THE EFFECTS OF ADMINISTERED INDIGENOUS MICROORGANISMS ON UPTAKE OF ^{125}I-GAMMA GLOBULIN IN *IN VIVO* INTESTINAL SEGMENTS OF NEONATAL CALVES

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

INTRODUCTION

Serum of the newborn calf contains only traces of humoral immunity. Immunity is acquired only after ingestion and absorption of colostral antibodies via pinocytosis by villus epithelial cells of the small intestine. The success of transfer of this immunity is important to calf health and development. Calves failing to absorb adequate quantities of colostral immunoglobulins (Ig) are predisposed to digestive and respiratory infections frequently leading to death or unthriftiness in later life. Absorption of colostral Ig occurs during the first 24 to 48 hours (h) of life. The age of the calf at the first colostral meal, amount of colostrum fed and the concentration of Ig in colostrum are important factors influencing the success of Ig transfer. Usually adequate absorption occurs when at least 3 to 4 kilograms (kg) of colostrum are fed within the first 24 h of life. In spite of early consumption of adequate quantities of colostrum, it is estimated that 10 to 20% of all calves born alive fail to attain adequate levels of immunity.

Cessation of Ig absorption in the neonate, termed closure, has been associated with increasing digestive activity, hormonal factors and the intestinal microflora. The role of abomasal pH or intestinal enzymatic activity in closure appears to be of minor importance. Isolation of the intestinal absorptive surface from the digesta flow or

administration of enzymatic inhibitors does not prolong absorption beyond 36 h of age.

In species of mammals receiving immunity via absorption of colostral Ig, a substantial increase in serum glucocorticoids precedes closure by 24 to 36 h. In the calf, maximal glucocorticoid concentration occurs at birth with completion of closure 24 to 36 h later. Furthermore, if the dam receives exogenous corticoids for a prolonged period of time preceding parturition, Ig absorption is impaired in the offspring. In the rat, maximal adrenocorticoid concentration occurs at day 18 with completion of closure by day 21. Administration of corticoids during the first or second week of life results in premature closure.. Conversely, adrenalectomy delays the onset of closure by several days. Thyroid hormone or other hormones may be involved in initiation of closure. It appears that glucocorticoids either induce a change in permeability of existing epithelial cells or signal the crypts of Lieberkuhns to produce epithelial cells incapable of macromolecular absorption.

Although a hormonal relationship with closure has been suggested, the fact that bilateral adrenalectomy does not prevent closure suggests involvement of other factors in closure. Comparisons of germ-free animals with animals exposed to environmental microorganisms demonstrates that intestinal microflora has a considerable influence on epithelial cell integrity and function, villus morphology, epithelial cell replacement rate and cell differentiation. Since calf intestine is sterile at birth, the manner of microbial colonization may have a pro-

found effect on absorptive capabilities. A relationship of early intestinal microflora with Ig absorption was suggested in studies where newborn calves receiving an inoculum of nonpathogenic intestinal bacteria possessed lower gamma globulin of serum than similarly managed uninoculated calves. The intestinal microflora might have influenced gamma globulin absorption by altering permeability of individual epithelial cells or by accelerating replacement of cells capable of macromolecular absorption by impermeable cells.

Considering the suboptimal calving conditions present on many farms and delayed colostrum feeding, it is likely that the gut might be colonized by undesirable microorganisms which would have considerable opportunity to interact with intestinal absorptive surface.

Therefore, the objectives of these experiments were to: 1) determine the distribution of uptake of gamma globulin in small intestine, and 2) measure the influence of known quantities of administered intestinal microorganisms on gamma globulin uptake.

LITERATURE REVIEW

It is generally accepted that there is little placental transfer of Ig from the dam to the fetus in the bovine (8). Therefore, the bovine is hypogammaglobulinemic at birth (86). Passive transfer of Ig from maternal colostrum to the neonatal calf by intestinal absorption is essential for immediate protection until the calf is capable of responding to antigenic stimuli. Several studies have shown a relationship between the failure to absorb adequate amounts of colostrum antibodies with neonatal diseases, chiefly diarrhea and septicemic infections (66, 72).

Colostrum Ig are absorbed unchanged by the intestinal epithelium for a period of 24 to 36 h after birth (8, 28). Studies by Kruse (50, 51, 52) indicated that serum Ig concentration during the first 24 h after colostrum feeding was a function of mass of Ig fed to the calf, age at colostrum feeding and birth weight of the calf. The two predominant factors were the mass of Ig fed and the age at feeding. Absorption was reduced linearly to about one-half by delaying colostrum feeding from two to 20 h. Birth weight was negatively related to gamma globulin absorption efficiency (8, 51). These findings reflect current recommendations that at least 2 kg of first milking colostrum be fed within the first 6 h of life (52).

Events Associated with Macromolecular Absorption

The region of the small intestine predominantly responsible for absorption of colostrum gamma globulin is uncertain. El-Nageh (26) has presented histological evidence that absorption of gamma globulin occurs mainly in the jejunum rather than the duodenum or ileum. Fluorescein-labelled gamma globulin was observed entering the lymphatics in jejunal tissue but not in duodenal or ileal tissue. However, Hardy (34) found that calves did not absorb ^{131}I-gamma globulin from colostrum whey infused through a loop of upper jejunal intestine. Comline et al. (18) found that absorption of intact protein from colostrum was restricted to the terminal small intestine as evidenced by the presence of large quantities of invacuolated protein within ileal epithelial cells. Staley et al. (84) examined the ultrastructure of neonatal calf intestine and absorption of heterologous proteins. His findings concurred with those of Hardy (34) and Comline et al. (18) in that cells of the ileum were most active in uptake of Ig G conjugated to ferritin. Upon injection of ferritin-Ig G into ligated loops of terminal ileum tissue, the protein was found in close association with the microvilli. After pinocytosis, ferritin-Ig G was observed entering the apical tubules that eventually terminated in vacuoles. Two to 6 h later, these vacuoles were located adjacent to the basal membrane of the villus epithelial cell. In contrast, ferritin-Ig G was restricted to the apical tubules of epithelial cells located in the mid-jejunum. The ferririn-Ig G conjugate was never found within the lamina propria or blood indicating that the

protein was not absorbed. Unconjugated Ig G was absorbed by adjacent loops of ileal tissue and transported into the blood. Staley also found that epithelial cells within the crypts and lower portion of the villus did not possess an apical tubular complex capable of macromolecular absorption. Macromolecular absorptive ability developed during migration of the epithelial cells up the villus. These studies (17, 26, 34, 84) indicated that the route of absorption was pinocytosis, entrance into the apical tubular complex, encapsulation within vacuoles, migration of the vacuole to the basal membrane and extrusion of entrapped globulins to the extracellular spaces. Upon extrusion from the epithelial cell, the globulins enter the villus lacteal and pass to the thoracic duct where they enter the blood 1 to 2 h after absorption (5, 8, 17). Contrary to previous findings (8, 34), the calf does appear to exhibit some selectivity in macromolecular absorption. Evidence for this was shown in findings of Staley et al. (84) that ferritin or ferritin-Ig G did not pass out of the epithelial cell to the lamina propria.

Factors Influencing Macromolecular Absorption

Certain compounds present in colostral whey are necessary for absorption of Ig by small intestine. Macromolecules presented to the absorptive surface in an electrolyte solution of similar composition to colostrum are internalized but are not transported out of the villus epithelial cell (5, 34). Balfour and Comline (5) observed that colostral factors required for absorption were a small molecular weight

protein, inorganic phosphate and glucose-6-phosphate. The addition of sodium chloride delayed absorption of globulins from test solutions or colostrum whey. Hardy (34) observed a stimulation of absorption by other compounds in colostrum whey such as pyruvate, lactate and salts of volatile fatty acids, especially potassium isobutyrate. However, these active compounds were found at much lower levels in fresh colostrum. It appeared that these factors were not necessary for uptake but acted to facilitate transport out of the cells, possibly by providing metabolic energy for the absorptive process.

Macromolecular Absorption in Pigs and Rats

The ultrastructural events associated with macromolecular absorption in the pig, rat and mouse have been more thoroughly studied than in the calf. These studies demonstrate striking differences between species in relation to region of optimum absorption and selectivity of absorption. In the pig, the region of optimal absorption appears to be the jejunum and duodenum with little absorption occurring in the ileum (83). Absorption occurs for the first 24 to 36 h of life in normally reared piglets. As in the calf, little specificity is observed in pinocytosis of macromolecules by pig intestine. Polyvinyl pyrrolidone (PVP) (M. W. 160,000) (35) and heterologous Ig (11) are readily pinocytosed and absorbed into the blood by neonatal pigs. In addition, presence of colostrum in the gut lumen stimulates development of the apical tubular complex required for macromolecular absorption. Pathways

involved in Ig absorption appear to be similar to that in the calf (83). Macromolecular absorption is dependent on factors contained in sow colostrum (35). However, neither phosphate, lactate, pyruvate nor volatile fatty acids, which were effective in the calf (5, 34), accelerated absorption in the pig.

Rats and mice differ considerably from the pig and calf. In these species there is both transplacental transfer of immunity and macromolecular absorption of colostrum Ig (77). Absorption of Ig occurs for the first 18 to 21 days of life (32). Ultrastructurally, absorption is best understood in the rat. Colostral Ig attaches to the glycocalyx in a non-selective manner. Upon pinocytosis, protein molecules enter and pass through the apical tubule system, ending in encapsulation within vacuoles (37, 77). These vacuoles move to the lateral or basal cell membrane and are believed to diffuse out of the cell to intercellular spaces (38). The rat exhibits selective macromolecular absorption (38, 68, 69, 77). Hemmings and Williams (37, 38), Morris and Morris (68, 69) and Rodewald (77) agree that the duodenum shows varying degrees of selectivity in preference of homologous Ig. However, there is considerable disagreement concerning the site within the cell where selectivity is expressed. Rodewald (77), using ferritin-labelled Ig, theorized that selectivity occurred upon pinocytosis. Homologous Ig became attached to receptor sites on the apical plasma membranes. After pinocytosis, the Ig are segregated within tubular vesicles and transferred to spherical, coated vesicles. These vesicles discharged the Ig at the lateral cell membrane by emieocytosis. Hemmings and Williams (37, 38) found no evidence of

selectivity during pinocytosis. He theorized that selectivity occurred in the extrusion of only homologous protein at the basal or lateral cell membrane. In these studies (38, 68, 69) both proximal and distal areas of the small intestine actively pinocytosed protein with transport in the duodenum appearing to be more efficient. Morris and Morris (68, 69) contended that selectivity was related to lysosomal degradation of heterologous proteins after pinocytosis within the cell. However, Jones (46) found that intraluminal degradation of colostral Ig occurs during transit through the small intestine. He observed that the greater part of the protein absorbed by the ileal tissue was of altered immunologic character, indicating degradation prior to absorption. This finding might account for the appearance of protein degradation products in the blood. It is obvious that certain similarities in the macromolecular absorptive process exist between speices. Differences are chiefly in regard to selectivity of absorption and in the region of intestine predominantly responsible for Ig absorption. It appears that the mechanisms of macromolecular absorption are similar across most species studied.

Closure

The termination of macromolecular absorption by intestinal epithelial cells in the mammalian neonate is termed closure. In the calf closure appears to be a one-step process, where uptake and transport of Ig out of the cell cease simultaneously by 24 to 36 h of age (26, 27).

Closure in the rat is also a one-step process beginning on day 18, with complete cessation of uptake and transport by day 21 of life (32). In the pig closure is a two-step process. Transport of Ig out of the intestinal cell to the lacteal ceases by 24 to 36 h of age in colostrum-fed pigs (76). However, uptake of macromolecules continues for a period of up to 18 days (63). Closure occurs in a proximo-distal direction with uptake ceasing in duodenal enterocytes shortly after birth, in the jejunum at about five to 10 days of age and in the ileum by 18 days of age (57, 63).

The onset of closure is both detrimental and advantageous to the neonate. It may be detrimental from the standpoint of not acquiring sufficient immunity from colostrum due either to delayed feeding or to factors which might shorten the absorptive period to less than 24 h. Closure is advantageous since apparently absorption of infectious agents may occur (19). What is of greater concern in the calf is the shortening of the absorptive period and the effect on transfer of passive immunity to the neonate.

Theories on the Causes of Closure

Onset of closure has been associated with the development of gastric and enzymatic function in the newborn, reduction in the permeability of individual epithelial cells on the villus, or replacement of the villus epithelium with a generation of cells incapable of pinocytosis of Ig.

Early workers theorized that closure was related to development of gastric and enzymatic activity in the newborn. Hill (39) measured pH in the abomasum of newborn lambs and found pH 7.0 two h after birth, pH 6.2 at 19 h, pH 4.0 at 24 h, pH 3.0 at 72 h and pH 2.0 five days after birth. Fey (28) found abomasum pH of eight experimental calves to range from pH 5.9 to 7.2 during the first 20 h of life. Owing to the high pH, it was believed that abomasal peptic activity would be low during this period of time and consequently little degradation of colostrum Ig would occur (54). However, Deutsch and Smith (24) failed to observe prolonged absorption of globulin after neutralization of gastric proteolysis by aluminum hydroxide administration. Smith et al. (81) ligated the duodenum distal to the abomasum, thus preventing gastric juices from entering the intestine. Globulins injected into intestines of six- and 18-h-old calves were normally absorbed but not in calves 48 or 60 h of age. They deduced that closure had no relationship with development of gastric enzymatic activity. Fey (28) examined the hypothesis that the fraction crystalizable (Fc) piece of Ig was required for binding to surface receptor sites on intestinal epithelium in order for absorption to occur. If sufficient intraluminal degradation had occurred, the Ig fraction containing the antibody binding sites (Fab) theoretically would not be absorbed. Using fractions containing only Fab fragments, Fey observed that these fragments were absorbed. Therefore, the Fc piece of Ig was not necessary in the sense of a receptor for uptake by epithelial cells. This work disproved the necessity of receptor sites and need for intact protein structure for pinocytosis

and transport of macromolecules out of the epithelial cell.

It appears that abomasal pH and enzymatic activity are not responsible for cessation of macromolecular absorption.

A brief description of small intestinal mucosa aids in visualizing the remaining hypotheses concerning mechanisms of closure. Intestinal mucosa consists of tubular crypts of Lieberkuhn in continuity with villi. Crypts are production zones where cell mitosis occurs. From them, epithelial cells migrate out and up the villi to extrusion zones where they are shed into the intestinal lumen (20). Two theories with greatest support are that closure is related to replacement of permeable cells by a generation of cells incapable of macromolecular absorption or to a change in permeability of existing cells in the villus.

Histological studies (15) of rat intestine have suggested that rapid decline in macromolecular uptake occurring between the 18th and 21st day of age consists of progressive reduction in the ability of small intestine to take up macromolecules. Macromolecular uptake by portions of small intestine is positively correlated with the extent to which the epithelium of villus is vacuolated (11, 14). Clarke and Hardy (15) later observed progressive displacement of vacuolated epithelial cells from the crypts towards the top of the villus. Autoradiographic estimates of turnover time of intestinal epithelium using tritiated thymidine revealed good agreement between time required for complete replacement of ileal epithelium (62 h) and duration of decline in macromolecular uptake (approximately 3 days) (20). Therefore, it was concluded that near the 18th post natal day there is a change in

functional characteristics of the apical plasma membrane of cells produced by crypts of Lieberkuhn in the distal small intestine. The stimulus is unknown, possibly hormonal, but epithelial cells subsequently produced in crypts are no longer capable of pinocytosing macromolecules. Support for this theory has been found in calves. El-Nageh (27) observed that all epithelial cells from the base to the tip of the villus actively pinocytosed fluorescently labelled gamma globulin in the 6-h-old calf. However, only cells at the villus tip continued to pinocytose gamma globulin in the 53-h-old calf. These findings suggest that, as in the rat, closure is related to replacement of epithelium by a generation of impermeable cells.

Other workers (10, 56, 78) theorize that closure is related to an alteration in permeability of the apical plasma membrane. Lecce et al. (59) found that closure in piglets was diet-dependent. Nursing piglets were unable to absorb macromolecules by 24 h of age. Conversely, neonatal piglets starved from birth retained the ability to pinocytose macromolecules for at least 86 h (58). Closure was induced by feeding 300 to 400 ml of colostrum or 300 mM glucose. Closure activity was located in a protein- and fat-free fraction of boiled colostrum whey (58). Electron microscopic examination of jejunal epithelium from neonatal pigs fed either colostrum or glucose revealed a stimulation of intense pinocytotic activity of intestinal epithelium (10). These studies suggested that closure occurred when the apical plasma membrane had been used up.

Rundell and Lecce (78) failed to observe the relationship of re-

placement of epithelium and closure in rabbits, hamsters or guinea pigs. However, they did concur with Clarke and Hardy (15) in observing replacement of distal intestinal epithelium between days 18 and 21 of age corresponding with onset of completion of closure. The rabbit (8), hamster (8) and guinea pig (16) actively pinocytose macromolecules but lack capabilities to transport measurable quantities of internalized Ig out of the epithelial cell. Since these species receive little immunity post partum via colostrum, it may not be justified to use these species for study of the macromolecular absortive process.

The theory that closure is related to depletion of the apical plasma membrane has a fault in that pinocytotic activity in the piglet does not cease by 24 to 36 h (55, 63). Ileal tissue actively pinocytoses macromolecules for the first 3 weeks of life (63). The process of blocking macromolecular absorption is not pinocytosis but transport of macromolecules out of the cell to the lymphatics, which is not supportive of this theory. Replacement of epithelium by a generation of cells incapable of pinocytosis or transport is more tenable based on studies conducted thus far.

Disparity in the hypotheses of closure (15, 58, 59) may be related to species or differing mechanisms of closure in different regions of the gut. Depending on experimental conditions, closure may occur as result of both a decrease in permeability of individual cells and to replacement of intestinal epithelium.

Factors Associated with Onset of Closure

Glucocorticoid hormones have been linked with onset of closure. Halliday (33) found that premature closure could be induced by administering deoxycorticosterone acetate either parenterally or orally. Closure was complete within 3 to 6 days. In neonatal rats serum glucocorticoid concentration reaches a maximum on day 18 of life followed by a decline in macromolecular absorption to nearly zero by day 22 (21). Bilateral adrenalectomy in 18-day-old rats results in depressed serum levels of corticosterone and extends the period of macromolecular absorption by approximately 4 days (23).

A similar relationship between decreased pinocytosis and increasing levels of plasma cortisol has been observed in rabbits 18 to 21 days after birth. Plasma cortisol is highest in the guinea pig at birth with complete closure 48 to 72 h later (61).

Structural changes associated with corticoid-induced closure in rats are similar to those normally occurring between day 18 and 22 of life (22). Daniels et al. (22) compared closure induced by administration of exogenous corticosterone acetate to cortisone acetate-induced closure. Rats receiving cortisone acetate ceased to internalize PVP 4 to 6 days after injection. Closure was associated with progressive replacement of epithelium by nonvacuolated impermeable cells. Corticosterone acetate-induced closure was transient, lasting only several days post injection and was not associated with any changes in histologic appearance by light microscopic examination. The time course of

cortisone-acetate-induced closure was longer than that normally occurring in untreated rats. This was not unexpected owing to longer villus epithelial cell turnover time of 7 days in 9-day-old rats as compared to 3 day turnover time in weanling rats (49). It appears that effect of corticoid hormones or closure may be twofold: 1) increasing cortisone levels may reach a threshold after which crypts no longer produce cells capable of macromolecular absorption, and 2) rising corticosterone levels may alter permeability of cells already occupying the villus. However, the glucocorticoids used in these studies produced unphysiologically high levels within the blood.

Newborn puppy intestinal epithelial cells are capable of macromolecular absorption during the first 24 h of life (30). Gillette and Filkins (30) found that puppies born from bitches treated 24 h prepartum with hydrocortisone or ACTH exhibited reduced antibody absorption. Progesterone, hydrocortisone or metopirone administered to puppies prior to antibody administration failed to affect absorption.

A relationship of glucocorticoids and Ig absorption has also been observed in the calf. Plasma cortisol concentration is highest (121 ng/ml) in the calf at parturition, decreasing rapidly by 12 h to 50 ng/ml and then slowly to 11 ng/ml by the 12th day of life (25). Peak corticoid levels are followed by closure 24 to 36 h later - similar to other mammalian species.

Injection of pregnant cows in the last 2 months of gestation with 40 mg of dexamethasone trimethylacetate induced early parturition and

reduced intestinal absorption of gamma globulin in the resulting calves. In addition, age of calves at onset of endogenous production of Ig A was delayed (42). Later work by Muller et al. (70) using dexamethasone and Langley and O'Farrell (53) using betamethasone to induce parturition failed to show differences in gamma globulin absorption between calves born of treated or untreated cows. A difference in these studies may be due to length of time the dam and calf were under influence of added corticoids since parturition does not occur until 14 days after dexamethasone trimethylacetate administration (4). Parturition occurs 30 to 50 h after dexamethasone administration (70) and 62 h after betamethasone administration (53).

