosed lamb of a pair of twins developed classical lesions of white muscle disease. As well as the pancreatic and kidney lesions the longissimus dorsi and several muscles of the hind limb developed bilaterally symmetrical pale to white lesions. In addition there were chalky-white subendocardial plaques present in the cardiac ventricles and these were more prominent on the left side of the heart (Plate 5.45).

Microscopic changes In the lamb in which gross lesions of white muscle disease were seen the microscopic changes were typical of those seen in this disease. In skeletal muscle the striations were lost and the individual muscle fibres became swollen and distorted and more densely eosinophilic. In severely affected fibres the cytoplasm exhibited a basophilia and the areas between fibres contained numerous mononuclear cells.

Discussion

One of the most significant lesions in this series of experiments was the presence of abomasal lesions in sheep and cattle that had been dosed with zinc sulphate (and chloride*). All evidence pointed to the fact that the lesions were produced locally by the presence of zinc sulphate in the lumen of the abomasum. The reticular groove experiments suggest that zinc stimulates the reticular groove reflex and results in channelling of the drench into the abomasum. The distribution of the lesions towards the dependent area of the abomasal mucosa suggested a local effect and the results of the gastric pouch experiment indicated that zinc was not excreted preferentially into the abomasum from the vasculature. Solutions of zinc instilled directly into the abomasum (C.F. Ramberg, personal communication) by catheter cause similar lesions.

Zinc solutions are known to have a caustic action on tissues. Aqueous zinc sulphate solutions (5%) are used to infuse the nasal cavity producing mucosal degeneration and subsequently anosmia (Sieck and Baumbach, 1974). Parenteral injections cause local necrosis (B.L. Smith, unpublished results), dilute ionic zinc in solution caused necrosis in tissue culture (B.L. Smith, unpublished results; P.H. Mortimer, personal communication) and causes lysis of red blood cells. In addition the emetic action of zinc solutions is believed to be due to its action as a gastric irritant (Esplin, 1970). The caustic property of zinc sulphate is probably sufficient to account for the observed abomasal lesions. However, it has been suggested (Morgan, 1978) that

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*tested in one sheep and produced severe lesions in abomasum.

in man zinc-sulphate-induced gastric lesions may be caused by conversion of the zinc sulphate to the chloride by the gastric acid.

These lesions of zinc sulphate may be compared with those produced in rats (Nayfield *et al.*, 1976) by ferrous sulphate, a substance which also causes occasional gastric erosions in man (Nayfield *et al.*, 1976). They demonstrated similar gastro-intestinal lesions to those experienced with zinc sulphate but also observed intestinal infarcts. They suggested that ferrous sulphate produces its acute effects by systemic toxicity with direct gastro-intestinal damage perhaps also contributing. Although intestinal infarcts were not observed with acute zinc toxicity, sufficiently high serum zinc concentrations were recorded to make systemic zinc toxicity a likely contributing factor in acute zinc toxicity.

There are several likely consequences of this local necrotising effect of zinc sulphate in the abomasum. Firstly, severe deep erosions of the abomasal mucosa were always present in cases of severe acute zinc sulphate toxicosis which resulted in deaths. As previously suggested it is most likely that these lesions and possibly the resulting very high serum zinc levels contribute significantly to death. Secondly, such changes have been shown to be associated with an exacerbation of the pancreatic lesions, an increase in organ zinc concentrations and greater weight losses, all of which are associated with zinc toxicity. The higher serum zinc concentrations probably arise from a diffusion of zinc down a concentration gradient from the abomasal lumen across the damaged mucosa to the vasculature of the submucosa and contribute to the exacerbation of zinc toxicity. The damaged abomasal mucosa itself may contribute substantially to the weight loss experienced in zinc toxicity. A possible third consequence of the abomasal lesion is in the aftermath of zinc toxicity. No studies were made of the effect of chronic abomasitis or its aftermath on subsequent digestion but several distorted abomasa were observed in surviving sheep and with such reduced mucosal areas that it seemed very likely that the functional capacity of the abomasum may have been severely reduced.

The pancreatic lesions of zinc toxicity are the most often recorded. The pancreas appears to be the organ most sensitive to zinc toxicity and often it is the only recorded lesion. In this series of experiments it occurred in zinc sulphate, oxide, acetate and, to a lesser extent, EDTA toxicity. The grossly visible change in the pancreas takes two to three weeks to develop properly, partly because of the fibrosis, a process which takes time to develop. Thus in an acute case of zinc toxicity the pancreatic lesion was not obviously apparent at postmortem examination. In the experiment in which the sequential changes of zinc toxicity in the pancreas of adult sheep were studied it appeared (a) that the primary lesion was in the pancreatic duct, (b) that the subsequent distribution of the parenchymal lesions was lobular, (c) that the full development of lesions took two to three weeks, and (d) that resolution of the lesion and regeneration appeared to take place after zinc dosing ceased.

In severe long standing cases of zinc toxicity the pancreatic mass may be sufficiently reduced to be detected visually at postmortem examination. In such cases the pancreatic mass may be reduced to less than 25% of the original mass when compared with the pancreas of contemporary controls. In such cases these changes are usually associated with body weight loss or reduced weight gain when compared with controls.

The extent to which pancreatic lesions affect gain or maintenance of body weight is still uncertain although an examination of lesions produced by zinc oxide (where gut lesions are not present) gives some indication. Unless the prevalance and severity of pancreatic lesions is extensive in a group of zinc oxide dosed sheep there does not seem to be a very great effect on body weight. However a positive weight response to zinc may oppose any pancreatic dysfunction effect on body weight. Nevertheless the lack of body weight loss associated with minor pancreatic lesions supports the statement of Tiertz (1976) that over 50% of pancreatic acinar cells must be destroyed before decreased functional capacity of the pancreas can be clearly demonstrated.

Studies on the effect of zinc dosing on the pancreatic exocrine secretion of sheep were limited by small numbers of successful surgical

preparations. However there was evidence that the secretion of the pancreas was most reduced in the pancreas with severe fibrosis and loss of mass. The between-sheep variation of the effects of zinc was seen in measurements of both pancreatic damage and secretion.

Fat necrosis, either within the pancreas or in the adjacent abdominal fat, was a common finding in the early stages of the pancreatic lesion. This lesion is commonly associated with pancreatitis and is believed to be caused by leakage of pancreatic lipase from the damaged pancreas (Smith *et al.*, 1972).

Studies on regeneration were not specifically made in this series of experiments. However in several experiments nodular hyperplasia, suggestive of acinar regeneration, was encountered in pancreata with severe lesions of long duration. The pancreas is capable of regeneration (Tiscornia and Dreiling, 1965) but at a slower rate than in damaged liver (McMinn, 1976). The relative importance of pancreatic regeneration repairing the pancreatic lesions of chronic zinc toxicity is not known. While it appears that regeneration may occur, it is by no means certain that it occurs in all cases or that when it does occur, fully compensates for the loss of pancreatic function.

Pancreatic lesions with some similarities to those seen in zinc toxicity have been described in selenium deficiency of chickens and mice (Thompson and Scott, 1970; De Witt and Schwarz, 1958) and the lesion can be corrected by supplementation of the diet with selenite. The result of one experiment in the present series does not support the contention that the pancreatic lesion is caused by a zinc-induced selenium deficiency.

Similar structural changes to those seen in the pancreata of zinc poisoned animals are seen also in kwashiorkor and it has been suggested (Davies, 1948) that kwashiorkor is essentially a pancreatic disorder due to malnutrition. The fatty liver seen in kwashiorkor is also seen in pancreatectomised dogs supplied with insulin and in sheep with damaged pancreata in the present series of experiments. Chronic pancreatic insufficiency is recognised as a contributor to fatty liver.

While the pancreatic lesion may be the cause of the fatty liver seen in zinc toxicity other causes of hepatic fatty change can not be ruled out.

The macroscopic and microscopic lesions of sheep and cattle suggestive of the occurrence of one or more haemolytic episode are indistinguishable from those seen in chronic copper poisoning. Interestingly the haemolytic crisis of copper poisoning is more readily seen in sheep than cattle whereas in our experiment with zinc toxicity the haemolytic crisis occurs more readily and is more dramatic in cattle than in sheep. There were also differences between the microscopic liver lesions of sheep and cattle that had suffered from haemolytic crises. In the case of sheep the randomly distributed focal hepatic necrosis was indicated mainly by small foci of polymorphs, most frequent and severe in the areas of fatty change but distinct from the sometimes considerable area of periacinar hepatic necrosis of the acute haemolytic crisis. In cattle however, the randomly distributed areas of hepatic necrosis were much more prominent and surrounded by an inflammatory reaction. In severe cases lakes of haemolysed blood could be distinguished in the centre of the necrotic centre.

The occurrence of such similar hepatic lesions of the haemolytic crisis in animals suffering from both zinc and copper poisoning suggests that the pathogenesis of each has factors or mediators in common. The presence of haemolytic crises in sheep dosed with zinc sulphate is surprising in view of the fact that zinc administration has been shown to prevent the accumulation of copper in the liver of lambs and has been suggested for the prevention of copper toxicosis (Bremner *et al.*, 1976). This anomaly and the apparent reversal of relative susceptibility of sheep and cattle to zinc and copper induced haemolysis may give rise to hypotheses for the pathogenesis of these haemolytic syndromes.

The kidney lesions seen in zinc induced haemolysis of sheep and cattle are also similar to those seen in ruminants suffering from the haemolytic crises of chronic copper poisoning. Intracytoplasmic granules in the proximal convoluted tubules, tubular degeneration and necrosis and haemoglobin casts in dilated tubules are all seen in both the zinc

and copper (Gopinath *et al.*, 1974) induced haemolysis. Of the above the intracytoplasmic granules alone are seen in sheep in which the haemolytic crisis has not occurred. Gopinath *et al.* (1974) have suggested that the kidney lesions of chronic copper poisoning are not due entirely to the haemolysis and haemoglobinuria. Certainly in the case of both copper and zinc poisoning the aggregation of intracytoplasmic granules occurs before haemolysis. Other causes of haemolysis (e.g. the plant *Allium validum*, Van Kampen *et al.*, 1970) do not cause such dramatic tubular changes and it has been shown (Lowe, 1966) that exacerbation of minor nephrotoxic effects are produced when haemoglobinuria is produced. Ionic copper is certainly known to be cytotoxic and experience with tissue culture has shown that ionic zinc at very low concentrations (P.H. Mortimer, personal communication; B.L. Smith, unpublished) is the same. When given intravenously ionic zinc is severely nephrotoxic (Brocks *et al.*, 1977; B.L. Smith, unpublished) and a simple shift from bound to unbound or ionic zinc in the plasma or tissues without a change in total zinc concentration could be sufficient to explain many of the lesions of zinc toxicity.

In the suckling-lamb the kidney lesions are the most dramatic lesion of zinc toxicity and are different to those experienced in adult ruminants with zinc toxicity. The main difference lies in the appearance of intracytoplasmic basophilic concretions in the degenerating tubular cells and the intestinal nephritis and kidney-distorting fibrosis which appears to follow from this. The difference may be the result of more rapid absorption of zinc in young ruminants, as has been shown (Miller and Cragle, 1965; Ballou and Thompson, 1961), but age-related changes in kidney tubular function may also be a factor.

The other lesions of accelerated thymus regression, adrenal enlargement and rumen papillary atrophy are regarded as non-specific and therefore of less consequence in the pathogenesis of zinc toxicity. The acute thymus involution and adrenal enlargement are part of the well known syndrome (Selye, 1936) resulting from physiological or pathological stimuli and mediated via the cortico-pituitary axis (Dougherty, 1952). It is seen in numerous disease conditions, inanition and malnutrition and its presence in zinc toxicity may simply be an indication that a

"stress" situation involving corticosteroid release had developed. The syndrome (thymus and adrenal) appeared to be related to the reaction to zinc toxicity rather than to the particular dose rate of zinc compound.

Rumen papillary atrophy which is discussed in Chapter 4.2, appears to be a sequel to the inappetance caused by zinc sulphate when administered by drenching gun. One other possibility is that the change represents a shift away from the parakeratotic tendency exhibited in rumen epithelium of ruminants fed certain diets (Lavker *et al.*, 1969). However the change to smaller papillae, loss of nuclei in outer layers and greater keratinisation (away from parakeratosis) occurred in the group of sheep receiving zinc by drenching gun. In this group much of the administered zinc would have bypassed the rumen because of the stimulation of the reticular groove reflex. However if the change was brought about by elevation of zinc in the blood rather than in the rumen lumen then this alternative explanation of the pathogenesis of this lesion appears more valid.

5.2 PATHOGENESIS OF THE PANCREATIC LESION OF ZINC TOXICITY

Introduction

From the work of previous authors (Ott 1966c; Veghelyi *et al.*, 1952; Scott and Fisher, 1937; Soffietti and Bestetti, 1975) and the present series of experiments in which zinc salts were dosed to ruminants it is obvious that the most sensitive organ to zinc toxicity, at least in the adult animal, is the pancreas. One of the outstanding features of this zinc induced pancreopathy of sheep is the presence of a lobular distribution of sharply defined parenchymal lesions at different stages of maturity throughout the organ. Additional significant features are the presence of severe necrosis and inflammation of the pancreatic ducts and a lack of lesions associated with the islets of Langerhans.

In man pancreatic atrophy due to ischemia results in lesions of the endocrine as well as the exocrine elements of the pancreas. However, when pancreatic atrophy is due to duct obstruction the islets are spared

and the exocrine elements only are affected (Robbins, 1974). These facts together with the lobular nature of lesion (embryological development of the lobule follows the pattern of duct bifurcation) and the presence of the duct necrosis and inflammation has led to the hypothesis that the primary lesion in zinc induced pancreatic injury is the duct necrosis.

In order to confirm this, it was decided to conduct a separate experiment in which sheep were dosed a zinc salt over a period of time. Groups of these sheep were slaughtered and the pancreas examined at different times during and after the period of zinc administration in order to determine the sequential development of the pancreatic lesions.

Materials and Methods

Sixty 10 month old Romney cross wethers were grazed on ryegrass/clover pasture throughout the experiment. All sheep were weighed before the commencement of treatments and the allocation of sheep to treatment. A group of 18 sheep were selected so that their mean weight was equal to the mean weight of all sheep in the experiment and designated as non-dosed controls. The remaining 42 sheep were dosed by intraruminal intubation on Mondays, Wednesdays and Fridays of each week with 240 mg Zn as ZnO/kg body weight/dose, giving approximately a mean daily intake of 100 mg Zn/kg. Dosing was continued for four weeks. Enough zinc oxide for each sheep dose was preweighed according to the previous weeks body weight, it was mixed with 200 ml water, poured down the intraruminal tube and washed down with a further 100 ml water.

The sheep were weighed and bled for plasma zinc analysis (Appendix A) on Fridays of each week. These weights and plasma concentrations (except on the first occasion) were used to select six sheep of the dosed group for slaughter on the subsequent Monday. Each six sheep for slaughter were selected so that their mean weight change and serum zinc concentrations were the same as that for the whole dosed group. The zinc dosed sheep were slaughtered after 4, 7, 14, 21 and 28 days of dosing and at four and twelve weeks after the end of the dosing. Six control sheep (selected similarly to the zinc dosed sheep) were

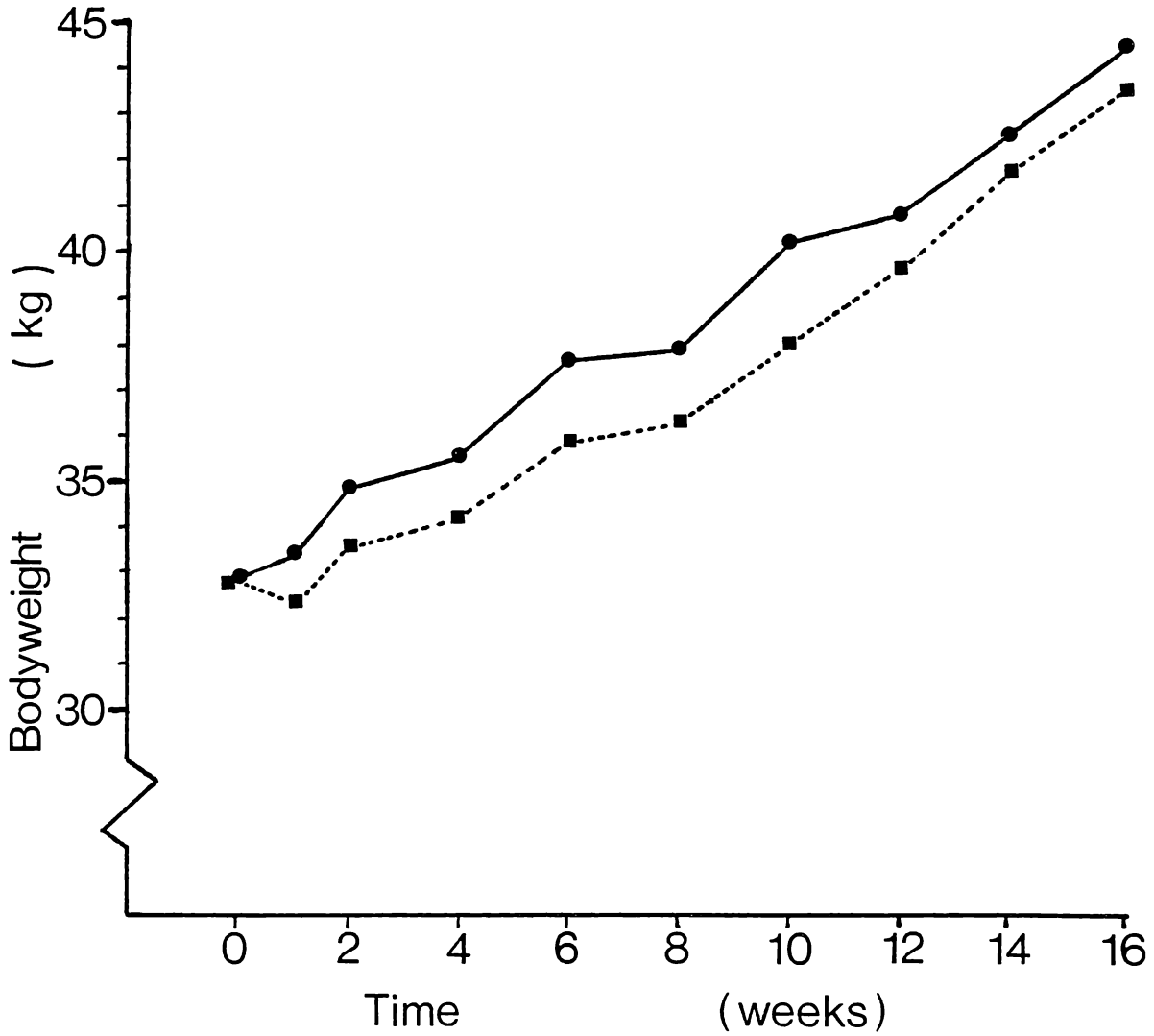


Figure 5.1 : Effect on bodyweight of intraruminally administered zinc oxide (approx. 100 mg Zn/kg b.wt/d) given thrice weekly to sheep for four weeks. Control o —— o; zinc oxide ■....■ .

also slaughtered at 4 and 28 days and 16 weeks after the start of dosing.

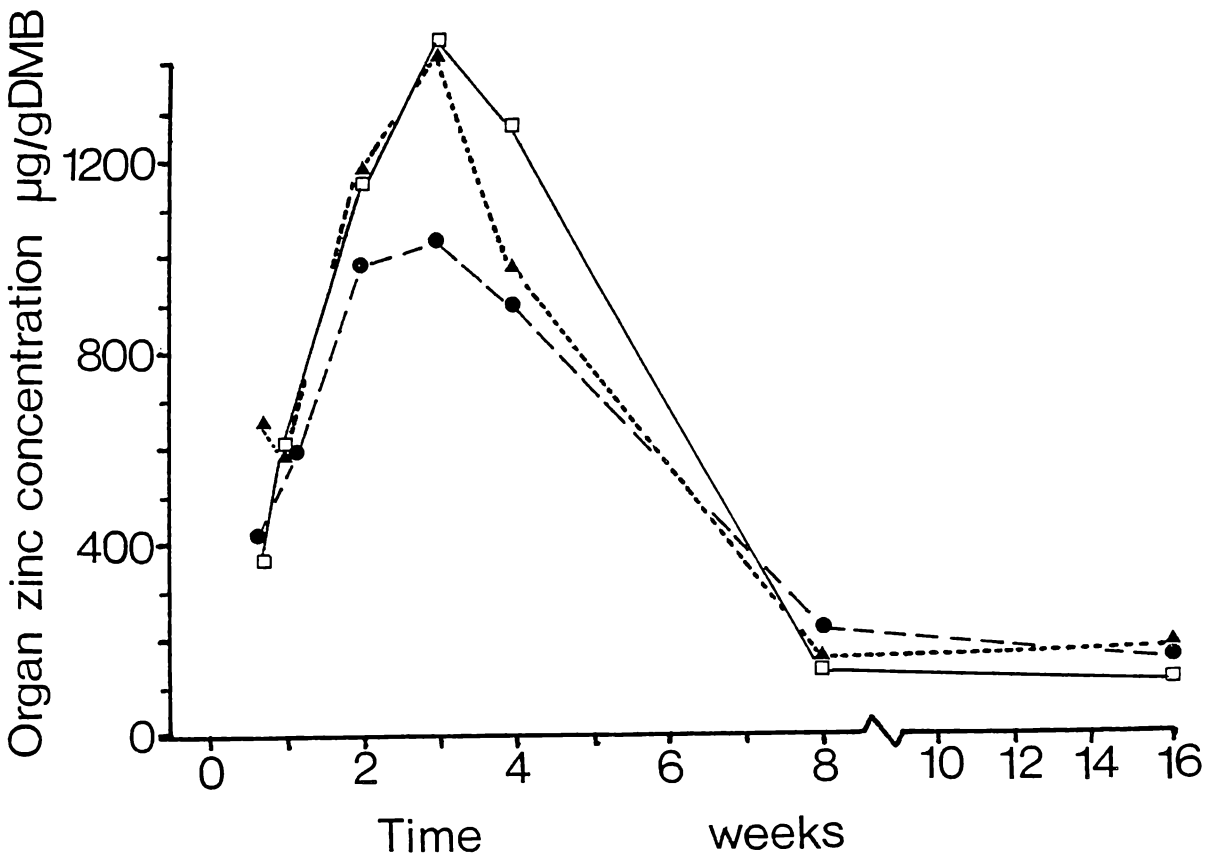
At slaughter, organs were examined and liver, kidney and pancreas tissue samples taken for zinc analysis (Appendix A). The pancreas was weighed and two separate samples taken for microscopic examination (Appendix B).

Results

No clinical signs were observed in any of the sheep dosed with zinc oxide except for a lower but statistically insignificant weight gain (Fig 5.1) recorded for those dosed sheep. Plasma zinc concentrations in the zinc dosed sheep were elevated from four days onwards until the end of the dosing period (Fig 5.3). By two weeks after the end of dosing the zinc concentrations in these sheep had declined to concentrations not significantly different from the control sheep ($p>0.05$).

Zinc concentrations in the liver, kidney and pancreas of zinc dosed sheep rose progressively over the first three weeks of zinc dosing and then declined reaching a level not significantly different from controls before four weeks after the cessation of dosing (Table 5.1; Fig 5.2). In the control sheep there was a slight but very significant ($p<0.001$) rise in serum zinc to the end of the dosing and then a sharp decline over the next week to previous levels. At postmortem examination pancreatic lesions were observed from day seven onwards and very occasional changes in liver and kidney were also seen. No abomasal lesions were seen.

In general it appeared (Table 5.1) that sheep with higher serum and organ zinc concentrations tended to have a greater amount of pancreatic damage although it was obvious that neither was a reliable indicator of the extent of pancreatic damage.


