TOXICITY AND PHYSIOLOGICAL MOVEMENT OF VANADIUM IN THE SHEEP AND RAT

By

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Experimental feeding trials involving 66 sheep and 142 rats were employed in conjunction with radioisotopic and chemical measurements to investigate the toxicity and physiological behavior of vanadium and quantitative relationships to animal performance.

In a growth study, 24 lambs, individually fed supplemental levels of 0, 10, 100, 200, 400 or 800 ppm dietary vanadium (ammonium metavanadate), showed clinical toxicity signs only on the two highest levels. Dietary quantities up to and including 200 ppm added vanadium for 84 days resulted in small initial negative effects on feed intake and tissue content was increased. No effects on hemoglobin, hematocrit or growth rate were detected. Lambs fed the two highest levels, 400 and 800 ppm, ceased eating after initial exposure, but subsequent

refeeding of the basal diet resulted in an apparent complete recovery within 8 days.

Fourteen sheep were fed 0, 50, or 200 ppm supplemental vanadium and were administered radiovanadium either orally or intravenously prior to a 144-hr blood-clearance and tissue-distribution study. Limited intestinal absorption was indicated but dietary vanadium did influence ^{48}V retention; less of the isotope was retained in kidney ($P < .01$) and major organs of sheep receiving the highest dietary vanadium level. Kidney, bone, liver and spleen retained (in decreasing order) the highest activity 144 hr after dosing, and patterns of deposition were similar for both methods of administration. Clearance of the isotope from circulating blood was initially rapid, was not significantly altered by dietary treatment and could be described by a model having three exponential components.

An experiment was conducted with 12 wether lambs which were administered three vanadium compounds by capsule. Daily dosage rate was increased by 50 mg vanadium at 2-day intervals to minimize delayed effects of toxicity. Onset of toxic effects did not differ significantly for calcium orthovanadate, calcium pyrovanadate or ammonium metavanadate when 25% reduction in feed intake was used as an indicator of response. Animals were adversely affected by dosage levels of 400 to 500 mg vanadium per day administered in this manner, corresponding to a range of 9.6 to 12 mg/kg body weight. Signs of toxicity included elevated tissue vanadium, fatty degeneration of

liver, diarrhea and mortality. An additional 16 lambs were fed 0, 50 or 200 ppm supplemental vanadium for 90 days; coccygeal vertebrae were sampled before and at 15-to 30-day intervals after imposing the dietary regimen to examine the effect of dietary intake on deposition of the mineral with time. Bone content of the element was elevated ($P < .05$) after feeding 200 ppm added vanadium for 15 days, but non-significant increases occurred thereafter. In a subsequent balance study, urinary vanadium excretion was directly related to dietary intake of the mineral.

Results of a series of investigations with the rat indicated that both feed intake and growth rate were depressed when 40 ppm vanadium was fed in combination with semi-purified diets. Vanadium content of bone and kidney was increased ($P < .01$) by 20 ppm added vanadium, but effects of elevated dietary protein on both performance and tissue content were non-significant. Hematocrit decreased linearly with increased dietary vanadium. When rats receiving 20, 40 or 80 ppm vanadium in semi-purified diets were compared to pair-fed controls, growth rates of the former group were negatively affected to a greater extent than the latter, although feed intakes of both were depressed with increasing dietary vanadium. Greater depression in growth at the 80 ppm level ($P < .05$) relative to pair-fed controls indicated that reduced feed intake was not the only mechanism in the growth impairment.

CHAPTER I

INTRODUCTION

Vanadium, V, is a bright white metal in the pure state, having a density of 5.9 and a melting point of 1900°C. It occurs in positive valencies of two, three, four or five (C.R.C., 1971-1972). Vanadium was discovered by Andrés Manuel del Rio in 1801. He named the new metal erythronium, but later decided it was only impure chromium. The element was "rediscovered" in 1830 by Nils Gabriel Sefström who named it after Vanadis, the Norse goddess of beauty (Busch, 1961).

Underwood (1962) noted that vanadium has been considered a rare element primarily because of the few commercially workable deposits. It is one of the more prevalent trace elements, however, occurring in larger quantities in the earth's crust than the essential elements, zinc, copper, cobalt, molybdenum and nitrogen; and the toxic elements cadmium and lead (Schroeder et al., 1963 Dunn et al., 1954).

Vanadium is found in some 50 different naturally-occurring minerals among which are carnotite, roscoelite, vanadinite and patronite. These four are important industrial sources of the metal (Faulkner-Hudson, 1964). Tool and cutting steel, high-strength structural steel and wear-resistant cast iron often contain .1 to .5% vanadium. By evenly dispersing

carbon, oxygen and nitrogen impurities, it makes the steel fine-grained and more homogeneous in quality, thus enhancing the desirable qualities of the steel. Natural vanadium occurs in igneous rock, in shales, in some uranium and iron ores and in certain deposits of phosphate rock. There is much vanadium in marine phosphorites of Montana, Wyoming and Idaho (Busch, 1961; Schroeder et al., 1963). It is found universally in the ash of coal, petroleum and asphalt; some samples contain 50 to 70 per cent vanadium pentoxide (Goldschmidt, 1958). Romoser et al. (1960) noted that many rock phosphates and colloidal clays contain as much as 6000 ppm of the element. Berg (1963) reported that a commercial phosphorus supplement, tricalcium phosphate, contained .25% vanadium pentoxide.

Vanadium compounds are toxic to all animal species that have been studied. The metal ranks as one of the more poisonous trace minerals. It has been shown, for example, to be slightly less toxic than selenium, but more toxic than arsenic when fed to rats (Franke and Moxon, 1937). Martinez and Church (1970) reported that the addition of 5 ppm vanadium significantly reduced cellulose digestion by washed suspensions of rumen micro-organisms. Vanadium was found to be less toxic than fluorine, but exhibited greater toxicity than selenium or chromium to these cells.

The metal is widely distributed in nature, occurring in many plants and animals (Curran and Burch, 1967; Underwood,

1971). Söremark (1967) has analyzed a few biological specimens, but there is little information in the literature on the concentration of vanadium in foods (N.R.C., 1974a). Ingredients used in commercial feeds for animals probably vary widely in vanadium content. Feed ingredient analyses, however, are rare. Levels in commercial phosphate sources range from low to very high, and dietary phosphorus sources containing vanadium have been shown to exhibit sufficient toxicity to reduce growth in poultry (Berg, 1963). Toxic effects of the mineral are decreased by other dietary factors, e.g., chromium and ascorbic acid (Wright, 1968; Berg and Lawrence, 1971), and are enhanced when it is fed with semi-purified diets (Mountain et al., 1959; Berg, 1966). Thus, diet composition influences the toxicity characteristics. New sources of nutrients are continually becoming available for use in formulating feeds. Vanadium may be, therefore, an unrecognized or potential hazard in other species.

Grazing animals are exposed to elevated levels of many minerals due to coincidental ingestion of soil (Thornton, 1974; Healy, 1973) and vanadium occurs in some soil types in quantities several orders of magnitude higher than in plants (N.R.C., 1974a). Additionally, both soil and herbage content may be enriched by fallout of vanadium-containing particulate matter from the air (Winchester and Nifong, 1970).

A practical problem due to the toxic nature of the element has been demonstrated in poultry. The potential for

decreased performance in other commercially important species is therefore apparent, yet little information is available with regard to tolerance, toxicity or physiological movement in domestic animals. In the present investigation, experiments were conducted to study the effects of vanadium on metabolism and performance. Chemical and radioisotopic procedures were employed to monitor the physiological movement of the absorbed metal in animals and ingestion trials conducted with both sheep and rats to quantitate absorption, toxic effects and retention following acute and chronic exposure.

CHAPTER II

REVIEW OF LITERATURE

Vanadium in the Environment

Interest in the quality of the environment and in the impact of environment on foods derived from plants and animals has contributed to the recent emphasis on minor element research. A knowledge of trace mineral movements through the food chain is necessary in order to differentiate beneficial from harmful effects. Many of the minor elements are known to be either acutely or chronically toxic to animals when present in the diet at abnormally high levels, while others, e.g., trivalent chromium, are relatively non-toxic. Well-known examples of the more toxic trace elements are fluorine and selenium. Less well known is the toxicity of vanadium, although the recent recognition of high concentrations in some phosphates, coals and petroleum products has heightened interest in the movement of the element from the environment to animals and man.

Attention has focused on vanadium with the finding of high concentrations in certain phosphate rocks used as sources of elemental phosphorous in domestic animal diets. In addition, the discovery of vanadium compounds in fly ash from the combustion of residual petroleum products and coal

from certain parts of the world has renewed interest in the toxic properties of the metal.

Vanadium in Soil and Mineral Deposits

Vanadium is one of the more abundant trace minerals. The level in the earth's crust has been variously estimated at 110 (Goldschmidt, 1958) to 150 ppm (Vinogradov, 1959), approximating the concentrations of zinc and nickel (N.R.C., 1974a). The mineral is widely distributed throughout a variety of soil and rock types and is more plentiful in the geosphere than two other notably toxic metals, cadmium and lead (Schroeder et al., 1963; Dunn et al., 1954).

In contrast to many metals, there are no naturally occurring, highly concentrated forms of vanadium. A few commercial deposits, chiefly carnotite and titaniferous magnetite ores, contain as much as 3% vanadium pentoxide (N.R.C., 1974a).

In the United States, vanadium has been extracted commercially from uranium-vanadium deposits (carnotite and roscoelite) in the Colorado plateau. Less productive sources are found in Arkansas. The metal has been commercially recovered as a co-product with elemental phosphorus from phosphate rock in Idaho (Griffith, 1970) and as a by-product of phosphoric acid and phosphate fertilizer production (Faulkner-Hudson, 1964).

Of special concern is the vanadium content of rock phosphates which may be used as phosphorus sources for animal

feed. Depending on the location, vanadium concentration may be as high as 6000 ppm in some deposits (Romoser et al., 1960). Berg (1963) reported that a commercial phosphorus supplement, tricalcium phosphate, contained .25% vanadium pentoxide (1400 ppm vanadium). The vanadium content of most marine phosphorites is low, although deposits in Idaho, Montana and Wyoming contain relatively high levels (Busch, 1961).

Soils vary considerably in content, but vanadium is usually present in measurable amounts (Goldschmidt, 1958). The concentration is generally higher in clays (300 ppm) and shales (200 ppm) than in other rocks or soils (Vinogradov, 1959). Sandstone and limestone, for example, may contain only 20 ppm (Schroeder, 1970). Chattopadhyay and Jervis (1974) found that a muck soil contained 10 to 32 ppm vanadium with the higher values at greater soil depths. Vanadium usually occurs in the earth's crust as relatively insoluble salts (N.R.C., 1974a), and is present in some sediments as oxovanadium (IV) anion bound to organic chelates (Yen, 1972).

In a recent review of the literature (N.R.C., 1974a), only five references were found giving vanadium content of foods. Thus, little is known of food content relative to soil levels. Bertrand (1950) indicated that the vanadium concentration of plants was lower in the aerial parts than in the roots; the roots having approximately the same content as the soil in which the plant was growing. However, Söremark

(1967) has investigated the uptake of ^{48}V from the soil by plants grown from seed and observed that differences in activity between roots and leaves were small. Vegetables were harvested 40 to 70 days after planting and the radioactivity was measured. Leaves and roots absorbed approximately 10^{-4} to 10^{-6}% of the isotope in the soil container (per gm of fresh vegetable).

The vanadium content of legumes appears to be higher than that of many other plants; the root nodules containing about three times as much as the remainder of the plant (Bertrand, 1950). Differences in vanadium absorption among grass species were noted by Fleming (1973), but the highest concentration of the mineral found in six common grasses was .13 ppm (dry weight basis). Furr et al. (1975) demonstrated that the vanadium content of yellow sweet clover was increased to .45 ppm (moisture-free basis) when grown on fly ash containing 255 ppm vanadium.

Vanadium in Air

The high concentration of vanadium in some fossil fuels combined with the increased usage of these fuels in recent years has led to speculation that vanadium could become a general air pollution problem (Athanassiadis, 1969; Lee and von Lehmden, 1973). Exposure to airborne metallic elements is of considerable toxicological interest since the absorption efficiency for most trace metals in the alveolar region of the lung is 50 to 80% (Natusch et al., 1973). Additionally,

vanadium has been shown to occur among the smallest particles collected from ambient air (Lee and von Lehmden, 1973) which would increase the probability of deposition in the alveolar region. Lee et al. (1972) observed that vanadium is associated with the submicron-sized fraction of particulate matter in air. Particles less than or equal to .5μm were found to contain .1 to .3% vanadium.

Concentration of the mineral is higher in air over urban than over rural areas. The air over New York City, for example, contained an average of 1320 ng/m^3, while the air content over many rural areas was less than 1 ng/m^3 (Kneip et al., 1970). The concentration of vanadium in the rural atmosphere of states on the eastern seaboard from Maine to South Carolina, however, was found to be significantly higher than in other rural areas of the United States (N.R.C., 1974a). Schwarz (1970) found 20 to 70 ppm vanadium in dust which accumulated on domestic furnace and laboratory air filters.

The combustion of coal and fuel oils contributes substantially to atmospheric vanadium. The Committee on Medical and Biological Effects of Environmental Pollutants (N.R.C., 1974a), in a report on vanadium, compiled data on air contamination from fossil fuels. It was estimated that 1,750 tons of vanadium were emitted into the air during 1969 from the combustion of bituminous coal. Concentration in coal is 15 to 34 ppm vanadium (Abernathy et al., 1969), whereas fly ash from the burning of coal may contain 180 to 2000 ppm vanadium (von Lehmden et al., 1974).

Petroleum products, principally crude oils and derivatives, are another source of atmospheric vanadium contamination (Lee et al., 1972). Residual fuel oils contain the highest-molecular-weight portions of the crude oil as well as most of the vanadium and other minerals present in oil as impurities (Yen et al., 1969). It has been estimated that residual fuel oils burned in this country in 1968 contained slightly less than 19,000 tons of vanadium and that 90% of this total was emitted into the air (N.R.C., 1974a).

The fate of airborne vanadium emitted from stationary sources is not known (Lee et al., 1975), but it would be expected that plants and animals in the immediate vicinity of some power production and heating facilities using oils would be exposed to higher than normal levels of vanadium. Vanadium levels in soil, forages and water in the vicinity of such facilities could be elevated through localized fallout since the vanadium content of petroleum ash is generally high (Goldschmidt, 1958). Winchester and Nifong (1970) estimated that there was a major input of vanadium and certain other trace elements into Lake Michigan due to fallout of aerosol particulates.

The physical characteristics of trace metal particulates emitted into the atmosphere determine in large part the residence time in air and the degree of dispersion from the point of emission (Motto et al., 1970). Vanadium, like lead, is associated predominantly with aerial particles less than

1.0μm in size (Lee et al., 1972). Both lead (Chow and Earl, 1970) and vanadium (N.R.C., 1974a) aerosols originate principally from combustion of petroleum products. The residence time for lead in air has been calculated to range from 7 to 30 days depending on environmental conditions (Burton and Steward, 1960; Francis et al., 1970), but comparable data for vanadium do not seem to be available. In view of the similarity of origin and particle size, it is probable that the two metals share a common fate relative to degree of dispersion and residence time in air.

Vanadium in Water

In a report of the National Academy of Sciences Subcommittee on Nutrient and Toxic Elements in Water, a vanadium concentration in drinking water of 100 μg/liter was recommended as a safe upper limit for livestock and poultry (N.R.C., 1974b). Recent studies by Linstedt and Kruger (1970) have shown that the mean vanadium concentration in river water ranges from .3 to 20.5 μg/liter. However, rivers in the vicinity of the Colorado Plateau may contain up to 70 μg/liter (Schroeder, 1970) and natural waters in Wyoming have been found to contain 30 to 220 μg/liter (Tank and Storvick, 1960). Vanadium content of drinking water from eastern Montana and the Dakotas varies from 7 to 150 μg/liter (N.R.C., 1974a). The concentration of vanadium in seawater is low, approximately 2 to 7 μg/liter (Ladd, 1974).

Weathering of vanadium-rich wastes and by-products of

metallurgic industries which process metals containing vanadium (e.g., ferrovanadium alloys) may elevate vanadium levels of water in some areas (N.R.C., 1974a). Except in cases of localized contamination, drinking water probably does not constitute an important source of vanadium (Hadjimarkos, 1966).

Vanadium Essentiality

Witz and Osmond in 1886 observed the interesting oxidation and reduction properties of vanadium and speculated that the element might later be shown to have a biological function. Based on a survey of the literature, Underwood (1971) noted that there was no clear indication that vanadium is required by the higher plants or higher animals and that evidence concerning the involvement of vanadium in lipid metabolism and in the calcification of bones and teeth was somewhat conflicting.

Hopkins and Mohr (1971a) and Hopkins and Mohr (1971b) have suggested the essentiality of vanadium by showing reduced feather growth and lower blood cholesterol levels in chicks fed a diet containing only 10 ppb of the element. More recently, Nielsen and Ollerich (1973) have shown that a vanadium deficiency induces changes in bone development and increases hematocrit values in chicks. Schwarz and Milne (1971a), Schwarz and Milne (1971b), and Schwarz (1974) reported that vanadium was necessary for the growth of rats

raised inside trace element-controlled, all-plastic isolators. Addition of vanadium to the diet enhanced growth by over 40% and optimum growth occurred with .1 ppm vanadium as sodium orthovanadate (Na_3VO_4). Hopkins and Mohr (1974) and Hopkins (1974) have reviewed the literature pertaining to the essentiality of vanadium and presented evidence that reproductive performance of rats is also impaired by a deficiency of the element.

Vanadium Toxicity in Animals

As early as 1876, Priestley showed that vanadium, given as sodium metavanadate, was highly poisonous to the pigeon, guinea pig, rabbit, cat and dog. These early experiments established that vanadium affected animals in two ways: first, a general systemic effect causing coma and convulsions, and second, an effect on the alimentary tract resulting in diarrhea and hemorrhage.

Toxicity of vanadium compounds in man was recognized more than a half-century ago. Exposure to vanadium used industrially, chiefly through breathing of particulate matter with a high vanadium content, was found to cause irritation of the nose and throat, anorexia, nausea and diarrhea in workers (Fairhall, 1949). There is considerable information on the toxicological aspects of vanadium exposure in man, but the main concern has been entry of the metal via the respiratory system. Vanadium toxicity in humans due to

ingestion is uncommon except as incidental to high aerial concentrations (N.R.C., 1974a).

Reviews on vanadium toxicity in man and animals have been published by Sjöberg (1950), Stokinger (1955), Stokinger (1963), Faulkner-Hudson (1964), Lillie (1970), Ammerman et al. (1973), and N.R.C. (1974a). Toxicity varies considerably with the nature of the compound administered, the route by which it enters the body and composition of the diet.

In Table 1 is presented a survey of the literature as it pertains to toxic levels of dietary vanadium. Chicks appear to be highly susceptible when it is administered orally. Because a potential practical problem exists, dietary vanadium has been extensively investigated in this species.

Daniel and Lillie (1938) first observed that some animals were able to adjust to vanadium when toxic amounts were gradually introduced into the diet. Strasia (1971) demonstrated that rats could be adapted to 500 ppm vanadium if the dietary level were gradually increased by small increments. In addition, chicks have been shown to recover partially from the initial toxic effect of vanadium, suggesting some adaptation to the metal when ingested (Williams, 1973).

Vanadium has been shown to be highly poisonous to all vertebrate species studied. The toxicity of the mineral as

TABLE 1. TOXIC LEVELS OF VANADIUM IN THE DIET.

Species	Age	Compound	Toxic level (ppm)	Diet	Physiological effect	Reference
Rat	Immature	$NaVO_3$ or V_2O_5	50-100	Semi-purified	Reduced growth and feed utilization	Mountain et al. (1959)
Rat	Immature	$NaVO_3$	250	Commercial	Reduced growth; mortality	Strasia (1971)
Rat	Immature	$NaVO_3$	25	Practical	Reduced growth; diarrhea	Franke and Moxon (1937)
Rat	Immature	$NaVO_3.4H_2O$	500	Semi-purified	Reduced liver coenzyme A	Mascitelli-Coriandoli and Citterio (1959)
Rat	Mature	$NaVO_3$	400	Commercial	Mortality	Strasia (1971)
Rat	Mature	V_2O_5	500	Practical	Reduced growth and feed utilization	Mountain et al. (1953)
Chicken	Immature	$Ca_3(VO_4)_2$	30	Practical	Reduced growth and feed utilization	Romoser et al. (1961)
Chicken	Immature	$Ca_3(VO_4)_2$	200	Practical	Mortality	Romoser et al. (1961)
Chicken	Immature	Natural source + NH_4VO_3	13	Practical	Reduced growth	Berg (1963)

TABLE 1 - continued.

Species	Age	Compound	Toxic level (ppm)	Diet	Physiological effect	Reference
Chicken	Immature	Natural source	20	Practical	Reduced growth	Berg (1966)
Chicken	Immature	NH_4VO_3	20	Semi-purified	Reduced growth	Berg (1966)
Chicken	Immature	NH_4VO_3, $VOSO_4$ or $VOCl_2$	10	Semi-purified	Reduced growth	Berg and Lawrence (1971)
Chicken	Immature	NH_4VO_3	20	Semi-purified	Reduced growth; mortality	Berg and Lawrence (1971)
Chicken	Immature	NH_4VO_3 or $VOSO_4$	25	Semi-purified	Reduced growth; mortality	Hathcock et al. (1964)
Chicken	Immature	NH_4VO_3	25	Semi-purified	Uncoupled oxidative phosphorylation	Hathcock et al. (1966)
Chicken	Immature	NH_4VO_3	20	Practical	Reduced growth and feed utilization	Nelson et al. (1962)
Chicken	Immature	$VOCl_2$	50	Practical	Reduced growth and feed utilization	Miller et al. (1961)
Chicken	Immature	Na_3VO_4	15	Semi-purified	Reduced growth; mortality	Williams (1973)
Chicken	Immature	NH_4VO_3	20	Semi-purified	Reduced growth	Wright (1968)

TABLE 1 - continued.

Species	Age	Compound	Toxic level (ppm)	Diet	Physiological effect	Reference
Chicken	Immature	-	10	Practical	Reduced growth	Summers and Moran (1972)
Chicken	Laying hen	NH_4VO_3	15	Practical	Depressed albumin quality	Berg et al. (1963)
Chicken	Laying hen	NH_4VO_3	20	Practical	Depressed albumin quality; reduced body weight	Berg et al. (1963)
Chicken	Laying hen	NH_4VO_3	30	Practical	Depressed egg production	Berg et al. (1963)
Chicken	Laying hen	NH_4VO_3	50	Practical	Depressed hatchability	Berg et al. (1963)
Chicken	Laying hen	NH_4VO_3 or $VOCl_2$	130	Commercial	Reduced body weight	Hernandez and Escoda (1971)
Sheep	Lamb	$NaVO_3$ or Na_3VO_4	7	Semi-purified	Reduced dry matter digestibility in vitro	Williams (1973)

it pertains to cattle, sheep, rats, mice and poultry will be considered in the present review.

Rats and Mice

Proescher et al. (1917), in observations on vanadium toxicity in mice, rats and other small animals, extended the early work of Priestley (1876). These authors reported that the LD_{50} in rats when injected subcutaneously as ammonium metavanadate (NH_4VO_3) was 20 to 30 mg vanadium per kg body weight. In many of these animals, necrosis of the convoluted tubules of the kidneys, fatty degeneration of the liver, adrenal hemorrhage, marked constriction of the visceral arteries and inflammatory lesions in the intestinal tract were noted.

Franke and Moxon (1937) used feed consumption and mortality as criteria in determining the toxicity of vanadium salts to rats. Vanadium as sodium metavanadate ($NaVO_3$) was found to be slightly toxic at 25 ppm and distinctly toxic at 50 ppm. In rat feeding experiments, Daniel and Lillie (1938) observed that animals receiving 11.5 and 22 ppm vanadium as sodium metavanadate appeared normal throughout a 12-week period. A dietary level of 92 ppm was very toxic, and 368 ppm usually caused death within 10 weeks. Muhler (1957) reported reduced weight gains and higher mortality in rats which were administered 20 ppm vanadium as vanadium pentoxide (V_2O_5) in drinking water. At 40 ppm, 100% mortality was observed within 65 days. Schroeder and Balassa (1967)

gave mice vanadyl sulfate ($VOSO_4$) in the drinking water at a level of 5 ppm vanadium from weaning until natural death. The authors found no toxicity in terms of growth, survival, life span or pathological changes. The metal was found to accumulate in heart and spleen. A similar long-term study using rats supplied with 5 ppm vanadium in drinking water showed the element to have no accumulative toxicity in this species (Schroeder et al., 1970). Moxon and Rhian (1943) observed that rats given 11 ppm selenium in drinking water died more quickly when the selenium was administered in combination with 5 ppm vanadium. At much higher levels, however, a well-defined species difference in response to vanadium was demonstrated by Pham-Huu-Chanh (1965) who noted that sodium metavanadate was an extremely toxic agent that affected rats more rapidly than mice.