The aforementioned studies have shown a relationship of endogenous levels of corticoids with onset of closure. However, closure is not entirely dependent upon corticoid stimuli since bilateral adrenalectomy does not prevent closure. Experimental induction of closure has been successful only through use of unphysiologically high levels of corticoid hormone. These observations suggest that other factors are acting on villus epithelium to terminate macromolecular absorption.

Integrity of intestinal mucosa, villus morphology and cell differentiation play an important part in intestinal absorptive function (47). Considering that calf intestine is sterile at birth (79, 80), the importance of type of microbial colonization on colostrum Ig absorption may be underestimated. It is likely that any factor altering rate of cell production in crypts of Lieberkuhn, cell migration up the villus or desquamation of cells from villus tips will have considerable influence

on absorptive capabilities (47). This is most evident in comparing germ-free animals with conventional animals allowed exposure to environmental microorganisms (1, 2, 60). Absence of microorganisms is associated with shallower crypts of Lieberkuhn, thinner mucosa and decreased rate of cellular renewal of ileal epithelium (1, 2). An increased renewal of villus epithelium might result in failure of cells to fully differentiate into a functional state upon reaching the upper villus. Experiments on initially germ-free piglets showed step-wise degradation of villus morphology with increasing microbial contamination (48). Uniformly symmetric finger-shaped villi existed in monocontaminated piglets while villi with considerable reduced height relative to crypt depth and fusing or clubbing of villi predominated in conventional piglets.

Mode of action of intestinal microflora on epithelial surface might be a pharmacological influence of toxic amines, phenols, ammonia or other bacterial products. By toxic and irritative actions, turnover rate is likely to increase, leading to distorted villus morphology and altered cell function (47).

There is also evidence for considerable interaction of intestinal microflora with absorptive surface of individual epithelial cells. Corley et al. (19) observed microbial attachment, exfoliation of microvilli and intracellular penetration of ileal epithelium when Escherichia coli 055 was administered to newborn colostrum-deprived calves.

A relationship of early intestinal microflora with absorption of colostrum gamma globulin was suggested in studies in Virginia (44, 45). Feeding intestinal fluid, as a source of microorganisms, with the

first colostrum meal to newborn calves resulted in lower gamma globulin of serum as compared to calves not receiving intestinal fluid (44). A second experiment (45) compared serum protein components of calves receiving colostrum and intestinal fluid concurrently, intestinal fluid followed 3 h later by colostrum and colostrum only. Total serum protein concentration was lower in calves receiving the intestinal fluid as compared to control calves. Calves receiving colostrum 3 hours after inoculum were lowest in serum protein, beta globulin and gamma globulin. These studies indicated a relationship between the early intestinal microflora and absorption of colostrum globulins.

Considering the unfavorable calving conditions present on many farms, the importance of the early intestinal microflora on Ig absorption may be underestimated. Under such circumstances, undesirable species of bacteria may be ingested by the calf at birth and multiply explosively in the small intestine. Any delay in colostrum feeding would further aid establishment of bacteria and interaction with the intestinal epithelium (19).

Undoubtedly, numerous factors influence closure in the bovine neonate. A hormonal stimulus in some manner appears to alter permeability of villus epithelial cells (22, 30, 42). Microflora present during the first hours of life might serve to enhance onset of closure by its effect on villus morphology, cellular renewal or cell function. Current knowledge of gamma globulin absorption and closure in the neonate indicates the need of information regarding: 1) the site of optimum gamma globulin absorption in the newborn calf, and 2) the relationship

of the intestinal microbial population to intestinal epithelium and macromolecular absorptive capabilities.

Techniques in the Study of Intestinal Absorption

Early techniques for study of macromolecular absorption involved measurement of the mass of Ig fed to the calf and subsequent determination of serum Ig levels (50, 51). Absorption was expressed as percentage of the Ig fed. This method involves somewhat tedious analyses and lacks accuracy and control of experimental conditions found with other techniques.

Balfour and Comline (5) and Hardy (34) described a procedure for the study of macromolecular absorption involving cannulation of the thoracic lymph duct and jugular vein. Gamma globulin was labelled with either ^{131}I or ^{125}I. Labelled globulins were administered in a variety of solutions by means of a surgically implanted duodenal cannula and absorption measured by monitoring radioactivity in lymph. These techniques: 1) increased accuracy of absorption measurements; 2) allowed examination of the influence of solution-carrier on absorption; 3) permitted study of route of absorption of different molecular weight proteins; and 4) enabled measurement of rate of globulin absorption. However, these techniques required considerable surgical proficiency, relatively aseptic working conditions and were suitable only for large species of animals.

Clarke and Hardy (16) found that pinocytosis or uptake of macro-

molecules was highly correlated with absorption and could be used in some species (e.g., rat, rabbit, guinea pig, calf) to measure macromolecular absorption. The macromolecule labelled with either ^{131}I or ^{125}I could be administered either orally or by injection into surgically exposed intestine. Uptake was determined by counting segments of intestine in a solid scintillation counter. These procedures reduced the amount of surgical skills needed and provided added information regarding the location of optimum macromolecular absorption in the small intestine. Lecce (57) modified these procedures by ligating small intestine into segments prior to injection of test solutions. In this manner the amount of globulin administered per unit of intestine could be controlled, allowing examination of absorptive ability throughout the intestine with greater accuracy. This procedure also allows comparison of effects of different treatments on intestinal tissue within the same animal. Due to the large variability in macromolecular absorption between animals, the ability to perform several replications of treatments within one animal is a power of this technique.

Testing of gamma globulin absorption with these procedures is possible in the calf since Hardy (34) found that gamma globulin administered in a simple electrolyte solution is pinocytosed but not transported out of the epithelial cell. The use of this technique in calves would allow study of distributional uptake of gamma globulin in small intestine and the effects of intestinal microflora on uptake with greater confidence than previously used methods.

EXPERIMENTAL PROCEDURES

The primary objectives of these experiments were to characterize distribution of macromolecular uptake in small intestine and to examine the relationship of microflora on gamma globulin uptake in the newborn calf.

Two experiments were conducted over a one-year period with newborn male Holstein calves from the Virginia Polytechnic Institute and State University Dairy Herd. Surgical and analytical procedures used in experiments I and II were identical except where noted in experiment II. Each of the experiments will be presented individually by stating objectives, materials and methods, and results and discussion. A general discussion will follow as a separate section.

Experiment I

Objectives

The objectives of experiment I were to:

1) Determine distribution of uptake of ^{125}I-gamma globulin by the small intestine of neonatal calf.
2) Determine effect of time of exposure of ^{125}I-gamma globulin to intestinal mucosa on amount of uptake.

Materials and Methods

Iodination of gamma globulin. Human gamma globulin[1] (Cohn fraction II - 99% gamma) was labelled with ^{125}I[2] by the chloramine t procedure of McConahey and Dixon (65) with the following modification. Free unbound iodine was removed by dialysis against 4 liters of phosphate buffer changed four times over a 24 h period. The resulting solution contained less than 6.7% unbound iodine as determined by precipitation with 20% trichloroacetic acid. An aliquot containing .182 ug of ^{125}I-gamma globulin was subjected to disc gel electrophoresis (64). The gel was cut into eight equal portions and each counted with a Searle[3] liquid scintilation counting system with B Gammavials[4]. B Gammavials consist of a vial with a sample well surrounded by a fluor that converts gammas of ^{125}I to beta emissions enabling counting with a liquid scintillation system at approximately 50% efficiency. Counts greater than background were not detected

[1]Sigma Chemical Co., St. Louis, Mo. 63178.
[2]New England Nuclear, Boston, Mass. 02118.
[3]Searle Analytic Inc., Des Plaines, Ill. 60018.
[4]Koch-Light Laboratories Ltd., Coinbrook, Bucks, England.

in any region of gel other than that region corresponding with gamma globulin. This, with results of trichloroacetic acid precipitation, indicated successful iodination. Specific activity of labelled gamma globulin was 140 uCi/milligram. Unlabelled gamma globulin, phosphate buffer and ^{125}I-gamma globulin were mixed to yield a final specific activity of 50 uCi/mg of gamma globulin. This solution was stored in small vials under an atmosphere of oxygen-free nitrogen gas to prevent self radiation damage (65) and frozen for later use.

Bovine gamma globulin was purchased from a commercial source. However, after completion of these experiments the supplier indicated that the globulin was of human rather than bovine origin. Staley (84) found that human gamma globulin was absorbed with equal facility as homologous gamma globulin in the newborn calf.

Preparation of ^{125}I-gamma globulin dose. Two h prior to surgery, one vial of frozen gamma globulin solution was removed and allowed to thaw at room temperature. Sufficient unlabelled gamma globulin, ^{125}I-gamma globulin solution and NaCl:KCl solution were mixed to yield 125 ml of 2% gamma globulin solution containing approximately 1 uCi/100 mg of gamma globulin. The NaCl:KCl solution contained 1.64 g/L of NaCl and 1.66 g/L of KCl. Concentration of salts in this solution was similar to colostrum whey (34). Administration of the dose in the NaCl:KCl solution restricted movement of the ^{125}I-gamma globulin dose to within the epithelial cell (34), thus facilitating measurement of absorptive ability.

Management of experimental calves. Ten newborn calves were immediately removed from their dams as soon as possible after birth to a sani-

tized holding box. Colostrum was not consumed. Calves were then weighed and transported to the laboratory where surgery was performed. Calves ranged in age from 2.5 to 12.5 h ($\bar{X}$ = 7 h) when surgery was initiated.

Surgical procedures. The abdominal area was prepared for surgery by clipping hair and scrubbing the area with iodine soap[5] and 70% ethanol alternatively with three repetitions.

Anesthesia was induced with Xylazine[6] and maintained thereafter with Pentobarbital Sodium[7]. A jugular catheter was established after induction of anesthesia. Sterile .9% saline was administered intravenously to maintain plasma volume during surgical manipulations and disconnected after closure of the incision. Each calf received approximately 450 ml of .9% saline solution. A 40 cm paramedial abdominal incision was made from the xiphoid process to the inguinal area. The cecum was located and the small intestine exteriorized. Beginning at the ileocecal junction and proceeding anteriorly, intestinal segments 10 cm in length were formed every 70 cm from the end of one segment to beginning of the next. Segments were formed by pushing a mosquito clamp with carpet thread or umbilical tape through the attached mesentery and tying the ligature around the entire intestine. The distal ligature, towards the cecum, was sterile carpet thread with a tag identifying incubation time and location of the segment. The proximal ligature was umbilical tape. Care was taken in

[5]Betadine - Purdue Frederick Co., Norwalk, Conn. 06856.

[6]Xylazine - Haver-Lockhart Laboratories, Shawnee, Ks. 66201.

[7]Pentobarbital Sodium - Fort Dodge Laboratories, Inc., Fort Dodge, Iowa 50501.

placing ligatures to avoid damage to blood vessels. Segments were formed at the described intervals until reaching the upper intestine, near the abomasum, where segment construction was difficult due to tight mesenteric attachments. The number of segments constructed in each calf varied due to the variable length of small intestine and mesentery attachments (Table 1). Due to variability in uptake observed in the first 4 segments, one additional segment was placed midway between segments 1, 2, 3 and 4 of the last 5 calves. The dose was administered to all segments immediately after formation with 23 gauge needles and 5 ml syringes. The aforementioned segments were exposed to ^{125}I-gamma globulin dose for 1.5 h. After completion of injections, intestines were returned to the abdominal cavity and the incision closed with interrupted sutures of Vetafil[8].

To assess the effect of time of exposure of ^{125}I-gamma globulin to intestinal absorptive surface, one additional segment was placed adjacent to segments 1, 5 and 10 and exposed to ^{125}I-gamma globulin for one-half h in the last six calves. The abdominal incision was reopened, segments formed and injected, the intestines returned to the abdomen and the incision closed as previously described. The number and location of segments in each calf are shown in Table 1.

One and one-half hour after injection of the first 1.5 h segment, the incision was reopened and zero time control (ZTC) segments were placed adjacent to segments 1, 5 and 10. The ZTC segments were formed, injected, agitated for 15 seconds and excised. These segments permitted

[8]Vetafil Bengen - S. Jackson Inc., Washington, D. C. 20014.

TABLE 1. Number and location of segments[a] exposed to ^{125}I-gamma globulin for 1.5 h, .5 h and .004 h.

Calf	Exposure time		
	1.5 h	.5 h	.004 h
1	1-15	none	1,5
2	1-15	none	1,5
3	1-15	none	1,5,10
4	1-14	none	1,5,10
5	1-14	1,5,10	1,5,10
6	1-13(1.5,2.5,3.5)[b]	1,5,10	1,5,10
7	1-12(1.5,2.5,3.5)	1,5,10	1,5,10
8	1-12(1.5,2.5,3.5)	1,5,10	1,5,10
9	1-13(1.5,2.5,3.5)	1,5,10	1,5,10
10	1-11(1.5,2.5,3.5)	1,5,10	1,5,10

[a]Segment 1 located at cecum terminus of small intestine and segment 15 located closest to abomasum terminus.

[b]Segments placed midway between segments 1 and 2, 2 and 3, and 3 and 4.

quantitation of ^{125}I-gamma globulin adhering to intestinal tissue after brief exposure and washing. After excision of ZTC segments, remaining segments were excised beginning with the most distal segment near the cecum and placed in 75 ml of .9% saline. The calf was euthanized, intestines were removed, length measured and location of segments determined. Location of each segment was expressed as percentage of total intestine length from the cecal terminus. Five ml of blood was removed via jugular catheter prior to euthanization for measurement of blood radioactivity. Approximately 1 g samples of thyroid, liver and mesenteric lymph node were removed for counting.

Measurement of uptake. Fluid volume remaining in each segment was determined by excising one ligature and draining contents into a graduated cylinder. Connective and mesenteric tissue were trimmed away from the segment. Segments were everted and rinsed by swirling for 15 seconds in each of two successive washes of 60 ml of .9% saline. Weight of wet tissue was measured, diluted 1:5 (W/W) with .9% saline and homogenized for 1 minute in a semi-micro Waring[9] blendor. Triplicate 1 ml aliquots of homogenate were counted in a Searle liquid scintillation system using B Gammavials. Uptake was expressed as milligrams of gamma globulin internalized per gram of intestinal tissue with correction for gamma globulin adhering to the tissue after washing (ZTC segments).

[9]Waring, A. H. Thomas, Philadelphia, Pa.

Presentation of data. Location of the segment was transformed from a given numerical identity to a percentage representing its relative position in the small intestine from the cecum. Regression analysis of uptake on position of segment was used to evaluate effects of segment location, age at initiation of surgery, birth weight and other parameters. Slopes representing incubation for 1.5 h and .5 h were tested for homogeneity by using a Student's t-test as described by Steel and Torrie (85). The least squares analysis of variance procedure (7) was used to test for significant effects of measured parameters on uptake. Correlation coefficients were determined between all dependent and independent variables.

Results and Discussion

Effect of location of segment and exposure time on uptake of ^{125}I-gamma globulin. Macromolecular absorptive ability of small intestine was determined by measuring uptake of ^{125}I-gamma globulin. Although previous studies (11, 14, 15) have shown high correlation between uptake and appearance of large molecular weight substances in blood, it should be noted that pinocytosis is only one step in the absorptive process. All substances pinocytosed by the epithelial cell are not necessarily transported to the serosal side of epithelium (84). However, in the calf, cessation of macromolecular absorption appears to be a one-step process whereby uptake and transport cease simultaneously (26, 27). Since there is high correlation between uptake and absorption of macro-

molecules (11, 34), it is reasonable to use uptake as a measure of absorptive ability in the calf.

Calves withstood surgery well with exception of two calves. Calves 1 and 2 died during excision of the last two segments. Average uptake of all 1.5 h segments was one standard deviation below the mean in calf 1 (Table 2). Uptake in calf 2 appeared unaffected. Information regarding effects of intestinal position on a within-calf basis was comparable to other calves.

Exposure of intestinal segments to ^{125}I-gamma globulin for 1.5 h. The mean uptake was 2.14 $\pm$ .97 mg/g of intestinal tissue (Table 2). Balfour and Comline (5) and Hardy (34) found that 12% to 25% of ^{125}I-gamma globulin administered to the intestine was absorbed into lymph. In this study uptake was expressed in milligrams protein internalized per gram of intestinal tissue to compensate for differing weights of the intestinal segments. Conversion of uptake per gram to uptake per segment yields an average of 22.22% of administered protein being pinocytosed (Table 2). Since uptake is highly correlated with absorption (34), the uptake values observed in this study are indicative of the absorption observed by Balfour and Comline (5) and Hardy (34).

An analysis of variance was performed on the data to evaluate the effect of segment location within the small intestine (PSEG) on uptake (Table 3). A significant calf effect was observed ($P<.01$). Although calves varied in terms of mean uptake, the distribution of uptake was similar among calves. Figures depicting uptake versus PSEG in indivi-

TABLE 2. Mean uptake of gamma globulin per gram of intestine tissue and average percentage of gamma globulin dose internalized per segment in calves in experiment I.

Calf	Mean uptake per gram of tissue	Average % internalized per segment
1	1.02 ± .26	8.85
2	1.87 ± 1.36	14.57
3	4.94 ± 2.09	43.58
4	2.45 ± 2.12	28.00
5	2.45 ± 1.41	25.72
6	.92 ± .58	12.71
7	1.37 ± .81	16.51
8	1.07 ± .78	12.56
9	3.48 ± 1.27	35.71
10	1.80 ± 1.18	20.69
Mean	2.14 ± .96	22.22

TABLE 3. Analysis of variance table for tissue uptake of gamma globulin after 1.5 h or .5 h exposure.

Source	1.5 h uptake		.5 h uptake	
	df	MS	df	MS
Calf	9	22.386**	5	.707*
PSEG[a]	1	9.258**	1	.796*
$PSEG^{2}$[b]	1	17.664**	1	1.124*
$PSEG^{3}$[c]	1	15.538**		
Error	134	.931	13	.168
Total	146		20	

[a]PSEG = position of the segment in the small intestine expressed as percentage of distance from cecum to abomasum.
[b]$PSEG^{2}$ = PSEG * PSEG.
[c]$PSEG^{3}$ = PSEG * PSEG * PSEG.
*(P<.05). **(P<.01).

dual calves are found in the Appendix. The calf effect was not unexpected since a great deal of variability has been observed in gamma globulin of serum in day-old calves (86).