Organ zinc concentration µg/gDMB
1200
800
400
0
0
2
4
6
8
10
12
14
16
Time
weeks

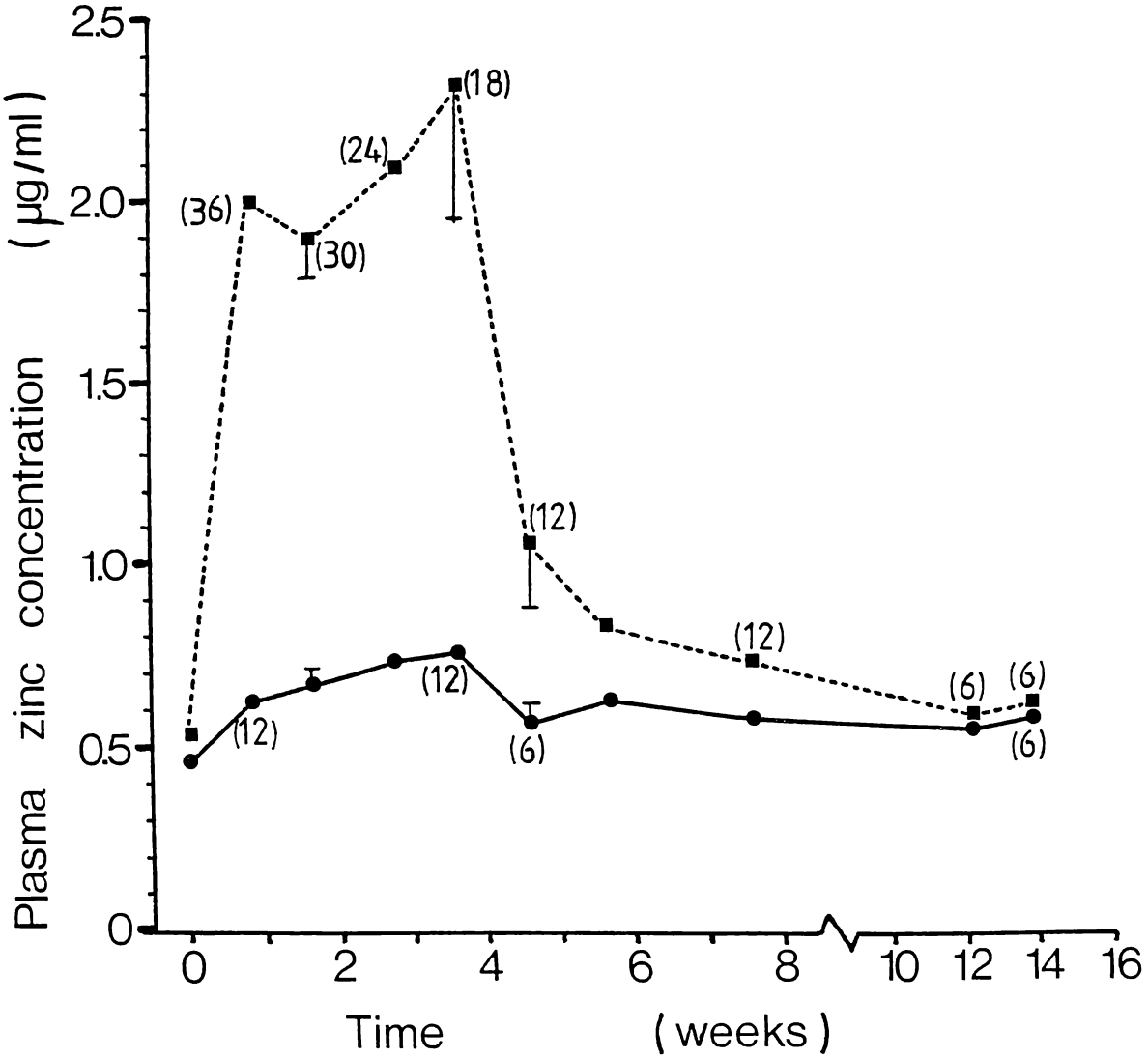

Plasma zinc concentration (µg/ml)
2.5
2.0
1.5
1.0
0.5
0
(18)
(24)
(36)
(30)
(12)
(12)
(12)
(6)
(6)
(12)
(6)
(6)
0
2
4
6
8
10
12
14
16
Time
(weeks)

Figure 5.2 : Organ zinc concentrations (±SEM) in sheep dosed with 240 mg Zn (as ZnO)/kg b.wt thrice weekly for four weeks. Control values for the same organs of non-dosed sheep slaughtered at 4, 28 and 112 days all lie within the 100-180 μg Zn/g DMB range. Pancreas ▲.... ▲; Liver ● ---- ●; Kidney □——□ .

Figure 5.3 : Plasma zinc concentrations (±SEM) in sheep during and after four weeks of thrice weekly dosing with zinc oxide (240 mg Zn/ kg b.wt/dose). Controls ● —— ●; zinc oxide ■----■ ; number of sheep at each bleeding shown in parenthesis.

Table 5.1 Serum and organ zinc concentrations and pancreas injury scores in individual sheep after being dosed with 240 mg Zn (as ZnO) /kg b.wt thrice weekly for up to four weeks.

Day	Sheep No.	Serum Zinc#	Organ zinc concentration µg Zn/g DMB			Pancreas score
			Liver	Kidney	Pancreas	
14	100	1.9	918	1580	1710	1
	171	1.8	904	1450	931	1
	193	1.7	709	1020	1130	0
	195	2.1	1280	799	1290	0
	211	2.9	1360	1510	1400	9
	259	1.0	603	598	598	0
21	361	2.3	1559	1399	3028	6
	184	2.4	1113	2109	2139	8
	311	1.9	1158	1160	1097	9
	268	2.0	672	1167	714	0
	271	2.3	966	1436	455	2
	260	2.6	917	1461	1203	4
28	183	2.5	881	1410	1280	8*
	200	0.9	813	884	753	6
	205	2.0	839	787	1040	6
	198	1.6	586	719	516	1
	335	2.9	1611	2920	1730	8*
	381	1.4	671	936	527	4
56	289	2.0	264	139	189	0
	295	3.8	341	490	335	6*
	223	1.6	178	114	128	0
	307	1.5	169	95	214	0
	252	2.3	225	117	143	3*
	226	1.9	135	112	117	3
112	322	2.6	162	183	208	2*
	167	1.4	166	114	90	0
	177	1.3	146	107	146	0
	194	2.1	147	120	255	0
	270	1.6	144	115	182	0
	280	3.6	243	140	324	2*

#Serum values are the mean of weekly serum zinc concentrations (µg Zn/ml) up to the sooner of the elected slaughter date or to 28 days;
*pancreas markedly reduced in size at slaughter.

LESIONS RECORDED IN PANCREAS AND OTHER ORGANS

DAY 4

Macroscopic - no lesions were seen

Microscopic - no lesions seen

DAY 7

Macroscopic - in three sheep a very few white opaque 2-5 mm well defined patches were seen on the serosal surfaces.

Microscopic - no tissue changes were seen in two sheep. In the other four sheep pancreata there were changes seen associated with the pancreatic ducts only. These were dilated in one sheep and in the others the ductular epithelium showed degenerative changes. The epithelium cytoplasm stained a darker red, the cells were slightly shrunken, intercellular differentiation was non-existent or indistinct and the nucleii failed to stain and were indistinguishable (Plate 5.46). In one sheep the epithelium on one side of the duct appeared completely necrotic and disorganised and an adjacent area of leucocyte infiltration was evident (Plate 5.47). Occasional areas of interlobular fat necrosis were present. No intralobular parenchymal changes were present.

DAY 14

Macroscopic - one pancreas was normal and three others showed white patches (1-5 mm) throughout. Oedema was present throughout or about the edges of two pancreata and two were pale and firm, some of these (with oedema) appearing swollen.

Microscopic - all pancreata had at least some change ranging from occasional necrotic ducts with and without periductular inflammation but with no parenchymal changes, to severe necrosis of the ductal epithelium and parenchymal

changes (Plate 5.48). The parenchymal changes consisted of a vacuolation of the acinar cells, dilatation of the acinar lumens to give a cystic appearance, invasion of the intralobular stroma by polymorphonuclear and mononuclear cells and the occasional presence of polymorphs and debris in the acinar lumen. In a very few of the lobules in which the cystic and inflammatory changes were evident there was some early fibroblast activity. Occasional areas of interlobular exudate were present and seen as a homogeneous moderately eosinophilic material.

DAY 21

Macroscopic - one pancreas appeared normal. Three pancreata had small sized white plaques (1-2 mm) covering one to three quarters of the serosal surface and two pancreata had severe changes in which the entire cut and uncut surfaces appeared very pale and firm. Oedema was present in two pancreata and was seen as gelatinous thickenings of the interlobular septa.

Microscopic - no changes were present in one pancreas. In the remainder, lesions varied in degree of severity and extent. Twenty to ninety percent of section area from sheep with lesions in their pancreata showed lesions at various stages of maturity. A lobular pattern of lesions was most evident with sharp contrast between adjacent lobules often being seen (Plate 5.49). The parenchymal acinar cells appeared to undergo a vacuolar (finely foamy) change and had less stain affinity. In some pancreata both intra and interlobular fibrosis was apparent. Some interlobular fat necrosis was also present (Plate 5.50).

DAY 28

Macroscopic - all pancreata exhibited some changes varying from the presence of 3-8 mm opaque white patches covering one quarter of the serosal and cut surfaces with oedema to the whole pancreas appearing pale very firm and shruken in appearance. Two kidneys were a much darker brown colour than is usual.

Microscopic - pancreatic lesions ranged from slight with only occasional lobules affected, to severe with almost the entire pancreas affected. In these cases the lobular pattern was most evident (Plate 5.51). A whole range of lobular changes was seen, the range being evident between lobules rather than within individual lobules where the stage of the lesions was remarkably even. Even in the most severely affected pancreata some lobules showed no changes or early parenchymal changes, with polymorph invasion, cystic dilatation of acini and loss of cytoplasmic stainability and architectural disruption while adjacent lobules showed the most severe changes of inter and intralobular fibrosis, complete loss of normal acinar tissue, severe duct lesions (Plate 5.51) and duct proliferation in the fibrous tissue mass.

DAY 56

Macroscopic - pancreatic lesions varied from none (two sheep) to very severe with the entire pancreas very small, pale, almost white, and firm with a smooth even surface. No oedema was present.

Microscopic - No pancreatic lesions were seen in three sheep. In two sheep only occasional islands of abnormal tissue were seen comprising mainly discreet nests of proliferating pancreatic ducts interlaced with fibrous tissue. In one pancreas these islands of fibrous tissue contained nests of both proliferating ductular tissue and areas of normal acini, giving the appearance of regenerating tissue. However no increased numbers of mitotic figures could be identified.

Table 5.2 Summary of histological lesions seen in sheep pancreata at different times after the start of zinc oxide dosing.

Pathological change	Days after start of 28 day dosing period						
	4	7	14	21	28	56	112
Duct necrosis & inflammation	-	++	+++	+++	+++	++	-
Fat necrosis	-	+	+	++	-	-	-
Oedema	-	-	+	++	++	-	-
Parenchymal necrosis & cystic change	-	-	+	+++	+++	++	-
Fibrosis	-	-	+	++	+++	+++	+
Duct hyperplasia & regeneration	-	-	-	-	-	+	+++

- no changes seen; +, ++, +++ mild moderate extensive changes seen respectively.

DAY 112

Macroscopic - three sheep had normal pancreata and two pancreata were reduced in size, pale and firm but had an irregular feel and appearance. The serosal surface had a nodular appearance. The remaining pancreas contained one discreet 1-2 cm firm lump.

Microscopic - No pancreatic lesions were seen in four sheep. In two there was an occasional area of fibrous tissue and duct hyperplasia, containing islands of normal acinar cells. Parenchymal tissue which covered nearly the whole section area, all appeared normal. While no mitotic figures were obvious it appeared in relation to changes seen in the pancreata of previously slaughtered groups, that there had been a resolution of damage and possibly considerable regeneration of parenchyma.

CONTROL SHEEP

Groups of six undosed sheep were killed on days 4, 28 and 112. No significant macroscopic or microscopic lesions were observed in any of these pancreata.

At each slaughter date there was considerable variation in the extent and severity of pancreatic lesions between the sheep of groups which received zinc oxide. However there were histological changes which were present at certain times but not at others. A summary to show the sequence of these changes, together with an indication of the extent of the changes, is given in Table 5.2.

Discussion

Embryological development of the pancreas arises from out-pocketing of the endodermal lining of the gut (Arey, 1954). The elongated axial duct bifurcates and the acinar tissue differentiates from side buds of the ducts. Connective tissue from the mesenchymal bed ensures the development of the lobular structure. Thus any injury resulting

in occlusion of the pancreatic ducts results in changes in the lobule or lobules draining into the damaged duct. This would be especially true in sheep where only a single duct drains the pancreas. In the case of experimental duct occlusion this pattern of lesion is produced. The early development of duct lesions seen in this experiment before the advent of the lobular parenchymal changes strongly supports the contention that the primary lesion in the zinc induced pancreopathy of sheep is pancreatic duct necrosis. Necrosis of the duct epithelium and periductular inflammation was present at seven days after the start of zinc administration and at 14 days occluded ducts, together with lobular distribution of parenchymal changes, were also present. The lack of any observed changes in the islets of Langerhans also support this hypothesis. If the pancreatic changes had resulted from vascular changes, which incidentally were not seen, then lesions of the islets might have been expected, judging from the pancreatic lesions observed in ischaemic pancreatic disease of man.

It is most likely that the periductular inflammation occurs in response to the epithelial necrosis but the cause of the initial duct damage is not known. It is known that zinc concentrations in the pancreatic juice can be high after zinc administration. Gooden and Grace (1976) record a greater than five fold increase in zinc concentration in pancreatic juice after administration of a 19 mg Zn/kg daily drench of zinc sulphate. However the relevance of high concentrations of zinc in pancreatic juice to the incidence of pancreatic duct damage is unknown. There is also a large increase in zinc concentration in pancreatic tissue and it is possible that the duct occlusion may exacerbate this. However large increases in zinc concentration also occur in the liver and kidney without any visible lesions occurring.

Serum zinc concentrations rapidly reached a peak at five days after the start of dosing and stayed at that level until zinc administration ceased. Organ zinc concentrations increased gradually to peak at 21 days and declined thereafter. Similar findings have been reported by Miller *et al.* (1978).

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS IN RELATION TO FACIAL ECZEMA

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS ON ZINC TOXICITY IN RELATION TO FACIAL ECZEMA

6.1 TOXIC EFFECT OF ZINC

Because the dose rates of zinc compounds required to prevent facial eczema are so high they do not fall far short of those which will cause zinc toxicity and other problems resulting from the use of zinc. The narrowness of the safety margin has been shown to depend on the form of zinc used, the length of time over which daily doses are maintained and the method of administration.

In the case of zinc sulphate the drenching gun concentrations necessary to deliver the dose of zinc required for facial eczema control are such that they cause reflex closure of the reticular groove. The resulting concentrations of zinc sulphate (or chloride) in the abomasum cause corrosive abomasitis and an exacerbation of zinc toxicity. Increases in volume aimed at avoiding either this problem or the requirement for daily drenching exceed the capacity of most drenching guns and appear to result in greater abomasal damage. The safety margin for the use of zinc sulphate by drenching gun ranges from two or three fold in the short term (over a few days) to no safety margin when dosing in long term (over several months).

The alternative method of administering zinc sulphate, by adding it to the drinking water of ruminants has other problems. The method applies only to cattle for sheep are known to be at best only sporadic drinkers of trough water and in the hill country situation, they are, for practical purposes inseparable from alternative water sources (e.g. seepages, creeks, ponds).

In the intensive dairy cattle grazing situation medication has been applied via drinking water (e.g. bloat prevention) and this method has been investigated with zinc sulphate. There are two main problems inherent in this method. The first is that daily intakes of drinking

water vary considerably from animal to animal. The variation has been shown to be up to four fold (Wright *et al.*, 1978) a situation which could allow some cattle to receive insufficient zinc to significantly prevent facial eczema while some receive double the required dose. Secondly it has been shown that rain or a new allocation of fresh pasture causes drinking water consumption to fall (Wright *et al.*, 1978; Chapter 3.2.2). This is a more serious problem as it has been shown that adequate zinc intakes are required during facial eczema 'danger periods' (when spore counts are high) and acutely toxic periods are known to occur most often after periods of rain. Attempts to overcome this decline in zinc intake via drinking water by temporarily raising zinc concentration have demonstrated that unpalatability of drinking water occurs causing a further decline in water and zinc intake. Theoretically, zinc sulphate medication via drinking water does not seem a viable proposition. However a field trial to test this method of controlling facial eczema has yet to be conducted.

Zinc oxide administration by paste gun and pasture spraying has a greater safety margin than does zinc sulphate. In zinc oxide toxicity there have been no lesions recorded in the gastrointestinal tract except those in the pancreas. Long term administration of zinc oxide via pasture spraying has been shown to cause pancreatic damage at double the intake which caused significant reduction of facial eczema liver injury (Smith, 1977). However the pancreatic lesions were minor and other trials with paste guns have shown that the safety margin for zinc oxide is approximately four fold in the short term (up to one week) but may be less in the long term (several months). Zinc oxide toxicity lesions appear to be confined to the pancreas and do not have a very big effect on body weight gains.

The zinc EDTA form has been shown to be much less toxic than either the sulphate or oxide and despite the fact that a considerable amount of zinc is excreted in the urine when this form is used it has also been shown to prevent facial eczema (N.R. Towers, personal communication). Unfortunately this form of zinc has the disadvantage of greater cost. For this reason greater research emphasis has been placed on the use of zinc oxide.

A further risk involved in the administration of high dose rates of zinc is its effect on the metabolism of other minerals. It has been shown that zinc interferes with the metabolism of copper (N.R. Towers, personal communication) and selenium (Chapter 4.6). In the case of selenium the risk of white muscle disease is lessened by the fact that zinc would be administered in the autumn and hence very young susceptible ruminants would not be at risk. Possible selenium responsive infertility of ewes on selenium marginal areas is a greater risk but this should be avoidable by ensuring that the selenium status of such animals is adequate before zinc dosing.

6.2 SIGNIFICANCE OF THE LESIONS AND EFFECTS OF ZINC TOXICITY

The most severe and devastating lesion of zinc toxicity recorded in these experiments has been the abomasitis associated with the drenching gun administration of concentrated zinc sulphate solutions. Severity may range from oedema or mild local corrosive lesions associated with lowered body weight gains to severe local or generalised corrosive necrosis penetrating the mucosa and often leading to the death of the animal. All forms of abomasal damage result in exacerbation of the pancreatic lesion of zinc toxicity and much higher concentrations of zinc in serum and offal organs. Ruminants surviving such lesions have been seen to have abomasa with reduced mucosal surface area and although the effect of this residual effect has not been formally investigated it probably has some deleterious effect on food conversion efficiency.

The significance of the reductions in size of ruminal papillae which accompanied abomasal damage and the disturbances in the rumen microflora, which are also known to occur (Martinez and Church, 1972), is not known. Such changes are thought to be reversible and the rumen probably returns to normal function after zinc sulphate dosing ceases.

The pancreatic lesions of zinc toxicity occurred with all the zinc compounds tested in these experiments and it appears that the pancreas is the organ most sensitive and indicative of zinc toxicity. The effect of this pancreatic change on ruminant health and productivity

does not appear to be as great as the abomasal lesion. Weight changes associated with chronic zinc oxide toxicity, which appears only to produce pancreatic lesions have been significant, but are not as dramatic as with zinc sulphate. It is possible that zinc oxide produces the body weight changes through changes in rumen metabolism as was shown by Ott *et al.* (1966c). The effect on pancreatic secretion is seen mainly as a reduction in secretion flow rate and concentration of protein but is only dramatic when severe damage to the pancreas is evident. The capacity of the pancreas to recover or regenerate after zinc induced injury has not been studied extensively in these experiments but there has been some evidence (Chapter 5.2) that the organ does regenerate. The pancreas is known to be capable of regeneration (Tiscornia and Dreiling, 1965).

Not all weight changes associated with zinc oxide toxicity have been deleterious. In one experiment (Chapter 3.1.2) zinc oxide dosed sheep showed weight gains which were significantly greater than those of control sheep despite the occurrence of moderate pancreatic lesions. In these sheep there was evidence of a change in fat metabolism (greater back fat thickness) but this was correlated with the greater weight of the sheep suggesting that it was due to the greater weight of these sheep. However more recent results (B.L. Smith, unpublished results) showed that a significantly greater back fat deposition occurred in zinc oxide dosed sheep which had minimal pancreatic damage. There was no difference from controls in body weight or carcass weight of the zinc dosed sheep. These results suggest that the greater fattiness of zinc oxide dosed sheep is neither related to the greater body weight of each group nor to some pathological manifestation of the pancreatic change. These latter findings were in lambs and did not result in a fat thickness which would have resulted in a down grading of the carcass.

The haemolytic syndrome recorded in several experiments was only recorded when zinc sulphate was administered by drenching gun and appeared to occur more readily in cattle than in sheep. This is a reversal of the susceptibilities of the two ruminants to the haemolytic crisis induced by copper. This syndrome constitutes a further

risk in zinc sulphate administration by drenching gun when doses higher than prophylactic are given in the short term or when prophylactic doses are maintained for a prolonged period of time (e.g. greater than two months). The risk of this syndrome occurring when zinc is added to the sole drinking water of cattle does not appear to be as great. It did not occur when zinc sulphate was added for nine weeks to the drinking water of cattle at concentrations high enough to cause unpalatability.

The kidney lesions (separate from those associated with the haemolytic crisis) which only occurred in young suckling-ruminants when zinc was added to their milk are not considered to be of any relevance to the autumn administration of zinc for facial eczema control. Ruminants this young (even autumn born ruminants) are not at risk to facial eczema and should not be exposed to zinc for the control of facial eczema.

High concentrations of zinc in the offal organs, liver, kidney and pancreas are a potential problem because of the limitations (40 ppm) imposed by the New Zealand Food and Drug Regulations 1973. There is no evidence that the zinc concentrations found in these organs during these experiments can cause any untoward effects in man and the problem can be overcome by withholding stock from slaughter for a suitable period after the end of zinc dosing. However this would not be a practical solution in the case of milk. High zinc concentrations in milk could also be of considerable significance especially where dried milk products were made from such milk. The values given (Fig 3.9) for whole milk are within the allowable maximum for food products. However when converted into a dried product many of these mean values will exceed this limit.

6.3 ACCEPTABILITY OF SAFETY MARGIN AND FUTURE OF ZINC FOR THE CONTROL OF FACIAL ECZEMA

In medical pharmacology and therapeutics the safety factor for various agents is referred to as the 'therapeutic index'. This is generally defined as the ratio of the median toxic dose to the median effective dose although some authorities prefer to use the median lethal dose rather than toxic dose. Some authorities insist on the more conservative ratio of minimal toxic dose to maximal effective dose, a ratio which, in the case of zinc for facial eczema control, would indicate virtually no safety margin at all.

It should be noted that median toxic dose (or any other measurement of toxicity) for most substances can have many values. In the case of zinc this would depend on many variables such as the form of zinc, method of administration and especially the type of toxic effect produced. Pancreatic lesions are common to all forms of zinc investigated in these experiments and, in the absence of more severe acute effects (such as the abomasal lesions) can be regarded as a useful monitor of zinc toxicity. As has been stated many other variables influence the 'therapeutic' (or more appropriately 'prophylactic') index and it has become most obvious that any meaningful ratio must only be quoted for a well defined situation surrounding the projected use of zinc. These experiments have given a rough guide for certain forms and uses of zinc, enough to exclude certain dosing regimes and indicate that all future experiments for testing prophylactic regimes should include groups receiving dose rates several times higher than those known to cause protection.

What then is a satisfactory safety margin for the use of zinc for facial eczema control? When toxicity and therapeutic testing is carried out on the same species of animal for which the drug is intended a therapeutic index of 10 is generally regarded as adequate. The therapeutic index for oral barbiturates is 10 but in the case of cardiac glycosides is only 3. When the toxic effects are an extension of therapeutic effects rather than side effects then, as in the case of cardiac glycosides, the therapeutic index is more meaningful. Not only must therapeutic and toxic effects be compared but the

relative importance of the two effects must also be considered and a professional judgement made using the available evidence. It is for reasons such as this that the Animal Remedies Board in New Zealand and equivalent bodies in other countries judge each case on its individual merits.

It seems then that the safety factor for the use of zinc sulphate by drenching gun (approximately two) is unsatisfactory. The magnitude of the toxic effect (severe abomasitis or death)outweighs any protective effect exerted by zinc. The other compounds tested, zinc oxide (Therapeutic Index approx. 4) and zinc EDTA (>5) have a more satisfactory safety margin. Furthermore the pancreatic lesions caused by the toxicity of these compounds appear to be of a less serious nature than those of facial eczema. There is some hope then that these less toxic forms of zinc may have some future for the control of facial eczema. Control of facial eczema by zinc has a similar effectiveness to that of benzimidazole fungicides sprayed on pastures and zinc prophylaxis may be more convenient and cheaper if satisfactory administration methods are available. It allows farmers greater freedom to utilise whatever pastures are available.

Finally, whatever the regime suggested in the case of a recommendation favouring the use of zinc, spore counting to detect danger periods will still be necessary to schedule and especially avoid the prolonged use of zinc. Any proffered recommendation should incorporate strict guidelines and limitations on the use of zinc. These are especially important because of the freedom with which zinc salts are available for use by farmers.

APPENDICES

APPENDICES

APPENDIX A

PREPARATION OF SAMPLES AND METHOD FOR ZINC ANALYSIS

Tissue collection

At postmortem examination particular care was taken to avoid contact of organs with gut contents or brass ear tags. Where possible organ samples were obtained before organs were removed from the carcass or the gut opened. An approximate 20 g sample of tissue was excised from the required organ with a stainless steel knife or scalpel and placed in a labelled plastic bag and deep frozen at -10 ^{o}C.

In the case of certain organs a standard method or site of collection was adopted.

Liver Because of the common occurrence of facial eczema lesions in the ventral lobe of the liver a standard procedure was adopted of always selecting the tissue sample from the right border of the dorsal lobe between the gall bladder attachment and the umbilical fissure.

Kidney The sample taken for analysis was always wedge shaped to include a similar proportion of medulla and cortex on each occasion.

Pancreas Sample taken from body of pancreas rather than tail.

Preparation of tissue* for zinc analysis

Approximately 2 g of thawed frozen sample was finely chopped on a glass plate and thinly distributed in a tared vitreosil crucible and weighed (±0.1 mg) to obtain 'wet' tissue weight. The sample and crucible were then oven dried overnight at 105-110 ^{o}C for approximately 15 hours.

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*Milk samples (10 ml) were weighed into crucibles and treated as for tissues above.

After cooling to room temperature in a desiccator, the sample and crucible were reweighed to obtain 'dry' tissue weight.

The dried samples in open crucibles were loaded into a muffle furnace and pre-ashed by raising the temperature slowly in a stepwise fashion as follows:

200-220 ^{o}C	2 hrs
260-265 ^{o}C	2 hrs
360-375 ^{o}C	2½ hrs

Thereafter ashing was completed by overnight ashing at 550 ^{o}C. The remaining ash was cooled and weighed with the crucible. Into the crucible was added 3.5 ml of approximately 4.5 N HCl (conc. 'Analar' HCl diluted 1:1), covered with a watchglass and placed over a boiling water bath for one hour to extract the zinc from the inorganic ash.

The crucible contents and distilled water washings were transferred to a stoppered measuring cylinder and the final volume recorded.

Serial five- or nine-fold dilutions of an aliquot of the digestate were made using distilled water in order to obtain a zinc concentration within the range of 0.2 to 2.0 µg Zn/ml which is the required range for the atomic absorption spectrophotometry (AAS).

The zinc concentration in the appropriately diluted solution was determined using a Varian Techtron Model 1200 Atomic Absorption Spectrophotometer operated in the concentration and integrated read-out modes which had been calibrated on each occasion against working standards containing 0.4 and 2.0 µg Zn/ml.