Roshchin et al. (1965), using mice, established the oral LD_{50} of vanadium trioxide (V_2O_3) at 130 mg vanadium per kg body weight. The LD_{50} values for the pentoxide and trichloride (VCl_3) forms were much lower — 23 mg per kg body weight for both compounds.

Mitchell and Floyd (1954) and Berg and Lawrence (1971) tested ascorbic acid and ethylenediaminetetra-acetate (EDTA) as antidotes in experimental vanadium poisoning. In mice and rats, both agents were effective antidotes, with ascorbic acid acting more quickly. The mode of action of these agents in reducing toxicity has not been fully established, although

EDTA may function by preventing absorption (Hathcock et al., 1964).

Ruminant Animals

Grazing cattle have been observed to ingest as much as 1.5 kg of soil per day (Thornton, 1974) and some soils contain high levels of vanadium (Vinogradov, 1959). However, there is little information in the literature concerning vanadium toxicity in larger domestic animals. Platonow and Abbey (1968) have studied the toxicity of vanadium in calves. The element was given orally in gelatin capsules as ammonium metavanadate (NH_4VO_3) at daily dosage levels of 1, 3, 5, 7.5, 10, 15 and 20 mg vanadium per kg body weight. The first four levels produced no clinical signs, but toxicity was observed at 10, 15 and 20 mg/kg. The dose of 20 mg/kg resulted in adverse effects within 3 days. Clinical signs of toxicity were, in order of occurrence, diarrhea, dehydration, emaciation, listlessness and prostration. No changes were observed in blood cell morphology, hemoglobin or hematocrit. Gross pathological changes in the calves that died included congestion of the liver and lungs, diffuse hemorrhage covering the kidneys and heart muscle, ulcers in the rumen and hemorrhagic inflammation of the intestinal tract. The greatest concentration of vanadium was found in kidney, followed by liver and spleen. None was detected in blood, lungs, skeletal muscle or testes.

Other studies suggest that alterations in rumen function could result from ingestion of the element. In vitro dry matter digestibility, for example, was significantly reduced in rumen fluid inoculum from lambs by 7 ppm added as sodium ortho- or metavanadate (Williams, 1973). Martinez and Church (1970) reported that the addition of 5 ppm or more of vanadium as sodium metavanadate in vitro significantly reduced cellulose digestion by washed suspensions of rumen microorganisms. These results were in general agreement with studies by Jha (1966).

Lillie (1970), citing a report by Heege (1964), noted that cows exposed to vanadium from fuel oil soot distributed over the grazing area showed weakness and ataxia. The vanadium content of liver tissue of the animals after exposure ranged from 1.5 to 4.7 ppm.

Poultry

Romoser et al. (1960) apparently were the first to show that dietary vanadium retarded the growth rate of chicks. These authors found that the LD_{50} for vanadium as the calcium salt [$Ca_3(VO_4)_2$] was between 300 and 350 ppm. Growth, however, was inhibited at levels above 20 ppm. Romoser et al. (1961) observed that chicks tolerated up to 20 ppm vanadium in a corn-soybean meal diet while Nelson et al. (1962) reported that 35 ppm vanadium was toxic for chick growth. The basal diets used in these studies contained

5 to 7 ppm vanadium. Hathcock et al. (1964) reported that the addition of 10 ppm vanadium to the diet as ammonium metavanadate did not affect growth of chicks, but 25 ppm significantly depressed growth and increased mortality. EDTA fed at twice the molar concentration of vanadium partially protected the chicks from toxicity.

Berg (1963) found that two commercial samples of tricalcium phosphate depressed chick growth in comparison to other phosphorus sources examined. The sample which caused the greater growth depression contained .25% vanadium pentoxide (1400 ppm vanadium) and contributed 28 ppm vanadium to the complete diet. A relatively non-toxic tricalcium phosphate sample contained only .025% vanadium pentoxide and contributed only 3 ppm vanadium when used in the same corn-soybean meal diet. In a separate experiment, this author found that a dietary vanadium level above 13 ppm caused growth depression in chicks. Berg and Lawrence (1971), adding vanadium as ammonium metavanadate to various diets at a level of 20 ppm, observed that tibial vanadium content ranged from 3.8 to 14.8 ppm, depending upon the dietary regimen. Increasing levels of ascorbic acid in the diet resulted in a progressive decrease in deposition of vanadium in bone. Berg et al. (1963) found that 60 ppm dietary vanadium (ammonium metavanadate) reduced hatchability of fertile eggs by 10%. Egg production was depressed with 30 ppm added vanadium and only 15 to 20 ppm were required to significantly lower egg albumin quality.

Several investigators (Mountain et al., 1959; Hatchcock et al., 1964; Berg, 1966) have observed that diet composition greatly affected the degree of dietary vanadium toxicity. Berg (1966) showed that 20 ppm vanadium as ammonium metavanadate added to a corn-soybean meal chick diet depressed growth 25 to 30%, whereas the same compound combined with a corn-fish meal diet depressed growth only 3 to 7%. However, when 20 ppm vanadium was added to a sucrose-fish meal diet, growth was depressed by 50% and high mortality resulted. Increasing the dietary protein level resulted in a linear decrease in the mortality rate of chicks fed the sucrose-fish meal diet.

Biochemical Aspects of Vanadium Toxicity

Proescher et al. (1917) observed that injections of vanadium compounds resulted in increased catabolism as indicated by increased output of nitrogen, sulfur and phosphorus constituents in the urine of animals. Vanadium was early suspected of altering the metabolism of liver tissue (Bernheim and Bernheim, 1938). They later found that vanadium increased the oxidation of phospholipids by rodent liver cells in vitro (Bernheim and Bernheim, 1939). Daniel and Lillie (1938) observed fat deposition in livers of rats exposed to vanadium compounds. Conversely, reduction in the lipid content of the adrenal cortex occurred at the same time. Snyder and Cornatzer (1958) administered vanadium sulfate solution to rats intraperitoneally and noted a depression of phospholipid synthesis in liver tissue.

Mountain et al. (1953) suggested that one of the primary modes of action of vanadium was an effect on reactions of sulfur-containing compounds. These authors fed diets containing varying levels of vanadium to rats and found that as little as 100 ppm of the metal reduced the cystine content of hair. Subsequently, Mountain et al. (1955) reported that the cystine content of fingernails was reduced in men occupationally exposed to vanadium. Snyder and Cornatzer (1958) have studied the effect of vanadium toxicity on the incorporation of isotope-labeled methionine into hepatic protein. A reduction of sulphydryl groups in the liver and an increased turnover of protein sulfur were reported to result from the administration of vanadium. In vitro studies by Bergel et al. (1958) showed that small quantities of vanadium activated pyridoxal phosphate and its dependent enzyme, desulphydrase, causing decomposition of cystine.

Curran (1954) was the first to demonstrate that vanadium inhibited cholesterol synthesis. Mountain et al. (1956) showed that dietary vanadium lowered the cholesterol content in the liver of rabbits. Salts of vanadium added to the diet were also observed to induce mobilization of excess aortic cholesterol in rabbits (Curran and Costello, 1956) and in chickens (Eades and Gallo, 1957) and to decrease serum free and total cholesterol in man (Curran et al., 1959). The metal was later found to inhibit cholesterol synthesis in vitro by interference with the formation and

utilization of the squalene synthetase complex (Azarnoff et al., 1961). However, Comar and Chevallier (1967) were unable to duplicate these findings in vivo. These authors concluded that the small quantity of the element absorbed was not sufficient to alter cholesterol metabolism.

Coenzyme A synthesis in rat liver has been shown to be decreased by high dietary vanadium (Mascitelli-Coriandoli and Citterio, 1959; Curran, 1954). In addition, Aiyar and Sreenivasan (1961) found that vanadium decreased the levels of both coenzyme Q and coenzyme A in rat liver tissue.

Wright et al. (1960) were the first to suggest that the mode of vanadium toxicity was uncoupling of oxidative phosphorylation. These authors reported that vanadyl sulfate increased the rate of disappearance of added adenosine triphosphate (ATP) in rat liver homogenate. They proposed that vanadium probably interfered with the maintenance of ATP levels in the system and that this could impair the synthesis of phosphorylated intermediates from melavonic acid, thus reducing cholesterol synthesis. Hathcock et al. (1966) showed that the addition of ammonium metavanadate to the diet of chicks at a level of 25 ppm vanadium uncoupled oxidative phosphorylation in mitochrondria subsequently isolated from liver tissue.

The effect of vanadium on oxidative phosphorylation and vanadium-chromium interrelationships were studied by Wright (1968). Supplemental chromium alleviated the toxicity of

vanadium; chromium at a level of 2000 ppm reduced mortality in chicks from 86.6% to 13.3% when fed with 20 ppm vanadium. This author suggested that chromium antagonized vanadium uncoupling of oxidative phosphorylation and retarded the intestinal absorption of vanadium.

DeMaster (1972) investigated the differential effects of vanadate, phosphate and arsenate on substrate-level oxidative phosphorylation and mitochondrial reactions. Vanadate was found to be an alternative substrate for phosphate in the glyceraldehyde-3-phosphate dehydrogenase reaction and the catalytic actions of vanadate and arsenate were found similar in this system. However, he was unable to confirm the uncoupling of oxidative phosphorylation by vanadate ion in rat liver mitochondria.

The biochemical or physiological basis of the lethal effect of vanadium is not clear. Enzymes involved in several vital processes are inhibited or otherwise affected by abnormal vanadium concentrations (Underwood, 1971) including those concerned with the metabolism of glucose (Meeks et al., 1971). Additional adverse effects in acute oral toxicity could result from cell damage through lysis as has been observed in vitro (Waters et al., 1974; Waters et al., 1975).

Chemical Forms and Comparative Toxicities of Vanadium Compounds

In the natural state, vanadium occurs with positive valencies of two, three, four and five. The pentavalent salts are vanadates, of which sodium and ammonium metavanadate

are best known; the quadrivalent ion forms vanadites (Faulkner-Hudson, 1964). Insoluble trivalent vanadium is oxidized to the pentavalent state in the soil during weathering and in the latter state it forms salts of heavy metals (Schroeder et al., 1963). The mineral in solution in sea water is probably in the +5 oxidation state; the principal forms are the anionic species HVO_4^{2-} and $H_2VO_4^-$ (Ladd, 1974).

High-temperature processing of phosphate ores converts the vanadium in the ore to orthovanadate (Romoser et al., 1961). The most important compound in industry is the pentoxide (V_2O_5). Other oxides of vanadium are hypovanadous oxide (VO or V_2O_2), hypovanadic oxide (VO_2 or V_2O_4) and vanadium trioxide (V_2O_3) (Fairhall, 1949).

As early as 1910, Ricciardi observed that anionic vanadium preparations, such as the vanadates, were more toxic than the cationic, like vanadyl sulfate. Hathcock et al. (1964), however, studied the relative toxicity of vanadium fed as either ammonium metavanadate (valence 5) or vanadyl sulfate (valence 4), and found the two equally toxic in chicks. Mountain et al. (1959) compared the oral toxicity of vanadium pentoxide and sodium metavanadate utilizing rats and rabbits. Sodium metavanadate, when fed to these animals, was found to be more toxic than vanadium pentoxide. The pentoxide form was not markedly poisonous at the 1000 ppm level. Roshchin et al. (1965) compared the toxicity of orally administered vanadium trioxide and vanadium pentoxide in mice. The latter

was six times as toxic as the former.

Proescher et al. (1917) investigated the toxicity of different vanadium preparations to both small and large animals. Ammonium metavanadate was more toxic than other compounds studied. Berg and Lawrence (1971) compared dietary NH_4VO_3 (valence 5) to $VOSO_4$ and $VOCl_2$ (valence 4) using growth depression and tibial deposition of vanadium in chicks as criteria of toxicity. These authors concluded that the three compounds were equally toxic to chicks.

Different oxidation states of vanadium also produced similar effects in the rat when used to inhibit cholesterol synthesis (Curran, 1954). This author attributed the similarity of actions to either a rapid conversion of the different oxidation states to a common one, or to the property of vanadium to be active in any of its oxidation states. The rate of organ uptake of pentavalent and tetravalent ^{48}V was more rapid than that of trivalent ^{48}V during the first 10 min after injection (Hopkins and Tilton, 1966). Differences in rate of uptake were not large, suggesting a minimal effect of oxidation state.

The relative toxicities of selenium, tellurium, molybdenum, vanadium and arsenic in rats were studied by Franke and Moxon (1936) and Franke and Moxon (1937), using feed consumption and mortality as criteria. These authors noted that vanadium as sodium metavanadate was highly toxic at 50 ppm, the level at which diarrhea and mortality were

observed. Selenium was the only element studied that was more toxic than vanadium. When Martinez and Church (1970) compared the effect of several minerals on cellulose digestion by washed suspensions of rumen micro-organisms, vanadium was found to be less toxic than fluorine, but exhibited greater toxicity than either selenium or chromium. Other *in vitro* studies using rabbit alveolar macrophages have shown that vanadium as ammonium metavanadate caused significant decreases in cell numbers through lysis at concentrations as low as 5.0 µg per milliliter (Waters *et al.*, 1974). Vanadium was thirty-seven times more toxic than nickel, manganese, or chromium. The cytotoxicities of cadmium and vanadium were comparable, although the modes of action were different; cadmium caused immobility without lysis while vanadium at low levels resulted in complete fragmentation of the cell membrane.

The bioavailability of various forms of vanadium has been investigated by Schwarz and Milne (1971b). In essentiality studies with mice, sodium orthovanadate was more effective in promoting growth than was sodium metavanadate. The pyrovanadate salt, however, was without activity.

Vanadium Absorption, Excretion and Tissue Distribution

Ballotta (1931) found that dietary vanadium, due to the low intestinal absorption of the metal, was largely lost in the feces. Radioactive tracer studies by Comar and

Chevallier (1967) have shown that vanadium is not readily absorbed from the alimentary tract of the rat. The average body concentration of stable vanadium was, however, found by these authors to be proportional to the dietary level. Faulkner-Hudson (1964), citing a report by Scott et al., (1951), indicated that in rats given radiovanadium intragastrically only .5% was absorbed. Curran et al. (1959) also found that less than 1% of the metal was absorbed from the human gastrointestinal tract.

Excretion of absorbed or injected vanadium is mainly by the kidneys. Talvitie and Wagner (1954) noted that vanadium as sodium metavanadate was rapidly excreted after intravenous injection with 60% of the dose being excreted via the kidneys within 24 hours. Hopkins and Tilton (1966) also observed that the main route of excretion of intravenously injected isotopic vanadium ($^{48}VOCl_2^+$) was through the kidneys. Approximately 45% of the injected isotope was lost in the urine and 9% in the feces. Principal organs of isotope retention were liver, kidney and spleen. In rats injected intraperitoneally with radioactive vanadium, removal from the soft tissues and bones was rapid; the molar teeth, however, retained vanadium in high concentration for at least 90 days (Thomassen and Leicester, 1964). Relatively high retention of ^{48}V in bones and kidneys of chicks following oral administration in the form of vanadyl dichloride was observed by Hathcock et al. (1964). Dietary EDTA significantly

reduced the concentration of ^{48}V in all tissues studied. Söremark et al. (1962) and Söremark and Üllberg (1962), reported that radioactive $^{48}V_2O_5$ was concentrated in bones and teeth of rats and mice 7 days after injection. Other tissues also retained the isotope; the decreasing order of activity was visceral yolk sac epithelium, lactating mammary gland, renal cortex, liver, lung, skin and salivary gland. Uptake by the fetal skeleton in pregnant females indicated placental transfer of the mineral.

CHAPTER III

DETERMINATION OF VANADIUM IN BIOLOGICAL MATERIALS

Introduction

The various analytical techniques for vanadium in biological materials have been reviewed by Athanassiadis (1969). Neutron activation is the method of choice for vanadium analysis at very low concentrations (Lambert et al., 1970), but measurement has been complicated by the high concentration of sodium in most biological materials and by the short (3.8 min) half-life of ^{52}V, the radioactive species produced. Instrumentation for flame atomic absorption spectrophotometry (AAS) is far simpler and more widely available, but except for a few metal ions such as zinc and magnesium, cannot be successfully employed for the direct determination of minerals below 1 ppm levels. Methods to improve the sensitivity of AAS and to concentrate the metal ions from dilute solutions are necessary for the determination of trace constituents.

Vanadium forms thermally stable oxides which are only partially atomized in air-acetylene flames commonly used in AAS. The development of the high-temperature nitrous oxide-acetylene flame considerably improved the detection limits for vanadium (Bowman and Willis, 1967; Pearton et al.,

1969). Willis (1965) demonstrated the advantages of the hotter flame and reported a sensitivity of 1.5 µg/ml for vanadium in aqueous solution.

The sensitivity of atomic absorption methods is also enhanced by the use of chelates and organic solvents. In addition, interferences from large amounts of commonly found ions such as sodium, calcium (II) and magnesium (II) are avoided (Allan, 1961; Sachdev et al., 1967a).

The increase in sensitivity gained by use of solvent extraction techniques, in which metal chelates are separated and preconcentrated into organic solvents, is well-established (Mulford, 1966; Allan, 1961). Sachdev et al. (1967a) and Sachdev et al. (1967b) used cupferron and various solvent systems to extract vanadium. Pearton et al. (1969) reported an AAS method for determination of vanadium in silicate materials using cupferron and n-butyl acetate to extract the metal from solution.

Crump-Wiesner and Purdy (1969) investigated the extraction of vanadium employing methylisobutyl ketone (MIBK). Cupferron was found to be the most suitable chelating agent because of ease and efficiency of extraction. The method was later applied successfully to the determination by AAS of vanadium in brine samples (Crump-Wiesner et al., 1971). The sensitivity of the method was reported to be 11 µg vanadium per liter for 1% absorption; 2.5 µg per liter could be detected in brine solutions.

When used in conjunction with the nitrous oxide-acetylene flame and newer solvent extraction procedures , flame AAS could be applicable to the determination of vanadium in biological materials, but the combined techniques have apparently not been tested for this use. The complexity of biological matrices necessitated a series of experiments to evaluate and validate procedures with animal and plant sources for subsequent toxicity studies.

Experimental Procedure

Reagents and Methodology

Reagent grade chemicals were used throughout. Nitric acid was distilled in an all-glass apparatus; other reagents were not further purified. Deionized water was employed for experimental purposes and for final rinsing of glassware and containers. Vanadium stock solution (atomic absorption standard — 1000 ppm vanadium pentoxide in hydrochloric acid) was obtained from Fisher Scientific, Fair Lawn, New Jersey, and was diluted just before use to obtain working standards of 2.0 and .2 µg/ml vanadium. A 5% (w/v) solution of cupferron (ammonium salt of nitrosophenylhydroxylamine) in deionized water was also prepared immediately prior to use. Metacresol purple indicator was prepared by dissolving .1 g metacresol purple reagent powder in 25 ml of .01 N sodium hydroxide and diluting to 250 ml with water. The organic solvent (MIBK), was not presaturated with water.

Crucibles for ashing were soaked in sulfuric-chromic acid and rinsed. Glassware was detergent cleaned. Both

crucibles and glassware were repeatedly rinsed in 10% (v/v) hydrochloric acid and deionized water before drying.

Apparatus

Absorbance measurements were made with a Perkin-Elmer Model 306 Atomic Absorption Spectrophotometer equipped with a nitrous oxide-acetylene burner assembly and Model 56 Recorder. The light source was a Perkin-Elmer Vanadium Hollow Cathode Lamp. Nominal operating conditions for the instrument were as shown in Table 2. Parameters not specified were those recommended by the manufacturer (Anonymous, 1973). A Corning Model 7 pH meter was used for final pH adjustments.

TABLE 2. OPERATING CONDITIONS FOR ATOMIC ABSORPTION SPECTROPHOTOMETER IN VANADIUM ANALYSIS.

Fuel:	Nitrous oxide 13.0 liters/min at 30 psig
	Acetylene 6.9 liters/min at 9 psig
Slit width:	1 mm (.7 nm bandpass)
Wavelength:	318.4 nm
Source current:	40 mA
Noise suppression:	2
Burner height:	Light beam 7 to 8 mm above burner head surface
Aspiration rate:	3.5-5.0 ml/min

Sample Preparation

Biological materials (animal tissue and plant samples) were weighed into porcelain crucibles, dried at 105°C and charred for 6 hr in a muffle furnace prior to dry ashing at 600°C for 15 to 20 hours. Dilute nitric acid solutions of the ash were prepared by successively evaporating to small volume 70-ml aliquots of 50% (v/v) nitric acid, 10% (v/v) nitric acid and deionized water added to the crucibles. The ash solution was then quantitatively transferred with filtration into 25 or 50 ml volumetric flasks, depending on vanadium concentration and size of sample. A reagent blank was processed in a similar manner.

Extraction of Vanadium

In brief, the procedure involved the extraction of vanadium as the cupferronate from the biological mineral matrix into methylisobutyl ketone (MIBK). The volume of the organic solvent was then reduced by heating to further concentrate the metal, thus improving the sensitivity of detection by AAS.

Appropriate aliquots of the mineralized solution containing .2 to 20 µg vanadium were pipetted into 100 ml flasks with ground-glass stoppers for extraction and vanadium analysis. A range of standards to bracket the vanadium concentration of samples was prepared at the same time. Reagent blanks were carried through both the ashing process and the analytical procedure.

To all flasks was added .8 µg vanadium to increase absorbance readings for later reagent blank correction, 100 µg aluminum (III), and 3 drops of metacresol purple indicator. The neck of the flask was rinsed with 2 ml of glacial acetic acid before pH adjustment (pH 2.2 to 2.4) with ammonium hydroxide. Cupferron solution (15 ml) was added and the solution swirled and allowed to stand 20 min before the addition of 15 ml of MIBK. Flasks were then stoppered and shaken vigorously for 1 minute. Deionized water was added to elevate the solvent layer into the neck of the flask. This layer was then transferred by suction into 25 ml vials and evaporated to approximately 2 ml on a 110°C hotplate under the strong air draft of a partially-closed hood. The sides of the vials were rinsed with 3 ml of MIBK and then evaporated to dryness. The residue was redissolved in 1.5 ml of MIBK and duplicate absorbance measurements were made by AAS.

After correction for reagent blank values, the absorbances of samples were compared to the least squares regression line plot of absorbance versus µg vanadium in standards. The amount of vanadium in an unknown sample was then determined by reference to the corresponding absorbance on the standard curve.

Tests for incomplete extraction of the metal were made by direct Flameless AAS analysis (Anonymous, 1974) of the extracted aqueous phase to test for residual vanadium.

Appropriate volumes (50 to 100 µl) were injected into the graphite furnace and atomized at 2700°C to obtain absorbance values. A Perkin-Elmer HGA-2100 graphite furnace with Deuterium arc background corrector was used in conjunction with previously described equipment for these analyses.

Results

A study was conducted to determine the proper pH for the most efficient extraction of vanadium (IV) with cupferron and MIBK. The results are shown in Figure 1. Optimal pre-extraction pH range was found to be 2.0 to 3.5. Only a fraction of the vanadium (IV) was extracted below pH 1.0. Above pH 7.5, the solvent layer was clear, indicating an apparent lack of extraction of the cupferronate into the organic solvent.

It was recognized that preconcentration of vanadium also increases the levels of certain other ions which may be extracted with vanadium (Furman et al., 1949). Detailed studies were therefore made of other metals which are potential interferents in the vanadium determination. In each case, the effect of various levels of concomitant ion on absorbance was measured while maintaining a constant vanadium concentration as shown in Table 3.

Under the conditions used, no serious interferences were found except at very high levels. Molybdenum and tin depressed absorbance readings of 5 µg vanadium at concentrations above 200 and 100 µg per sample, respectively,

Figure 1. Effect of pH on MIBK-cupferron extraction of 6 μg of vanadium (IV). Each point represents the average of two independent determinations. Solid circles, pH before extraction; open circles, pH after extraction.

