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THE UNIVERSITY OF ALBERTA

PROTEIN METABOLISM AFTER *E. coli* INFECTION

by

SHIQI TIAN

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled PROTEIN METABOLISM AFTER *E.coli* INFECTION submitted by SHIQI TIAN in partial fulfilment of the requiements for the degree of MASTER OF SCIENCE in ANIMAL BIOCHEMISTRY.

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ABSTRACT

A controlled experimental *Escherichia coli* septicemia was developed in broiler chicks. Systemic infection with *E. coli* significantly reduced feed intake and slowed growth of the whole body, eviscerated carcass and skeletal muscles. Muscle decreased as a proportion of body weight while liver and heart increased when expressed in this way. Protein accumulation in the eviscerated carcass, extensor digitorum communis and sartorius muscles was severely inhibited by infection, to a greater extent than body weight. Failure of muscle tissue to accumulate protein was associated with a significant fall in protein synthesis, as measured in vitro (-48%; $P<0.05$) or in vivo (-42%; $P<0.001$). Protein degradation also fell (-28.7%), but to a lesser extent than protein synthesis. Although the infected chicks showed no viable bacteria at day 12 after infection, chicks did not reach the same body weight as controls by day 30 after infection.

Treatment with naproxen (6-methoxy-α-methyl-2-napthalene acetic acid), an inhibitor of prostaglandin production, reduced weight losses of body and muscle, and significantly inhibited muscle protein wasting in infected chicks and rats. *E. coli* infection significantly increased net protein degradation by 44.8% ($P < 0.05$) and PGE_2 production by 148% ($P <0.05$) in isolated extensor digitorum communis muscle from chicks on day 2 after infection. Naproxen treatment significantly reduced net protein degradation and PGE_2 production in infected chicks to levels seen in muscles of healthy controls.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PROTEIN METABOLISM AFTER *E. coli* INFECTION

I. INTRODUCTION

PROTEIN METABOLISM AND ITS REGULATION

The objective of this chapter is to introduce the topic of protein turnover with specific reference to skeletal muscle, liver, and heart, and to consider the effect of infection on protein turnover.

A. Protein Turnover

Proteins are the most abundant macromolecules in living cells and constitute 50 % or more of their dry weight. They are found in all cells and all parts of cells. Proteins are fundamental to all aspects of cell structure and function since they are the molecular instruments by which genetic information is expressed. Proteins continuously undergo degradation and resynthesis in the body. Synthesis and degradation of protein are fundamental processes in the metabolism in living organisms, and are collectively termed protein turnover.

Although turnover includes synthesis and secretion of proteins, and cell replacement, only the intracellular synthesis and degradation of proteins will be considered here. Proteins are turned over when their levels are changed as well as when the levels are constant. The level may either increase, decrease or remain the same, but regardless of the direction of change, replacement or turnover, is still going on

during a given time interval. When there is a net accumulation of a protein in a tissue, the rate of synthesis is greater than the rate of degradation. If there is a net decrease in tissue protein, then the degradation rate exceeds the synthesis rate. These phenomena can be illustrated as follows:

Protein Synthesis = Protein Degradation -----> Steady State

Protein Synthesis > Protein Degradation -----> Growth

Protein Synthesis < Protein Degradation -----> Atrophy

Protein turnover may also be expressed as the flux between the amino acids bound in protein and the amino acids that are free. Only 1% of all amino acids are free and only 7% of these free amino acids are present in the plasma pool that is 0.07% of total amino acids in the body (Furst, 1983). Tissue proteins break down into amino acids and are released into the free amino acid pool where some amino acids used to resynthesize tissue proteins and others can be irreversibly lost to synthesize glucose or oxidized to provide energy. This amino acid loss can be replenished by dietary proteins (Waterlow et al., 1978; Figure 1). Under steady state physiological conditions, protein turnover is in a dynamic equilibrium, the free amino acid pool and all tissue proteins are in balance.

Protein turnover occurs in all tissues, however rates of protein turnover in different sites can be very different. The relative importance of a tissue in whole body protein turnover depends upon the

turnover rate of proteins in that tissue, and its size. For example, skeletal muscles account for 25%, liver for 24%, kidney for 2.2%, and heart account for 0.4% of total protein turnover per day in rats (Waterlow, 1984). Fractional rates of protein synthesis (% per day) in rats are 13.0 in muscle, 67.5 in liver, 47.9 in kidney, and 7.2 in heart (Garlick et al., 1976). In addition, protein turnover is much slower in organs of large animals than in those of small. In general, for example, the fractional rates of protein synthesis in tissues of rat appear to be 2 to 3 times faster than those in the pig (Garlick et al., 1976).

Numerous direct experiments have shown that the gain or loss of weight and protein content of the whole body is caused in a large part by changes in muscle because skeletal muscle is the most abundant tissue and major protein reservoir in the body. A very small change in muscle protein turnover can lead to a large change in muscle protein deposition or loss over time. Similarly, tissues such as liver, gastrointestinal tract and skin, which have high turnover rates, can make a major impact on overall protein metabolism.

Protein turnover is an essential process for cell survival and may have several important functions. Protein turnover can participate in the regulation of flux through a metabolic pathway by influencing the quantities of rate-limiting enzymes (Schimke, 1977). The relatively high rates of turnover of enzyme proteins would appear to confer sensitivity to this form of regulation. Secondly, during periods of nutritional deprivation or disease, when carbohydrates are either unavailable or not properly utilized, body proteins are called upon as fuel, increased protein breakdown provides an important energy

substrate for oxidation in the TCA cycle (Wassner et al. 1977; Garlick et al. 1975; Beisel, 1984). Thirdly, protein turnover is also required for the removal of abnormal or damaged proteins generated within the cell which could affect proper cell functions (Bradley et al., 1976). Finally, protein turnover is essential during the growth and the restructuring of cells resulting from anatomical changes during development and with activities such as exercise (Booth and Watson 1985; Mohan et al. 1985).

B. Protein Synthesis and Degradation

Protein synthesis takes place in the cell cytoplasm and involves interaction between ribosomes and messenger ribonucleic acid (mRNA), transfer RNA (tRNA), amino acids, adenosine triphosphate (ATP) and guanosine triphosphate (GTP) and various enzymes (Lehninger, 1982; Waterlow et al, 1978). There are usually four interrelated steps in the process of protein synthesis. The first step is activation of aminoacyl-tRNA. Amino acids are first esterified to their corresponding tRNA for protein synthesis by specific aminoacyl-tRNA synthetases in the cell plasma (Dang, 1986). The second step is initiation of the polypeptide chain; this occurs at the NH_2-terminal end. Initiation requires the formation of an initiation complex, involving ribosome subunits, messenger RNA, the initiating N-formylmethionyl-tRNA, initiation factor (IF) proteins IF1, IF2, and IF3 , ATP and GTP. The third step is the elongation of the polypeptide chain. This step requires the initiation complex, aminoacyl-tRNA, and elongation factors (EF) EF-Tu, EF-Ts, and EF-G and GTP. The last step is termination of the polypeptide chain. During termination the

completed peptide chain and the tRNA detach from the ribosome (Lehninger, 1982).

Systems for carrying out intracellular protein degradation are extensive (Schimke, 1973). The processes of intracellular protein degradation are generally classified into two pathways according to the cellular compartment in which they occur; nonlysosomal (cytosolic) and lysosomal (Waterlow et al., 1978). Lysosomes are membranous vesicles which contain a range of peptide hydrolases with a wide enough spectrum of endo- and exopeptidase activity to degrade most, if not all, cellular proteins (Hershko & Ciechanover, 1982). The lysosomal proteases include carboxypeptidase cathepsin A, thiol carboxypeptidase cathepsin B_2, endopeptidases cathepsins B, D, H, L, and dipeptidylpeptidase I (cathepsin C), II (Bird and Carter, 1980). The lysosome surrounds a cytoplasmic component containing protein in the process known as autophagy (Beynon and Bond, 1986). The enzymes digest the proteins into polypeptides, tripeptides, dipeptides, and free amino acids under low pH within the lumen of the autophagic vacuole. The nonlysosomal pathway is made up of cytosolic proteases and peptidases (Pontremoli and Melloni, 1986), which continue to be identified. These enzymes include calcium-dependent proteinases (CDP), ATP-ubiquitin dependent proteinases, and ATP-dependent-ubiquitin-independent proteinases (Hershko and Ciechanover, 1982). However, the participation of these enzymes in the degradation of specific proteins remains to be clarified. In some studies the importance of the lysosomal and nonlysosomal systems has been compared. For example, the nonlysosomal pathway is in charge of the breakdown of short-lived, abnormal proteins (Hershko and

Ciechanover, 1982), cytoskeletal (Hatanaka et al., 1984), and membrane (McGowan et al, 1983) and responsible for the majority of protein degradation during normal metabolic conditions in fibroblasts (Amenta and Brocher, 1981). However, according to Ciechanover et al. (1984), this pathway, is not sensitive to nutritional degrivation or hormonal action (Amenta and Brocher, 1981). The lysosomal pathway contributes to the degradation of extracellular proteins brought into the cell by endocytosis long-lived proteins under conditions of physiological and nutritional stress (Hershko and Ciechanover, 1982).

C. Methods of Determination of Protein Turnover

Isotopic tracer methods have been employed to determine the rate of protein turnover in the whole body and individual tissues. These involve the use of an isotope to label a given metabolite, such as an amino acid, and measurement of the incorporation of the labelled precursor into tissue protein. Radioisotopically labelled molecules are chemically indistinguishable from unlabelled molecules but can be detected through radioactivity decay. The direct precursor for protein synthesis, aminoacyl-tRNA, is technically difficult to determine. In addition, it is not known whether the tRNA is charged exclusively with amino acids derived from the extracellular or from intracellular pools (Airhart et al., 1974). The assumption is usually made that the system is in steady state-i.e. that pool sizes do not change. When a labelled amino acid is injected to an animal it is rapidly distributed in the body fluids, from which it is incorporated into proteins. Methods based on the incorporation of labelled amino acids by proteins demand the measurement of precursor pool specific radioactivity (S.R)

(disintegrations per minute, DPM/nMol). If a labelled amino acid reachs a plateau specific radioactivity (DPM appearing in precursor pool over time stays constant), the specific radioactivity of amino acid in the precursor free amino acid pools can be used as a value for the specific radioactivity of the precursor pool for protein synthesis. The calculation would be:

$$\text{Rate of Protein Synthesis} = \frac{\text{S.R of protein-bound labelled amino acid}}{\text{S.R of free labelled amino acid pool} \times t}$$

The rates of protein degradation are usually determined from the rate of loss of radioactivity from protein previously labelled by the injection of a single dose of radioisotopic tracer (Waterlow et al., 1978). In most cases, however, radioactive amino acids derived from protein breakdown persist in the precursor pool and are reutilized for protein synthesis. Such reutilization results in underestimate of protein degradation rate. Protein degradation rate can also be estimated as the difference between net protein accumulation and protein synthesis (Waterlow, 1984).

The methods for the measurement of protein synthesis can be classified into in vivo and in vitro methods. Here we focus on an in vivo method. Two commonly used in vivo methods to reach constant values of specific radioactivity of the precursor amino acid are continous infusion and single flooding dose techniques.

i. Continuous Infusion technique

The technique of constant infusion of radioactive amino acids to measure rates of protein synthesis in both the whole body and in individual tissues has been widely adopted following the pioneer development work on this method by Waterlow and his colleagues (Waterlow and Stephen, 1967; Garlick et al., 1973). The theory behind this method for measuring rates of protein synthesis in vivo rests on the observation that the specific activity of the free amino acid in blood and tissues reach a constant or plateau value (Waterlow and Stephen, 1968). Because of the rapid change in the specific activity of labelled amino acid after a single injection (Henriques et al. 1955; Simon et al., 1978), continuous infusion of labelled amino acid was employed to reach the constant specific radioactivity of labelled amino acid in plasma and tissues (Waterlow and Stephen, 1967, 1968; Dietz et al, 1982; Lobley et al., 1980; Garlick, 1969; Garlick et al., 1976). Constant specific radioactivity can be reached either by continuous intravenous infusion (Garlick et al, 1973) or by inclusion in a diet which is continuously fed (Harney et al.,1976). Measurement of the specific radioactivity of protein bound and free amino acids was used to calculate the rate of protein synthesis when a plateau was reached (Garlick et al, 1973). The constant specific activity of labelled amino acids has been demonstrated by using a number of different amino acids in small animals such as rats (Gan and Jeffay, 1967; Garlick et al., 1973; Seta et al., 1973; Waterlow and Stephen, 1968), and also in large farm animals such as pigs (Simon et al., 1978; Garlick et al., 1976), cattle (Lobley et al., 1980), and man (James et al., 1974; Waterlow, 1967).

