IMMUNOLOGIC EFFECTS OF EXPERIMENTAL IODINE TOXICITY IN CATTLE

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
DALE L. HAGGARD
1978

ABSTRACT

IMMUNOLOGIC EFFECTS OF EXPERIMENTAL
IODINE TOXICITY IN CATTLE

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This project was designed to evaluate the effects of 4 levels of daily iodine supplementation on the defense mechanisms in 40 calves. Immune and inflammatory responses were measured by titers to brucellosis, leptospirosis, and infectious bovine rhinotracheitis vaccinations; stimulation of lymphocyte mitoses by pokeweed, phytohemagglutinin (PHA) and con-A mitogens; intradermal PHA response; *in vitro* phagocytosis by white blood cells (WBC); WBC counts; and inflammatory response to trauma. Calves dosed with 1250 mg iodine daily had significant decreases in persistence of antibody titers to brucellosis and leptospirosis, lymphocyte mitotic activity, PHA injection induration, phagocytosis by WBC, and WBC counts. Calves on the 2 lower iodine levels tended to have decreased leptospirosis titers, lymphocyte mitoses, PHA injection induration, and *in vitro* phagocytosis by WBC compared to controls. Results suggest high dietary iodine interferes with titer maintenance to some antigens, lymphocyte DNA synthesis, and phagocytic activity of WBC.

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Large Animal Surgery and Medicine

1978

Dedicated to Sunny, Jane, and Martha

ACKNOWLEDGEMENTS

I wish to express my thanks to Dr. Gabel Conner, my major professor, for counsel and guidance in this investigation, and to Dr. Howard Stowe, who guided me to completion after Dr. Conner's retirement. I also wish to thank Dr. Virginia Mallmann for advice on the immunological aspects of this project; Dr. Sam Getty for editing the manuscript; Dr. James Main for his cooperation on the project; Drs. Roger Nietzel and John Gill for statistical analyses; Ms. Roberta Milar for technical assistance; Dr. Charles Muscoplat, University of Minnesota, for doing the lymphocyte blastogenesis studies; Dr. Soesanto Mangkoewidjojo for reading skin window slides; and Ms. Janice Fuller for typing the manuscript.

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INTRODUCTION

Iodine compounds are commonly used as feed additives by veterinarians and livestock producers to treat or prevent cattle diseases such as infectious pododermatitis, bovine respiratory disease complex, mastitis, infertility, and actinomycotic infections. Sometimes iodine levels in the feed are greatly in excess of the recommended daily dietary requirements. The potential of iodine toxicosis resulting from excessive iodine intake stimulated this investigation.

Some associations have been made between the excessive clinical use of iodine and increased incidence of infectious diseases in cattle. The signs that have been observed in field cases from increased iodine feed levels include slower growth rates, rough dry hair coats, decreased milk production, and decreased fertility. Acute cases associated with high levels of dietary iodine have had persistent coughing, excessive salivation, nasal discharge, ocular discharge, inappetence, increased incidence of respiratory disease, and slower response to treatment.

It was believed that the prevalence of excessive iodine utilization was sufficient to create a severe economic problem. Essentially no work has been done in this area; therefore, a multifaceted research project was designed in which several aspects of this problem were investigated. The approach included endocrinology (Mr. Kwan-Yee-Leung, Dairy Science), clinical pathology (Dr. James Main, Large Animal Surgery and Medicine), histopathology (Dr. Soesanto Mangkoewidjojo,

Pathology), hematology, and immunology. The latter aspects were investigated because of observations of increased incidence of infectious diseases associated with iodism and are the topics of this thesis.

LITERATURE REVIEW

Historical

Ancient Greek history indicated that burnt sponges were successfully used in the treatment of human goiter. Iodine was found to be abundant in sponges early in the 19th century. Following this discovery iodine was successfully used in human goiter therapy (Underwood, 1977). Further research indicated that there were endemic goiter areas in every continent and most countries (Kelly and Sneeden, 1960). Goiter in man and farm animals was established to be associated with an iodine deficiency in the food and water of affected areas (Allman and Hamilton, 1948). Rising living standards and the use of iodized salt has resulted in a substantial decline in the occurrence of goiter (Scrimshaw, 1960).

Iodine Metabolism

All body tissues, cells, and secretions contain iodine. The total amount of iodine (inorganic iodide and organically bound iodine) in the healthy adult human is estimated to be 10 to 25 mg. Most of the total iodine (70 to 80%) is concentrated in the thyroid gland. The skeletal muscles rank next in total iodine because of their large mass, but the concentration per unit weight is less than in the ovaries, bile, hair, and pituitary gland. This type of iodine distribution applies to all animal species so far studied (Maurer and Dugrue, 1928).

Iodine occurs in food predominantly as inorganic iodide and is absorbed in this form at all levels of the gastrointestinal tract. Nearly all of the absorbed iodine appears in the thyroid and little appears in the feces. The kidney is the main pathway of excretion of iodine apart from the small amounts lost in perspiration and feces. Some iodine is excreted by the stomach but little iodine is recovered in the feces; therefore, it must be reabsorbed further down the gastrointestinal tract (Leblond, 1942).

In lactating females iodine is also excreted in the milk. The iodine content of milk is extremely variable in most animals. Iodine concentration in milk is influenced by the iodine concentration of the diet and by the stage of lactation. Iodine is unique among the trace elements in the ease with which it passes the mammary barrier and the extent to which the level in the milk can be influenced by raising or lowering the dietary intake of this element (Orr and Leitch, 1926).

The major function of iodine in animals is its presence in thyroid hormone. Thyroid hormone is concerned with a variety of metabolic processes. It exercises control over the rate of energy metabolism, influences physical and mental growth and differentiation or maturation of tissues, affects the hypophysis and gonads, influences neuromuscular functioning, affects circulatory dynamics, affects the integument and its outgrowths--hair, fur, wool, and feathers, and influences the metabolism of various food nutrients. Most of these activities are believed to derive from the fundamental effect of thyroid hormone on cellular oxidation (Underwood, 1977).

Daily iodine requirement for the adult human has been estimated to be 200 µg to maintain normal thyroxine synthesis (Wolff, 1969).

The daily iodine needs of a cow producing 20 kg of milk per day are: maintenance - 2 mg, pregnancy - 1 mg, and milk production - 6 mg, for a total of 9 mg (Wallace, 1976).

Mason (1976) reported that the iodine content of ewe milk was a reliable indicator of dietary iodine intake. Milk iodine concentrations of ewes below 8 µg/dl indicated an iodine deficiency. Iodine milk concentrations from cow colostrum were several times higher than later milk, and there was a further decrease in iodine concentration at the end of lactation (Matovinovic, 1976). Cow milk has been found to contain in excess of 2.0 ppm iodine (Hillman et al., 1975).

Ullberg and Ewaldson (1964) found higher levels of iodine in fetuses than in maternal tissues of mice, rats and cats using autoradiographic techniques. Brown-Grant (1961) believed that fetuses from dams with hemochorial or hemoendothelial types of placentae tended to have the greater iodine concentration.

Metabolism studies have been done on dairy cows using ethylenediamine-dehydroiodide (EDDI) and radioactive sodium and potassium iodide. EDDI was absorbed equal to or greater than sodium or potassium iodide. Iodine from EDDI was retained in most organs and tissues longer than iodine from sodium iodide (Miller and Swanson, 1972).

Forbes et al. (1932) reported that there were no appreciable benefits from feeding iodinated linseed meal to growing swine or fattening lambs. Also, no effect was observed in the growth rate, mortality rate or egg-laying rate of female chicks fed iodized linseed meal.

Sources of Iodine

Most land plants contain iodine in varying concentrations. The iodine content of hays and straws has been reported to be in the range of 300-500 ppb. Dietary supplementation of iodine is usually accomplished by adding iodine to salt, mineral mixtures, or concentrates. Seaweed, which has been used as a feed additive, may contain levels of iodine in excess of 6000 ppm (Underwood, 1977).

Iodine used therapeutically and prophylactically contributes to excess iodine intakes. Forbes et al. (1932) reported on the therapeutic value of dietary iodine on infectious diseases. They fed iodized linseed meal to cows infected with *Brucella abortus*. Iodine dosage was based on recommendations for the treatment of actinomycotic infection and ranged from 0.65 to 2.13 grams per day.

Iodine-containing feed additives are also used to treat or prevent infectious pododermatitis, mastitis, infertility, and actinomycosis (McCauley et al., 1972a). The EDDI dosage recommended for prevention of infectious pododermatitis in cattle is 50 mg/head/day. The therapeutic dosage for the same condition is 200 mg/head/day (Miller and Swanson, 1972).

Toxic Levels of Iodine in Cattle Feeds

Forbes et al. (1932) found that tolerance of cattle to iodized linseed meal was variable. Certain cows maintained normal production consuming 2.13 g of iodine per day for a maximum of 139 days. Some cows had decreased production and feed consumption when fed 1.00 g of iodine per day. Ingestion of 5.00 g of iodine in iodized linseed meal per day caused cows to decrease feed consumption on the second day. Feeding iodine to calves at the rate of 30 mg of iodine per hundred

pounds of body weight daily caused a decrease in feed consumption. With the feeding of iodine to cattle at the rate of 10 mg per hundred pounds per day, there were no harmful effects observed. Calves fed iodine consumed less hay than did those not fed iodine. There was less gain in weight but greater gain in height in calves consuming iodized linseed meal.

Miller and Swanson (1972) stated that the recommended dose of EDDI (50 mg/head/day) for cattle infectious pododermatitis prevention will produce elevated serum iodine with consequent high iodine excretions. The therapeutic dose of iodine (200 mg/day) produced serum iodine concentrations approximately 10 times the levels found in control animals. Cattle fed a therapeutic level of iodine excreted high levels of iddine in the milk and urine. Miller and Swanson (1972) stated the minimum toxic dietary iodine level for calves was near 50 ppm. Some calves had decreased growth rates being fed 50 ppm iodine. McCauley et al. (1972a) reported on increased mammary and respiratory infections in 4 herds of cattle fed high levels of EDDI. Total iodine consumption in these herds ranged from 250 to 1700 mg per head per day.