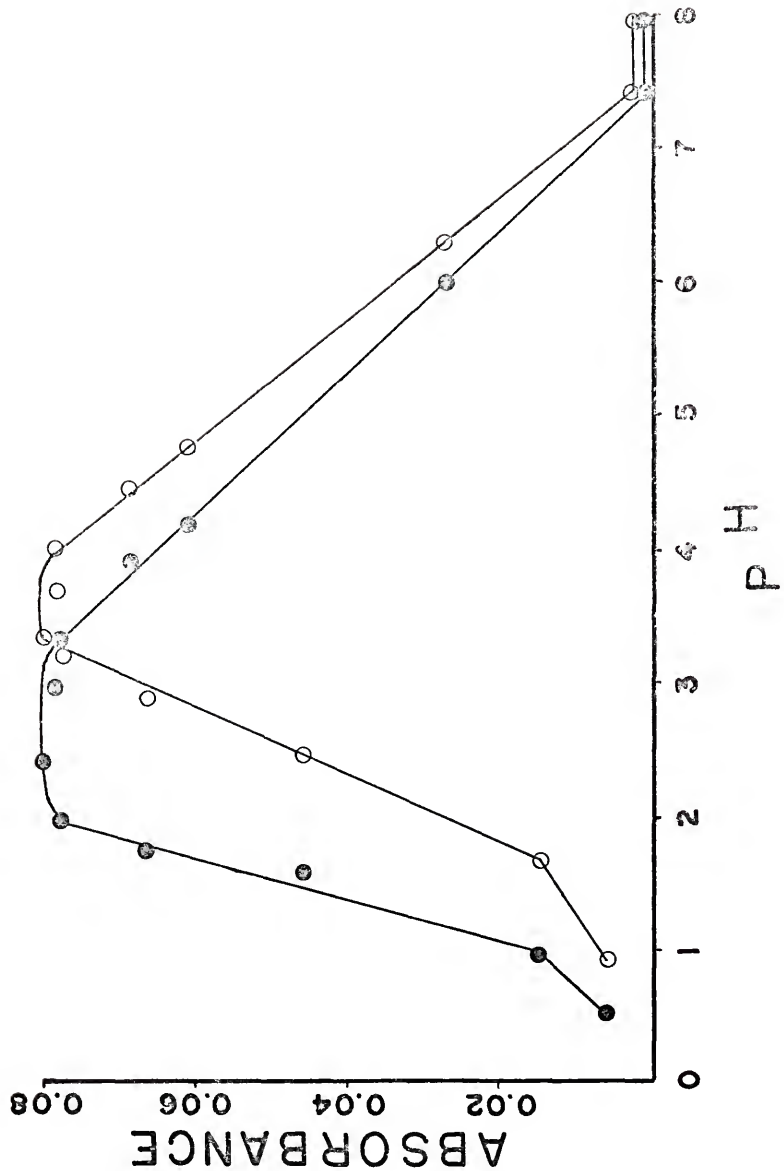

ABSORBANCE
0.02
0.04
0.06
0.08
0
1
2
3
4
5
6
7
8
pH

TABLE 3. EFFECT OF ADDED ALUMINUM, TIN, CALCIUM, MOLYBDENUM AND TUNGSTEN ON ABSORBANCE VALUES OF VANADIUM.[a]

Element	Source	Concomitant level µg/sample	Percent relative interference[b]
Aluminum	metal	10	0
		20	+4
		30	+10
		40	+14
		80	+14
		100	+14
		200	+19
		300	+17
		400	+12
		1000	+10
Tin	$SnCl_2.2H_2O$	50	+2
		100	+2
		200	+2
		400	-19
		1000	-21
Calcium	$CaCl_2$	1000	-4
		5000	0
		10000	+2
Molybdenum	Na_2MoO_4	50	+5
		100	0
		1000	-15
		2000	-28
Molybdenum	metal	50	+4
		100	0
		1000	-5
		2000	-12
Tungsten	$Na_2WO_4.2H_2O$	20	-2
		50	0
		70	-5
		100	-7
		200	-8
		300	-17

[a] Average of two independent determinations; 5 µg vanadium per sample.

[b] Percent relative interference =

$$\frac{\text{Change in absorbance with concomitant} \times 100}{\text{Absorbance of vanadium}}$$

while aluminum above 10 μg per sample enhanced the absorbance by 14 to 19%.

However, these levels are higher than those normally encountered in biological samples. The presence of iron in many animal tissues was observed to cause depression of the vanadium absorbance values, but the suppression of absorbance due to iron could be alleviated by the use of additional cupferron, as shown in Figure 2. When cupferron concentration was increased from .01 to .10 M, the suppression due to 14000 μg of added iron (III) was completely eliminated. Percent relative interference for these comparisons was calculated as:

$$\frac{\text{Change in absorbance with interferent}}{\text{Absorbance of 5 μg vanadium}} \times 100$$

The concentration of vanadium in NBS standard reference materials bovine liver 1577 and orchard leaves 1571 (National Bureau of Standards, Washington, D.C.) was determined by both the AAS method and by instrumental neutron activation analysis. The latter determinations were performed by the Environmental Trace Substances Research Center, University of Missouri, Columbia, using the method of Kaiser and Meinke (1963).

In addition, liver and bone samples from sheep fed high levels of dietary vanadium were analyzed for vanadium by both methods. Results of the comparisons for bone, liver and plant tissue are shown in Table 4. Similar concentration values were obtained by both analytical methods for liver and bone. In the NBS orchard leaves (SRM 1571), however,

Figure 2. Effect (percent relative interference) of increasing iron (III) levels on the AAS determination of 5 μg vanadium at three concentrations of cupferron (squares, 1.0×10^{-2} Molar cupferron, open circles, 7.0×10^{-2} Molar cupferron, solid circles, 10.0×10^{-2} Molar cupferron).

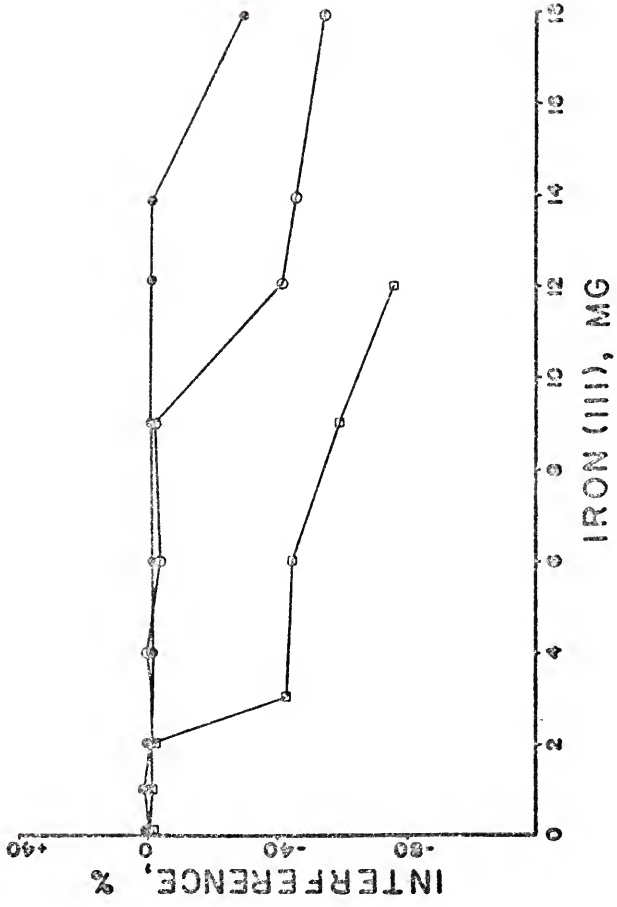

INTERFERENCE, %
+40
0
-40
-80
0
2
4
6
8
10
12
14
16
18
IRON (III), MG

TABLE 4. COMPARISON OF RESULTS OBTAINED BY AAS METHOD AND INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS FOR BIOLOGICAL MATERIALS[a]

Sample number	Material	Vanadium analysis (dry weight basis)[b] AAS method µg/g	Activation method µg/g
V-72	Orchard leaves (SRM 1571)[c]	.63 ± .04	1.23
V-73	Bovine liver (SRM 1577)[c]	.17 ± .02	.21
V-71	Treatment liver[d]	2.11 ± .10	2.38
V-74	Normal bone, ovine	.41 ± .03	.34
V-75	Treatment bone[d]	2.5 ± .17	1.9

[a]Mean plus standard error. AAS values are means of three determinations.

[b]Dried at 90°C; bone, ash weight basis.

[c]Standard reference materials, National Bureau of Standards, Washington, D.C.

[d]Treatment liver and bone from sheep in experimental vanadium toxicity study.

the level of vanadium determined by INAA was approximately twice the value obtained by AAS analysis. The reason for the disagreement in results is not known. The value measured by AAS (.63 µg/g) is in better agreement with the finding of Morrison and Potter (1972), who reported that the vanadium content of SRM 1571 was less than or equal to .7 µg per gram (dry weight basis). Certified vanadium concentrations in these biological standards have not yet been established (N.B.S., 1975).

Extraction of vanadium added to plant and animal material was also examined by flameless atomic absorption spectrophotometry. The average recoveries of vanadium added were 96 and 98% for NBS Orchard leaves (1571) and ovine liver tissue respectively. Extraction from deionized water standards with added vanadium was essentially complete (99%).

Linearity of the standard curve was tested for a series of concentrations from .2 to 40 µg vanadium per sample and found to be linear throughout this range. The coefficient of determination (r^2) averaged .99 for 15 independent analyses. The mean slope (b) of the least squares regression line for vanadium standards was .103 µg vanadium per absorbance unit with a relative standard deviation of 4.7%.

Seven samples containing 2.8 µg vanadium were analyzed to assess the precision of the method for standards in deionized water. The relative standard deviation was 5.2%. Six 6.5-gram ashed subsamples of liver tissue were also

analyzed for vanadium content. The standard deviation was .025 µg of vanadium (standard deviation 7.6%). This latter value, while higher than that for analysis of synthetic vanadium standards, was relatively small compared to the mean value of .32 µg vanadium per gram of dry liver tissue.

Discussion

Sample Preparation

Preparation of biological samples for vanadium analysis has been surveyed by the National Academy of Sciences Committee on Medical and Biologic Effects of Environmental Pollutants (N.R.C., 1974a). While low-temperature ashing is generally recommended for more volatile elements, high temperature dry ashing in porcelain crucibles was used to prepare samples prior to analysis by the method described. Recoveries of the metal following ashing were high (greater than 95%), so losses of vanadium by volatilization during ashing appear to be small for vanadium added to liver and bone tissues.

Other authors have reported minimal losses of vanadium during dry ashing of biological samples. Dry ashing at 500°C and wet ashing of airborne particulate matter samples were compared by Kometani et al. (1972) and recoveries of vanadium obtained by the two methods compared favorably. Welch and Allaway (1972) found that dry ashing of biological materials was a satisfactory alternative to wet ashing prior to analysis for vanadium content. Additionally, Lambert et al. (1970), using a neutron activation technique, reported essentially no

vanadium loss through volatilization when animal diets were dry ashed at 650°C.

Christian (1971) recommended quartz ashing vessels because of the presence of vanadium in porcelain glaze. The crucibles used for ashing in the present study had been in use for several years and thus inner surfaces were rarely glazed. When nitric acid blanks were prepared in glazed (new) and unglazed crucibles, however, the same low absorbance values were obtained for both using the sensitive flameless AAS technique. Thus, under these conditions, the crucibles were not a significant source of vanadium contamination.

Robertson (1968) surveyed trace metal concentrations in glass and plastic containment materials, reagents and other laboratory items and observed that normally vanadium was an infrequent contaminant. Nitric acid may contribute appreciable quantities of vanadium, but this source of systematic error was controlled with adequate blanks when vanadium analyses in the ppm range were performed. At very low levels of vanadium, however, purification of nitric acid becomes necessary. A redistillation step removes all but a trace of vanadium from the acid (Söremark, 1967). The mineralization procedure contributed very small amounts of vanadium to reagent blanks (less than .2 µg/sample). Levels were reduced still further by distillation of the nitric acid.

Polyphosphates may be formed in biological materials during high-temperature ashing (Talvitie, 1953). Vanadate

ions may also be complexed with phosphate (Russel et al., 1961) or other vanadate ions (DeMaster, 1972). However, the complexes thus formed are apparently hydrolyzed by slow evaporation of an ash solution in the presence of excess nitric acid (Talvitie, 1953). Nitric acid probably also served to maintain or convert the vanadium to higher oxidation states since the valence of the element is easily changed in response to changes in the redox potential of the environment (Crump-Wiesner et al., 1971).

Extraction and Interfering Ions

Both vanadium (IV) and vanadium (V) are extracted by cupferron in MIBK at the proper pH. This effect could be due to the chelating ability of cupferron and/or a conversion to the higher valence through a redox reaction (Crump-Wiesner and Purdy, 1969; Dean and Herringshaw, 1963). The Oxidation-reduction potentials of metals of variable valence states are altered by chelation (Christian and Feldman, 1970).

Reducing agents such as hydrochloric and formic acid lead to low recoveries of vanadium by similar extraction schemes (Pearton et al., 1969; Welch and Allaway, 1972). The interference by hydrochloric acid was verified. Solubilizing the ash in hydrochloric acid with a final concentration of the acid above 4 to 5% resulted in very low absorbance values; hence the less pure nitric acid was used.

Sachdev et al. (1967a) found that the sensitivity of the AAS determination for vanadium as the cupferrate in MIBK using

the nitrous oxide flame was 1.0 part per million. A number of substances were found to enhance the vanadium signal, but when aluminum was added in excess, the interferences were eliminated. Goecke (1968) also described the enhancement of vanadium absorbance by aluminum and used added aluminum to control interfering ions in ore samples.

Tungsten and tin have been reported to interfere in vanadium analyses when in excess of 5 and 10 µg/ml, respectively (Crump-Wiesner et al., 1971), but both occur at very low concentrations in animal tissues (Christian and Feldman, 1970).

Molybdenum also depressed vanadium absorbance when present in amounts above 1000 µg/sample. In biological materials, molybdenum concentration is usually less than 1 ppm, but plants may contain up to 300 ppm on a dry weight basis (Welch and Allaway, 1972).

In atomic absorption, acetone has been shown to offer greater sensitivity for some metals than MIBK and other solvents. Robinson (1960) found enhancement of absorbance with acetone in a study with nickel. Manganese absorption is also maximum in this solvent (Feldman et al., 1967), but normal solvent extraction procedures cannot take advantage of this enhancement due to the miscibility of acetone with water. The enhancement in acetone was about twice that in MIBK in these studies, but the acetone-cupferron mixture adhered to the burner chamber, making cleaning difficult.

Detection of very low levels of vanadium using Scale Expansion were made possible by the stability of the baseline

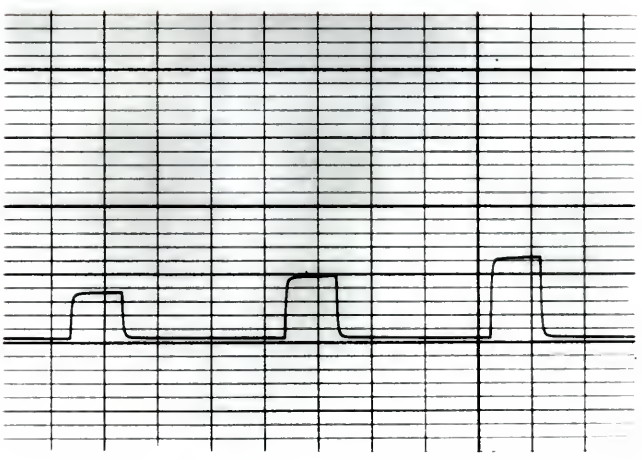
resulting from the comparatively low background noise of the aspirated vanadium solution. Shown in Figure 3 is the recorder tracing of .2, .4 and .6 µg vanadium extracted and concentrated as described. The detection limit for 1% absorption was .5 µg vanadium per sample (in 1.5 ml MIBK); but as little as .2 µg can be detected under the conditions of the procedure.

Reduction of the volume of separated organic phase by evaporation eliminated potential error caused by variations in the volume of solvent which occur due to solubility in the aqueous phase (Christian and Feldman, 1970).

Summary

A method is described for the determination of vanadium in animal tissue and plant material by AAS with a nitrous oxide-acetylene flame. Following dry ashing and solubilization in nitric acid, sample pH is adjusted (2.2 to 2.4) and the vanadium is extracted with 5% cupferron and methylisobutyl ketone. The ketone layer is evaporated to dryness; the residue is then redissolved in MIBK and aspirated for AAS measurements. Aluminum is utilized to control interferences. As little as .2 µg vanadium per sample could be detected by the method described. Tungsten, tin and molybdenum interfere, but the concentrations normally found in biological samples are well below interference levels. Iron at high levels also interferes in the determination, but interference due to high iron concentration can be overcome by additional cupferron.

Figure 3. Photograph of recorder tracing of vanadium absorbance response. Peaks represent .2, .4 and .6 µg vanadium added to samples and extracted as described. Instrument was in Integrate-2 mode with scale expansion X 30.



CHAPTER IV

DIETARY VANADIUM TOXICITY IN SHEEP

Introduction

Dietary vanadium has been shown to be toxic to livestock and poultry at very low levels (N.R.C., 1974a; Faulkner-Hudson, 1964). The mineral is known to occur in phosphate supplements in amounts sufficient to cause growth depression in chicks (Berg, 1963). Additionally, signs of toxicity in cows exposed to grazing areas contaminated by ash from combustion of petroleum products have been linked to the vanadium content of the ash (Lillie, 1970). Martinez and Church (1970) demonstrated that vanadium in trace amounts inhibited *in vitro* rumen cellulose digestion. Diarrhea, emaciation and other pathological changes in calves following oral administration of ammonium metavanadate have been observed by Platonow and Abbey (1968). The average concentration in soils of the United States (200 ppm) is high (N.R.C., 1974a); the potential of the metal for decreasing production through excessive ingestion and for accumulating in animal tissues used as foods for man is therefore considerable. Experimental dietary toxicity of vanadium in ruminant animals and response to the mineral when consumed in excess have been little explored. The present study was conducted to evaluate

the effect of graded levels of dietary vanadium on performance and tissue vanadium levels of sheep.

Experimental Procedure

Twenty-four Florida native wethers weighing 37 kg initially were randomly allotted to six dietary treatments of either five or two lambs each. Treatment diets containing supplemental levels of 0, 10, 100, 200, 400 and 800 ppm vanadium as reagent-grade ammonium metavanadate (NH_4VO_3) were formulated using the basal diet shown in Table 5. All animals were housed individually in elevated pens. Appropriate experimental diets were fed once daily in amounts of at least 10% more than the previous daily consumption. Water containing, by analysis, .01 ppm vanadium was available *ad libitum*. Sheep were weighed initially and weekly thereafter for the 84-day feeding period. Performance data were calculated individually for those animals that continued on treatment. The four animals initially offered the diets containing 400 and 800 ppm vanadium refused feed after the first days' intake, and so were returned to the basal diet 48 hr after initial exposure. Feed intake was recorded during the subsequent recovery period. This exposure and recovery sequence was repeated twice for additional data. The animals were then allowed 2 weeks of readjustment to the basal diet and were used in a series of short-term experiments testing response to high vanadium level and voluntary intake of the mineral.

TABLE 5. COMPOSITION OF BASAL DIET.

Ingredient	Percent
Corn meal, yellow	57.50
Cottonseed hulls	21.00
Soybean meal (50% protein)	12.50
Alfalfa meal (17% protein)	3.00
Corn oil[a]	3.00
Defluorinated phosphate (31% Ca, 18% P, .018% V)	1.00
Corn starch	1.00
Trace mineralized salt[b]	1.00
Vitamins A, D and E[c]	+
	100.00
Vanadium analysis, ppm[d]	2.2
Crude protein, %	12.8

[a]Santoquin added at .0125% of total diet.

[b]Analysis in percent listed as follows: NaCl, 97.5; Fe, .25; Mn, .23; S, .05; Cu, .03; Zn, .01; Co, .01; and I, .01 (Gordy Salt Co., New Iberia, Louisiana).

[c]Vitamins added per kilogram of diet: 4,500 USP units of vitamin A palmitate, 600 USP units of vitamin D_3 and 11 mg DL-α-tocopherol.

[d]Dry matter basis.

The four sheep were randomly reassigned, with two animals per treatment, to a high-vanadium diet (400 ppm) initially; after 48 hours, when feed intake was severely reduced, two sheep were returned to the basal diet for a 5-day period while the remaining two were offered only the diet containing 400 ppm vanadium for 5 days.

An additional short study, for the purpose of investigating voluntary intake of vanadium-containing diets, was initiated using these four sheep. The animals were offered, in identical containers, equal amounts of either the basal diet or the diet supplemented with 200 ppm vanadium for a period of 14 days. The relative positions of the containers in the pens were changed daily. Intake of each diet was recorded for determination of selective consumption by the sheep.

Blood samples were taken weekly by jugular vein puncture. Hemoglobin was determined by the acid hematin method (Cohen and Smith, 1919) and hematocrit values were obtained by centrifuging the whole blood in micro-capillary tubes for 10 min in a clinical centrifuge. Sheep were killed by exsanguination following the experimental period and samples of liver, metacarpal bone, kidney and portions of semitendinosus and adjoining muscles were removed and frozen in plastic bags until aliquot sampling for chemical analysis.

Vanadium in feed, water and tissues was determined by atomic absorption spectrophotometry after a preparatory

procedure (Chapter III) in which the metal was preconcentrated by chelation and extraction with cupferron and methylisobutyl ketone. Data were analyzed for treatment differences by least squares analysis of variance (Draper and Smith, 1966) using a generalized least squares computer program (Barr and Goodnight, 1972).

Results

Animal Performance

Performance was similar for lambs receiving levels of added vanadium up to and including 200 ppm, as shown in Table 6. Average daily gain and average daily feed intake for the 84-day period were not affected ($P > .05$) by increasing supplemental vanadium levels from 0 to 200 ppm. Feed intakes of animals fed 0 and 200 ppm supplemental vanadium were 1.60 and 1.55 kg, respectively. Feed conversion ratios were slightly but not significantly reduced at the highest level (200 ppm) consumed.

Blood Parameters

Mean values for the effect of dietary vanadium, with time, on blood parameters are given in Table 7. Dietary vanadium at 200 ppm or less did not significantly affect hemoglobin or hematocrit values of blood. Hemoglobin values were 10.6 and 11.2 g/100 ml for sheep fed 0 and 200 ppm, respectively.

TABLE 6. EFFECT OF DIETARY VANADIUM ON FEED INTAKE AND PERFORMANCE OF LAMBS[a]

	Supplemental Vanadium, ppm					
	0	10	100	200	400	800
Number of Lambs	5	5	5	5	2	2
Initial Body Weight, kg	39.7	39.6	36.2	35.6	35.6	36.3
Average Daily Feed Intake, kg	1.60 ± .15	1.64 ± .05	1.61 ± .03	1.55 ± .09	-	-
Average Daily Gain, g	214 ± 34	221 ± 44	216 ± 18	225 ± 30	-	-
Feed per Unit Gain	7.49 ± .64	7.42 ± .84	7.45 ± .64	6.89 ± .53	-	-

[a]Mean plus standard error, values based on 84-day feeding period.

TABLE 7. INFLUENCE OF DIETARY VANADIUM ON HEMOGLOBIN AND HEMATOCRIT OF SHEEP.[a]

Supplemental vanadium, ppm	Time, Weeks 0	2	4	6	8	10	12	Average
				(Hemoglobin, g/100 ml)				
0	10.9 ±.48	10.4 ±.38	9.6 ±.21	10.2 ±.69	10.6 ±.67	10.8 ±.58	11.6 ±.36	10.6
10	11.9 ±.19	10.9 ±.54	9.9 ±.17	11.3 ±.33	11.0 ±.28	11.5 ±.54	10.9 ±.45	11.1
100	11.0 ±.58	10.7 ±.79	10.2 ±.65	11.0 ±.58	10.7 ±.78	11.2 ±.85	11.2 ±.40	10.9
200	11.8 ±.48	11.0 ±.37	10.7 ±.61	11.0 ±.35	11.4 ±.43	10.8 ±.33	11.5 ±.45	11.2
				(Hematocrit, %)				
0	34.6 ±1.31	34.3 ±1.37	33.7 ±1.21	35.3 ±1.53	36.0 ±2.54	33.9 ±1.56	32.0 ±.42	34.3
10	36.6 ±1.24	37.3 ±.44	35.4 ±1.30	39.8 ±.20	37.6 ±1.76	35.3 ±.66	31.4 ±.97	36.2
100	33.3 ±1.44	35.5 ±2.10	35.6 ±1.51	36.8 ±1.45	36.7 ±2.48	35.9 ±.88	34.2 ±1.61	35.4
200	35.8 ±2.23	36.6 ±1.44	32.7 ±1.32	37.8 ±1.37	38.0 ±.96	32.4 ±1.58	31.6 ±1.01	35.0

[a]Mean plus standard error. Means represent data from five sheep.

Tissue Vanadium

Mean values for the vanadium content of muscle, liver, kidney and bone are shown in Table 8. The vanadium deposited in tissues, in general, reflected the higher levels of dietary vanadium, but the effects on tissues of feeding 10 ppm supplemental vanadium were not large.

Significant increases in vanadium concentration of muscle were observed only with the 200 ppm level. Dietary vanadium levels of 100 and 200 ppm significantly ($P < .01$) increased vanadium concentrations in kidney, bone and liver. Tissues of sheep receiving 10 ppm supplemental vanadium contained essentially the same levels as animals fed the basal diet, although kidney levels were slightly, but not significantly, elevated.