The linear, quadratic and cubic effects for PSEG were all highly significant ($P<.01$) (Table 3). The regression of PSEG on uptake was best described as a cubic function with $r^2=.72$ as shown in Figure 1. The data indicated that uptake increased from the cecum to a maximum in an area 15% of the distance from the cecum to abomasum (Figure 1). Uptake progressively declined through the midgut until reaching an area corresponding to the 70% of the cecum-abomasum distance. The slight increase in uptake seen in the regression curve from the 70% area towards the abomasum may be related to several factors. First, there were few observations in this region of the gut near the abomasum. Therefore, errant values in several calves may be responsible. Secondly, the increase may be mathematical - relating to solution of the prediction equation - and have no biological meaning. Most workers (26, 34, 84) agree that the duodenum is incapable of measurable macromolecular absorption. Previous studies (26, 27, 84) evaluated macromolecular absorption by histological techniques, which are difficult to quantify. El-Nageh (26) contended that the jejunum was the primary absorptive site as evidenced by the appearance of fluorescein-labelled globulins in jejunal lamina propria and not in ileal tissue. However, Staley et al. (84) observed that ferritin-labelled Ig did not pass beyond the apical tubular complex in jejunal tissue. In the ileum ferritin-Ig passed out of the tubular complex and was located adjacent to the lateral plasma mem-

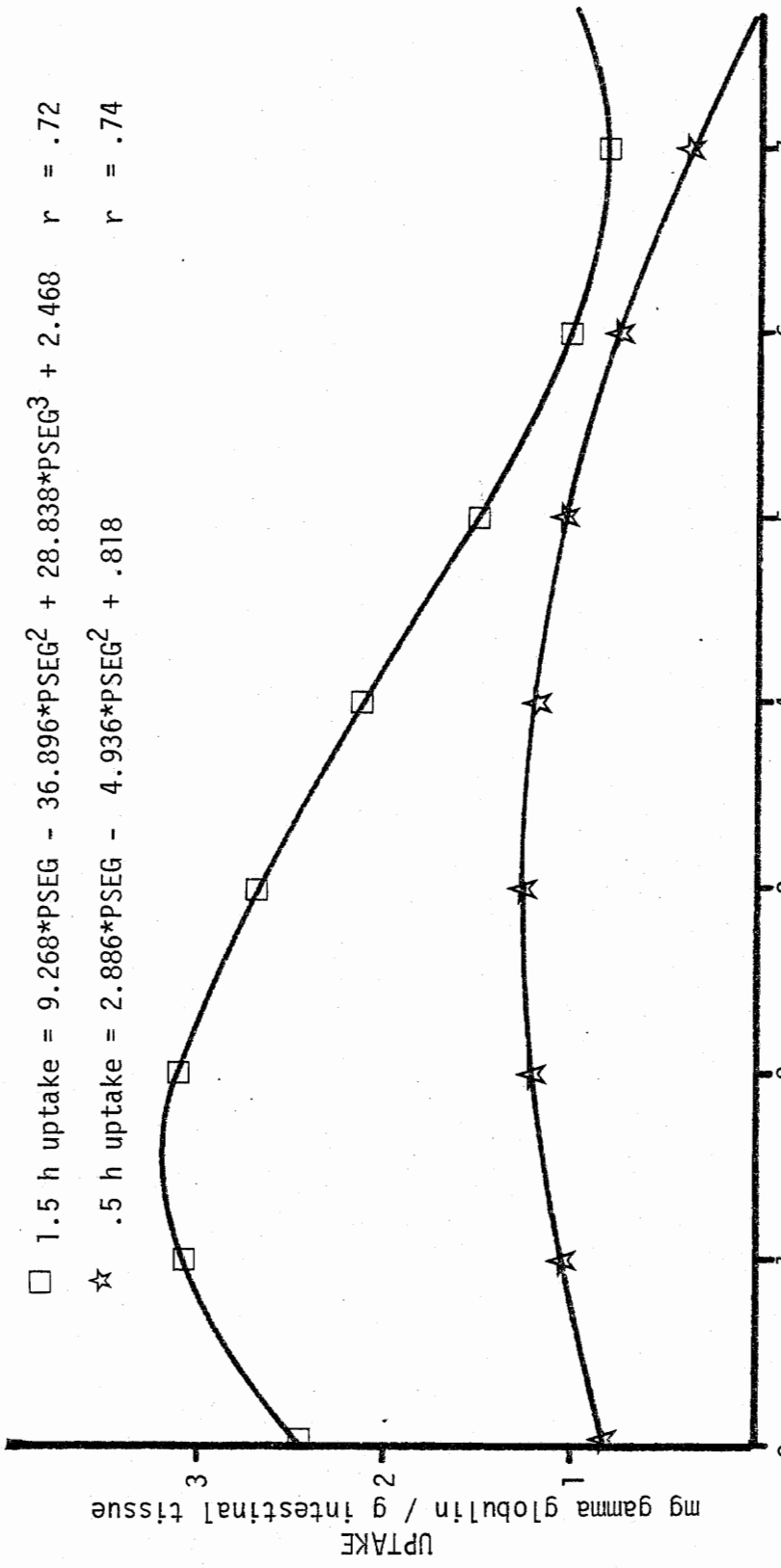

Figure 1. Prediction equations for regression of uptake on PSEG in segments exposed to ^{125}I-gamma globulin for 1.5 h (□) and .5 h (☆).

brane awaiting extrusion from the cell. Jejunal cells appeared to lack development of the apical tubular complex apparently necessary for absorption. A shortcoming of previous studies was limited samples of tissue obtained for examination, thus tissue obtained may not have been representative of absorptive ability. In this study considerable variability was observed between consecutive segments, especially in the lower ileum, indicating that pinocytotic ability is not uniform along a given length of intestine.

Clarke and Hardy (15, 16) observed that macromolecular uptake is greatest in the distal half of small intestine in the rat, rabbit, ferret and guinea pig and becomes progressively restricted to the distal ileum with increasing age. These findings (15, 16, 84) concur with the data of this experiment suggesting that the distal small intestine is the primary area of macromolecular uptake.

An analysis of variance including weight of the intestinal segment (tissue weight) and interactions of tissue weight with the linear, quadratic and cubic effects of PSEG (Table 4) revealed that tissue weight did not affect uptake ($P>.05$). The correlation coefficient of tissue weight and uptake (-.13, $P>.05$) (Table 5) indicated that heavier segments internalized less gamma globulin per unit of tissue although this effect did not alter distribution of uptake. The PSEG*tissue weight interaction was nonsignificant.

The volume of contents remaining in the segment after excision was highly correlated with uptake ($r = -.638$, $P<.01$). When net fluid absorption was impaired, gamma globulin uptake was reduced. It is diffi-

TABLE 4. Analysis of variance table including tissue weight for uptake of gamma globulin after 1.5 h or .5 h exposure.

Source	1.5 h uptake		.5 h uptake	
	df	MS	df	MS
Calf	9	21.898**	5	.301
PSEG[a]	1	3.608*	1	.075
$PSEG^2$	1	4.913	1	.249
$PSEG^3$	1	4.674*		
Tissue weight[b]	1	.041	1	.122
PSEG*tissue weight	1	2.783	1	.036
$PSEG^2$*tissue weight	1	3.095	1	.170
$PSEG^3$*tissue weight	1	3.005		
Error	130	.887	10	.098
Total	146		20	

[a]PSEG = position of the segment in the small intestine expressed as percentage of istance from cecum to abomasum.
[b]Tissue weight = weight of excised intestinal segment in g.
*(P<.05). **(P<.01).

TABLE 5. Correlation coefficients for age, birth weight, intestine length, segment contents, segment weight, location of segment and uptake of gamma globulin in segments exposed for 1.5 h.

	Age	Birth weight	Intestine length	Volume of segment contents	Tissue weight of segment	PSEG
Birth weight	-.360**					
Intestine length	-.225**	.363**				
Volume of segment contents	.196*	-.043	.142			
Tissue weight of segment	-.098	-.108	-.112	-.153		
PSEG	.036	.006	-.048	.527**	-.130	
Uptake	-.218**	.314**	-.005	-.638**	-.133	-.484**

N = 147. *(P<.05). **(P<.01).

cult to ascertain whether greater fluid content was due to decreased absorption or to an increased efflux of fluid into the lumen.

Exposure of ^{125}I-gamma globulin for .5 h. To determine uptake after a .5 h exposure to the absorptive surface, one segment was formed adjacent to segments 1, 5 and 10. An analysis of variance was performed on these data with the main effects being calf, PSEG and PSEG^2 (Table 3). A calf effect was observed ($P<.01$) as was seen in the segments exposed to ^{125}I-gamma globulin for 1.5 h. Both linear and quadratic effects for PSEG were significant ($P<.05$). The regression of PSEG on uptake was a quadratic function with $r^2 = .74$ (Figure 1). The data indicated that in this case uptake was greatest in an area located 30% of the distance from the cecum to the abomasum (Figure 1). This region of the gut would probably be located in the lower jejunum. In his studies El-Nageh (26) failed to describe the length of time that fluorescein-labelled globulins were exposed to the absorptive surface. However, the values he reported concur with our findings that the lower jejunum is the region of optimum uptake of gamma globulin after .5 h exposure.

When the slopes of 1.5 h versus .5 h exposure were tested for homogeneity, a significant ($P<.05$) difference in uptake was shown. A relationship between time of exposure of macromolecules to the absorptive surface and uptake has not been previously documented. However, Balfour and Comline (5) observed that gamma globulin was not detected in the

thoracic lymph duct fluid until 60 to 120 minutes after administration in the duodenum. Maximum concentration of gamma globulin was observed 180 to 200 minutes after duodenal administration. Considering the time required for transit of gamma globulin to absorptive areas of distal small intestine and movement of lymph to the thoracic duct, it appears that 1.5 h may approximate the time when maximal uptake has occurred.

An analysis of variance including tissue weight (Table 4) revealed that tissue weight was nonsignificant ($P>.05$). However, inclusion of this variable in analysis of variance negated the linear and quadratic effect of PSEG. Tissue weight was negatively correlated with uptake ($r = -.601, P<.01$) indicating that heavier segments internalized less gamma globulin per unit of tissue (Table 6). This relationship was not observed in segments exposed to gamma globulin for 1.5 h. Tissue weight was not significantly correlated with PSEG ($r = -.065$). It appears that, with the shorter exposure time, segment size influenced uptake more than PSEG.

Volume of contents in the intestinal segments was negatively correlated with uptake ($r = -.628$, $P<.01$) as was seen in segments exposed for 1.5 h (Table 6).

Effects of age, birth weight and length of intestine on uptake of ^{125}I-gamma globulin. Values for age, birth weight, intestine length and mean uptake after 1.5 h exposure for each calf are shown in Table 7. An analysis of variance (Table 8) was performed on data from the 1.5 h segments with main effects PSEG, $PSEG^2$, $PSEG^3$, age, birth weight,

TABLE 6. Correlation coefficients for age, birth weight, intestine length, segment contents, segment weight, location of segment and uptake of gamma globulin in segments exposed for .5 hours.

	Age	Birth weight	Intestine length	Volume of segment contents	Tissue weight of segment	PSEG
Birth weight	-.404					
Intestine length	-.229	.544*				
Volume of segment contents	.023	.088	-.049			
Tissue weight of segment	.283	-.246	-.555**	.099		
PSEG	.078	-.074	-.159	.369	-.065	
Uptake	-.379	.085	.417	-.628**	-.601**	-.286

N = 21 *(P<.05). **(P<.01).

TABLE 7. Body weight, age, intestine length and uptake in 10 calves of experiment I.

Calf	Body weight (kg)	Age (h)	Intestine length (m)	Mean uptake[a] ($\frac{\text{mg gamma globulin}}{\text{g tissue}}$)
1	50.5	5.00	12.0	1.02 ± 0.26
2	49.8	12.50	12.0	1.87 ± 1.36
3	60.0	6.00	11.5	4.94 ± 2.09
4	50.5	6.50	10.0	2.45 ± 2.12
5	51.4	7.00	12.7	2.45 ± 1.41
6	50.9	12.00	12.3	0.92 ± 0.58
7	40.0	11.50	10.3	1.37 ± 0.81
8	55.9	3.25	15.1	1.07 ± 0.78
9	48.6	2.50	14.0	3.48 ± 1.27
10	48.6	4.00	8.2	1.80 ± 1.18
Mean	50.6	7.02	11.9	2.14
S.D.	4.9	3.58	1.9	0.96

[a]Mean uptake after 1.5 h exposure to ^{125}I-gamma globulin.

TABLE 8. Analysis of variance table for tissue uptake of gamma globulin among calves with 1.5 h exposure.

Source	df	MS
PSEG[a]	1	3.032
$PSEG^2$	1	12.124*
$PSEG^3$	1	8.914*
Age	1	7.040*
Birth weight	1	19.977**
Intestine length	1	9.592*
PSEG*birth weight	1	1.304
PSEG*age	1	2.326
PSEG*intestine length	1	1.817
Error	137	1.905
Total	146	

[a]PSEG = position of the segment in the small intestine expressed as percentage of distance from cecum to abomasum.
*(P<.05). **(P<.01).

intestine length and interactions of age, birth weight and intestine length with PSEG. The quadratic and cubic effects for PSEG were significant ($P<.05$).

In this experiment, a lower age at initiation of surgery was associated with greater ($P<.05$) uptake of gamma globulin (Table 8). Figure 2 shows isoquant regressions of uptake on PSEG at the mean age (7 h) and plus or minus one standard deviation, holding birth weight constant at 50 kg and intestine length at 12 meters. The PSEG*age interaction was not significant ($P>.05$) although a trend is evident indicating that uptake was progressively restricted to the distal small intestine with increasing age. A restriction of pinocytosis to the terminal ileum with increasing age has been observed in the pig (55, 63), ferret (16) and rabbit (16). Kruse (51) found that the absorption coefficient for gamma globulin was reduced linearly to about one-half by delaying colostrum feeding from 2 h to 20 h of age. The relationship of age with uptake, although significant, was not clear cut in this study. Two calves less than 5 h of age failed to internalize an average of 1.5 mg of gamma globulin per gram of intestinal tissue. These findings are in agreement with those of Tenant et al. (86) who found that, in spite of adequate consumption of colostrum early in life, approximately 20% of all calves fail to absorb sufficient quantities of gamma globulin. These findings suggest that there may be other factors that predetermine absorptive capabilities of intestinal epithelium in early life.

Uptake of gamma globulin from the electrolyte solution was greater ($P<.01$) in heavier calves (Table 8). Uptake was greater in all regions

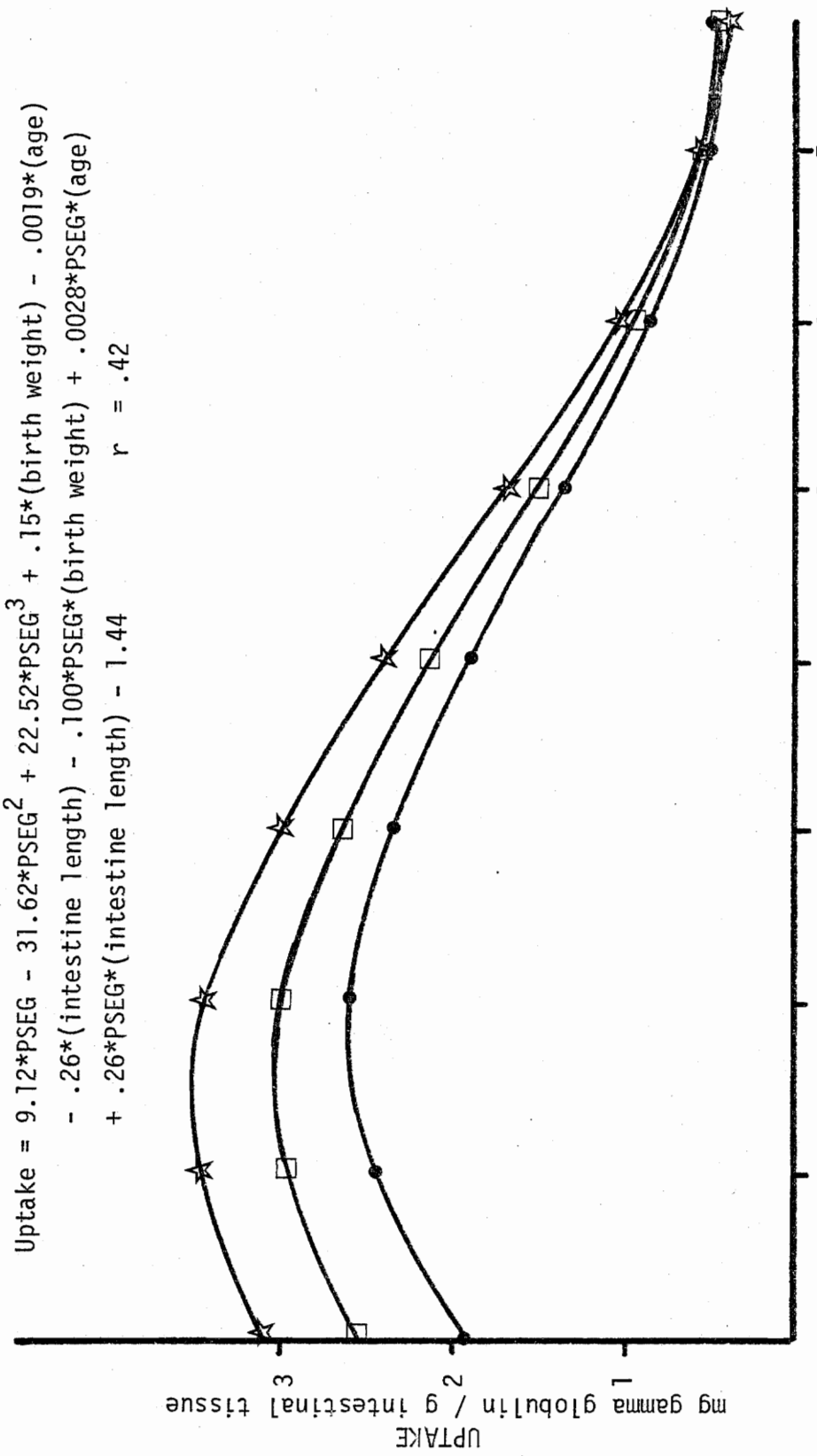

Figure 2. Prediction equations for regression of uptake on PSEG in segments exposed to gamma globulin for 1.5 h in calves 2 h (●), 7 h (□) and 12 h (☆) of age.

of the gut in larger calves as the PSEG*birth weight interaction was nonsignificant ($P>.05$). Kruse (51) found a negative relationship between birth weight and gamma globulin absorption in 72 Black and White Danish breed calves and no relationship in eight Jersey or 61 Red Danish calves. The relationship of birth weight to macromolecular uptake may be minor, considering the limited number of calves used in this study. Kruse (51) found that birth weight contributed very little to the r^2 of a prediction equation in which mass of Ig fed, age of the calf at first feeding and birth weight were considered. He found that the greatest determinant of absorption efficiency was the age of the calf at first feeding, followed by mass of Ig fed.

Length of intestine was negatively related ($P<.05$) to uptake of gamma globulin (Table 8). In previous studjes the author has noted exceptional cases of variability in intestinal length (44, 45). The finding that calves with shorter intestines internalized more gamma globulin per unit of intestinal tissue is difficult to explain. Possibly, it serves as a compensatory mechanism to assure adequate colostrum absorption. However, these findings must be considered in knowledge of the limited number of calves used in the experiment.

Relationships of age, birth weight and intestine length to uptake must also be considered with the understanding that there was considerable uncontrollable confounding of these variables as shown by correlation coefficients in Table 5. Birth weight was negatively correlated with age ($r = -.360$, $P<.01$), meaning that larger calves were also younger calves. Since the relationship of age with gamma globulin ab-

sorption has been established with large numbers of calves (51, 52), it is questionable whether or not the assocaition of birth weight and uptake is real.

Effects of age, birth weight and intestine length on uptake after .5 h exposure of designated intestine segments to ^{125}I-gamma globulin were determined by analysis of variance. Quadratic effects for PSEG on uptake were significant ($P<.05$). However, age, birth weight and intestine length effects on uptake were nonsignificant ($P>.05$).

Disposition of ^{125}I-gamma globulin dose. One g samples of thyroid, liver and mesenteric lymph node tissue and two 1 ml aliquots of jugular blood were counted to achieve an estimate of the quantity of ^{125}I passing through the intestinal epithelium. Total activity residing within all intestine tissue and fluid contents of each segment were measured. One ml aliquots of solutions used to rinse the luminal surface of representative segments were counted. Blood volume was calculated by multiplying birth weight by .08 (42). Values for these measurements are found in Appendices XII through XV at the end of the text. An average of 1.43% of the total amount of ^{125}I administered was found in blood 1.5 h after the first segment was injected. Balfour and Comline (5) found approximately 3% of the ^{131}I-gamma globulin introduced into the duodenum was recovered from lymph when gamma globulin was administered in an identical electrolyte solution as that used in this study. As expected, thyroid tissue contained the highest amount of ^{125}I per gram of tissue (7.8×10^{-4} uCi). Liver and lymph node tissue

contained an average of 1.7×10^{-4} uCi and 2.5×10^{-4} uCi per g of tissue, respectively. The low levels of blood and tissue activity encountered in this experiment indicate that most of the administered ^{125}I remained within luminal fluid or tissue of the segment. Total recovery of ^{125}I from tissue, washings and luminal contents averaged 78% of the total amount administered.

Techniques used in this experiment to measure uptake have been thoroughly tested by British workers (5, 34). A major concern of this procedure was stability of ^{125}I-gamma globulin within intestinal lumen and during absorption. The possibility exists of transfer of iodine label from gamma globulin to another protein fraction or the hydrolysis of labelled globulin into smaller fragments. Balfour and Comline (5) investigated this by dialyzing an aliquot of lymph containing high levels of radioactivity against phosphate-borate buffer pH 8.6 at 5 C for 24 h. No change in radioactivity was detected after corrections for dilution and decay. Another aliquot of lymph was subjected to zone electrophoresis. Of the radioactivity applied to the column, 92% was recovered in the fraction corresponding to gamma globulin. Later work by Hardy (36) supported the findings of Balfour and Comline (5). Gel filtration of lymph and plasma from calves fed ^{131}I-gamma globulin confirmed that proteolysis before and during absorption was slight as only low quantities of low molecular weight labelled material were found.

There are several possible contributors to the radioactivity found in blood and tissues. Disc gel electrophoresis of commercially obtained gamma globulin revealed the presence of a probable albumin contaminant.