It should be noted that the constant infusion methodology has subsequently received considerable criticism, although it has some advantages (Waterlow et al., 1978). The main concern is the identity of the precursor pool of amino acids for protein synthesis. With most tissues the choice of conveniently measured pools lies between the extracellular pool (blood), the intracellular pool or a combination of both pools. This problem is particularly acute when using an amino acid as a tracer which, following a continuous infusion, shows marked differences between the specific activities in the extracellular fluid and the intracellular fluid (Edmunds et al., 1980; McNurlan et al., 1979, 1982; McNurlan and Garlick, 1980). In tissues where appreciable recycling of free amino acids occurs, the difference between the specific radioactivity measured in the extracellular and intracellular may be sufficiently large to make estimates of protein synthesis based on either specific radioactivity unreliable (Pomposelli et al., 1985). Furthermore, during long infusion periods (several hours), recycling of tracer (labelled amino acids incorporate by synthesis into protein and subsequently release by degradation into the free amino acid pool) can occur due to rapid turnover of proteins. This recycling of tracer has been postulated to be an additional error of the constant infusion method (James et al., 1971). In addition, constant intravenous infusion involves of stress on the animal which could result in an alteration of rate of protein synthesis (Preedy and Garlick, 1984).

ii. Single 'Flooding Dose' Technique

A successful way to attempt to avoid the problems caused by constant infusion has been to administer the labelled amino acid as a

very large dose (Garlick et al., 1980a). The injection of a single very large dose of labelled amino acid is sufficient to flood all possible precursor pools (extracellular, intracellular and aminoacyl-tRNA precursor amino acid) so that they reach nearly the same specific radioactivity, which is then maintained almost constant during the period of incorporation into protein (Garlick et al., 1980a; McNurlan et al., 1979). The flooding dose method has some advantages compared to the constant infusion, due to the short labelling period, this serves to limit the amount of recycling of labelled amino acid and reduces the stress on the animal due to a short period of restraint and study. Scornik (1974) demonstrated that, when the free leucine pools were flooded with large amount of labelled leucine, all leucine pools would tend to attain the same specific radioactivity rapidly, which would be equal to that injected. The synthesis rate would be subsequently derived from the incorporation of labelled amino acid into protein during a brief period when intracellular specific radioactivities are at plateau or declining linearly (McNurlan et al., 1979).

The flooding dose technique could be used for most tissues, but the need to use the relatively large quantities of isotope makes its application to the large species difficult (Buttery, 1980). The reliability of the results requires, in part, that the large injection dose does not influence the rate of synthesis of the tissue being investigated. Some studies have demonstrated that some amino acids, such as leucine, alanine and glutamine, have some regulatory function in protein turnover. For example, leucine has been reported to stimulate protein synthesis and inhibit protein degradation in muscle

and heart independently of its function as a precursor of protein synthesis (Tischler et al., 1982). The most commonly used amino acid for the flooding dose technique is ^{3}H-phenylalanine. It is quite soluble so that a large dose may be given, and the pool of free phenylalanine in tissues is relatively small. It is relatively inexpensive and does not have any direct effect on protein turnover (Garlick et al., 1980a). Furthermore, an automated high-performance liquid chromatographic (precolumn derivation) method for quantitative analysis of phenylalanine has developed by (McAllister, 1987). This improved method makes the assay for the specific activity of free and protein-bound phenylalanine rapid and sensitive. Thus this method is ideally suited for studies of in vivo protein turnover in which the specific radioactivity of free and protein-bound phenylalanine must be accurately determined in large numbers of samples (McAllister, 1987).

D. Regulation of Protein Turnover

a). Role of hormones and feed intake

Muscle protein turnover varies widely under physiological conditions and can be influenced directly by hormonal and nonhormonal agents. It has been recognized that protein metabolism is under strict endocrine control and many hormones and factors could be involved in regulation of protein synthesis and degradation (Young, 1980). Some factors, such as growth hormone, thyroid hormone (normal level), insulin, Insulin-like growth factor-1, prostaglandin $F_{2\alpha}$, dietary protein and muscle activity (contraction, stretch) are anabolic and some others such as somatostatin, thyroid (high level), prostaglandin E_2 and glucagon, muscle disuse (inactivity), are

catabolic. All of these have a regulatory role in muscle protein turnover (Table 1).

i. Insulin

Of all the hormones known to influence protein turnover, the effects of insulin have been most thoroughly characterized (Waterlow et al., 1978). Insulin is the most predominant, and promotes anabolism in tissues by increasing protein synthesis and decreasing protein degradation (Goldberg et al., 1980). Insulin is capable of both stimulating the transport of amino acids into the cell and their incorporation into protein by increasing protein synthesis. Insulin also decreases protein degradation in muscle and heart (Mortimore and Mondon 1970; Stirewalt and Low 1983). In the perfused liver, insulin added alone (Mortimore and Mondon,1970) or with amino acids (Woodside and Mortimore, 1972) had little effect on the rate of synthesis of fixed liver proteins, but markedly suppressed protein breakdown.

ii. Glucagon

One of glucagon's most documented functions appears to be in the regulation of glucose production by the liver (Alford and Chisholm, 1979). Its effect on protein turnover has also been reported in recent studies. Glucagon acts mainly on the breakdown of protein in the liver, but it also seems to have some inhibitory effect on synthesis (Waterlow et al., 1978). Administration of a pharmcological dose of glucagon decreased protein synthesis in skeletal muscle, and did not influence protein synthesis in heart. Liver protein synthesis was inhibited by glucagon treatment for 10 minutes, but stimulated after 6

hours (Preedy et al., 1980; Preedy and Garlick, 1985).

iii. Glucocorticoids

It has been known for many years that increased circulating levels of glucocorticoids induce a generalized decrease in body mass and skeletal musculature (Long, 1940). Some studies suggested that glucocorticoids may cause a change in ribosomal concentration resulting in reduced protein synthesis (Rannls et al., 1978). While others have suggested that glucocorticoids stimulate muscle protein breakdown directly (Goldberg 1975). However, glucocorticoids do not always decrease protein synthesis and increase protein degradation under normal physiological conditions. Goldberg (1980) reported that cortisol stimulated muscle protein breakdown and the release of amino acids from muscle in fasting animals markedly but did not have effect on muscle protein degradation in fed animals. In addition, in contrast to their catabolic effect on skeletal muscle, glucocorticoids are anabolic on the heart. Glucocorticoid treatment induced early cardiac hypertrophy by increasing cardiac protein synthesis (Clark et al., 1986). Administration of gluocorticoids has also been reported to induce an initial trancient supression of protein synthesis, followed by an increase (Kim and Kim, 1975; Jeejeebhoy et al., 1973)

iv. Gonadal Steroids

Both androgens and estrogens have been used to increase muscle protein deposition in farm animals because theses hormone stimulate protein synthesis in many tissues including skeletal muscle, in

addition to their specific action on the accessory sex organs (Lu & Rendel, 1976). As would be anticipated, castration of males results in the reduction of plasma testosterone concentration, the regression of many skeletal muscles (Michel and Baulieu, 1980) and retarded growth (Rubinstein et al., 1939). Testosterone acts on muscle chromatin to allow more rapid synthesis of specific mRNAs, which in turn regulate protein synthesis (Peeters, et al., 1980; Rarker, et al., 1980; Ostrowski, et al., 1979, 1982).

v. Nutritional state

In addition to circulating hormones discussed above, many other factors such as muscle activity and nutritional state, e.g., availability of carbohydrates and amino acids have been shown to affect rate of protein synthesis and degradation and every factor has its own pathway of regulating protein turnover. It is well known that fasting inhibits protein synthesis in several tissues such as skeletal muscle and livers (Emery et al., 1986; Preedy et al., 1984; El Haj et al., 1986). Wassner et al. (1977) and (Goodman et al., 1980) demonstrated that skeletal muscle proteolysis increases in rats fasted for 24 h. Emery et al. (1986) and Garlick et al. (1975) found that there was an abrupt rise in the rate of protein degradation of smooth muscle and liver of rats fasted for one day. The decline in protein synthesis associated with short-term fasting is maintained during long-term fasting (El Haj et al., 1986; Golden et al., 1977). Urinary nitrogen excretion decreases in men fasted for 6 d (Saudek and Felig, P., 1976) and protein degradation decreases in chronically malnourished children (Golden et al., 1977). Some researchers have

suggested that a reduction in thyroid activity may be responsible for the decreased rate of protein degradation in long-term fasting (Vignati et al., 1978; Goldberg et al., 1978). In addition, it is suggested that protein synthesis in the skeletal muscle is more sensitive to nutritional change and muscle protein deposition is also more sensitive to nutritional deprivation than it is in other tissues (Reeds and Fuller, 1983), .

b). Prostaglandins

The prostaglandins (PG), a family of lipid-soluble organic acids containing 5-carbon rings, are cyclopentane derivatives formed from polyunsaturated fatty acids (e.g., arachidonic acid) and released by most animal cell types in response to a variety of physiological and pathological changes (Samuelsson et al., 1978). They are regulators of hormone action, and are made by, and function in, all organs. For example, skeletal muscles in human and rats have been reported to produce prostaglandins which include PGE_2, PGF_{2a}, PGD_2, 6-keto-$PGF_{1\alpha}$ and thromboxane B_2 (Berlin et al, 1979; Freund et al., 1985; Nowak et al., 1983; Rodemann and Goldberg, 1982).

Mononuclear phagocytes are a major cellular component of the reticuloendothelial system and include a circulating form (monocytes) and a fixed tissue form (macrophages). Mononuclear phagocytes are central in host defence responses, and exhibit anti-tumour, anti-bacterial and inflammatory activity (Dinarello, 1984; Dinarello et al., 1984). Mononuclear phagocytes synthsize and release more than 75 defined molecules (Table 1.2) which collectively mediate host defence. Prostaglandin E has been shown to have a clear role in the

regulation of cellular and humoral immune responses (Goodwin and Ceuppens, 1983). PGE_2 acts as a feed back inhibitor of cellular immune responses and these responses can be enhanced *in vivo* as well *in vitro* by the administration of PG synthesis inhibitors. However, under certain experiment conditions PGE_2 can deliver activating signals. For example, PGE_2 induces immature thymocytes to differentiate into mature T cells (Bach et al., 1975). In multiple sclerosis patients, an increased production of PGE_2 by the monocytes causes increased adherence of lymphocytes to measles-infected cells (Dore-Duffy and Zurier, 1979). PGE has also been found to have dual effects (inhibition or activaion) on B cells, natural killer cells, and macrophages. Therefore, PGE_2 is a regulator rather than a universal suppressor for immune responsese.