Hillman et al. (1976), in field observations, associated an increased incidence of infectious diseases and thyroid malfunctions with an excessive iodine intake in Michigan dairy cattle. The histories of feed purchases indicated that 70 to 150 mg of iodine per head per day had been fed to cattle for a period of several months prior to the development of health problems.

Excessive quantities of iodine have been fed to cattle without producing toxicity. These findings were reported on non-stressed adult dairy cattle, dairy and beef calves (Long et al., 1953).

Signs of Iodine Toxicity in Cattle

Cattle consuming excessive iodine over long periods of time have been reported to have a sluggish and lethargic attitude (McCauley et al., 1972). Decreased feed consumption and increased gain in height are signs of chronic iodine toxicity in calves (Forbes et al., 1932). Other signs of iodine toxicity are rough hair coat, a brownish discoloration of the skin about the eyes, nose and tail, an exfoliation of dandruff-like epidermal scales (Smith et al., 1974), and alopecia (Hillman et al., 1976). A mucopurulent nasal discharge (Forbes et al., 1932; Newton et al., 1972; McCauley et al., 1972; Clark and Myers, 1967), persistent coughing (Newton et al., 1972), excessive lacrimation and conjunctivitis (McCauley et al., 1972; Matovinovic, 1976; Hillman et al., 1976; Smith et al., 1974), and blindness (Clark and Myers, 1967) are also associated with iodine toxicity. Digestive signs include excessive salivation (McCauley et al., 1972), decreased feed consumption (Newton et al., 1974; Clark and Myers, 1967), and decreased weight gains (Newton et al., 1974; Hillman et al., 1976; Bostrom et al., 1964). There may be decreased milk production (Matovinovic, 1976; Wallace, 1976; Hillman et al., 1976), and objectionable odor of the cream (Forbes et al., 1932), and an increased iodine content of the milk (Matovinovic, 1976). Abortions may occur in the second or third trimester (Hillman et al., 1976) and there may be an atrophy of the testicles or mammae (Clark and Myers, 1967). Chronic lameness has been reported in cattle being fed high levels of iodine (Wallace, 1976). Prolonged administration of iodides may cause posterior paralysis (Clark and Myers, 1967).

Signs of Iodine Toxicity in Other Species

Finkelstein et al. (1936) found few references to iodine poisoning in humans and found edema of the glottis as the most frequent manifestation of fatal iodine toxicity in humans. These cases resulted from the ingestion of iodine by mistaking it for another medication or from the ingestion of iodine with suicidal intent. Iodine-toxic goiter is produced in infants born to asthmatic women taking iodine compounds for their expectorant action during pregnancy (Martin and Rento, 1962). Murray (1953) reported that long-term oral administration of sodium iodate to rabbits (1 mg/kg twice weekly) produced no signs of ill health in 1 year. Arrington et al. (1965) reported that feeding 250 to 1000 ppm iodine for 2 to 5 days to does caused increasing mortality of newborn rabbits. Drew et al. (1975) stated that mares eating 83 mg daily produced foals with enlarged thyroids and weakness of the legs. Eklund et al. (1973) produced nephrocalcinosis in rats by feeding casein-containing diets and found the condition was more severe when these diets contained high amounts of iodine. McCauley et al. (1972b) reported on hyperthermia occurring in lambs fed EDDI (94 to 785 mg iodine/lamb/day). The elevated body temperature persisted for 7 days after iodine administration had ceased. Blaxter (1948) reported severe bronchopneumonia and acute deaths in sheep after administration of iodinated casein. Because some sheep receiving high levels of iodine did not die, Blaxter (1948) concluded that both infection and high dietary iodine must be present to produce clinical disease in animals.

Clinical Pathology

Hillman et al. (1976), under field conditions, noted that a tendency towards hypoplastic anemia characterized by low red blood cell counts, decreased hemoglobin values and decreased hematocrit values occurred in dairy cows on high dietary iodine. Blood hemoglobin levels in calves fed 100 and 200 ppm iodine were lower than in calves fed 10 ppm iodine or diets with no added iodine. Blood serum calcium levels in cows from 6 herds fed high levels of iodine were significantly lower than in control herds (Hillman et al., 1976). Serum calcium levels were significantly lower in calves fed 200 ppm iodine in the diet, but no differences were found when the highest level of iodine fed was 100 ppm (Newton et al., 1974). The mean serum cholesterol of 13 lactating cows in a herd on high dietary iodine was 95 mg per deciliter. The average serum cholesterol in normal lactating cows was 191 $\pm$ 30 mg/dl and 97 $\pm$ 22 mg/dl in non-lactating cows. This suggested a hypocholesterolemia in lactating cows fed a high iodine diet. Thyroid impairment was believed by Hillman et al. (1976) to have been induced by excessive iodine intake in 15 herds of dairy cattle in Michigan. Symptoms of exophthalmic goiter were evident, including bilateral exophthalmos, nervousness, and loss of hair. The thyroxine blood levels of 3 cows examined were 1.2, 2.8, and 4.4 µg/dl (Hillman et al., 1976). Elevated iodine intakes tended to increase the size of the adrenal and thyroid glands in calves, causing thyroid hypertrophy and adrenocortical hyperplasia (Newton et al., 1974; Wallace, 1976).

Effect of Iodine on Inflammation

In 1916, Kolmer et al. found that a positive pustular or nodular lutein reaction could be obtained in most persons, irrespective of the presence of syphilis, by the administration of potassium iodide simultaneously, before, or after the intradermal lutein test. Other substances, such as agar and starch, when injected intradermally, gave a similar reaction when potassium iodide or other drugs containing iodine were administered. Clinically, these reactions resembled the ordinary lutein reaction closely. However, they were more distinct under the influence of iodides. The amount of potassium iodide capable of producing these results among non-syphilitic individuals varied considerably.

Bostrom et al. (1964) found that the anti-inflammatory agents, salicylate, phenylbutazone, oxyphenylbutazone, and 2,4-dinitrophenol, all depress the excretion of sulfate esters in the urine. It was concluded that these drugs uncouple oxidative phosphorylation in the animal tissues which are concerned with the biosynthesis of sulfate esters. Whitehouse (1964) reported that sodium salicylate, which uncouples oxidative phosphorylation at low drug levels in liver and muscle mitochondria, selectively inhibits the incorporation of inorganic phosphate and sulfate into polysaccharide sulfates. This action, which occurred in bovine cartilage *in vitro* without inhibiting the oxidation of glucose or octanoate, will uncouple oxidative phosphorylation in connective tissue, muscle, kidney, and liver. This property of the drugs to uncouple phosphates may underlie their anti-inflammatory activity and be similar to the anti-inflammatory effect of iodine.

Middlebrook et al. (1955) reported that a low concentration of inorganic iodide will uncouple oxidative phosphorylation in mitochondria. The iodide suppresses completely the formation of adenosine triphosphate (ATP) without inhibiting oxidation. Thyroxine has a similar action. Thyroxine could be considered as being essentially iodide linked to an organic radical enabling the iodide to exert its action in a low concentration at specific points.

McCauley et al. (1972) concluded that the hyperthermia which occurred in sheep on high dietary iodide level may have been due to the action of iodide to uncouple oxidative phosphorylation. This would result in the inability of energy to be captured in the high energy bonds of ATP and its dissipation in oxidative metabolism. The reduction of ATP may be detrimental to any biosynthetic process of the body in a non-specific manner.

Stone and Willis (1967) reported on the effect of systemic and topical iodides on the response to induced inflammation. Guinea pigs (200-400 gm) were dosed with 33 mg of potassium iodide daily. Seventy-two hours after the initial dose of oral potassium iodide, the animals were injected with 0.1 ml of collagenase. The diameter of inflammation at the injection site was recorded at timed intervals. Orally administered potassium iodide significantly enhanced the inflammatory response present at 24 and 48, but not 72, hours after local injection of collagenase. It was concluded that iodine may influence the leukocytes and facilitate the phagocytosis of injected foreign material in skin reactions. Stone and Willis (1967) also stated that in discussing any inflammatory reaction two factors must be considered. The first is the inducing factor and the second is the enhancing factor. Enhancing factors are substances present in concentrations that are not

capable of inducing inflammation but are capable of enhancing inflammation. Iodide is one such enhancing factor. Enhancement could occur by stimulation of inflammation or by decreasing defense mechanisms. It was the opinion of Stone and Willis (1967) that iodides have a direct influence on the inflammatory response rather than influencing systemic mechanisms.

Meilens et al. (1968) investigated the effects of potassium iodide on granuloma pouch formation, cotton granuloma formation, carageenan and croton oil edema in rats, and turpentine abscesses in monkeys. In the studies on granuloma pouch formation in rats, potassium iodide administered orally at 116, 233, or 466 mg/kg daily inhibited exudation and wall formation. Inhibition of exudate increased proportionally with dosage of potassium iodide. All doses of potassium iodide in the granuloma pouch test were well tolerated. Iodide did not inhibit cotton granuloma formation at 466 mg/kg nor inhibit carageenan or croton oil edema at 800 mg/kg. Potassium iodide at 100 mg/kg was also ineffective as an inhibitor of turpentine edema and abscess formation in monkeys. Meilens et al. (1968) were of the opinion that the most likely effect of potassium iodide was suppression of the thyrotropin-thyroxine axis. However, the activity of the thyroids did not appear to be suppressed following its administration at granuloma pouch inhibitory doses. Also, there appeared to be a separation of doses of potassium iodide that inhibited granuloma pouch formation from doses that affected the rats adversely. The activity of iodides appeared to be directed against fibrotic tissue and a specific "antifibrotic" effect was conceived. The "antifibrotic" effect was not contradictory to enhancement of

inflammation (Stone et al., 1967) because the enhancement of inflammation was at the acute (22-48 hours) stage of inflammation.

Evaluation of Toxic Effects on Body Defenses

Antibody Response

Forbes et al. (1932) found iodized linseed meal fed to cows (0.65 to 2.13 g per day) did not change titers of brucellosis-infected cows.