The quantity of zinc recovered from each tissue sample was determined by the following calculation:

Zinc recovered (µg) = AAS readout (µg/ml) x dilution x digest volume (ml)

Working zinc standards were obtained by dilution of a stock standard, prepared by dissolving dried 'Analar' zinc oxide in 1% HCl (1 g Zn/ℓ). Solutions were prepared and dilutions made using single glass distilled water. Distilled water was used as a blank solution in the AAS. No zinc could be detected in the concentrations of acid aspirated during AAS. All glassware used was steeped in 10% HCl and rinsed in distilled water.

The above method was arrived at by consultation with D. Harrison and S.R. Solly, Meat Monitoring Laboratory, Wallaceville Animal Research Centre and by reference to a number of sources (Gorsuch, 1959; Micak-Devic, 1970).

Zinc analysis of body fluids (serum, plasma, urine, pancreatic juice, and bile)

Body fluids were prepared by simple serial five or nine fold dilutions with distilled water to establish a zinc concentration within the range of 0.2 to 2.0 µg Zn/ml. In the case of each fluid it was determined that the viscosity of the lowest dilution was not such that it reduced the flow rate of the aspirator nebuliser of the AAS. In the case of serum it was found that at five fold dilutions and above flow rate was not affected.

With serum two special standards were prepared so that the standard concentrations of zinc (0.4 and 2.0 µg/ml) were contained in a five fold dilution of sheep serum. The use of these standards remove any chance of errors due to possible nebulisation characteristics of diluted serum.

At the outset of this investigation this method had only been used for the determination of zinc in chicken plasma (Varian Techron Methods Manual). However it has since been found that other investigators (M. Chvapil, personal communication; Hackley *et al.*, 1968) also use this simple dilution technique for the determination of zinc in serum.

APPENDIX B

PATHOLOGICAL PROCEDURES

Autopsy methods

Postmortem examinations were performed in a number of circumstances. Complete postmortem procedures (Carter, 1962) were carried out on animals which died during the course of an experiment as soon as possible after death or immediately after slaughter *in extremis* (for humane reasons) or at the completion of the trial. Young sheep were slaughtered by rapid transection of the vital neck vessels and spinal cord and in older sheep and cattle by exsanguination after stunning with a captive bolt pistol.

Where there was any doubt about the cause of death, selected samples were submitted to the Ruakura Animal Health Laboratory. On nearly all occasions however the lesions or deaths were undoubtedly caused by the experimental treatment and the postmortem procedure was tailored to an examination of the effects of treatment, e.g. samples or organs for zinc analysis were always removed before the gut was incised or the viscera removed.

At the completion of some experiments the trial animals were sent to the Ruakura abattoir for slaughter. In these instances the abdominal and thoracic organs were removed complete from the carcass and examined. When required, the adrenal glands and kidneys were examined and samples taken before removal of viscera from the abdomen.

Histological procedures

Tissue samples were removed as soon as possible after death and small 3-5 mm thick slices of tissue placed in 10% buffered neutral formalin (4% formaldehyde) so that tissue volume did not exceed more than 10% of total volume. After a minimum of 48 hours tissues were processed to be embedded in paraffin wax. Sections were cut 5 µm thick with a rotary microtome and after mounting on glass slides, stained by haematoxylin and eosin. Occasionally other methods and stains were utilised as indicated in the separate Chapters.

APPENDIX C

MEASUREMENT METHODS FOR LESIONS

Method of recording pancreatic lesions

Samples of pancreas from the body of the organ were fixed in buffered neutral formalin, processed and embedded in paraffin block, sectioned and stained with haematoxylin and eosin.

On microscopic examination of the section a visual assessment was made of the percentage of area of pancreas affected by zinc toxicity. In damaged pancreata acinar cell necrosis, duct proliferation and fibrosis together with occasional islands of inflammatory cells were usually evident. In practise it was often easier to estimate the percentage of normal parenchyma remaining and take this from 100%. Estimates were made to the nearest 10% and to obtain a 'score' the percentage area damaged was divided by 10. A score of 0 indicated no damage and 10 total damage to the pancreas. This system was probably a conservative method of estimating damage as in the long term severely affected pancreata there was considerable shrinkage of pancreatic mass (to 25% of original mass remaining).

In early experiments it was found that sections taken from different regions of the pancreas gave similar pancreatic damage scores and it was found that a single section from the body of the pancreas was sufficient to give a reliable indication of damage. All estimates of pancreatic injury were made without knowledge of the treatment origin of the animal concerned.

Method of recording the severity of abomasal lesion

Abomasal damage was recorded by visual appraisal of the extent of the damage. As soon as possible after the death of an animal (usually sheep) the abomasum was removed from the abdomen by incising through the omasal/abomasal junction and just posterior to the pylorus, the omentum and fat from the serosal surface was removed and the abomasum opened by incising along the length of the greater curvature.

The mucosa was observed, wiped lightly with a hand and if necessary washed lightly with water. Where necrosis of the mucosa was present, the damage was scored according to the estimated percentage area of the mucosa affected by the zinc dose.

Abomasal damage scores were recorded on a 0 to 5 scale where 0 indicates no detectable damage, 1 less than 10% affected, 2, 10-20% affected, 3, 30-50% affected, 4, 50-80% affected and 5 more than 80% affected. Where oedema of the mucosa was present this was scored according to the estimated severity of the oedema where 1 represented mild, 2 moderate and 3 severe oedema of the mucosa.

All estimations of severity of abomasal damage, although subjective, were carried out without knowledge of the prior treatment of the sheep from which each abomasum was taken.

Measurement of ruminal papillae

An objective measurement of the size of ruminal papilla was taken from a piece of formalin fixed rumen wall.

A square of rumen wall (approximately 3 x 3 cm) was taken soon after death of the sheep from the centre of the anterior wall of the ventral sac of the rumen and placed in 10% formalin. Twelve random papillae were measured for length and greatest width and the means of each of these two measurements were multiplied to give a L X W product used as an indication of the size of the rumen papillae for each sheep. The measurement was carried out using a taper gauge with an accuracy of ±0.1 mm.

It has been shown (Moon and Campbell, 1973) that formalin shrinkage of papillae is about 3%.

APPENDIX D

HAEMATOLOGY METHODS

Blood samples for haematology were collected in evacuated glass tubes ('Venoject') containing a suitable anticoagulant (disodium ethylene diamine tetraacetate), immediately mixed with a gentle rocking action and the determinations carried out as soon as possible after collection.

Packed cell volumes (Haematocrit, PCV)

For packed cell volume determination blood samples from the rocking anticoagulant tubes were drawn into capillary tubes, heat sealed and centrifuged at 12 000 g for five minutes on an International Micro-capillary centrifuge, Model M8 and the microhaematocrit readings done on an International Micro-capillary Reader. Occasionally paired samples were read but it was always found that paired determinations were within 0.5% of one another.

Haemoglobin values

Haemoglobin determinations were carried out on the same blood sample haemolysed by an impregnated applicator stick. The haemoglobin concentration was determined using a comparator method incorporating a standardised glass wedge (American Optical Co.).

Total red cell counts

Red cell counts were made using a suitably calibrated electronic particle counter after dilution of the blood sample.

Red blood cell indices (standard ratios)

These were calculated by using the well established formulae (Archer, 1977) for mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC).

APPENDIX E

INTRAVENOUS GLUCOSE TOLERANCE TEST (Kaneko, 1970)

Sheep were not fasted prior to the performance of the intravenous glucose tolerance test. A sterile 40% w/v dextrose solution was injected into the jugular vein over a period of approximately two minutes.

Blood samples with a fluoride oxalate anticoagulant were collected in evacuated tubes ('Vacutainer') at 5, 10, 20, 30, 40 and 50 m and 1, 2 and 3 h after the intravenous injection. After centrifugation and separation, the plasma samples were deep frozen (-20 ^{o}C) to await glucose determinations.

The glucose analyses were performed by the Chemical Services Laboratory, Ruakura Animal Research Station, using an autoanalyser (Technicon) adaptation of the method of Hill and Kessler (1961) which involves the detection, by a dye, of the glucose oxidase oxidation of glucose.

APPENDIX F

STATISTICAL ANALYSES

Statistical evaluation of results was either carried out or checked by members of the Biometrics Section, Ruakura Animal Research Station.

For comparing the means from groups containing small numbers of animals Students 't' test was carried out according to the formula:

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{\Sigma(x - \bar{x}_1)^2 + \Sigma(x - \bar{x}_2)^2}{n_1 + n_2 - 2}} \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

For rapid routine analysis use was made of a programme written for a Sharp 'ELSI MATE' pocket computer PC-1201. For closer examinations, the data were checked to see that the variances of the groups did not differ significantly by calculating the variance ratio or F test and determining its significance by means of a two-tailed table of F values at the 5% significance level.

In addition the following statistical procedures were used as required:

Analysis of variance and/or covariance, F-test, Duncans multiple range test

These tests were used for comparing two or more sets of observations from different experimental treatment groups.

Non parametric tests e.g. Wilcoxsons two sample test

Used in comparing means when the parameters of the sample are unknown, i.e. the distribution of data is not known.

Goodness of Fit Tests e.g. Contingency table analysis χ^2 tests

Were used for problems involved in the classification of data into groups and comparing the observed and expected (theoretical) frequencies.

Calculation of Regression line equations and correlation coefficients

These calculations were used to deal with association between pairs or groups of variables and to show how changes in one particular variable are related to changes in other variables.

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PLATES

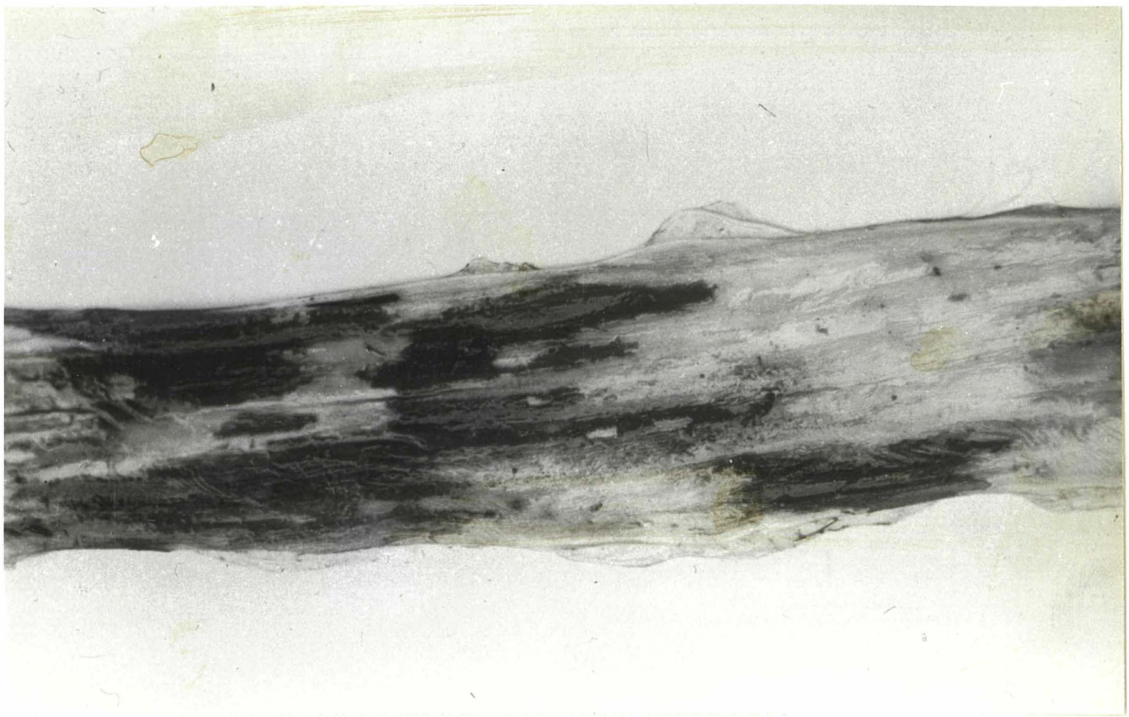


Plate 3.1 : Sheep oesophagus showing severe ulceration (dark areas) of mucosa in lower third of oesophagus after long term dosing with zinc sulphate.

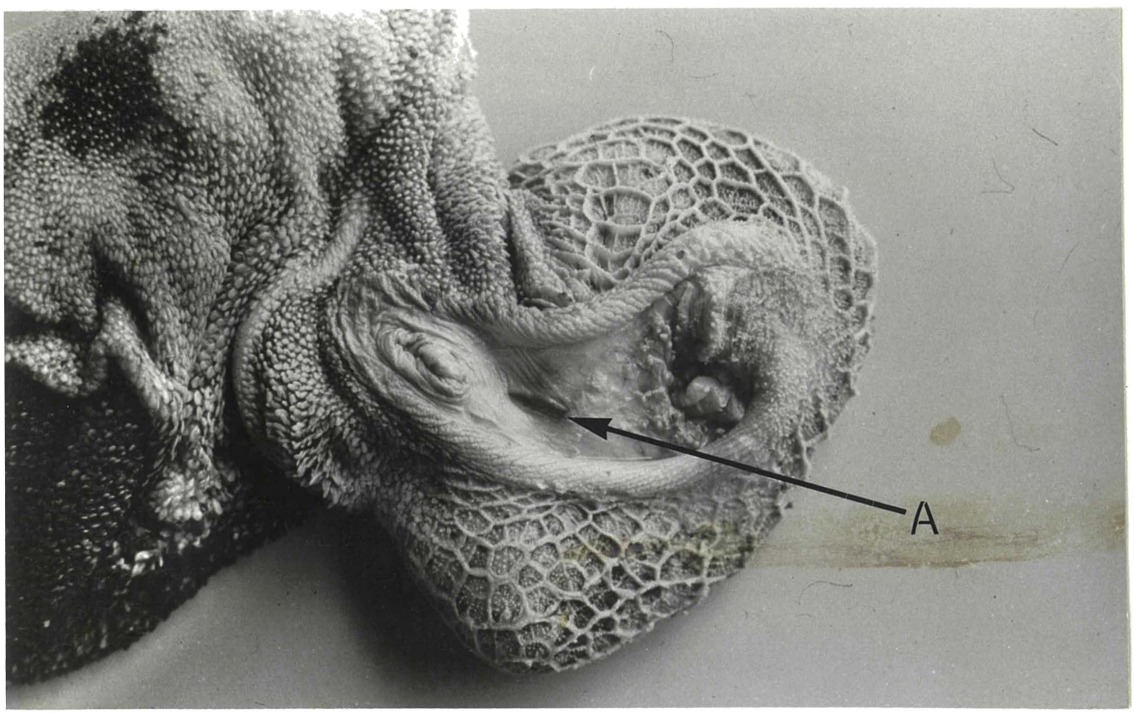


Plate 3.2 : Reticular groove of sheep showing ulceration (A) in a sheep drenched with zinc sulphate.

Plate 4.1 : Crystal violet staining of abomasum after drenching gun administration to sheep of a mixture of 20% (W/V) zinc sulphate and 0.4% (W/V) crystal violet. Staining score 5.

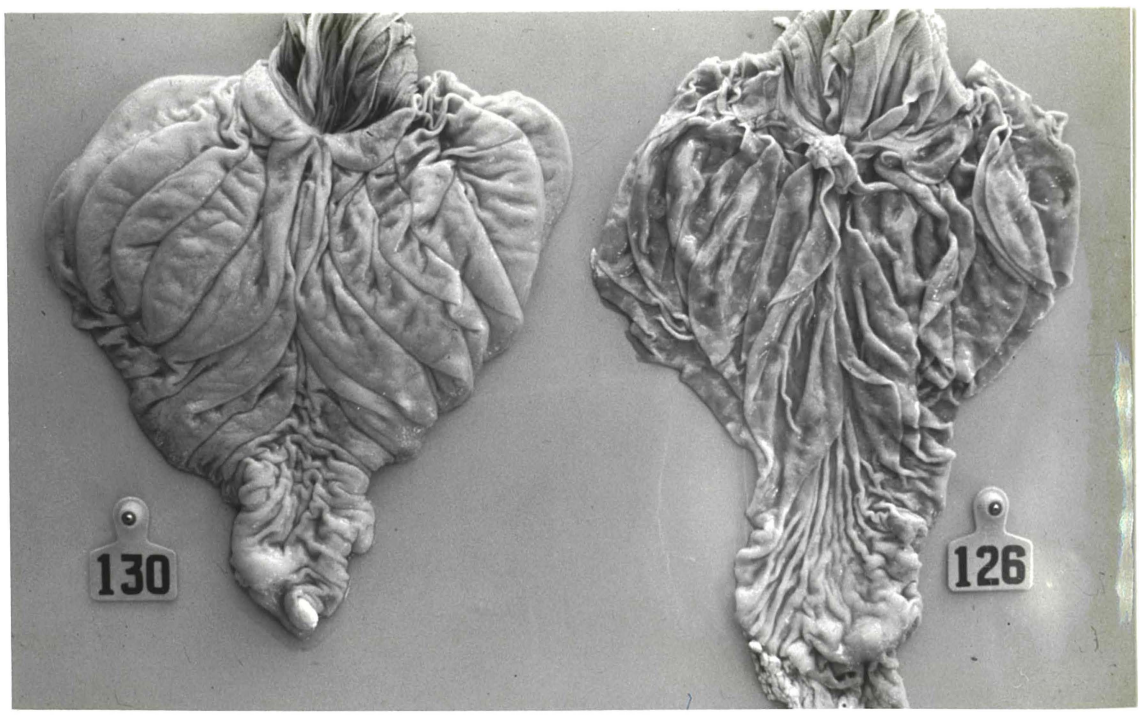


Plate 5.1 : Ovine abomasum (130) showing oedema of mucosa. Fundic folds are thickened and turgid with rounded edges. Normal abomasum (126) for comparison.

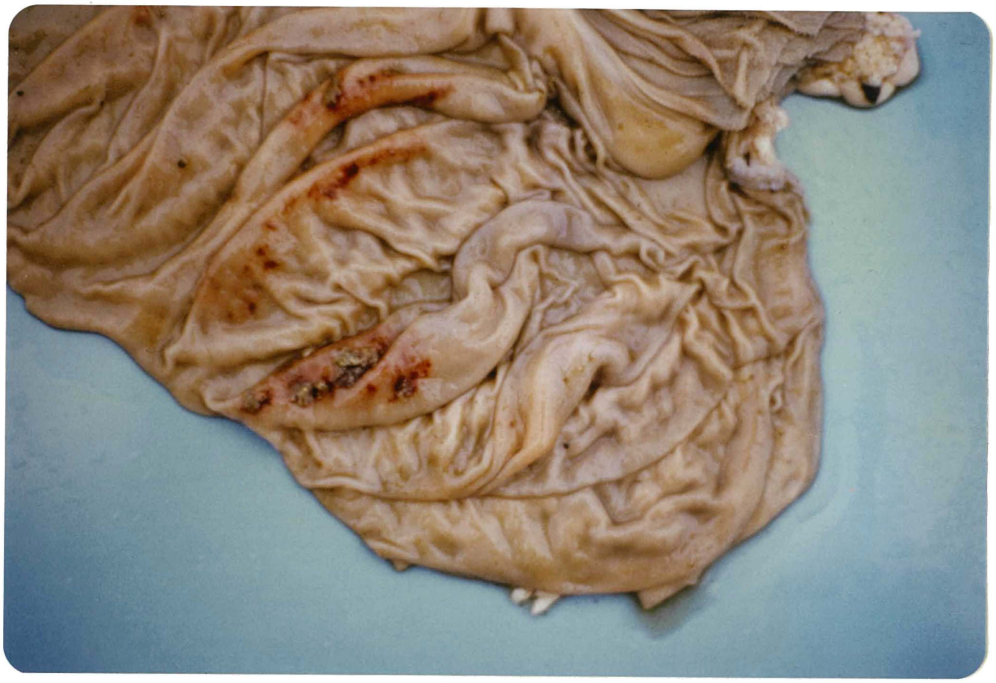


Plate 5.2 : Abomasum from sheep drenched with zinc sulphate. Focal necrosis and haemorrhage present on dependent parts of fundic folds.

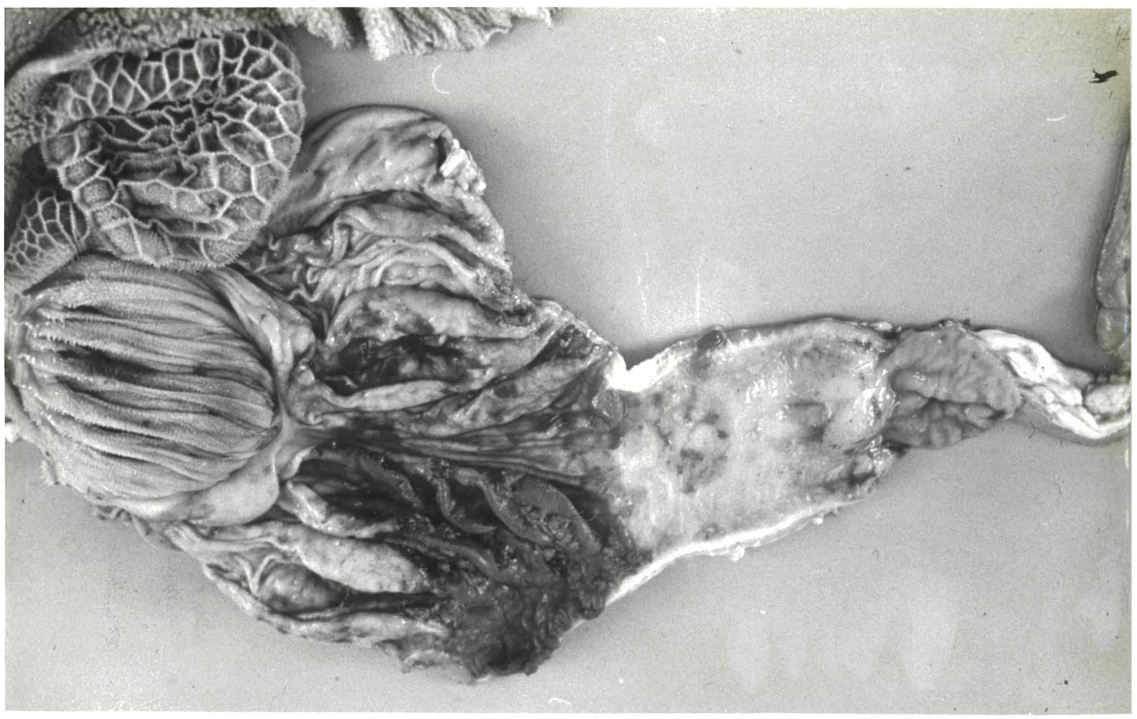


Plate 5.3 : Opened abomasum from a sheep dosed with zinc sulphate by drenching gun. The large haemorrhagic ulcerative area had almost perforated the stomach wall and was visible on the serosal surface.

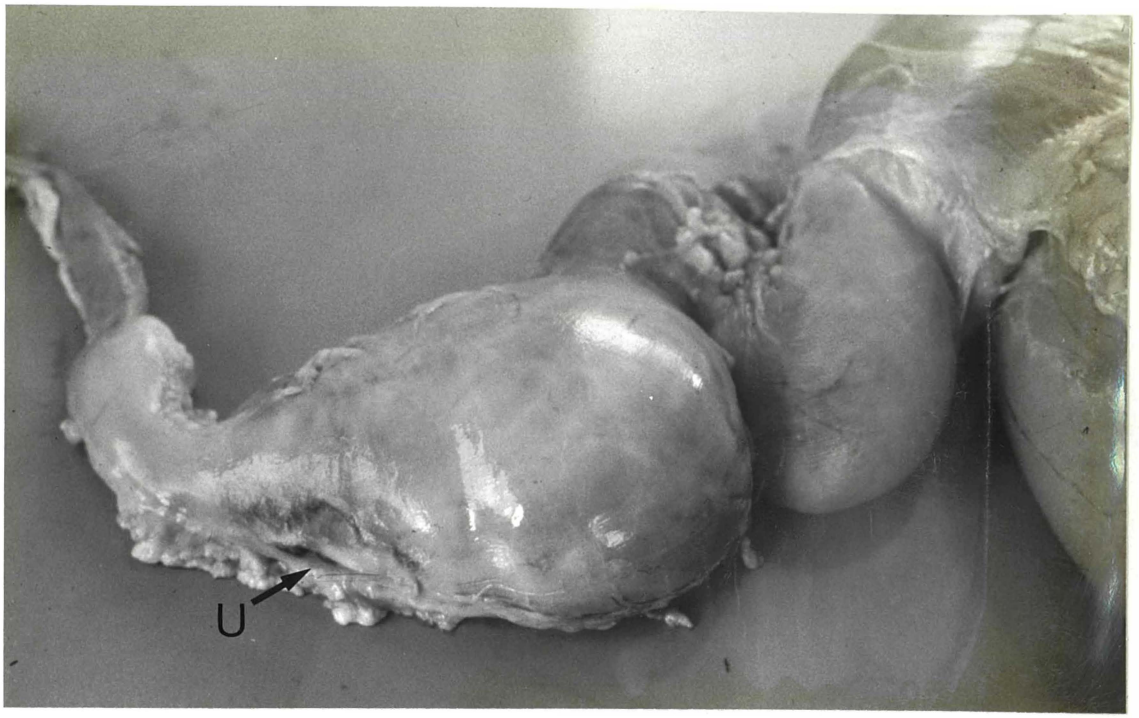


Plate 5.4 : Unopened abomasum from a sheep dosed with zinc sulphate by drenching gun. A haemorrhagic, almost penetrating ulcer (U) can be seen on the serosal surface of the greater curvature (dependent part) of the abomasum.