Apart from their regulatory function on immune responses, recently evidence has been obtained that metabolites of arachidonic acid affect protein turnover in muscle. $PGF_{2\alpha}$ has been reported to increase the rate of protein synthesis in skeletal muscle *in vitro* (Rodemann and Goldberg, 1982; Smith et al., 1983), and mediate the stimulating effect of insulin on protein synthesis in skeletal muscle *in vitro* (Reeds and Palmer, 1983) and *vivo* (Reeds et al., 1985). On the other hand, PGE_2 has been shown to stimulate protein degradation in skeletal muscle *in vitro* and mediate the stimulating effect of leukocytic pyrogen (Interleukin-1) on protein degradation in skeletal muscle (Baracos et al., 1983). For example, when muscles were treated with PGE_2, protein synthesis was not affected, but protein degradation increased about 22% (Rodemann and Goldberg, 1982). In contrast to PGE_2, $PGF_{2\alpha}$ had no effect on

protein degradation but did reproducibly increase protein synthesis (Rodemann and Goldberg, 1982). Injection of live *E. coli* into rats caused fever, and increased both protein degradation and PGE_2 production in muscle. When the rats were pre-treated with indomethacin which is an inhibitor of prostaglandin production the increase in protein degradation and prostaglandin production were inhibited (Fagan and Goldberg, 1985). Indomethacin also was found to inhibit promoted rapid muscle protein loss induced by Streptococcus pneumoniae infection in rats (Ruff and Secrist, 1984). In addition, Reeds et al. (1985) reported that insulin increase muscle $PGF_{2\alpha}$ production and also protein synthesis, dexamethasone decrease muscle protein synthesis and also $PGF_{2\alpha}$ production both in vivo and in vitro. These results suggest that prostaglandins may have regulatory function on protein turnover in sksletal muscle *in vivo*. However, whether prostaglandins affect protein turnover in other tissues or whether prostaglandins affect other aspects of protein metabolism remain to be fully understood.

It should be noted that protein turnover is regulated by coordinate action of hormones and other factors. For example, glucocorticoids and insulin have their own individual effects and also interplay with each other. Physiologically glucocorticoids and insulin exhibit inverse effects, constituting a very sensitive regulatory mechanism for protein metabolism in response to dietary supply (Goldberg, 1980; Preedy et al, 1984). The way in which corticosterone and insulin influence protein synthesis appears to involve a primary role for insulin which is modulated by corticosterone, the opposite seems the case for proteolysis-i.e a

primary role for corticosterone which is modulated by insulin. (Millward et al., 1985).

Some of mechanisms of regulation of protein turnover have been clarified and others remain unclear. Further understanding of the regulation of protein turnover calls for more research.

Most of stuides about regulation of protein turnover in muscle have been down in rats and other mammals, and very little is known about other species. It remaineds to be seen whether they have the same regulation pathway or they have some different regulation pathway in muscle protein turnover.

E. Physiological Control of Protein Turnover

Aspects of whole body protein turnover which are of physiological interest include its relation to body size, growth and development, energy metabolism, exercise and food intake (Waterlow, 1984). The changes of protein turnover that occur in infection are begining to be explored.

i. EFFECT OF INFECTION ON PROTEIN TURNOVER

Infection results in wide metabolic changes in host biochemical pathways and control. Clinically, the most visible metabolic effects of an infection are catabolic. It has been recognized that body weight and total body protein can be lost markedly during infection (Beisel, 1966; 1984). When infections are chronic or complicated by injuries, surgery or burns, up to a third of total body proteins can be lost (Wilmore and Kinney, 1981). This negative nitrogen balance results in impaired growth in a young growing individuals. The change of protein turnover and the rearrangement of protein synthesis and degradation in

different tissues would be difference during infection.

Skeletal muscle is a major reservoir of protein in animals, so it must be a major contributor to whole-body protein turnover. There is growing evidence that the gain or loss of weight and protein content of whole body is caused in a large part by changes in muscle and skeletal muscle is the primary tissue of the carcass that undergoes protein catabolism under stress (Moldawer et al., 1987). Generalized muscle weight loss and protein depletion are common clinical signs associated with infectious diseases. Several possible alterations in protein turnover which may resulted in the negative nitrogen balance in skeletal muscle during infection have been suggested : 1). Increased protein degradation; 2). decreased protein synthesis; 3). increased protein degradation higher than increased protein synthesis; 4). increased protein degradation and decreased protein synthesis. All of the above-mentioned responses have been reported after injection of experimental animals with live bacteria or bacterial toxins (Table 3). For example, when rats were infected with *E. coli*, protein degradation increased significantly but protein synthesis did not change in incubated skeletal muscle (Fagan and Goldberg, 1985). Fern et al.(1985) and Lust (1966) found significantly decreased protein synthesis in rats infected with malaria or *Diplococcus pneumoniae*. Klasing and Austic (1984a, 1984b) found increased protein degradation but decreased protein synthesis in chick muscle during *E. coli* infection. It has also been suggested that both synthesis and degradation, to a greater extent, increase (Long, 1977; Garlick et al, 1980b; Tomkins, 1983). Pomposelli et al (1985) found increased protein synthesis in the early stages of infection by *Pseudomonas*

aeruginosa in rats. These incongruous results on the responses of muscle protein turnover to infectious diseases reported by different researchers may be due to: 1). the different methods they used in the determination of protein synthesis, 2). the different subjects and disease models they chose, 3). the different time points of observation, and 4). the different nutritional state the subjects were in.

F. Objectives of the present study

So far, many investigators have studied the host protein metabolic responses to infection including muscle repletion when growth resumes after an infection or fever. It has been recognized that depressed food intake is one of the factors which cause negative nitrogen balance during infection; anorexia is a prominent symptom of acute infection. Acute infectious diseases are accompanied by a complex variety of interrelated nutritional and metabolic responses within the host. However, more information is needed to understand the nutrient requirement of different tissues during infection and subsequent recovery. Baracos (unpublished) found that when growth resumed after fever, food intake recovered to normal level, rates of muscle protein synthesis returned to normal and rates of protein degradation fell; and net degradation was significantly less in birds which had been febrile, than in control birds. However, these responses to infection are not yet fully understand, and very little is known about the time course of change in protein synthesis and degradation due to infection or the factors contributing to resumption of growth after infection. Therefore, further research is needed to fully understand protein

metabolism during infection and recovery. The objectives of the present study were:

1. To investigate the effect of infection on whole body and muscle growth and the changes of protein turnover due to *Escherichia coli* infection in broiler chicks.
2. To test for a prostaglandin-dependant mechanism in muscle wasting during systemic infection in the young animals.

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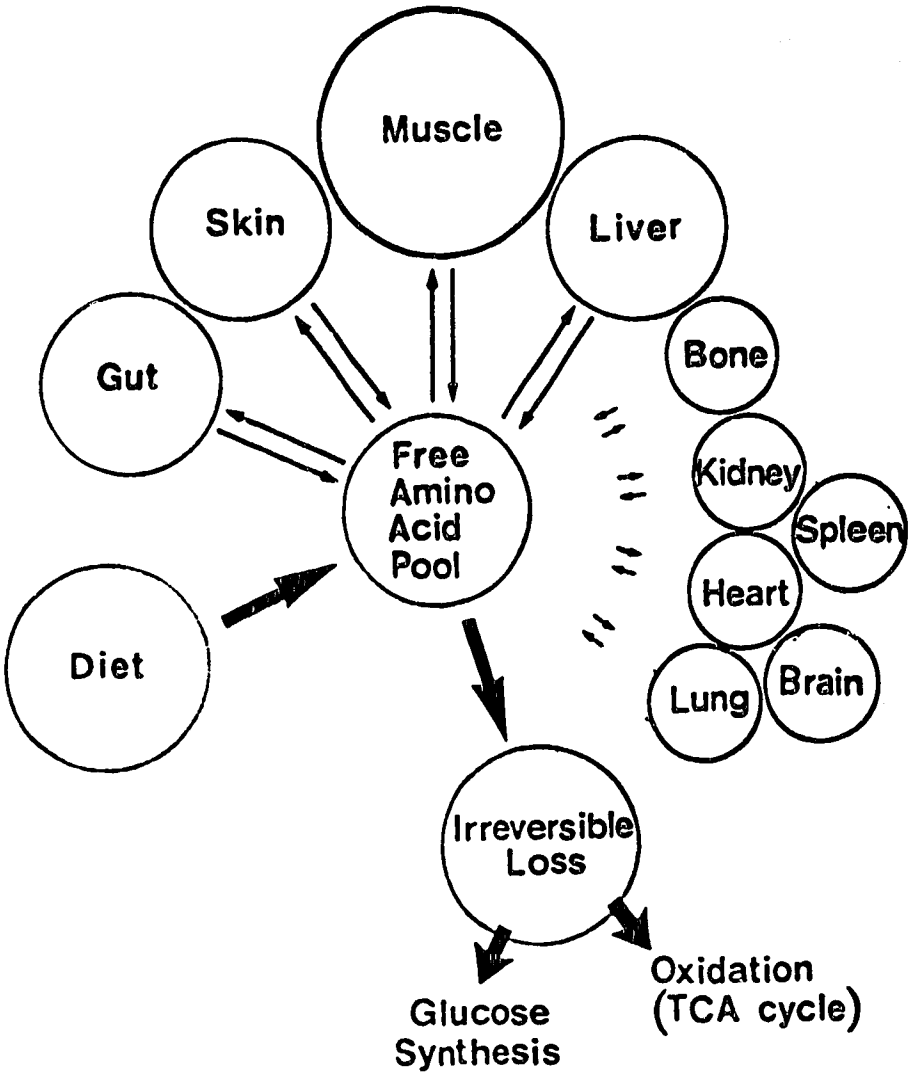
Figure 1.1.

PROTEIN TURNOVER IN THE BODY



Amino acids in tissue protein are in dynamic equilibrium with those in the free pool (intracellular and extracellular). In the steady state, irreversible amino acid loss is replaced by the diet.

Table 1.1.

EFFECT OF FACTORS ON PROTEIN TURNOVER IN SKELETAL MUSCLE

Factors	Protein Synthesis	Protein Degradation	Overall
Growth Hormone	+	/	Anabolic
Thyroid Hormone (normal level)	+	?	Anabolic
Thyroid Hormone (high level)	?	+	Catabolic
Insulin	+	-	Anabolic
Insulin-like Growth-factorl	+	/	Anabolic
Prostaglandin E_2	/	+	Catabolic
Prostaglandin F_{2a}	+	/	Anabolic
Glucagon	-	+	Catabolic
Glucocorticoids	-	+	Catabolic
Androgens	+	?	Anabolic
Estrogens	+	?	Anabolic
Muscle Activity	+	?	Anabolic
Muscle Disuse	-	?	Catabolic

+: Stimulation
-: Inhibition
?: Unclear
/: No effect

Table 1.2.

Secretory Products of Activated Macrophages

1. Lysosomal Hydrolases
2. Lysozome
3. Neutral Proteases
   - -elastase
   - -collagenase
   - -plasaminogen activator
   - -cytolytic protease
4. Arachidonic Acid Metabolites

   PGI_2, TXB_2, PGE_2, $PGF_{2\alpha}$, LTC_4, LTB_4
5. Reactive Oxgen Metabolites

   $-O_2-$, H_2O_2, OH.
6. Complement Components
7. Cytokines Cellular Growth Factors
   - -interleukin-1
   - -interferon
   - -tumour necrosis factor

Table 1.3.

EFFECT OF INFECTION ON PROTEIN TURNOVER IN SKELETAL MUSCLE

Species	Condition	Synthesis	Degradation	Reference
Rat	Pseudomonas aeruginosa *in vivo*	↑	ND	Pomposelli, (1985)
Rat	Malaria *in vivo*	↓	ND	Fern et al., (1985)
Mice	Diplococcus pneumoniae *in vivo*	↓	ND	Lust & George, (1966)
Chick	E. coli *in vivo*	↓	ND	Klasing & Austic, (1984a)
Chick	E. coli *in vitro*	ND	↑	Klasing & Austic, (1984b)
Rat	E. coli *in vivo*	↓	↑	Fagan & Goldberg, (1985)
Chick	E. coli endotoxin *in vitro*	↓↓	↓	Baracos, unpublished observations
Chick	Vaccination *in vitro*	↓	ND	Hentges et al., (1984)

ND: Not determined

II. EFFECT OF *Escherichia coli* INFECTION ON GROWTH AND PROTEIN METABOLISM IN BROILER CHICKS (*Gallus domesticus*)

EFFECT OF *Escherichia coli* INFECTION ON GROWTH AND PROTEIN METABOLISM IN BROILER CHICKS (*Gallus domesticus*)

1. A controlled experimental *Escherichia coli* septicemia was developed in broiler chicks.
2. Systemic infection with *E. coli* significantly reduced feed intake of chicks and slowed growth of the whole body, eviscerated carcass and skeletal muscles. Muscle decreased as a proportion of body weight while liver increased when expressed in this way.
3. Protein accumulation in the eviscerated carcass, extensor digitorum communis and sartorius muscles was severely inhibited by infection, and to a greater extent than body weight.
4. Failure of muscle tissue to accumulate protein was associated with a significant fall in protein synthesis, as measured in vitro (-48%; $P<0.05$) or in vivo (-42%; $P<0.001$). Protein degradation also fell (-28.7%), but to a lesser extent than protein synthesis.
5. Although the infected chicks showed no viable bacteria at day 12 after infection, chicks did not reach the same body weight as controls by day 30 after infection.

