NITRATE — A FACTOR IN ANIMAL HEALTH

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SUMMARY

Nitrate as a substance potentially lethal to animals is widely recognized, and the pathway of its action understood. However, no definite intake of nitrate per animal or concentration in a ration can be described in absolute terms. Less widely recognized is the subclinical nitrate effect on animals. This paper reviews conditions leading to nitrate accumulation in feedstuffs, and the consequences in terms of animal physiology. It appears that nitrate *per se* is not directly involved, but manifests its action by altering the iodine-vitamin A-thyroid metabolism. Further studies are indicated on the physiological effects of nitrate apart from the classical nitrate poisoning syndrome (methaemoglobinaemia).

INTRODUCTION

NITRATE NITROGEN occupies a major position in the classical nitrogen cycle of nature. Modern agricultural technology has led to methods by which the potential nitrate nitrogen availability to plants can, as a routine, be increased in both grassland and non-grassland agriculture. From the animal physiology point of view it is not important whether the nitrate reaching the plant in excessive amounts is due to clover association, commercial fertilizers, stocking rate or application of natural manures, pasture or crop genetic composition, environmental factors, etc. The important question to be answered becomes: "Is nitrate a factor in animal health regardless of concentration?" Once a satisfactory answer is obtained, the technological innovations or agricultural practices contributing to the accumulation of nitrate must be re-evaluated. If these practices warrant continuance from economic considerations, then it becomes necessary for agricultural scientists to find antidotes and prophylactic treatments to minimize the detrimental physiological action. In this paper an attempt will be made to move from the areas of more definite knowledge toward the area of newly developing knowledge of the relationship of nitrate to animal health.

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CLASSICAL NITRATE POISONING

Synonymous with classical nitrate poisoning is methaemoglobinaemia. Relatively few other compounds give rise to methaemoglobin formation in domestic animals. Outbreaks of classical nitrate poisoning have been described in many countries, indicating that a wide range of conditions can enter its aetiology. In the United States, classical nitrate toxicity was formerly considered a problem of the Western Plain States owing to the practice of feeding oats grown during drought periods (oat hay poisoning). The same geographic or crop factors cannot be held liable for more recent occurrences owing to the changes in agricultural practice which have taken place.

Although the number of farms or ranches affected is usually quite small, the severity of loss to the individual is often high. Because of the severity and the ease of detecting methaemoglobinaemia in the field the mechanism involved within the animal and the antidotes which are successful are well known. Though the mechanism has been described many times (Sapiro *et al.*, 1949; Lewis, 1951a; Holtenius, 1957; Pfander *et al.*, 1957), a brief review seems important in order that the latter part of the paper can be considered in proper perspective.

Nitrate is converted to nitrite by the microbial population of either the rumen or intestine, depending on the animal species. The nitrite is absorbed into the bloodstream where it combines with the haemoglobin to form methaemoglobin. This reaction oxidizes the iron of haemoglobin to the ferric state, rendering the compound incapable of carrying oxygen to the tissues. Thus, the animal dies of anoxia if not properly treated. Methylene blue given intravenously has proved to be effective in restoring the oxygen-carrying potential to the haemoglobin. Other than damage to specific tissues caused by the anoxia, there is little recent evidence to suggest nitrite damage *per se* (Heuper and Landsberger, 1940).

Since ruminants ingest more nitrate by the nature of their feed, it is of interest to understand what can take place in the rumen with respect of nitrate, nitrite and ammonia (Lewis, 1951a, b). Failure of various workers to agree on a dose which will produce the same level of methaemoglobin consistently can partly be explained by the variations in metabolism in the rumen. Animal adaptation or rumen microflora adaptation resulting in lower methaemoglobin values has been demonstrated (Davison *et al.*, 1962). Sheep fed nitrite for extended periods were found to have micro-