Hardy (34) found that, when albumin or polyvinyl pyrollidone (M. W. - 40,000) were administered to the gut in an electrolyte solution, sizable quantities passed through the blood and into the portal capillaries. Another source of radioactivity in the blood is free ^{125}I. Precipitation of ^{125}I-labelled gamma globulin with trichloroacetic acid indicated that 6.7% of the total activity was found in the supernatant. Free iodine would probably pass through the intestinal epithelium and into portal blood. Finally, it is possible that small quantities of ^{125}I-gamma globulin were degraded either within the intestinal lumen, during pinocytosis, or during freezer storage as a result of radiolysis. Being of low molecular weight, these fragments would also pass directly into the portal venous system.

Considering the low levels of radioactivity found in blood and tissues, and the knowledge of free ^{125}I and albumin existing within the ^{125}I-gamma globulin solution, the techniques used in this experiment allowed accurate measurement of uptake.

Experiment II

Objective

The objective of experiment II was to determine the effect of administered indigenous bacteria on uptake of ^{125}I-gamma globulin in *in vivo* intestinal segments of neonatal calves.

Materials and Methods

Donor calf management. Two male Holstein calves from the University herd were used as donors of intestinal fluid. Intestinal fluid was used as a source of intestinal microorganisms for live bacterial inoculum and autoclaved bacterial inoculum. Calves were removed from their dams within 2 h of birth, fed 4 liters of colostrum and placed in individual pens bedded with shavings. Precautions were taken to isolate these calves from any diseased calves. Whole milk was fed at eight percent of body weight daily during the first week and 10% thereafter but not exceeding 5.5 kg per day. A commercial calf starter grain was offered after 2 months to minimize consumption of bedding.

Single duodenal cannulae were surgically placed (43) 1 meter distal of the abomasum in one calf at 2 weeks of age and one calf at 5 weeks of age. Recovery from surgery was uneventful. Procedures for collection of intestinal fluid have been decribed (43, 44).

Procedures for microbial analysis. All procedures and media formulation were as described in the V.P.I. Anaerobe Laboratory Manual (40). Culturable anaerobic counts were determined using rumen fluid-glucose-cellobiose agar (RGCA) in roll tubes (41) with 100% nitrogen atmosphere incubated at 38 C for 24 h. Dilutions yielding 30 to 300 colonies per tube were counted using a dissecting microscope. Microbial counts were expressed as whole numbers.

Growth characteristics of intestinal fluid bacteria from donor calves. Intestinal fluid was studied to determine amounts of fluid and length of incubation needed to achieve an active growing microbial culture of 10^8 viable bacteria per ml of culture broth media. This concentration represents approximate numbers found in gut contents of day-old calves (80). Three tenths or one tenth ml of intestinal fluid was added to 9 ml rumen fluid-glucose-cellobiose broth (RGCB) and incubated at 38 C. One ml aliquots of this culture were obtained at 2 h intervals up to 8 h, serially diluted with dilution solution and microbial counts determined as previously described. Four h incubation with .3 ml inoculum of intestinal fluid yielded 10^8 bacteria in the shortest time and was the procedure followed in this experiment.

Inoculum preparation. Preparation of inocula for injection of intestinal segments began after collection of intestinal fluid and transportation of the newborn calf to the laboratory. Fifty ml of intestinal fluid was collected in order to obtain a representative sample. Ten

tubes of RGCB were inoculated with .3 ml of intestinal fluid and incubated at 38 C. After 4 h, all tubes were removed from the incubator. Four tubes were autoclaved at 15 p.s.i., 12 C, for 15 minutes and allowed to cool. After injection of the segments, each inoculum - live bacteria, sterile broth and autoclaved bacteria - was cultured to determine numbers of viable bacteria. Sterile broth and autoclaved bacteria were checked for sterility.

Iodination of gamma globulin. Human gamma globulin was labelled with ^{125}I as previously descrived. Examination of ^{125}I-gamma globulin solution after dialysis revealed less than 2.5% of the iodine remaining in the supernatant after precipitation with 20% trichloroacetic acid, thus indicating successful iodination. Specific activity was 262 uCi per mg of gamma globulin. Sufficient unlabelled gamma globulin, ^{125}I-gamma globulin and phosphate buffer were added to achieve a final specific activity of 50 uCi per mg of gamma globulin. This solution was stored as previously described. A 50 ul aliquot of unlabelled gamma globulin solution (1 mg/ml) was subjected to disc gel electrophoresis (64). Based on this, the commercial source contained 74% gamma globulin. Three additional peaks were detected corresponding with albumin, $alpha_1$ globulin and $alpha_2$ globulin. The relative percentage of the contaminants was albumin, 17.1%; $alpha_1$ globulin, 6.8%; and $alpha_2$ globulin, 1.8%. A chart of the densitometer tracing of this gel is located in Appendix table I.

Management of experimental calves. Ten male Holstein calves handled

as described in experiment I were used in this experiment. Calves ranged from 4.5 h to 14 h ($\bar{X}$ = 8.6 h) of age when surgery was initiated.

Surgical procedures. After induction of anesthesia, a sterile solution of .9% NaCl and 2.5% dextrose was administered intravenously during surgical procedures and disconnected when the incision was closed. Each calf received a maximum of 450 ml of solution. A 30 ml sample of blood was obtained prior to the initiation of surgery for later analysis. An endotracheal tube was placed to maintain a patent airway and minimize anorexia as occasionally observed in calves in experiment I. Sterile techniques prevailed throughout the duration of surgery.

Segments 10 cm in length at 3 cm intervals were constructed beginning approximately 1.8 meters above the ileocecal junction. The location was determined to be the area of greatest and most uniform uptake in the small intestine. Seven treatments were repeated in three successive sections of small intestine. Treatments, as shown in Table 9, were assigned randomly along the intestine within each section. Each section was injected with the respective inoculum immediately after formation. The injection procedures were: 1) Anaerobic inoculum was withdrawn from the roll tube into a three ml syringe. 2) The needle was replaced with another sterile needle and the segment injected. 3) The injection site on each segment was wiped with gauze pads soaked in 70% ethanol. These precautions were taken to prevent microbial contamination of the abdominal cavity. One syringe was used for each inoculum. Possible effects of live microorganisms, microbiological media

TABLE 9. Treatment protocol for inoculum and gamma globulin administration to *in vivo* intestinal segments[a].

Treatment	Inoculum	Gamma globulin
1	Live bacteria (10^8/ml)	^{125}I
2	Live bacteria (10^8/ml)	Unlabelled
3	Sterile broth	^{125}I
4	Sterile broth	Unlabelled
5	Autoclaved bacteria	^{125}I
6	Sterile rumen fluid-glucose-cellobiose broth	^{125}I
7	Live bacteria (10^8/ml)	^{125}I

[a]Inoculum was allowed four hours incubation within the segment followed by 1.5 hour exposure to gamma globulin solutions, except in treatments 6 and 7 where gamma globulin was exposed to tissue for 15 seconds.

and microbial toxins, end products or killed bacteria, on macromolecular uptake were determined in treatments 1, 3 and 5, respectively. Treatments 6 and 7 were ZTC segments used to assess adherence of dose to mucosal surfaces after brief exposure and thorough washing procedures. After inoculation of the last segment in section 3, the intestines were returned to the abdominal cavity and the incision closed with an internal series of continous sutures and, externally, interrupted sutures. Four h after injection of the first segment, the incision was reopened and gamma globulin solutions were administered by injection with 23 gauge needles and syringes. Treatments 2 and 4 received cold gamma globulin to allow measurement of microbial growth associated with intestinal mucosa. Microbial assay of treatment 2 segments measured growth in those segments receiving added bacteria. Number of endogenous microorganisms residing within the gut prior to surgery were measured in treatment 4 segments. It was assumed that microbial populations in treatment 4 segments represented the microbial growth in treatment 3 and treatment 5 segments. After injection of all segments of treatments 1 through 5 with gamma globulin, the intestines were returned to the abdominal cavity and the incision closed. One and one-quarter h after injection of first segment, 30 ml of blood was obtained from the jugular catheter. The incision was reopened and treatments 6 and 7 injected with ^{125}I-gamma globulin, immediately excised and placed in beakers containing .9% NaCl. The remaining segments were then excised and processed for counting as previously described. Segments receiving treatments 2 or 4 were placed in tared 25 x 150 mm test tubes under an atmos-

phere of 100% nitrogen for later microbial analysis.

After euthanizing the animal, 1 g samples of thyroid, liver and mesenteric lymph node were obtained for counting. Distance from the cecum to the first segment of section 1 and distance from the last segment of section 3 to the abomasum was measured.

A subjective evaluation of gut motility, blood supply and integrity of the mesentery was conducted during initial inoculum injections, gamma globulin injections and excision of intestinal segments. Heart rate and respiration rate were monitored at frequent intervals throughout the experiment.

Preparation of intestinal tissue for microbial analyses. Intestinal tissue was prepared for culture by excising one ligature, measuring volume of luminal contents and flushing the lumen with 15 ml of anaerobic dilution solution (40). Excess mesenteric tissue and fat were removed, tissue weighed and homogenized for one minute under 100% nitrogen in a semi-micro Waring blendor with nine volumes of dilution solution.

Intestinal tissue homogenate was serially diluted 10^3, 10^5, 10^7 with dilution solution. Determination of viable bacteria per gram of tissue proceeded as previously described using RGCA media.

Blood analyses. Serum was harvested from blood obtained before and after surgery. Serum protein concentration was determined by the biuret method (73). Albumin and alpha, beta and gamma globulin of serum were determined by cellulose acetate electrophoresis and den-

sitometry (29). Total glucocorticoids in serum were quantified using the competitive protein binding assay (71) as modified by Gwazdauskas et al. (31).

Intestinal fluid analyses. One ml or less aliquots of intestinal segment contents were counted using a Searle liquid scintillation system with B Gammavials. Proteins were extracted from intestinal fluid of segments receiving unlabelled gamma globulin by the procedure of Marsh et al. (62). Intestinal fluid proteins were separated by disc gel electrophoresis (64).

Presentation of data. Treatment effects on uptake were measured using treatment 1, 3 and 5 segments and microbial growth measured in segments of treatments 2 and 4. Adherence of ^{125}I-gamma globulin to mucosal surface was corrected for by subtracting uptake values of sterile broth - ZTC segments from uptake values for segments receiving treatments 3 (sterile broth) and 5 (autoclaved bacteria). Similarly, uptake values of bacterial inoculum-ZTC segments (treatment 6) were subtracted from values of bacterial inoculum-^{125}I-gamma globulin (treatment 1). All data were subjected to analysis of variance using the procedure of least squares as described by Steel and Torrie (85). Treatment effects for uptake were compared by partitioning degrees of freedom into:

1) C1 = live bacterial inoculum versus sterile broth and autoclaved bacterial inoculum.

2) C2 = sterile broth versus autoclaved bacterial inoculum.

Results and Discussion

Owing to the longer duration of the surgical manipulations in this experiment, new problems were encountered. A severe perotinitis developed in the first calf two h after initiation of surgery. This calf was euthanized and data not used in the statistical analysis. The infection was attributed to contamination of the abdominal cavity with the bacterial inoculum as a result of nonsterile injection procedures. The procedures were modified to changing the needle prior to injection of the intestinal segment followed by swabbing the injection site with 70% ethanol. Further problems of this nature were not encountered.

Effects of added microorganisms on uptake of ^{125}I-gamma globulin. Treatment means for uptake, tissue bacteria populations and number of bacteria per ml of inoculum for each calf are shown in Table 10. An analysis of variance was performed on the data (Table 11) to evaluate effects of added live bacteria, sterile rumen fluid-glucose-cellobiose broth (RGCB) and autoclaved bacteria on uptake of ^{125}I-gamma globulin. The main effects were C1, C2, calf, section, section*treatment, microorganisms per gram tissue, fluid volume within the lumen of the segment and tissue weight of the segment. Figure 3 shows mean uptake by treatment within each calf in ascending order of age.

Uptake of ^{125}I-gamma globulin was lower ($P<.05$) in segments receiv-

TABLE 10. Treatment means for uptake, bacterial numbers in tissue and inoculum within each calf.

Calf	Uptake[a]			Bacteria ($\times 10^6$)			
				Tissue[e]			Inoculum[f]
	1[b]	3[c]	5[d]	1	3	5	1
1	0.45±0.40	0.88±0.75	1.47±0.58	261± 42	23± 29	23± 29	50.00
2	2.89±1.29	3.76±1.40	5.24±0.64	742±220	29± 28	29± 28	150.00
3	2.69±0.37	2.09±1.00	2.26±0.85	246±272	228±230	228±230	3.65
4	0.97±0.07	2.41±0.53	3.02±0.78	818±497	260±180	260±180	22.70
5	2.56±0.81	5.60±0.46	5.43±1.29	810± 99	149± 90	149± 90	165.00
6	1.72±0.41	3.70±0.22	3.53±0.44	560±222	5± 9	5± 9	325.00
7	3.31±0.56	4.44±0.43	4.11±0.55	634±509	2± 2	2± 2	505.00
8	2.80±0.78	3.37±0.40	4.07±0.55	442±167	25± 15	25± 15	105.00
9	0.38±0.15	1.10±0.41	1.43±0.36	706±327	0± 0[g]	0± 0[g]	455.00
10	2.59±0.69	3.64±0.42	4.34±1.15	602±217	143±237	143±237	420.00
Mean	2.09±1.15	3.18±1.50	3.56±1.49	594±316	75±140	75±140	220.00

[a] mg gamma globulin internalized / g intestinal tissue ± S.D.
[b] Treatment 1 = live bacterial inoculum.
[c] Treatment 3 = sterile microbiological broth.
[d] Treatment 5 = autoclaved bacterial inoculum.
[e] Tissue bacteria = number of bacteria / g homogenized tissue.
[f] Inoculum bacteria = number of bacteria / ml culture. Treatment 3 and 5 inocula were sterile.
[g] Contained 10^3 bacteria / g homogenized tissue.

TABLE 11. Within calf analysis of variance table for uptake of gamma globulin.

Source	df	MS
C1[a]	1	31.509*
C2[b]	1	2.134
Calf(treatment)	27	4.938**
Section	2	.396
Section*treatment	4	.351
Tissue microorganisms	1	.965§
Volume of segment contents	1	5.188**
Segment tissue weight	1	2.731*
Error	48	.335
Total	86	

[a]C1 = live bacteria inoculum versus sterile broth and autoclaved bacteria.
[b]C2 = sterile broth versus autoclaved bacteria inoculum.
§(P<.10). *(P<.05). **(P<.01).

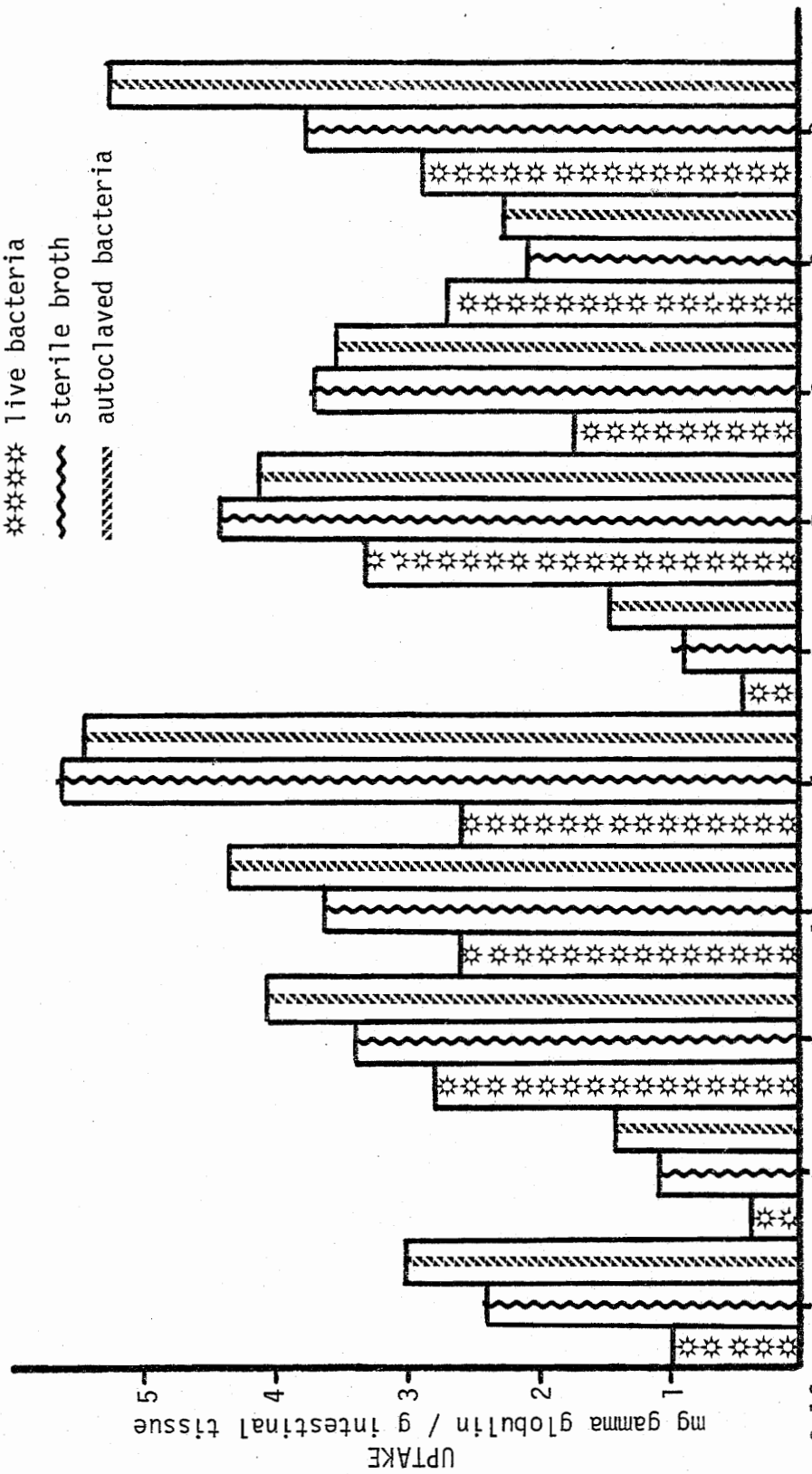

Figure 3. Treatment means for uptake in individual calves shown in order of ascending age.

ind added live bacteria as compared to segments receiving sterile inoculum (Table 11). The live bacteria might have acted to decrease uptake by decreasing the permeability of existing epithelial cells, by enhancing the replacement of permeable cells or by a combination of the two. Corley et al. (19) found considerable alteration of mucosal surface when E. coli was fed to newborn colostrum-deprived calves. Since calves in experiment II had not received colostrum, it is possible that the additional bacterial load acted to alter the brush border and apical tubular system resulting in decreased pinocytotic activity by the epithelial cell. The influence of bacterial inoculum on turnover rate of epithelium is probably of lesser importance in this study due to the relatively short exposure (4 h) to absorptive surface. El-Nageh (27) found that intestinal epithelium is usually replaced within 24 h. Restriction of intestinal peristalsis and digesta flow through must be considered in lieu of presence of intestinal ligatures. Depressed motility of the gut would allow greater interaction of bacterial flora with intestinal epithelium. Mechanisms with which the live bacterial inoculum affected macromolecular uptake are uncertain without histological observation.

Heat-stable components of bacterial cells or bacterial end products were probably not instrumental in altering gamma globulin uptake as evidenced by the nonsignificant ($P>.05$) C2 comparison (Table 11). According to Broughton and Lecce (10), cessation of pinocytotic activity in piglets is related to nonspecific utilization of the apical plasma membrane. It would be expected that dead bacterial cells, or even components of microbiological media would act to depress uptake since the

macromolecular absorptive process is relatively nonspecific in the calf (34). However, such a relationship was not observed.

Volume of fluid remaining in the lumen after excision of the segments was related to uptake ($P<.01$). Lumen fluid volume was negatively correlated with uptake ($r = -.787$, $P<.01$) (Table 12). It appears that bacterial flora inhibited fluid absorption as well as macromolecular uptake. This might suggest that microflora did alter the absorptive surface of individual epithelial cells rather than enhance replacement of the intestinal epithelium. If the latter process was involved, fluid absorption would probably not be impaired. Absorption of fluid must be considered as the net result of influx and efflux of fluid across the epithelial cell barrier. Possibly the bacteria altered the epithelial surface to the extent that fluid movement towards intestinal lumen was no longer inhibited resulting in increased fluid contents.

Microbial analysis of tissue after thorough washing with dilution solution demonstrated that bacteria rapidly colonize the gut of the newborn calf (Table 10). Segments receiving sterile inoculum had a mean of 8.6×10^7 bacteria per g of tissue. These bacteria were probably obtained from the immediate environment at calving and prior to surgery. In 8-h-old colostrum-fed calves, Smith (80) found 10^4 to 10^6 organisms per g of intestinal chyme in the upper and lower small intestine of colostrum-fed calves. The higher levels of bacteria observed in this experiment may be related to restricted gut motility previously discussed as well as the absence of colostrum. Age of the calf was not significantly correlated ($r = -.089$, $P>.05$) with the num-

TABLE 12. Correlation coefficients for age, birth weight, segment contents, segment weight, microbial growth data, serum corticoids and uptake in calves of experiment II.