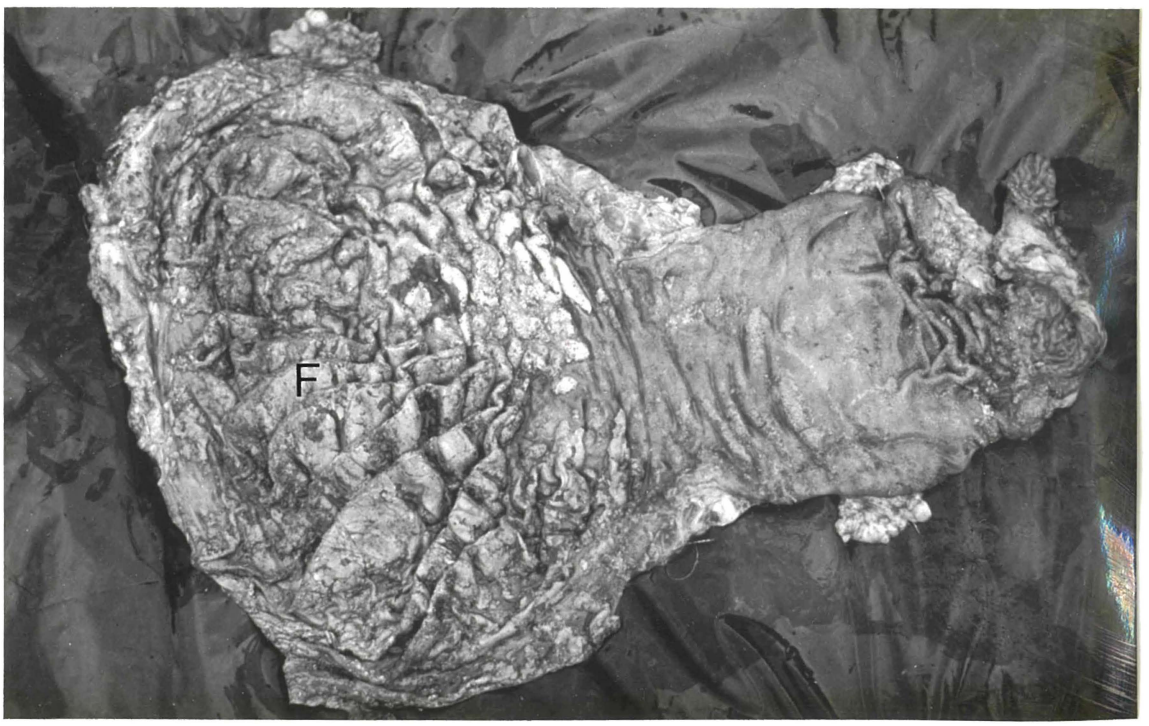


Plate 5.5 : Abomasum from a cow which had been dosed with zinc sulphate by drenching gun. Severe caseous necrosis of mucosa over fundic area (F).

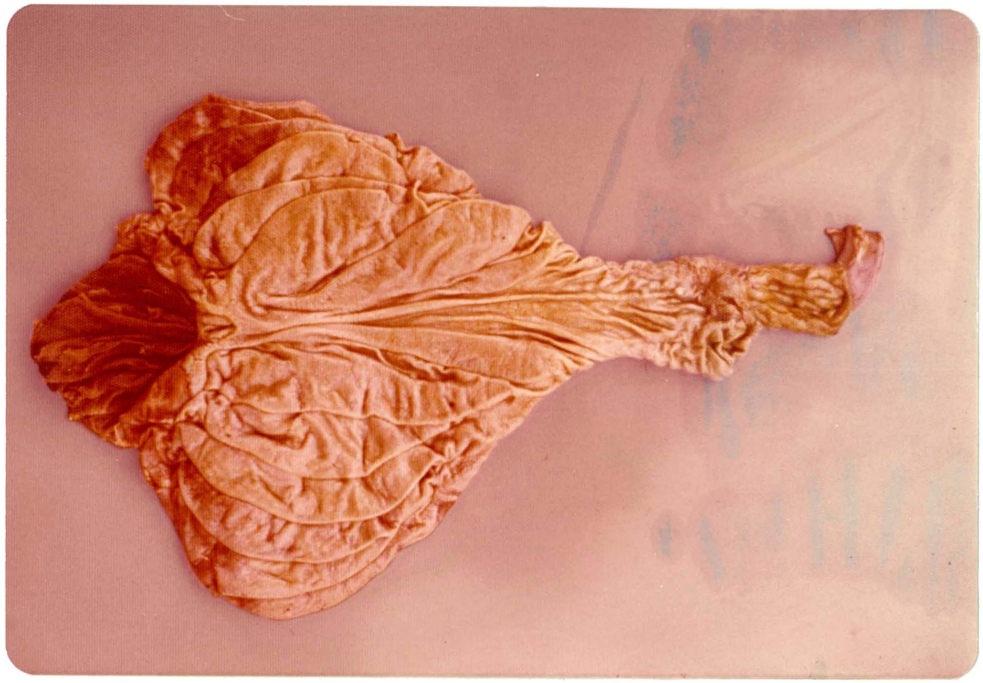


Plate 5.6 : Severe generalised corrosive abomasitis in sheep receiving zinc sulphate by drenching gun. Anterior mucosa of abomasum fundus is haemorrhagic.

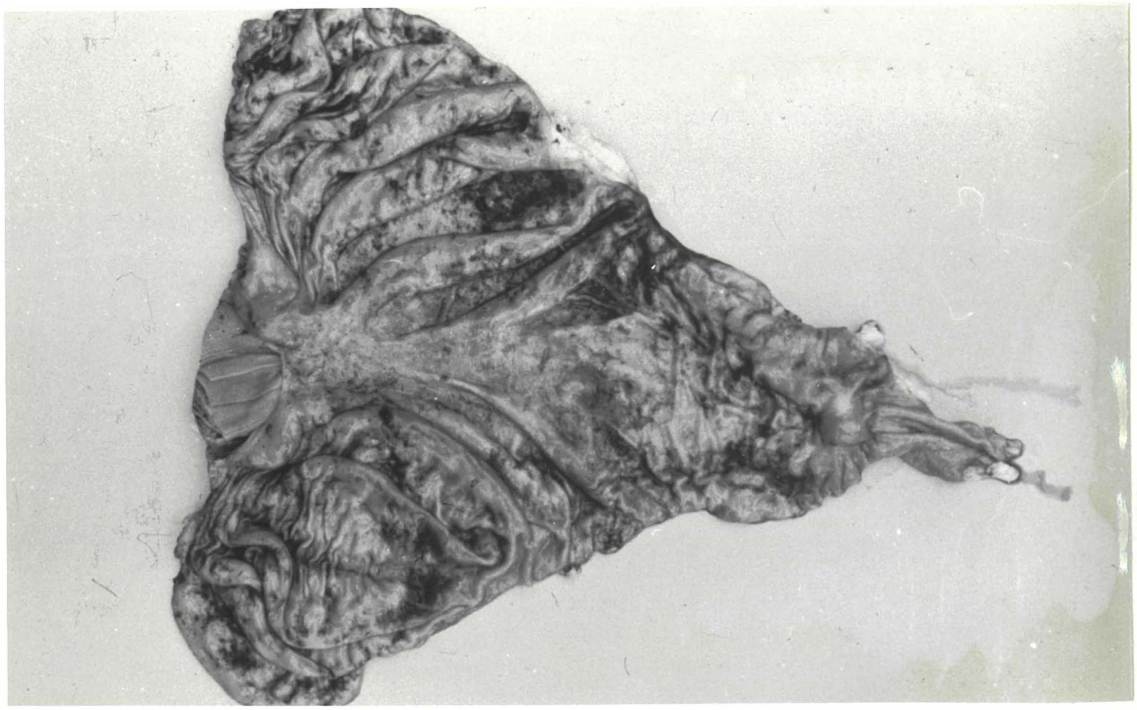


Plate 5.7 : Severe abomasal necrosis in a sheep after dosing with zinc sulphate by drenching gun. Rounded thickened fundic folds and dark haemorrhagic areas are present.

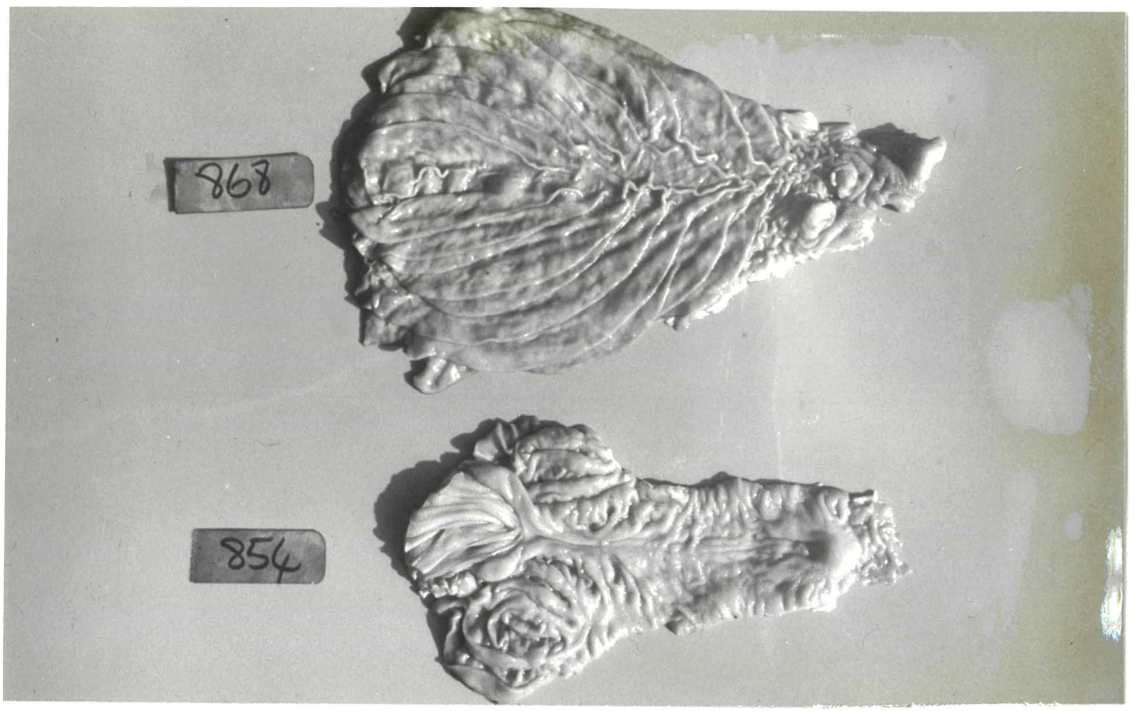


Plate 5.8 : Sheep abomasum after long term zinc sulphate dosing by drenching gun (854). Abomasum is small and fundic folds are shortened and distorted. Normal abomasum (868) for comparison.

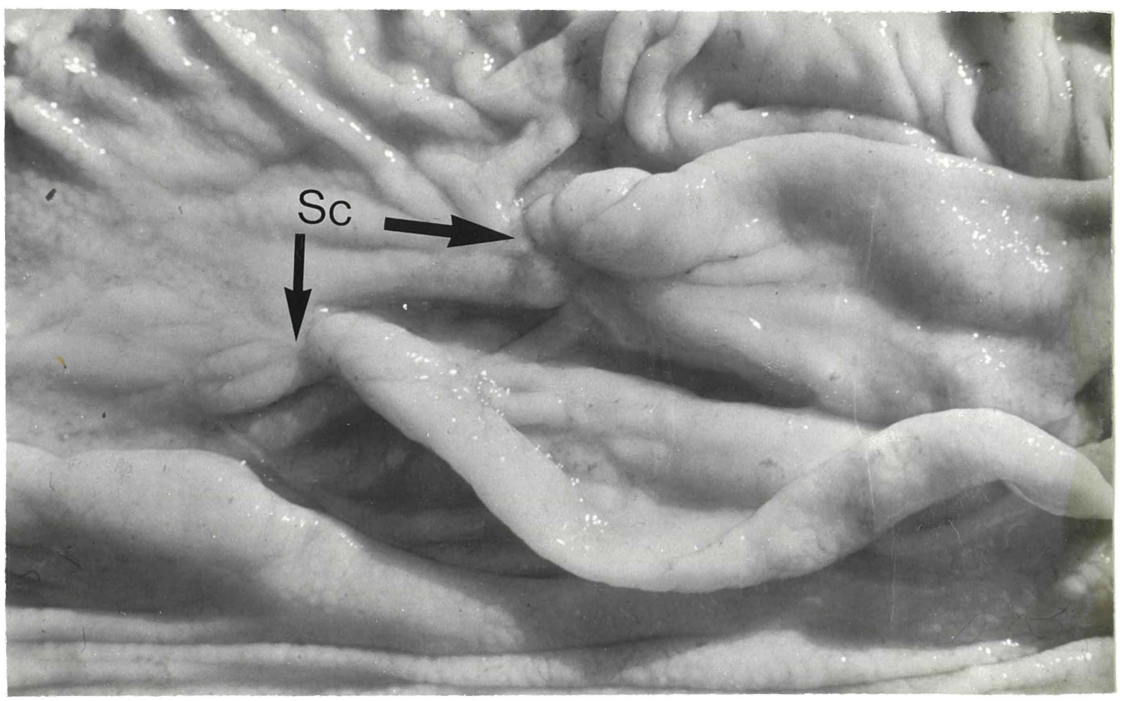


Plate 5.9 : Fundic folds of abomasum of zinc dosed (drenching gun) sheep. Distortion and scarring of fundic folds is evident (Sc).

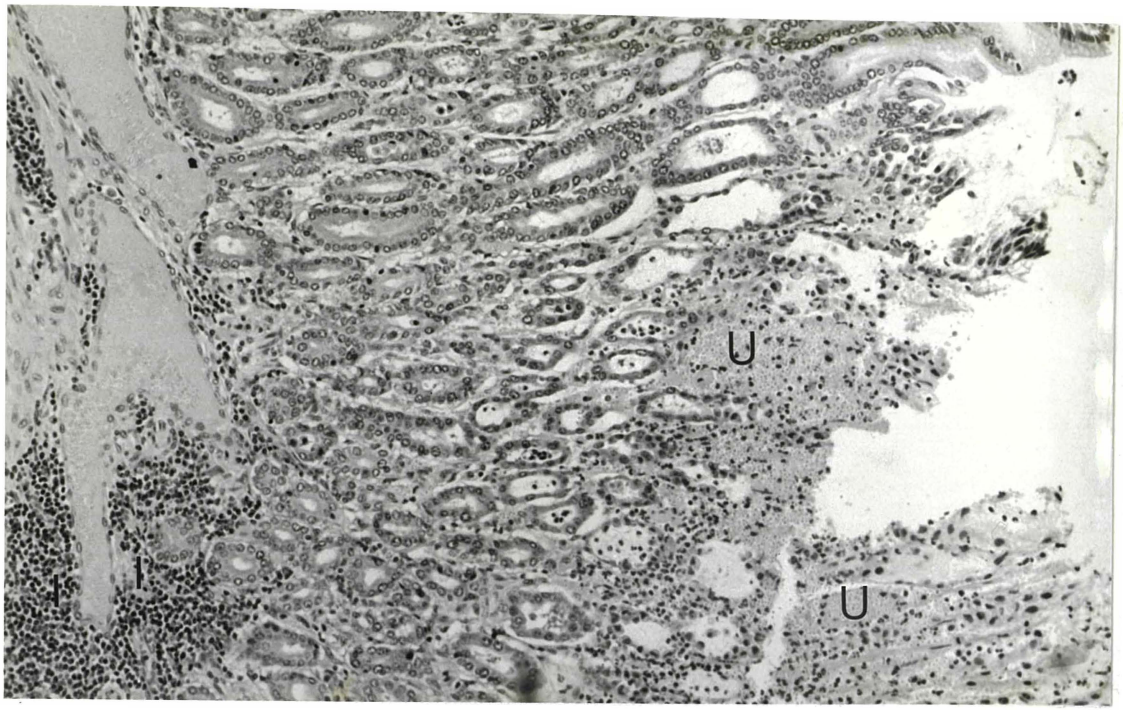


Plate 5.10 : Sheep abomasum after administration of zinc sulphate by drenching gun. Focal area of mucosal ulceration (U) and some focal submucosal leucocyte (I) infiltration is visible. (H & E)

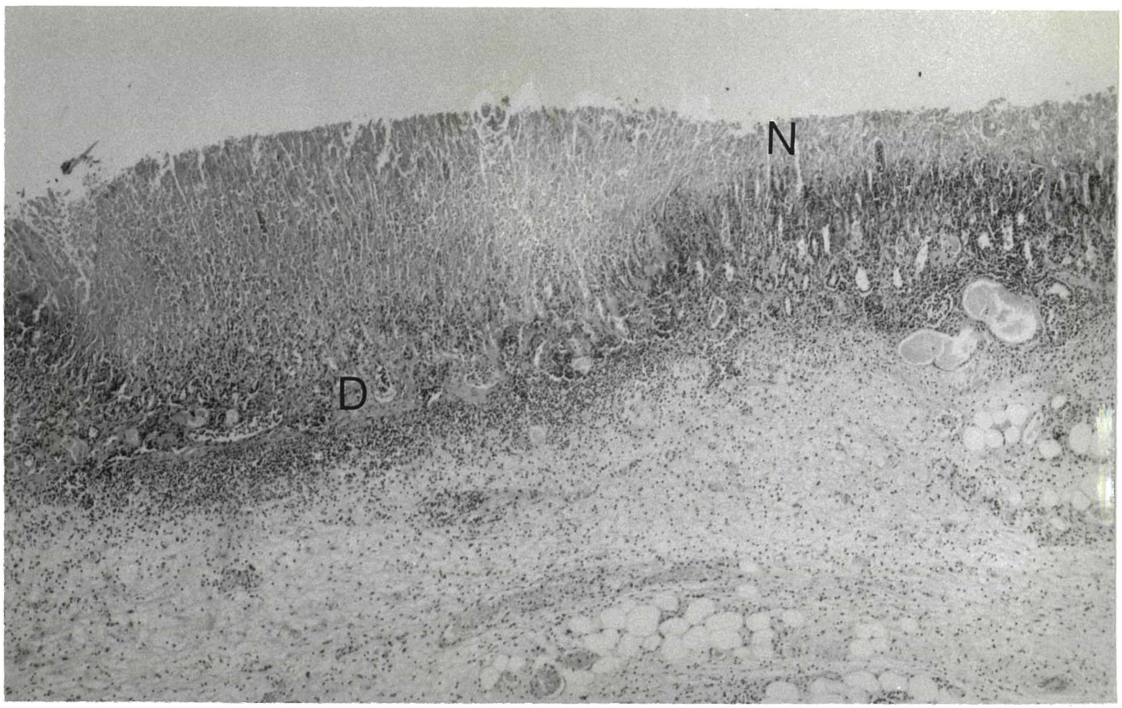


Plate 5.11 : Sheep abomasum after drenching gun administration of zinc sulphate. Severe necrosis of mucosal epithelium (N) with a deeper area of necrosis (D) penetrating into the submucosa. An inflammatory reaction surrounds the area of necrosis and there are some focal areas of inflammation about some vessels in the submucosa. (H & E)

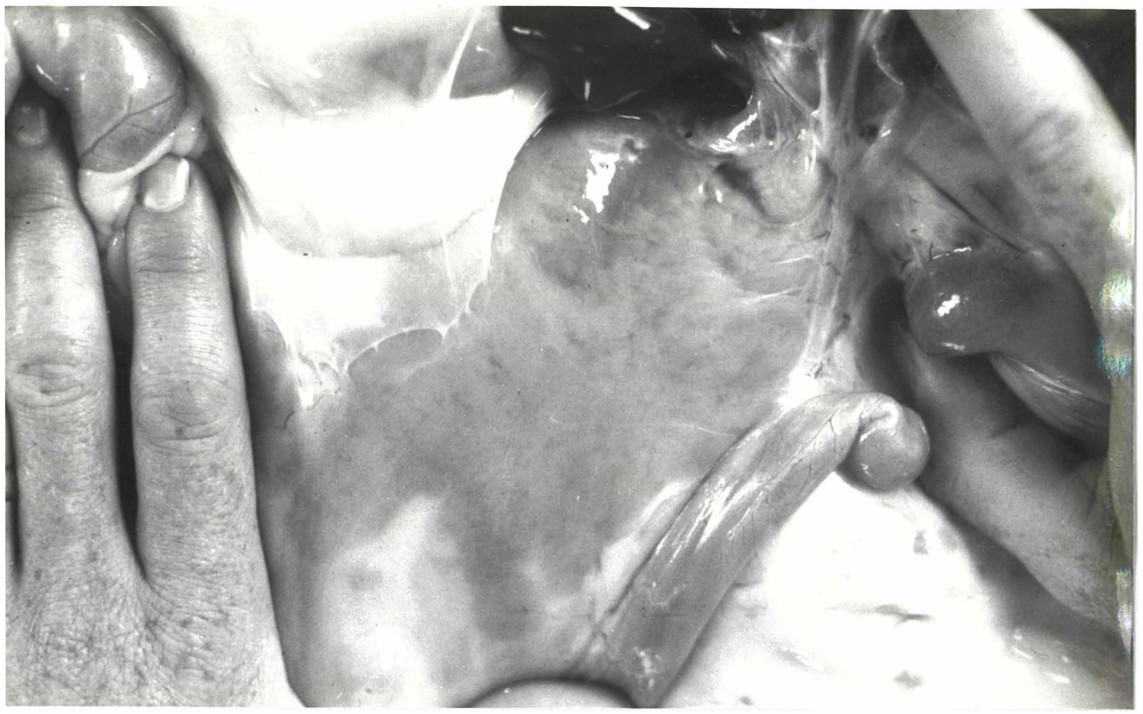


Plate 5.12 : Normal pancreas from control sheep undosed with zinc salts.

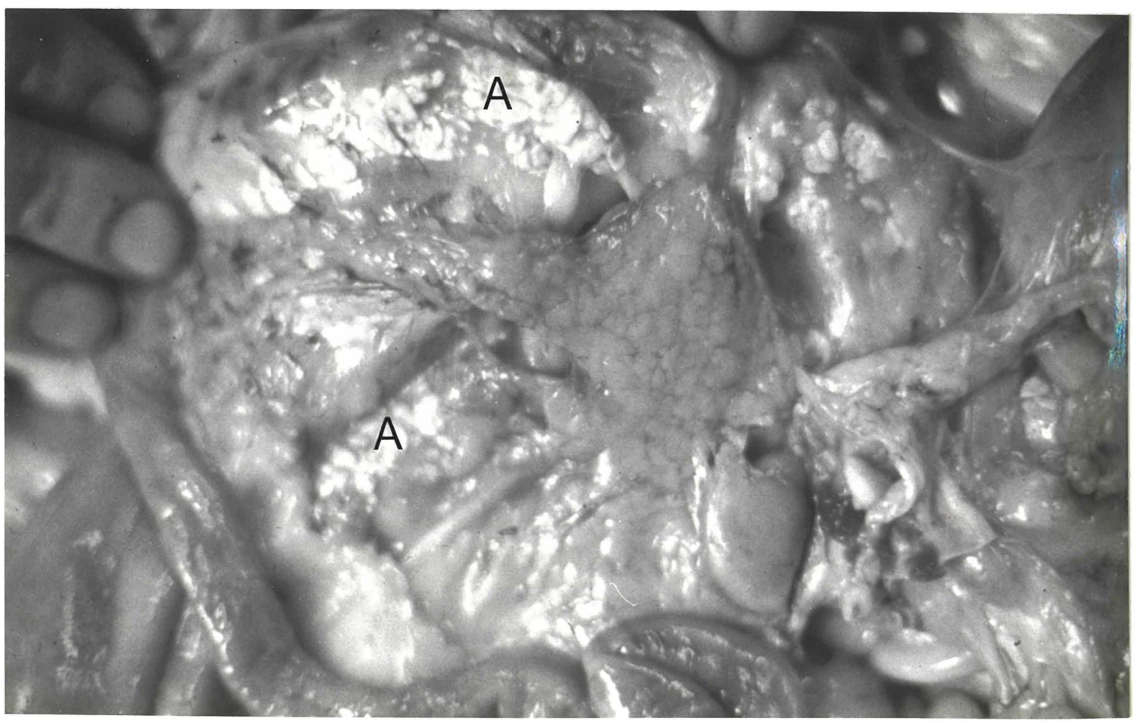


Plate 5.13 : Pancreas from zinc dosed sheep. Early pancreatic lesion with fat necrosis (A) visible about margins of pancreas.

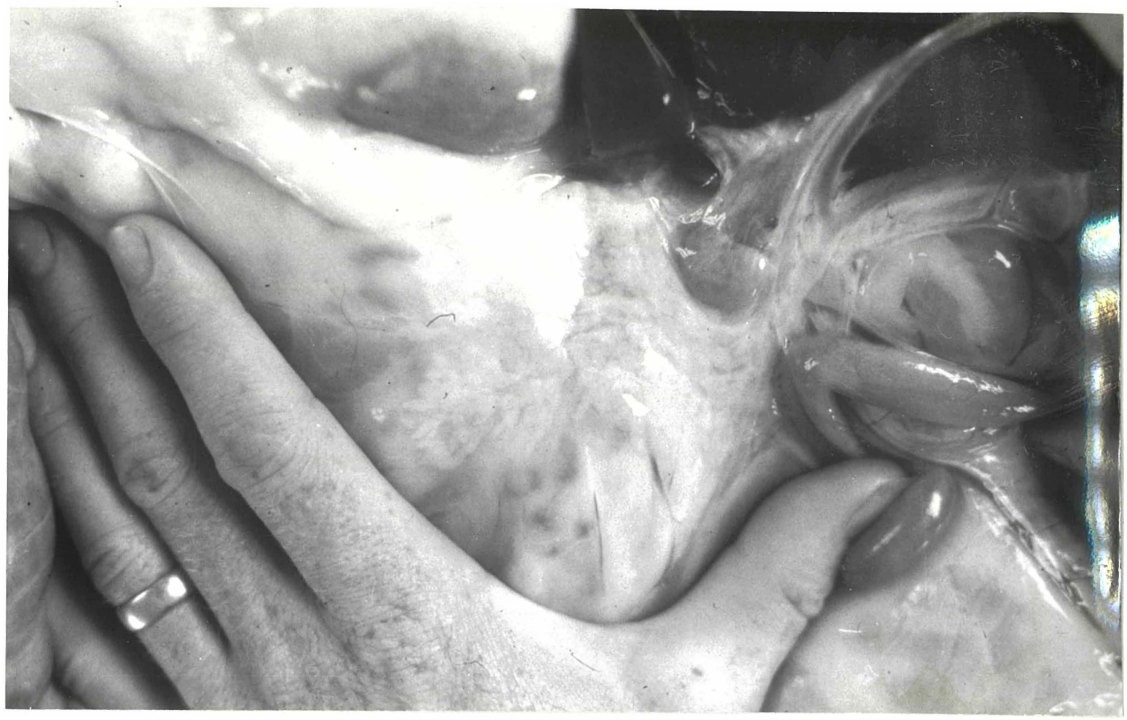


Plate 5.14 : Pancreatic atrophy and fibrosis following long term dosing of sheep with zinc sulphate.