EFFECT OF *Escherichia coli* INFECTION ON GROWTH AND PROTEIN METABOLISM IN BROILER CHICKS (*Gallus domesticus*)

INTRODUCTION

Extremely rapid growth at early stages of life is characteristic of broiler strains of domestic chickens (*Gallus domesticus*). Chicks may increase ten fold in body weight within the first few weeks of life. The rate of this growth makes the chick an attractive model for study of factors affecting growth and growth disorders. Failure of growth may be attributable to many causes: malnutrition, injury, disease, or hormonal excess or deficiency. When growth failure is repaired, such as in the repletion of nutrients to a malnourished individual, growth resumes, but often at a rate well above that to be expected for the age or level of maturity (Tanner, 1986). Rapid growth may also be associated with increased voluntary feed intake. This phase of recovery has been called catch-up or compensatory growth (MaCance and Widdowson, 1974; Wilson and Osbourn, 1960). The type, degree and duration of growth failure would appear to influence whether complete catch-up growth occurs, or whether a permament stunting effect endures (Wilson and Osbourn, 1960; Smart et al., 1977; McCance, 1976). It has hence been suggested that the conditions for optimizing catch-up are different in different clinical conditions, and that each condition has to be considered separately (Wilson and Osbourn, 1960).

The influence of feed restriction and dietary protein restriction on growth and catch-up by domestic poultry (Washburn and Bondari, 1978; Wilson, 1952; Auckland and Morris, 1971; Brody et al., 1980) and other species (Szepesi and Oney, 1977; McMeekan, 1940, 1941; Bohman and Torrel, 1956; Palsson and Verges, 1952) have been extensively studied. Comparatively little information exists concerning the implications of a systemic infection on growth; thus one objective of this work was to examine the effect of infection on whole body growth, and feed intake in the broiler chick.

Modified growth of the whole body may be associated with changes in body composition (Forbes, 1986). Infectious disease is known to result in whole body nitrogen loss which is proportional to the duration and severity of the disease (Beisel, 1984; Garlick, et al., 1980a; Keilman, 1977). Catabolic losses of skeletal muscle protein appear to be a substantial component of this nitrogen loss (Beisel, 1984; Rennie, 1985). The loss of muscle weight and protein content may result from increased protein degradation, decreased protein synthesis, or both. Some studies have suggested that the negative nitrogen balance results from an accelerated degradation of cellular protein, particularly in skeletal muscle (Garlick et al.,1980a). However, a number of other studies indicate that in infection, net protein loss results from a marked reduction in protein synthesis (Fern et al., 1985; Lust, 1966). Thus a second objective of the present studies has been to determine the extent of tissue protein loss, and to identify the underlying changes in protein synthesis and degradation which account for alterations in protein content.

Escherichia coli septicemia is a common infection of domestic poultry, resulting in significant loss of production (Coutts, 1981). To conduct these studies, a controlled experimental model of systemic *E. coli* infection was developed.

Materials and Methods

Experimental design:

Chicks were randomly assigned to two treatment groups, *Control* or *Infected*, such that the mean initial body weight and SEM of each group were similar. Two main themes were studied: 1) effects of experimental *Escherichia coli* (*E. coli*) infection on the growth of the whole body, skeletal muscle, liver, and heart, and on protein accumulation by these tissues. The time course of these parameters after infection was studied to determine whether catch-up growth would occur within the time frame of typical broiler production (6 weeks). 2) determination of changes in protein synthesis and degradation in muscle, liver and heart, which underlie the response of these tissues to infection.

Animals and diet:

Day-old male broiler chicks of the Hubbard strain which had been vaccinated for Marek's disease were obtained from Co-op Hatchery (Edmonton, Alberta). The chicks were housed (13 or 14/pen) in electrically heated batteries (33-35^{o}C) with raised wire-mesh floors and continuous lighting. Numbers of birds in each treatment group were more than that of studied because of expectant mortalyty during study. The chicks were fed a broiler starter diet based on wheat and soybean meal containing 2795 kcal metabolizable energy / kg and 23.3% crude protein until 4 weeks of age (Appendix I), and in experiments where chicks were raised beyond 4 weeks of age, a grower diet containing 2863 kcal ME / kg and 20.2% crude protein was fed (Appendix II). Feed

and water were provided *ad libitum* throughout the study. In different experiments, chicks were studied for different time periods ranging from 2 to 30 d after infection. Feed intake was measured daily on a per pen basis (13 or 14 birds/pen), and individual body weights were measured every other day. Eight to ten birds from each treatment group were sacrificed at the selected time points after the injection and tissues dissected: *extensor digitorum communis* (EDC) muscle, *sartorius* muscle, liver, heart, and eviscerated carcass (after removal of head, feet, skin, feathers, gastrointestinal tract, and contents of abdominal and thoracic cavities). Tissues were weighed immediately and stored frozen at -30 ^{o}C until analyzed.

Experimental Infection:

Chicks were infected by intraperitoneal (i.p.) injection of live *E. coli*. The cells used were of a single strain which was obtained from an outbreak of coliform septicemia in turkey poults, and was provided by Dr. D. Onderka (Animal Health Division, Alberta Agriculture). The Serotype of these cells is not known. Cell stocks were stored frozen in sheep blood at -70^{o}C. To determine a dose which would result in infection with limited mortality, preliminary experiments were carried out. A single i.p. injection containing 10^{6} to 10^{14} colony forming units was given to each bird on day 8 *ex ovo*. Control birds were sham injected with 1.0 ml of sterile, non - pyrogenic saline. To determine the time course of infection, liver samples were taken on days 2, 6, and 12 after injection from each treatment group to culture for *E. coli*.

Analyses

Frozen muscles were thawed at room temperature and dissolved with 1.0 N NaOH for 4 to 8 hours. Muscle protein content was measured by a modified Bradford Method (Bradford, 1976), according to the changes suggested by Read and Northcote (1981). Bovine serum albumin (BSA) was used as protein standard in the assay. The frozen eviscerated carcasses were thawed at room temperature and digested with 6 N HCL for 8-48 h. The carcass nitrogen content was then measured by the Kjeldahl method. The number of mg of N obtained by the Kjeldahl method was multiplied by 6.25 to give the number of mg of protein in the sample. For determination of tissue water content, carcasses and muscles were dried to constant weight at 110°C. It took approximately 30 h for muscles and 96 h for carcasses to attain constant weight. The concentration of phenylalanine (Phe) and other amino acids in the incubation medium and in the intracellular fraction were determined by High Performance Liquid Chromatography (HPLC;Jones and Gilligan, 1983).

Measurement of Muscle Protein Synthesis *In Vivo*

The rate of protein synthesis in vivo was determined using the 'flooding dose' method (Garlick et al., 1980b). The method was prefered to other available methods because it has shown to reduce the difference in specific radioactivity between tissue compartments as well as changes with time within them (Garlick et al., 1980b). The overall error of measurement is, therefore, likely to be smaller than other methods (Fern et al., 1985). Chicks were given a single i.p. injection of a sterile solution containing Phe (150 mM) and

[U-^{3}H]-Phe (150 μCi/ml; 1.0 ml per 100 g body weight). After 15, and 30 min, chicks were sacrificed by decapitation, and the sartorius muscle, heart and liver were rapidly excised and frozen. The specific radioactivity of Phe in the tissue intracellular pool and in the tissue protein were determined after the enzymatic conversion of phe to β-phenethylamine by the action of L-tyrosine decarboxylase (Garlick et al., 1980b). Concentrations of β-phenethylamine were determined by HPLC. The fractional rate of protein syntheisis (Ks) in the tissues was calculated with the formula:
$Ks = (100 \times S_B) / (S_A \times t)$ given by Garlick et al. (1980b), where S_B is specific radioactivity of phenylalanine in tissue protein in chicks killed at 30 min; S_A is the mean specific radioactivity of phenylalanine in the tissue free pool between 15 and 30 min; and t is the incorporation time in days (for example, 30 min=0.0208 d). The units of Ks are % per day.

Measurement of protein turnover *in vitro*:

In separated experiments, 6 to 9 chicks from control and infected groups were sacrificed and the extensor digitorum communis (EDC) muscles dissected and mounted at their resting length *in situ* on a stainless steel wire support by means of their tendons (Baracos, 1989 in the press). Tissues were incubated in 4 ml Krebs-Ringer Bicarbonate medium: NaCl 119mM; KCl 4.82 mM; $MgSO_4$ 1.25 mM; $CaCl_2$ 1.0 mM; NaH_2PO_4 1.24 mM; $NaHCO_3$ 25 mM; HEPES 2 mM/NaOH, pH 7.4; glucose 15 mM; insulin 0.01U/ml. Muscles from right wings were used to determine the rate of protein synthesis and muscles from left wings were used to determine amino acid release and intracellular amino

acids (see below). Muscles were incubated for 30 min, then transferred to fresh medium and incubated for 3 h. Protein synthesis and net protein degradation were determined as described previously (Baracos & Goldberg, 1986) using phenylalanine. In these methods, the sum of rates of protein synthesis and protein degradation equals the net protein balance, which may be a positive term (anabolism) or a negative term (catabolism). Rates of protein degradation and net protein balance are measured, and rates of protein degradation determined by difference. The validity of this approach has been discussed previously (Tischler et al., 1981).

Statistics:

Results are expressed as means ± standand error of the mean. All experiments used at least 6 birds/treatment and each experiment were repeated at least twice. The results were compared by the paired or unpaired Student's T test.

Results

Experimental *E. coli* Infection

Injection of 10^6 colony forming units did not have any observable effect on the growth of 8 day old broiler chicks, while injection of 10^{14} cells resulted in 100% mortality. Doses in the range of 10^9 to 10^{11} resulted in growth failure with limited mortality (36 - 70% in different experiments) and were used throughout the study, as indicated in the Figure and Table legends. Infected birds showed loss of appetite and depression of growth; most died infected birds died within 48 hours after injection. Thirty - eight birds which succumbed to the infection were the subjects of postmortem examination. Typically, birds showed prominent fibrinous peritonitis. Large areas of ecchymotic hemorrhage were seen on the intestines, particularly on the serosal side, and on other visceral organs including the gizzard and proventriculus. Most birds showed congested, oedematous lungs. Other organs appeared grossly normal, including the liver, heart, spleen and kidneys. Culture of liver samples showed heavy *E. coli* growth in infected birds, but scant or no *E. coli* growth in control birds on days four and six after the injection. The *E. coli* growth was not evident in either infected or control birds on day 12 after the injection, showing that the birds had overcome the injected bacteria.

Feed Intake

The absolute feed intake of control birds increased continuously over 30 d (Fig. 2.1). The feed intake of infected birds fell rapidly

within 24 h after the injection by 27-30% in different experiments. When birds died overnight after injection of bacteria, average feed intake/bird was calculated based upon the maximum number of the live birds in the pen on any given interval during study. Therefore the data shown reflect, if anything, an underestimate of feed intake. Feed intake was depressed continously for 25 d after infection by 11-38%. By 25 d after injection, feed intake reached control values (Fig. 2.1). As seen in Fig. 2.2, when feed intake was expressed per 100 g of body weight, the intake of infected birds was lower than that of control birds for only the first 5 d following the injection. Thereafter, feed intake/100 g body weight was similar to that of healthy controls.