Lymphocyte Stimulation by Mitogens

Fitzgerald (1972) developed a quantitative test of lymphocyte response to phytohemagglutinin (PHA) for the recognition of immunological defects in humans. The technique was used to investigate patients with suspe :ted immunological disorders and deficiencies. Park and Good (1972) developed a micromethod for testing PHA stimulation of human lymphocytes. The method provided a useful evaluation of thymus-dependent lymphocyte (T cell) populations in man and experimental animals and quantitated the deficiency of the T cells. Alhaji et al. (1974) studied peripheral blood lymphocytes of a calf artificially infected and a cow naturally infected with *Mycobacterium paratuberculosis*. The lymphocytes of the cow and calf and those of an uninfected control animal were incubated with purified protein derivatives (PPD) of *M. paratuberculosis* and other mycobacterium species. The degree of lymphocyte stimulation by PPD was determined by the incorporation of radioactive thymidine in desoxyribonucleic acid (DNA). Peripheral blood lymphocytes from infected cattle were stimulated as much as 15-fold by the homologous PPD prepared from the mycobacterial species causing the infection. Heterologous PPD

induced little or no stimulation. Lymphocytes from control cattle were unaffected.

Muscoplat et al. (1974b) studied the mitogenic activity of PHA and pokeweed mitogen (PWM) on lymphocytes from normal calves and calves with acute lymphocytic leukemia (ALL). Lymphocytes from calves with ALL were unresponsive to PHA and PWM. In another study, Muscoplat et al. (1974a) reported that lymphocytic responses to PHA and PWM in cows with persistent lymphocytosis were greater than in normal cows. Janossy and Greaves (1971) stated the responsiveness to PHA is predominantly a property of thymus dependent (T cell) lymphocytes and responsiveness to PWM is mainly a property of thymus independent (B cell) lymphocytes. PHA- and PWM-induced lymphocyte stimulations are indicative of functional competence of the cell-mediated and humoral immune systems, respectively.

Phytohemagglutinin Intradermal Test

The work of Lawlor et al. (1973), Bonforte et al. (1972) and Blaese et al. (1973) indicated that normal humans without prior sensitization responded to intradermal injections of PHA. There were erythema and induration at 24 hours and a perivascular infiltration of mononuclear cells characteristic of cutaneous delayed hypersensitivity. Intradermal injection of PHA was used as an assessment of cell-mediated immunity. Lawlor et al. (1973) stated that there was a correlation of *in vivo* and *in vitro* tests with PHA in 22 of 23 normal humans tested and believed the PHA intradermal test to be a simple and useful screening test for cellular immune function.

Dinitrochlorobenzene Topical Test

Catalona et al. (1972) developed a method to measure human cell-mediated immune responses using dinitrochlorobenzene (DNCB). A quantitative clinical evaluation included topical application of a sensitizing dose of DNCB (2 mg) and recording reaction to a challenge dose (50 µg) 14 to 21 days later. Sensitization was expressed by the occurrence of a spontaneous reaction after application of the challenge dose. Maximal response occurred at 4 days after application of the challenge dose. A positive response had to include induration and/or vesicle formation. Erythema alone did not constitute a positive response (Spitler, 1976). Gross (1965) studied DNCB sensitivity in different groups of humans and found decreased delayed hypersensitivity response in chronically or actively infected individuals. Another report stated that it was difficult, if not impossible, to create contact dermatitis with DNCB in dogs (Baker, 1966).

In vitro Phagocytosis

Stossel and Taylor (1976) reported that neutrophil hypofunction was a cause of recurrent pyogenic infection. Many microbes resist ingestion because they are not recognized by neutrophils. Serum interacts with most of these microorganisms and deposits opsonins on them which render them palatable to the neutrophils.

Stuart (1967) described a procedure for measurement of phagocytic activity of polymorphonuclear cells and monocytes. White blood cells were incubated in serum. After incubation the white blood cells were stained and the percentage of cells active in phagocytosis was determined. Stossel and Taylor (1976) stated that when all reagents, i.e., cells, serum and microorganisms, were incubated simultaneously,

the measured rate of ingestion was actually a combination of two rates, opsonization and ingestion. Lencotactic assays have been performed to determine the extent to which leukocytes can respond to chemotactic stimuli (Ward, 1976).

Measurement of Skin Inflammatory Reaction

Rebuck and Crowley (1955) described a procedure by which the cellular exudate of lesions could be sampled at desired times by taping glass coverslips over induced human skin lesions. At intervals the coverslips were removed from the lesions and the adhering cellular exudate was dried and stained. This allowed detailed comparison of the exudative leukocytes from acutely inflamed tissues with the leukocytes of blood smears. The participation of blood monocytes in local inflammation was in proportion to the number of vascular monocytes available for mobilization at the time of inflammation. This procedure was used to determine the extent to which leukocytes can respond to chemotactic stimuli. Neutrophilic leukocytes of man migrated at an early stage into the lesions and, after performing their functions of phagocytosis and enzyme elaboration, they shrank and degenerated. Before degeneration they lost their granule-containing cytoplasm to the exudate in the form of small fragments, which for a time were free in the inflammatory fluids but which ultimately were ingested by the hypertrophied lymphocytes and tissue macrophages. This was an original procedure for studying leukocyte functions *in vivo*.

Ward (1976) stated that the unidirectional migratory response of cells to an increasing chemical gradient of attraction was chemotaxis. Leukocytes have the ability to respond to chemotactic stimuli.

Attractant factors are the complement proteins of plasma. Enzymatic cleavage of the third and fifth components of complement results in the release of chemotactic peptides.

iodine. The iodine was in the form of ethylenediaminedihydroiodide (EDDI).

Antibody Titer Measurements

Brucellosis Titers

Strain 19 brucellosis vaccine[b] was administered by subcutaneous injection after a negative brucellosis test on all calves. Brucellosis titers were determined at Geagley Laboratories, East Lansing, Michigan, to study the antibody response to a live bacterial antigen. The titers were run to end-point dilutions using the plate agglutination test 1, 2, 3, 4, 6 and 10 weeks post-vaccination.

Ten weeks after the first vaccination, a second 2 cc dose of Strain 19 brucellosis vaccine was administered by subcutaneous injection to all calves. Titers were then determined 1, 2, 3-1/2, 4-1/2, 5-1/2, 6-1/2, 7-1/2, 10 and 13 weeks following this vaccination.

Leptospirosis Titers

Leptospirosis bacterin[c] was administered by subcutaneous injection to all calves that were negative for antibodies to *Leptospira pomona*. Leptospirosis titers were determined by Dr. Norma Greiner at Geagley Laboratories to study the antibody response to a killed bacterial antigen. The titers were run to end-point dilutions using the plate agglutination test 1, 2, 4, 5 and 6 weeks post-vaccination.

Six weeks after the first vaccination a second dose of leptospirosis bacterin was administered by subcutaneous injection to the

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[b]Professional Biological Laboratories (2 cc dose/animal).

[c]Norden Laboratories (IBR-Lepto vaccine) (2 cc/dose).

MATERIALS AND METHODS

Animals

Forty Holstein heifer calves between 4 and 5 months of age and weighing an average of 120 kg were used to study the effects of 4 different levels of supplemental iodine consumption on several aspects of body defenses during a 6-month period. The same animals were used by 3 investigators to study selected parameters of clinical pathology, endocrinology, and histopathology. The calves were housed in pens of 2 to 5 animals at Veterinary Research Barn 1 on the Michigan State University campus. Feed consisted of pelleted corn and oats[a] (2.25 kg/head/day) fed twice daily and free choice second-cutting alfalfa hay. Fresh water was provided *ad libitum*.

Experimental Groups

The 40 calves were numbered as they were received. They were then divided by the random number method into 4 groups of 10 calves each. Calves in group I were dosed daily with 20 ml of water containing no iodine. Calves in group II were dosed daily with 20 ml of water containing 50 mg of iodine. Calves in group III were dosed daily with 20 ml of water containing 250 mg of iodine. Calves in group IV were dosed daily with 100 ml of water containing 1250 mg of

[a]Purina custom order.

same calves. Titers were then determined 1, 2, 3 and 5-1/2 weeks following this vaccination.

Infectious Bovine Rhinotracheitis Titers

A modified live infectious bovine rhinotracheitis (IBR) vaccine[c] was administered by subcutaneous injection to all calves that were negative for antibodies to IBR. Antibody titers for IBR were determined at the National Animal Disease Center, Ames, Iowa, to study the antibody response to a modified live virus antigen. The titers were run to end-point using the serum neutralization test 1, 2, 4, 5 and 6 weeks post-vaccination.

Six weeks after the first vaccination a second dose of modified live IBR vaccine was administered by subcutaneous injection to the same calves. Titers were then determined 1, 2, 3, and 5-1/2 weeks following this vaccination.

Lymphocyte Mitogen Blastogenesis

Measurement of thymidine uptake of bovine lymphocytes in blastogenic responses to mitogens was done on 6 calvesof each group at intervals during the project. Increased thymidine uptake indicates increased synthesis of nucleic acids and increased mitotic activity by the stimulated lymphocytes. Heparinized blood (10 units/ml of blood) samples were diluted using a culture medium to 1/10, 1/20 and 1/30 dilutions. These dilutions were then incubated at 30°C for 3 to 6 days in 5% CO_2 and 100% humidified culture termination; cultures were pulsed with tritiated thymidine.[d] Radioactivity was counted on a liquid scintillation spectrometer. The degree of lymphocyte deoxyribonucleic acid (DNA) synthesis was expressed as the mean of

[d]New England Nuclear Corporation, Boston, MA.

triplicate culture counts per minute (cpm). The mitogens used were pokeweed mitogen (PWM), phytohemagglutinin (PHA) and concanavalin-A (CON-A). These procedures were performed by Dr. Charles Muscoplat of the University of Minnesota Veterinary Immunology Laboratory, St. Paul, Minnesota. Blood samples were drawn, packed in an insulated box with a can of 100°F water and shipped by air freight. Cultures were started within 18 hours after blood was drawn.

Intradermal and Topical Skin Tests

Phytohemagglutinin (PHA) Intradermal

The dosages of PHA (1, 2, 5 and 10 µg) used in humans for the evaluation of cell-mediated immune response (Lawler, 1973) produced diameters of induration of insufficient size (<8 mm) to clearly interpret differences in the project calves. It was therefore necessary to establish the most appropriate dose of PHA for interpreting this test in the bovine. Six Angus calves weighing approximately 270 kg were injected with 20, 40, 80 and 160 µg of PHA. Diameters of induration at 24 hours measured 18 to 24 mm for the 80 and 160 µg dosages and were measurable (5 to 12 mm) at 96 hours. Based on these determinations, a dose of 100 µg was used for intradermal testing of 6 calves per group in the project.