	Age	Birth weight	Volume of segment contents	Tissue wt. of segment	Tissue bacteria number	Inoculum bacteria number	Pre-surgery corticoids	Post-surgery corticoids	Treatment
Birth weight	-.075								
Volume of segment contents	-.229*	.397**							
Tissue weight of segment	-.057	.077	-.034						
Tissue bacteria number	-.089	.120	.221*	.129					
Inoculum bacteria number	-.016	-.024	.300**	.001	.767**				
Pre-surgery corticoids	-.205*	-.477**	-.541**	.122	.074	.005			
Post-surgery corticoids	.108	-.469**	-.478**	.081	.010	.014	.835**		
Treatment	.000	.000	-.269**	-.009	-.655**	-.861**	.000	.000	
Uptake	.226*	-.440**	-.787**	-.170	-.410**	-.402**	.429**	.435**	.400**

*(P<.05). **(P<.01).

ber of bacteria per gram of tissue, indicating that older calves did not necessarily have higher levels of bacteria within the gut (Table 12).

Correlation of treatment with bacteria numbers in the tissue ($r = -.655$, $P<.01$) indicated that segments receiving live bacterial inoculum (treatment 1) possessed higher levels of bacteria in their tissue. Consequently, the higher bacterial concentration was related to lower uptake ($P<.10$). Although tissue bacteria effect on uptake was significant only at the .10 level, it is justified in considering these findings meaningful. Greater differences in microbial numbers may have been obscured by inherent inaccuracies of methods used for determining microbial numbers. Considering the effects observed with apathogenic populations of microorganisms used in this experiment, pathogenic species or a microflora associated with undesirable calving conditions would probably be a greater detriment to gamma globulin absorption.

The live bacterial inoculum was relatively consistent in terms of numbers across all calves with exception of that used in calves 1, 3 and 4 (Table 10). However, differences in tissue bacteria levels were evident except in calf 3. The combination of low levels of bacterial growth in inoculum plus high endogenous microbial populations resulted in little difference in microbial growth between segments receiving added bacteria or sterile inocula. As a result, uptake appeared unaffected by treatment. Greater numbers of bacteria in inoculum were highly correlated (Table 12) with tissue bacteria levels ($r = .767$, $P<.01$). The correlation of inoculum bacterial populations and uptake was highly significant ($r = -.400$, $P<.01$).

Section of small intestine had no effect (P>.05) on uptake (Table 11). Utilization of this portion of gut provided the area of uniformity desired according to experimental design. Section was correlated with tissue weight (r = .370, P<.01), meaning that heaviest segments were located in section 3, most distal to the cecum. Tissue weight of the segments affected uptake (P<.01) in a negative manner. Heavier segments internalized less gamma globulin per gram of tissue. The increased weight is probably due to greater segment length. The correlation coefficient of tissue weight and uptake over all calves and treatments was -.170 and nonsignificant (P>.05).

Values for age, birth weight and pre- and post-surgery serum glucocorticoids are shown in Table 13. An analysis of variance was performed on this data (Table 14). Unlike the previous experiment, age had a positive effect on uptake (P<.01) in this study. Although Kruse (51) observed a linear relationship between age of the calf and gamma globulin absorption, previous studies by the author (44, 45) failed to observe this relationship in calves less than 7 h of age. Age was positively correlated (r = .226, P<.05) (Table 12) with uptake, indicating that in these calves, age was not a limiting factor in gamma globulin uptake. Smaller calves internalized more (P<.01) gamma globulin than larger calves. Significance of this finding is questionable due to the small number of calves (10) involved. This observation disagrees with the previous experiment in which birth weight was positively related to gamma globulin uptake. However, Kruse (51) found a similar low but significant relationship of birth weight and gamma globulin ab-

TABLE 13. Values for age, birth weight, pre-surgery corticoids, post-surgery corticoids, intestine length from cecum to first segment and length from last segment to abomasum, total intestine length and mean uptake in individual calves.

Calf	Age[a] (h)	Birth weight (kg)	Serum corticoids (ng/ml) pre	Serum corticoids (ng/ml) post	Average corticoids (ng/ml)	Intestine length (m) c	Intestine length (m) d	Intestine length (m) e	Mean uptake[b] (mg gamma globulin / g tissue)
1	8.50	46.8	19.6	---	19.6	2.37	11.30	15.07	.937 ± .647
2	14.00	45.9	70.4	107.6	88.8	2.10	10.35	14.55	3.967 ± 1.436
3	12.50	48.6	33.4	31.8	32.6	1.80	11.85	15.75	2.346 ± .735
4	4.25	53.2	91.0	91.3	91.2	2.35	8.25	12.70	2.131 ± 1.024
5	7.00	46.4	58.7	66.1	62.4	2.85	8.55	13.50	4.531 ± 1.678
6	12.25	47.3	54.1	86.9	70.5	.85	11.55	14.50	2.984 ± 1.004
7	11.00	41.4	107.6	103.0	105.3	1.20	11.50	14.80	3.953 ± .671
8	5.50	40.0	105.3	102.5	103.9	1.35	10.35	13.80	3.412 ± .757
9	5.00	50.0	39.0	36.2	37.6	1.73	10.80	14.63	.969 ± .542
10	6.25	40.0	74.7	84.7	79.7	1.20	12.90	16.20	3.525 ± 1.039
Mean	8.63	45.9	66.9	78.9	74.7	1.78	10.74	14.55	2.942
S.D.	3.4	4.2	27.8	26.9	25.1	.64	1.44	1.03	1.509

[a]Age = hours of age when surgery was initiated.
[b]Mean uptake = average uptake of all segments receiving ^{125}I-gamma globulin.
[c]Length of intestine from cecum to first segment.
[d]Length of intestine from last segment to abomasum.
[e]Total intestine length.

TABLE 14. Among calves analysis of variance table for uptake of gamma globulin.

Source	df	MS
Section[a]	2	.821
Treatment	2	16.542**
Section*treatment	4	.186
Age	1	8.900**
Birth weight	1	11.555**
Average corticoid	1	7.764**
Error	69	1.202
Total	80	

[a]Section = section of the intestine.
**(P<.01).

sorption. Intestine length was not related (P>.05) to uptake in experiment II and was not included in the analysis of variance.

Relationship of serum glucocorticoids and uptake. Mean pre-surgery corticosteroid concentration was 66.9 ± 27.8 ng/ml of serum (Table 13). Eberhart and Patt (25) found maximum plasma corticosteroid concentration of 121 ± 11 ng/ml at birth declining to 49 ± 11 ng/ml by 12 h of age. The mean age at time of sampling was 8.63 ± 3.44 h in calves in this study, indicating agreement with the findings of Eberhart and Patt (25). Corticosteroids of serum declined with increasing age of calves as evidenced by the negative correlation ($r = -.205$, $P<.05$) (Table 12). Mean post-surgery serum corticosteroid concentration was 78.9 ± 26.9 ng/ml and not significantly different (P>.05) from pre-surgery values as determined by Student's t-test (85). The time interval between pre- and post-surgery corticosteroid values was approximately 5 h. Lack of further decreases in serum corticosteroid concentration was not unexpected owing to trauma induced by the surgical procedures which probably serve to maintain adrenal secretion of glucocorticoids at high levels. Due to the lack of significant differences in pre- and post-surgery corticoids, measurements were averaged for statistical analysis purposes.

Low serum corticosteroid values (Table 13) were associated with low gamma globulin uptake (P<.01). This was unexpected since previous workers (21, 22, 30, 33, 42) observed a relationship between elevated serum glucocorticoids and onset of closure. However, Patt and Eberhart (75) witnessed a different association between corticosteroids and Ig

absorption in newborn pigs. Plasma cortisol levels in Caesarian-derived piglets were either suppressed with metyrapone or increased with ACTH immediately after birth. Post colostrum feeding increases of serum Ig were lower in piglets receiving metyrapone than either untreated piglets or piglets receiving exogenous ACTH. Serum concentration of Ig did not differ between untreated or ACTH-treated piglets.

Examination of corticosteroid values for individual calves (Table 13) reveals that three calves (1, 3 and 9) had corticoid levels below 40 ng/ml of serum. These calves were also lowest in terms of uptake.

The data of Patt and Eberhart (75) and other previously discussed observations in this experiment suggests that the actions of corticosteroids in some species may be twofold: 1) A threshold level of corticosteroid concentration may need to be reached before the epithelial cells are capable of macromolecular absorption. 2) Concurrently, the crypts of Lieberkuhn may be stimulated to produce epithelial cells with a diminished capacity for macromolecular absorption.

Serum protein concentrations pre- and post-surgery. Total protein, albumin, and alpha, beta and gamma globulin of serum were determined prior to and immediately preceding termination of surgery (Table 15). Pre-surgery values for total protein and serum protein components were within normal limits (86). Gamma globulin of serum indicated that colostrum had not been consumed prior to surgery, as all values were less than .3 g/100 ml. Calf 1 had the highest concentration of gamma globulin, .19 g/dl. However, according to Tennant et al. (86),

TABLE 15. Mean pre-surgery and post-surgery values for serum protein components.

Calf	Serum protein		Albumin		Alpha globulin		Beta globulin		Gamma globulin	
	pre	post	pre	post	pre	post	pre	post	pre	post
1	5.47[a]		2.67		1.85		.76		.19	
2	4.40	4.76	2.24	2.36	1.32	1.50	.79	.84	.04	.06
3	4.79	3.90	2.54	2.24	1.19	.90	1.00	.68	.06	.07
4	4.46	3.34	2.68	2.05	1.16	.84	.54	.39	.08	.06
5	4.43	4.04	2.38	2.22	1.52	1.28	.53	.52	.02	.02
6	4.38	4.44	2.67	2.55	1.23	1.42	.41	.44	.06	.02
7	3.95	4.08	2.49	2.46	1.00	1.11	.46	.43	.01	.06
8	4.41	4.09	2.61	2.38	1.15	1.05	.58	.59	.06	.06
9	4.25	3.90	1.93	1.51	1.76	2.02	.49	.31	.07	.05
10	5.03	4.80	2.44	2.44	1.95	1.68	.60	.65	.04	.06
Mean	4.52[c]	4.15[c]	2.46[d]	2.26[e]	1.40[f]	1.31[f]	.61[g]	.54[g]	.06[h]	.05[h]
S.D.	.38	.43	.23	.30	.31	.37	.17	.16	.04	.02

[a]All components expressed in g/dl.
[b]Pairs of means with different superscripts are significantly different (P<.05).

this is within the normal range prior to colostrum feeding. Mean post-surgery values for serum protein components were lower than pre-surgery values, indicating a probable hemodilution caused by administration of intravenous fluids. These differences were tested by Student's t-test (85) and were not significantly different ($P>.05$) with exception of albumin in which pre-surgery values were higher ($P<.05$) than post-surgery values.

Disc gel electrophoresis of intestinal fluid protein. Separation of protein components in luminal fluid from intestinal segments receiving unlabelled gamma globulin was not successful. Two peaks, probably corresponding to albumin and prealbumin, were evident. However, slower migrating protein fractions did not separate clearly, making it difficult to visualize the region corresponding to gamma globulin. In our studies it was not possible to detect differences in either amount (as determined by the density of staining) or protein fractions in fluid of intestinal segments receiving added bacteria or sterile inoculum.

Relationship of blood supply, gut motility, integrity of mucosa and physiological abnormalities on uptake. Venous and arterial supply, gut motility and integrity of the small intestine were subjectively evaluated during initial and final surgical procedures. These observations are shown in Table 16 with age, mean uptake and other pertinent remarks. Depressed gut motility did not appear to be related to lower uptake, based on visual observation and comparison with mean uptake. Two calves

TABLE 16. Gut motility, blood supply, integrity of the mesentery, age at initiation of surgery and mean uptake in calves.

Calf	Age (h)	Mean uptake mg gamma globulin / g tissue		Gut motility	Blood supply	Mesentery integrity	Remarks
1	8.50	.937	0.647	0	+	-	Respiratory difficulties 2 h after initiation of surgery.
2	14.00	3.967	1.436	-	0	+	
3	12.50	2.346	.735	0	0	-	Small diameter intestine.
4	4.25	2.131	1.024	+	-	-	Difficult birth - first calf heifer.
5	7.00	4.531	1.678	+	+	+	
6	12.25	2.984	1.004	+	+	-	
7	11.00	3.953	.671	+	+	+	
8	5.50	3.412	.757	+	+	0	
9	5.00	.969	.542	+	+	-	Grossly enlarged thyroid.
10	6.25	3.525	1.039	0	+	+	

+ = good. 0 = fair. - = poor.

with the least motility internalized gamma globulin well. Both propulsive and segmentative contractions were observed in most intestinal segments. The significance of gut motility is questionable due to restrictions imparted by intestinal ligatures. In unrestricted intestinal tissue one major benefit of peristalsis is the reduction in exposure of intestinal microorganisms to epithelium. Typically, reduced motility is associated with bacterial overgrowth in intestine (3) as occurs in the large intestine where motility is lower.

Development of venous and arterial blood supply to the intestine was difficult to assess. In appeared that mesenteric blood supply had little obvious influence on uptake. Provided that tissue is adequately maintained with nutrients, altered blood flow to the tissue should not affect uptake. In vitro techniques produce considerable trauma and are frequently used to measure absorption, yielding valuable information demonstrating that temporary deprivation of blood supply does not abolish macromolecular absorption.

Evaluation of the mesentery integrity involved examining the mesenteric lymph nodes and thickness of the mesentery, particularly in the distal small intestine. A thickened mesentery that was not translucent, but creamy white was nearly always associated with lower mean uptake. All calves possessing a mesentery exhibiting these characteristics had a mean uptake of less than 3 mg of gamma globulin per g of intestinal tissue (Table 16). It appeared that increased thickness may have been related to excessive fat accumulation. Frequently, the kidneys were completely obscured from vision by fat deposits.

Several other observations were made prior to knowledge of the uptake measurements. Calf 1 experienced extreme respiratory difficulties approximately 2 h after initiation of surgery. Respiratory rate dropped to less than six per minute and heart rate to approximately 40 beats per minute. Reinsertion of the endotracheal tube alleviated the problem and both respiration and heart rate returned to pre-surgery levels. Mean uptake was lowest in this calf, however, whether this influenced uptake of gamma globulin administered 2 h later is unknown. At the time of gamma globulin injection, the tissue appeared normal.

The intestine in calf 3 was observed to be of reduced diameter (about 1.5 cm) as compared to other calves (2 to 3 cm). Absorptive surface area per unit of tissue may have been reduced. This would not affect the comparison of segments receiving added live bacteria or sterile inocula but might have prejudiced values for uptake in the statistical analysis involving age, birth weight or glucocorticoids.

Calf 4 was very weak prior to surgery probably due to a difficult calving since the dam was a first calf heifer and the calf weighed 53 kg. Low mean uptake observed may have been related to this condition.

After completion of surgery in calf 9, the thyroid was observed to be grossly enlarged. Subsequent determination of gamma globulin uptake revealed values more than one standard deviation below the mean. Chan et al. (13) demonstrated a relationship between thyroxine and closure in young rats. Exogenous administration of thyroxine (2 ug thyroxine per g body weight) on the fifth postnatal day resulted in complete cessation of macromolecular absorption by day 13 of life. Possibly, the enlarged

thyroid observed in this calf is related to subnormal uptake.

The aforementioned observations are intended to fully describe conditions present in experimental animals which may have had a bearing on uptake results of this experiment.

Disposition of ^{125}I-gamma globulin dose. Values for radioactivity of ^{125}I in the blood, thyroid, liver and lymph tissue are shown in Appendix XVI. Aliquots of solution used to rinse the mucosal surface were not counted as in experiment I. Average percentages of the dose remaining in intestinal segment tissue and lumen contents together were 53.8. This compares favorably with 59.8% found in segment tissue and lumen contents in experiment I. Thyroid, liver and lymph tissue contained 6.38×10^{-4}, 4.5×10^{-4}, and 1.54×10^{-4} uCi of activity per gram of tissue, respectively. Similar levels of activity were found in corresponding tissues of calves in experiment I. The average radioactivity found in the blood of the 10 calves was .736% of the total activity administered. In experiment I blood activity was 1.43% of the dose. The lower levels of activity found in the blood of experiment II calves may be due to lower levels of free ^{125}I found in the gamma globulin dose used in this experiment. Only 2.46% of the dose was found in the supernatant after trichloroacetic acid precipitation compared to 6.7% in the gamma globulin dose used in experiment I. Activity remaining in the supernatant is either free

^{125}I or ^{125}I bound to low molecular weight proteins (65). As discussed previously, these components would be readily absorbed by intestinal epithelium. The very low levels of radioactivity found in thyroid, liver, lymph and blood indicated total transport had not occurred, giving validity to the techniques used.

GENERAL DISCUSSION

Mean uptake by segments in experiment II was 2.94 ± 1.50 mg gamma globulin per gram of intestinal tissue. This agrees with the value of 3.06 mg gamma globulin per gram of intestinal tissue obtained by extrapolation from the prediction equation representing a similar region of the gut in calves of experiment I.

Data from experiment I indicated that the distal small intestine was the region of greatest uptake when allowed 1.5 h exposure to gamma globulin. These findings concur with those of Staley et al. (84). When tissue was exposed to gamma globulin for .5 h, an area corresponding to the lower midgut internalized more of the labelled protein. This might indicate that the midgut internalized macromolecules at a greater rate. Documentation of this is not available. Pinocytosis of more gamma globulin by lower gut may be related to several factors. 1) Ileal epithelial cells may possess a greater hoolding capacity for macromolecules. Staley et al. (84) noted that the apical tubular system in jejunum was not as developed as in ileal enterocytes, indicating diminished capacity for macromolecular pinocytosis. In the rat (18), cytoplasmic vacuoles containing gamma globulin in ileal cells are more prominent than in jejunal or duodenal cells. 2) Increased uptake may be related to progressive restriction of pinocytosis to the distal small intestine with increasing age as observed in the rabbit (16), ferret (16) and pig (55, 63). Al-

through the PSEG*age interaction for uptake was nonsignificant ($P>.05$), a trend was evident.

No effect of tissue weight was evident ($P>.05$) in segments exposed to gamma globulin for 1.5 h. This may have been the result of epithelial cells reaching their maximum capacity for internalized protein. Hardy (34) reported that 6 h after duodenal infusion of gamma globulin in electrolyte solution identical to that used in this study, ileal enterocytes contained large quantities of invacuolated protein. Staley et al. (84) observed a similar relationship after 2 h exposure. In segments exposed to gamma globulin for .5 h, tissue weight did not affect ($P>.05$) uptake but linear and quadratic effects for PSEG were no longer significant ($P>.05$). Since tissue weight of the segment was negatively correlated ($P<.01$) with uptake, it was deduced that the amount of absorptive surface area may have affected uptake more than position of the segment. Information obtained from this experiment enabled selection of a region of intestine to evaluate the effects of added microorganisms on uptake.

Exposure of intestinal tissue to added bacteria early in life resulted in reduced uptake ($P<.01$) of gamma globulin as compared to tissue exposed to either sterile broth or autoclaved bacteria. Without microscopic examination of affected tissue, it is unknown how bacteria acted to inhibit pinocytosis. According to Corley et al. (19), E. coli are capable of exfoliating microvilli and penetrating the apical plasma membrane in colostrum-deprived calves. Undoubtedly, these actions would impair macromolecular absorption. Possibly the microflora existing within segments of the calves of this experiment was acting in a similar

manner. The actions of the fostered microflora on replacement of villus epithelium are probably minor, owing to the short duration of exposure of epithelium to added microorganisms. Considering the restrictions to intestinal motility imparted by segment ligatures, microbial interactions with the epithelial surface may have been increased. As a result, enterocytes permeable to gamma globulin would be progressively restricted to the villus tip as observed by El-Nageh (27).

It is not known whether sterile microbiological media or autoclaved bacteria affected uptake. Mean uptake for segments receiving sterile broth or autoclaved bacteria was 3.18 $\pm$ 1.50 and 3.56 $\pm$ 1.49 mg gamma globulin per gram tissue, respectively. These values compare favorably with uptake of 3.06 mg gamma globulin per gram tissue found in a similar region of gut in calves of experiment I. In the last calf of experiment II, one segment in each section was formed but was not inoculated. Uptake in these three segments was not different from segments receiving sterile broth or autoclaved bacteria. Considering these observations, it appeared that sterile broth or autoclaved bacteria did not act to inhibit uptake. The nonspecific utilization of the apical plasma membrane as a cause of closure is not a plausible theory in calves (10).