Short-Term Effects

The four animals offered the two highest levels of vanadium (400 and 800 ppm) ceased eating and exhibited diarrhea after an exposure of 1 day to the experimental diets. This effect of vanadium ingestion on feed intake and subsequent recovery from depressed intake for these four sheep when returned to the basal diet is illustrated in Figure 4. Feed intake is shown as percent of initial or first-day feed intake which averaged 1.65 and 1.83 kg for the animals receiving the diets containing 400 and 800 ppm supplemental vanadium, respectively. Following consumption of the high-vanadium diets offered initially, the sheep ceased eating

TABLE 8. EFFECT OF DIETARY VANADIUM ON TISSUE VANADIUM OF SHEEP.[a]

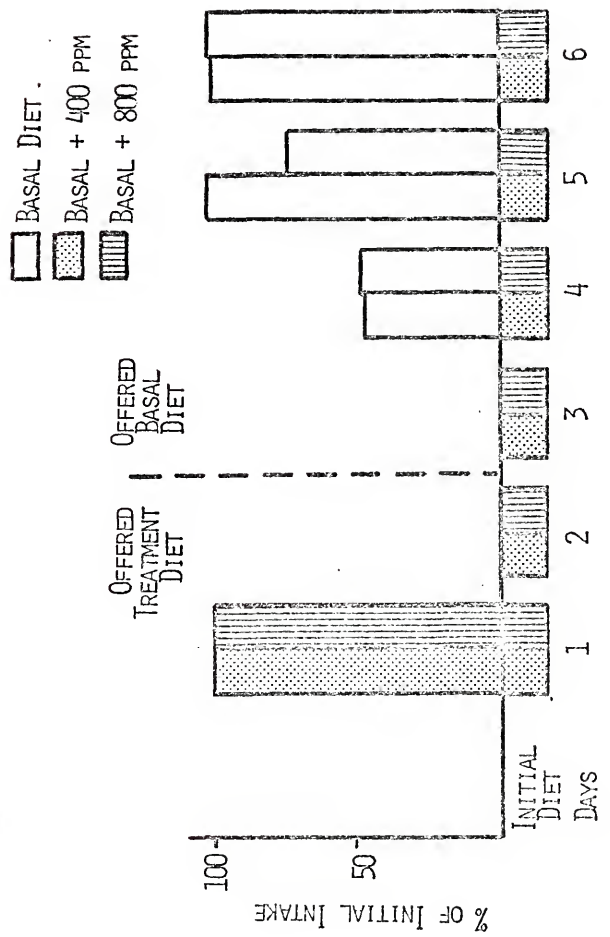
Supplemental vanadium, ppm	Tissue, ppm Dry Matter Basis[b]			
	Bone	Liver	Kidney	Muscle
0	.19 ± .04[c]	.12 ± .03[c]	.23 ± .04[c]	.04 ± .00[c]
10	.22 ± .05[c]	.18 ± .04[c]	.41 ± .06[c]	.05 ± .01[c]
100	1.50 ± .34[c]	.96 ± .12[c]	3.62 ± .19[d]	.12 ± .01[c]
200	3.32 ± .23[d]	2.81 ± .24[d]	11.13 ± .46[e]	.41 ± .04[d]

[a]Means plus standard error. Means represent data from five sheep.

[b]Bone, ash weight basis.

[cde]Means in the same column with different superscripts are different ($P < .05$).

Figure 4. Short-term feed intake effects and recovery in sheep following ingestion of diets containing 400 and 800 ppm vanadium.


BASAL DIET
BASAL + 400 PPM
BASAL + 800 PPM
OFFERED TREATMENT DIET
OFFERED BASAL DIET
INITIAL DIET
DAYS
1
2
3
4
5
6
100
50
% OF INITIAL INTAKE

and refused further feed until the basal diet was substituted. When the substitution was made 48 hours after exposure to high vanadium levels, the regular recovery pattern illustrated in Figure 4 was followed. The sheep began to consume the low-vanadium basal diet after a delay of 1 day. Feed consumption improved progressively and, by the sixth day, all animals were eating normally. This pattern of recovery from dietary insult was demonstrated repeatedly in these four animals.

Allowing only two of the sheep access to the basal diet during recovery while the other two remained on the 400 ppm diet confirmed the intake-depressing effect of the mineral. At the end of the 6-day test period, feed intake of the sheep which continued on the high-vanadium diet was only 4.2% of that of the control animals.

Voluntary Intake

To further test the ability of the sheep to differentiate between the basal diet and the diet containing 200 ppm vanadium, a voluntary intake trial was conducted. The sheep did not appear to consume either diet selectively, although slightly more of the basal diet was ingested during the 14-day test period. Consumption of the two diets as a percentage of total intake was 52.4 and 47.6% for the basal and vanadium-supplemented diets, respectively. Total intake was normal for these animals.

Discussion

Daily weight gains of sheep receiving up to 6.6 mg vanadium per kg body weight (200 ppm in diet) for 84 days were not severely affected, although a non-significant decrease in daily feed intake was observed at the highest level consumed. Feed intake values for the sheep fed 200 ppm vanadium during the first 2 weeks of the experiment, however, averaged 17% less than controls. This was a significant ($P < .05$) decrease, but the difference was masked in the longer feeding period. By the third week of the experiment, feed intake approximated that of control sheep. Platonow and Abbey (1968) administered orally 7.5 mg of vanadium per kg body weight to calves for 30 days and noted that live weight was not affected by treatment. At daily levels above 10 mg per kg body weight, however, the animals died. Strasia (1971) found that rats developed a tolerance to the mineral and that if dietary vanadium were gradually increased to 500 ppm, feed consumption and weight gain were unaffected. Williams (1973) showed that chicks were able to partially recover from the initial toxic effect of 30 ppm dietary vanadium, suggesting a certain degree of adaptation.

No significant effects of dietary vanadium on hemoglobin and hematocrit were observed in the present study. Strasia (1971) found that packed cell volume was significantly depressed in rats by prolonged consumption of high

levels of vanadium. Lewis (1959), however, indicated that men exposed to vanadium did not show a change in hematocrit. When vanadium was administered orally to 6-month-old calves at dosage levels up to 20 mg per kg body weight (Platonow and Abbey, 1968), no changes were observed in either the hemoglobin or hematocrit values.

Vanadium in the tissues generally reflected vanadium level in the diet in the present studies, although 10 ppm supplemental vanadium caused no significant changes in tissue concentration when compared to the basal diet. This may have been due to the similarity of vanadium content of the two diets as fed (2.2 and 8.5 ppm, by analysis).

There seem to be few values in the literature for tissue levels of vanadium in the ovine. The early analyses reported by Proescher et al. (1917) suggested that kidney vanadium content was elevated in toxic states. Sheep bone was found by Morrison (1967) to contain .2 ppm vanadium (dry weight basis). The few reported values for vanadium concentration in other animals are generally in the ppb range. Welch and Allaway (1972), for example, found 2-20 ppb (wet weight basis) in rat liver, kidney, muscle and bone. The samples of tissue were from animals fed an experimental diet primarily composed of corn and torula yeast, presumably low in vanadium.

The present values for sheep fed the basal diet are slightly higher than those reported by Söremark (1967) for

calf liver. He found only small amounts in bovine liver (2 to 10 ppb wet weight basis), but the dietary vanadium level was not reported.

Platonow and Abbey (1968) showed that tissue vanadium concentrations in calves could be elevated by administering vanadium orally; liver vanadium content, following dosing, ranged from .3 to 5.1 ppm (fresh weight basis), while the range for kidney tissue was higher, up to 40 ppm, depending on dosage level.

Hopkins and Mohr (1971b) found that vanadium in kidneys of chicks was increased from a few ppb when fed a deficient diet to 760 ppb (dry weight basis) when supplied with 2 ppm vanadium (as NH_4VO_3) in water. Liver and heart muscle concentrations were also elevated, but the increases were smaller.

Schroeder et al. (1963), in reporting on vanadium in deer tissue, found .07 and 2.07 ppm (wet weight basis) in liver and kidney, respectively. Bertrand (1950) reported a range of .02 to .3 ppm, with a mean of .1 ppm vanadium, for various vertebrate tissues (wet weight basis).

Autoradiographic (Söremark and Üllberg, 1962) and isotopic (Hathcock et al., 1964) studies with rats, mice and chicks indicated kidney and bone to be sites of vanadium accumulation. Little dietary vanadium is apparently absorbed and injected or absorbed vanadium is excreted rapidly by the kidney (Talvitie and Wagner, 1954).

Retention in liver may be due in part to movement of vanadium into mitochondria (Hopkins and Tilton, 1966).

Phosphate (PO_4^{-3}) and vanadate (VO_4^{-3}) have identical ionic configurations and it has been suggested that vanadium may exchange with phosphorus in the apatite matrix of teeth (Lowater and Murray, 1937). Söremark et al. (1962) showed that isotopic vanadium concentrated in the areas of rapid mineralization in tooth dentine and bone of mice.

In the series of short feeding studies with sheep, vanadium at 400 and 800 ppm in the diet caused an abrupt decrease in feed intake. The diets were generally consumed in normal amounts during the first day they were offered. However, intake on the following day was often nil. Because normal intake of the high-vanadium feed was a regular occurrence, it is apparent that either the physiological consequences resulting from ingestion of diets high in the mineral did not cause the sheep to develop an aversion to vanadium-containing diets or that the animals were unable to detect vanadium in the feed at these levels.

In an experiment to determine if sheep could differentiate between the basal diet and the same diet supplemented with 200 ppm vanadium, only slightly more of the basal diet was consumed. Intake of the combined total of the two diets was normal for these sheep. Although data were limited, it was clear that the diet containing 200 ppm was not completely rejected because of vanadium content, since substantial amounts were ingested. A larger number of animals would be required to determine if the slight favoritism shown the

low-vanadium diet in this voluntary intake study illustrated a significant trend.

Summary

Twenty-four Florida native wethers averaging 37 kg initially were used in an 84-day study of dietary vanadium toxicity. Supplemental levels of 0, 10, 100, 200, 400 and 800 ppm vanadium as ammonium metavanadate were fed in a roughage, corn-soy diet to treatment groups of either five or two lambs each. The four animals offered the two highest levels of vanadium ceased eating and exhibited diarrhea after an exposure of 1 day to the experimental diets. Subsequent feeding of the control diet to these sheep resulted in an apparent complete recovery within 5 to 8 days. No outward indications of toxicity were seen in sheep fed the lower levels of vanadium. For those fed 0, 10, 100 and 200 ppm vanadium, average daily gains were 214, 221, 216 and 225 g per day; values for feed per unit gain were 7.49, 7.42, 7.45 and 6.89, respectively, but these parameters were not altered significantly by treatment. Hemoglobin (10.6, 11.1, 10.9 and 11.2 g/100 ml) and hematocrit (34.3, 36.2, 35.4 and 35.0%) values were not significantly different. Metacarpal bone, liver, kidney and muscle vanadium concentrations were higher ($P < .05$) when 200 ppm dietary vanadium was fed, with the greatest concentration in kidney. Data suggest that vanadium is not markedly toxic when fed to growing lambs for an 84-day period at a level of 200 ppm or less in the diet.

CHAPTER V

INFLUENCE OF DIETARY VANADIUM ON METABOLISM OF VANADIUM-48 IN SHEEP

Introduction

Vanadium occurs in appreciable quantities in soils (N.R.C., 1974a) and is widely distributed in plants and animals, although in trace amounts (Söremark, 1967). The essentiality of the mineral in bone and feather development of chicks (Hopkins and Mohr, 1971b; Nielsen and Ollerich, 1973) and for rat growth (Schwarz and Milne, 1971b) has been shown.

Selective retention of injected vanadium-48 (^{48}V) in kidney, bone and liver tissue has been reported for mice (Söremark and Üllberg, 1962) and for rats (Hopkins and Tilton, 1966). The biological and toxicological aspects of vanadium in man and animals have been reviewed by Faulkner-Hudson (1964) and more recently by Underwood (1971).

Proescher et al. (1917) reported that vanadium was toxic to sheep as well as to other animals and Platonow and Abbey (1968) observed that the toxic oral dose of the mineral was as low as 10 mg per kg body weight in calves. Although in vitro studies by Martinez and Church (1970) have shown intense cytotoxicity of the mineral to rumen

micro-organisms, the metabolism of vanadium in ruminant animals does not appear to have been previously studied.

The present experiment was conducted to investigate the influence of dietary vanadium level on the metabolism of trace amounts of orally and intravenously administered ^{48}V in sheep.

Experimental Procedure

Fourteen rams weighing 58 kg and one lactating ewe weighing 42 kg at the time of isotope administration were housed individually in metabolism crates (Figure 5) and allowed a 10-day predose adjustment period. Tap water containing, by analysis, 8 ppb vanadium was provided ad libitum. Intake of treatment diets containing 0, 50 and 200 ppm supplemental vanadium as ammonium metavanadate (NH_4VO_3) formulated from the basal diet (Table 9) was restricted to 800 g per head daily. The number of rams receiving each of the respective diets was 4, 2 and 8 sheep per treatment; ^{48}V was administered orally to half of the animals and intravenously to the remainder. The rams had been fed the experimental diets ad libitum for 25 weeks prior to dosing.

The carrier-free radioactive ^{48}V (dioxovanadium chloride in 1 M HCl) with a specific activity of approximately 2 X 10^5 mc per g was obtained from Amersham Searle, Arlington Heights, Illinois. A calibrated dosage level of 105 μc of the isotope was administered by injection with .9% NaCl

Figure 5. Photograph of metabolism unit used in radioisotope studies.


CAUTION

TABLE 9. COMPOSITION OF BASAL DIET.

Ingredient	Percent
Corn meal	57.5
Cottonseed hulls	21.0
Soybean meal (50% protein)	12.5
Alfalfa meal (17% protein)	3.0
Corn oil[a]	3.0
Corn starch	1.0
Defluorinated phosphate (31% Ca, 18% P)	1.0
Trace mineralized salt[b]	1.0
Vitamins A, D and E[c]	+
	100.0
Vanadium analysis, ppm[d]	2.6
Crude protein, %	12.7

[a]Santoquin added at .0125% of total diet.

[b]Analysis in percent listed as follows: NaCl, 95.0; Zn, 1.0; Fe, .30; Mn, .20; S, .10; Cu, .08; Co, .01 and I, .01 (Carey Salt Company, Hutchinson, Kansas).

[c]Vitamins added per kilogram of diet: 2200 USP units of vitamin A, 440 USP units of vitamin D and 11 mg of DL-α-tocopherol.

[d]Dry matter basis.

(approximately .2 ml per kg body weight) into the jugular vein of seven rams maintained on the various treatment diets. An additional seven rams were dosed orally with 1015 μc of ^{48}V given in a gelatin capsule.

A single lactating ewe was also dosed orally with 310 μc of the radioactive vanadium to obtain additional data on distribution and for preliminary information about milk transfer of the element. The ewe was nursing a 7-kg lamb which was housed separately and allowed to nurse four times per day; milk samples were taken concurrently.

At the time of administration, reference standards were prepared from the isotope solution. Standards with geometry and activity levels similar to samples were used to relate sample activity to dose.

Blood samples were obtained by jugular vein puncture at intervals from 4 min to 144 hr after dosing. Total daily fecal and urine collections were made during this period.

At 144 hr postdosing, animals were stunned and exsanguinated. Organs and selected tissues were sampled, rinsed and blotted to remove excess blood before weighing and freezing.

The ^{48}V activity in blood, tissue and excreta was determined either in a well-type NaI (Tl) crystal scintillation counter or a large-capacity detector with 4-π geometry. The counting chamber of the latter detector was

11.5 cm in diameter, permitting radioassay of activity in whole organs or 300 g samples. Activity was calculated as percent of dose per kg sample:

$$= \frac{\text{tissue counts per min/dose counts per min}}{\text{tissue weight in kg}} \times 100$$

The radioactivity was further expressed, for comparative purposes, as percent relative concentration:

$$= \frac{\text{tissue counts per min per kg tissue}}{\text{dose counts per min per kg body weight}} \times 100$$

This treatment enabled comparisons among organs and tissues since uniform distribution of the isotope would theoretically result in equivalent relative concentration values.

For sheep dosed intravenously, blood activity at time zero was estimated from a semilog plot of initial values for net counts per min per ml by extrapolating the curve thus obtained to the zero time intercept. Blood volume was then estimated by dividing total injected activity by counts per min per ml at zero time.

Stable vanadium in feed and water was determined by atomic absorption spectrophotometry. A single extraction procedure (Chapter III) was employed which involved chelation of the mineral in ash solution with cupferron and extraction of the chelate into methylisobutyl ketone.

Calculations of blood clearance kinetics by a graphical logarithmic analysis were accomplished essentially as described by Mertz et al. (1965). By the graphical technique, however, the procedure is lengthy, so a Fortran IV program

using a linear regression subroutine was applied for individual animal data. From these values, estimates of biological half-lives of ^{48}V and compartmental constants were made based on the mathematical model of Aubert et al. (1963).

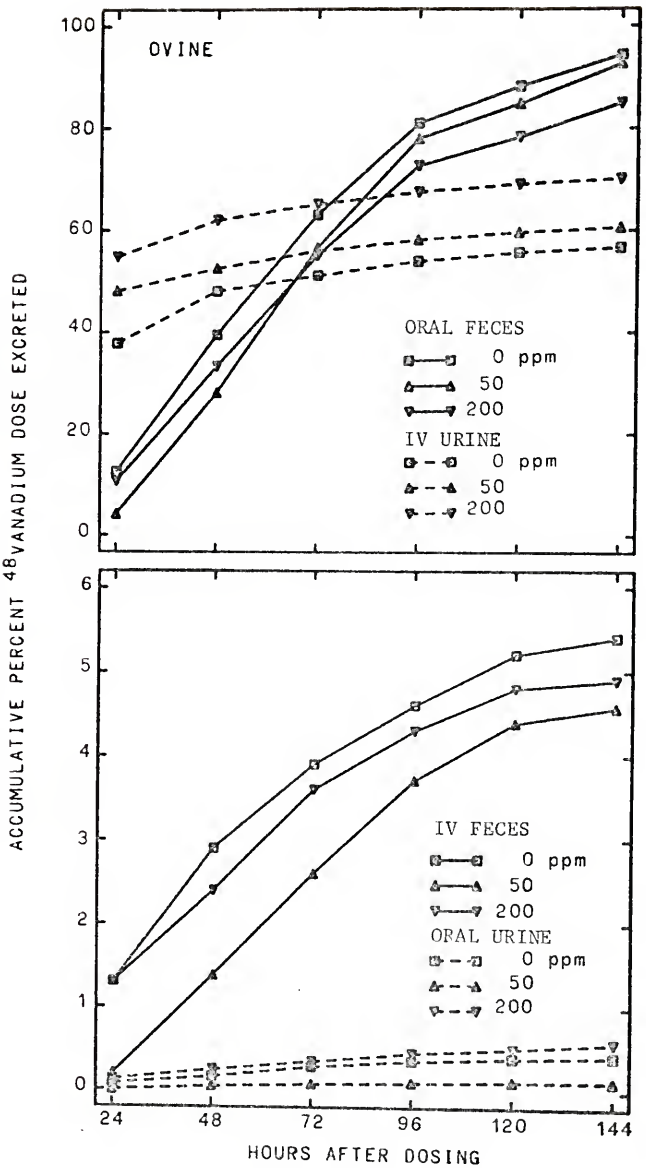
The data were analyzed statistically by least squares analysis of variance (Draper and Smith, 1966) using a generalized least squares program (Barr and Goodnight, 1972).

Results and Discussion

Excretion

The cumulative percentages of the ^{48}V dose excreted in feces for 144 hr following oral and intravenous administration are shown in Figure 6, and reflect the ultimate body retention of the element. Comparative treatment effects are presented in Appendix Tables 30 and 31. Pathway of isotope administration had a significant ($P < .01$) effect on the percent of dose excreted in the feces throughout the experimental period. Fecal excretion of ^{48}V during 6 days after isotope administration in sheep fed the low vanadium level totaled 94% after oral administration of ^{48}V and 5.3% after intravenous dosing. Animals fed the high vanadium level (200 ppm V) excreted 85% of the dose in the feces after oral ^{48}V administration and 4.9% after intravenous administration. The sheep receiving 50 ppm supplemental dietary vanadium and dosed orally excreted an intermediate amount (93% of the dose) in feces. Excretion values for

Figure 6. Accumulative percent of ^{48}V dose excreted in the feces and urine of sheep after oral and intravenous (IV) administration.


OVINE
ORAL FECES
0 ppm
50
200
IV URINE
0 ppm
50
200
IV FECES
0 ppm
50
200
ORAL URINE
0 ppm
50
200
ACCUMULATIVE PERCENT ^{48}VANADIUM DOSE EXCRETED
HOURS AFTER DOSING
0
20
40
60
80
100
1
2
3
4
5
6
24
48
72
96
120
144

the single animal dosed intravenously were lower at all time intervals than the means for animals on other dietary treatments, but the lower fecal excretion rate was within the range observed for other animals dosed intravenously. Intravenously dosed rats excreted 8.6% of the labeled vanadium in feces (Hopkins and Tilton, 1966). In sheep fed the diet without supplementary vanadium, both routes of administration resulted in slightly, but not significantly, higher percentages of the ^{48}V dose excreted than in the vanadium-supplemented diets. Dietary vanadium level had no significant effect on percent of the radiovanadium dose excreted in the feces.

Total urinary excretion of ^{48}V represented 57, 61 and 70% of the dose during 6 days following intravenous administration for the sheep fed 0, 50, and 200 ppm supplemental vanadium, respectively (Figure 6). The difference between animals receiving high and low dietary treatments was significant ($P < .05$) at 24 hr and at subsequent time intervals. The significance for the intermediate values of urinary excretion was not determined since the observed data were for one animal. Oral administration resulted in lower ($P < .01$) urinary ^{48}V excretion with a mean daily 6-day excretion of .08% of the dose for all dietary treatments. Individual animal variation within dietary treatments, however, was large. There was an 11-fold difference between low and high values for urinary excretion of the isotope in

sheep dosed orally; however, no significant differences attributable to dietary vanadium were observed.

The results indicated that ^{48}V orally administered was excreted mainly by way of the intestinal tract. This is consistent with the findings of Comar and Chevallier (1967) in isotope studies with rats. Balance studies in man have shown stable vanadium to be excreted largely unabsorbed (Proescher et al., 1917). A similar pattern of elimination was found in rabbits fed diets containing ammonium metavanadate (Ballotta, 1931).

The present studies also indicated that clearance of intravenously administered ^{48}V was rapid with the principle route of excretion being via the kidneys. Rapid urinary clearance has been previously reported for other species. Hopkins and Tilton (1966) observed that isotopic vanadium ($^{48}VOCl_2^+$) excretion in urine of intravenously dosed rats amounted to 46% of the dose after 4 days, most of this total (42%) being excreted within 24 hours. Kent and McCance (1941) found that, in man, 84% of the injected dose was excreted in the urine and 7% in feces. The predominantly renal excretion pattern following intravenous administration of vanadium has also been observed in rabbits (Talvitie and Wagner, 1954).

Blood Clearance

The changes with time in the whole blood activity of sheep receiving high and low levels of stable dietary vanadium

and dosed intravenously are shown in Figure 7. The rate of clearance from blood was similar irrespective of dietary treatment. That portion of the dose retained in blood decreased rapidly to less than 10% by 24 hr after administration.

Although clearance from circulating blood was generally rapid, it was of interest that actual increases in blood activity occurred in two of the sheep 2 to 3 hours after dosing. This phenomenon has also been observed in rats given ^{48}V intravenously (Hopkins and Mohr, 1971b).

Whole blood retention of the injected dose was plotted against time on semilogarithmic coordinates for further comparisons. The curve was reduced to a minimum of three exponential components (Figure 8) by graphical logarithmic analysis. The general model for activity in blood as a function of time may be represented by the equation:

$$Y = A_1 e^{-k_1 t} + A_2 e^{-k_2 t} + A_n e^{-k_n t}$$

where: Y is the concentration of radioactivity at time t, A_1, A_2, and A_n are fractional parts of the total amount of ^{48}V injected and k_1, k_2, and k_n are coefficients representing the rate of change in activity for each fractional part.

The biological half-life ($t_{1/2}$) of ^{48}V, defined as the time in which one-half of the activity was cleared from whole blood for each compartment is given by:

$$t_{1/2}\ ^{48}V = \frac{\ln 2}{k}$$

Figure 7. Changes with time in whole blood ^{48}V levels of sheep fed 0 and 200 ppm supplemental vanadium after intravenous ^{48}V administration.