Measurements of induration were made at 24, 48, 72, 96 and 120 hours post-injection. Diameters of erythema could not be accurately measured because some of the calves had pigmented skin at the sites of injection.

Dinitrochlorobenzene Contact Sensitization

Dosages of dinitrochlorobenzene (DNCB) used in humans to measure cell-mediated immune responses (2 mg topically followed in 14-21 days by a 50 µg challenge) (Catalona et al., 1972) produced no measurable response when used on the project calves. Therefore, an attempt was made to determine sensitizing and test dosages for cattle using Angus calves. The procedure included use of topical sensitizing doses of 5, 10 and 15 mg of DNCB which were applied to 3 cm^2 prepared areas of skin. Two calves were used for each dose. Two weeks later, test doses of 12.5, 25, 50, 100 and 200 µg DNCB were applied to shaved, washed and dried areas of skin on each calf. Test areas were observed daily for 5 days and no measurable erythema or induration occurred. The same test doses were similarly applied 4 weeks following application of the varying sensitizing doses. Observation for 5 consecutive days revealed no measurable response. The DNCB contact sensitization test for measurement of cell-mediated immune response was therefore not used in this research.

Measurements of Inflammatory Response

In vitro Phagocytosis

A modification of the procedure of Territo et al. (1976) was used to measure neutrophil function. Heparinized Vacutainers[e] were used to draw 10 ml of venous blood; additional sodium heparin (2 drops) was added and mixed. Blood was pipetted into sedimentation rate tubes and centrifuged at 2000 rpm for 5 minutes. Two-thirds of the plasma was removed and the buffy coat and the rest of the plasma

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[e]Becton-Dickinson, Inc.

were harvested. White blood cells were counted and diluted with Hank's balanced salt solution (HBSS) and plasma to maintain 15% plasma with white blood cells standardized at 10^6 per milliliter. Standard solutions, one containing 10^6 *Candida albicans* organisms per milliliter and the other containing 10^6 *Brucella abortus* organisms per milliliter, were prepared. Two tubes, each containing 100,000 WBC, were prepared from cell suspensions for each calf. To one tube was added a suspension containing 300,000 *C. albicans* organisms and to the other tube was added a suspension containing 300,000 *B. abortus* organisms. These mixtures were incubated at 37°C for 30 minutes. Following incubation the tubes were centrifuged at 300 rpm for 5 minutes. The supernate was pipetted off. A slide was prepared from the cellular sediment in the tube, smeared, and stained. For washed cell preparations, the buffy coat cells were washed twice in HBSS and the process was completed without the presence of plasma. One hundred cells were counted and the number of particles phagocytized and percentage of cells active in phagocytosis were determined.

Skin Windows

A measurement of the inflammatory response in acute cutaneous inflammation as described by Rebuck and Crowley (1955) was modified and used on the calves. A site on the skin over the dorsal process of a sacral vertebra was shaved and cleaned with alcohol. With a sterile scalpel, the epithelium was scraped away from an area 4 to 6 mm in diameter. When the papillary layer of the skin was reached, fine bleeding points were in evidence. If excess bleeding occurred, another site was chosen. The trauma of the technique itself served as the inflammatory stimulus. The lesion was then covered with

adhesive surgical tape. At intervals of 2, 4, 8, 12 and 24 hours the tape was removed and a glass slide was pressed against the lesion and rapidly air dried. Another piece of tape was applied to cover the lesion for the time until the next slide was to be made. Cells on the prepared slides were then stained with Giemsa stain. Observations included differential counts of cells to determine the percent of polymorphonuclear leukocytes, lymphocytes, and monocytes. Necrotic cellular changes were also recorded.

RESULTS

Antibody Titers

The brucellosis antibody titers were elevated by vaccination in all treatment groups (Table 1). Maximal antibody titers were obtained 2 weeks following the first vaccination. The antibody response to brucellosis vaccination in all groups was generally greater following the first vaccination. The titers obtained following the first vaccination of the calves given 1250 mg iodine per day declined more rapidly than those of the other groups. At 10 weeks post-vaccination, titers for this high iodine group were significantly lower ($P<.05$) than the titers of the groups fed lesser quantities of iodine. The calves given 1250 mg iodine per day also had brucella titers, 1 week following the second vaccination, significantly lower ($P<.05$) than those of the other groups for the same time period.

The leptospirosis antibody titers following the first vaccination for *Leptospira* were inconsistent (Table 2). The leptospira antibody response after the second vaccination was greater (anamnestic response) than after the first vaccination and was greatest 1 week following the second vaccination. Five and one-half weeks following the second vaccination, the means of leptospira titers of all iodine-supplemented calves were significantly lower ($P<.05$) than the mean titer of the control group at that sampling period.

Table 1. Logarithms of brucella antibody titers after 2 brucellosis vaccinations of calves fed different levels of iodine

Daily Iodine Dosage (mg)	Weeks Post-Vaccination														
	1	2	3	4	6	10*	11	11½	13	14	15	16	17	20	22
0	2.151[1] ±.227	2.903 ±.383	2.542 ±.298	2.271 ±.231	1.932 ±.054	1.659[a] ±.078	1.532[a] ±.434	2.005 ±.077	2.359 ±.141	2.336 ±.123	1.883 ±.466	1.907 ±.030	1.924 ±.086	1.837 ±.083	1.762 ±.084
50	2.060 ±.056	2.843 ±.197	2.469 ±.074	2.211 ±.202	1.859 ±.064	1.600[a] ±.027	1.805[a] ±.124	2.065 ±.113	2.432 ±.132	2.366 ±.125	1.985 ±.054	1.889 ±.074	1.877 ±.056	1.788 ±.104	1.803 ±.037
250	1.927 ±.217	2.722 ±.247	2.572 ±.150	2.301 ±.242	1.907 ±.085	1.626[a] ±.016	1.955[a] ±.062	2.078 ±.106	2.504 ±.058	2.417 ±.105	2.106 ±.037	2.015 ±.044	1.944 ±.040	1.910 ±.082	1.823 ±.083
1250	2.011 ±.736	2.783 ±.326	2.401 ±.185	2.134 ±.126	1.762 ±.020	0.947[b] ±.548	1.282[b] ±.580	1.946 ±.099	2.395 ±.041	2.195 ±.036	2.057 ±.023	1.937 ±.037	1.893 ±.036	1.724 ±.075	1.587 ±.112

[1]Mean ± SEM (10 animals).

[a,b,c]Values with different letter superscripts within columns are significantly different ($P<.05$).

*Second vaccination after blood sample obtained on this date.

Table 2. Logarithms of leptospira antibody titers after 2 leptospirosis vaccinations of calves fed different levels of iodine

Daily Iodine Dosage (mg)	Weeks Post-Vaccination								
	1	2	4	5	6*	7	8	9	11½
0	.100	.260	0	0	0	1.535[1] ±.251	1.261 ±.357	0.901[a] ±.296	1.140[a] ±.250
50	.460	.200	.100	0	0	1.626 ±.568	1.381 ±.433	0.720[a] ±.438	0.660[b] ±.356
250	.222	.444	.111	.111	0	1.715 ±.243	1.361 ±.097	0.860[a] ±.241	0.820[b] ±.378
1250	.375	.200	0	0	0	1.595 ±.162	1.240 ±.097	0.300[b] ±.233	0.360[c] ±.363

[1]Mean ± SEM (10 animals).

[a,b,c]Values with different letter superscripts within columns are significantly different ($P<.05$).

*Second vaccination after blood sample obtained on this date.

The antibody titers following 2 infectious bovine rhinotracheitis vaccinations are presented in Table 3. Antibody response to the first vaccination was slow. One calf in the control group and one calf in the 50 mg iodine per day group had a measurable antibody titer 1 week following vaccination. Measurable titers were not evident from calves given the 2 higher levels of iodine at that time. IBR antibody titers for all groups were not significantly different from 1 week following the first vaccination to 5-1/2 weeks following the second vaccination.

Table 3. Logarithms of antibody titers after 2 infectious bovine rhinotracheitis vaccinations of calves fed different levels of iodine

Daily Iodine Dosage (mg)	Weeks Post-Vaccination							
	1	2	4	6*	7	8	9	11½
0	0.120[1] ±.380	1.064 ±.410	0.993 ±.510	0.993 ±.550	1.114 ±.400	1.114 ±.400	1.203 ±.380	0.946 ±.440
50	0.167 ±.500	0.803 ±.600	0.669 ±.630	0.702 ±.540	1.137 ±.250	1.070 ±.430	0.836 ±.490	0.753 ±.630
250	0	0.978 ±.470	0.870 ±.510	0.769 ±.600	1.304 ±.150	1.237 ±.100	1.003 ±.400	0.865 ±.540
1250	0	1.054 ±.430	1.016 ±.420	1.016 ±.420	1.317 ±.160	1.242 ±.250	1.054 ±.160	0.817 ±.380

[1]Mean ± SEM (10 animals).

*Second vaccination after blood sample obtained on this date.

Lymphocyte Blastogenesis

Pokeweed Blastogenesis

The results of the thymidine uptake studies on calf lymphocytes stimulated by PWM are presented in Table 4. The mean thymidine uptake rates, in counts per minute (CPM), for lymphocytes from the control calves ranged between 10,553 and 36,726 for the 3 sampling periods of 7, 17, and 25 weeks of the experiment. The lymphocytes from calves

Table 4. Thymidine uptake rates of pokeweed mitogen stimulated lymphocytes from calves given different levels of iodine

Daily Iodine Dosage (mg)	Number of Calves	Weeks on Experiment 7	17	25
		(counts per minute)		
0	4	36,726[1,b] ±17,357	13,622[a] ±6,239	10,553[2,a] ±1,305
50	4	43,197[a] ±11,780	9,714[a,b] ±1,776	9,700[3,a,b] ±4,126
250	4	49,296[a] ±22,318	9,518[a,b] ±3,743	8,083[b,c] ±755
1250	4	27,082[c] ±15,816	3,797[c] ±736	7,480[c] ±3,282

[1] Mean ± SD.

[2] Three determinations.

[3] Two determinations.