organisms which reduced nitrite rapidly, resulting in low methaemoglobin values (Holst *et al.*, 1961). The final nitrite level fed to these sheep was close to or in excess of the lethal amount for non-adapted animals. Careful evaluation is required to distinguish whether the rumen flora or the metabolic action of the animal are involved. Some animals can withstand two high doses of nitrate but not a third. This supports the idea of a purely animal capacity which may adapt with time. The dose required to produce acute toxicity ranges from 15 g nitrate per 100 lb of body weight, administered by capsule (Bradley *et al.*, 1940), to 45 g nitrate per 100 lb of body weight, administered by sprinkling on to hay (Crawford and Kennedy, 1960). Thus the earlier work (Sapiro *et al.*, 1949; Lewis, 1951a, b; Clark and Quin, 1951), indicating that type of forage and energy level of the ration are related to the amount of nitrate converted to nitrite and the amount of nitrite utilized by the bacteria, must be seriously considered in determining the nitrite available for absorption into the bloodstream.

Data from an experiment where $K^{15}NO_3$ was directly added to the rumen of cattle (Wang *et al.*, 1961) suggest the direct absorption of nitrate and nitrite from the rumen. Although absorption did take place from the ligated rumen of sheep under anaesthesia, the rate of absorption did not seem compatible with intact animals as measured by the increase in blood level of nitrate or methaemoglobin (Pfander *et al.*, 1957). This latter work also demonstrated that in classical nitrate poisoning nitrate does not contribute to the death of the animal beyond being the source of nitrite.

In practice the assessment of the toxic level of nitrate in the feed becomes a matter of interpretation of local conditions. The writer, from field experience, believes the 1.5% potassium nitrate equivalent of the ration dry matter (0.9% nitrate) should be considered borderline with respect to acute nitrate toxicity until data for specific areas can demonstrate it to be low or high. In concluding this part of the paper it should be pointed out that most of the studies have been carried out on dry rations under stall feeding.

AGRONOMIC FACTORS ASSOCIATED WITH NITRATE ACCUMULATION

Whitehead and Moxon (1952) and Butler (1959) presented conditions known to be associated with nitrate accumulation in plants. These are nitrogen fertility level of soil available for plant growth; nitrification rate of the soil bacteria during plant growth; fertilizers applied which con-

tain nitrogen in relation to the major plant nutrients (K, P); soil texture and rainfall contributing to the degree of leaching; soil moisture; temperature; light intensity; and trace element deficiency (Mo, Fe, Cu, Mn and S).

It is well known that certain crops and weeds accumulate more nitrate than others when grown under similar conditions (Dodd and Coup, 1957). More recently the genetic composition of a strain or variety has been shown to be a factor in nitrate accumulation in ryegrass (Butler *et al.*, 1962) and in corn (Hageman and Flesher, 1960). Subject to the many conditions mentioned above, the grasses and brassicas are more prone to nitrate accumulation than are the legumes.

Grazing habits or harvesting methods are important also, because the nitrate is not equally distributed in leaves, stems and roots. The stage of maturity of the plant has a definite effect. Immature plants tend to be higher in nitrate than more mature plants, and stems contain more nitrate than leaves (Flynn *et al.*, 1957; Butler, 1959). In chopped forage, or during the ensiling procedure the plant leaf enzymes are freed and reduce the total nitrate. The association of micro-organisms present during ensiling reduces still further the nitrate level (Barnett, 1954). Silage made with preservatives, or rapidly dried forage, may contain essentially the same concentration of nitrate as when cut but the total digestible nutrients (T.D.N.) are higher, which would help alleviate the nitrate effect in the animals.

The rate of nitrate intake and the composition of the ration are subject to management practices. Therefore, with the aid of simple chemical diagnostic tests and knowledge of the factors involved in nitrate accumulation for a given locality (Durham, 1962), acute nitrate poisoning can be avoided with good management.

Without going into detail, the water supply must also be considered to be important as a source of nitrate and in some cases it contains enough nitrate to be toxic (Campbell *et al.*, 1959; Garner, 1959).