Whole Body and Tissue Growth

Healthy young broiler chicks showed rapid rates of growth from d 8 to 38 after hatching (Fig. 2.3). After injection with *E. coli*, the growth of infected birds was significantly slowed or stopped for four days. However, after day four, growth resumed. No catch-up growth was found within the time frame of these studies. The weights of the infected birds were depressed by 15-25% from day 2 through day 15 after the injection and 12-14% from day 15 through day 30 (Fig. 2.3). This waas constant between xperiments, where body composition and protein turnover were measured. By 30 d after injection, body weight of infected birds was still significantly depressed by 12.1% ($P < 0.01$). Similar results were seen in a second experiment carried out for 12 d after injection. The carcass of control birds also showed rapid growth (Fig. 2.3). The weight of eviscerated carcass of infected

birds were significantly lower by injection of *E. coli* by 23-33% from day 2 through day 15 after injection and 12-13% from day 15 through day 30 after injection. Carcass weight, like body weight, was depressed by -12.9% ($P < 0.01$) at day 30 after injection.

Weights of skeletal muscles from control and infected chicks are shown in Table 2.1. Both the EDC and sartorius muscles showed rapid growth over the 30 d period of study, increasing by 27 and 17-fold, respectively, in weight. The weights of sartorius muscle of infected birds were significantly lower than those of control birds by 30-41% from day 2 through day 15 after the injection and 13-19% from day 15 through day 30 after injection. The response of *extensor digitorum communis* (EDC) muscle was quantitatively similar to that of *sartorius* muscle (Table 2.1). Muscle growth of infected birds seemed more affected by the injection than the whole body growth. For example, the mean weight of the sartorius muscle expressed per 100 g of body weight, was significantly lower than that of the control birds for the first two weeks following the injection ($P < 0.01$), but approached control values at day 20 (Fig. 2.4). Similar results were obtained for the EDC muscle (not shown).

The liver of rapidly growing broiler chicks also increased greatly in size over the 30 d study period, from 4.3±0.3 g at day 0, to 32.5±1.4 g (n=10) at day 30. Liver weights of control and infected birds were not significantly different from each other on days 2, 4, 10, 20, 25, or 30 after injection (n=10). However, if the liver weight was expressed per 100 g of body weight, the liver from infected birds was a significantly higher proportion of body weight than in control birds for 10 days after the injection ($P < 0.05$). After day 10 of

injection, liver weight/100 g body weight was similar for the 2 treatments.

Heart weights of control and infected birds are shown in Table 2.3. Heart weights of infected birds were not significantly different from those of control birds on day 2 but fell significantly by day 4 after injection. However, when expressed per 100 g of body weight, heart weights of infected birds were higher than those of control birds on both day 2 and day 4.

Body composition

Because infection may be accompanied by changes in tissue and body composition, the protein contents of the EDC, the sartorius muscle, and the eviscerated carcass were measured over 12 days after infection (Table. 2.2). In addition to the absolute weight, the total protein content and percent protein of eviscerated carcass and the muscles of infected birds were significantly lower than those of control birds.

To test the involvement of changes in water content on the observed alterations in whole body and tissue weight, the water content of the whole body and muscles was determined. The results revealed no difference between infected birds and control birds. The water contents, as a percentage of the total weight, were 72-76% in eviscerated carcass, 76-78% in EDC muscle, and 77-78% in sartorius muscle (n=8).

Protein Turnover

When chicks were injected with ^{3}H-phe, the intracellular specific activity of intracellular Phe rose rapidly in heart,

sartorius muscle and liver. In a preliminary experiment, intracellular specific radioactivity was seen to reach a plateau value from 10 min to 40 min after injection (Appendix III). This was no significant differences in the level of specific radioactivity attained in the three tissues, amongst control and infected birds (n=7). In subsequent experiments (Table 2.3), chicks were sacrificed at 15 and 30 min after injection, and the average intracellular specific radioactivity at the two time points used in the calculation of protein synthesis. The fractional rates of protein synthesis of sartorius muscle in infected birds were significantly lower than those in control birds on both day 2 (-42%; $P < 0.001$) and day 4 (-33%; $P < 0.05$) after infection (Table. 2.3). However, no significant differences were found in liver and heart protein synthesis between infected birds and control birds on days 2 or 4 after infection.

Rates of protein synthesis , degradation, and net degradation of EDC muscle determined *in vitro* at day 2 after injection are shown in Table 4. The fall in protein synthesis (-48%; $P < 0.05$) was quantitatively similar to that seen *in vivo* in sartorius muscle. Incubated EDC muscle also showed an increase net release of phenylalanine (+56%; $P < 0.03$). Protein degradation, fell (-28.7%), but to a lesser extent than protein synthesis. The total intracellular amino acid concentration in isolated muscle of infected birds was not significantly different from that of control birds, although some amino acids such as aspartic acid, glutamic acid, asparagine, serine, glutamine, glycine, tyrosine, and methioine were significantly lower, and tryptophan, isoleucine, and lysine were present at significantly higher concentrations than in control birds (Table 2.5). Incubated

muscles also released amino acids into incubation medium. The total amino acids released from isolated EDC muscle of infected birds increased significantly above that of control birds by 37.9 % ($P < 0.05$; Table 2.6). The net release of taurine (+75%), alanine (+118%), tyrosine (+66%) and phenylalanine (+56%) was significantly increased in muscles from infected birds ($P < 0.05$). Although most other amino acids showed increased net release ranging from +25% to + 108%, this trend was not significant ($P > 0.05$).

Discussion

The results of this study describe modified growth and protein metabolism following *E. coli* infection. Infection significantly reduced feed intake and depressed the growth of the whole body, eviscerated carcass, and especially skeletal muscles but did not affect the growth of liver and heart in broiler chicks. Although growth resumed 2 to 4 days after infection, catch - up to healthy controls did not occur within the subsequent 30 d period. The rate of muscle protein synthesis 2 to 4 days after infection was significantly decreased by 42-48%; this was much higher than the reduction of protein degradation (28.7%) suggesting that decreased protein synthesis would be a major contributor to the negative nitrogen balance following infection.

E. coli is ubiquitous in poultry production systems and outbreaks of *E. coli* septicemia can cause significant losses of production (Coutts, 1981). The strain used here predictably produced a severe infection with limited mortality, within a dose range of 10^9 to 10^{11} cells / bird. Birds showed significant clinical signs, including fibrinous peritonitis, hemorrhage on the visceral organs, loss of appetite and growth depression of whole body and individual tissues. Thus treatment with a single i.p. injection of *E. coli* provides a simple experimental model for study of effects of infection on growth and protein metabolism.

Depressed growth of the whole body is characteristic of host response to infection in rapidly growing young of all species (Beisel, 1984). The large majority of work in this area has been done in mammals and man (Beisel, 1984; Tanner, 1986) and relatively little

work has been reported in avian species. The present study demonstrated that *E. coli* infection severely inhibited body growth, and that this effect was large and of long duration. Within 30 days after infection, birds were still 12% lighter than healthy controls ($P<0.01$). It has been previously demonstrated that an animal whose growth has been retarded exhibits, when the restriction is removed, a rate of growth greater than that which is normal in animals of the same chronological age (Brody, 1926). Such catch-up growth after undernutrition has been extensively studied in mammals and birds (Beane et al., 1977; McCartney and Brown, 1976; Wilson and Osbourn, 1960; Brody et al., 1980). Very little information, however, appears to be available concerning catch up after infection, which usually involves both a pathogenic process, and some measure of supression of voluntary feed consumption. In the case of feed or protein restriction, the nature, duration and severity of the stress influence whether or when complete catch up occurs. For example, when broiler chicks were feed - restricted for 22 weeks after hatching, resulting in a body weight one third of the normal weight, then refed *ad libitum*, catch up growth did not occur within the subsequent 22 weeks (Brody et al.,1980). In another study, (Washburn and Bonderi, 1978), when broiler chicks were restricted to 85% of their ad libitum intake for periods ranging from 3 to 5 weeks, from 3 - 8 weeks of age, weight gains were 10 - 20% below controls and complete catch-up growth did not occur within the 8 week study period. By contrast, in studies by Auckland and coworkers (Auckland and Morris 1971; Auckland et al.,1969), when turkeys were provided restricted protein intake for 4 to 6 weeks duration during the first 10 weeks after hatching, then

refed *ad libitum*, complete catch up growth was seen by 14 weeks after removal of the restriction. In the present study, although feed intake was restricted by about 30% for only a few days, growth was halted, then slowed for the whole period of study. This suggests that after infection chicks would not reach weight of healthy controls by 6 weeks of age, at which time broilers are usually marketed. In a related study (Baracos, V.E. and Robinson, F.E. unpublished observations), chicks of a layer strain infected at 8 days of age, caught up to control birds by 15 weeks after infection.

Each tissue has a characteristic growth rate, which, like the whole body, is target - seeking. Infection modified the normal pattern of tissue growth and was different for different tissues. *E. coli* infection significantly slowed the growth of muscles and carcass (consisting primarity of muscle and bone) but did not affect the growth of liver and heart. The influence of infection in different tissues is clarified from data expression per 100 g body weight. Liver constituted a larger proportion of body weight from one to ten days after infection. By contrast, muscle constituted a smaller proportion of the body for up to fifteen days after infection. Heart was also increased as a percent of body weight by 48% and 56% on day 2 and 4 after infection. After infection, chicks returned rapidly to proportional body composition, as judged, by the percent of body weight accounted for by the different tissues. Having rapidly achieved the correct proportions, body size *per se* was much slower to catch up.

The losses of body and muscle weight due to infection are usually ccompanied by changes in tissue and body composition (Forbes, 1986).

It is well known that increased nitrogen loss occurs during infection in mammals and man (Beisel, 1984; Fern et al., 1985); this also occurs in birds (Squibb and Grun, 1966). The present study showed that the protein contents of muscles of two major fibre types (EDC, fast twitch muscle from the wing; sartorius, slow twitch muscle from the leg) and eviscerated carcass decreased dramatically during *E. coli* infection. For example, on day 12 after infection, the weight losses (-36% in EDC muscle and -43% in sartorius muscle) seen in the study was accompanied by an even much larger fall in tissue protein (-47% in EDC muscle, -50% in sartorius muscle). Thus during infection, body protein, especially muscle protein, are more severely affected than the weights. By contrst, water content of muscles, as a percentage of muscle weight, from infected birds was not different from healthy controls.

Numerous studies have demonstrated that the changes of protein content of whole body result from changes in muscle protein turnover to a large extent. However, these responses are not fully understood, and very little is known about the time couse of change in protein synthesis and protein degradation due to infection and recovery. Considerable debate exists concerning the relative importance of protein synthesis and degradation in muscle protein catabolism during infection.During infection, four possible alterations of protein turnover have been reported: 1. increased both protein synthesis and protein degradation (Long, 1977; Garlick et al., 1980a; Tomkins, 1983); 2. decreased protein synthesis (Fern, et al., 1985; Lust, 1966)); 3. increased protein degradation (Fagan and Goldberg, 1985) and 4. increased protein degradation and decreased protein synthesis

(Klasing and Austic, 1984a; 1984b). Some authors have reported increased protein synthesis during infection (Pomposelli et al., 1985). The discrepancies amongst the studies may due to the time points, type of infection, or animal species selected. The present study found that *E. coli* injection depressed fractional rate of protein synthesis in sartorius muscle by 42.0% on day 2, and 33.4% on day 4 after the infection. Similar results were seen in muscles of infected rats (Fern et al., 1985; Lust, 1966) and chicks (Klasing and Austic, 1984a). The fall in muscle protein can be explained by falling rates of protein synthesis; by contrast, rates of liver and heart protein synthesis were maintained. A second method was used *in vitro*, to support the *in vivo* results, as well as to estimate protein degradation. Protein synthesis in isolated EDC muscle decreased significantly by 47.8% on day two after the infection which was similar to the 42.0% decreases seen in sartorius muscle *in vivo* on the same day. Protein degradation also fell(-28.7%), but to a lesser extent than protein synthesis. Thus, our data would appear to support the view that protein synthesis falls and protein degradation rates either fall or be unchanged during infection (Rennie, 1985; Schonheyder et al., 1954).