[a,b,c] Values with different letter superscripts within columns are significantly different ($P<.05$).

in the 50 and 250 mg iodine per day groups had greater thymidine uptake ($P<.05$) at 7 weeks on experiment than lymphocytes from calves of the control group. Lymphocytes from control calves had significantly greater thymidine uptake than those cells from calves on all levels of iodine ($P<.05$) at 17 and 25 weeks on experiment. Also, at 17 and 25 weeks of experiment the thymidine uptake of lymphocytes from calves on the highest level of daily iodine feeding (1250 mg) was significantly less ($P<.05$) than the thymidine uptake by lymphocytes of calves fed 0, 50, and 250 mg of iodine.

Phytohemagglutinin (PHA)

The results of the thymidine uptake studies on calf lymphocytes stimulated by PHA are presented in Table 5. The mean CPM of thymidine uptake for lymphocytes from the control calves ranged between 35,868 and 84,585 for the 3 sampling periods of 7, 17, and 25 weeks of the experiment. The lymphocytes from 1 iodine supplemented group (50 mg/day) after 7 weeks on experiment and another iodine supplemented group (250 mg/day) after 17 weeks on experiment had greater thymidine uptake than lymphocytes from control calves. On the tests after 7 and 17 weeks on experiment, the thymidine uptake of lymphocytes from calves receiving 1250 mg iodine per day was significantly less ($P<.05$) than the thymidine uptake by lymphocytes from calves of the other 3 groups. The thymidine uptake by lymphocytes of control calves at 25 weeks on experiment was significantly greater ($P<.05$) than the thymidine uptake of lymphocytes from calves on each level of daily iodine intake.

Table 5. Thymidine uptake rates of phytohemagglutinin stimulated lymphocytes from calves fed different levels of iodine

Daily Iodine Dosage (mg)	Number of Calves	Weeks on Experiment 7	17	25
		(counts per minute)		
0	4	84,585[1,a] ±21,336	35,868[a] ±19,267	50,072[a,3] ±440
50	4	100,659[a] ±47,520	22,689[b] ±12,048	24,740[b,2] ±21,509
250	4	76,215[a] ±19,739	41,025[a] ±21,983	22,228[b] ±10,845
1250	4	44,452[b] ±17,456	12,765[c] ±7,640	25,775[b] ±18,031

[1]Mean ± SD.

[2]Three determinations.

[3]Two determinations.

[a,b,c]Values with different letter superscripts within columns are significantly different ($P<.05$).

CON-A Mitogen

The results of the thymidine uptake studies on calf lymphocytes stimulated by CON-A mitogen are presented in Table 6. The mean thymidine uptake rates (CPM) for cells from control calves ranged between 117,980 and 125,681 for the 3 sampling periods of 7, 17, and 25 weeks of the experiment. The lymphocytes from calves dosed daily with 50, 250 and 1250 mg iodine, after 7 weeks on experiment, tended to be mitotically stimulated by CON-A mitogen more than lymphocytes from the control calves. After 17 and 25 weeks on experiment, the calves fed 1250 mg iodine per day had lymphocytes which were mitotically

stimulated by CON-A mitogen significantly less (P<.05) than lymphocytes from any other group of calves.

Table 6. Thymidine uptake rates of CON-A stimulated lymphocytes from calves fed different levels of iodine

Daily Iodine Dosage (mg)	Number of Calves	Weeks on Experiment 7	17	25
		(counts per minute)		
0	4	120,750[1,a] ±33,431	117,980[a] ±20,446	125,681[3,a] ±18,331
50	4	177,674[b] ±34,645	105,182[a] ±11,210	117,180[2,a] ±13,370
250	4	161,109[b] ±15,259	122,074[a] ±28,355	118,257[a] ±16,668
1250	4	133,310[c] ±41,732	53,154[b] ±17,316	94,584[b] ±42,460

[1]Mean ± SD.

[2]Three determinations.

[3]Two determinations.

[a,b,c]Values with different letter superscripts within columns are significantly different (P<.05).

Intradermal Phytohemagglutinin (PHA)

The mean diameters of the areas of induration caused by intradermal PHA injections of the calves 15 weeks on experiment are presented in Table 7. The size of the induration sites of the control calves at this test were maximal in size (22.5 mm) within 24 hours after injection and diminished to 4 mm in diameter at 120 hours post-injection. The level of iodine supplementation was not

Table 7. Diameters of induration sites following intradermal injection of phytohemagglutinin to calves fed different levels of iodine for 15 weeks

Daily Iodine Dosage (mg)	Hours of Injection				
	24	48	72	96	120
			(mm)		
0	22.5[1] ±4.2	22.3 ±3.3	16.3 ±3.7	12.2[a] ±6.5	4.0 ±6.3
50	24.3 ±3.2	20.3 ±2.0	12.3 ±6.1	6.0[b] ±9.3	4.7 ±5.3
250	24.9 ±3.9	23.8 ±2.6	16.5 ±4.3	5.7[b] ±6.3	0
1250	20.1 ±4.6	21.3 ±1.5	12.5 ±8.4	0[c]	0

[1]Mean ± SD.

[a,b,c]Values with different letter superscripts in columns are significantly different (P<.05).

associated with significant differences in the intradermal reactions of calves to PHA during the first 72 hours after injection. At 96 hours post-injection, the mean diameters of the induration sites of all iodine-fed calves were significantly less (P<.05) than those of the control calves. The induration sites disappeared by 96 hours post-injection in the calves fed 1250 iodine per day and by 120 hours post-injection in the calves fed 250 mg iodine per day.

The mean diameters of the areas of induration caused by intradermal PHA injections of the calves 23 weeks on experiment are presented in Table 8. The results were similar to those obtained at the 15 week period. The induration sites were significantly less (P<.05) than the control groups within 24 hours post-injection in the calves

Table 8. Diameters of induration sites following intradermal injection of phytohemagglutinin to calves fed different levels of iodine for 23 weeks

Daily Iodine Dosage (mg)	Hours after Injection				
	24	48	72	96	120
			(mm)		
0	23.2[1,a] ±2.9	22.2[a] ±2.3	13.2[a] ±2.2	10.7[a] ±1.0	0
50	23.2[a] ±3.6	17.7[b] ±2.6	11.0[a] ±1.7	5.0[b] ±4.0	0
250	23.5[a] ±3.8	17.0[b] ±3.5	6.0[b] ±4.9	1.8[b] ±2.9	0
1250	16.3[b] ±6.4	13.0[c] ±4.2	0[c]	0[c]	0

[1]Mean ± SD.

[a,b,c]Values with different letter superscripts in columns are significantly different ($P<.05$).

fed 1250 mg iodine per day, within 72 hours post-injection in the calves fed 250 mg iodine per day, and within 96 hours post-injection in the calves fed 50 mg iodine per day. The induration sites associated with the intradermal injection of PHA were nondetectable by 72 hours in the 1250 mg group of calves and by 120 hours in all other groups.

Measurements of Inflammatory Response

In vitro Phagocytosis of *Candida albicans*

The results of *in vitro* phagocytosis of *C. albicans* organisms with plasma and without plasma by WBCs of the experimental calves are listed in Table 9. The mean number of *C. albicans* organisms

Table 9. Numbers of phagocytized *Candida albicans* particles per white blood cell (with and without plasma) from calves fed different levels of iodine

Daily Iodine Dosage (mg)	Number of Calves Sampled	Weeks on Experiment: 10 with plasma	10 without plasma	15 with plasma	15 without plasma	20 with plasma	20 without plasma	23 with plasma	23 without plasma
		(phagocytized particles per WBC)							
0	6	2.02[1,a] ±.29	0.57[a] ±109	3.05[a] ±.52	1.45[a] ±.48	2.28[a] ±.16	0.82[a] ±.22	1.65[a] ±.18	0.47[a] ±.03
50	6	1.53[b] ±.13	0.32[b] ±.06	1.33[b] ±.37	0.15[b] ±.01	1.62[b] ±.33	0.53[b] ±.07	1.05[b] ±.07	0.53[a] ±.08
250	6	1.13[c] ±.15	0.28[b] ±.07	0.93[c] ±.25	0.20[b] ±.06	1.08[c] ±.66	0.42[b] ±.06	1.07[c] ±.05	0.32[b] ±.08
1250	6	0.97[c] ±.06	0.31[b] ±.06	1.05[c] ±.11	0.04[b] ±.00	1.00[c] ±.32	0.48[b] ±.07	0.60[c] ±.48	0.22[b] ±.03

[1] Mean ± SEM.

[a,b,c] Values with different superscripts within columns are significantly different ($P<.05$).

phagocytized with plasma by WBCs from the control calves ranged from 1.65 $\pm$ .18 to 3.05 $\pm$ .52 at the sampling periods between 10 and 23 weeks after the onset of the experiment. The ability of the WBCs from calves fed 50 mg iodine per day to phagocytize *C. albicans* was significantly less ($P<.05$) than the control calf WBCs at each of the sampling periods. The WBCs of calves fed 250 and 1250 mg iodine per day were significantly less phagocytic ($P<.05$) for *C. albicans* than were WBCs from the calves fed 50 mg iodine per day.

The mean number of *C. albicans* organisms phagocytized without plasma by WBCs of the experimental calves showed differences similar to those found in the presence of plasma. The one exception was at 23 weeks on experiment cells from calves fed 50 mg iodine per day phagocytized more *C. albicans* organisms than cells from the control calves. The number of organisms phagocytized without plasma was less than the number phagocytized in plasma.

The data on percent of calf WBCs phagocytizing *C. albicans* organisms *in vitro*, with and without plasma, are presented in Table 10. The percentage of WBCs with plasma from control calves found phagocytizing *C. albicans* organisms *in vitro*, at the sampling periods between 10 and 23 weeks on experiment, ranged between 73.3 and 82.0%. The percentage of WBCs from the calves fed supplemental iodine, phagocytizing *C. albicans* organisms, was significantly less ($P<.05$) than for control calf WBCs at all sampling periods. After 23 weeks on experiment, the calves fed the highest level of iodine had WBCs which were significantly less capable of phagocytizing *C. albicans* organisms than cells from calves being fed the 2 lower levels of iodine. The differences in percent phagocytosis were similar without plasma, but percentages of phagocytosis were lower.