A concern of previous studies (44, 45) was that the inhibition of post-colostrum feeding increases in serum gamma globulin was related to non-microbial components of duodenal fluid inoculum. Results of experiment II indicate that bacteria are the likely cause of inhibition rather than substances of intestinal fluid.

Low serum corticosteroid was associated ($P<.01$) with depressed

uptake in calves of experiment II. These findings must be considered with respect to hemodilution caused by intravenous fluid administration, trauma resulting from the surgical procedures and the limited number of calves used in this study. Hemodilution was indicated by nonsignificant ($P>.05$) reduction in serum protein concentration of post-surgery blood samples as compared to pre-surgery values. Since colostrum was not fed, it was assumed the serum protein would remain constant. The observation that serum albumin was lower ($P<.05$) post-surgery could have considerable importance since corticosteroids are bound to albumin during transport in blood (9).

Mean post-surgery corticosteroid values were generally higher than pre-surgery values although the difference was nonsignificant ($P>.05$). Three calves with lowest corticosteroid of serum, both pre- and post-surgery, also had lower uptake as compared to remaining calves. The depressed values could not be attributed to age of calf. It is possible that depressed uptake in those calves is explained by findings of Patt and Eberhart (75). Piglets having depressed corticosteroid of plasma absorbed less colostrum Ig as compared to untreated or ACTH-treated littermates, suggesting that a threshold level of plasma corticosteroid may need to be reached before cells are capable of macromolecular absorption. Possibly hypogammaglobulinemia in the colostrum-fed newborn calf is related to an adrenal insufficiency.

The possible relationship of corticoids with gamma globulin must be considered with respect to observations made in two calves exhibiting low serum corticosteroids. Calf 1 had severe anorexia two h after ini-

tiation of surgery. Possibly this affected either uptake or corticosteroid levels. Calf 9 had an enlarged thyroid gland which may have had considerable bearing on macromolecular absorption based on studies by Chan et al. (13). Further studies examining corticosteroid and thyroid hormone status of newborn calves and colostrum Ig absorption are needed.

Volume of fluid remaining within the segment was negatively correlated with uptake ($P<.01$) in experiments I and II. When uptake was reduced, net fluid absorption was lower. Bacterial growth in tissue was positively correlated ($P<.05$) with volume of segment contents. Possibly bacteria acted to increase net efflux of fluid into the segment lumen through alteration of the plasma membrane as observed by Corley et al. (19). In experiment I restrictions on intestinal motility may have allowed endogenous bacteria to increase, resulting in considerable interaction with the intestinal epithelium. Volume of segment fluid was also negatively correlated ($P<.01$) with PSEG which concurs with the finding that the ileum is the site of greatest water absorption (9). Interaction of transport systems for ion absorption and macromolecular absorption have not been studied. Therefore, the effects of NaCl and KCl absorption on pinocytosis of gamma globulin as related to osmotic pressure and hydrostatic pressure gradients are unclear.

Relationship of age with uptake was unclear in these studies. Age was negatively related ($P<.05$) to uptake in experiment I and positively related to uptake ($P<.01$) in experiment II. Kruse (51) found that gamma globulin absorption was reduced in a linear manner when first colostrum

feeding was delayed from 2 h to 20 h. However, results of experiments I and II as well as previous studies by the author (44, 45) fail to support a linear relationship between age and serum gamma globulin status in the calf less than 14 h old. It appears that unknown factors other than age influence gamma globulin absorption more in the newborn calf.

Our results agree with Kruse (51) that birth weight is of minor importance to gamma globulin absorption or uptake. In experiment I larger calves internalized more ($P<.05$) gamma globulin but in experiment II the opposite was true. Previous studies (44, 45) failed to detect a relationship between birth weight and serum gamma globulin levels.

It is obvious from the results of these experiments that the early intestinal microflora may influence macromolecular absorption as evidenced by reduced uptake of gamma globulin in segments receiving added bacteria. However, microscopic examination of tissue exposed to added microorganisms is necessary before this relationship can be confirmed. Sufficient evidence has been presented to suggest a relationship of hormone status with macromolecular absorption. Possible steroid or thyroid hormone levels at birth predetermine the absorptive capabilities of the intestine for Ig. Cessation of macromolecular absorption is not exclusively related to any one factor, it is likely the interplay of early intestinal microflora and its physical effects on absorptive surface, hormonal status of the neonate, age at first feeding of colostrum and mass of colostrum Ig fed.

SUMMARY AND CONCLUSIONS

Two experiments were conducted using newborn colostrum-deprived calves to establish the distribution of uptake of ^{125}I-gamma globulin in the small intestine and to investigate the effects of added microorganisms on ^{125}I-gamma globulin uptake.

In experiment I, 10 calves less than 12.5 h of age ($\bar{X}$ = 7 h) were anesthetized and intestines exteriorized through a paramedial abdominal incision. Beginning at the ileocecal junction and proceeding anteriorly, intestine was ligated into 10 cm segments at 70 cm intervals. Segments were injected after formation with ^{125}I-gamma globulin in an electrolyte solution that permitted pinocytosis but not transport out of the epithelial cell. Segments were exposed to ^{125}I-gamma globulin for 1.5 h. In the last six calves one additional segment was placed adjacent to segments 1, 5 and 10 to assess effects of .5 h exposure of ^{125}I-gamma globulin on uptake by the epithelium. Intestines were returned to the abdomen between 1.5 h and .5 h injections and the incision closed. One and one-half h after injection of the first 1.5 h segment, zero time control segments were formed adjacent to segments 1, 5 and 10, injected with ^{125}I-gamma globulin, agitated and immediately excised. These segments measured gamma globulin adhering to the external surface of the epithelium after brief exposure and repeated washing. Remaining segments were then excised, homogenized and uptake determined. Volume of

segment contents was measured. Location of each segment was expressed as percentage of the distance from cecum to abomasum. Uptake was expressed as milligrams gamma globulin internalized per gram of segment tissue after correction for residual gamma globulin. Age of calf at initiation of surgery, birth weight and intestine length were recorded.

Regression analysis of uptake on position of segment (PSEG) was used to evaluate effects of PSEG, age, birth weight and other parameters on uptake. Slopes representing gamma globulin incubation for 1.5 h and .5 h were tested for homogeneity by Student's t-test. Least squares analysis procedure tested for significant effects of measured parameters on uptake.

Distribution of uptake of gamma globulin after 1.5 h incubation was a cubic function of PSEG. Uptake was greatest in a region 15% of the distance from cecum to abomasum and declined progressively towards the abomasum. Uptake was greater ($P<.05$) for segments exposed to gamma globulin for 1.5 h than .5 h. For segments exposed to gamma globulin for .5 h, regression of uptake on PSEG was a quadratic function of PSEG. Uptake was greatest in an area located 30% of the cecum-abomasum distance.

Calves were different ($P<.01$) in mean uptake of segments at both 1.5 h and .5 h exposure, but distribution of gamma globulin uptake by the small intestine was similar. Volume of segment contents remaining after excision was negatively correlated ($P<.01$) with uptake, indicating a relationship between net fluid absorption and gamma globulin uptake. Weight of segment did not affect ($P>.05$) uptake in either 1.5 h or .5 h gamma globulin exposure. However, in .5 h gamma globulin exposure seg-

ments, inclusion of segment weight in the analysis of variance caused linear and quadratic effects of PSEG to become nonsignificant ($P>.05$). Possibly segment weight affected uptake more than PSEG in segments exposed to gamma globulin for .5 h. Age was negatively related to uptake ($P<.05$), indicating that uptake was lower in intestines of older calves. Birth weight was associated with uptake ($P<.01$) in a positive manner.

Ten calves less than 14 h of age ($\bar{X}$ = 8.6 h) were used in experiment II to study effects of added intestine origin bacteria, sterile microbiological broth and autoclaved bacteria on uptake of ^{125}I-gamma globulin in an electrolyte solution by small intestine tissue.

Beginning approximately 1.8 meters above the ileocecal junction, intestine was ligated into segments 10 cm in length at 3 cm apart. Seven treatments were assigned in random order to segments in three successive sections of small intestine. Three treatments measuring uptake consisted of inoculation with 1 ml of either live bacteria culture, sterile broth or autoclaved bacteria culture followed by 4 h incubation then exposure to ^{125}I-gamma globulin for 1.5 h. Two treatments measured anaerobic microbial growth after 4 h incubation with either live bacteria or sterile broth. These segments received unlabelled gamma globulin for 1.5 h. Residual ^{125}I-gamma globulin was measured in segments receiving either live bacteria or sterile broth with 5.5 h incubation followed by 15 seconds exposure to ^{125}I-gamma globulin. After 1.5 h exposure to gamma globulin solutions, segments were excised and tissue radioactivity measured. Uptake was expressed as in experiment I. Microbial growth was measured using roll

tube techniques.

All data was analyzed by least squares analysis of variance. Treatment effects for uptake were compared by partitioning degrees of freedom into: 1) C1 = live bacterial inoculum versus sterile broth and autoclaved bacterial inoculum and 2) C2 = sterile broth versus autoclaved bacterial inoculum.

Uptake was lower ($P<.05$) in segments receiving live bacteria than in segments receiving sterile broth or autoclaved bacteria. Greater numbers of bacteria in segment tissue were associated with reduced uptake ($P<.10$). Numbers of bacteria in inocula were positively correlated ($P<.01$) with bacterial growth in segment tissue. These findings indicate that live bacterial inoculum influenced tissue bacterial growth resulting in reduced uptake of gamma globulin. Volume of contents in the segment was negatively correlated ($P<.01$) with uptake, indicating a relationship between uptake and net fluid absorption.

Low serum corticosteroid values were associated ($P<.01$) with low gamma globulin uptake. Three calves possessing the lowest serum corticosteroid concentration were lowest in uptake of gamma globulin. Possibly corticosteroid status of the newborn calf may predetermine gamma globulin absorptive ability.

Lower body weight ($P<.01$) and greater age ($P<.01$) were associated with increased gamma globulin uptake. However, these parameters were probably of minor significance in regard to uptake.

Visual evaluation of the intestines, blood supply and motility did not reveal any relationship with uptake. However, a thickened mesentery

that appeared to contain large amounts of adipose tissue was associated with reduced uptake.

From these experiments with newborn calves, the following conclusions were made:

1) It appears that the distal small intestine is the site of greatest gamma globulin uptake per gram of intestinal tissue, provided that exposure to gamma globulin is not limited.
2) Exposure of intestinal epithelium to added bacteria results in reduced uptake of gamma globulin.
3) Levels of serum corticosteroids in the newborn calf may influence uptake of gamma globulin in a negative manner.
4) Age and body weight are factors of minor importance in regard to gamma globulin uptake in the calf less than 14 h of age.

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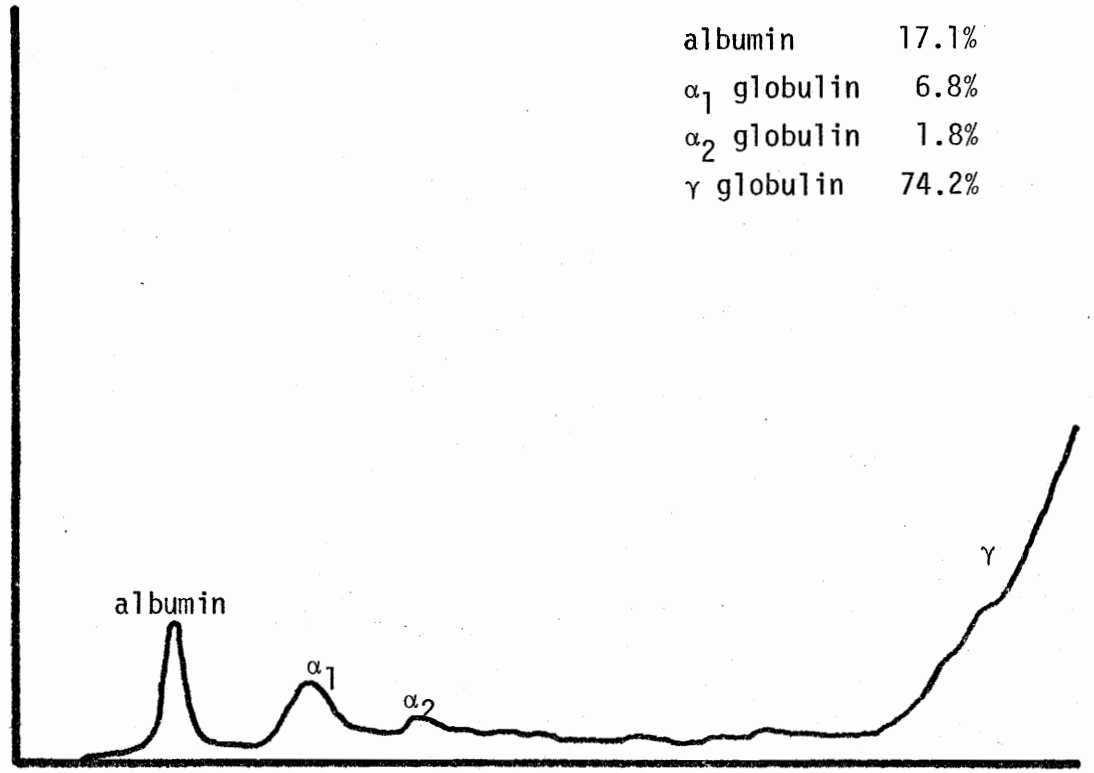
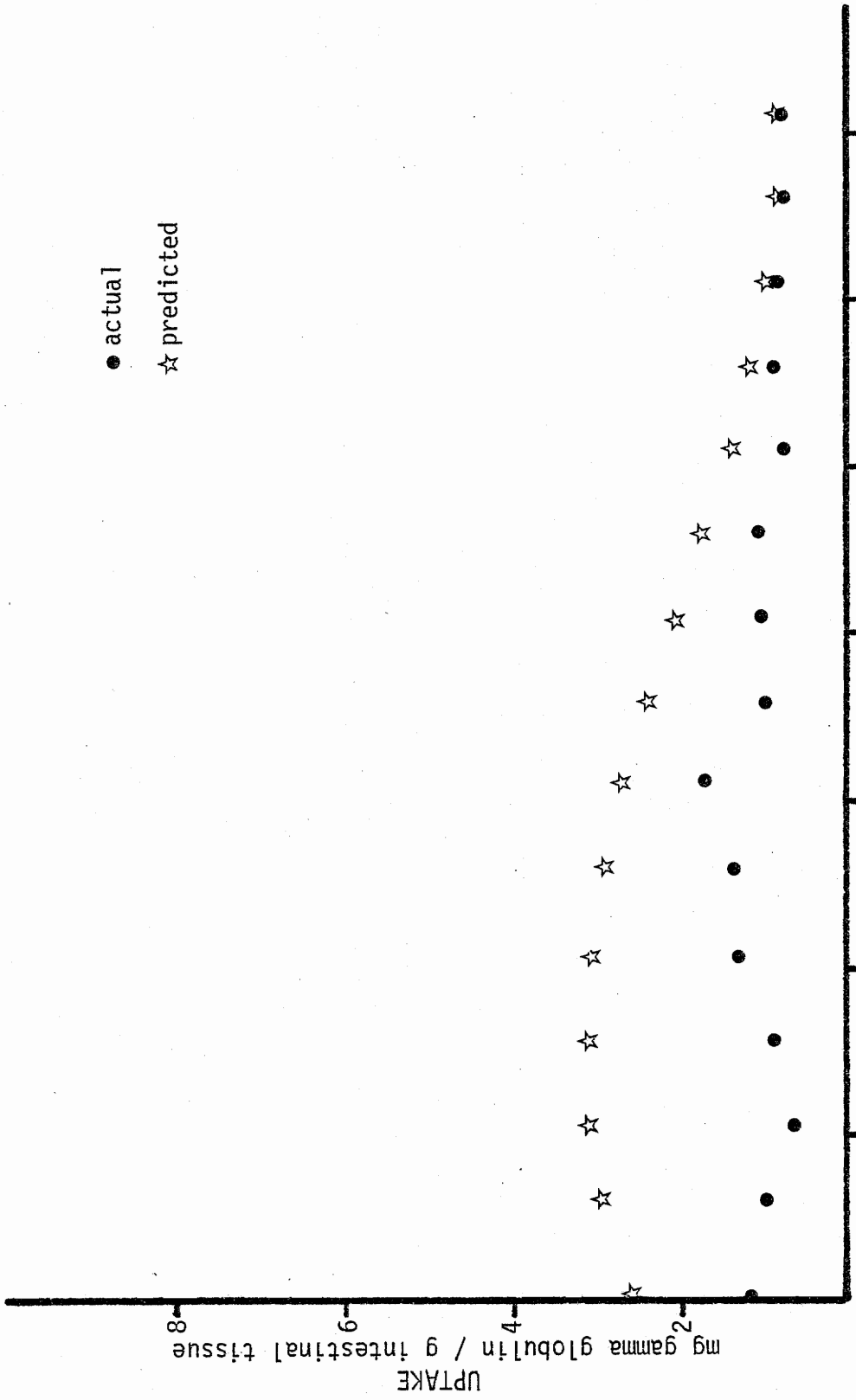
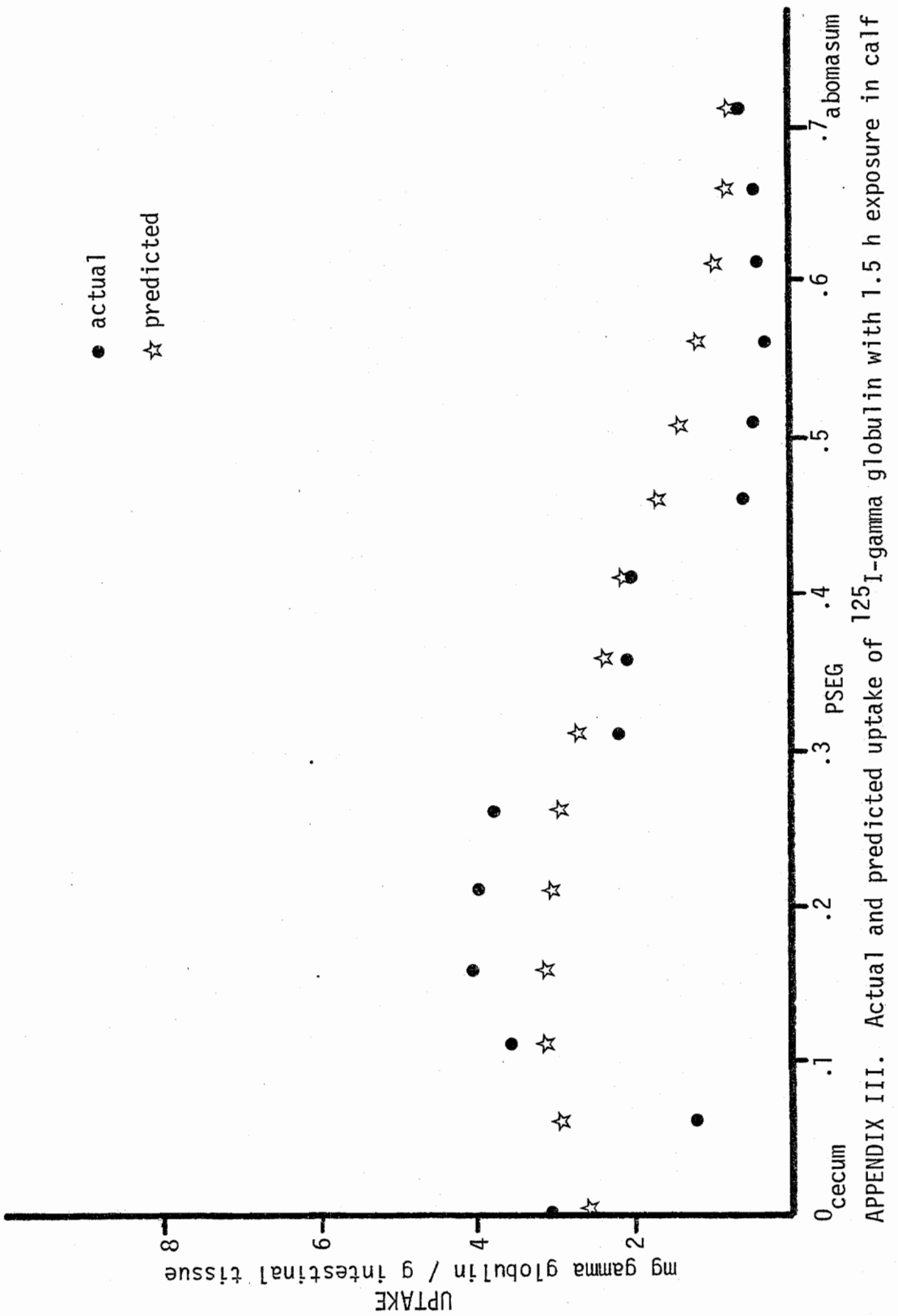