SUBCLINICAL EFFECT OF CHRONIC NITRATE INGESTION

Early literature (1850–1917) has been reviewed. Experiments were conducted with humans to determine any deleterious effect of nitrate arising from meat cured with saltpetre (Grindly and Mitchell, 1917). They found no detectable effect. A summary of the early literature can be

found in the U.S. Dispensatory, 24th edition. A summary of this information for *potassium nitrate* shows:

(1) It does not alkalize the system as organic potassium compounds do.
(2) It will increase urine flow.
(3) It is not a very potent poison.
(4) Long exposure to small amounts may result in anaemia and low blood pressure.
(5) It can cause cyanosis in milk-fed infants.

A similar summary for *sodium nitrite* shows:

(1) It increases urine flow.
(2) It diminishes reflex activity.
(3) It relaxes all smooth muscles.
(4) It converts haemoglobin to methaemoglobin.
(5) It is not a very potent poison.

With the exception of methaemoglobin formation (acute toxicity) little attention was paid to sub-lethal amounts of nitrate or nitrite in animal feeds until about ten years ago. The changes in agricultural practices from 1945 to 1955 in technically developed countries resulted in greater availability of nitrogen to plants and the potential for nitrate accumulation thus increased. In the U.S.A. a severe drought seriously stunted corn, leading to heavy losses of cattle when this corn was used for grazing (Muhrer *et al.*, 1955). These losses were not of the classical nitrate syndrome as methaemoglobin was absent, however petechiation of the œsophageal region, rumen impaction or apparent rumen stasis were present and there was a correlation between the nitrate content of the forage and the death losses. In addition, the ensiling of the corn grown during the drought resulted in a phenomenon called "fuming silo" (due to oxides of nitrogen being released) which led to animal and human deaths when the gases were inhaled (Peterson *et al.*, 1952; Grayson, 1956).

Feeding the preserved, drought-stricken forage caused further animal difficulties such as milk production loss, reproductive failure and weight loss (Case, 1957; Muhrer *et al.*, 1956). Abortion in cattle associated with the nitrate content of pasture weeds (Sund *et al.*, 1957) increased the interest in the physiological response to nitrates. Field evidence of full term but dead calves, swollen briskets, rough hair coats, and scours in various combinations led practising veterinarians to use vitamin A therapy and to increase the ration carbohydrates (Case, 1957).

Using available experimental evidence, field data and personal observations, a bulletin *Learn to Live With Nitrate* was prepared for use in Missouri (Garner, 1958). A concentration of 0.5% potassium nitrate equivalent (0.3% nitrate) of the ration dry matter was suggested as a safer level than the 1.5% normally accepted, and the addition of pre-formed vitamin A was recommended, even in cases which should have had ample carotene. The outcome of these recommendations has been generally satisfactory to date.

Data reported for ruminant feeding trials with naturally occurring nitrate or with added nitrate salts have, with respect to weight gains and hepatic vitamin A stores, been conflicting. A study with fattening steers (Hale *et al.*, 1962) demonstrated a significant interaction between T.D.N. level (54.3% versus 71.3%) and nitrate (0 versus 1% KNO_3). Nitrate did affect intake significantly, but not average daily gain. There was a significant positive relationship between ration T.D.N. level and depletion of vitamin A. Nitrate increased the depletion but not significantly. Field observations of vitamin A deficiencies in feed-lot cattle in the U.S.A. may be more related to high concentrate feeding than to nitrate, except on low energy, roughage rations. Decreased retention of nitrogen in a ration high in non-protein nitrogen (Bloomfield *et al.*, 1961b) could also be a factor in growth when nitrate is present.

The evidence of nitrate induced abortions in cattle (Simon *et al.*, 1958; Garner, 1958) and foetal resorption in rats (Muhrer *et al.*, 1956) are not substantiated in cattle (Davison *et al.*, 1962) or in swine (Tollett *et al.*, 1960).

Experimental evidence for loss of milk production in dairy cows fed nitrate (Muhrer *et al.*, 1956; Stewart and Merilan, 1958) are not compatible with the experimental data of Davison *et al.* (1962). Other dietary factors may be involved besides total T.D.N. (especially the sugar and starch levels) and vitamin A. In the experiment of Davison *et al.* (1962) the ration was composed of a full feed of first cutting lucerne-grass hay plus 4 lb of a concentrate composed of 80% ground shelled corn, 18% soybean oil meal, 1% trace mineralized salt, 1% dicalcium phosphate and 2,000 IU vitamin A palmitate per lb. This ration is generally superior to rations being fed in practical conditions from the point of view of nutritional balance. This illustrates the growing body of evidence that sub-clinical nitrate toxicity is associated with a degree of malnutrition, and if the plane of nutrition is sufficiently high even acute nitrate poisoning occurs less readily.