Table 10. Percentage of white blood cells, with and without plasma, from calves fed different levels of iodine phagocytizing *Candida albicans in vitro*

Daily Iodine Dosage (mg)	Weeks on Experiment							
	10		15		20		23	
	with plasma	without plasma	with plasma	without plasma	with plasma	without plasma	with plasma	without plasma
	(percent)							
0	77.5[1,a] ±13.4	30.0[a] ±12.7	82.0[a] ±9.3	34.5[a] ±17.7	77.5[a] ±10.7	46.7[a] ±21.5	73.3[a] ±11.8	28.8[a] ±7.7
50	58.7[b] ±11.3	21.3[b] ±15.1	57.7[b] ±16.1	9.5[b] ±7.5	63.0[b] ±15.5	35.5[b] ±16.2	61.3[b] ±17.3	31.7[a] ±12.6
250	51.7[b] ±12.3	17.7[b] ±14.6	50.0[b] ±11.5	16.0[b] ±17.6	50.7[b] ±31.7	25.5[b] ±9.9	53.5[b] ±15.2	21.8[b] ±14.6
1250	49.5[b] ±10.0	17.7[b] ±12.8	56.5[b] ±5.9	2.8[c] ±2.4	54.5[b] ±19.7	26.0[b] ±11.7	29.7[c] ±33.0	14.7[c] ±10.9

[1]Mean ± SD.

[a,b,c]Values with different superscripts within columns are significantly different ($P<.05$).

In vitro Phagocytosis of *Brucella abortus*

The results of *in vitro* phagocytosis of *B. abortus* organisms, with and without plasma, by WBCs of the experimental calves are listed in Table 11. The phagocytic indices (PI) of *B. abortus* organisms by WBCs with plasma from control calves ranged from 1.39 ± .74 to 2.17 ± .44 at the sampling periods between 10 and 23 weeks after the onset of the experiment. The ability of the WBCs from control calves to phagocytize *B. abortus* was greater ($P<.05$) than the ability of WBCs from calves on all levels of iodine on all tests with the exception of that of the WBCs from calves being fed 50 mg of iodine per day at 20 weeks on experiment. At 15 and 23 weeks on experiment, the PI of WBCs for *B. abortus* from calves on the highest level of iodine was significantly less ($P<.05$) than the phagocytic indices of WBCs from calves of the other groups.

The PI of *B. abortus* organisms by WBCs (without plasma) of the control group were similar to those found in the presence of plasma at tests on 15, 20 and 23 weeks on experiment. At 10 weeks on experiment WBCs from calves receiving 250 mg of iodine per day had a significantly higher ($P<.05$) PI than WBCs from calves of all other groups.

The data on percent of calf WBCs phagocytizing *B. abortus* organisms *in vitro*, with and without plasma, are presented in Table 12. The percentage of WBCs with plasma from control calves found phagocytizing *B. abortus* organisms *in vitro*, at the sampling periods between 10 and 23 weeks on experiment, ranged between 60.7 ± 28.6 and 80.0 ± 15.9%. The percentage of WBCs from calves of the control group phagocytizing *B. abortus* was significantly higher ($P<.05$) than that from the calves on the highest level of iodine on all test dates. At 23 weeks on experiment, WBCs from calves receiving 50 mg of iodine per day

Table 11. Phagocytic indices of *Brucella abortus* organisms per white blood cell (with and without plasma) from calves fed different levels of iodine

Daily Iodine Dosage (mg)	Number of Calves Sampled	Weeks on Experiment: 10 with plasma	10 without plasma	15 with plasma	15 without plasma	20 with plasma	20 without plasma	23 with plasma	23 without plasma
		(phagocytic indices)							
0	6	$1.87^{1,a}$ ±.44	0.27^{b} ±.18	2.17^{a} ±.44	1.15^{a} ±.58	1.39^{b} ±.74	0.77^{a} ±.38	1.64^{a} ±.19	0.75^{b} ±.19
50	6	1.41^{b} ±.41	0.27^{b} ±.14	1.50^{b} ±.33	0.65^{b} ±.29	1.65^{a} ±.56	0.70^{a} ±.59	1.13^{b} ±.55	1.21^{a} ±.52
250	6	1.03^{b} ±.53	0.34^{b} ±.29	1.75^{b} ±.31	0.87^{b} ±.64	1.03^{c} ±.41	0.39^{c} ±.22	1.05^{b} ±.34	0.76^{b} ±.30
1250	6	1.24^{b} ±.41	0.29^{b} ±.23	1.08^{c} ±.42	0.36^{c} ±.25	0.89^{c} ±.51	0.61^{b} ±.41	0.68^{c} ±.36	0.43^{c} ±.23

[1]Means ± SD.

[a,b,c]Values with different superscripts within columns are significantly different ($P<.05$).

Table 12. Percentage of white blood cells, with and without plasma, from calves fed different levels of iodine phagocytizing *Brucella abortus in vitro*

Daily Iodine Dosage (mg)	Weeks on Experiment							
	10		15		20		23	
	with plasma	without plasma	with plasma	without plasma	with plasma	without plasma	with plasma	without plasma
	(percent)							
0	$78.7^{1,a}$ ±14.1	17.4^{b} ±9.5	80.0^{a} ±15.9	53.8^{a} ±26.2	60.7^{b} ±28.6	40.3^{a} ±16.0	67.7^{a} ±6.5	31.6^{b} ±7.7
50	69.3^{b} ±17.5	17.2^{b} ±8.7	73.8^{a} ±14.1	36.0^{b} ±15.2	72.7^{a} ±16.4	34.7^{b} ±25.0	50.5^{b} ±24.2	53.0^{a} ±21.9
250	48.3^{b} ±21.8	22.0^{a} ±17.3	76.0^{a} ±11.5	44.7^{b} ±22.0	51.3^{c} ±18.7	28.0^{b} ±11.5	50.0^{b} ±13.9	37.5^{b} ±13.5
1250	57.0^{b} ±16.4	20.7^{a} ±15.7	59.7^{b} ±24.2	19.8^{c} ±13.4	39.5^{d} ±19.6	31.0^{b} ±16.2	35.2^{c} ±19.2	22.3^{c} ±12.3

[1]Mean ± SD.

[a,b,c]Values with different superscripts within columns are significantly different ($P<.05$).

had a significantly greater ($P<.05$) percentage of phagocytosis than WBCs from calves of the control group. The percentage of WBCs from control calves phagocytizing *B. abortus* without plasma was significantly greater than that from calves on the highest level of iodine at the 15, 20, and 23 week tests. On the 10 week test WBCs from calves on the 2 higher levels of iodine had a greater percentage of phagocytosis of *B. abortus* than cells from control calves.

Results of the WBC counts done on the *in vitro* phagocytosis test dates are listed in Table 13. The control group calves and calves on the 50 mg level of daily iodine intake had significantly higher ($P<.05$) WBC counts than calves on the 2 higher levels of iodine intake.

Table 13. Means of white blood cell counts of calves fed different levels of iodine

Daily Iodine Dosage (mg)	Weeks on Experiment				Mean of Four Counts
	10	15	20	23	
0	11,616[1] ±2,670	10,650 ±2,536	9,517 ±1,683	12,900 ±2,475	11,171[a]
50	11,033 ±2,114	8,833 ±1,347	12,450 ±3,478	11,500 ±1,950	10,954[a]
250	10,300 ±1,774	8,717 ±618	10,967 ±1,317	11,533 ±2,667	10,379[b]
1250	8,433 ±1,515	9,667 ±3,318	10,517 ±4,172	8,600 ±1,314	9,304[c]

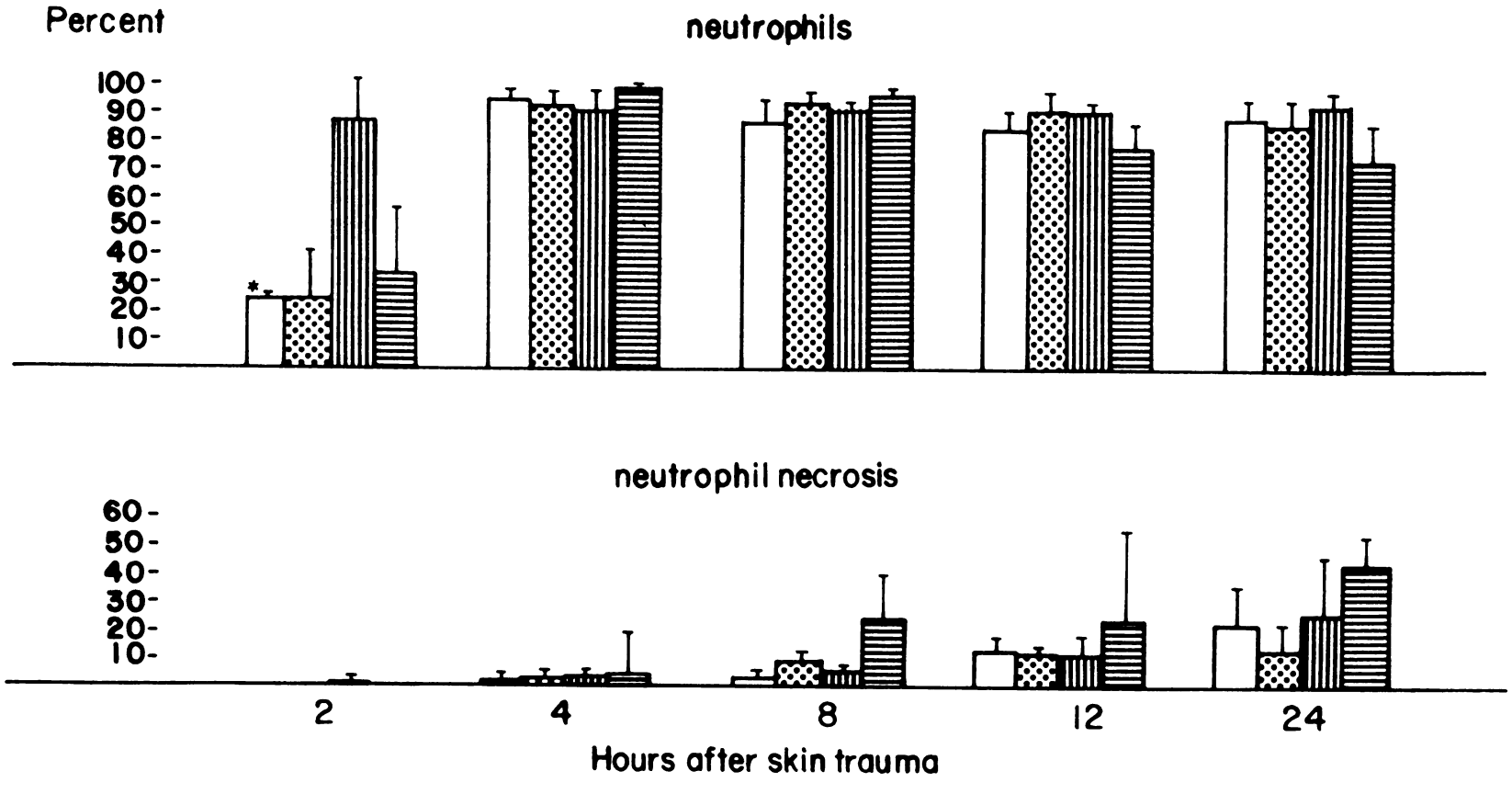
[1]Mean ± SD.