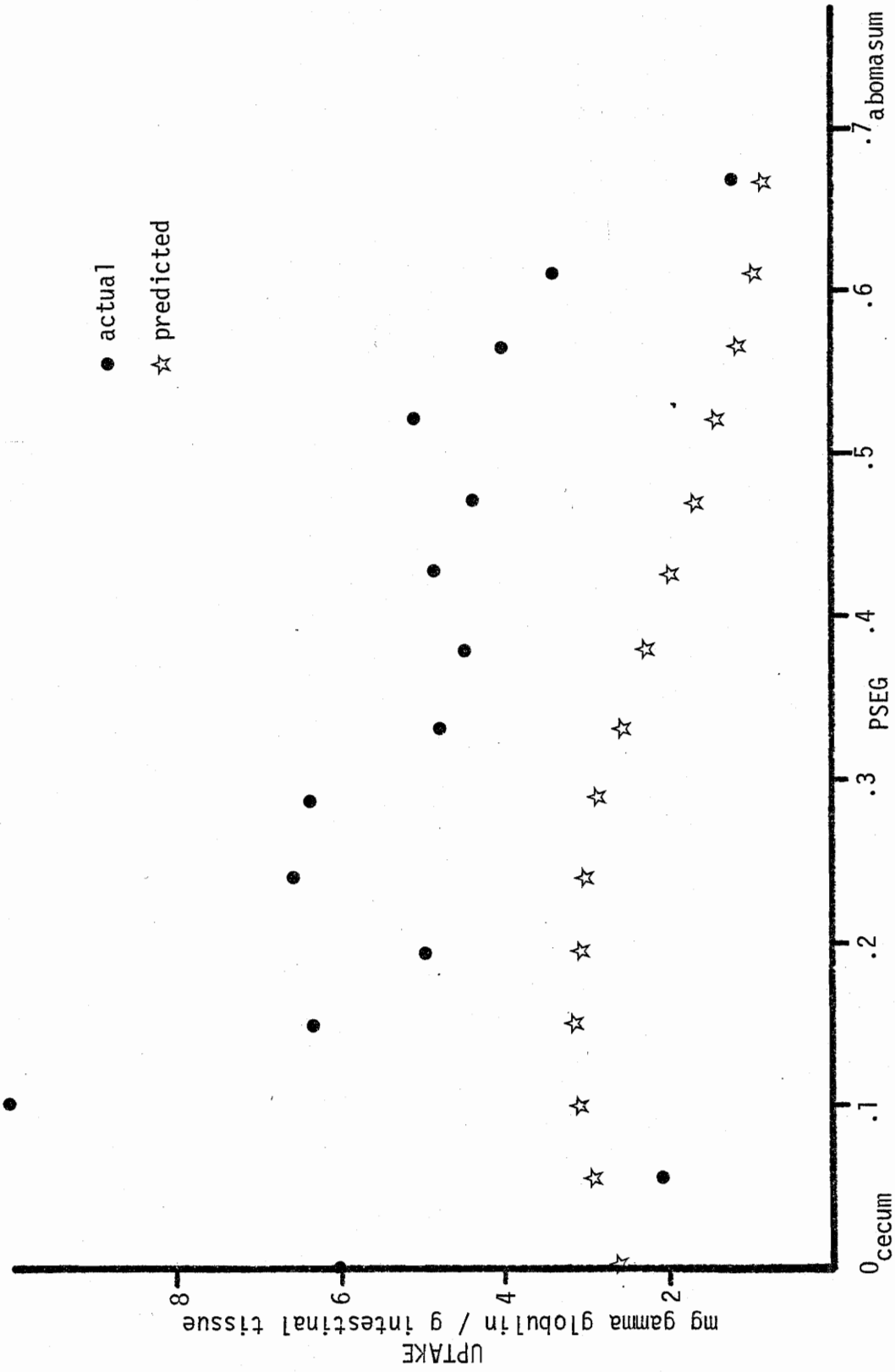
Appendix I. Densitometer tracing of gamma globulin used in experiment II.



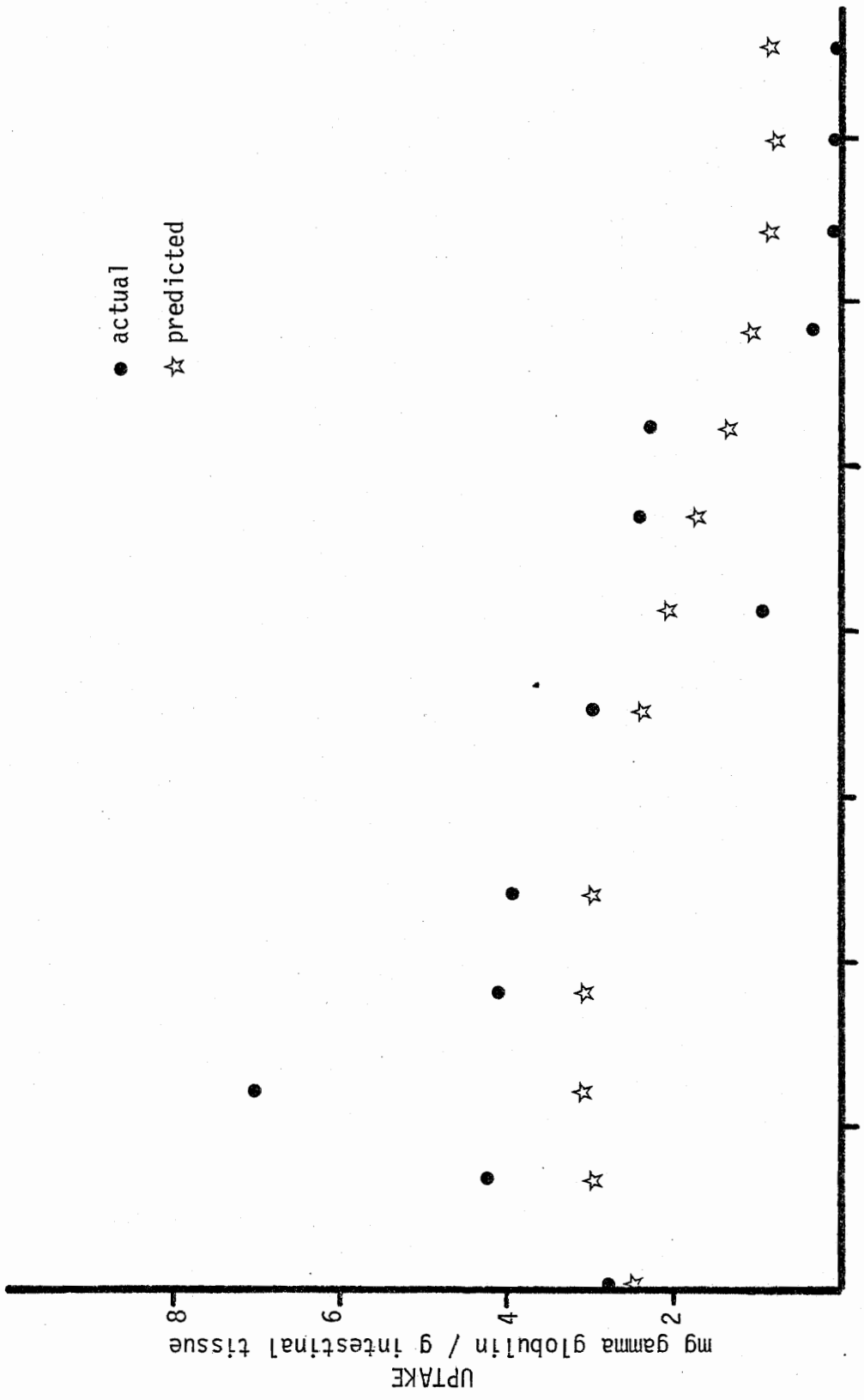
APPENDIX II. Actual and predicted uptake of ^{125}I-gamma globulin with 1.5 h exposure in calf 1.



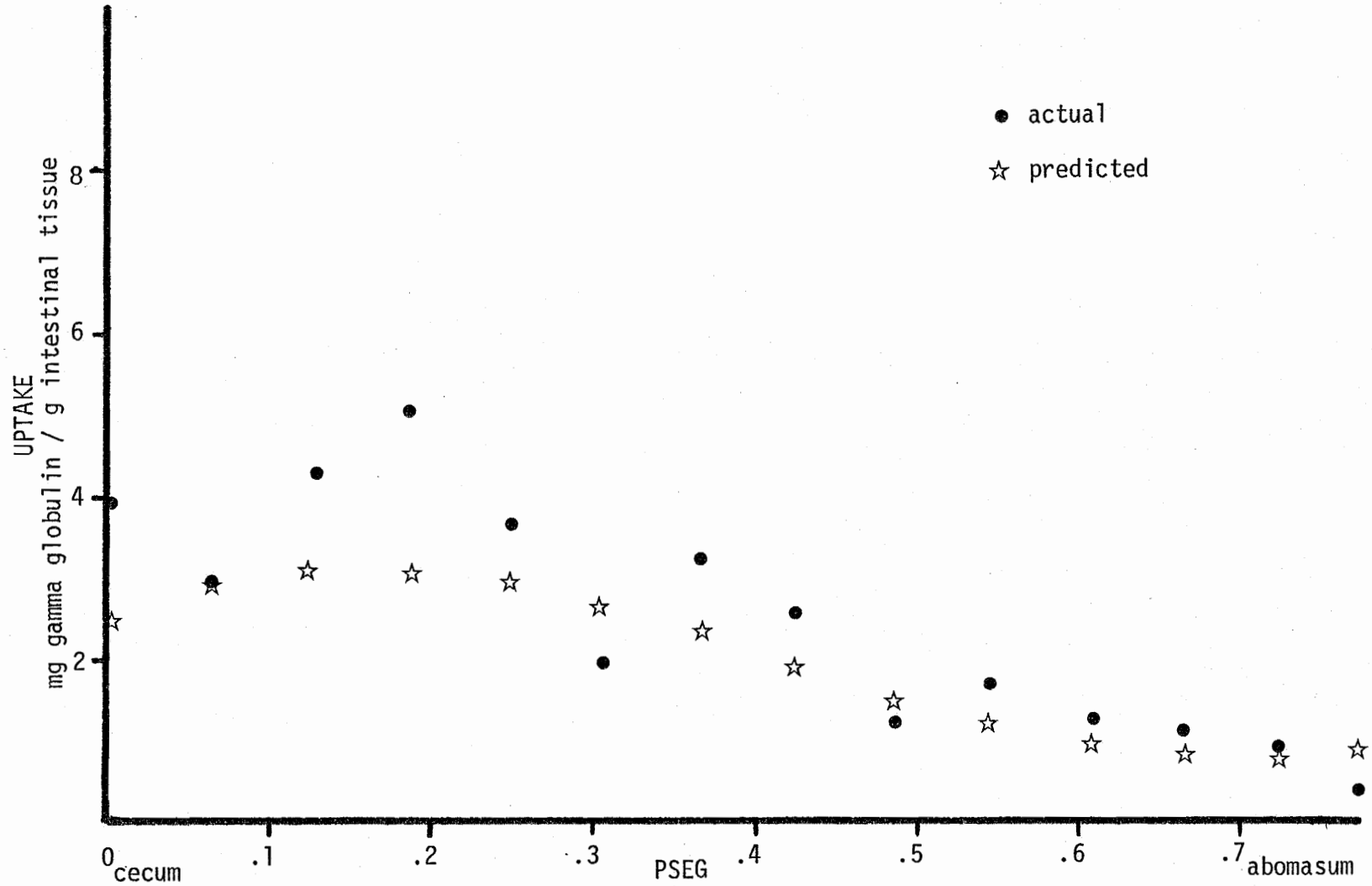
APPENDIX III. Actual and predicted uptake of ^{125}I-gamma globulin with 1.5 h exposure in calf 2.



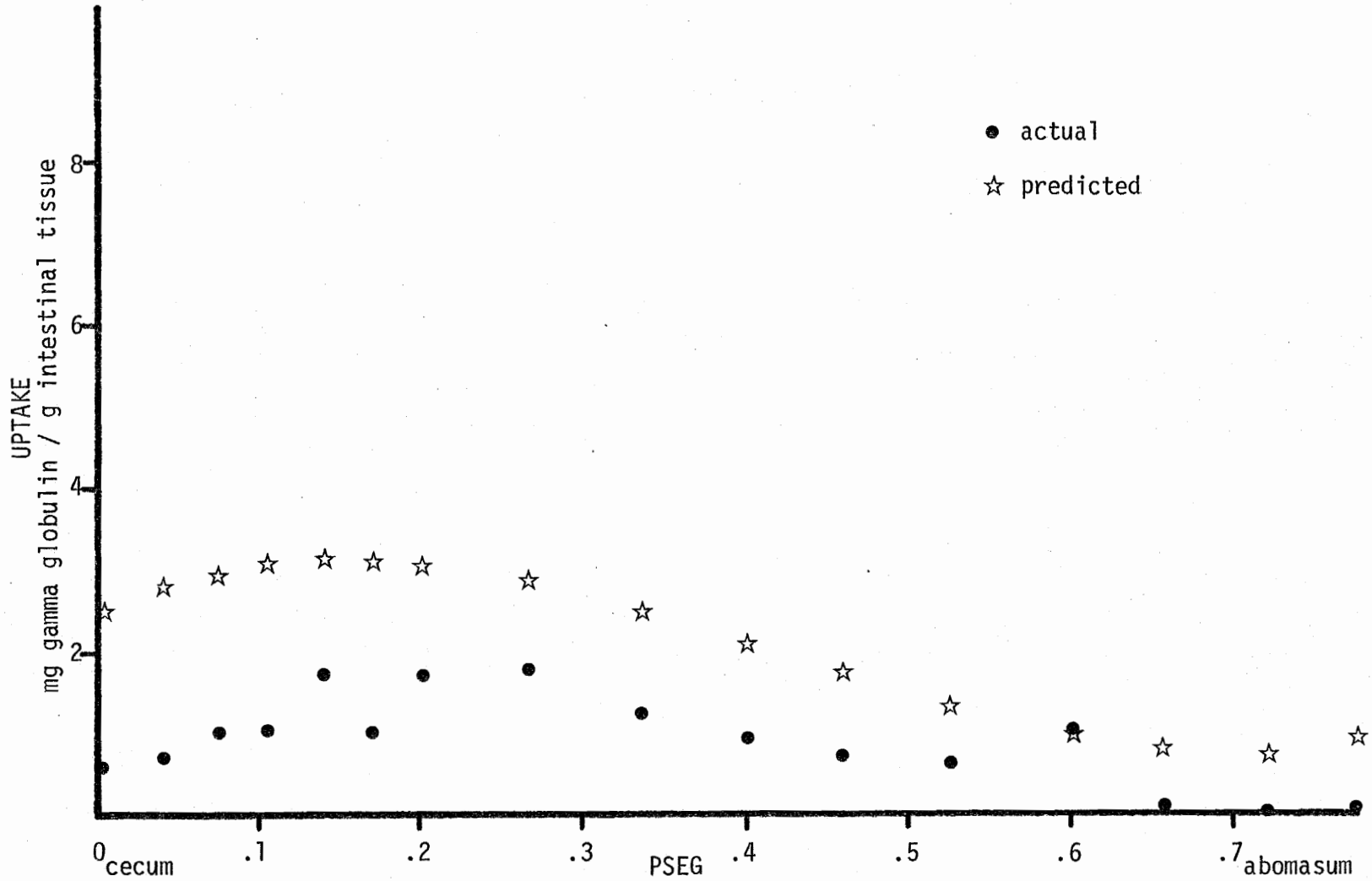
APPENDIX IV. Actual and predicted uptake of ^{125}I-gamma globulin with 1.5 h exposure in calf 3.



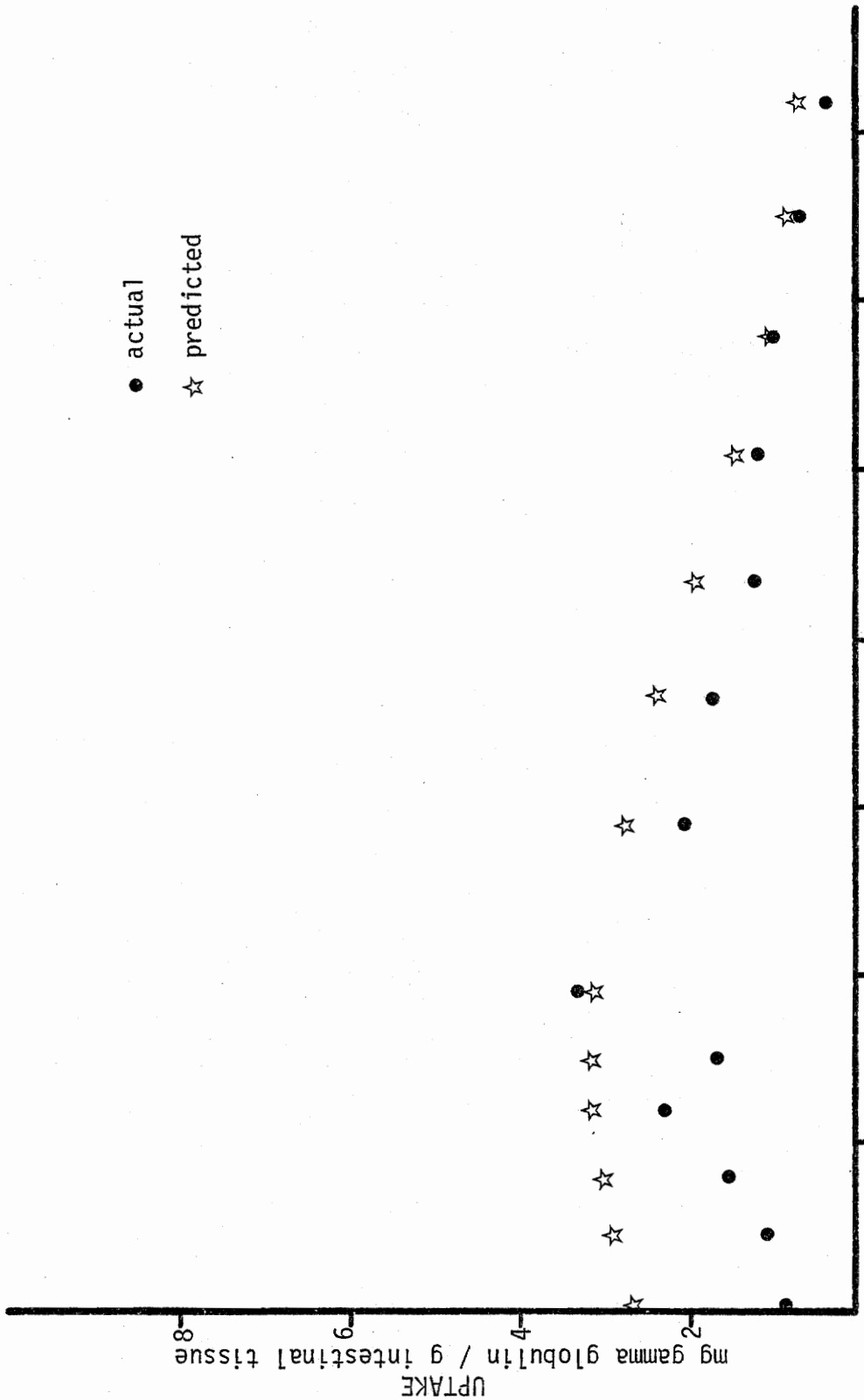
APPENDIX V. Actual and predicted uptake of ^{125}I-gamma globulin with 1.5 h exposure in calf 4.