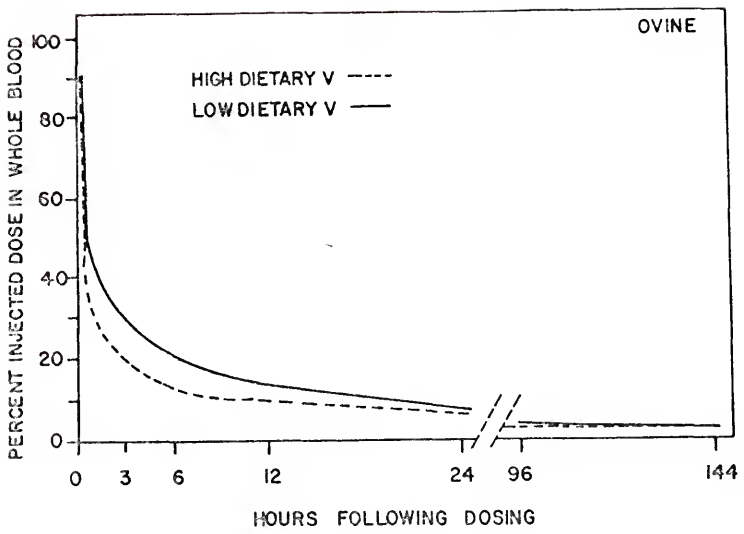
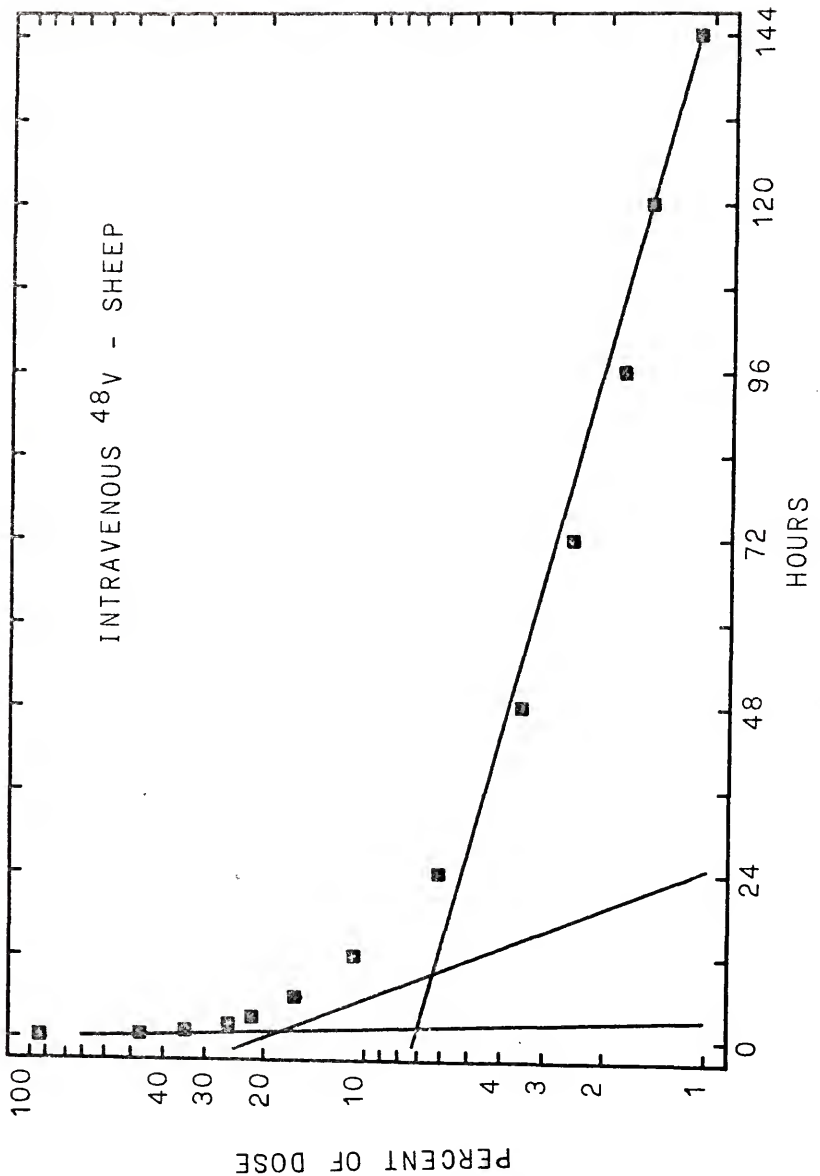

OVINE
HIGH DIETARY V
LOW DIETARY V
PERCENT INJECTED DOSE IN WHOLE BLOOD
100
80
60
40
20
0
0
3
6
12
24
96
144
HOURS FOLLOWING DOSING

Figure 8. Graphical logarithmic analysis of whole blood ^{48}V disappearance curve of sheep fed basal diet.


INTRAVENOUS ^{48}V - SHEEP
PERCENT OF DOSE
100
40
30
20
10
4
3
2
1
0
24
48
72
96
120
144
HOURS

where: k is the rate coefficient k_1, k_2 or k_n, for the three respective components. The data for sheep fed the basal diet could be satisfactorily described by an equation having at least three terms:

$$Y = 73.40\ e^{-3.05t} + 20.83\ e^{-.038t} + 5.16\ e^{-.012t}$$

where: Y is the percent of dose at time t (hr) after intravenous ^{48}V administration.

Components for sheep fed 0 and 200 ppm supplemental vanadium are mathematically described in Table 10. The biological half-lives for the three components A_1, A_2, and A_3 in sheep fed the non-supplemented basal diet were .23, 7.9 and 59.9 hr, respectively. Timed ^{48}V distribution studies with rats (Hopkins and Tilton, 1966) and present data suggest that the two compartments (A_1 and A_2), characterized by the shortest half-lives, probably represent rapid clearance into tissues and urine, whereas the compartment of longer duration (A_3) shows graphically the slower efflux of radioactivity from the animal body.

Slightly but not significantly more rapid initial (A_1 and A_2) blood clearance rates were observed in animals receiving 200 ppm supplemental vanadium, while the half-clearance times associated with the A_3 component were approximately the same for both dietary treatments.

Similarities in ionic configuration (Hill and Matrone, 1970) and biological distribution pattern (Hopkins and Tilton, 1966) between vanadium and trivalent chromium have been noted.

TABLE 10. EQUATIONS DESCRIBING REMOVAL OF INTRAVENOUSLY ADMINISTERED ^{48}V FROM THE CIRCULATING BLOOD OF SHEEP RECEIVING 0 AND 200 PPM DIETARY STABLE VANADIUM.[a]

Supplemental vanadium, ppm	Compartment	Compartmental coefficients A	k	r^2	Biological half-life hr
0 (2)[b]	A_1	73.40 ± 1.32	-3.05 ± .31	.99	.23
	A_2	20.83 ± 1.16	-.088 ± .01	.98	7.86
	A_3	5.16 ± .23	-.012 ± .00	.95	59.9
200 (4)	A_1	72.12 ± 1.11	-3.39 ± .33	.98	.20
	A_2	22.21 ± .96	-.095 ± .01	.99	7.33
	A_3	4.40 ± .31	-.013 ± .00	.96	54.1

[a]Mean plus standard error. Compartmental analysis based on generalized model:

$$Y = A_1e^{-k_1t} + A_2e^{-k_2t} + A_3e^{-k_3t}.$$

[b]Refers to number of observations.

Mertz et al. (1965) observed that previous dietary history had very little effect on whole-body retention of intravenously injected chromium-51 (III) in rats. Additionally, ^{48}V clearance in chicks was not altered by 1000 ppm dietary chromium (Wright, 1968).

Isotope levels in the total circulating blood of sheep for 144 hr after oral administration are shown in Figure 9. Activity in blood was low in the majority of the animals dosed orally. As in the case of urinary excretion by the orally dosed animals, variability among sheep was also high for blood activity. No significant differences ($P > .05$) in activity between the low and high dietary vanadium levels were observed.

Tissue Retention and Distribution

Treatment effects on tissue retention of radioactivity 144 hr after intravenous dosing are shown in Table 11. The percent dose per kg of fresh tissue ranged from a high of 15.4 in kidney of sheep fed the low-vanadium basal diet to a low of .08 in muscle. Thomassen and Leicester (1964) observed that rat liver retained 31% of the dose per kg dry tissue 72 hr after ^{48}V and ^{49}V injection.

In the present experiment, the high vanadium dietary treatment resulted in reduced ($P < .01$) retention of the isotope by kidney tissue when compared to sheep fed the basal diet. Supplemental vanadium at a level of 200 ppm also significantly ($P < .05$) affected the percent of dose

Figure 9. Percent of orally administered ^{48}V in total circulating blood of sheep fed diets containing 0 (A) and 200 (B) ppm supplemental vanadium. Total blood volume calculated as 57 ml per kg body weight (Hansard et al., 1953).

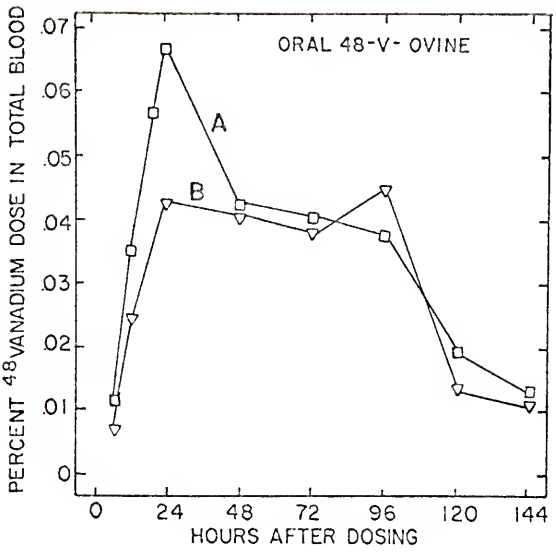

ORAL 48-V- OVINE
A
B
PERCENT ^{48}VANADIUM DOSE IN TOTAL BLOOD
.07
.06
.05
.04
.03
.02
.01
0
0
24
48
72
96
120
144
HOURS AFTER DOSING

TABLE 11. EFFECT OF DIETARY VANADIUM LEVEL ON TISSUE RADIOVANADIUM RETENTION 144 HR AFTER INTRAVENOUS ADMINISTRATION

Supplemental vanadium, ppm		Tissue									
		Kidney	Bone Metacarpal	Liver	Spleen	Bone Vertebra	Testicle	Lung	Heart	Brain	Muscle
		(Percent of dose per kg fresh tissue)[b]									
0	(2)[a]	15.40**	2.01	1.78*	1.29*	1.26	.537*	.438	.269	.144	.135*
50	(1)	9.45	2.04	1.61	1.38	1.11	.501	.535	.193	.157	.085
200	(4)	7.32**	1.80	1.27*	.840*	1.10	.330*	.460	.192	.141	.077*
		(Percent relative concentration)[c]									
0	(2)	860*	111	99	72	70	30*	25	15	8	7
50	(1)	510	110	87	75	62	27	28	10	8	5
200	(4)	446*	105	75	51	62	20*	28	12	8	5

[a]Refers to number of observations included in mean.

[b]Percent of dose per kg fresh tissue = $\frac{\text{Tissue counts per min/dose counts per min}}{\text{Kg fresh tissue}}$ X 100.

[c]Percent relative concentration = $\frac{\text{Tissue counts per min per kg fresh tissue}}{\text{Dose counts per min per kg body weight}}$ X 100.

*Means in the same column under each main heading are different ($P < .05$). Sheep fed 50 ppm supplemental vanadium was not considered in the statistical analysis.

**Means differ ($P < .01$).

per kg fresh tissue retained in liver, spleen, testicle and muscle. In the animals fed the basal diet, the total organ ^{48}V activity for liver, both kidneys and spleen was 1.6, 1.4 and .1 percent of the dose, respectively.

The data are also shown as percent relative concentration, a form which reduces variation due to size of animal. The percent relative concentration of ^{48}V in kidney and testes was higher ($P < .05$) for animals fed the basal diet compared to those receiving the high vanadium diet. Intermediate values were generally observed for tissue activity of the single sheep fed 50 ppm supplemental vanadium, notably in kidney, liver, testicle and muscle. Calculating from the data of Hopkins and Tilton (1966), percent relative concentration of ^{48}V in the rat 96 hr after intravenous administration ranged from 254 in kidney to 1.7 in brain. These authors found that kidney, liver, spleen and bone were highest in activity after 4 days. In mice, however, ^{48}V tissue retention was found to be higher in bone and liver than in kidney at 3, 5 and 7 days after intravenous dosing, although a different vanadium compound was used (Söremark and Üllberg, 1962).

Activity retained in the tissues of orally dosed sheep was lower than that of intravenously dosed sheep by a factor of approximately 100 (Table 12), even though the administered dose was 10 times greater. Values are expressed times 10^{-2} for comparison to tissue activity in sheep dosed intravenously.

TABLE 12. INFLUENCE OF DIETARY VANADIUM LEVEL ON TISSUE RADIOVANADIUM RETENTION 144 HR AFTER ORAL ADMINISTRATION

Supplemental vanadium, ppm		Tissue									
		Kidney	Bone Metacarpal	Liver	Bone Vertebra	Spleen	Lung	Testicle	Heart	Brain	Muscle
		(Percent of dose per kg fresh tissue X 10^{-2})[a]									
0	(2)[b]	10.2	1.23	1.14	.923	.581	.424	.324	.168	.080	.076
50	(1)	7.03	.814	.838	.812	.464	.302	.210	.116	.061	.063
200	(4)	12.5	1.62	1.82	1.04	.923	.698	.400	.221	.117	.091
		(Percent relative concentration X 10^{-2})[c]									
0	(2)	595	72	66	52	34	25	19	10	5	4
50	(1)	476	56	56	45	32	20	14	8	4	4
200	(4)	680	89	99	58	51	38	22	12	6	5

[a]Percent of dose per kg fresh tissue = $\frac{\text{Tissue counts per min/dose counts per min}}{\text{Kg fresh tissue}}$ X 100.

[b]Refers to number of observations included in mean.

[c]Percent relative concentration = $\frac{\text{Tissue counts per min per kg fresh tissue}}{\text{Dose counts per min per kg body weight}}$ X 100.

Kidney, bone, spleen and liver evidenced the highest activity at 144 hr following oral dosing, paralleling the tissue distribution observed after intravenous dosing. However, tissue activity values were quite variable within dietary treatments. The coefficients of variation among liver and kidney tissues, for example, were 105 and 87%, respectively. No significant differences in tissue activity due to dietary treatment were observed for orally dosed sheep.

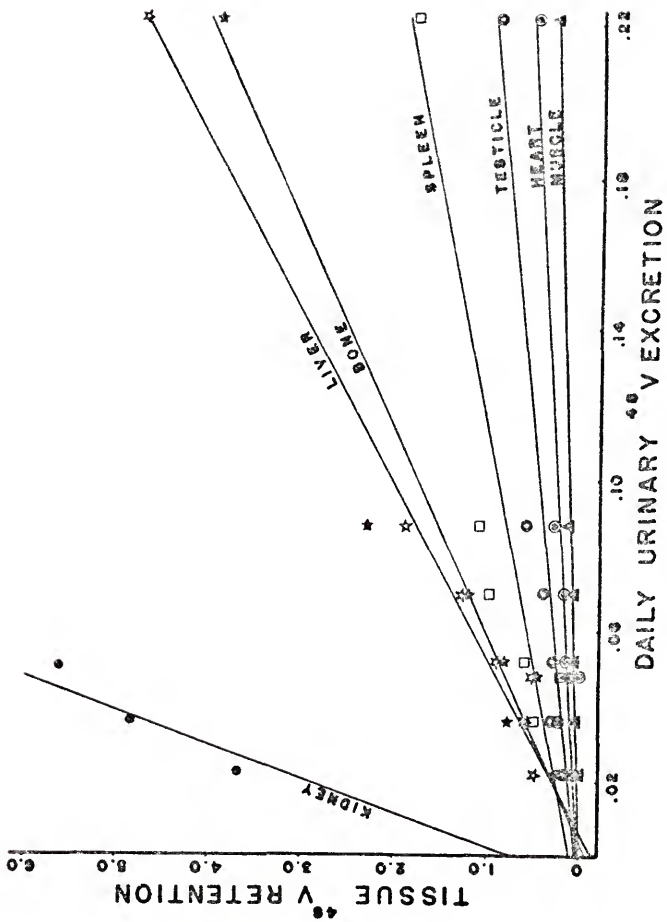
Hathcock et al. (1964) studied ^{48}V distribution in chick and rat tissues 2 days after oral administration. In chicks, bone and kidney were observed to have the highest retention values, followed by liver and spleen. The bone activity in adult rats, on the other hand, was relatively low, while kidney and liver were higher in ^{48}V concentration. In rats dosed intragastrically with ^{48}V, only .5% was absorbed (Scott et al., 1951).

Comar and Chevallier (1967), giving ^{48}V orally in the drinking water to mature rats, found that kidney was enriched most, while spleen, teeth, bone and liver tissues were relatively high and muscle and blood were noted to be very low in activity. Calculations from the data of these authors indicate that kidney retention was on the order of .08% of the dose. In the present study, both kidneys retained .01% of the dose. In contrast to the findings with intravenously dosed sheep, there was low correlation of ^{48}V tissue retention after oral dosing with dietary vanadium level ($r^2 = .47$).

Paradoxically, tissue activity was found to be positively related to urinary ^{48}V excretion. Equation components for the simple linear regression of tissue retention after oral ^{48}V administration on mean daily urinary excretion of the isotope are shown in Appendix Table 32. Sufficient numbers of observations were also available for comparisons within the high dietary vanadium level. All tissue activities were found to correlate highly with urinary excretion of the isotope, the relationship having correlation coefficients, across dietary treatments, of .93 or greater. The close association of urinary excretion and tissue retention is illustrated for various tissues in Figure 10 and suggests that individual absorption and/or retention patterns exerted a more pronounced effect than dietary treatment on tissue activity. Dimond et al. (1963) observed that stable vanadium excretion in the urine of man was highly variable following oral administration and suggested that the variability may be due to differing rates of absorption among individuals.

Tissue activity values (Appendix Table 33) for the orally dosed ewe were similar in magnitude to those for rams fed the control diet. Mammary gland tissue contained .04% of the ^{48}V dose per kg of tissue (wet-weight basis), which was 36% that of liver activity. Söremark and Üllberg (1962), using autoradiographic techniques, demonstrated that vanadium was concentrated in the mammary gland of pregnant mice. In

Figure 10. Tissue ^{48}V retention (percent of dose per kg fresh tissue X 10^{-2}) 144 hr after oral administration *versus* mean daily urinary ^{48}V excretion (percent of dose). Regression equation components for linear relationships shown are given in Appendix Table 32.


TISSUE 48V RETENTION
6.0
5.0
4.0
3.0
2.0
1.0
0
KIDNEY
LIVER
BONE
SPLEEN
TESTICLE
HEART
MUSCLE
.02
.06
.10
.14
.18
.22
DAILY URINARY 48V EXCRETION

the present study, radioactive vanadium was not detected in milk, but blood activity was very low, indicating that a higher dosage level or alternate route of administration would be needed for accurate estimation of transfer into milk. The isotope was not detected in kidneys, liver, bone or muscle of the nursing lamb.

Summary

Radio-tracer techniques were used to investigate the influence of dietary stable vanadium on the excretion, distribution and blood clearance kinetics of ^{48}V in fifteen sheep. The animals were fed a basal corn-soybean meal diet with added levels of 0, 50 or 200 ppm vanadium (NH_4VO_3) prior to either oral or intravenous administration of the isotope.

Dietary effects on blood clearance following intravenous administration were small, with less than 1% of the dose remaining in whole blood 144 hr after dosing. Tissue ^{48}V activity, however, was affected by dietary treatment; the highest level of dietary vanadium reduced ^{48}V concentration in kidney by 52% and significantly lowered ($P < .05$) activity in liver, spleen, testicle and muscle tissue of intravenously dosed sheep.

Oral administration of the isotope resulted in much lower tissue activity than did intravenous dosing. No effect of diet on tissue retention was observed following oral dosing. Liver and other tissue activities, however,

were highly correlated with mean daily urinary ^{48}V excretion, suggesting that individual absorption rates influenced tissue levels of the isotope more strongly than dietary history. Kidney, bone, liver and spleen retained the highest levels of ^{48}V activity 144 hr after dosing; distribution patterns were similar for both administration pathways. The principal route of excretion of intravenously administered ^{48}V was through the kidneys, whereas the isotope given orally was excreted almost entirely by way of the feces, resulting in low tissue and urinary radiovanadium levels.

Preliminary results indicated that mammary gland tissue of the lactating ewe retained appreciable concentrations of ^{48}V, but detectable activity was not transferred into milk.

CHAPTER VI

COMPARATIVE AND ACUTE TOXICITY OF VANADIUM COMPOUNDS IN SHEEP

Introduction

Concern for toxicity in domestic animals due to ingested vanadium is comparatively recent, although the toxicology of vanadium in man has been of interest for many years because of industrial exposure to the metal (Faulkner-Hudson, 1964).

Vanadium compounds vary considerably in toxicity (Roshchin et al., 1965), ammonium metavanadate being one of the most toxic (Ricciardi, 1910). Different species respond differently to the metal, mice being generally the most resistant to the toxic effects of vanadium and rabbits and horses the most sensitive (Proescher et al., 1917); but few reports concerning ruminant animals are available.

Vanadium occurs in phosphate sources at levels toxic to some species (Berg, 1963). Processing of phosphate ores converts the vanadium to orthovanadate (Romoser et al., 1961). The ortho- and pyrovanadates are alternate forms of vanadic acid, usually found in nature complexed with alkali elements or other metals (Hawley, 1971). Vanadium is a metal of variable valences and is chemically complexed with

many other elements, including numerous compounds with oxygen (Fairhall, 1949).

Previous studies (Chapter IV) indicated that vanadium at sufficiently high levels in the diet severely affected feed intake. The present investigation was conducted to compare, using appetite as an indicator, the relative toxicities of calcium orthovanadate, calcium pyrovanadate and ammonium metavanadate and to examine the effects of acute vanadium toxicity in sheep.

Experimental Procedure

Experiment 1

Twelve Florida native wethers, averaging 41 kg initially, were housed in individual pens with raised wire flooring and were provided tap water *ad libitum*. Consumption of the basal diet (Table 13) was restricted to 1300 g per head daily. After an adjustment period of 2 weeks, the sheep were randomly allotted to a control and three treatment groups of three animals each. Gradually increasing amounts of either purified sand, ammonium metavanadate, calcium orthovanadate or calcium pyrovanadate were administered once daily in a gelatin capsule 1 hr after feeding. The weight of sand given control animals was equal to the maximum weight of vanadium compound administered to animals on treatment, the granular sand being utilized to standardize the effect of handling procedures. Soluble vanadium in the

TABLE 13. COMPOSITION OF BASAL DIET

Ingredient	Percent
Corn meal	57.5
Cottonseed hulls	21.0
Soybean meal (50% protein)	12.5
Alfalfa meal (17% protein)	3.0
Corn oil[a]	3.0
Corn starch	1.0
Defluorinated phosphate (31% Ca, 18% P)	1.0
Trace mineralized salt[b]	1.0
Vitamins A, D and E[c]	+
	100.0
Vanadium analysis, ppm[d]	2.6
Crude protein, %	12.7

[a]Santoquin added at .0125% of total diet.

[b]Analysis in percent listed as follows: NaCl, 95.0; Zn, 1.0; Fe, .30; Mn, .20; S, .10; Cu, .08; Co, .01 and I, .01; Carey Salt Company, Hutchinson, Kansas.

[c]Vitamins added per kilogram of diet: 2200 USP units of vitamin A, 440 USP units of vitamin D and 11 mg DL-α-tocopherol.

[d]Dry matter basis.

sand, by analysis, was less than 250 µg at the levels employed. The calcium salts of the ortho- and pyrovanadates were obtained from K and K Laboratories, Inc., Plainville, New York.

The vanadium compounds were independently analyzed for vanadium and calcium in another laboratory[1] and were administered on the basis of the average analytical determination of elemental vanadium content. The chemical composition for the three compounds is shown in Table 14. The chemical structure of ammonium metavanadate was confirmed by X-ray diffraction analysis, but the calcium vanadates were not crystalline and therefore could not be identified by this method. Chemical analyses, however, demonstrated that calcium and vanadium were present in the proper ratios.

The daily dosage level was increased progressively by 50 mg vanadium at 2-day intervals in order to minimize the delayed effect of toxicity. When feed intake declined to 25% of that of control animals, no further vanadium was given. The objective of the study was to compare the toxic effect of the compounds under these conditions and to determine the level of vanadium which would cause a cessation of feed intake. Thus, responses to vanadium compounds were measured chiefly in terms of onset of inappetance.

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[1] International Minerals and Chemical Corporation, Libertyville, Illinois.

TABLE 14. CHEMICAL COMPOSITION OF VANADIUM COMPOUNDS

Compound	Chemical formula[a]	Vanadium, %[b]	Composition, %[c]	
			Calcium	Vanadium
Ammonium metavanadate	NH_4VO_3	43.5	-	41.6
Calcium pyrovanadate	$Ca_2V_2O_7$	34.7	23.4	30.8
Calcium orthovanadate	$Ca_3(VO_4)_2$	29.1	26.5	23.7

[a]Structure of ammonium metavanadate confirmed by X-ray diffraction analysis.

[b]Calculated from chemical formula.

[c]Values represent average of two independent determinations.

Experiment 2

In a study of acute toxicity, three sheep were given a single lethal oral dose (40 mg per kg body weight) of vanadium (NH_4VO_3) administered in a gelatin capsule. Two sheep were given comparable amounts of purified sand as a procedural control. Prior to dosing, all animals had been individually fed 1000 g of the basal diet (Table 13) per day for a period of 3 weeks. Time of death was recorded for acutely affected animals; remaining sheep were killed at 96 hr postdosing and tissues were sampled for vanadium analysis.