[a,b,c]Values with different superscripts are significantly different ($P<.05$).

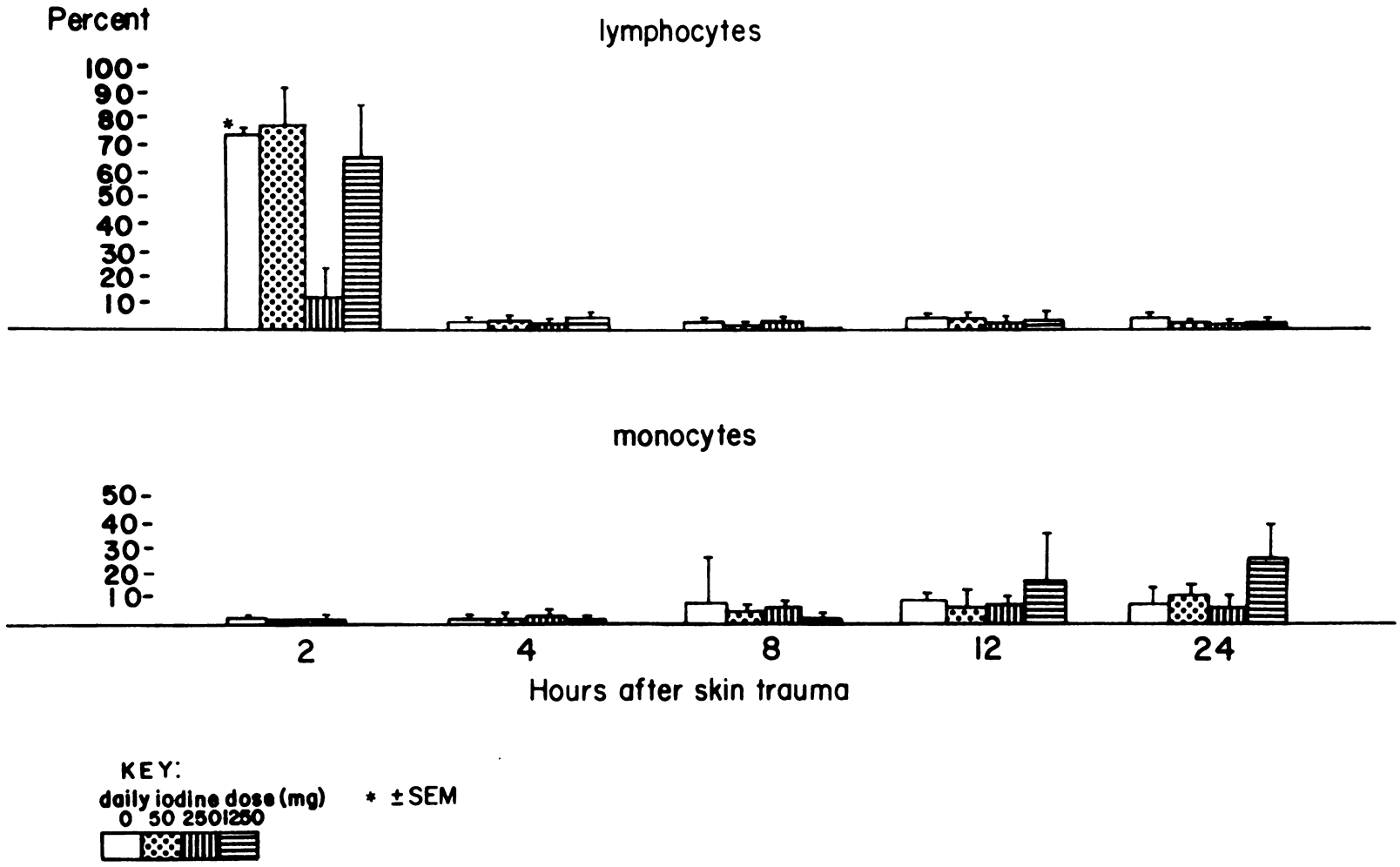
Skin Windows

The results of the skin window tests done at 18 and 23 weeks on experiment are listed in Graphs 1 through 4. There are no significant differences in percent neutrophils or percent lymphocytes in either test. There were significant differences ($P<.05$) in percent macrophages at 24 hours in both tests, higher in the group consuming 1250 mg iodine daily than in all other groups. There was a significantly ($P<.05$) higher percent neutrophil necrosis in the group consuming 1250 mg iodine daily in the test at 18 weeks on experiment. There were no significant differences in percent neutrophil necrosis in the test at 23 weeks on experiment.

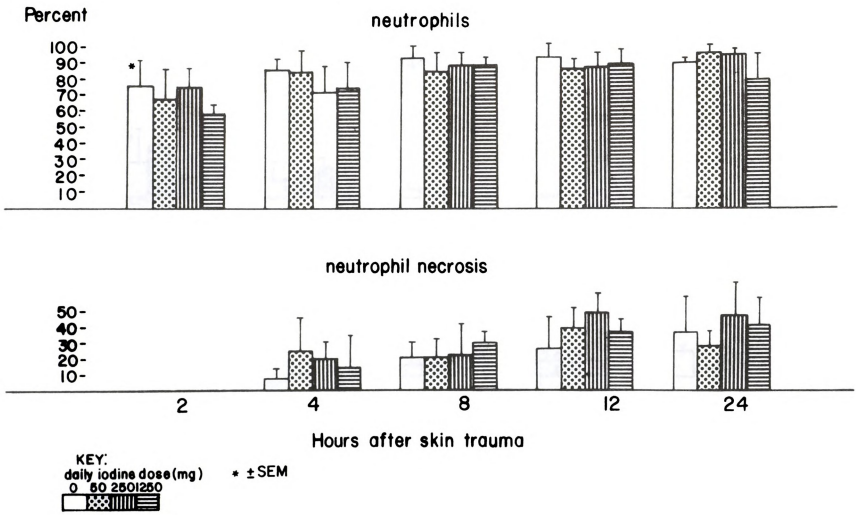
Graph - 1 Percent Neutrophils and Neutrophil Necrosis in Skin Windows After 18 Weeks on Test



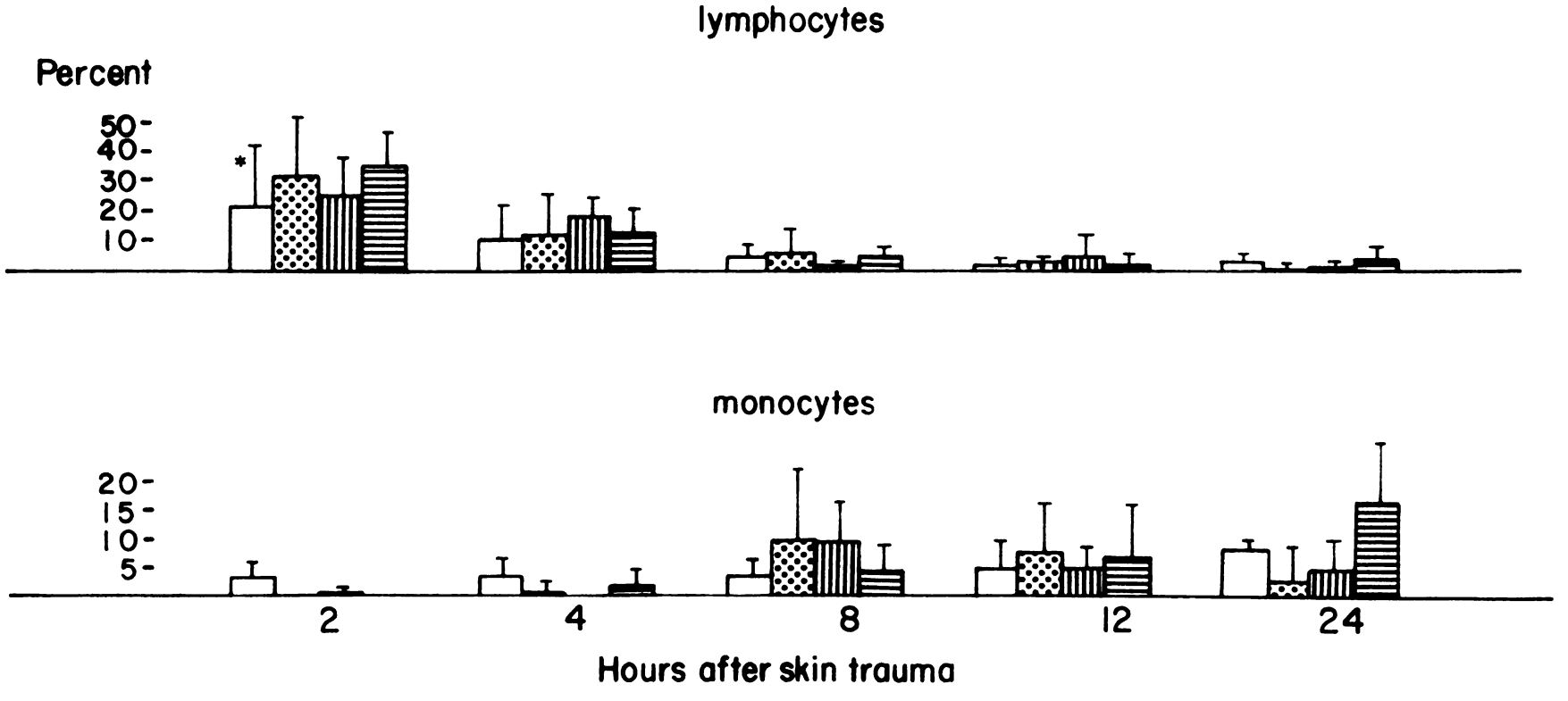
Graph - 2 Percent Lymphocytes and Monocytes in Skin Windows After 18 Weeks on Test