A characteristic of the metabolic response to infection is that decreased muscle protein synthesis or increased muscle protein breakdown is associated with an accelerated release of amino acids from the skeletal muscle and uptake by the liver and other viseral tissues (Wannemacher et al., 1974; Beisel, 1984). In the present study, decreased muscle protein synthesis due to infection resulted in increased total amino acid release from the muscle by 37.9% ($P <$

0.05), but the total intracellular amino acid concentration did not change significantly. The release of individual amino acids release increased to different degrees during infection, although some of the increases were not significant. It is interesting to note that alanine release doubled the control value among those which increased dramatically during infection such as taurine, alanine, tyrosine, and phenylalanine. This agreed with the comment that there usually is a corresponding increase in the flux of alanine from muscle into plasma with the accelerated proteolysis of skeletal muscle protein following infection (Beisel, 1984). In perfused liver *in vivo*, alanine has been found to be the best substrate for gluconeogenesis in adult rat (Ross et al., 1967; Exton and Park, 1967). During infection, the role of alanine in glucose production becomes important, because it can serve as a substrate for gluconcogenesis which is a important energy source for infected birds. In fact, each amino acid may have its own role to play in host defence (Beisel, 1984), but the mechanism to control the uptake of free amino acids from plasma by specific single cells or tissues and the function of each amino acid in host defence remains unclear.

It would appear in keeping with the vital role of the liver in host defence, that this organ have maintained rates of protein synthesis. The increased liver protein synthesis seen in rats infected with malaria (Fern et al., 1985) and in chicks infected with *E. coli* (Klasing and Austic, 1984a) was not found in this study. No significant differences were found in liver protein synthesis between infected birds and control birds. However, liver protein synthesis was maintained in the face of very catabolic state in muscle. Heart

protein synthesis also showed no significant difference between infected and control birds in the present study, in agreement with the results obtained from infected rats (Fern et at., 1985). The retention of heart protein synthesis may be because heart is a vital organ, and resistant to a number of catabolic stresses. The functions of each organ may explain why different tissue have different responses to infection. Muscle is to some extent a disposable tissue which is major protein reservoir in the body and it can supply amino acids during infection. Liver plays a highly important role in orchestrating the metabolic responses to infection by generating substrate fuels for energy, by producing proteins for its own use as well as for secretion into plasma, by detoxifying microbial toxins as well as bacateria metabolites, and by sequestering metals such as iron and zinc (Beisel, 1984). Heart is not disposable and possibly also responding to increased work load.

Negative nitrogen balance of body and muscle due to infection or other pathological states is usually accompanied by a reduction of feed intake, as also demonstrated here. Many studies have shown that nutritional restriction inhibits growth and protein accumulation (Wilson and Osbourn, 1960; Tanner, 1986). Therefore, feed intake is thought to be a important factor affecting the nitrogen loss involved in infection. Pair feeding studies would be required to evaluate the importance of depressed feed intake in the present study. In related studies, when chicks were infected with Newcastle disease-virus, their nitrogen retention was significantly depressed. However, when infected chicks were forced fed, their nitrogen retention was higher than that of infected chicks without forced feeding (Squibb and Grun, 1966). The

present study showed no overshoot of intake, so that after infection birds did not eat more than healthy controls. Feed intake depression was small, short term, especially when expressed per unit body weight. It seems unlikely that this relatively small effect would entirely account for the differences in growth seen here between control and infected birds. Some factors apart from feed intake would be involved in regulating protein turnover during infection.

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Table 2.1.

EFFECT OF E.coli INFECTION ON MUSCLE WEIGHT

TIME AFTER INFECTION (days)	MUSCLE WEIGHT (mg) EDC		SARTORIUS	
	CONTROL	INFECTED	CONTROL	INFECTED
0	14.6± 1.0		255.5± 13	
2	25.1± 0.1	18.3± 0.2**	365.1± 16	251.8± 17**
4	35.4± 2.1	23.2± 3.2**	522.6± 29	306.0± 39**
6	46.6± 2.3	34.8± 2.5**	626.0± 33	441.0± 34**
10	72.5± 5.0	53.4± 4.0*	961.0± 44	669.0± 46**
15	128.6± 5.6	93.6± 5.3**	1726.0± 56	1186.0± 73**
20	184.5± 7.3	160.1± 7.0**	2326.0±109	2013.0± 85*
25	258.4± 7.5	224.6±11.0*	3509.6±184	2830.7±140**
30	399.1±13.0	324.6±18.0*	4408.0± 82	3744.0±273*

Values are means ± SEM of 8-10 birds. * $P < 0.05$ significantly different from control. ** $P < 0.01$ significantly different from control.

Control birds were injected with 1.0 ml sterile phosphate buffered saline and infected birds were injected with 1.0 ml 1.0×10^9 E. coli (i.p), at day 8 ex ovo. Extensor digitorum communis (EDC) and Sartorius muscle were dissected and weighed at the time points indicated.

Table 2.2. EFFECT OF *E.coli* INFECTION ON TISSUE PROTEIN CONTENT

TIME AFTER INFECTION (DAYS)	TISSUE PROTEIN CONTENT			
	TOTAL PROTEIN		% OF TISSUE WEIGHT	
	CONTROL	INFECTED	CONTROL	INFECTED
	CARCASS (g)			
0	6.30±0.20		12.37±0.19	
2	9.78±0.56	6.92±0.47**	13.00±0.15	12.45±0.15*
4	12.36±0.47	6.38±0.63**	13.06±0.25	11.98±0.17*
6	15.57±0.49	8.80±1.28**	12.83±0.18	12.28±0.21
12	28.10±1.35	16.61±2.05**	12.94±0.24	12.50±0.24
	Extensor digitorum communis (EDC) MUSCLE (mg)			
0	3.63±0.21		15.22±0.38	
2	5.56±0.44	3.31±0.50*	15.93±0.25	15.43±0.24
4	7.22±0.36	3.60±0.69*	16.18±0.22	17.70±1.06
6	14.76±0.50	5.07±0.83*	24.44±0.91	18.56±0.60**
12	23.12±1.14	12.35±1.78*	21.35±0.43	18.08±0.44**
	SARTORIUS MUSCLE (mg)			
0	40.90± 1.98		13.22±0.36	
2	63.55± 5.29	41.37± 4.60*	16.67±0.86	14.39±0.71
4	90.34± 5.30	39.20± 3.10**	16.89±0.24	15.14±0.87
6	113.70± 3.80	52.79± 8.36**	17.12±0.49	14.99±0.50*
12	231.01±15.47	116.46±19.89**	18.70±0.78	16.25±0.79*

Values are means ± SE of 8-10 birds.
*P < 0.05 significantly different from control
**P < 0.001 significantly different from control
a. Eviscerated carcass after removal of head, feet, skin, feathers, gastrointestinal tract and contents of abdominal and thoracic cavities.
Muscle protein content was measured by the Bradford method, and carcass protein content was measured by the Kjeldahl method.

Table 2.3.

EFFECT OF *E. coli* INFECTION ON TISSUE WEIGHT AND PROTEIN SYNTHESIS IN VIVO

TISSUE	TIME AFTER INFECTION (days)	TISSUE WEIGHT (g) CONTROL	TISSUE WEIGHT (g) INFECTED	FRACTIONAL RATE OF PROTEIN SYNTHESIS (Ks % / day) CONTROL	FRACTIONAL RATE OF PROTEIN SYNTHESIS (Ks % / day) INFECTED
SARTORIUS MUSCLE	2	0.38±0.01	0.27±0.01**	28.8±1.5	16.7±1.5**
	4	0.53±0.01	0.24±0.01**	22.7±2.3	15.2±2.6*
LIVER	2	6.36±0.27	6.33±0.38	118.0±3.0	109.7±3.6
	4	7.96±0.23	7.63±0.33	105.2±3.9	95.3±4.5
HEART	2	1.16±0.04	1.32±0.10	52.3±1.2	51.3±2.5
	4	1.65±0.05	1.41±0.06	51.2±2.9	53.7±1.6

Values are means ± SEM of 7-8 birds.
** $P < 0.001$ significantly different from control.
* $P < 0.05$ significantly different from control.

Control birds were injected with 0.5 ml sterile phosphate buffered saline and infected birds were injected with 0.5 ml 5.0×10^9 *E. coli* (i.p), at day 8 *ex ovo*. At 2 and 4 days after the injection, birds were sacrificed and tissue dissected. Protein synthesis was determined by the method of Garlick et al. (1980b).

Table 2.4.

EFFECT OF *E. coli* INFECTION ON PROTEIN TURNOVER IN ISOLATED EDC MUSCLE

TREATMENT	PROTEIN SYNTHESIS	NET PROTEIN BALANCE	PROTEIN DEGRADATION[a]
	(nmol phe/mg muscle/ 3h)		
CONTROL	0.316±0.03	0.070±0.017	0.386
INFECTED	0.165±0.02*	0.110±0.025**	0.275
DIFFERENCE	-0.151 (-48%)	+0.040 (+57%)	-0.111 (-29%)

Values are means ± SEM of 6-9 birds.
* $P < 0.05$ significantly different from control
** $P < 0.025$ significantly different from control.

Control birds were injected with 1.0 ml sterile phosphate buffered saline and infected birds were injected with 1.0 ml 2.2×10^9 *E. coli* (i.p), at day 8 *ex ovo*. Measurements were carried out on day 2 after the injection. Protein turnover was determined in isolated chick EDC muscles, incubated in Krebs-Ringer bicarbonate medium containing 15 mM glucose and 0.01 U /ml insulin.

a. Calculated value = protein synthesis + net protein degradation.

Table 2.5.

EFFECT OF *E. coli* INFECTION ON INTRACELLULAR AMINO ACID CONCENTRATION IN ISOLATED EDC MUSCLE
(nmol amino acid/mg muscle)

Amino Acids	CONTROL	INFECTED	INFECTED/CONTROL
Asp	0.5574	0.3976**	0.71
Glu	1.3707	1.0119**	0.74
Asn	0.3359	0.2026*	0.60
Ser	0.5957	0.3487**	0.59
Gln	2.8254	1.8186*	0.64
Gly	1.1484	1.1081	0.96
Thr	0.4900	0.1790**	0.37
Arg	0.3119	0.3400	1.09
Tau	7.2693	9.2086	1.27
Ala	0.8277	0.7700	0.93
Tyr	0.1190	0.0784*	0.66
Trp	0.2800	0.6870*	2.45
Met	0.0643	0.0094*	0.10
Val	0.0327	0.0183	0.56
Phe	0.0746	0.0649	0.87
Ile	0.1369	0.2124**	1.55
Leu	0.1447	0.1550	1.07
Lys	0.1427	0.2747**	1.93
Total	16.56	16.12	0.97

Values are means of 7-9 birds in each group.
* $P < 0.01$ significantly different from control.
** $P < 0.004$ significantly different from control.

Table 2.6.

EFFECT OF *E. coli* INFECTION ON NET AMINO ACID RELEASE
From Isolated EDC Muscle
(nmol amino acid released/mg muscle/3h)

Amino Acids	CONTROL	INFECTED	INFECTED/CONTROL
Asp	0.0312	0.0400	1.28
Glu	0.0724	0.1013	1.40
Asn	0.1054	0.1082	1.03
Ser	0.2772	0.3543	1.27
Gln	0.8594	1.0622	1.24
Gly	0.3158	0.4470	1.42
Thr	0.4128	0.3157	0.76
Arg	0.1360	0.1818	1.34
Tau	0.0994	0.1742*	1.75
Ala	0.2726	0.5947*	2.18
Tyr	0.1210	0.2010*	1.66
Trp	0.0218	0.0310	1.42
Met	0.0134	0.0280	2.09
Val	0.0566	0.1090	1.93
Phe	0.0704	0.1098*	1.56
Ile	0.0228	0.0645	2.83
Leu	0.0364	0.0865	2.38
Lys	0.0692	0.1108	1.60
Total	2.9938	4.1200*	1.38

Values are means of 5-6 birds in each group.
* $P < 0.05$ significantly different from control.