Post-mortem examinations were performed on all sheep. Kidney and liver tissue samples from each animal were preserved in 10% buffered neutral formalin for histological comparisons, while additional samples were stored frozen until vanadium analyses could be performed.

Histological sections of liver and kidney were prepared and stained in hematoxylin and eosin by standard procedures (Luna, 1968). Vanadium was determined in tissues, feed, sand and vanadium compounds by an atomic absorption method. Sample preparation prior to AAS absorbance determination involved a solvent extraction of the vanadium from a dilute nitric acid solution using cupferron and methylisobutyl ketone (Chapter III).

Data were analyzed for significance by analysis of variance where appropriate, and differences between means were determined using Duncan's multiple range test (Steel and Torrie, 1960).

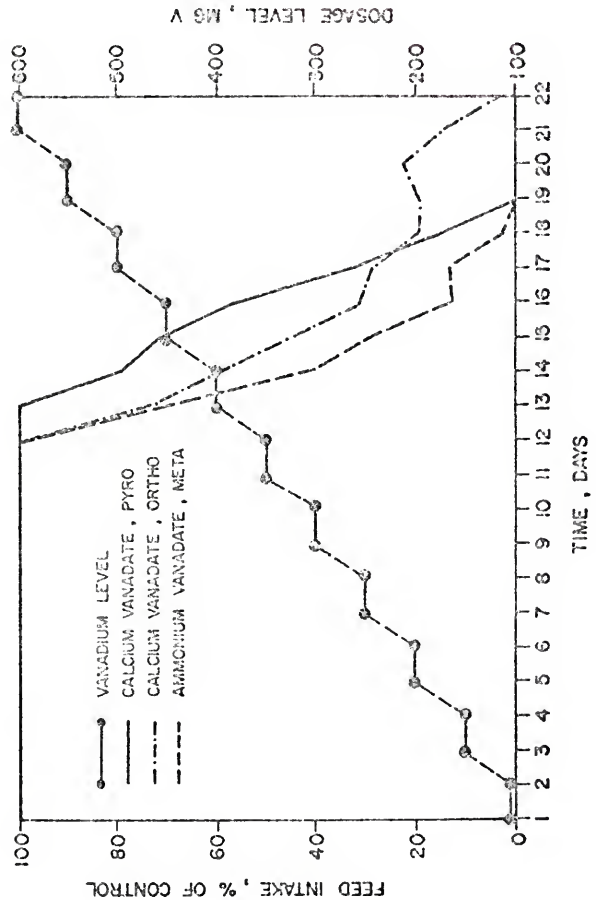
Results and Discussion

Experiment 1

Figure 11 graphically illustrates the reduction in feed intake with time (days) in response to increasing levels of vanadium in the form of ammonium metavanadate, calcium orthovanadate and calcium pyrovanadate. Although there was a probable cumulative effect in this type of design, response is depicted for the day following dosing. As the elemental vanadium level was increased from 100 to 350 mg per day by increments of 50 mg, feed intake of all animals remained unaffected. A threshold level, however, was apparently reached equivalent to a dosage level of 400-450 mg vanadium. At this point feed intake of sheep receiving ammonium metavanadate and calcium orthovanadate began to decline. Feed consumption of the sheep receiving calcium pyrovanadate was adversely affected a day later and within 4 days, intake for all treatment animals averaged 34% that of controls. Feed intake of sheep receiving ammonium metavanadate and calcium orthovanadate declined rapidly to zero by the 19th day of the experiment.

Response in the sheep dosed with calcium pyrovanadate was slightly slower than for the other treatments. A non-hemorrhagic diarrhea accompanied the rapid initial decline in feed intake. There were no significant differences among vanadium treatments for feed intake.

Figure 11. Reduction in feed intake with time in sheep administered increasing levels of ammonium metavanadate, calcium orthovanadate and calcium pyrovanadate.


DOSAGE LEVEL , MG V
600
500
400
300
200
100
22
21
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
TIME , DAYS
FEED INTAKE , % OF CONTROL
100
80
60
40
20
0
VANADIUM LEVEL
CALCIUM VANADATE , PYRO
CALCIUM VANADATE , ORTHO
AMMONIUM VANADATE , META

Proescher et al. (1917) observed that sodium pyrovanadate was less toxic than sodium orthovanadate when administered intravenously to rabbits, but the difference was not marked. These authors also compared the toxicities of ammonium metavanadate, sodium orthovanadate and sodium pyrovanadate when injected subcutaneously in mice, rats and guinea pigs. In rats and mice, ammonium metavanadate was the most toxic, whereas the three compounds were similarly poisonous to the guinea pig.

Accumulation of vanadium in the rumen probably occurred, since isotope studies have indicated feed passage time in sheep to be approximately 6 days (Fick, 1974). Therefore, separate effects of a build-up of vanadium in the rumen could not be defined from these data. Precise toxicity in terms of weight of vanadium relative to body weight likewise could not be determined except under progressively increasing dosage levels as used in this trial. Toxic levels of the three vanadium compounds when administered as described are given in Table 15. Since decline in feed intake was rapid once a threshold value of vanadium dosage was reached, initial toxicity was defined in the present case as the level resulting in a 25% reduction in feed intake relative to the control sheep. The amount of vanadium required to induce signs of toxicity was not significantly different among the various compounds; the daily quantity, in terms of mg per kg body weight, ranged from 9.6 to 12.0.

TABLE 15. TOXIC LEVELS OF THREE VANADIUM COMPOUNDS WHEN ADMINISTERED TO SHEEP IN PROGRESSIVELY INCREASING DOSAGES (EXPERIMENT 1)[abc]

Compound administered	Body weight, kg ±S.E.	Toxic elemental vanadium level		
		mg/day	mg/kg body weight	Accumulative vanadium, g
Ammonium metavanadate	40.2 ± .55	400-450	10.0 - 11.2	3.5 - 3.95
Calcium pyrovanadate	41.7 ± .77	400-500	9.6 - 12.0	3.50 - 5.40
Calcium orthovanadate	41.4 ± .90	400	9.7	3.50

[a]Elemental vanadium level increased in 50 mg increments at 2-day intervals.

[b]Toxicity defined as level resulting in 25% decline in feed intake relative to control sheep given purified sand.

[c]Data for three sheep per treatment.

Platonow and Abbey (1968), giving vanadium (NH_4VO_3) daily by capsule, obtained comparable results in 6-month-old calves. Levels of 10 to 20 mg per kg body weight caused pronounced diarrhea and other signs of toxicity which appeared in 3 to 14 days.

Previous studies in which high levels of vanadium were given in the diet (Chapter IV) demonstrated that sheep ceased eating, but recovered promptly within 3 to 8 days following ingestion of toxic amounts of the mineral. The death of one of the sheep in the present study 3 days after dosing with 550 mg vanadium (calcium orthovanadate) was therefore unexpected. One animal from each of the other groups was killed and autopsied for immediate comparison. Remaining sheep were examined and tissue samples were taken at the time of death, or alternatively, if unable to rise for 2 consecutive days, the animals were killed and gross pathology recorded. Post mortem examination of sheep from each treatment group showed extensive hemorrhage in the small intestine and diffuse hemorrhage (Figure 12) on kidney surfaces, although these were not constant findings. Hemorrhage in the small intestine was seen on autopsy in six of the nine treatment sheep, whereas petechial or diffuse hemorrhage on the surfaces of the kidneys was seen in four of the animals. The incidence of signs of toxicity could not be related to treatment compound given.

Figure 13 shows photomicrographs of liver tissue of control and calcium orthovanadate-treated sheep. The

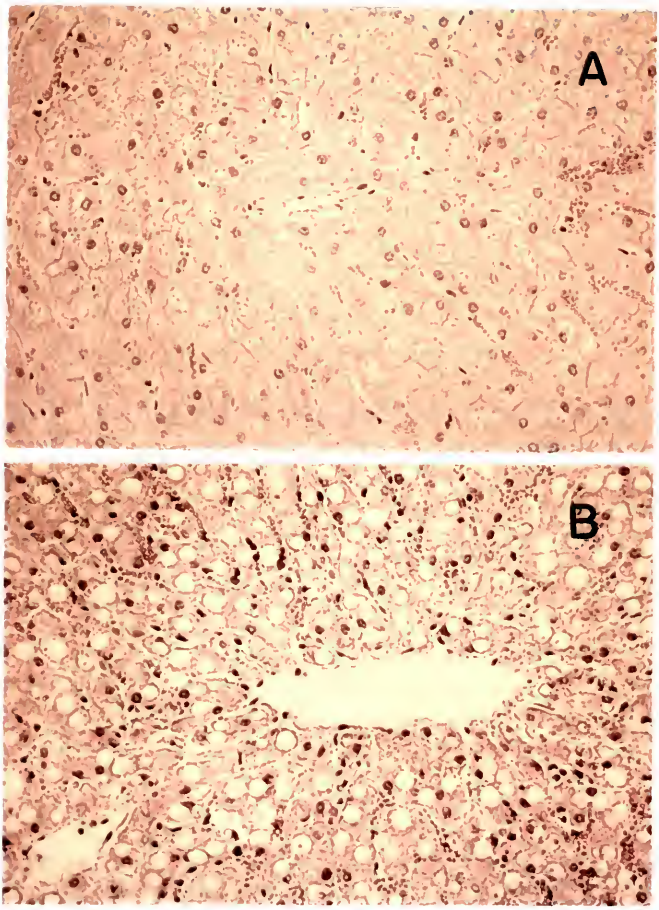
Figure 12. Photograph of sheep kidney and exposed lumen of small intestine.

A. Photograph of kidney from treatment sheep showing diffuse hemorrhage on surface.

B. Photograph of exposed lumen of small intestine showing mucosal hemorrhagic enteritis.


A
101
B

Figure 13. Photomicrograph of histological sections of sheep liver tissue. Liver of control sheep (A) and liver of sheep given calcium orthovanadate showing distinct vacuoles and fatty infiltration of tissue (B). Both sections stained with hematoxylin and eosin. Magnification is X 35.


A
B

enlarged non-staining vacuoles indicated severe fatty degeneration of the hepatic tissue. Differing degrees of fatty infiltration were also observed on examination of livers of sheep given calcium pyrovanadate and ammonium metavanadate, while enlarged vacuoles were absent in liver tissue of control animals. Due to varying survival times of sheep in the present case, strict comparisons among forms of vanadium could not be made. Well-marked fatty changes in the livers of dogs and cats poisoned by ammonium metavanadate have been described by Faulkner-Hudson (1964).

Levels of vanadium in kidney, liver, muscle and lung tissue are shown in Table 16. All tissue levels were significantly elevated ($P < .05$) compared to control levels. No differences attributable to vanadium compounds were observed.

The ammonium metavanadate levels that resulted in appetite depression in the present study were higher than those observed to affect feed intake when the vanadium was given mixed in the feed (Chapter IV). While this difference may be due to mode of administration or other variables, it has been demonstrated that tolerance to vanadium was developed by rats when increasing doses were given (Daniel and Lillie, 1938; Strasia, 1971).

Experiment 2

The effect of a single oral dose of vanadium (40 mg per kg body weight) on vanadium content of sheep tissues is shown

TABLE 16. EFFECT OF ADMINISTRATION OF THREE VANADIUM COMPOUNDS ON TISSUE VANADIUM COMPOSITION OF SHEEP (EXPERIMENT 1)[a]

Vanadium compound	Tissue, ppm dry matter basis			
	Muscle	Lung	Liver	Kidney
Control	.08 ± .03[b]	.13 ± .05[b]	.42 ± .12[b]	.68 ± .23[c]
Ammonium metavanadate	.83 ± .07[c]	1.42 ± .18[c]	3.51 ± .56[c]	22.74 ± 5.32[d]
Calcium pyrovanadate	.75 ± .12[c]	1.48 ± .26[c]	2.83 ± .23[c]	20.24 ± 5.02[d]
Calcium orthovanadate	.97 ± .09[c]	2.60 ± .22[c]	3.68 ± .42[c]	24.37 ± 6.47[d]

[a]Mean plus standard error. Values represent data from three sheep.

[bcd]Means in the same column or row with different superscripts are different ($P < .05$)

in Table 17. Two of the sheep died as a result of vanadium administration at 68 and 80 hr postdosing. The level of tissue vanadium, especially in the kidney, was closely related to length of time prior to death. Tissue levels of animals given vanadium were greatly elevated in comparison to control sheep. Calves given orally 20 mg vanadium per kg body weight evidenced signs of toxicity in 3 days (Platonow and Abbey, 1968); kidney and other tissue vanadium levels were increased. Proescher et al. (1917) injected an ewe intravenously with approximately .5 mg vanadium per kg body weight as sodium tetravanadate ($Na_2V_4O_{11}$) and noted the extreme toxicity of the element to this species.

Diarrhea, non-hemorrhagic in nature, was observed in treated sheep in the present study as early as 15 hr after dosing. Although the sheep were given the vanadium 1 hr after feeding, feed was entirely consumed during the ensuing 24 hr, but feed intake was zero thereafter. No gross pathology of liver, kidney, or intestine was observed, lending support to the observation of Proescher et al. (1917) that, in acute poisoning, the gastrointestinal tract is not severely affected.

Microscopic examination of liver showed some evidence of toxic hepatitis and fatty degeneration in the animals given vanadium, but the distinct vacuoles observed in sheep given increasing amounts of vanadium over a longer time period (Experiment 1) were not observed.

TABLE 17. EFFECT OF A SINGLE LETHAL ORAL DOSE OF VANADIUM ON SHEEP TISSUE VANADIUM COMPOSITION (EXPERIMENT 2)[a]

Item	Time from dosing until death, hr	Sheep wt, kg	Tissue, ppm dry matter basis[b]						
			Kidney	Liver	Bone	Spleen	Lung	Heart	Muscle
NH_4VO_3	68	45.4	38.3	7.92	4.54	3.61	-	1.14	.97
NH_4VO_3	80	40.8	20.4	2.61	2.11	1.82	4.22	.82	.53
NH_4VO_3	96	54.4	18.8	2.04	1.23	.89	2.31	.56	.40
Control	96	53.1	.31	.30	.11	.09	.18	.15	.13
Control	96	53.5	.44	.35	.15	.10	.09	.06	.06

[a]Vanadium (40 mg per kg body weight) administered by capsule.

[b]Bone, ash weight basis.

Histopathology of kidney tissue involved sloughing of tubular epithelium of the proximal convoluted tubules, but these signs were not prominant.

Fatty degeneration of liver tissue of rats and rabbits following chronic poisoning with airborne vanadium has been reported (Roshchin et al., 1965), but marked changes occurred only with prolonged exposure (Faulkner-Hudson, 1964).

Summary

Twelve Florida native wethers, averaging 41 kg initially, were used in a study of the comparative toxicity of three vanadium compounds. Feed intake was restricted to 1300 g per head daily of a roughage-corn-soy diet. After an adjustment period of 2 weeks, the sheep were allotted to a control and three treatment groups. Gradually increasing doses of purified sand, ammonium metavanadate, calcium orthovanadate or calcium pyrovanadate were administered orally, by capsule. The daily dosage of elemental vanadium was increased progressively by 50 mg at 2-day intervals to compare the effects of toxicity under these conditions, and to determine the level that would cause a cessation of feed intake.

Initial decline in feed intake was observed at a level of 400 to 500 mg vanadium per day (9.6 to 12 mg per kg body weight) when administered in this manner, but intake was not affected at lower levels. The rapid decline in feed intake was accompanied by diarrhea.

Extensive hemorrhage in the mucosa of the small intestine and diffuse or petechial hemorrhages on kidney surfaces were observed during postmortem examination of sheep from each treatment group. The three vanadium compounds appeared to be similar in toxicity when abrupt decline in feed intake and pathological examination were used as criteria of comparison.

In an investigation of acute toxicity, three sheep were given 40 mg of vanadium per kg body weight in gelatin capcules. Two of the animals died within 80 hr after administration. Vanadium content of kidney, liver, bone, spleen, lung and muscle was elevated by treatment. Gross pathological changes were not observed, although histological examination showed slight evidence of liver degeneration and nephritis.

CHAPTER VII

EFFECT OF VANADIUM INTAKE ON EXCRETION AND DEPOSITION IN BONE OF SHEEP

Introduction

Vanadium content of bone and other tissues of small laboratory animals and poultry has been shown to increase in response to increasing dietary vanadium (Hopkins and Mohr, 1971b, Berg and Lawrence, 1971). A deficiency of the metal in the diet of chicks affects bone development, causing alterations in the epiphyseal plate and primary spongiosa (Nielsen and Ollerich, 1973).

Although a large portion of absorbed or injected vanadium is rapidly excreted, principally in the urine (Ballotta, 1931; Kent and McCance, 1941), some degree of retention has been observed in numerous species. Relatively high whole-body and bone retention of the mineral was noted in chicks (Wright, 1968). Talvitie and Wagner (1954) observed that retention of intravenously administered vanadium was approximately 10% in rats and rabbits and suggested that the skeleton was the locus of deposition. Experiments in which radioactive vanadium was administered to chicks (Hathcock *et al.*, 1964) and mice (Söremark and Üllberg, 1962) indicated that bone was relatively high in activity in comparison to

other tissues. Little information is available on physiological accumulation or deposition of the mineral in hard structures with dietary exposure, although in a related study, Thomassen and Leicester (1964) observed that isotopic vanadium deposited in soft tissues of rats during growth was very mobile, whereas it was much less so in bones and teeth.

The experiments presented here were conducted to investigate the effect of dietary vanadium on deposition of the mineral with time in the coccygeal vertebrae of lambs. The relationships of diet and urinary and fecal excretion of vanadium were also studied under controlled balance conditions.

Experimental Procedure

Two experiments were conducted with sheep, in which the basal diet described previously (Table 9, Chapter V) was used. In both studies, vanadium was added as ammonium metavanadate (NH_4VO_3) to formulate three diets containing 0, 50 and 200 ppm supplemental vanadium. Growing lambs were maintained on treatment diets for 140 days. During the first 90 days (Experiment 1), samples of bone (coccygeal vertebrae) were taken periodically for vanadium analysis. At the conclusion of this study, a 7-day balance (Experiment 2) was conducted, using these sheep, to examine urinary and fecal excretion of the mineral. Tap water was provided *ad libitum* throughout.

In the first experiment, 16 intact male lambs with an average body weight of 30 kg were randomly assigned to the three dietary treatments and were housed individually in elevated pens during the 20-day adjustment and 90-day experimental phase. Body weights were recorded at approximate 2-week intervals throughout the experimental period. The sheep were fed the appropriate experimental diets once daily and intake was recorded; the quantity of feed offered each day was 10% more than intake on the preceding day to ensure ad libitum intake.

The number of coccygeal vertebrae in "long-tailed" sheep is approximately 16 to 18, although variation in number is common (Sisson and Grossman, 1956). To examine skeletal deposition of vanadium, samples of the relatively accessible coccygeal vertebrae were obtained surgically at intervals for analysis. A relationship between these vertebrae and metacarpal bone was established when these sheep were later administered ^{48}V both intravenously and orally and vertebrae and metacarpal bone were assayed for radioactivity (CHAPTER V).

The sheep were divided into two groups on the basis of differing surgical treatments. Group I was composed of 12 animals, evenly distributed among the three dietary assignments. Distal tail sections of lambs in this group were removed surgically, under local anesthesia, prior to initiation of dietary treatment to establish baseline data. Additional segments were sequentially removed after 15, 30, 60 and 90

days of treatment. Divisions made in the length of tail during sampling were nessarily approximate due to variations in the number of vertebrae among sheep; however, a minimum of two vertebrae were obtained from lambs in each instance. Since actively mineralizing or growing bone apparently concentrates vanadium (Söremark et al., 1962), the terminal vertebrae were detached and only those remote from sites of healing were retained for vanadium analysis.

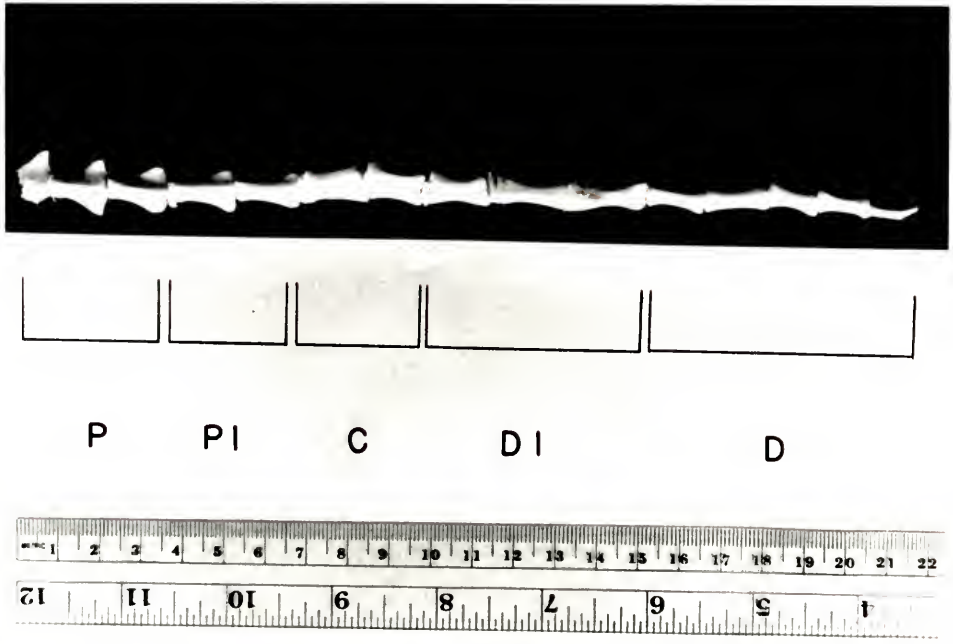
Conclusions from a timed study of this design are partly dependent on even distribution of vanadium regardless of vertebrae position. Therefore, sheep fed 200 ppm supplemental vanadium in Group II were utilized to examine the uniformity of accretion patterns among vertebrae. The entire tail from one of the four animals in Group II was docked at 15, 30, 60 and 90 days. The length of tail was comprised of 13 to 18 coccygeal vertebrae and was divided into sections and labeled as illustrated in Figure 14. Each section was analyzed for vanadium content. Thus, from the combined data it was possible to differentiate between the effect of vanadium exposure with time and the effect of vertebrae position on the mineral content of bone. The sheep were given tetanus antitoxin (Fort Dodge Laboratories, Inc., Fort Dodge, Iowa) subcutaneously at the time of surgical treatment according to manufacturer's recommendations. Procedures used to remove vertebral samples were simple and required little time. Local anesthesia was accomplished

by infiltration injection of 2% Lidocaine hydrochloride (Elkins-Sinn, Inc., Cherry Hill, New Jersey). The tail was then sheared and merthiolate used to disinfect the surrounding skin and wool. An incision was made between adjoining vertebrae after application of a tourniquet. Following separation, a topical antiseptic (Keraspray, S.E. Massengill Co., Bristol, Tennessee) was applied and nylon monofilament was then used to suture overlapping skin to close the wound. The antiseptic was analyzed for vanadium and no trace was found under conditions in which 20 ppb would have been detected.

In preparation for vanadium analysis, samples of excised vertebrae were first divested of skin and adhering tissues. Since there was the possibility of surface contamination, they were washed briefly in successive portions of acetone, 10% hydrochloric acid and deionized water and then stored frozen in plastic bags.

In Experiment 2, 14 of the sheep which were assigned to dietary treatments of 0, 50 or 200 ppm supplemental vanadium in Experiment 1 were transferred to individual metabolism stalls and fed 800 g per head daily of the assigned experimental diets. A 10-day preliminary period was followed by a 7-day total urinary and fecal collection period. Fecal samples were pooled within each 7-day collection period for each animal and aliquots saved for vanadium analysis. Because of the very low level of vanadium in urine relative

Figure 14. Photograph of coccygeal vertebrae of sheep. Labels show approximate divisions made when vertebrae were sectioned for vanadium analysis: D, distal; DI, distal intermediate; C, central; PI, proximal intermediate; P, proximal.


P
PI
C
DI
D

to feed and feces, contamination was considered to be a problem. Therefore, analysis of daily urine samples for each animal was performed so that contaminated samples could be identified by comparison within collection periods.

Feed, feces and bone were ashed in a muffle furnace at 575°C prior to vanadium analysis, while urine samples were prepared by a low-temperature method similar to that described by Slavin et al., (1975). Vanadium in bone (Experiment 1) and in urine (Experiment 2) was analyzed by means of flameless atomic absorption spectrophotometry (Anonymous, 1974). A Perkin-Elmer Model 306 spectrophotometer equipped with an HGA-2100 graphite furnace and deuterium arc background corrector was used for these analyses. Bone vanadium content was also determined by instrumental neutron activation analysis.[1] A limited number of vertebrae samples from sheep fed either 0 or 200 ppm supplemental vanadium were analyzed by this technique and composition values were compared to adjacent vertebrae assayed by flameless atomic absorption spectrophotometry using the method of standard additions (Anonymous, 1973). The flame AAS method described in CHAPTER III was employed in the assay of vanadium content of feed and feces.