Graph-3 Percent Neutrophils and Neutrophil Necrosis in Skin Windows After 23 Weeks on Test



Graph 4 Percent Lymphocytes and Monocytes in Skin Windows After 23 Weeks on Test



DISCUSSION AND CONCLUSIONS

The effect of iodine on the brucellosis antibody titers only occurred in calves on the highest supplemental level of iodine (1250 mg/calf/day). The results indicate there may be a decrease in ability to maintain antibody titers and a slower anamnestic response to this attenuated live bacterial antigen in calves fed this level of iodine. This finding is in contrast with that of Forbes et al. (1932), who found no change in antibody titers of brucellosis infected cows fed up to 2.13 grams of iodine as iodized linseed meal per day. The effect of iodine on the leptospirosis antibody titers also indicates a decreased ability to maintain anamnestic antibody titers. This response was observed in calves being fed all levels of supplemental iodine. The lowest level of iodine (50 mg/calf/day) is a level fed over long periods of time for the prevention of infectious pododermatitis in calves (Miller and Swanson, 1972). There were no significant differences in infectious bovine rhinotracheitis (IBR) titers associated with iodine intake following two vaccinations with a modified live virus vaccine.

These findings indicate high levels of dietary iodine may enhance antibody decay and depress the anamnestic response to some antigens. Differences were observed in titers to attenuated live bacterial (brucellosis) and bacterin (leptospirosis) antigens. There were no differences observed in titers to the modified live virus vaccine

(IBR). Further investigation using greater numbers of animals may be necessary for substantiation of these findings and determination of the site of the depressive effects of iodine. These changes may be caused by decreased lymphocyte mitosis, decreased plasma cell receptivity, or a direct effect on the antigen.

The effect of iodine on the mitotic activity of lymphocytes stimulated by mitogens suggests that on exposure to an antigen or infectious agent calves consuming high levels of iodine for several months would have fewer lymphocytes to react with the agent. The effect is the same on cells stimulated by phytohemagglutinin (PHA) as on cells stimulated by pokeweed mitogen (PWM). Janossy and Greaves (1971) stated that responsiveness to PHA was predominantly a property of thymus dependent lymphocytes (T cells) which are functional in cell-mediated immunity. They also stated that responsiveness to PWM was primarily a property of thymus-independent (B cells) lymphocytes which are functional in humoral immunity. In either case the decreased mitotic activity of lymphocytes would provide fewer of these cells to react with the infectious agent. These investigators also suggested that the morphologic and biochemical characteristics of mitogen-induced lymphocyte response *in vitro* are very similar to antigen-induced immune reactions *in vivo*. The decreased number of lymphocytes could be a factor in decreased resistance to infection and decreased response to treatment of cattle on high levels of iodine reported by other investigators (McCauley et al., 1972).

The effect of iodine on the persistence of induration following intradermal injection of PHA indicates a decreased function of the cell-mediated immune system in the calves given all levels of iodine.

There were no significant differences between the control group and the groups fed the two lower levels of iodine at 24 hours post-injection, the time periods used in testing human cell-mediated immune function (Lawlor et al., 1973). The differences in diameters of intradermal induration occurred at 96 hours in this experiment. These differences correlate with the differences found in the *in vitro* mitotic activity of PHA mitogen stimulated lymphocytes as has been described by Lawlor et al. (1973). Blaese et al. (1973) reported that PHA skin testing may provide useful information in the evaluation of cellular immunodeficiency in infants and children although PHA skin testing was not usually effective in indicating these deficiencies in adult humans. This work indicates that the PHA intradermal test could be used as a screening test to evaluate cellular immune competence in calves.

The demonstrated effect of iodine on the phagocytic activity of white blood cells suggests an effect on the white blood cell and not on the complement system or opsonins. There was a direct relationship between the level of iodine fed and the decrease in number of phagocytized particles. There was also a direct relationship between the level of iodine fed and the decrease in percent phagocytosis. The total number of phagocytized particles and the percentage of cells active in phagocytosis was decreased without serum but the relative differences between the groups were the same as with serum. These findings are at variance with those of Kolmer et al. (1916), who reported that iodides may stimulate leukocytes and facilitate the phagocytosis of particles. The decreased white blood cell counts from calves on the two highest levels of iodine indicate a quantitative

effect, there being decreased numbers of white blood cells as well as decreased cellular function. Further work could differentiate between the phagocytic cells (polymorphonuclear cells and monocytes), which was not done in this experiment. The short (30 minute) period of incubation is actually a measurement of initial particle uptake. It was reported by Stossel and Taylor (1976) that to also measure phagocytic capacity there must be a sufficiently long incubation period and sufficiently high organism concentration to allow the cells to surfeit themselves. Further and more involved work would also be necessary to determine the efficiency of intracellular killing of the microorganisms. This would be a determination of intracellular enzyme function which might possibly be interfered with or affected by iodine toxicity.

The demonstrated effect of iodine on cutaneous inflammation suggests a decrease in inflammatory response in calves on the highest level of iodine. The changes were not constant, but there was an increase in percent macrophages at 24 hours in lesions on calves consuming the highest level of iodine in both tests. The increased percentage of macrophages could be caused by the necrosis of polymorphonuclear cells and a decrease in their number. There was increased neutrophil necrosis in lesions at 24 hours on calves consuming the highest level of iodine in one test. Further work is necessary to determine the reliability of skin windows for interpretation of inflammatory response in cattle. These results differ from those of Rebuck and Crowley (1955), who found increased lymphocytes in skin lesions on normal human subjects at 6, 9 and 12 hours after origination of cutaneous lesions.

There were several possible sites of complications in the interpretation of this work. Blood samples used in lymphocyte blastogenesis studies were shipped via air freight from Lansing, Michigan, to St. Paul, Minnesota, for determinations. Though the blood samples were packed in an insulated box, possible differences in temperature and time allow only comparison between groups at the time of each test. Comparison of results between different tests would not be valid. Varying environmental temperatures and possible differences in time from bleeding to completion of laboratory procedures could be a factor in variability of results between different test dates in the WBC *in vitro* phagocytosis work. Skin window tests were performed on 24 animals. Time variability in slide preparation could be the reason for the difference in percentages of neutrophils and lymphocytes from lesions on calves in group 3 observed on the 2-hour slides in the first test.

This work indicates that excessive dietary iodine intake can cause a decrease in number and functional capability of white blood cells in calves. These decreases could be a factor in decreased resistance to infection. Stossel and Taylor (1976) stated that susceptibility to infection is as much determined by the exposure to the pathogen as by the status of the host and clinical presentation of patients with neutrophil abnormalities may vary enormously. Clinical case reports of amounts of iodine greater than those used in this project are being fed to cattle. If a veterinarian should experience increased infection and decreased response to treatment in a herd of cattle, it may be beneficial to consider and investigate the iodine consumption of that herd.

SUMMARY

Forty Holstein heifer calves were used to determine the effects of iodine on several biological defense mechanisms. The calves were divided into 4 groups which were dosed with 0, 50, 250 and 1250 mg of supplemental iodine per head per day.

Determinations included antibody titer measurements at intervals following 2 vaccinations for brucellosis, leptospirosis and IBR; measurement of thymidine uptake of lymphocytes stimulated to mitose using PWM, PHA and CON-A mitogens; intradermal response to PHA injection; *in vitro* phagocytosis by WBC; WBC counts; and cellular response to cutaneous inflammation.

The brucellosis titers of calves of the control group and the 2 lower levels of iodine were significantly higher 10 weeks following the first vaccination and 1 week following the second vaccination than those of calves dosed with 1250 mg of iodine per day. The leptospirosis titers of control calves and calves on the 2 lower levels of iodine were significantly higher 9 weeks following the second vaccination than titers of calves dosed with 1250 mg of iodine. Eleven and one-half weeks following the second vaccination, leptospirosis titers of control calves were significantly higher than those of calves on all levels of iodine. There were no significant differences between group IBR titers from 1 week following the first vaccination to 11-1/2 weeks following the second vaccination. These results indicate that high levels of dietary iodine may have a

deleterious effect on the maintenance of antibody titers to some antigens.

In all lymphocyte blastogenesis tests, with the exception of the CON-A mitogen test at 7 weeks on experiment, lymphocytes from calves of the control group demonstrated more mitotic activity than lymphocytes from calves on the highest level of iodine. These results suggest possible decreased DNA synthesis by lymphocytes from calves on the highest level of iodine.

The diameters of induration sites on control calves 96 hours following intradermal injection of PHA were greater than those on calves consuming all 3 levels of iodine. The differences of this test were comparable to those of *in vitro* mitogen stimulation of lymphocytes which indicates that the intradermal PHA test could be used as a screen test for cell-mediated immune function in calves.

White blood cells with plasma and without plasma from calves of the control group demonstrated greater *in vitro* phagocytic activity than WBC from calves on all levels of iodine. The phagocytic activity of WBC from calves on all levels of iodine was less without plasma, but the proportional differences between groups was the same as with plasma. It was concluded that high levels of dietary iodine decrease the phagocytic activity of WBC of calves. The white blood cell counts of calves dosed with the 2 higher levels of iodine were less than control calves and calves dosed with 50 mg of iodine per day.

Skin window tests of response to cutaneous inflammation had increased macrophages and increased necrosis of polymorphonuclear cells present in lesions on calves consuming 1250 mg of iodine daily. Further work is necessary to determine the value of skin windows in measuring inflammatory response in calves.

This work indicates that excessive dietary iodine can depress humoral immunity, cell-mediated immunity, and inflammatory responses in calves.

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