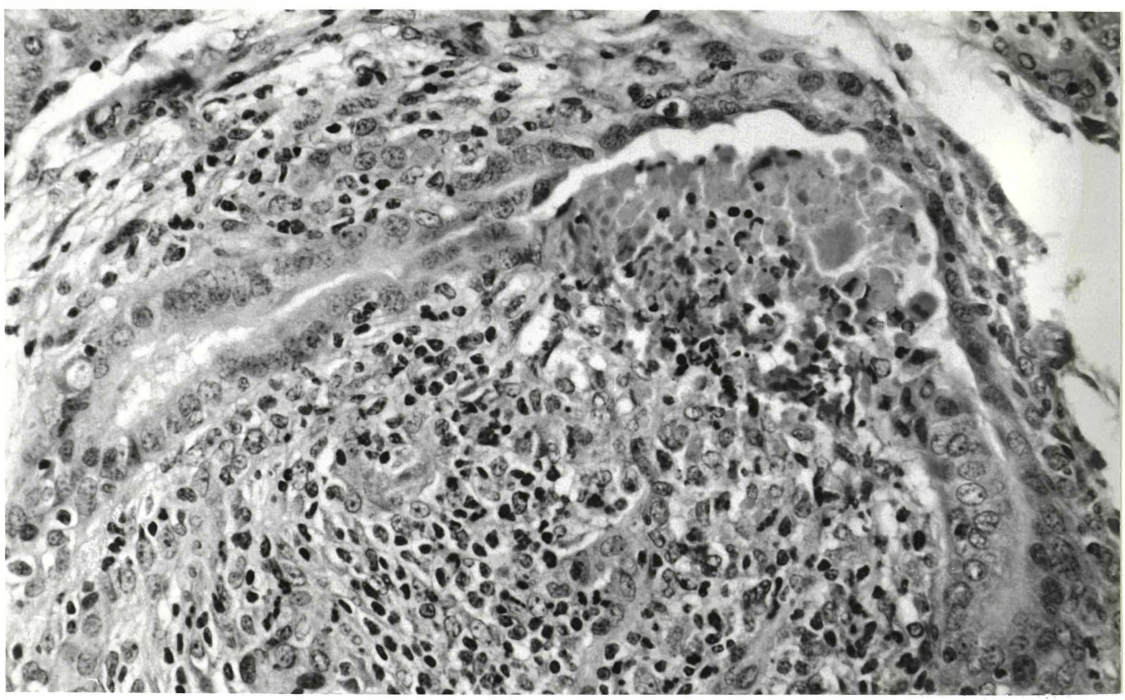


Plate 5.15 : Focal necrosis of one side of a major pancreatic duct 28 days after the start of zinc oxide administration. Severe coagulative change with pyknosis and karyorrhexis is accompanied by massive cellular reaction in the adjacent tissue causing distortion and possible blockage of duct. (H & E)

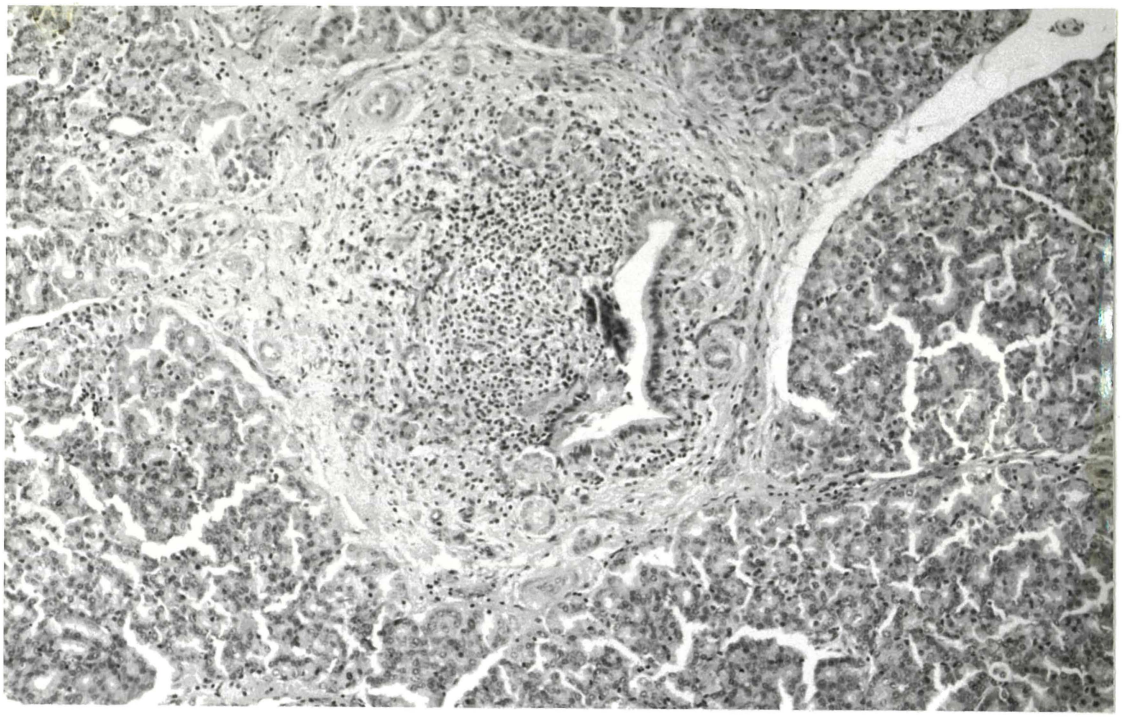


Plate 5.16 : Section of ovine pancreas. Zinc sulphate toxicity. Early changes in a pancreatic duct. Degeneration of ductular epithelium and accompanying inflammatory changes are adjacent on one side of the duct. (H & E)

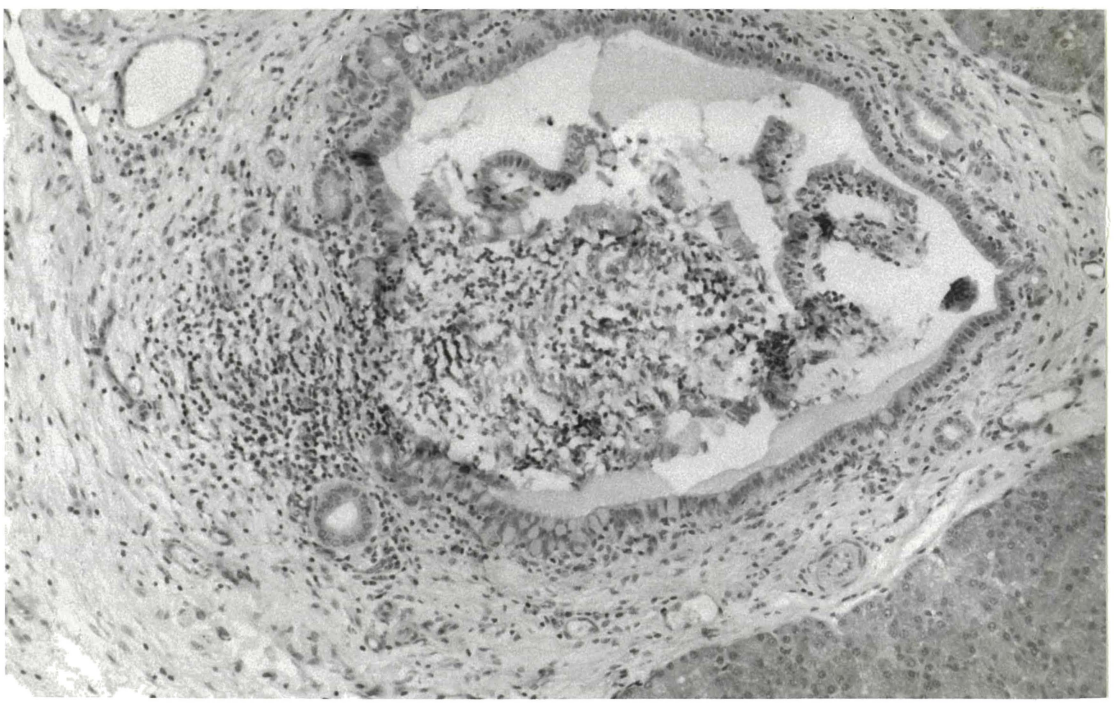


Plate 5.17 : Major pancreatic duct of sheep after zinc sulphate dosing by drenching gun. Disruption of one side of duct epithelium evident with prolapse of subepithelial tissue into duct lumen. Local inflammatory cell accumulation present in prolapse and adjacent tissue. (H & E)

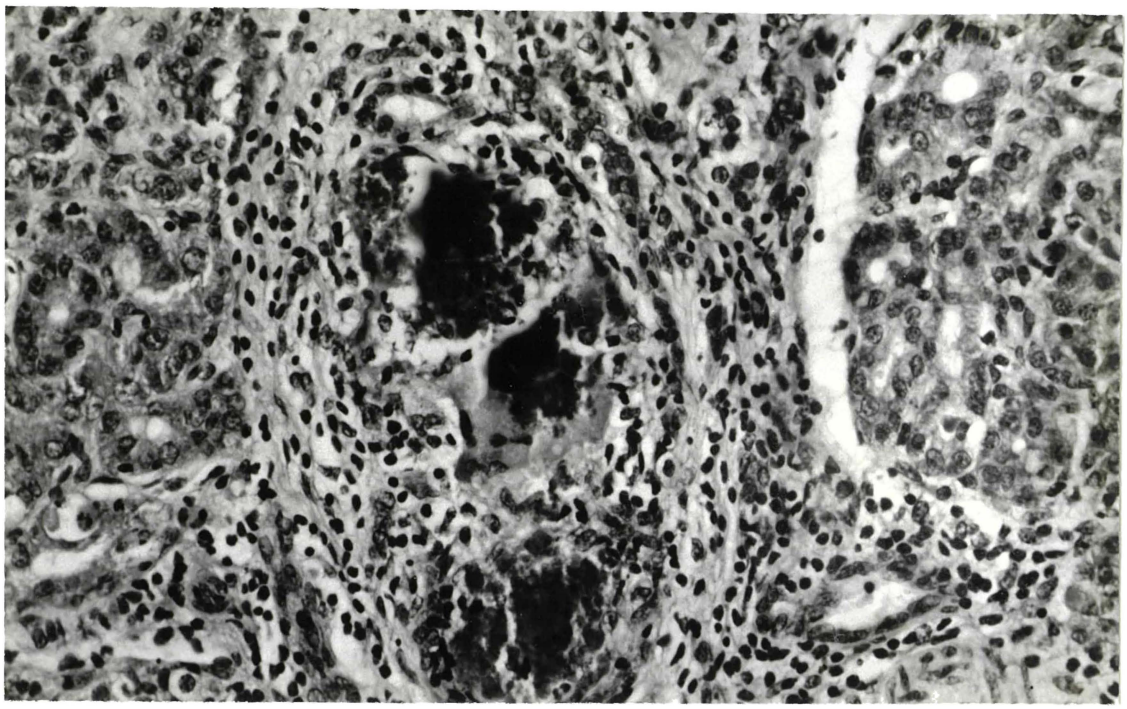


Plate 5.18 : Sheep pancreatic duct after zinc administration. Strongly basophilic concretion surrounded by inflammatory cell reaction appears to be all that remains of duct. (H & E)

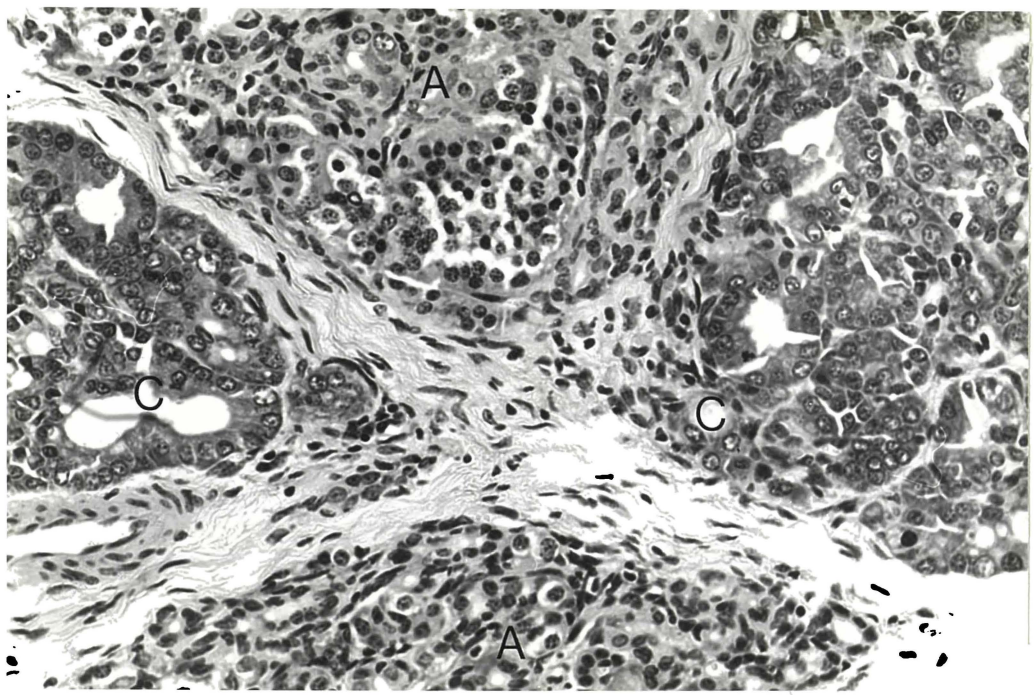


Plate 5.19 : Sheep pancreas after zinc sulphate administration. Both intralobular and interlobular fibrosis is present and the four different lobules visible are at different stages of degeneration. The changes range from early transformed acini showing cystic appearance (C) to a more advanced change (A) with fibroblast activity, inflammatory cell infiltration and possibly duct proliferation. (H & E)

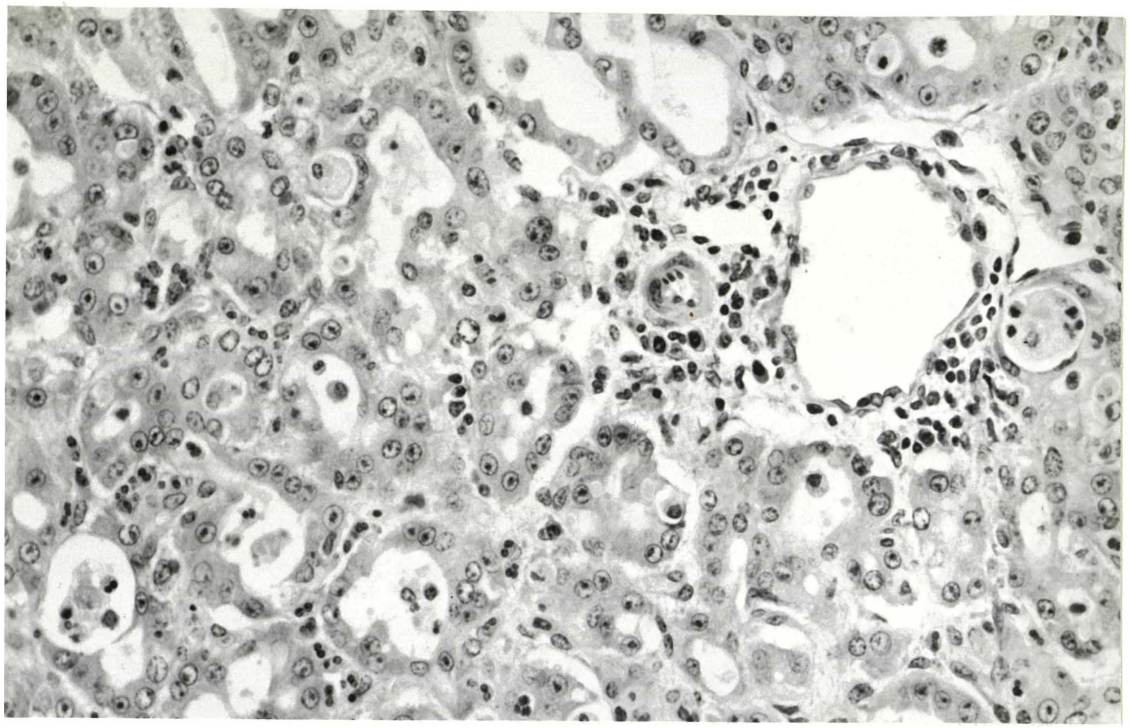


Plate 5.20 : Pancreas from zinc sulphate dosed cow. An apparent transformation of acini into tubular structures without zymogen granules is evident giving the parenchyma a cystic appearance. Degenerating cells in the tubular epithelium and lumens and the presence of inflammatory cells may be seen. (H & E)

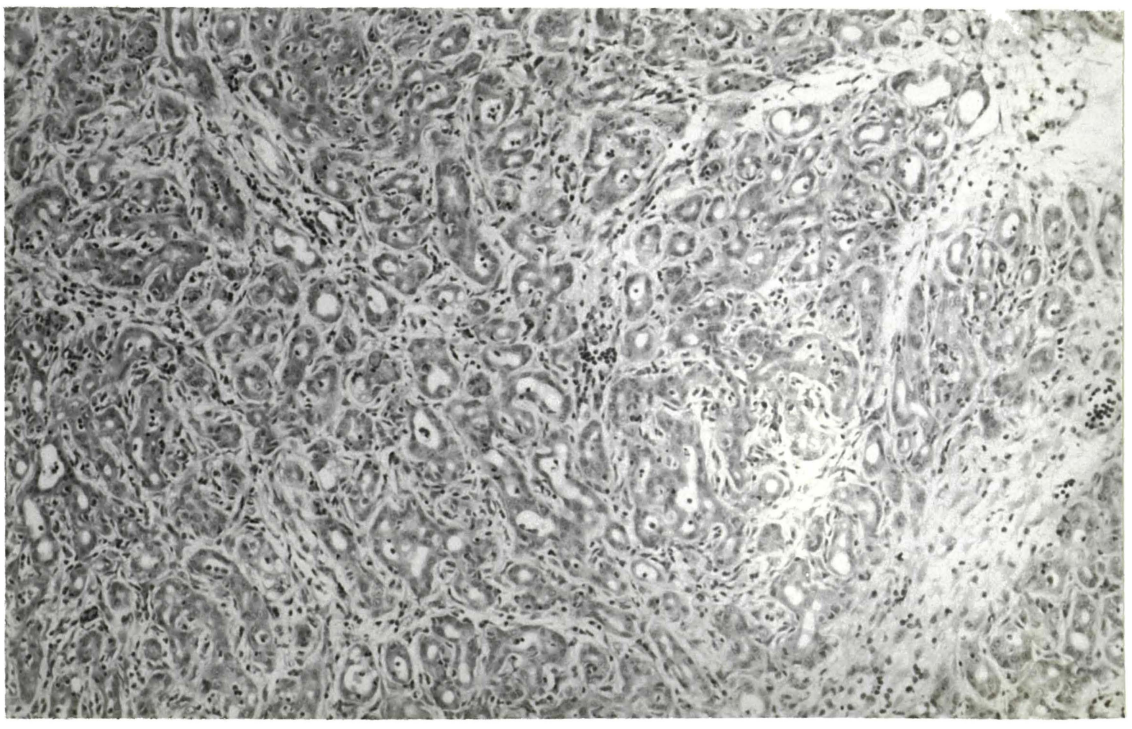


Plate 5.21 : Sheep pancreas after zinc sulphate administration. Intralobular fibrosis, interstitial leucocyte infilatration and parenchymal cystic changes are evident. (H & E)

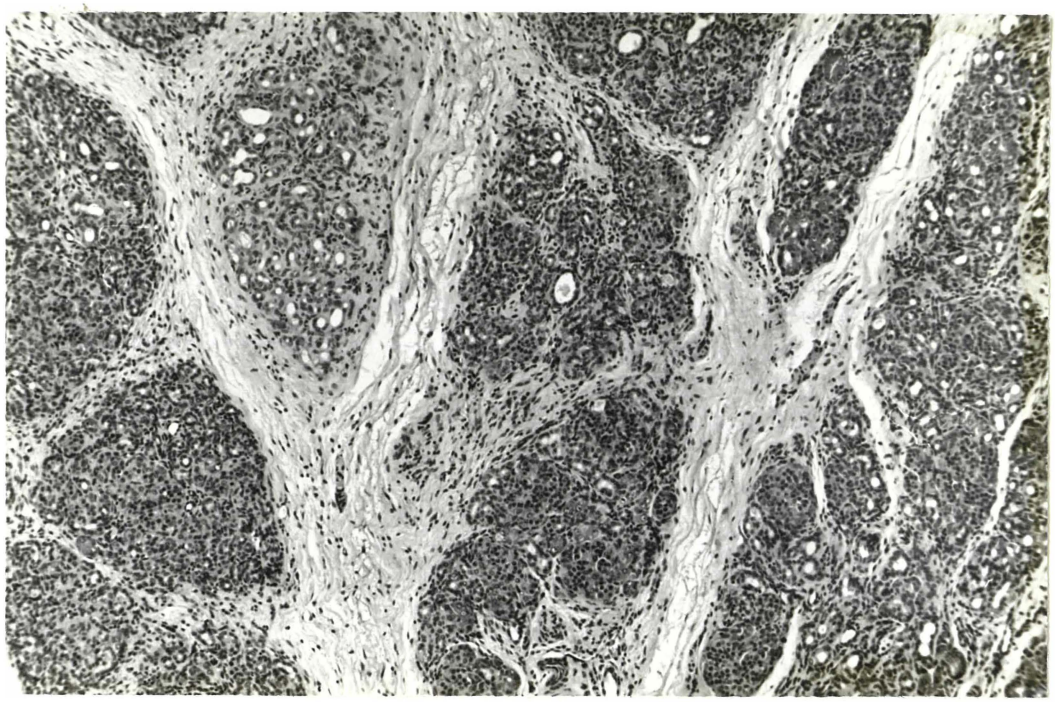


Plate 5.22 : Sheep pancreas after zinc sulphate administration. Extensive intra and interlobular fibrosis and a moderate cystic change were evident. (H & E)

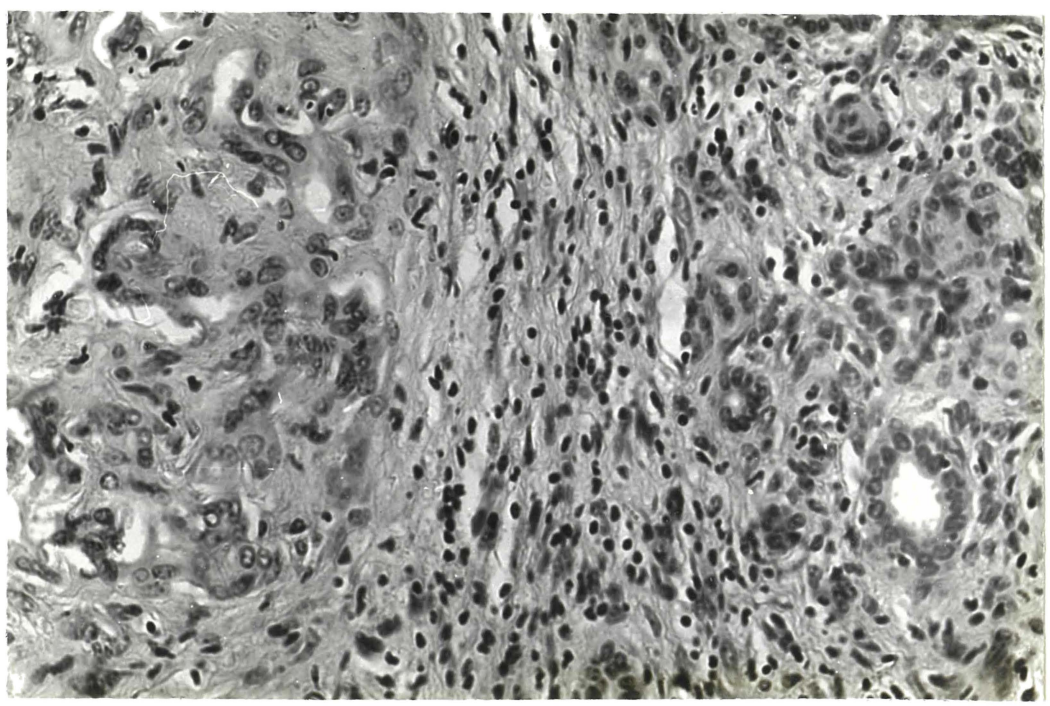


Plate 5.23 : Ovine pancreas after toxicity caused by zinc sulphate administration. Fibroblast activity and mononuclear cell infiltration is evident. (H & E)

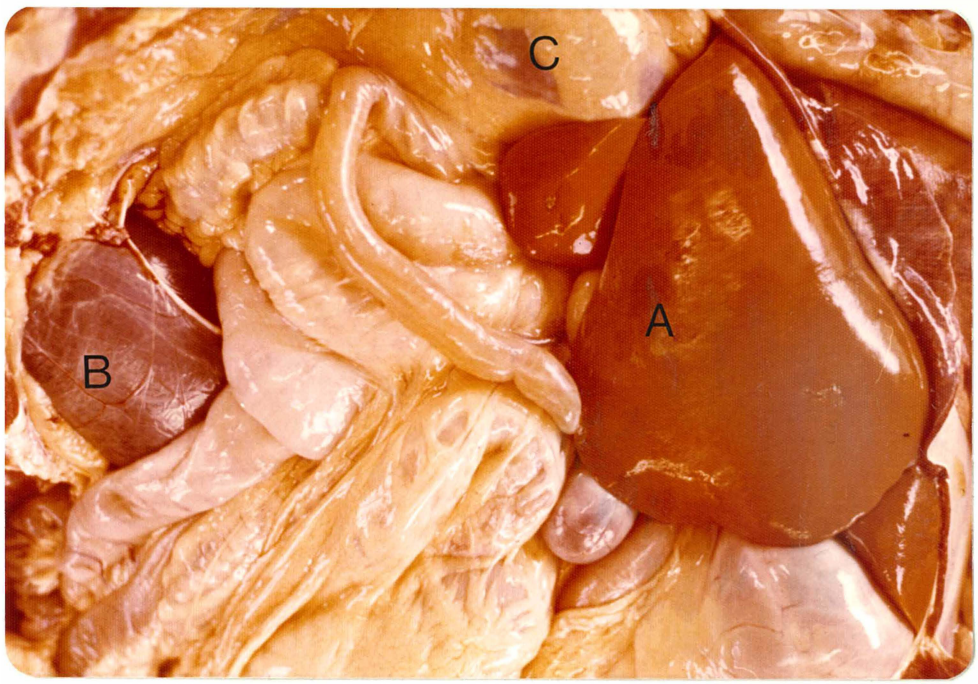


Plate 5.24 : Sheep viscera after chronic zinc sulphate administration. Generalised icterus, orange liver (A), dark urine-filled urinary bladder (B) and dark kidneys (C) are present.