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[1]Environmental Trace Substances Research Center, 223 Research Reactor Facility, University of Missouri, Columbia.

Data were analyzed statistically by least squares analysis of variance using a generalized least squares program (Barr and Goodnight, 1972).

Results and Discussion

Experiment 1

Animal Performance. The effects of combined dietary and surgical treatment on weight gains and feed intakes of sheep during the 90-day experimental period are shown in Table 18. All animals in Group I were handled identically with regard to surgical sampling of coccygeal vertebrae. Thus, although the added stress may have altered the responses to some extent, observed differences in weight gains and feed intakes were, at least in part, attributable to dietary vanadium level. Performance of sheep in Group I indicated that 200 ppm supplemental vanadium in the diet was not detrimental. Average daily feed intakes of those fed 0 and 200 ppm added vanadium were 1.26 and 1.40 kg/day, respectively. A trend toward improved daily gains and feed intakes with increasing vanadium was observed, although the differences were not significant ($P > .05$).

It is problematical whether the four sheep fed 200 ppm supplemental vanadium in Group I can be compared to the four animals in Group II fed an identical diet, since handling procedures differed. Noteworthy, however, is the fact that daily gains were considerably lower (164 g/day) for sheep

TABLE 18. EFFECT OF DIETARY AND SURGICAL TREATMENT ON WEIGHT GAIN AND FEED INTAKE OF SHEEP. (EXPERIMENT 1)[a]

Surgical treatment	Supplemental dietary vanadium, ppm	Number of sheep	Initial weight kg	Daily feed intake, kg	Daily Gain g
Group I[b]	0	4	30.0	1.26 ± .08	191 ± 15
Group I	50	4	30.4	1.26 ± .14	207 ± 31
Group I	200	4	31.3	1.40 ± .10	232 ± 11
Group II	200	4	30.4	1.25 ± .16	164 ± 36

[a]Mean plus standard error. Values represent data from four sheep fed 90 days.

[b]Sheep in Group I were surgically manipulated three times during the experimental feeding period; those in Group II, only once, as explained in text.

in Group II than for those in Group I (232 g/day) even though surgical manipulation was more frequent in the latter group. The difference was due principally to the low feed intake (.80 kg/day) and daily gain (56 g/day) of one animal in Group II. Moreover, feed intake of this animal was even lower (.34 kg/day) during the first 45 days, but later improved, suggesting an apparent adaptation to 200 ppm dietary vanadium. Subsequent examination of daily urinary vanadium excretion (Experiment 2) revealed that this sheep excreted far more of the mineral (1133 µg/day) than did the others in Group I or II. Furthermore, an inverse relationship between initial feed intake and urinary excretion of the metal was also noted in other sheep fed the diet containing the highest level of vanadium. During the first 14 days of the study, before the stress of surgery was superimposed, three of the eight animals were found to be adversely affected; feed intakes averaged 53% of those of sheep fed the basal diet. Thus, vanadium in the diet apparently resulted in signs of toxicity in some, but not all, of the sheep, suggesting variable responses to the mineral by individual animals and an acquired tolerance following dietary exposure.

Vanadium in Coccygeal Vertebrae. Table 19 shows changes with time in vanadium content of coccygeal vertebrae of sheep fed 0, 50 or 200 ppm supplemental vanadium (Group I). Content of the metal in vertebrae was higher ($P < .05$) in

TABLE 19. CHANGES WITH TIME IN VANADIUM CONTENT OF COCCYGEAL VERTEBRAE OF SHEEP FED 0, 50 or 200 PPM SUPPLEMENTAL VANADIUM . (EXPERIMENT 1)[a]

Supplemental vanadium, ppm	Time on treatment, days				
	0	15	30	60	90
	[Vanadium in vertebrae, ppm (ash weight basis)]				
0	.45 ± .06[b]	.32 ± .05[b]	.56 ± .08[b]	.44 ± .09[b]	.38 ± .05[b]
50	.39 ± .07[b]	1.61 ± .46[b]	1.70 ± .23[b]	1.65 ± .45[b]	1.70 ± .29[b]
200	.51 ± .09[b]	3.59 ± .79[c]	3.68 ± 1.4[c]	3.94 ± .83[c]	4.13 ± 1.2[c]

[a]Mean plus standard error. Values are means of analytical data for four sheep (Group I).

[bc]Means in the same column or row with different superscripts are different ($P < .05$).

sheep fed 200 ppm added vanadium than in controls. Additionally, the level was elevated ($P < .05$) 15 days after imposition of the highest dietary vanadium treatment. Following the initial increase, however, the content was only slightly increased by feeding vanadium for longer periods of time.

Bone vanadium content generally reflected vanadium in the diet, but response was not uniform among animals. The level in vertebrae of sheep fed 200 ppm added vanadium ranged from 1.7 to 6.8 ppm (ash weight basis). Values shown were determined mainly by flameless atomic absorption spectrophotometry. Comparable results were obtained by instrumental neutron activation analysis (INAA). When vertebrae of sheep fed 0 and 200 ppm supplemental vanadium were analyzed by INAA in another laboratory, values of .34 and 1.9 ppm, respectively, were reported. Adjacent vertebrae from the same sheep were analyzed by flameless AAS; the samples contained, on an ash weight basis, .41 and 2.5 ppm vanadium.

Evidence that the observed changes were due to dietary exposure rather than to location of vertebrae is given in Table 20. Although tails of animals in Group II were docked at 15, 30, 60 and 90 days, no trend toward increasing vanadium with time was observed. Content of vertebrae was relatively uniform, with only small differences among divisions of the tail.

Comparisons with data in the literature are difficult due to the small number of pertinent references. Bone from

TABLE 20. DISTRIBUTION OF VANADIUM AMONG COCCYGEAL VERTEBRAE OF SHEEP FED 200 PPM SUPPLEMENTAL VANADIUM. (EXPERIMENT 1)

Time on treatment, days	Division[a]				
	P	PI	C	CI	D
	[Vanadium in vertebrae, ppm (ash weight basis)]				
15	4.16	4.28	4.42	3.82	4.33
30	2.81	2.71	3.30	3.14	3.45
60	3.13	3.63	3.46	3.76	3.68
90	3.36	3.19	2.82	3.21	3.31
Average[b]	3.37 ± .29	3.45 ± .33	3.50 ± .34	3.48 ± .18	3.69 ± .23

[a]Divisions made in tail are illustrated in Figure 14.

[b]Mean plus standard error. Values are means of analytical data from four sheep (Group II).

normal sheep was observed by Morrison (1967) to contain .2 ppm vanadium (dry weight basis). Berg and Lawrence (1971) found that vanadium in the tibia of chicks increased three-fold when 20 ppm supplemental vanadium was fed. Strasia (1971) also noted a response of bone to vanadium in the diet. This author found that the bone of rats fed 0 or 200 ppm added vanadium contained 5.9 and 17.2 ppm, respectively. These values were expressed on a wet weight basis and were, therefore, much higher than those observed in the present study. Lower values, however, have been reported by others. Welch and Allaway (1972), for example, observed that bone from rats fed a semi-purified diet contained only 9 ppb (wet weight basis).

Experiment 2

Table 21 shows the effect of three levels of dietary vanadium on fecal and urinary excretion of the mineral. Excretion of the metal in the urine was found to be a sensitive indicator of dietary intake. When dietary content was increased from 0 to 200 ppm, urinary excretion of vanadium increased by a factor of sixty-seven. A proportional rise in urinary excretion was also observed in sheep receiving 50 ppm supplemental vanadium. Statistical differences in urinary excretion were noted at the 1% level. Wide variations in excretion were observed, however, especially among sheep exposed to large amounts of the mineral. Among those fed 200 ppm supplemental vanadium, a six-fold difference in daily urinary excretion was found.

TABLE 21. EFFECT OF DIETARY VANADIUM LEVEL ON DAILY VANADIUM EXCRETION AND URINE VOLUME OF SHEEP. (EXPERIMENT 2)[a]

Supplemental vanadium, ppm	Vanadium intake mg/day	Fecal vanadium mg/day	Urinary vanadium µg/day	Urine volume ml/day
0(4)[b]	2.01 ± .08	1.93 ± .12[c]	7.38 ± .60[c]	433.3 ± 66.5
50(2)	37.61 ± 2.3	32.22 ± 4.41[d]	83.1 ± 13.7[d]	790.0 ± 128.3
200(8)	153.24 ± 7.4	159.34 ± .96[e]	497.5 ± 111.9[e]	976.8 ± 162.1

[a]Mean plus standard error. Values are based on data from 7-day balance period.

[b]Refers to number of observations included in mean.

[cde]Means in the same column with different superscripts are different ($P < .01$).

An inverse relationship between urinary vanadium and animal performance has been discussed previously (Experiment 1). Adverse effects on appetite of sheep, especially at the time of initial exposure, were apparent. The severity and duration of the effects appeared to be related to the amount of vanadium reaching the urine. When urinary excretion exceeded 600 µg/day, intake of feed was depressed; below this level adverse effects were not observed. Feed intake of one animal excreting more than 1100 µg of vanadium per day was depressed 70% initially, but increased to normal after 45 days despite continued exposure. Urinary levels of 630 and 800 µg/day were associated with decreases in feed intake of 32 and 58%, respectively, but recovery was much more rapid. A direct relationship between excretion at all levels and performance was not observed. Rather, the data suggest that dietary exposure which resulted in excretion of the metal in amounts exceeding a critical level resulted in detrimental effects on feed intake under these conditions.

These data are in general agreement with results from a limited number of studies with other animal species and man. Less than 8 µg of vanadium per day is normally excreted in the urine of man (Schroeder et al., 1963). The level can be increased greatly, however, when vanadium salts are given orally in subtoxic amounts (Dimond et al., 1963). Relationships between the amount of vanadium passed in the

urine and aerial exposure to the metal have also been noted in dogs (Rockhold and Talvitie, 1956; Stokinger, 1963).

Incremental increases in dietary vanadium also resulted in corresponding increases ($P < .01$) in fecal vanadium in the present study (Table 21). The relationship of dietary to fecal vanadium with regard to absorption and retention, however, was not well defined. The improbability of obtaining accurate data for apparent absorption by difference between intake and excretion for the trace and "ultratrace" minerals has been noted by Miller (1973) and Carter et al. (1974). Therefore, apparent absorption was not calculated by difference from the balance data. Less than .5 mg of the mineral was excreted daily in the urine of sheep fed the highest level. For the following reasons it is probable that this amount approximately represents the daily quantity of vanadium absorbed by these sheep: a) mean daily urinary excretion of stable vanadium in the present balance study was .34% of the dietary vanadium intake for animals fed the highest (200 ppm) dietary level; b) clearance of orally administered ^{48}V by these animals was similar (CHAPTER V), with a daily urinary excretion range from .02 to .22% of the dose; c) intravenous administration of ^{48}V to sheep fed 200 ppm supplemental vanadium (CHAPTER V) resulted in lower tissue activities in major organs, suggesting partial saturation due to the dietary stable vanadium; and d) endogenous fecal vanadium, measured following intravenous

^{48}V administration, was almost inconsequential, amounting to approximately 5% of the injected activity.

Thus, with due consideration of the small amount of endogenous fecal vanadium, urinary excretion should be approximately equivalent to absorption if the dietary level is constant, as it was in the present case. On this basis, absorption of vanadium by these sheep was estimated to range from .13 to .75% of ingested vanadium with a mean of .34 percent. The estimates for sheep fed 200 ppm supplemental vanadium may not apply to animals fed the basal diet, since Venchikov (1974) has observed that when the concentration of trace elements is increased above physiological levels, entry into the animal is delayed or reduced. Curran et al. (1959) estimated that between .1 and 1% of vanadium given orally was absorbed by man. There is evidence of a small degree of absorption in the rat as well; only .5% of radiovanadium administered intragastrically was absorbed (Scott et al., 1951).

Urine volumes of sheep fed three levels of dietary vanadium are also shown in Table 21. Mean daily volumes for sheep fed 0 or 200 ppm supplemental vanadium were 433 and 977 ml/day, respectively. Although the mean urine volume of sheep fed the highest level of the metal was higher, the difference was not significant ($P > .05$).

In essentiality studies with sheep, Williams (1973) observed alterations in water balance of sheep which were

attributed to vanadium. Jackson (1912), in agreement with the trend noted in the present study, observed that small quantities of the mineral caused diuresis in the dog. Other species are similarly affected (Proescher et al., 1917). Dehydration was observed by Strasia (1971) to be a sign of vanadium toxicity in rats.

Summary

Sixteen male lambs were fed 0, 50 or 200 ppm supplemental vanadium for 90 days and coccygeal vertebrae were sampled before and at 15, 30, 60 and 90 days after imposing dietary treatments to investigate the effect of dietary intake on deposition of the mineral with time. Vertebral content was elevated ($P < .05$) after exposure to 200 ppm added vanadium in the diet for 15 days, but non-significant increases occurred thereafter. Increases of the mineral in the diet resulted in corresponding increases in bone vanadium level; content in vertebrae of sheep fed 0, 50 and 200 ppm supplemental vanadium was .4, 1.7 and 3.8 ppm (ash weight basis), respectively.

In a subsequent balance study conducted with these sheep, urinary levels of the element were also directly related to dietary intake; with dietary intakes of 32 and 159 mg vanadium per day, 83 and 498 µg/day, respectively, were excreted in the urine. A trend toward elevated urine volume with increased intake of the element was also observed.

CHAPTER VIII

DIETARY VANADIUM TOXICITY IN THE RAT

Introduction

The toxicity of airborne and intravenous vanadium at low levels to rats and other laboratory animals has been amply verified (Muhler, 1957; Pham-Huu-Chanh, 1965; Roshchin et al., 1965), but reports concerned with vanadium in the diet are limited.

Several specific dietary factors have been shown to decrease the toxicity of vanadium since Stockinger (1955) observed that enriched diets, in general, counteracted toxicity in rats. Dietary factors in excess such as chromium (Wright, 1968) and ascorbic acid (Berg and Lawrence, 1971), have been shown to be protective. Other investigators (Mountain et al., 1959; Hathcock et al., 1964) have also observed that diet composition greatly affected the degree of toxicity of dietary vanadium. Berg (1966) showed that increasing dietary protein levels resulted in linear decreases in the mortality rate of chicks exposed to excesses of the element.

The studies reported here were conducted to investigate the effects of diet composition and dietary vanadium level on performance and vanadium deposition in rats.

Experimental Procedure

Experiment 1

Sixty male Long-Evans rats weighing 52 g initially were randomly assigned from weight outcome groups to ten dietary treatments in a 2 X 5 factorial arrangement. The treatments consisted of semi-purified diets calculated to contain either 20 or 30% protein (Table 22) with added levels of 0, 5, 10, 20 or 40 ppm vanadium as sodium metavanadate ($NaVO_3$). Pairs of rats were housed in stainless-steel cages; feed and deionized water were provided *ad libitum*. Weekly body weight and daily feed consumption records were maintained throughout the experimental period. After 56 days, rats were euthanized and blood was collected in lithium citrate-treated containers. Liver and kidneys were excised and weighed immediately and kidney, muscle and bone (femur plus tibia) were excised, weighed and reserved for vanadium analysis.

Experiment 2

An additional 36 rats (18 pairs) with an average initial weight of 62 g were used in an equalized paired feeding experiment in which rats fed diets with 20, 40 or 80 ppm supplemental vanadium were paired with rats fed the basal diet containing no added vanadium. Additions of the mineral as sodium metavanadate were made to the diet containing 20% protein (Table 22). The amount of feed offered rats

TABLE 22. COMPOSITION OF BASAL DIETS[a]

Ingredient	Crude Protein, % 20	30
Casein (vitamin free)[b]	22.98	34.48
Sucrose	35.02	23.52
Corn starch	25.00	25.00
Mineral mixture[c]	5.00	5.00
Corn oil[d]	5.00	5.00
Alphacel[e]	5.00	5.00
Vitamin mixture[f]	2.00	2.00
	100.00	100.00

[a]Contained .25 and .29 ppm vanadium (dry matter basis) for the 20 and 30% protein levels, respectively.

[b]Vitamin free casein, Nutritional Biochemicals Corporation, Cleveland, Ohio, Crude protein, minimum 87%.

[c]Salt mixture USP XIV, Nutritional Biochemicals Corporation, Cleveland, Ohio. The mineral mix provided the following quantities of nutrient in grams per kilogram of diet: calcium citrate, 15.525; dibasic potassium phosphate, 10.940; potassium chloride, 6.235; calcium biphosphate, 5.640; sodium chloride, 3.855; calcium carbonate, 3.430; magnesium sulfate, 1.915; magnesium carbonate, 1.760; ferric ammonium citrate, .764; sodium fluoride, .025; manganese sulfate, .010; ammonium alum., .0046; cupric sulfate, .0038; potassium iodide, .002.

[d]Stabilized with Santoquin (ethoxyquin) at level of 2 ml/liter of oil.

[e]Alphacel, Nutritional Biochemicals Corporation, Cleveland, Ohio.

[f]Vitamin Diet Fortification Mixture, Nutritional Biochemicals Corporation, Cleveland, Ohio. The vitamin mix provided the following per kilogram of diet: vitamin A, 18000 IU; vitamin D, 2000 IU; tocopherol, 100 mg; ascorbic acid, 900 mg; inositol, 100 mg; choline chloride, 1.5 g; menadione, 45 mg; P-aminobenzoic acid, 100 mg; niacin, 90 mg; riboflavin, 20 mg; pyridoxine HCl, 20 mg; thiamin, 20 mg; calcium pantothenate, 60 mg; biotin, .40 mg; folic acid, 1.8 mg; and vitamin B_{12}, .027 mg.

receiving the basal diet was restricted to the quantity consumed by corresponding paired rats fed the three diets with 20, 40 or 80 ppm added vanadium. All animals were caged and fed individually; deionized water was provided ad libitum. At the end of the 35-day feeding period, the rats were euthanized and terminal blood samples were taken. Liver and kidneys were removed and fresh weights recorded.

Experiment 3

Eighteen weanling male and 18 female rats of the Long-Evans strain were randomly assigned from weight outcome groups to three dietary treatments utilizing a commercial rat diet with supplemental vanadium. Treatments were comprised of three supplemental vanadium levels (0,100 and 150 ppm) added as sodium metavanadate to a commercial laboratory chow (Ralston Purina Company, St. Louis, Missouri). Vanadium toxicity was measured in terms of effect on growth rate and feed consumption for the 15-week period. The rats were housed in groups of three. Both feed and water were supplied ad libitum.

Experiment 4

A voluntary intake study was conducted using 10 male rats fed for 14 days. The rats were housed singly in stainless-steel cages and were provided deionized water. A commercial laboratory chow (Ralston Purina Company, St. Louis, Missouri), to which was added either 0 or 50 ppm supplemental vanadium ($NaVO_3$), was fed from two identical

containers. Daily feed consumption of each diet was recorded and used as an indicator of selective intake by individually fed rats. The positions of the feed containers within the cages were changed daily.

Hematocrit was determined by a micro-hematocrit method using a clinical centrifuge. Vanadium content of muscle, kidney and bone (Experiment 1) was determined by means of atomic absorption spectrophotometry. A preparatory extraction procedure which involved chelation with cupferron and extraction of the chelate into methylisobutyl ketone was used for concentrating vanadium from solutions of ashed material (CHAPTER III).

The data were analyzed statistically by analysis of variance and significant differences between means were determined using Duncan's multiple range test (Steel and Torrie, 1960).

Results and Discussion

Experiment 1

Statistical analysis of the data obtained did not show significant protein X vanadium level interactions; therefore, only data for main effects are presented.

Feed intake, body weight gain and feed efficiency for rats receiving four levels of supplemental vanadium are shown in Table 23. The growth rate of control rats in the present case was only slightly less than typical for Long-Evans strain rats fed non-purified diets (N.R.C., 1972).

TABLE 23. EFFECT OF DIETARY PROTEIN AND VANADIUM LEVEL ON FEED INTAKE AND GROWTH OF RATS. (EXPERIMENT 1)[a]

Protein %	Supplemental vanadium, ppm	Feed Intake g/day	Daily Gain g	Feed Per Unit Gain
Protein Main Effect				
20		13.07 ± .23	4.01 ± .13	3.25 ± .11
30		13.66 ± .22	4.03 ± .11	3.41 ± .07
Vanadium Main Effect				
	0	13.85 ± .20[b]	4.26 ± .14[b]	3.26 ± .07
	5	13.91 ± .23[b]	4.30 ± .20[b]	3.28 ± .14
	10	13.56 ± .28[b]	4.26 ± .10[b]	3.18 ± .06
	20	13.33 ± .33[b]	4.00 ± .09[b]	3.34 ± .09
	40	12.06 ± .36[c]	3.28 ± .11[c]	3.67 ± .20

[a]Mean plus standard error. Means represent 30 rats for protein main effect and 12 rats for vanadium main effect.

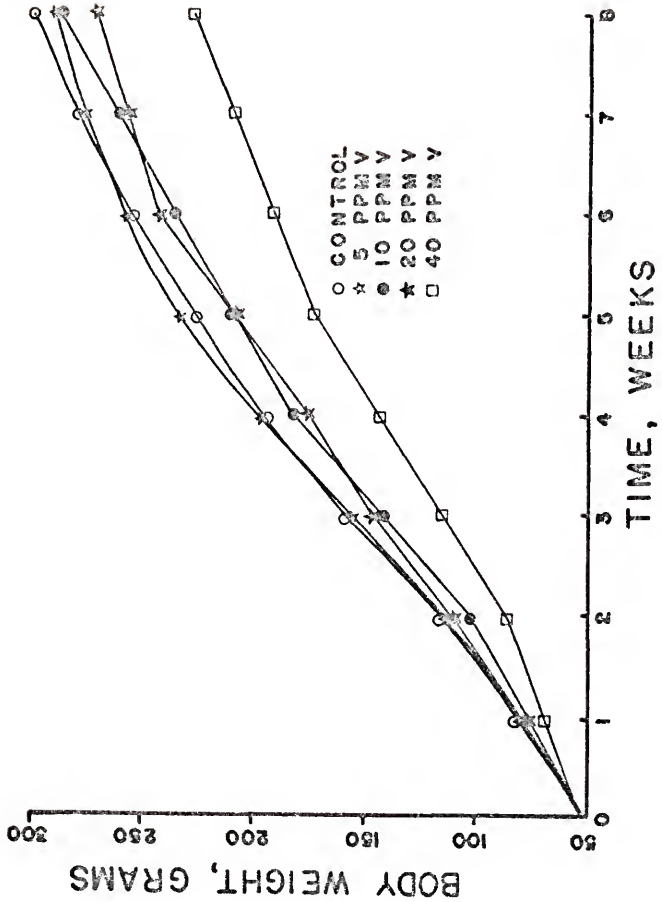
[bc]Means in the same column with different superscripts are significantly different ($P < .05$).

Decreased feed intakes and weight gains in rats fed 20 ppm vanadium were evident, but differences in these parameters became significant ($P < .05$) only at the highest supplemental level, 40 ppm vanadium. The decrease in feed intake approximately paralleled the decline in growth rate; thus, a slight, but non-significant decrease was observed in the feed efficiency of these rats. The animals receiving 20% protein consumed slightly less feed, but body weight gain was essentially equal to that of rats receiving 30% protein in the diet. Daily gain was slightly, but not significantly lower (3.17 g/day) when 40 ppm vanadium was combined with the 20% protein diet than when fed in combination with 30% protein (3.37 g/day).

Figure 15 shows the growth curve for rats receiving the five treatment diets. Since protein effect on growth was minimal in this case, only the effect of vanadium is illustrated. The reduced growth rate of rats fed 40 ppm vanadium is apparent; body weight was sufficiently depressed to differ from control animals ($P < .05$) after 4 weeks.

The effect of vanadium in relation to protein intake has been studied with chicks (Berg, 1966). He investigated the effect of 20 ppm vanadium fed in combination with 18, 22, 26 or 30% protein. Although protein in the diet and growth depression were inversely related, only a 6.6% difference in body weight between chicks fed the low and high protein levels was observed.

Figure 15. Effect of 0, 5, 10, 20 and 40 ppm supplemental dietary vanadium on growth of rats.


BODY WEIGHT, GRAMS
50
100
150
200
250
300
0
1
2
3
4
5
6
7
8
TIME, WEEKS
CONTROL
5 PPM V
10 PPM V
20 PPM V
40 PPM V

The influence of dietary vanadium on liver and kidney weights and on hematocrit of rats is shown in Table 24. A protein effect was not observed. Hematocrit decreased linearly ($r = .85$) with increasing dietary vanadium; the effect was significant ($P < .05$) only at the 40 ppm vanadium dietary level. Strasia and Smith (1970) noted a similar inverse relationship between vanadium in the diet and hemoglobin in rats, although adverse effects were observed with lower vanadium levels. Kidney and liver weights were depressed slightly, but not significantly, by 40 ppm dietary vanadium.