MECHANISMS WHERE NITRATE CAN BE A FACTOR

It is of interest to establish what happens to nitrate once it is absorbed into the animal's body. Monogastric animals are easier to study with dietary nitrate sources than are ruminants, although intravenous infusion of nitrate solution can offset part of the rumen effect. In dogs (Hiatt, 1940) hypochloraemia could be produced by nitrate administration with apparent replacement of chloride in extracellular fluid by nitrate. Recoveries of 92% of an infused dose in dogs in 72 hours indicate little metabolic action (Greene and Hiatt, 1954). Similar measurements have been made with rabbits (Kilgore *et al.*, 1959). In measuring the excretion rate of nitrate in cattle and sheep the "nitrate space" was found to correspond to the "chloride or thiocyanate space" (Garner *et al.*, 1961). A marked rise in excretion of chloride in cattle and sheep at the start of nitrate infusion has been observed (E. O. Kearley, *pers. comm.*). A recovery of 4.8 to 14.3% in the urine of sheep given 15 g of sodium nitrate orally compared with 8.0 to 21.4% recovery when given intravenously (Setchell and Williams, 1962) corresponds to average values of 16% and 27% for sheep and cattle, respectively (Garner *et al.*, 1961).

Having established that nitrate was in equilibrium in the extra-cellular fluids and could replace chloride in body fluids, nitrate in gastric secretion was measured. Bloomfield, *et al.* (1962) demonstrated a concentration gradient of 20 between gastric content and plasma in the rat. Kearley *et al.* (1962), using a Pavlov pouch in a sheep, were able to show a concentration gradient of 9.96 with a plasma level of 2.2 mg per cent. nitrate. Nitrite was found sporadically in both studies but its quantitative physiological relationship cannot be evaluated at this time. What role nitrate in gastric contents might have on animal health awaits further investigation, while nitrite may reduce the availability of carotene and vitamin A (Pugh *et al.*, 1962; Emerick and Olsen, 1962). Carotene destruction by nitrite is chemical in nature and has been shown *in vitro* to be pH dependent, suggesting the site of action is the true stomach. However, the possible destruction of vitamin A by nitrite at a tissue level is not ruled out as nitrite-fed rats on a vitamin A- and carotene-free diet became depleted more rapidly than the controls (O'Dell *et al.*, 1960). Nitrate has less effect on the depletion rate but reduces vitamin A storage in rat liver when vitamin A-deficient rats are given carotene.

The mechanism by which nitrate depresses liver storage of vitamin A in rats may be twofold, but by a single action. One effect may be due to a goitrogenic action (Wyngaarden *et al.*, 1952; Bloomfield *et al.*, 1961a) resulting in less absorption of carotene from the gut, and the second effect that of reducing the total body iodine (Bloomfield, *et al.*, 1962a). The storage of vitamin A in rat liver is related to available dietary iodine (Yadav *et al.*, 1962). This latter effect must be tested more thoroughly. However, the depletion of vitamin A reserves to the point of xerophthalmia was delayed by increasing iodate supplementation from 0.2 μg to 2.0 μg iodine per gram of ration. When nitrate was added at these levels of iodine it increased the incidence of xerophthalmia in the depletion period and depressed vitamin A storage in repletion.

Further evidence of thyroidal involvement in the non-acute nitrate syndrome has been presented by Reddy and Thomas (1962) showing a thyroxine or tri-iodo-thyronine-nitrate-carotene conversion relationship with duodenal homogenates. Both sheep and rats have shown thyroidal responses to nitrate as measured by ^{131}I uptakes or gland size and the difference between treatments is increased with decreasing environmental temperature (Welsch *et al.*, 1962; Bloomfield, *et al.*, 1962b).