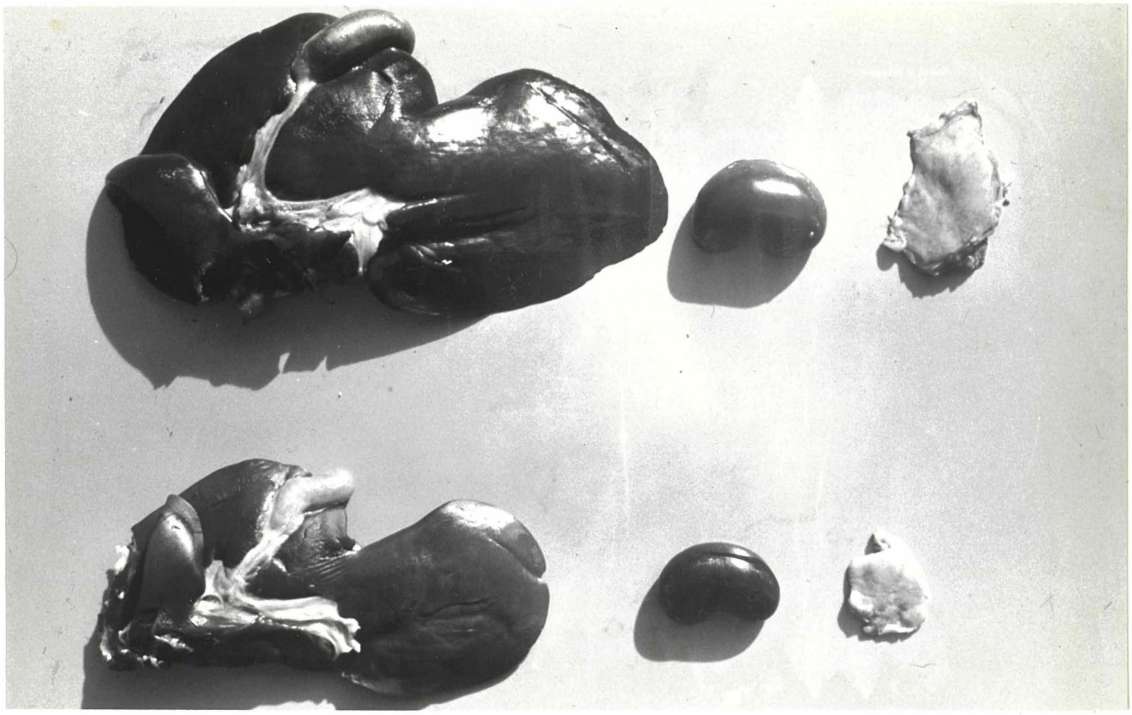


Plate 5.25 : Liver, kidney and pancreas from a normal sheep (top) are contrasted with those from a sheep which has been dosed with zinc sulphate for several weeks (bottom). The liver is lighter (icterus), the kidney darker (haemobloginuria) and the pancreas smaller and lighter (fibrosis and atrophy).

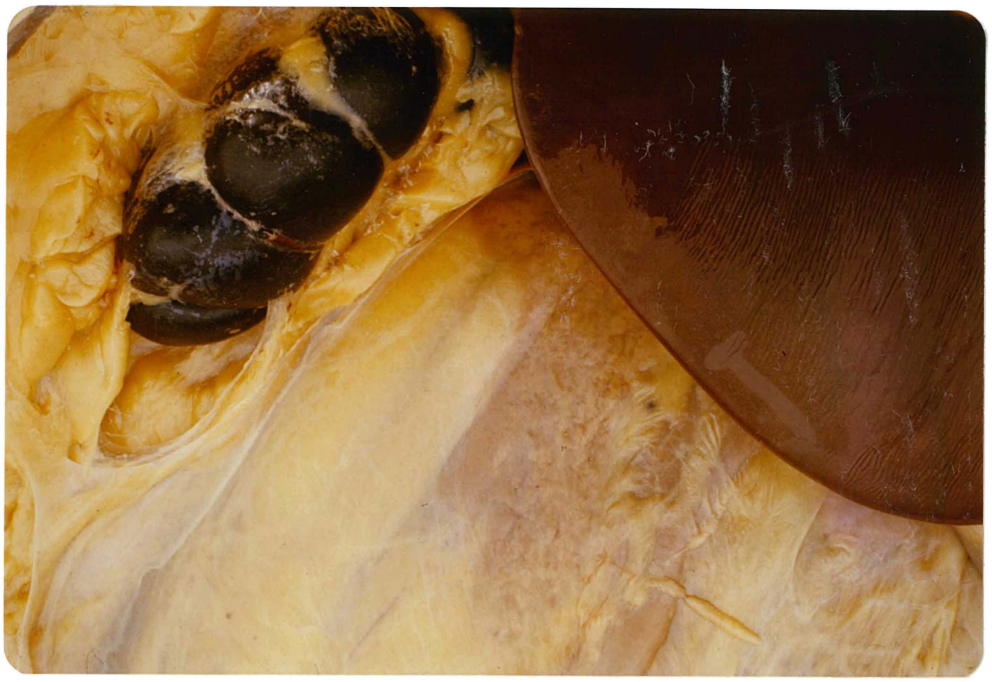


Plate 5.26 : Lesions indicating occurrence of haemolytic crisis in dairy cow drenched with zinc sulphate. Icterus, brown liver and black kidneys are evident.

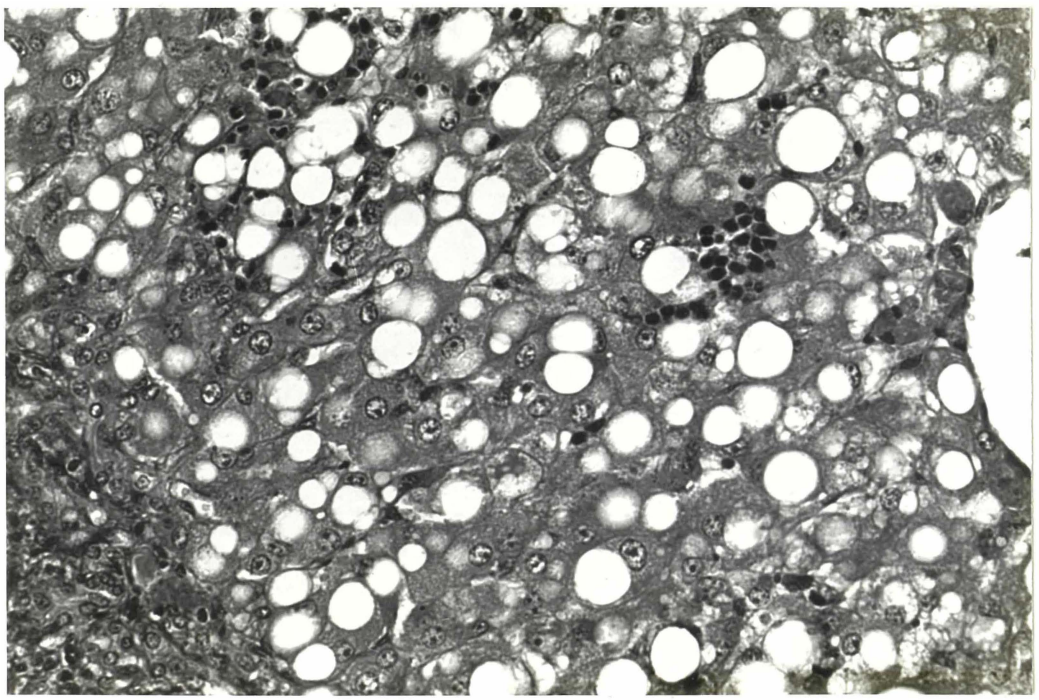


Plate 5.27 : Liver from sheep after drenching with zinc sulphate. A fatty change and focal areas of necrosis and leucocyte infiltration are seen in this section (Masson Trichrome).

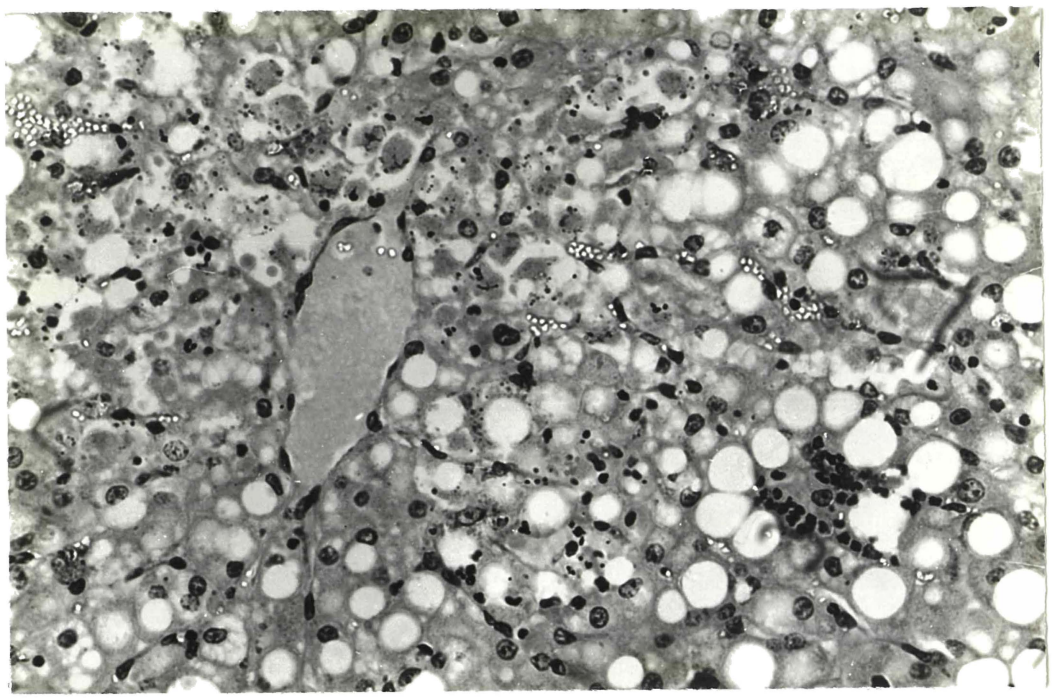


Plate 5.28 : Section of sheep liver after administration of zinc sulphate by drenching gun. A severe fatty change, focal necrosis and inflammation and a large periacinar area of parenchymal necrosis with karyorrhexis, pyknosis and other cellular debris is visible. (H & E)

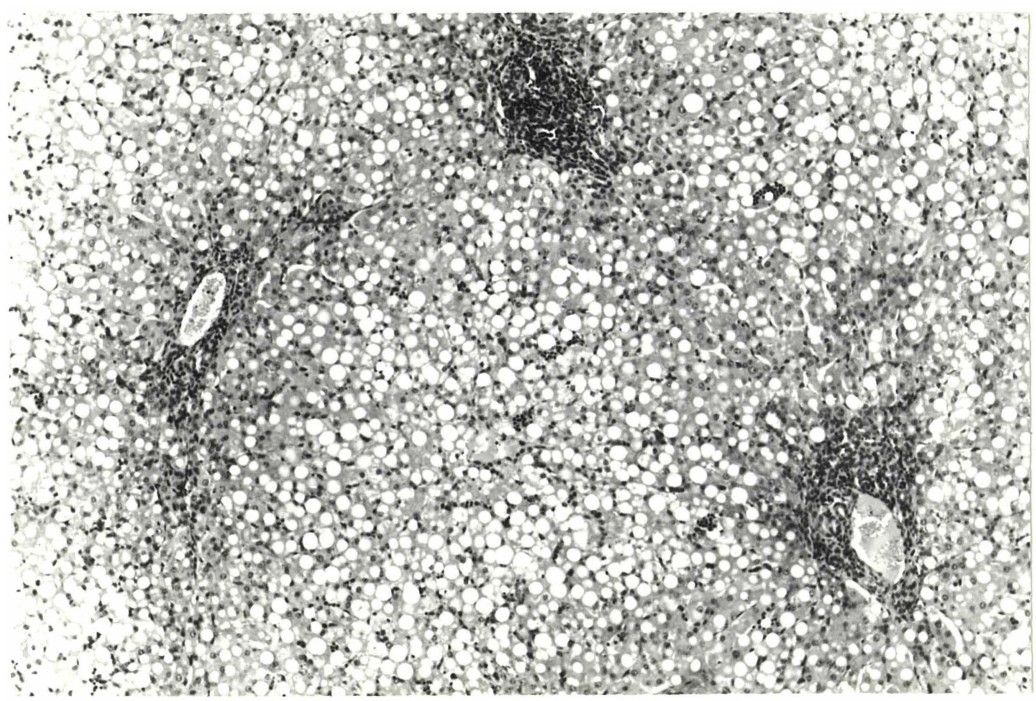


Plate 5.29 : Liver from zinc dosed sheep showing widespread periacinar fatty change with numerous small foci of polymorphs scattered randomly throughout the parenchyma. (H & E)

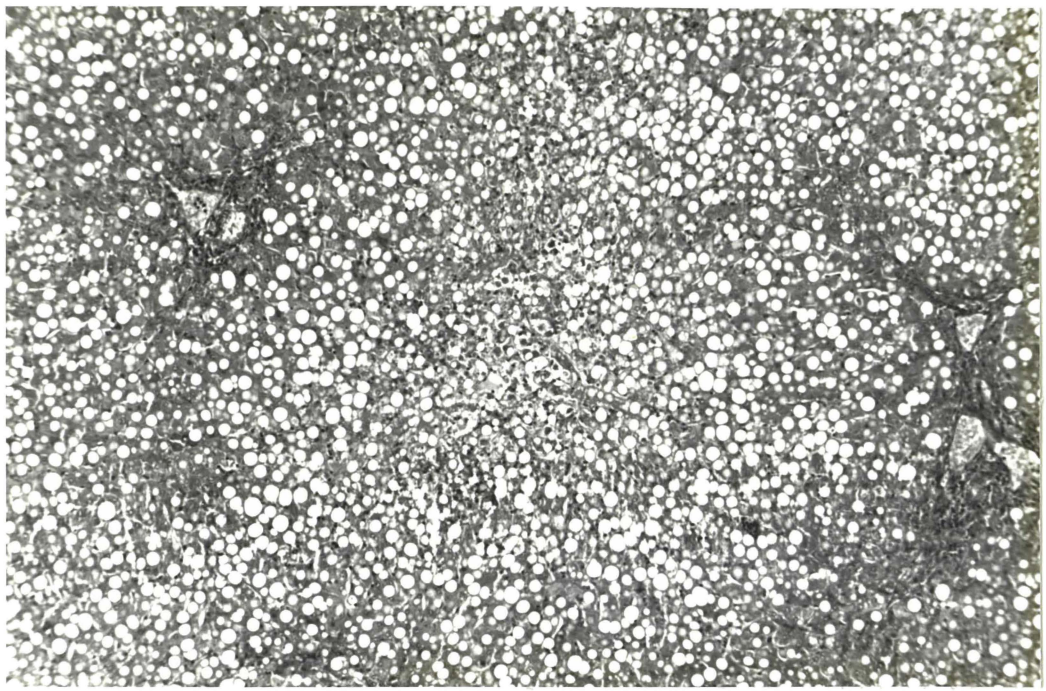


Plate 5.30 : Liver from sheep dosed with zinc sulphate by drenching gun for several weeks. Extensive fatty change throughout lobules, tending to be more severe towards the periphery of the acinus where a large area of parenchymal necrosis is also present. (H & E)

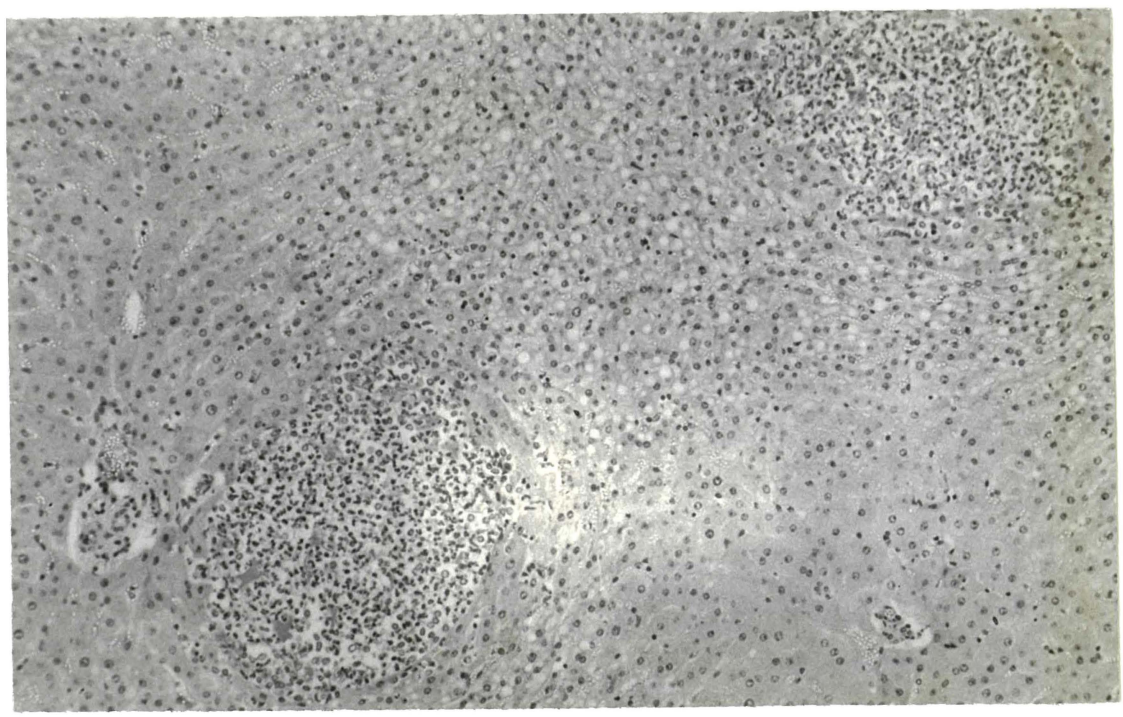


Plate 5.31 : Liver from cow which had received zinc sulphate by drenching gun and had evidence of one or more haemolytic episodes. Large focal areas of hepatic necrosis and inflammation are present and in addition a parenchymal fatty change with some polymorph infiltration is present. (H & E)

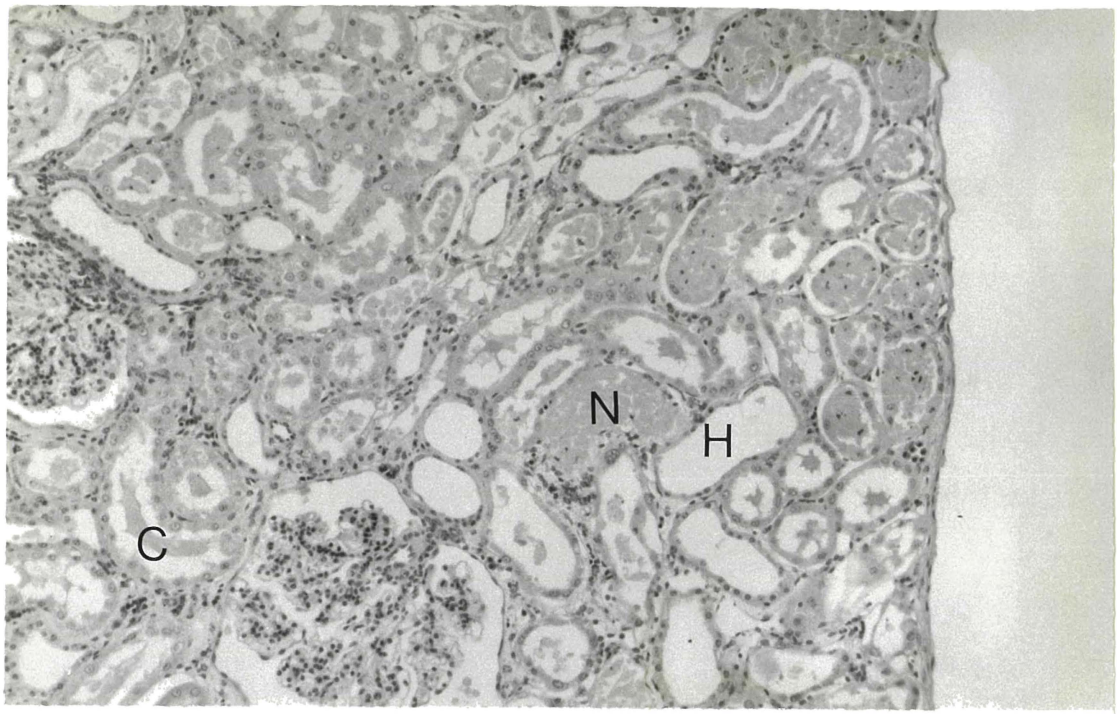


Plate 5.32 : Section of sheep kidney after long term zinc sulphate administration. Severe hydronephrosis (H), tubular necrosis (N) and intratubular haemoglobin casts (C) are present. (H & E)

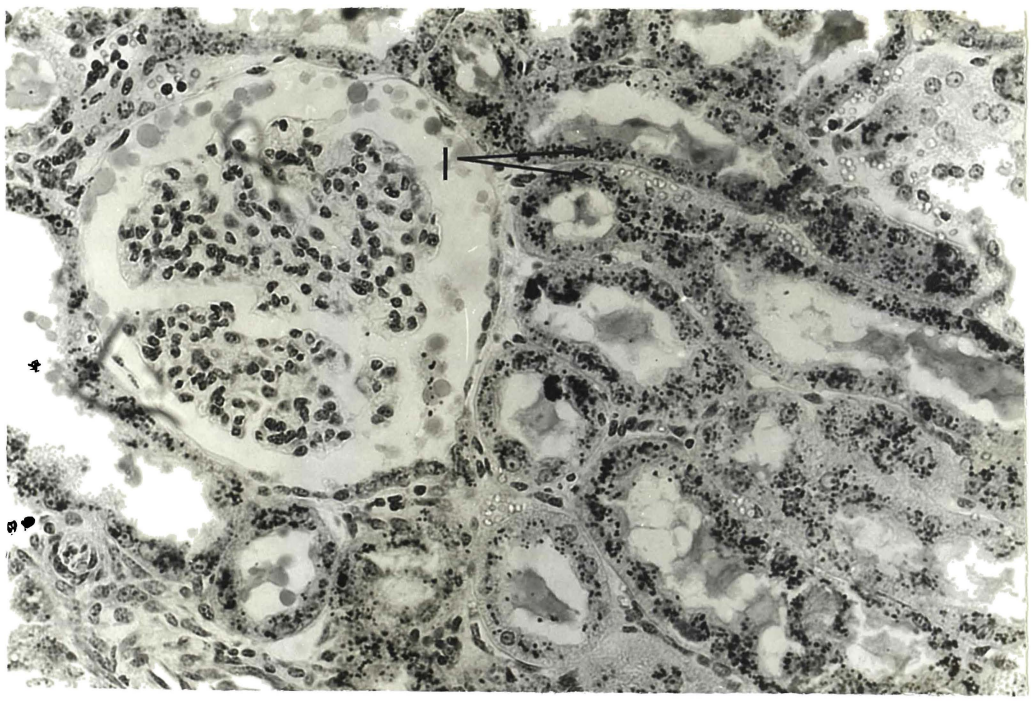


Plate 5.33 : Kidney from zinc dosed sheep showing acidophilic homogeneous material in periglomerular space and tubular lumens and an intracellular (iron positive) granular material (I) throughout the epithelial cells of the convoluted tubules. (Perls)

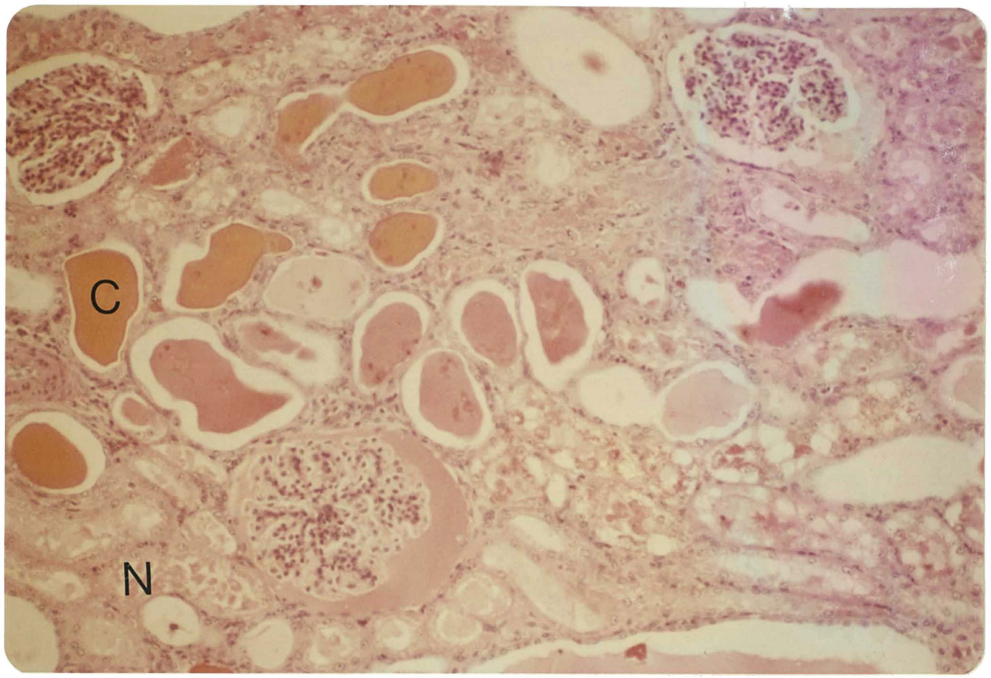


Plate 5.34 : Kidney of cow which had suffered an haemolytic crisis after drenching with zinc sulphate. Tubular necrosis (N) and haemoglobin casts (C) are visible. (H & E)

Plate 5.35 : Cut and subcapsular surfaces of kidneys from lambs which received zinc sulphate in their milk. Note misshapen kidneys and fine striations visible on cut surface. Divisions are cm.