The effects of protein and supplemental vanadium levels on concentrations of vanadium in bone, kidney and muscle are shown in Table 25. Vanadium in skeletal muscle of rats consuming the basal diet was not detected. Protein intake did not influence ($P > .05$) the amount of vanadium in tissues. However, organ and bone levels generally reflected dietary vanadium with kidney vanadium content more responsive to diet. Higher ($P < .01$) vanadium concentrations in kidney were observed at the 10 ppm dietary level and vanadium intakes of 20 ppm substantially increased ($P < .01$) bone vanadium content.

Experiment 2

Table 26 shows feed intake and performance values for rats fed 20, 40 and 80 ppm dietary vanadium and for the corresponding pair-fed controls receiving diets without

TABLE 24. EFFECT OF DIETARY PROTEIN AND VANADIUM LEVEL ON ORGAN WEIGHTS AND HEMATOCRIT OF RATS. (EXPERIMENT 1)[a]

Protein %	Supplemental vanadium, ppm	Liver weight g	Kidney weight g	Hematocrit %
Protein Main Effect				
20		10.79 ± .31	2.18 ± .05	39.09 ± .63
30		10.65 ± .36	2.15 ± .05	36.71 ± .62
Vanadium Main Effect				
	0	10.65 ± .49	2.23 ± .09	41.64 ± .72[b]
	5	11.57 ± .61	2.25 ± .07	39.46 ± .71[b]
	10	11.79 ± .43	2.25 ± .07	36.63 ± .69[b]
	20	10.12 ± .43	2.11 ± .06	36.61 ± .80[b]
	40	9.38 ± .40	1.97 ± .07	35.18 ± 1.12[c]

[a]Mean plus standard error. Means represent 30 rats for protein main effect and 12 rats for vanadium main effect.

[bc]Means in the same column with different superscripts are significantly different ($P < .05$).

TABLE 25. EFFECT OF DIETARY PROTEIN AND VANADIUM ON BONE ASH AND TISSUE VANADIUM CONCENTRATION OF RATS. (EXPERIMENT 1)[a]

Protein %	Supplemental vanadium, ppm	Bone ash %	Tissue vanadium, ppm dry matter basis[b]		
			Bone	Kidney	Muscle
Protein Main Effect					
20		57.22 ± .40	2.21 ± .22	3.48 ± .32	.34 ± .02
30		54.71 ± .36	2.30 ± .28	3.62 ± .36	.28 ± .03
Vanadium Main Effect					
	0	55.32 ± 1.01	.14 ± .01[d]	.20 ± .02[d]	nd[c]
	5	56.08 ± .89	.86 ± .30[d]	1.42 ± .24[d]	.21 ± .02
	10	55.05 ± .94	1.43 ± .17[d]	2.42 ± .32[e]	.42 ± .03
	20	56.05 ± 1.03	3.34 ± .62[e]	5.12 ± .36[e]	.42 ± .04
	40	55.51 ± .91	5.51 ± 1.08[e]	8.61 ± .96[f]	.56 ± .16

[a]Mean plus standard error. Means represent 30 rats for protein main effect and 12 rats for vanadium main effect.

[b]Bone, ash weight basis.

[c]nd: not detected.

[def]Means in the same column and group with different superscripts are significantly different ($P < .01$).

TABLE 26. EFFECT OF DIETARY VANADIUM LEVEL ON FEED CONSUMPTION AND GROWTH OF RATS COMPARED TO PAIR-FED CONTROLS. (EXPERIMENT 2)[a]

Supplemental vanadium, ppm[b]	Feed intake g/day	Daily gain g	Feed per unit gain
0	13.28 ± .22[c]	4.44 ± .25[c]	3.02 ± .18[c]
20	13.71 ± .33[c]	4.78 ± .11[c]	2.87 ± .07[c]
0	12.44 ± .25[d]	3.75 ± .18[d]	3.35 ± .14[c]
40	12.66 ± .24[d]	3.69 ± .26[d]	3.43 ± .22[c]
0	11.84 ± .32[e]	3.52 ± .16[d]	3.36 ± .10[c]
80	12.02 ± .29[e]	3.38 ± .34[e]	3.56 ± .43[d]

[a]Mean plus standard error. Values represent means of six observations.

[b]Feed intake of controls (0 ppm) restricted to intake of corresponding paired rats fed diets supplemented with 20, 40 or 80 ppm vanadium.

[cde]Means in the same column bearing different superscripts are different ($P < .05$).

without added vanadium. Average daily feed intakes of animals offered diets without added vanadium (pair-fed controls) were slightly lower due to the method used for restricting feed intake; feed offered the control animals was limited to the amount of vanadium-supplemented diet consumed on the previous day by paired treatment rats.

Progressive decreases in average daily gain with increasing vanadium were noted. Daily gain in body weight of rats receiving 80 ppm dietary vanadium was severely depressed ($P < .05$) when compared to animals fed 20 or 40 ppm vanadium. The reduction in growth rates of both treatment rats and pair-fed controls that accompanied increased dietary vanadium indicated that decreased growth was due, in part, to feed intake depression by the mineral. Daily gains, however, were lower ($P < .05$) for rats fed 80 ppm added vanadium than for corresponding pair-fed controls. Thus, the data suggest that the mechanism of vanadium action under these conditions involved a toxic effect as well as an effect on feed intake. Mountain et al. (1953) also observed that weight gains of rats receiving vanadium as vanadium pentoxide was less than for control rats pair-fed as a group, indicating that feed restriction was not the sole factor in the growth impairment.

In Table 27 is shown the effect of dietary vanadium on organ weights and hematocrit of rats compared to pair-fed controls. Kidney and liver weights were depressed both

TABLE 27. EFFECT OF DIETARY VANADIUM LEVEL ON ORGAN WEIGHTS AND HEMATOCRIT OF RATS COMPARED TO PAIR-FED CONTROLS. (EXPERIMENT 2)[a]

Supplemental vanadium, ppm[b]	Liver weight g	Kidney weight g	Hematocrit %
0	9.64 ± .90	1.89 ± .10	45.17 ± 1.41[c]
20	10.52 ± .26	1.99 ± .05	47.92 ± 1.55[c]
0	7.50 ± .34	1.65 ± .07	38.60 ± .59[d]
40	9.39 ± 1.02	1.82 ± .11	37.25 ± 1.96[d]
0	7.63 ± .26	1.63 ± .05	35.90 ± .98[d]
80	7.53 ± .99	1.59 ± .12	36.00 ± 1.37[d]

[a]Mean plus standard error. Values represent means of eighteen observations for main effects and six observations for vanadium-level effects.

[b]Feed intake of controls restricted to intake of corresponding paried rats fed diets containing 20, 40 or 80 ppm supplemental vanadium.

[cd]Means in the same column bearing different superscripts are different ($P < .05$).

in rats fed higher levels of vanadium and in corresponding control animals. Differences were not significant and probably reflect the lower body weights observed for these groups.

Hematocrit was lower ($P < .05$) in rats receiving vanadium in the diet above 20 ppm, but differences between paired rats fed diets with and without added vanadium were not well-marked.

Experiment 3

The influence of vanadium on male and female rat growth and feed intake when fed in combination with a commercial laboratory chow is shown in Table 28. Male rats consumed more of this diet than of the semi-purified diet used in Experiment 1 and Experiment 2. Daily gains were generally lower for these animals than for rats in previous experiments, due to the faster rate of growth of the younger rats used previously. Growth rate was similar to that of rats fed under approximately the same conditions (Arrington and Ammerman, 1966). The added vanadium did not significantly ($P > .05$) affect growth rate or feed consumption measured at time intervals of 5, 10 or 15 weeks. The results are similar to those reported by Strasia (1971). This author observed that feeding 150 ppm vanadium in a commercial laboratory chow did not markedly affect feed intake of immature rats; the minimum toxic level of vanadium for older rats was also above 150 ppm in this type of diet. The small

TABLE 28. INFLUENCE OF DIETARY VANADIUM ON FEED INTAKE AND WEIGHT GAIN OF MALE AND FEMALE RATS (EXPERIMENT 3)[abc]

Supplemental vanadium, ppm	Male		Female	
	Feed intake g/day	Daily gain g	Feed intake g/day	Daily gain g
	5 Weeks			
0	18.82 ± .24	3.64 ± .17	13.80 ± 1.09	2.20 ± .09
100	18.24 ± .75	3.55 ± .28	13.13 ± .51	2.01 ± .17
150	18.88 ± .15	3.31 ± .18	13.35 ± .38	2.12 ± .21
	10 Weeks (Accumulative)			
0	21.59 ± 1.15	3.42 ± .17	14.45 ± .57	1.77 ± .07
100	20.01 ± 1.02	3.08 ± .22	12.91 ± .35	1.70 ± .11
150	20.21 ± .27	3.13 ± .14	13.11 ± .32	1.73 ± .03
	15 Weeks (Accumulative)			
0	21.03 ± .70	2.43 ± .10	14.20 ± .46	1.34 ± .05

TABLE 28. CONTINUED.

Supplemental vanadium, ppm	Male		Female	
	Feed intake g/day	Daily gain g	Feed intake g/day	Daily gain g
100	20.78 ± .08	2.27 ± .15	13.18 ± .45	1.25 ± .09
150	19.98 ± .12	2.25 ± .07	12.83 ± .24	1.24 ± .07

[a]Vanadium ($NaVO_3$) added to laboratory chow, Ralston Purina Company, St. Louis, Missouri. Listed analysis: protein, minimum 23%; fat, minimum 4.5%; fiber, maximum 6.0%; Ca, 1.20%; P, .86%; K, .90%; Mg, .24%; Na, .45%; Cl, .54%; F, 3.5 ppm; Fe, 198 ppm; Zn, 58 ppm; Mn, 51 ppm; Cu, 18 ppm; Co, .4 ppm; and I, 1.7 ppm.

[b]Mean plus standard error. Weight gain values are means of six observations. Values for feed intake are means of two observations representing six rats.

[c]Average initial body weights of male and female rats were 113 and 95 g, respectively.

effect on gain of 150 ppm in the present study contrasts with the marked experimental effect of 40 ppm vanadium fed in a semi-purified diet (Experiment 1). The toxic effect of vanadium was greater in chicks when fed with a semi-purified sucrose-fishmeal diet than when fed in combination with a practical corn-soybean meal diet (Berg, 1966). Vanadium at 100 ppm enhanced growth of rats when fed with a commercial diet, but resulted in adverse effects when fed in a semi-purified diet (Mountain et al., 1959).

Experiment 4

The rats offered two levels of vanadium (0 and 50 ppm) in separate but identical containers did not consistently avoid either diet. As shown in Table 29, the animals consumed daily 8.9 g of the basal diet and 5.5 g of the vanadium-containing diet. The difference in intake was significant only at the 10% level. No adverse effects were noted in those rats that consumed more of the diet with 50 ppm supplemental vanadium. Although only 20% of the rats selected more of the diet with added vanadium than without, the diet was readily consumed by all of the animals. Thus, under these conditions, growing rats showed a slight preference when offered a commercial laboratory chow without added sodium metavanadate.

Summary

Sixty weanling rats were used in a 2 X 5 factorial arrangement of treatments to study the effect of dietary

TABLE 29. VOLUNTARY INTAKE BY RATS OF DIETS CONTAINING 0 and 50 PPM SUPPLEMENTAL VANADIUM. (EXPERIMENT 4)[a]

Initial weight, g	Selective feed consumption, g/day		Total feed g/day	Daily gain g
	Basal Diet	Basal + 50 ppm vanadium		
74 ± 1.1	8.9 ± .96[†]	5.5 ± .99[†]	14.4 ± .64	4.7 ± .35

[a]Mean plus standard error. Values represent means of 10 rats fed for 14 days.

[†]Means differ ($P < .10$).

protein and vanadium ($NaVO_3$) on feed intake, performance and vanadium concentration of muscle, kidney and bone. Semi-purified diets containing 20 or 30% protein, supplemented with 0, 5, 10, 20 or 40 ppm vanadium, were fed for 56 days. Dietary protein level did not significantly affect the parameters studied; but feed intakes and weight gains were depressed ($P < .05$) with the 40 ppm vanadium level. Vanadium concentrations were elevated ($P < .01$) in bone when 20 ppm vanadium was fed and were increased in kidney by 10 ppm dietary vanadium. High dietary vanadium also decreased hematocrit values.

An equalized paired-feeding experiment was conducted which involved 36 rats fed 0, 20, 40 or 80 ppm supplemental vanadium. Higher levels of vanadium progressively depressed weight gains and feed intakes of rats. However, growth rate of pair-fed controls was also decreased, although to a lesser extent, suggesting that decreased growth rate was due in part to the effect of feed intake restriction.

In an additional study, vanadium ($NaVO_3$) fed to growing male and female rats in combination with a commercial laboratory chow at levels up to 150 ppm resulted in only slight depression of growth rate and feed consumption. Rats offered either 0 or 50 ppm added vanadium in a commercial diet indicated a slight preference for the basal diet.

CHAPTER IX

GENERAL DISCUSSION AND SUMMARY

Vanadium is widespread in nature (N.R.C., 1974a), is absorbed by plants (Söremark, 1967) and animals (Comar and Chevallier, 1967) and is associated with phosphates (Berg, 1966), making it generally available in feedstuffs and consequently accessible to the food chain. The wide variations in distribution, toxicity differences in elemental combinations (Proescher et al., 1917), adaptability of animals (Strasia, 1971; Williams, 1973) and difficulties of measurement in biological materials have complicated specific toxicity studies with this element. Relationships to dietary combinations (Berg, 1966; Mountain et al., 1959) and animal species (Pham-Huu-Chanh, 1965) have also rendered mode of action reports less conclusive and more perplexing for explaining the biochemical and physiological bases of vanadium toxicity.

Recent improvements in analytical instrumentation and procedures (Pearton et al., 1969; Davidson and Secrest, 1972), the development of solvent extraction techniques (Crump-Wiesner et al., 1971; Christian and Feldman, 1970) and validation of procedures with biological materials have clarified interpretation of results of animal investigations and aided in establishing parameters for vanadium toxicity in diets and tissues.

Experiments using chemical and concurrent radioisotopic procedures were conducted to gain information concerning dietary toxicity, tissue distribution and physiological movement of vanadium in sheep and to add to the limited amount of available information relative to ruminant animals. Further studies were directed toward obtaining additional data on the response of the rat to vanadium in the diet, interrelationships of intake and growth depression and distribution in tissues subsequent to ingestion of mineral.

In a growth trial, twenty-four lambs, individually fed supplemental levels of 0, 10, 100, 200, 400 or 800 ppm vanadium (NH_4VO_3) in a corn-soybean meal diet showed clinical toxicity signs only on the two higher levels. Dietary levels up to and including 200 ppm added vanadium for 84 days resulted in only small initial negative effects on feed intake, although tissue content was increased. No effects on hemoglobin, hematocrit or growth rate were detected in these animals. Lambs fed the two highest levels, 400 and 800 ppm, ceased eating after initial exposure, but subsequent feeding of the basal diet resulted in an apparent complete recovery within 8 days.

Fourteen sheep were fed 0, 50 or 200 ppm supplemental vanadium and were administered radiovanadium either orally or intravenously prior to a 144-hr blood-clearance and tissue-distribution study. Limited intestinal absorption was indicated but dietary vanadium did influence ^{48}V retention;

less of the isotope was retained in kidney ($P < .01$) and major organs of sheep receiving the highest dietary vanadium level. Kidney, metacarpal bone, liver and spleen retained (in decreasing order) the highest levels of ^{48}V 144 hr after dosing, and patterns of deposition were similar for both methods of administration.

Vanadium occurs in phosphate sources in quantities toxic to some animal species. It is a metal of variable valences, is complexed by many elements and varies in toxicity characteristics among species (Faulkner-Hudson, 1964). A study was conducted in which 12 Florida native wethers were administered three vanadium compounds by capsule. Daily dosage rate was increased by 50 mg vanadium at 2-day intervals to minimize delayed effects of toxicity. Onset of toxic effects did not differ significantly for calcium orthovanadate, calcium pyrovanadate or ammonium metavanadate when 25% reduction in feed intake was used as an indicator of response. Animals were adversely affected by dosage levels of 400 to 500 mg vanadium per day administered in this manner, corresponding to a range of 9.6 to 12 mg/kg body weight. Signs of toxicity included greatly elevated tissue levels, fatty degeneration of liver, diarrhea and mortality.

In further studies, 16 male lambs were fed 0, 50 or 200 ppm supplemental vanadium for 90 days; coccygeal vertebrae were sampled before and at 15, 30, 60 and 90 days after imposing

the dietary regimen to examine the effect of dietary intake on deposition of the mineral with time. Bone content of the element was elevated ($P < .05$) after feeding 200 ppm added vanadium for 15 days, but non-significant increases occurred thereafter. In a subsequent balance study with 14 of these sheep, urinary vanadium excretion was directly related to dietary intake of the mineral. Urinary excretion of more than 600 µg/day by individual sheep was also associated with depressed feed intake.

A series of investigations with the rat to study the effects of dietary vanadium ($NaVO_3$) on performance and tissue deposition of the metal were conducted with: a) 60 growing male rats for 8 weeks in a 2 X 5 factorial experiment using two levels of protein (20 and 30%) and 0, 5, 10, 20 and 40 ppm supplemental vanadium in the diet; b) 36 rats receiving 0, 20, 40 or 80 ppm added dietary vanadium in an equalized paired feeding study; c) 36 rats fed 0, 100 or 150 ppm supplemental vanadium for 15 weeks in a commercial laboratory chow; and d) a voluntary intake study in which 10 rats were offered a choice of 0 or 50 ppm added vanadium for 2 weeks.

Results indicated that both feed intake and growth rate were depressed when 40 ppm vanadium was fed in combination with a semi-purified diet. Vanadium content of bone and kidney was increased ($P < .01$) by 20 ppm added vanadium, but effects of elevated protein level on both performance and tissue levels were non-significant. Hematocrit decreased

linearly ($r = .85$) with increased dietary vanadium. When rats receiving 20, 40 or 80 ppm vanadium in a semi-purified diet were compared to pair-fed controls, growth rates of the former group were negatively affected to a greater extent than the latter, although feed intakes of both were depressed with increasing dietary content of the element. Greater depression in growth at the 80 ppm level ($P < .05$) relative to pair-fed controls suggested that reduced feed intake was not the only mechanism in the growth impairment; the inclusion of vanadium in the diet resulted in an additional reduction in growth. Rats consumed more laboratory chow than semi-purified diets but vanadium toxicity appeared to be above 150 ppm in contrast to 40 ppm found to be severely toxic in the semi-purified diets. Vanadium added to the diet affected preference only to a small extent when voluntary intake was tested with either 0 or 50 ppm vanadium in a standard laboratory chow. These data are consistent with the findings of other investigators (Berg, 1966; Mountain et al., 1953) where similar comparisons were made.

Vanadium concentrations in standard diets examined in the course of these investigations and quantities in mixed diets and forages reported by others (Nelson et al., 1962; Söremark, 1967; Fleming, 1973) have been generally low. Thus, while exceptions have been noted, present evidence indicates only limited problems with vanadium as a toxic element in conventional feed ingredients.

APPENDIX

TABLE 30. MAIN AND SIMPLE EFFECTS OF DIETARY VANADIUM LEVEL AND PATHWAY OF RADIOISOTOPE ADMINISTRATION ON ACCUMULATIVE PERCENT OF ^{48}V DOSE EXCRETED IN THE FECES OF SHEEP.

Supplemental vanadium, ppm	^{48}V Administration pathway	Days[a] 1	2	3	4	5	6
				(percent of dose)			
Main effects							
0 (4)[b]		6.91	21.20	33.45	42.78	46.59	49.77
50 (2)		2.17	14.81	29.61	40.90	44.60	48.62
200 (8)		5.93	17.84	29.27	38.49	41.52	45.00
	IV (7)	1.12	2.39	3.50	4.36	4.82	4.98
	ORAL (7)	10.23	34.36	57.54	75.77	82.01	88.79
Simple effects							
0 (2)	IV	1.30	2.91	3.93	4.65	5.06	5.30
0 (2)	ORAL	12.53	39.49	62.98	80.90	88.13	94.24
50 (1)	IV	0.19	1.38	2.60	3.77	4.38	4.51
50 (1)	ORAL	4.14	28.25	56.62	78.04	84.82	92.74
200 (4)	IV	1.26	2.38	3.50	4.35	4.80	4.94
200 (4)	ORAL	10.61	33.32	55.05	72.63	78.25	85.07

[a]Days following intravenous (IV) or oral administration.
[b]Refers to number of observations included in mean.

TABLE 31. MAIN AND SIMPLE EFFECTS OF DIETARY VANADIUM LEVEL AND PATHWAY OF RADIOISOTOPE ADMINISTRATION ON ACCUMULATIVE PERCENT OF ^{48}V DOSE EXCRETED IN THE URINE OF SHEEP.

Supplemental vanadium, ppm	^{48}V Administration pathway	Days[a] 1	2	3	4	5	6
					(percent of dose)		
Main effects							
0 (4)[b]		19.92	24.13	25.72	27.15	28.07	28.56
50 (2)		24.07	26.36	28.07	29.19	29.90	30.40
200 (8)		27.45	31.10	32.74	33.97	34.74	35.33
	IV (7)	49.54	56.68	59.85	62.31	63.87	64.93
	ORAL (7)	.09	.20	.29	.37	.41	.46
Simple effects							
0 (2)	IV	39.78	48.10	51.16	53.94	55.75	56.71
0 (2)	ORAL	.08	.17	.29	.36	.39	.41
50 (1)	IV	48.13	52.67	56.07	58.29	59.67	60.68
50 (1)	ORAL	.03	.05	.08	.12	.18	.23
200 (4)	IV	54.77	61.96	65.15	67.51	68.99	70.10
200 (4)	ORAL	.13	.24	.34	.44	.49	.56

[a]Days following oral or intravenous (IV) administration.

[b]Refers to number of observations included in means.

TABLE 32. SIMPLE LINEAR REGRESSION OF SHEEP TISSUE ^{48}V RETENTION 144 HRS AFTER ORAL ADMINISTRATION ON MEAN DAILY URINARY EXCRETION[ab]

Tissue	n	b	a	r	r^2	$S_{y \cdot x}$
			Across Dietary Treatments			
Kidney	7	128.095	.825	.953	.909	8.409
Bone	7	18.249	-.050	.957	.916	1.194
Liver	7	22.076	-.253	.989	.979	1.396
Spleen	7	7.580	.151	.925	.856	.513
Lung	7	7.018	.009	.967	.935	.454
Testicle	7	4.008	.032	.940	.883	.267
Heart	7	2.101	.023	.948	.898	.139
Muscle	7	.937	.004	.960	.922	.061
Brain	7	1.655	-.032	.978	.956	.106
			200 ppm Supplemental Vanadium			
Kidney	4	122.310	1.010	.988	.976	9.228
Bone	4	17.525	-.020	.999	.997	1.306
Liver	4	21.916	-.236	.999	.999	1.635
Spleen	4	6.641	.301	.970	.941	.510
Lung	4	6.487	.089	.999	.997	.484
Testicle	4	3.723	.051	.998	.996	.278
Heart	4	1.935	.039	.999	.999	.144
Muscle	4	.875	.008	.989	.977	.066
Brain	4	1.716	-.043	.979	.959	.130

[a]Mean daily urinary excretion calculated as total percent of oral dose excreted in urine/number of days (6).

[b]Tissue retention expressed as percent of dose per kg of tissue.

TABLE 33. TISSUE RETENTION OF ORALLY ADMINISTERED ^{48}V IN A LACTATING EWE 168 HR AFTER DOSING[a]

Tissue								
Kidney	Liver	Bone	Spleen	Mammary gland	Lung	Heart	Muscle	Adipose
9.423	1.311	.682	.540	.473	.401	.134	.112	trace

[a]Activity expressed as percent of dose per kg fresh tissue X 10^{-2}.

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BIOGRAPHICAL SKETCH

Samuel Leroy Hansard II was born February 20, 1944 at Fort Sill, Oklahoma. He attended primary and secondary schools in Tennessee, Florida and Louisiana and was graduated with honors from Robert E. Lee High School, Baton Rouge, Louisiana. He attended the University of Tennessee, Knoxville, and was awarded the Bachelor of Science degree in Zoology and Chemistry in 1966. He entered the Graduate School, University of Florida, and received the Master of Science degree in 1968. He spent two years as a First Lieutenant in the armed forces and served as artillery forward observer with the 1st Infantry Division in Vietnam and Cambodia, receiving several citations and combat medals, including the Bronze Star and Air Medal with oak leaf clusters.

Following service with the U. S. Army, he completed the Radioisotopes in Research course at Oak Ridge, Tennessee and was afterward granted a Research Assistantship in Animal Science at the University of Florida to continue studies toward the Doctor of Philosophy degree with a major in nutrition. Supplementing his academic program, he served as President of the Student Agricultural Council, Chancellor

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

C. B. Ammerman, Chairman
Professor of Animal Science

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Charles M. Allen, Jr
Associate Professor of
Biochemistry

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This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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