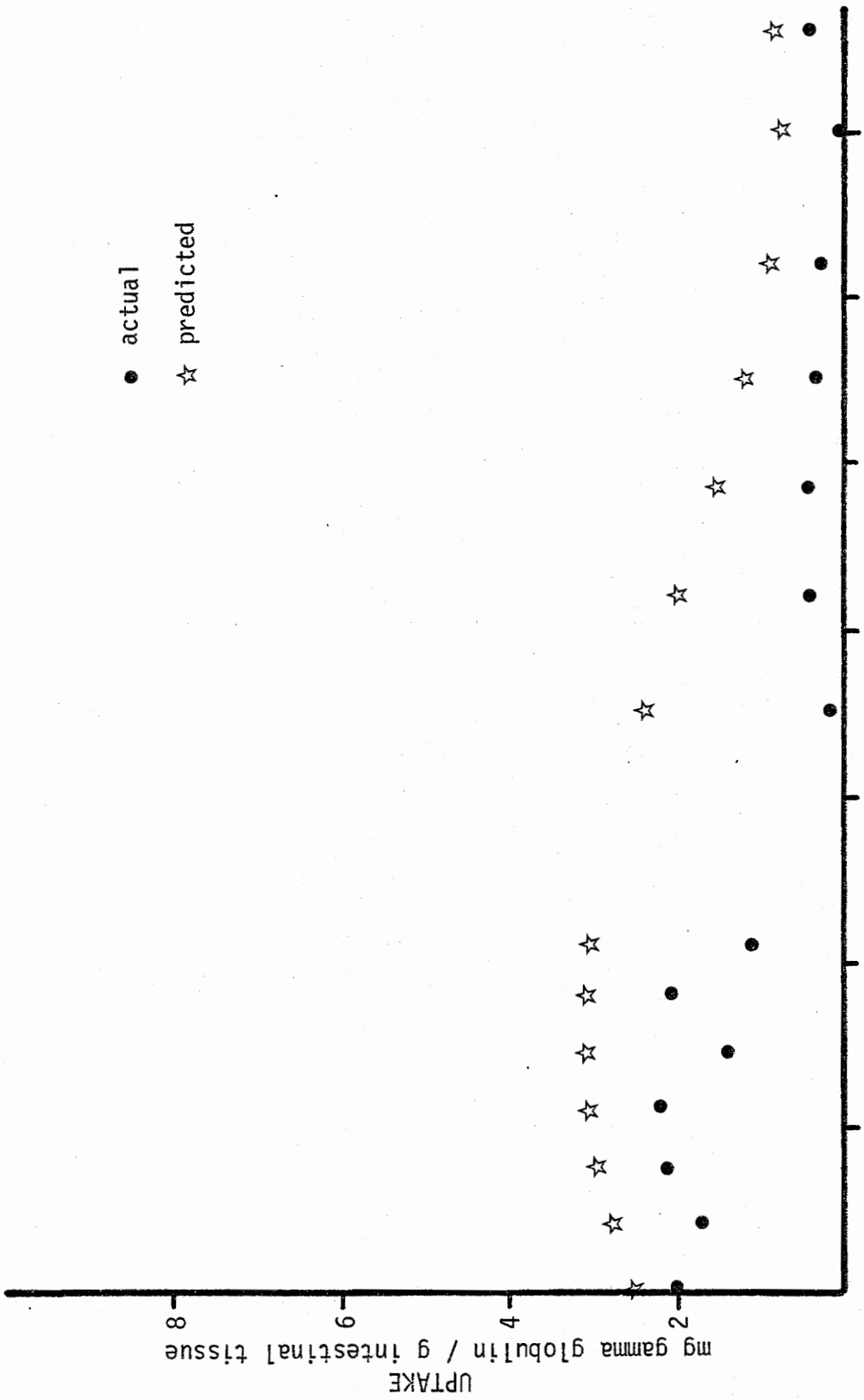
APPENDIX VI. Actual and predicted uptake of ^{125}I-gamma globulin with 1.5 h exposure in calf 5.



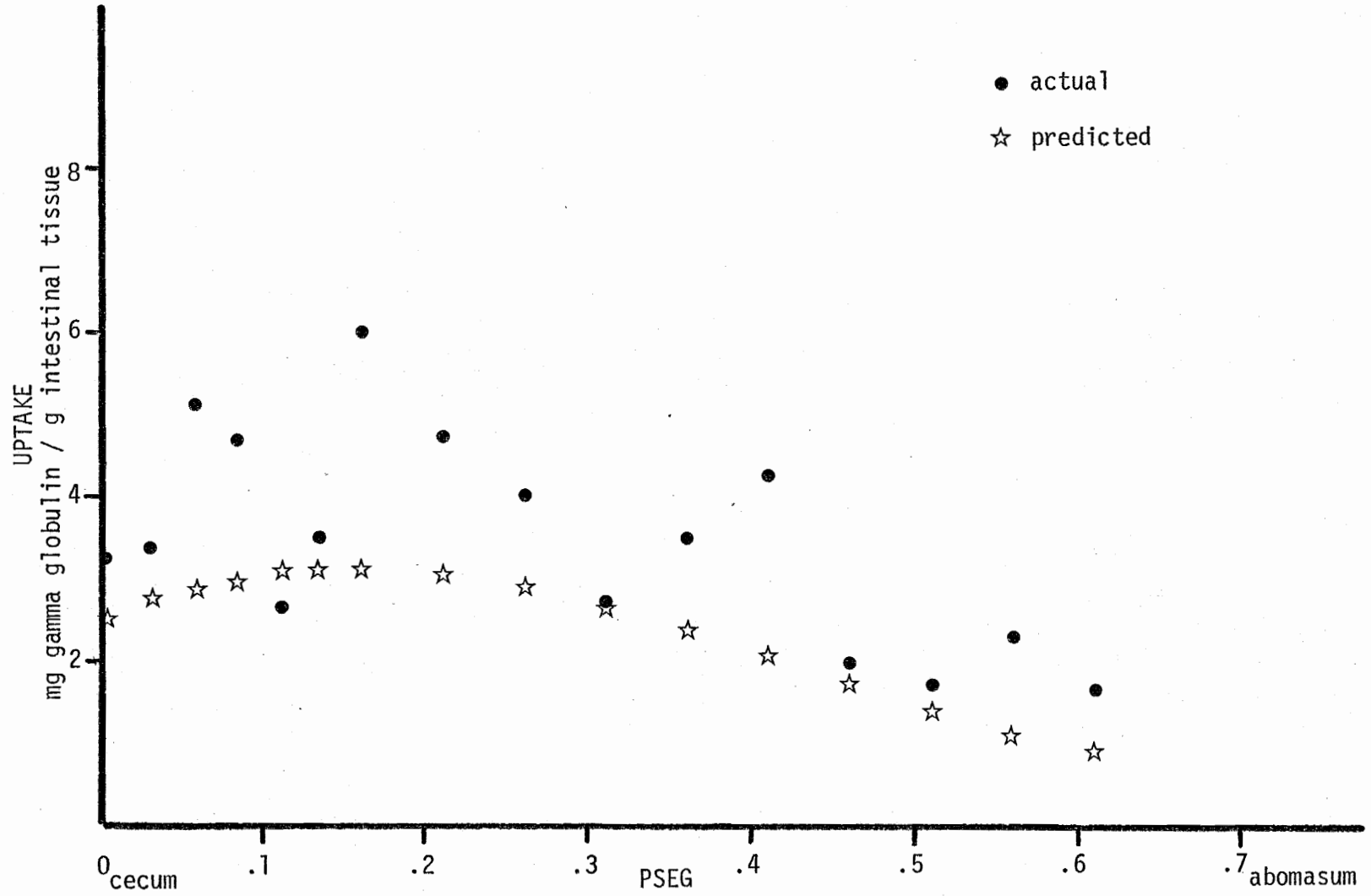
APPENDIX VII. Actual and predicted uptake of ^{125}I-gamma globulin with 1.5 h exposure in calf 6.



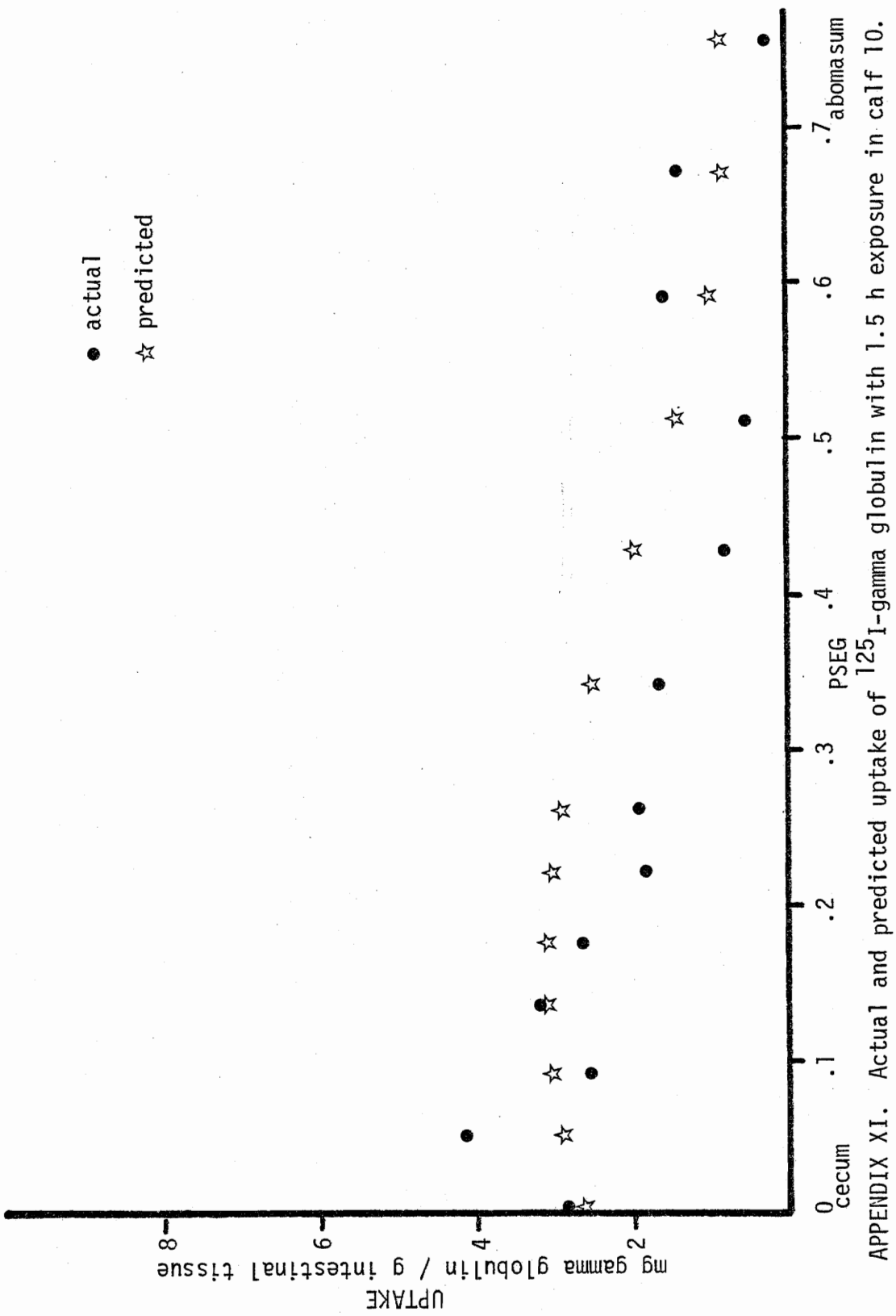
APPENDIX VIII. Actual and predicted uptake of ^{125}I-gamma globulin with 1.5 h exposure in calf 7.



APPENDIX IX. Actual and predicted uptake of ^{125}I-gamma globulin with 1.5 h exposure in calf 8.



APPENDIX X. Actual and predicted uptake of ^{125}I-gamma globulin with 1.5 h exposure in calf 9.



APPENDIX XI. Actual and predicted uptake of ^{125}I-gamma globulin with 1.5 h exposure in calf 10.

APPENDIX XII. Levels of radioactivity in blood, thyroid, liver and mesenteric lymph node tissue and average percent recovery of ^{125}I-gamma globulin dose in intestinal segment tissue and contents in experiment I calves.

Calf	% recovery of dose in segment tissue and contents[a]	Total uCi administered[b]	Blood-total uCi in estimated blood volume[c]	% dose in total blood volume	Thyroid (uCi/g x 10^{-4})	Liver (uCi/g x 10^{-4})	Lymph (uCi/g x 10^{-4})
1	63.1 ± 12.3	24.21	.27	1.09	1.50	---	---
2	74.9 ± 13.6	35.44	.22	.61	17.00	4.00	1.30
3	55.7 ± 26.9	11.23	.30	2.68	6.00	.75	1.35
4	74.1 ± 13.6	10.93	.31	2.88	6.00	1.70	1.90
5	70.2 ± 12.5	18.82	.15	.79	15.50	1.00	1.45
6	61.2 ± 14.3	33.30	.32	.97	13.50	.60	.70
7	57.0 ± 16.0	18.57	.21	1.13	6.00	1.45	6.50
8	64.8 ± 16.6	23.30	.31	1.32	6.00	4.65	.95
9	47.4 ± 9.6	28.47	.31	1.09	5.50	.42	9.00
10	41.0 ± 17.5	22.52	.39	1.75	5.50	.80	2.25
Mean	59.8 ± 17.6		.28	1.43	1.95	1.70	2.54

[a]Average percentage recovery of all segments within each calf.
[b]Total uCi administered = number of segments injected x uCi per segment.
[c]Blood volume = birth weight x .08.

APPENDIX XIII. Isotope recovery from selected lower intestine segments - tissue, luminal fluid and rinses.

Calf	Segment[a]	Tissue	Fluid	0 Rinse	1 Rinse	2 Rinse	Dose	% Dose
1	1	.269[b]	.532	.004	.234	.127	1.424	82.0
2	1	.655	.543	.005	.316	.140	2.085	79.6
3	2	.154	.000	.105	.015	.011	.624	47.4
4	1	.241	.082	.006	.091	.064	.643	75.3
5	1	.510	---	.004	.221	.076	.941	86.1
6	1	.203	.570	.011	.219	.088	1.449	75.3
7	1	.098	.482	.100	.021	---	.884	79.2
8	1	.295	.195	.001	.130	.008	1.059	59.3
9	1	.449	.281	.002	.069	.043	1.238	68.2
10	1	.408	---	.003	.133	.212	1.073	70.4
Mean								72.3

[a] All segments exposed to gamma globulin for 1.5 h except where noted by §. § = .5 h exposure.
[b] Expressed as uCi ^{125}I in total segment weight, lumen fluid volume or total rinse volume.

APPENDIX XIV. Isotope recovery from selected middle intestine segments - tissue, luminal fluid and rinses.

Calf	Segment[a]	Tissue	Fluid	0 Rinse	1 Rinse	2 Rinse	Dose	% Dose
1	3	.075[b]	.876	.093	.240	.034	1.424	92.0
2	3	.378	.627	.030	.804	.093	2.085	92.7
3	5	.260	---	.005	.114	.037	.624	66.5
4	5	.250	---	.003	.147	.039	.643	68.2
5	5	.312	---	.012	.176	.068	.941	60.4
6	5	.227	.828	.010	.239	.060	1.448	94.8
7	5	.189	.328	.004	.175	.038	.884	83.0
8	5§	.040	.670	.011	.064	.017	1.059	75.7
9	5	.563	---	.003	.233	.118	1.238	74.1
10	5	.217	---	.005	.335	.097	1.073	60.9
Mean								76.8

[a] All segments exposed to gamma globulin for 1.5 h except where noted by §. § = .5 h exposure.

[b] Expressed as uCi ^{125}I in total segment weight, lumen fluid volume or total rinse volume.

APPENDIX XV. Isotope recovery from selected upper intestine segments - tissue, luminal fluid and rinses.

Calf	Segment[a]	Tissue	Fluid	0 Rinse	1 Rinse	2 Rinse	Dose	% Dose	Aver.% recovery[c]
1	5	.069[b]	.891	.004	.288	.056	1.424	92.0	88.7
2	5	.468	.876	.017	.660	.061	2.085	99.8	90.7
3	8	.287	---	.006	.180	.037	.624	81.7	65.2
4	8	.126	---	.063	.111	.029	.643	51.2	64.9
5	5§	.113	.358	.006	.125	.029	.941	67.0	71.2
6	10	.214	.686	.013	.215	.061	1.448	80.4	83.5
7	10	.101	.492	.009	.130	.049	.884	88.3	83.5
8	10	.054	.694	.099	.040	---	1.059	83.8	72.9
9	10	.372	.316	.006	.241	.081	1.238	82.0	74.8
10	10	.077	.588	.014	.144	.041	1.073	80.5	70.6
Mean								80.7	76.6

[a]All segments exposed to gamma globulin for 1.5 h except where noted by §. § = .5 h exposure.
[b]Expressed as uCi ^{125}I in total segment weight, lumen fluid volume or total rinse volume.
[c]Average % recovery = average of lower, middle and upper intestine segment % recovery (from Appendices XIII, XIV and XV).

APPENDIX XVI. Levels of radioactivity in blood, thyroid, liver and mesenteric lymph node tissue and average percent recovery of ^{125}I-gamma globulin dose in intestinal segment tissue and contents in experiment II calves.

Calf	% recovery of dose in segment tissue and contents[a]	Total uCi administered	Blood-total uCi in estimated blood volume[c]	% dose in total blood volume	Thyroid (uCi/g x 10-4)	Liver (uCi/g x 10-4)	Lymph (uCi/g x 10-4)
1	76.9 ± 2.9	19.28	.14	.73	4.35	1.70	1.25
2	46.4 ± 7.8	21.29	.12	.58	9.00	3.50	2.00
3	47.2 ± 16.8	22.68	.13	.56	10.00	26.50	3.35
4	53.6 ± 13.7	22.78	.16	.71	3.65	1.40	.70
5	48.6 ± 10.1	18.27	.12	.67	5.00	.40	1.65
6	59.1 ± 9.8	26.00	.09	.34	6.00	2.25	2.50
7	42.9 ± 9.1	19.09	.12	.63	7.00	.30	1.35
8	46.6 ± 11.6	15.94	.13	.82	8.00	2.90	.55
9	62.1 ± 12.9	20.73	.17	.80	1.80	1.20	.85
10	53.8 ± 9.5	22.58	.34	1.51	9.00	4.95	1.15
Mean	53.7 ± 20.4		.15	.74	6.38	4.50	1.54

[a]Average percent recovery of all segments within each calf.
[b]Total uCi administered = number of segments injected x uCi / segment.
[c]Blood volume = birth weight x .08.

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Robert E. James

THE EFFECTS OF ADMINISTERED INDIGENOUS MICROORGANISMS ON UPTAKE OF ^{125}I-GAMMA GLOBULIN IN *IN VIVO* INTESTINAL SEGMENTS OF NEONATAL CALVES

by

Robert Elliott James

(ABSTRACT)

Two experiments were conducted using newborn colostrum-deprived calves to establish the distribution of uptake of ^{125}I-gamma globulin in small intestine and to investigate effects of added microorganisms on ^{125}I-gamma globulin uptake.

Ten calves less than 12.5 h of age ($\bar{X}$ = 7 h) were anesthetized and intestines exteriorized through an abdominal incision. Intestine was ligated into 10 cm segments at 70 cm intervals beginning at the ileo-cecal junction, injected with ^{125}I-gamma globulin in an electrolyte solution and incubated for 1.5 h. One additional segment was formed adjacent to segments 1, 5 and 10 to assess effects of .5 h exposure to ^{125}I-gamma globulin on uptake by epithelium. After prescribed gamma globulin exposure, segments were excised, volume of lumen contents, segment weight and tissue activity were determined. Age, birth weight and intestine length were recorded. Location of each segment (PSEG) was expressed as percentage of distance from cecum to abomasum. Uptake

was expressed as milligrams gamma globulin internalized per gram of segment tissue.

Distribution of gamma globulin uptake after 1.5 h exposure was a cubic function of PSEG. Uptake was greatest in a region 15% of cecum-abomasum distance, declining progressively towards the abomasum. After .5 h exposure, regression of uptake on PSEG was a quadratic function with greatest uptake at 30% of cecum-abomasum distance. Uptake after 1.5 h exposure was greater than after .5 h.

In experiment II 10 calves less than 14 h of age ($\bar{X}$ = 8.6 h) were anesthetized and intestines surgically exteriorized. Intestine was ligated into segments 10 cm in length at three cm intervals beginning 1.8 m above the ileocecal junction. Seven treatments were assigned in random order to segments in three successive sections of small intestine. Three treatments compared uptake in segments receiving one ml of either live intestine origin bacteria culture, sterile microbiological broth or autoclaved bacteria culture with four h incubation followed by 1.5 h exposure to ^{125}I-gamma globulin. Two treatments measured anaerobic microbial growth after four h incubation with one ml of either sterile broth or live bacteria culture. Residual ^{125}I-gamma globulin was measured in segments receiving one ml of sterile broth or live bacteria culture with 5.5 h incubation followed by 15 second exposure to ^{125}I-gamma globulin.

Measurements were as described for the first 10 calves. Serum corticosteroids, total protein and protein components were measured at 0 h and 5.5 h later.

Uptake was lowest in segments receiving live bacteria as compared to segments receiving sterile inocula. Number of bacteria per gram of segment tissue was negatively correlated with uptake. Low serum corticosteroids were associated with low gamma globulin uptake. Body weight and age were not related to uptake in either experiment in a decisive manner.