Figure 2.1.

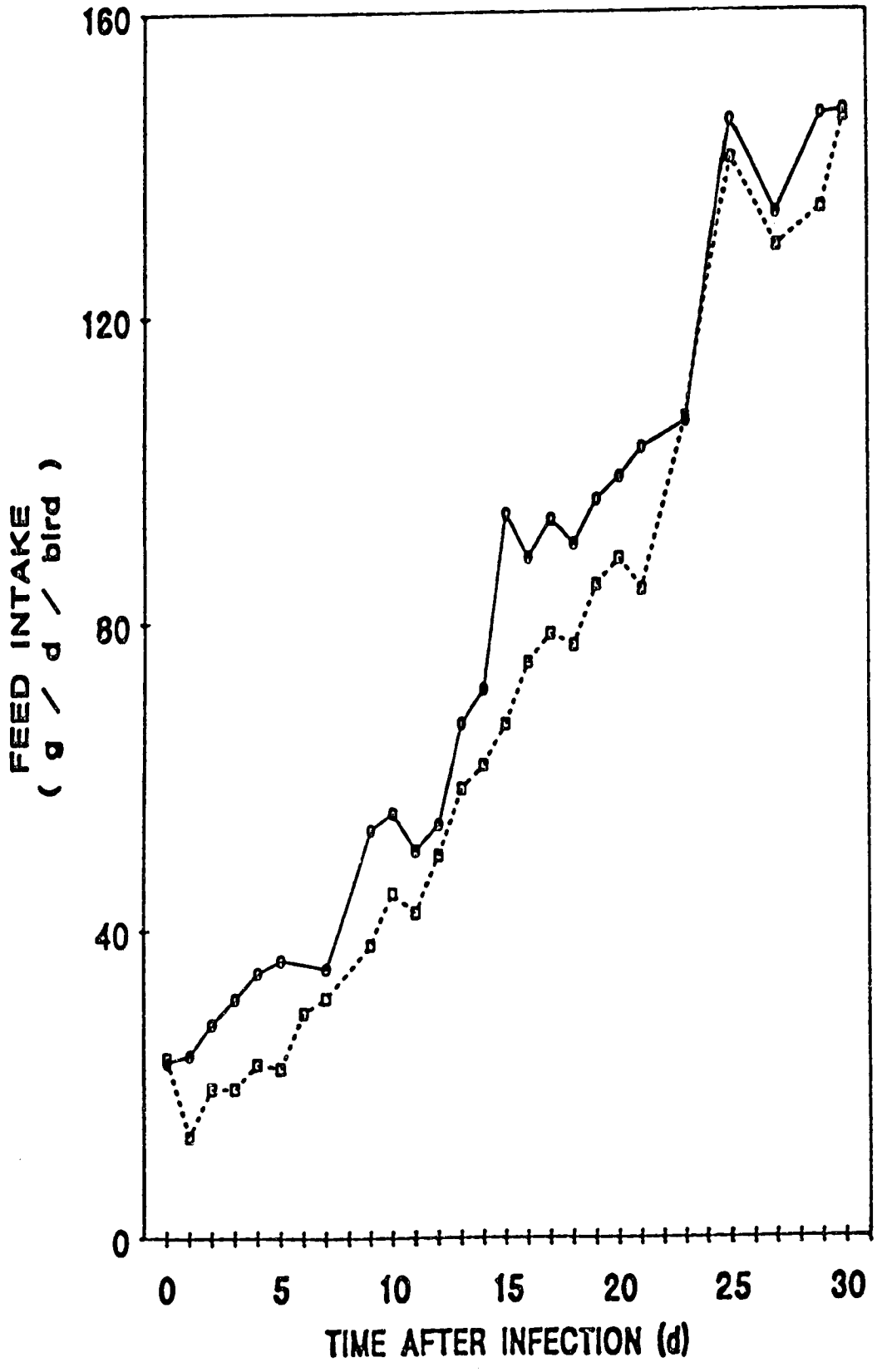
160
120
80
40
0
FEED INTAKE
(g / d / bird)
0
5
10
15
20
25
30
TIME AFTER INFECTION (d)

Figure 2.2.

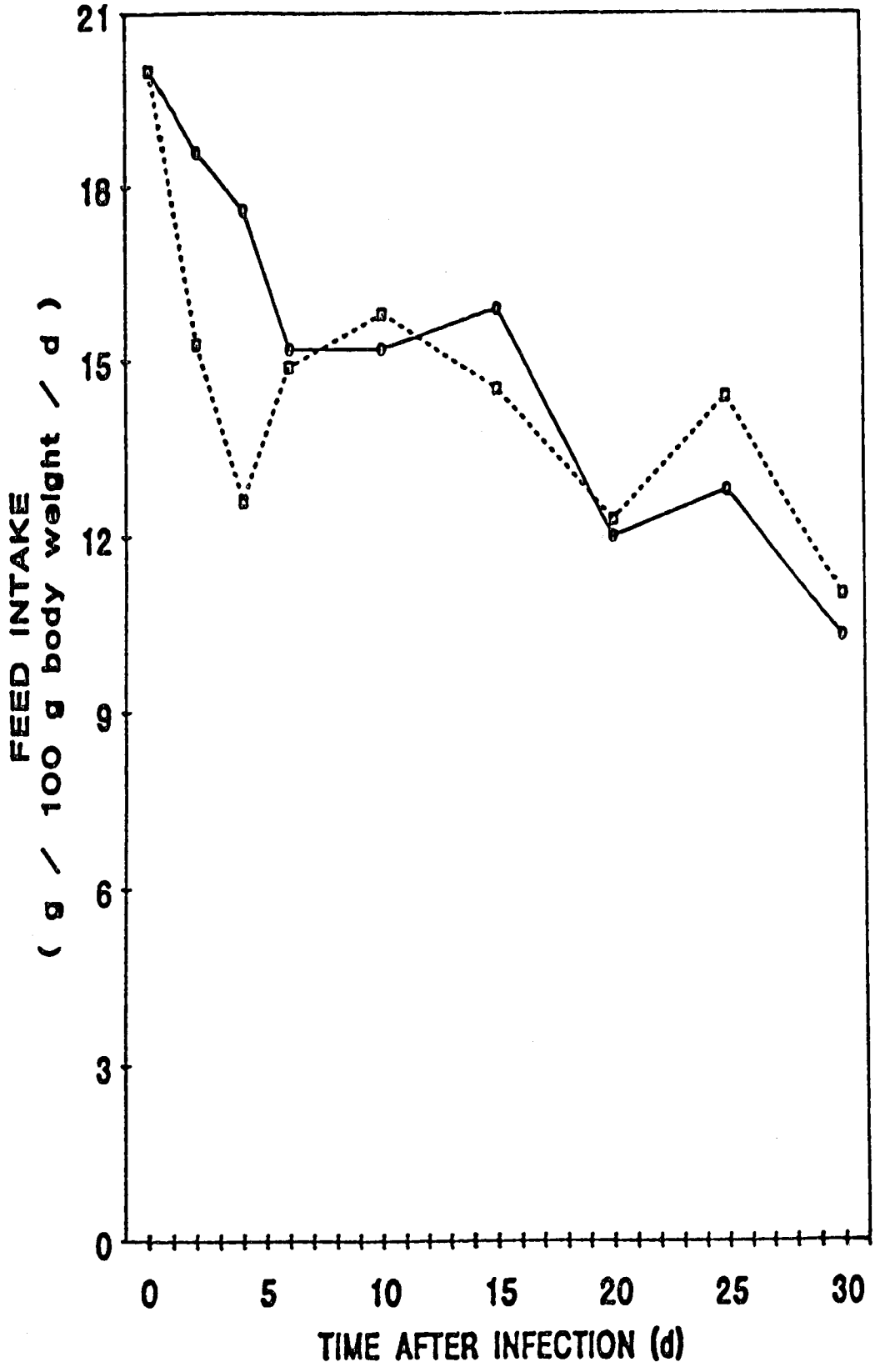
21
18
15
12
9
6
3
0
FEED INTAKE
(g / 100 g body weight / d)
0
5
10
15
20
25
30
TIME AFTER INFECTION (d)

Figure 2.3.

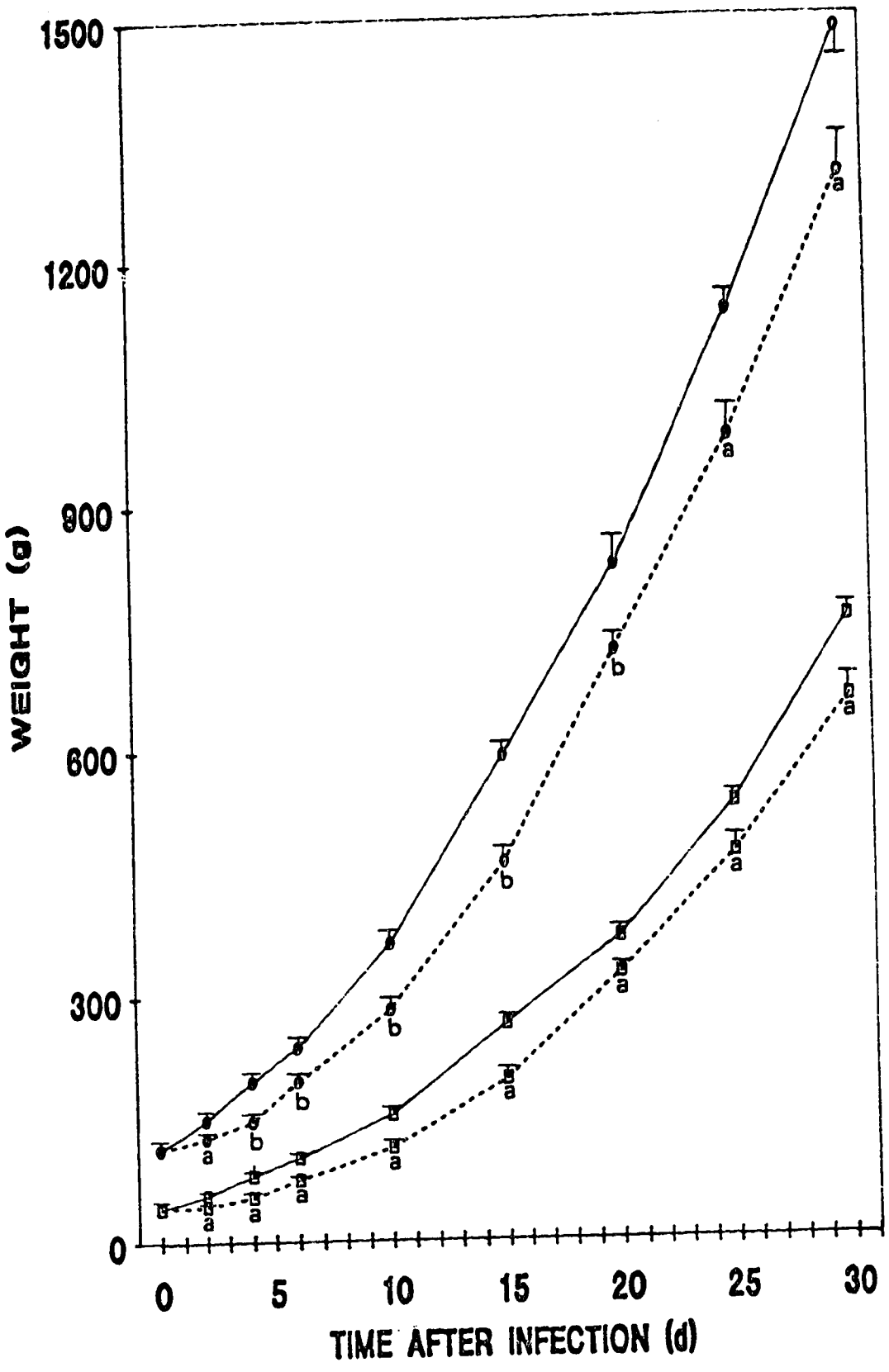
1500
1200
900
600
300
0
WEIGHT (g)
0
5
10
15
20
25
30
TIME AFTER INFECTION (d)

Figure 2.4.