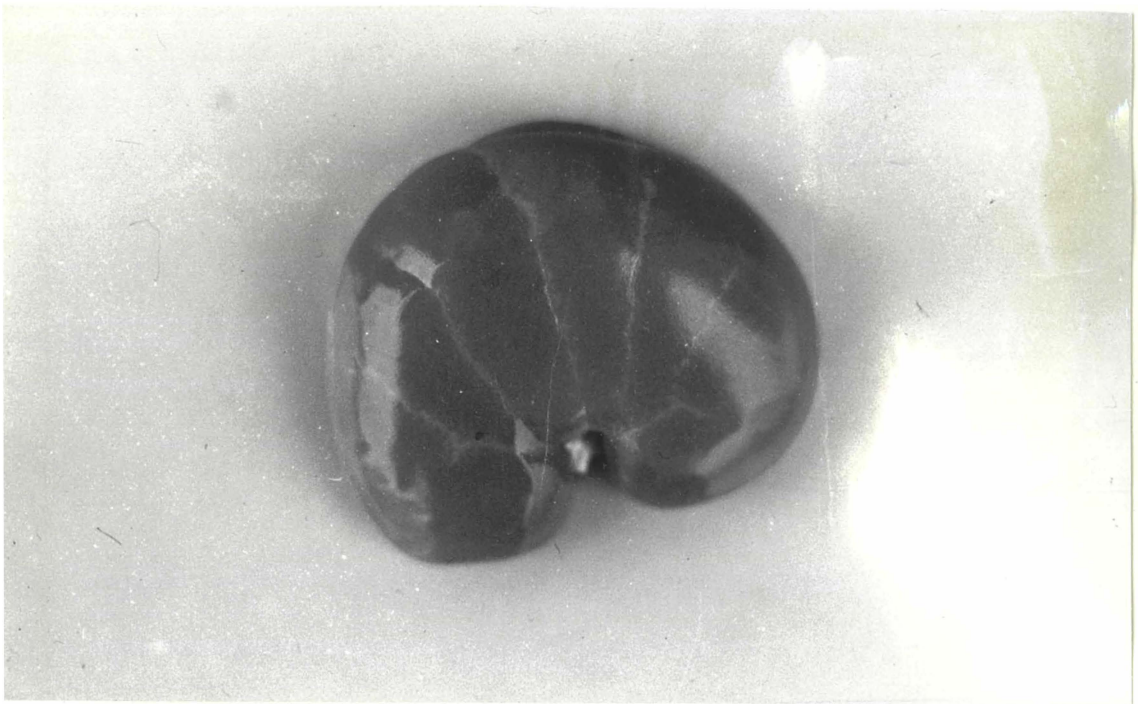


Plate 5.36 : Kidney from lamb after administration of zinc sulphate in bottle-fed milk. Prominent lines of scarring appear to inhibit expansion of kidney parenchyma causing distortion and misshapen appearance.

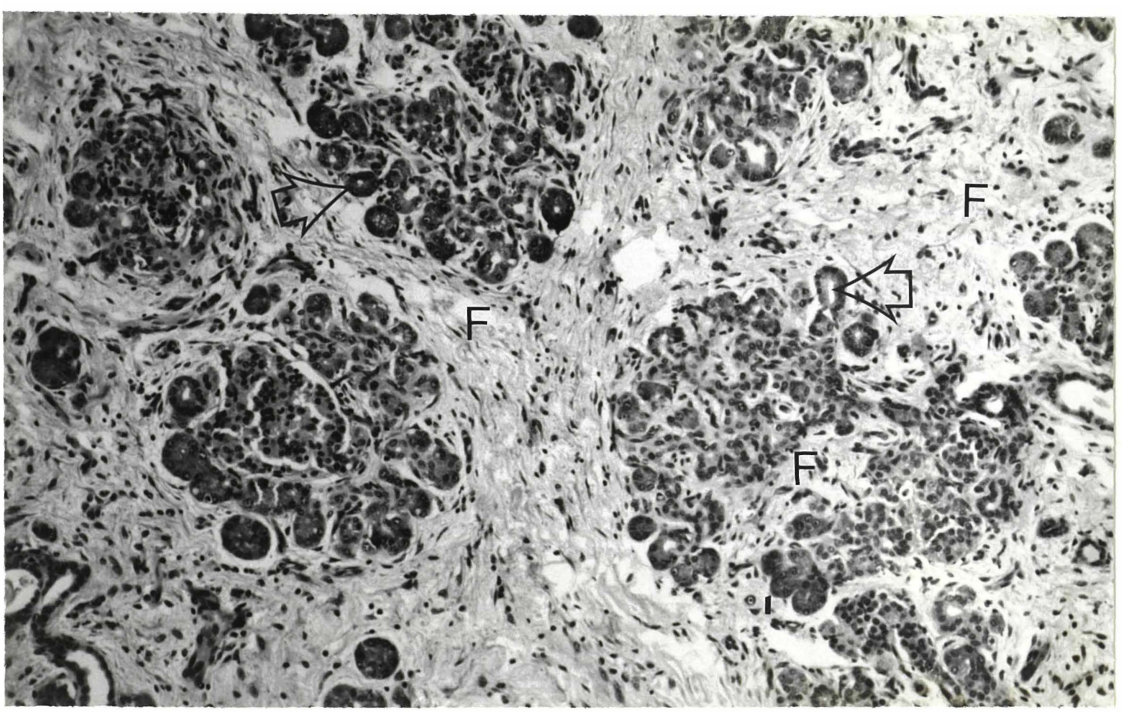


Plate 5.37 : Pancreas from lamb after receiving zinc sulphate in bottle-fed milk. Extensive inter- and intralobular fibrosis (F), loss of normal exocrine structure and an apparent transformation to ductular hyperplasia (arrows) is evident. (H & E)

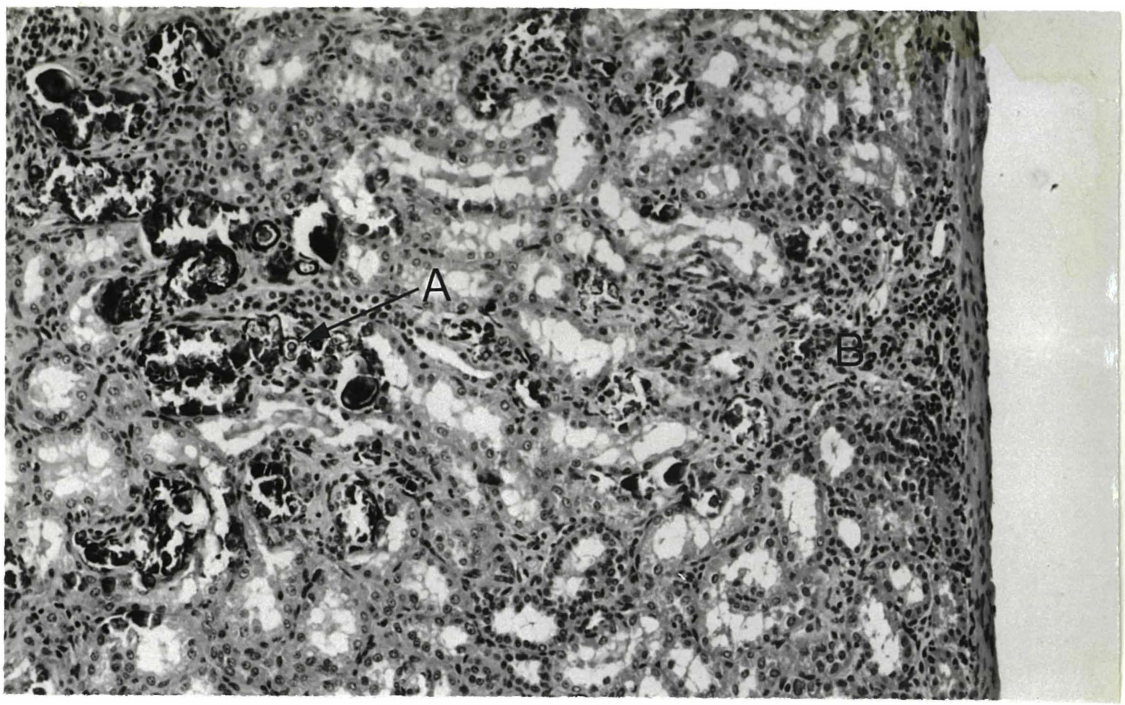


Plate 5.38 : Kidney cortex from lamb after receiving zinc sulphate in bottle-fed milk. Numerous basophilic concretions are all that remain of convoluted tubules. Many of these have a concentric onion-like structure (A) and there are areas of interstitial nephritis (B) extending in from the capsule. (H & E)

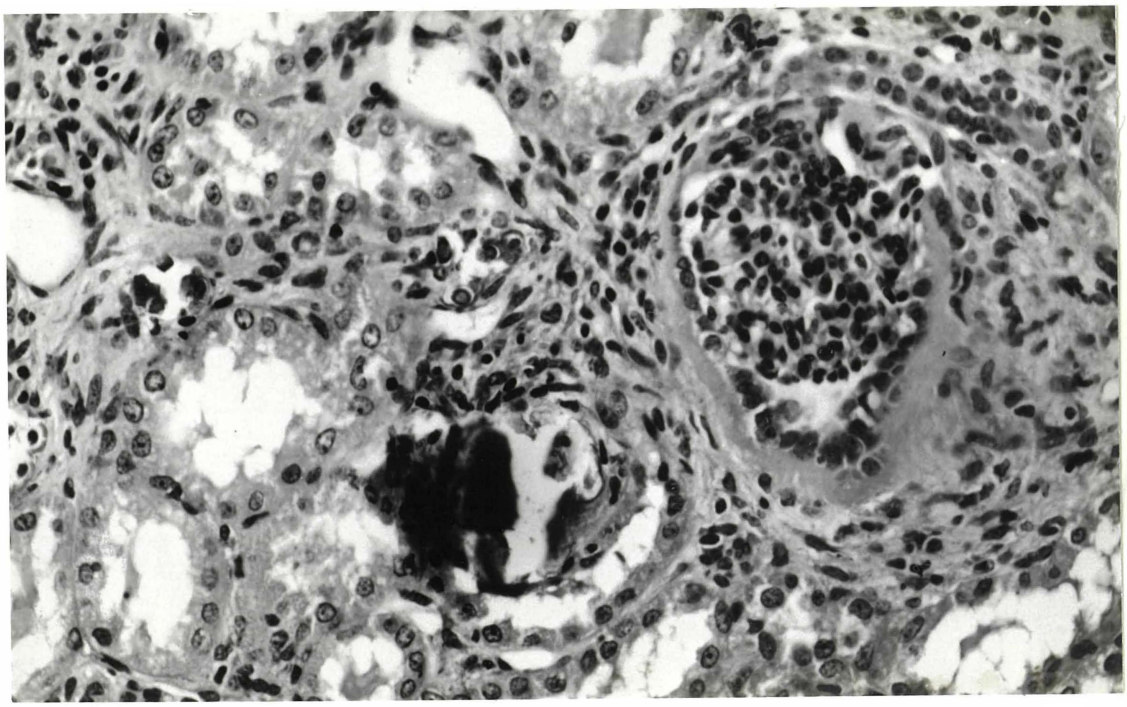


Plate 5.39 : Basophilic mass replaces a convoluted tubule in kidney cortex of lamb after administration of zinc sulphate in bottle-fed milk. Thickening of Bowman's capsule is also present. (H & E)

Plate 5.40 : Reticulum, reticular groove and rumen (anterior sac) from a sheep in which severe abomasitis had resulted from drenching gun administration of zinc sulphate. Note the smoothness of the epithelium and the lack of prominent papillae.

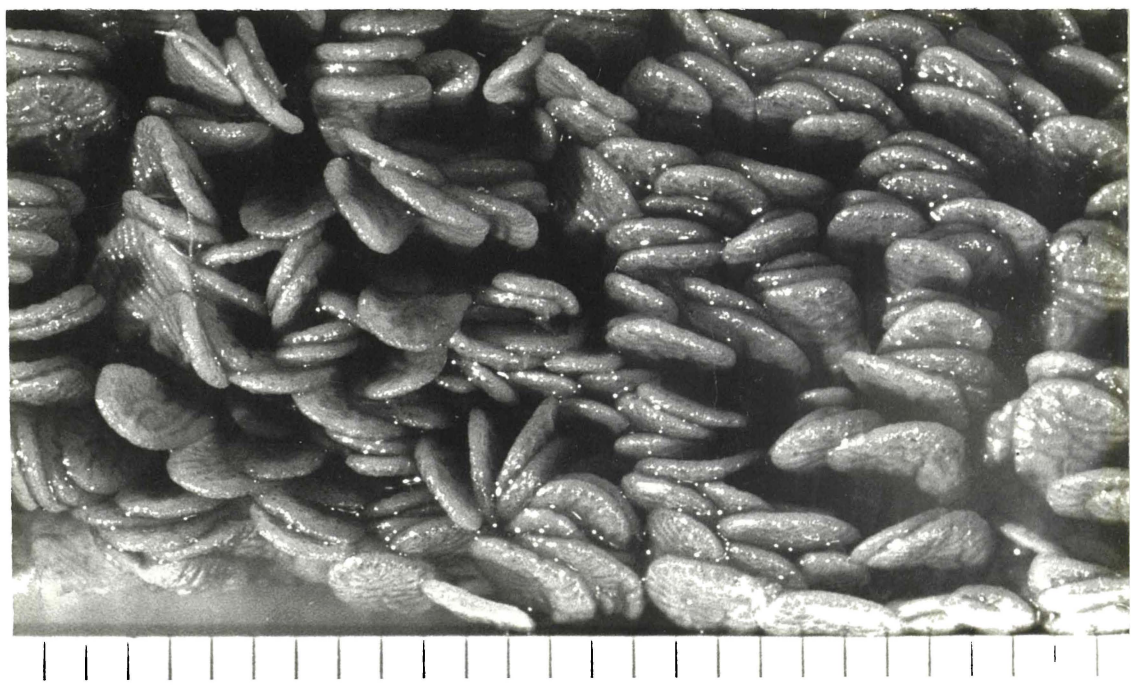


Plate 5.41 : Large spathulate papillae from the anterior ventral rumen sac from a control sheep not dosed with any zinc compound. Scale mm units.

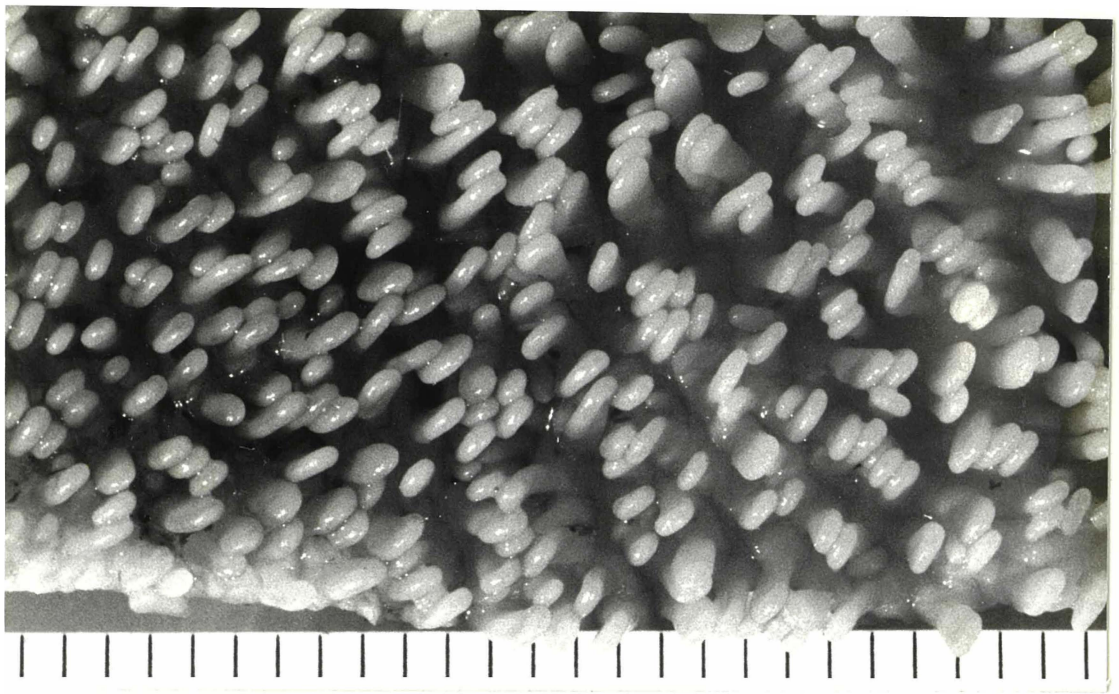


Plate 5.42 : Small shrunked smooth surfaced papillae from the anterior ventral rumen sac from a sheep which had been drenched zinc sulphate by drenching gun and had severe abomasal lesions. Scale mm units.

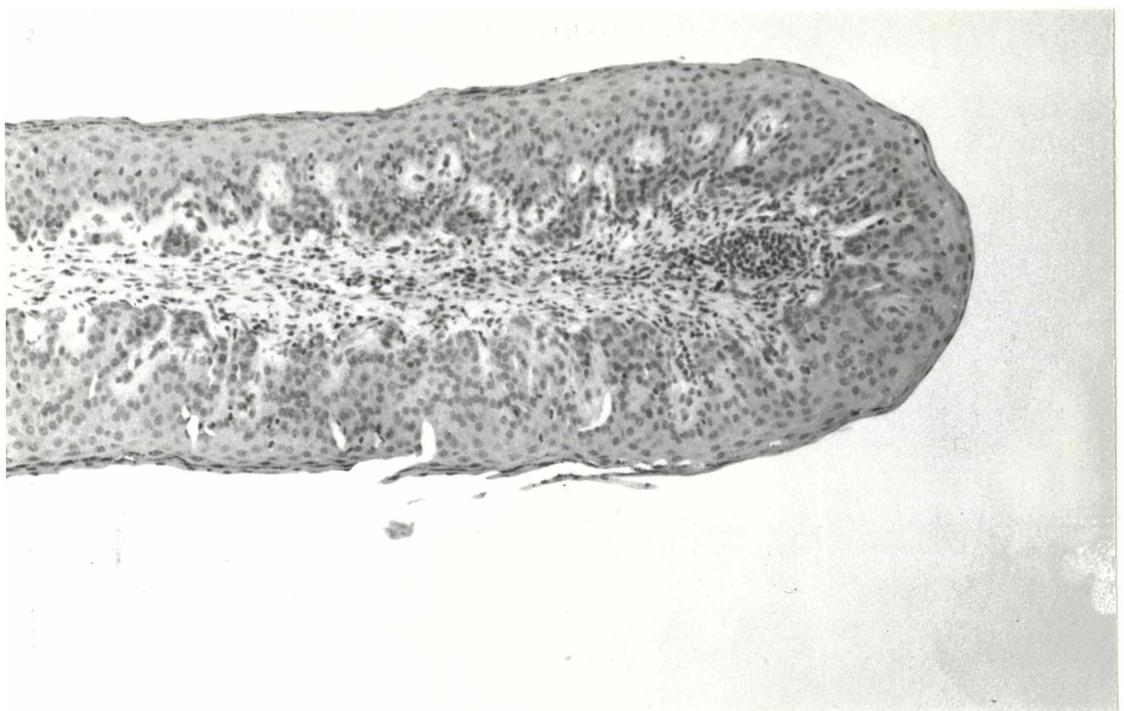


Plate 5.43 : Section of rumen (anterior ventral sac) papilla from a sheep dosed with zinc sulphate by drenching gun, resulting in a severe gastric lesion. The epithelial cells and their nuclei flatten; keratinisation although present is not prominent. (H & E)

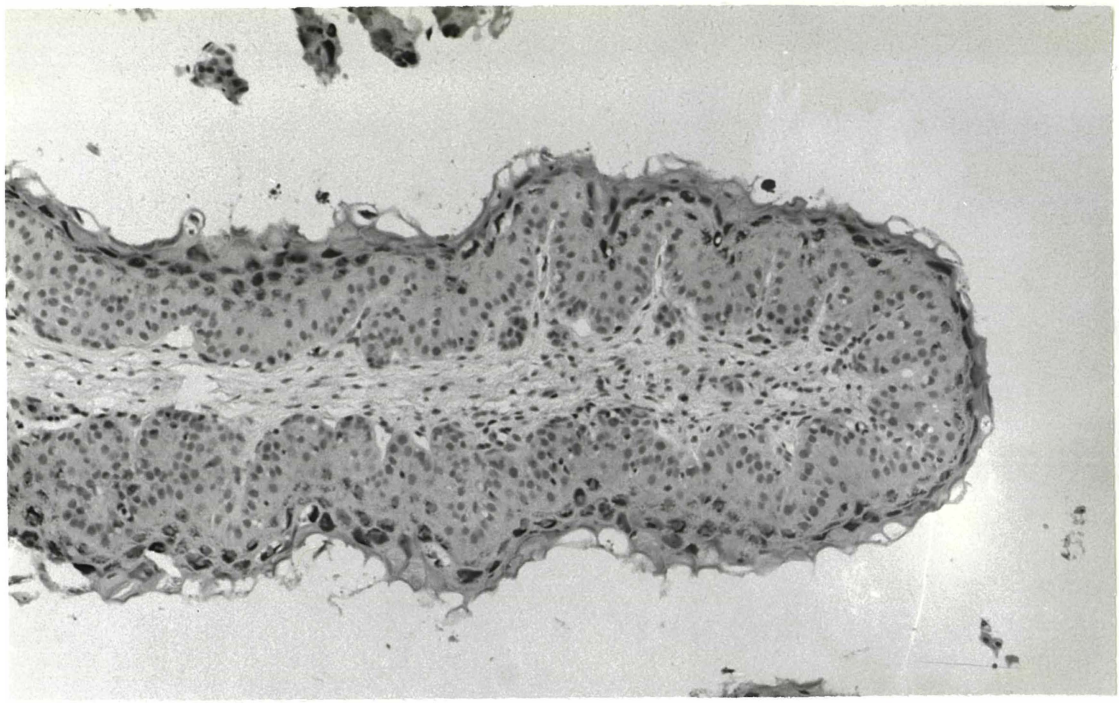


Plate 5.44 : Rumen (anterior ventral sac) papilla from sheep dosed with zinc sulphate by intraruminal intubation. At post-mortem examination these papillae were of normal size and shape. The epithelial cells show normal parakeratotic tendency with nuclei enlarged and maintained to the outermost stratified layers. At the outermost layer vacuolation, a scalloped appearance and incomplete keratinisation are evident. (H & E)

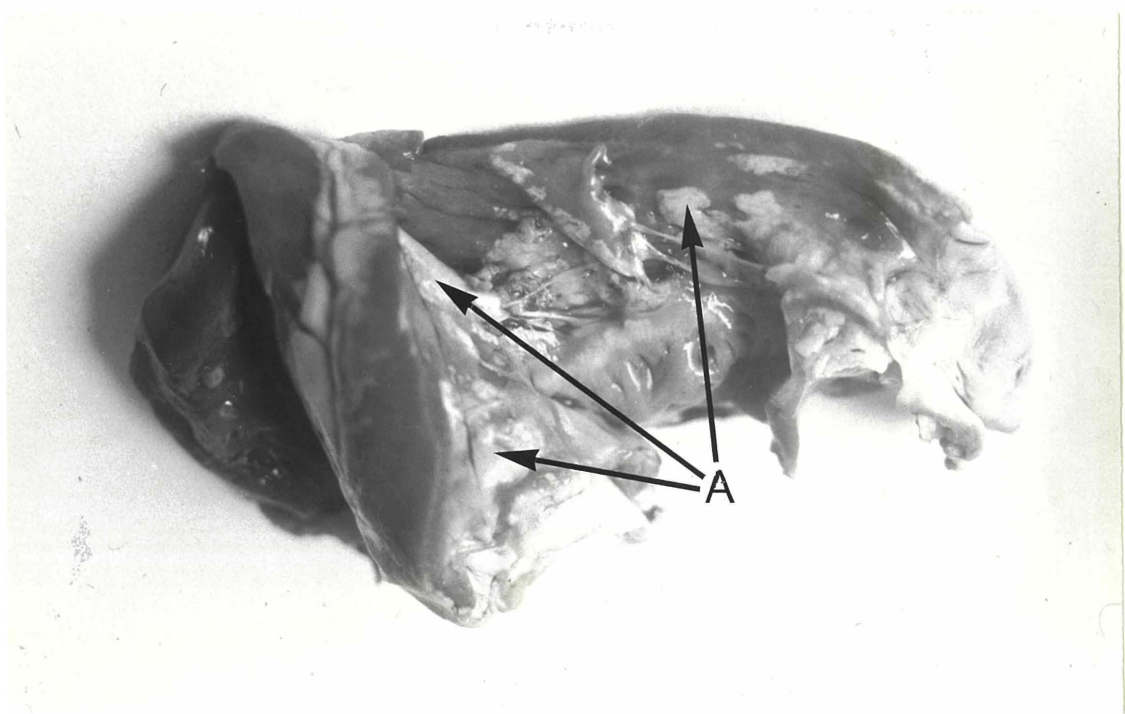


Plate 5.45 : Right ventricle of lamb's heart at post-mortem examination after being fed zinc sulphate in milk for 28 days. White subendocardial plaques (A) typical of white muscle disease are present.