Whether these mechanisms, together with the action of nitrate on the rumen flora, explain the field conditions or not is difficult to evaluate. Certainly it is well known that hypothyroidism is not conducive to milk production and growth, and vitamin A deficiency can lead to lowered survival at birth and other reproductive difficulties. In any case, more research is required before the answer to the question of sub-clinical nitrate toxicity can unequivocally be given.

NITRATE AND ENDEMIC DISORDERS IN NEW ZEALAND

The mechanism of nitrate/nitrite action in the rat and sheep so far established should be of interest in evaluating nitrate accumulation in the ryegrass, ryegrass-clover and brassica fodders used in ruminant production. The possible relationship of nitrate to hogget ill-thrift should be pursued (Jamieson, 1958; Clarke, 1959; Butler, 1959). The assessment of the contribution of nitrate as well as the cyanoglucosides to the overall pasture goitrogenicity (Flux *et al.*, (1960) represents a challenge in view of the responses noted to injected iodine. In addition, there is the role nitrate might play in precipitation of goitre in lambs in the iodine deficient areas of New Zealand (Andrews and Sinclair, 1962). In all of these cases the vitamin A status should not be overlooked.

CONCLUSION

(1) Classical nitrate poisoning cases will increase with higher per acre yields of crops or meat unless farmers, veterinarians and researchers remain vigilant and implement sound practices.
(2) The existence of sub-clinical nitrate toxicity may be more a matter of malnutrition or increased requirement of a given nutrient (iodine and vitamin A) than nitrate toxicity *per se*.
(3) Usual manifestations of sub-clinical nitrate toxicity under practical conditions are poor growth, milk production loss, symptoms of vitamin A deficiency and reproductive difficulty. Experimental evidence both for and against can be quoted.
(4) The level of nitrate *per os* required to produce classical nitrate poisoning symptoms in ruminants not adapted to it is near 15 g nitrate per 100 lb of body weight by drench or rapid ingestion of feedstuffs (0.9% nitrate of diet dry matter). The minimum level for causing no measurable physiological change appears to be less than 0.3% nitrate of the diet dry matter for some rations.
(5) A mechanism of action for nitrate in relation to thyroid metabolism and vitamin A storage has been demonstrated.

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DISCUSSION

Q: *Is there any evidence that nitrates can have an antirachitic effect in animals?*

DR G. B. GARNER: Not to my knowledge.

Q: *According to Dr Garner, levels of 1% nitrate on a feed dry weight basis may be toxic to stock, yet green pasture may contain up to 7% nitrates and show no toxic effects. What might be the reason for this?*

DR GARNER: First, one must be sure the animals are consuming this herbage, secondly, one needs to know the amount they are eating in terms of body weight and, thirdly, that toxic effect spoken of here refers only to classical symptoms of methaemoglobin and possibly abortion. Most of the research reports in the literature are on dried herbage or silage, not green pasture. Just what part the plant enzyme may play has not been evaluated quantitatively but it is conceivable that nitrite arising from nitrate is quickly converted to other compounds by nitrite reductase of plant origin. Thus, nitrite concentration would be low and the grass appear to be non-toxic. Certainly a quantitation of this plant enzyme role in the rumen should be done.

DR A. T. JOHNS: Some standardization of method of dosing with nitrate in experiments carried out by different workers would be desirable. For example, the effect of a dose given all at once may be quite different from the effect of the same dose given over a day as a number of sub-doses.

DR GARNER: This is true if we are all measuring the same response or trying to elucidate a given pathway. Unfortunately, the rate of ingestion of feedstuffs varies between animals in the practical case, resulting in a lack of standardization for a single experimental ration.

DR G. W. BUTLER: It would seem particularly important for workers in this field to measure the total soluble carbohydrate content of the feed as well as the nitrate level, since it is well established that the ratio of these constituents is of prime importance with respect to nitrate poisoning.

DR GARNER: Dr Butler's comment is very important at nitrate levels approaching 1% of the dry matter. Certainly if all the nitrate can be converted to a more reduced form (*i.e.*, NH_3) in the rumen no physiological response would be expected.