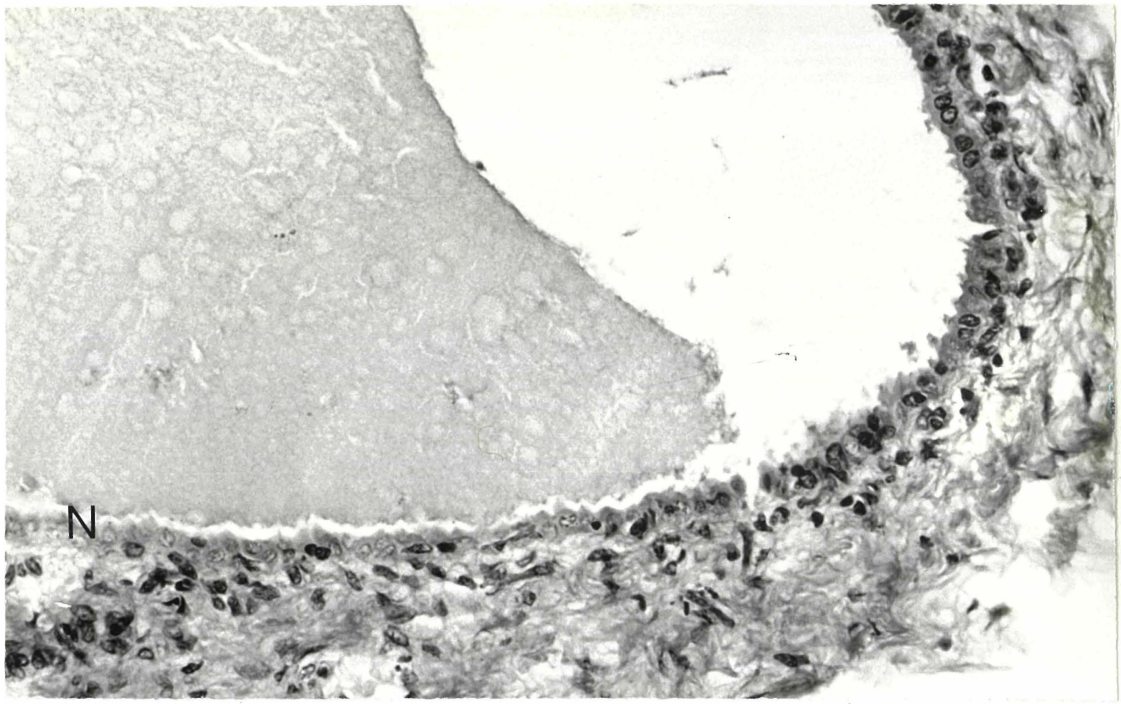


Plate 5.46 : Sheep pancreatic duct after zinc sulphate administration. Very early duct lesion. Many such ducts were seen in zinc dosed animals. i.e. loss of nuclear detail on one side of duct (N) and the presence of an eosinophilic homogeneous material adherent to the changed duct wall. (H & E)

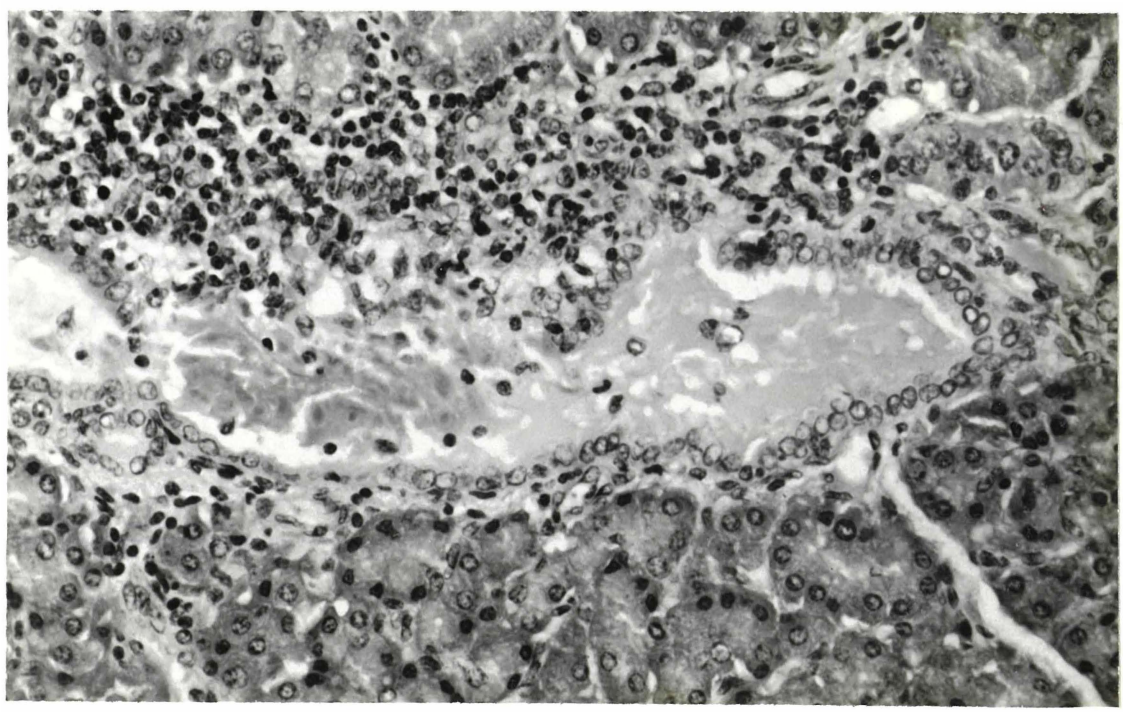


Plate 5.47 : Very early disruption of pancreatic duct epithelial integrity at seven days after start of zinc oxide administration. Leucocyte infiltration has begun in tissue adjacent to necrotic epithelium. (H & E)

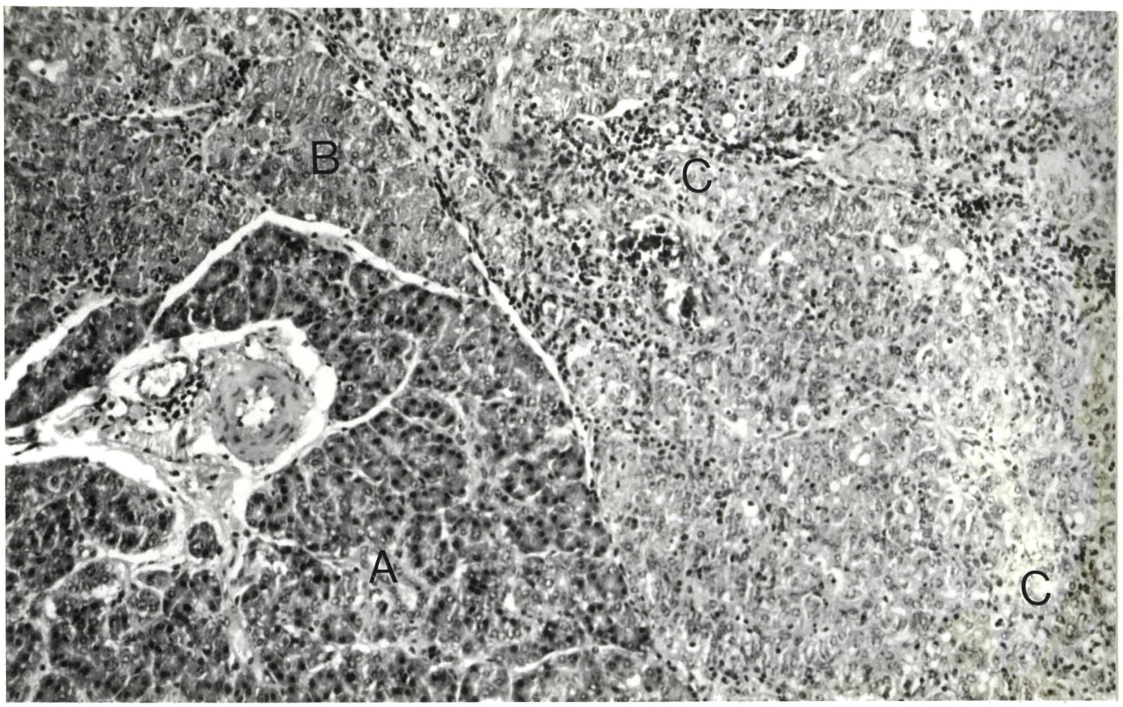


Plate 5.48 : Early pancreatic change in zinc oxide toxicity in sheep. Three adjacent lobules show different stages of degeneration. (A) none (B) early and minor changes (C) loss of zymogen granules, some parenchymal necrosis and inflammation. (H & E)

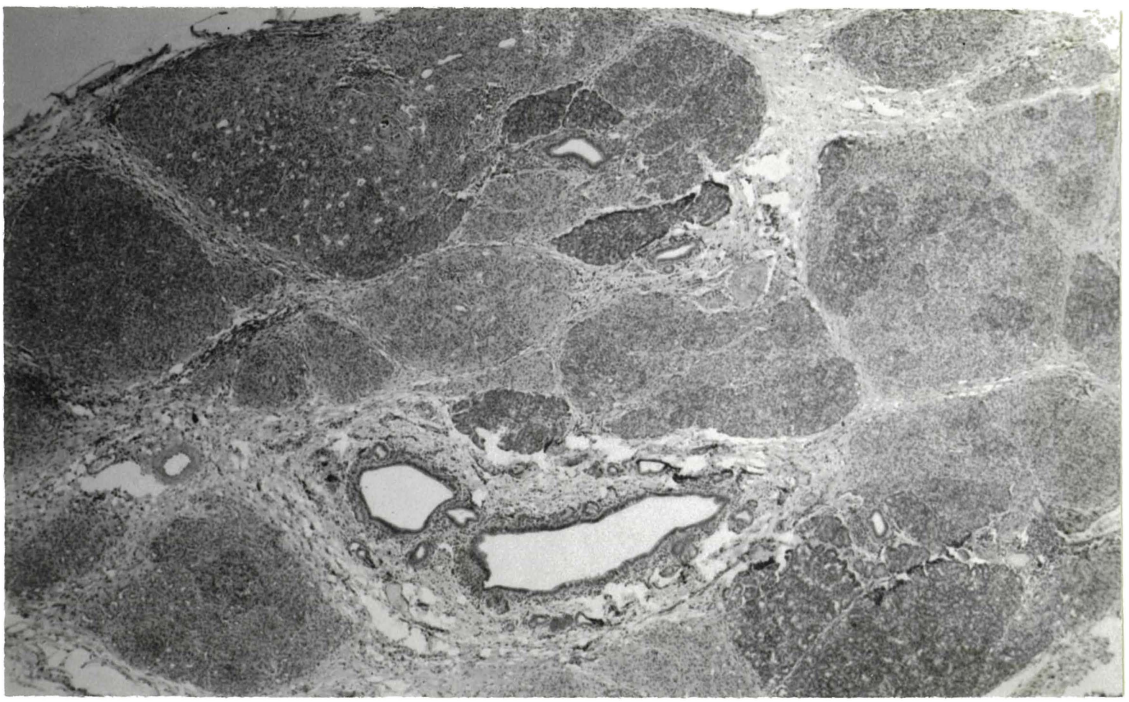


Plate 5.49 : Pancreas from sheep after administration of zinc oxide by paste gun. Early interlobular fibrosis is separating the different lobules. The differences in the development of changes within different lobules is evident. (H & E)

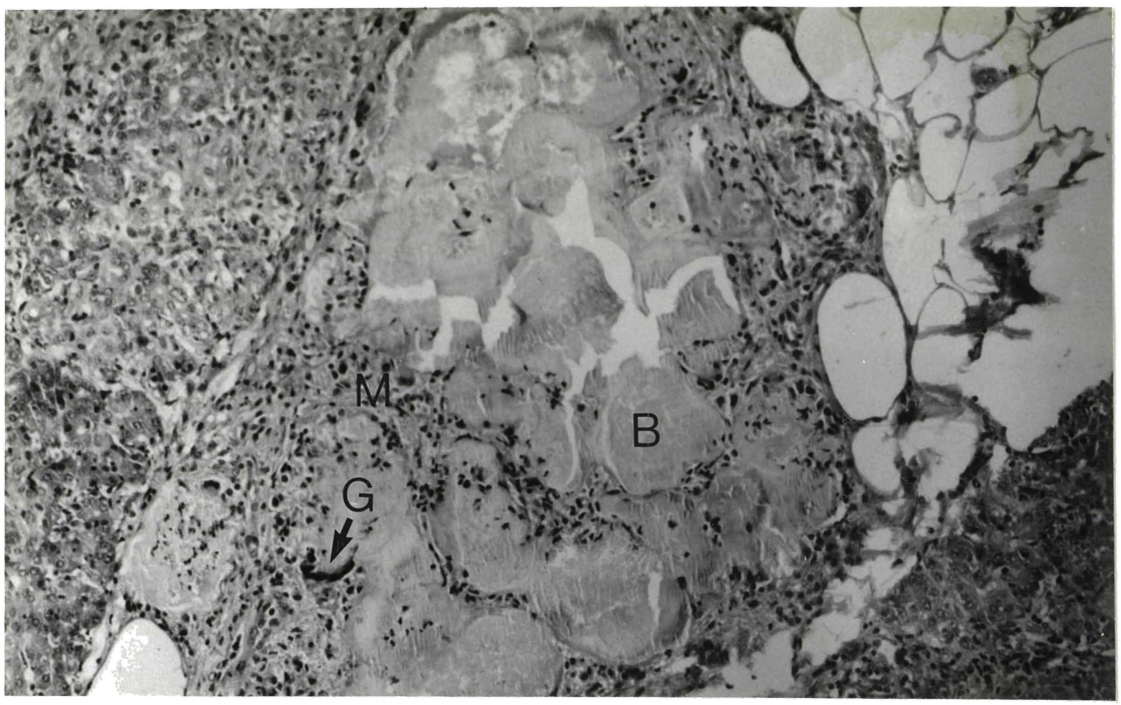


Plate 5.50 : Pancreas of sheep after zinc oxide administration. A faintly basophilic homogenous material remains in lipocytes (B) after routine processing for paraffin embedding and H & E staining, contrasts with the large clear spaces of normal fat vacuoles. Mixed inflammatory cell reaction includes macrophages (M) and multinucleated giant cells (G). Typical fat necrosis. (H & E)

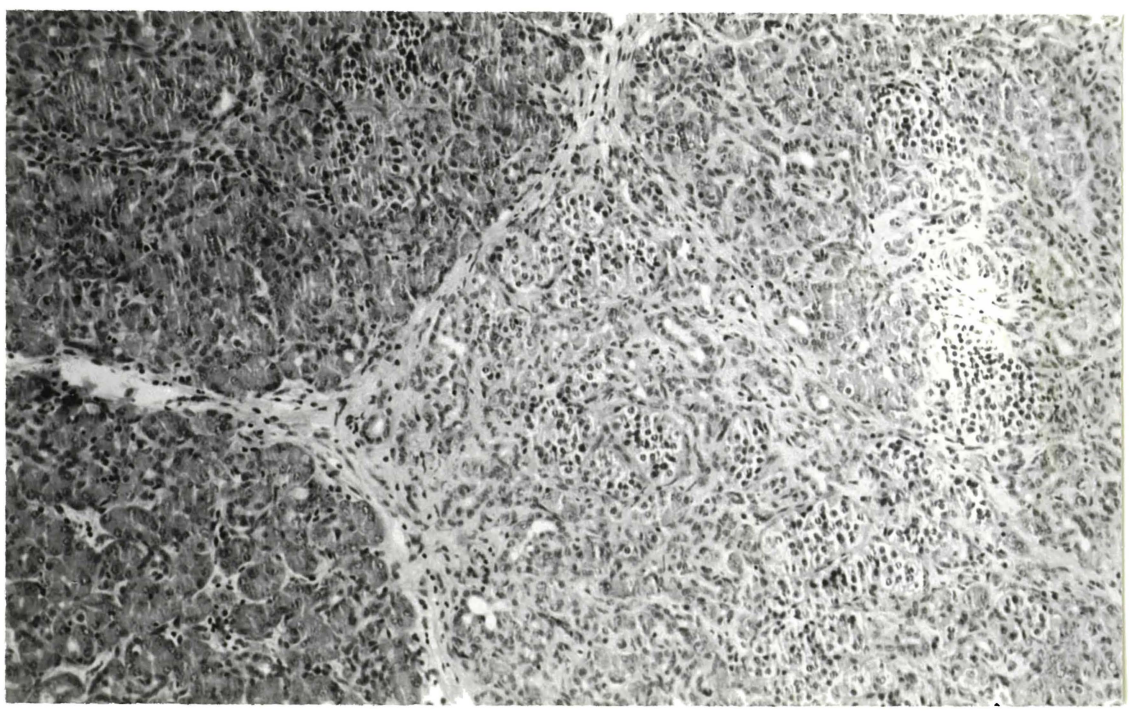
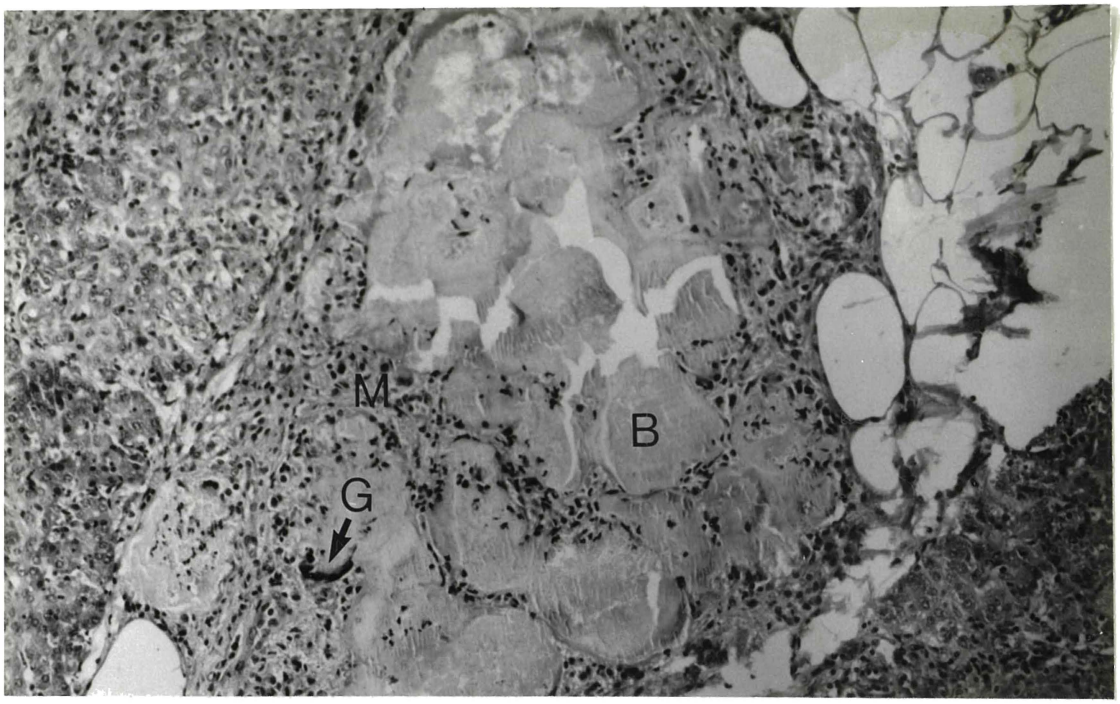


Plate 5.51 : Lobular differences in pancreatic lesions at 28 days after the start of zinc oxide dosing of sheep. Inflammatory cell infiltration, cystic appearance and fibrosis contrast with absence or minor nature of lesions in adjacent lobule. (H & E)



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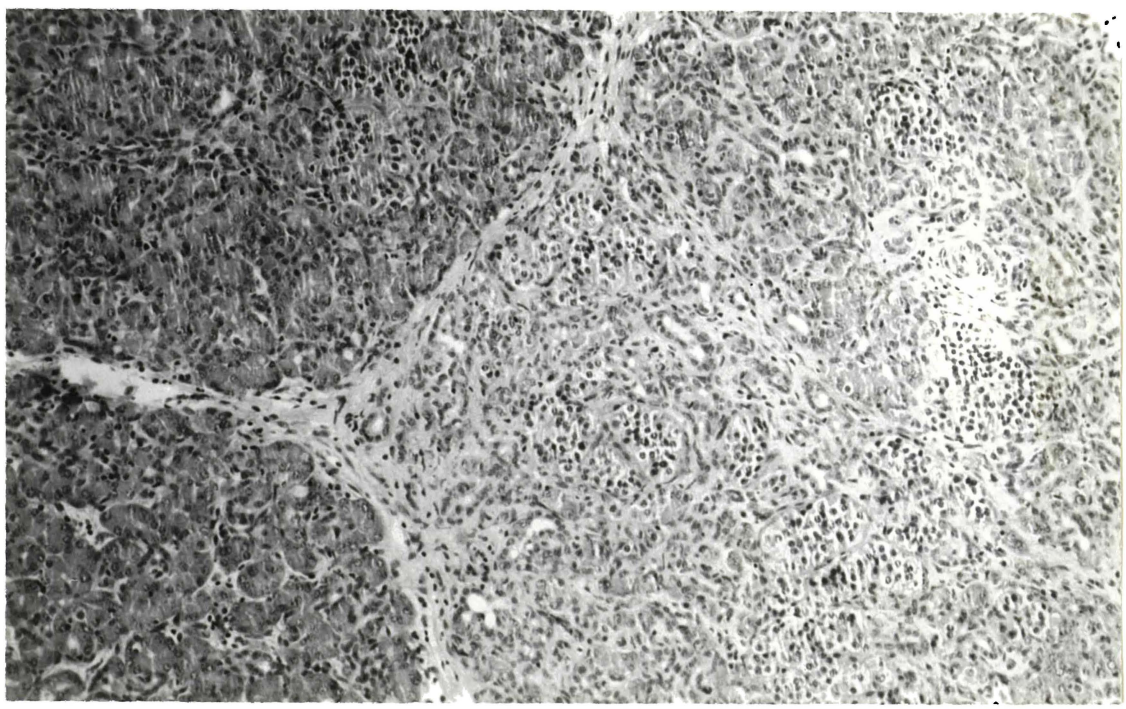

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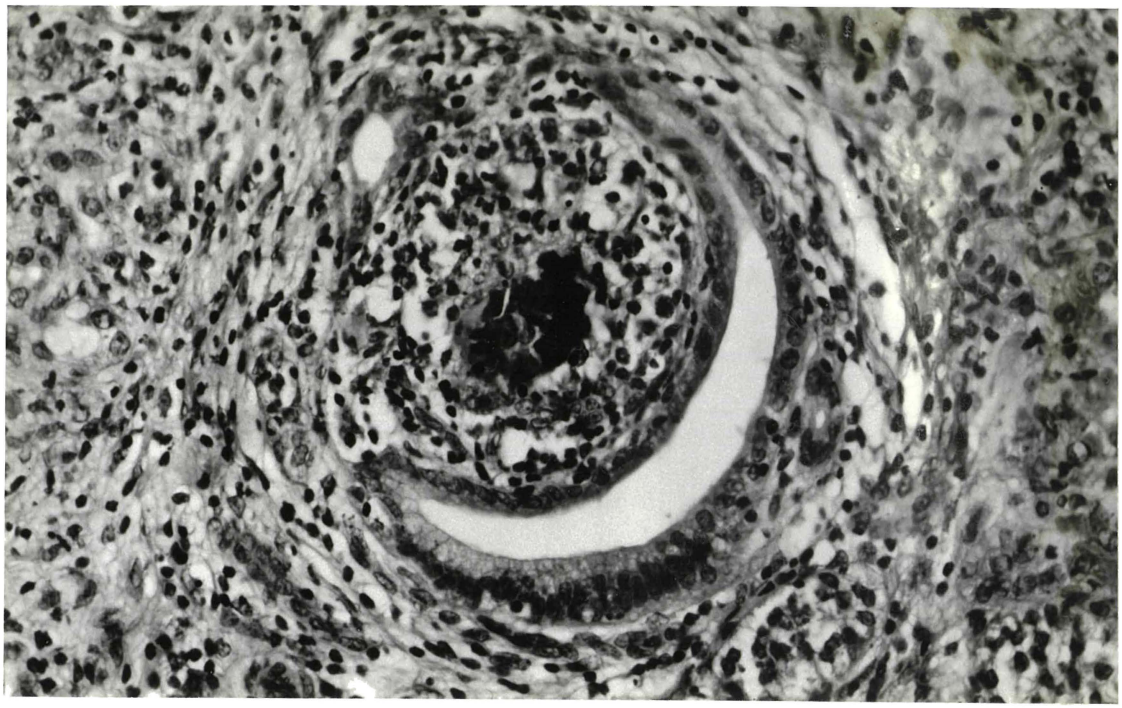

Plate 5.52 : Ovine pancreatic ducts after zinc toxicity. A basophilic concretion is all that remains of pancreatic duct. Its neighbour from which it has just branched is unaffected except for a pressure effect from the inflammatory change surrounding the degenerated duct. (H & E)