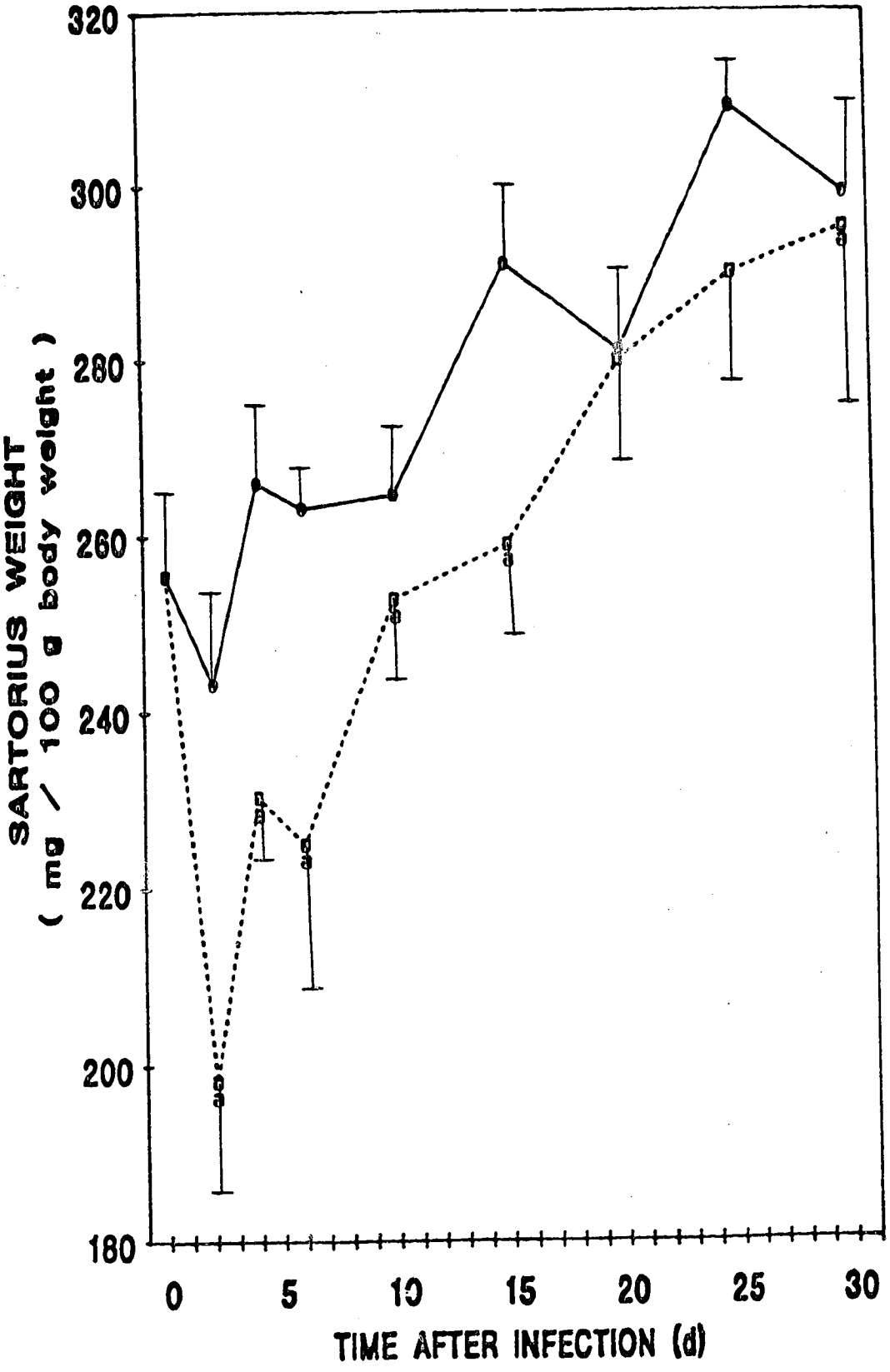
320
300
280
260
240
220
200
180
SARTORIUS WEIGHT
(mg / 100 g body weight)
0
5
10
15
20
25
30
TIME AFTER INFECTION (d)

Figure 2.5.

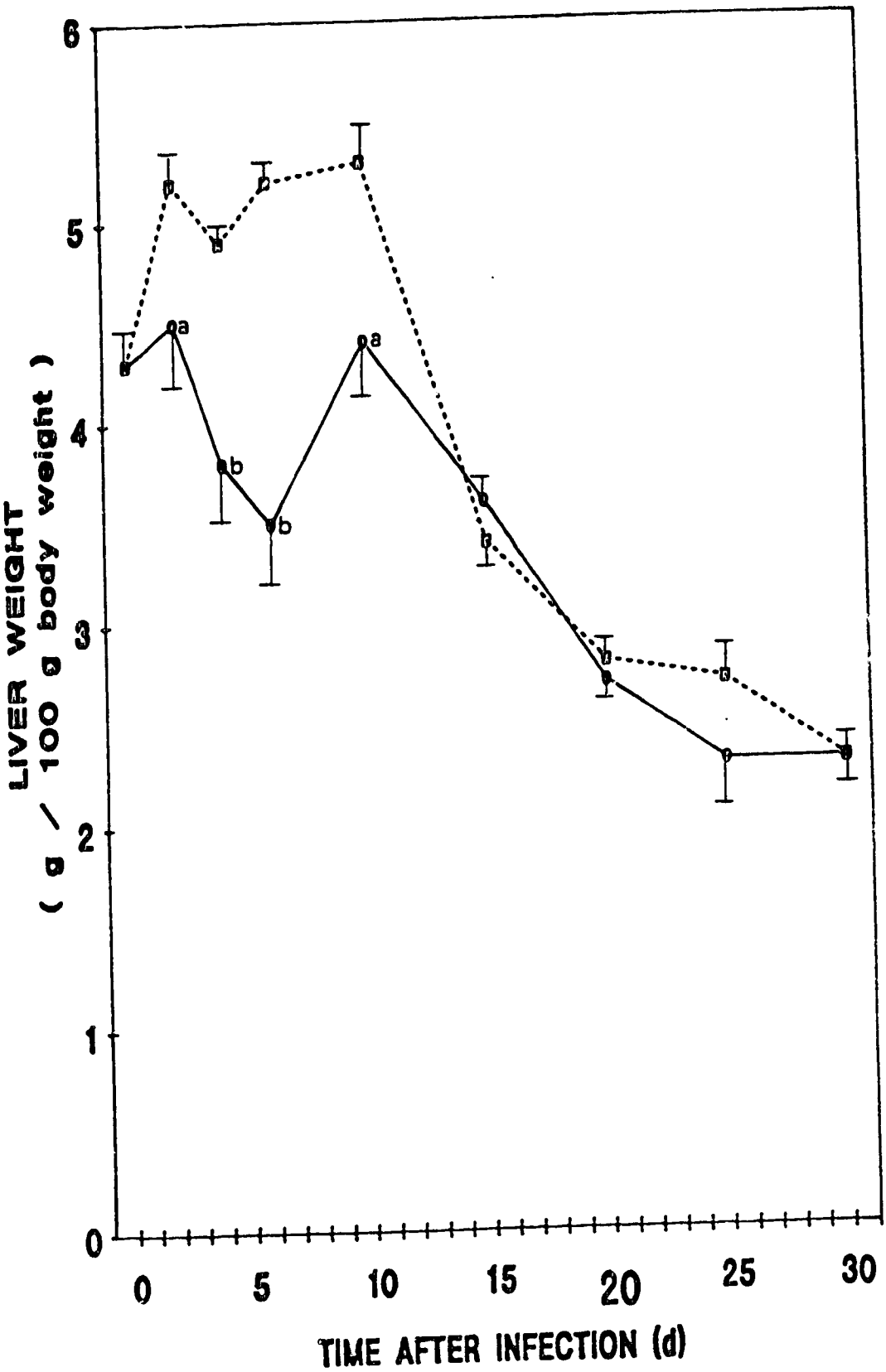
LIVER WEIGHT
(g / 100 g body weight)
TIME AFTER INFECTION (d)
0
1
2
3
4
5
6
0
5
10
15
20
25
30
a
b
a
b
b
b
b
b

Figure 2.1: Effect of *E. coli* infection on feed intake in broiler chicks

o——o Control; □····□ Infected.

Broiler chicks were housed in battery cages (13-14 birds/ pen). *Ad libitum* feed intake for each group was determined daily. Chicks were fed broiler starter ration which contains 23.3% CP and 2795 kcal ME/Kg. The numbers given represent average values of at least 2 pens of birds.

Figure 2.2: Effect of *E. coli* infection on feed intake as proportion of body weight.

o——o Control; □····□ Infected.

Broiler chicks were housed in battery cages (13-14 birds/ pen). *Ad libitum* feed intake for each group was determined daily. Chicks were fed broiler starter ration which contains 23.3% CP and 2795 kcal ME/Kg. The numbers given represent average values of at least 2 pens of birds.

Figure 2.3: Effect of *E. coli* infection on body and eviscerated carcass weight.

o——o Control body weight; o····o Infected body weight;

□——□ Control carcass weight; □····□ Infected carcass weight.

Values are means of 10 birds. a - $P < 0.01$ and b - $P < 0.001$ significantly different from control. Eviscerated carcass was carcass after removed of the head, feet, skin, feathers, gastrointestinal tract and contents of abdominal and thoracic cavities were removed. Control birds were injected with 1.0 ml sterile phosphate buffered

saline and infected birds were injected with 1.0 ml containing 1.0 x 10^9 *E. coli* (i.p.), at day 8 *ex ovo*.

Figure 2.4: Effect of *E. coli* infection on sartorius muscle weight as proportion of body weight.

o——o Control; □····□ Infected.

Values are means 8-10 birds. a - $P < 0.01$ significantly different from control. Control birds were injected with 1.0 ml sterile phosphate buffered saline and infected birds were injected with 1.0 ml containing 1.0 x 10^9 *E. coli* (i.p.), at day 8 *ex ovo*.

Figure 2.5: Effect of *E. coli* infection on liver weight as proportion of body weight.

o——o Control; □····□ Infected.

Values are means of 8-10 birds. a - $P < 0.03$ and b - $P < 0.001$ significantly different from control. Control birds were injected with 1.0 ml sterile phosphate buffered saline and infected birds were injected with 1.0 ml containing 1.0 x 10^9 *E. coli* (i.p.), at day 8 *ex ovo*.

III. PROSTAGLANDIN-DEPENDANT MUSCLE WASTING DURING SYSTEMIC INFECTION IN THE BROILER CHICK (*Gallus domesticus*) AND LABORATORY RAT (*Rattus norvegicus*)

PROSTAGLANDIN-DEPENDANT MUSCLE WASTING DURING SYSTEMIC INFECTION IN THE BROILER CHICK (*Gallus domesticus*) AND LABORATORY RAT (*Rattus norvegicus*)

1. Systemic infection with *Escherichia coli (E. coli)* significantly reduced feed intake, slowed growth of the whole body and skeletal muscles, and severely inhibited muscle protein accumulation in both chicks and rats.
2. Treatment with naproxen (6-methoxy-α-methyl-2-napthalene acetic acid), an inhibitor of prostaglandin production, reduced weight losses of body and muscle, and significantly inhibited muscle protein wasting in infected chicks and rats.
3. *E. coli* infection significantly increased net protein degradation by 44.8% ($P < 0.05$) and PGE_2 production by 148% ($P < 0.05$) in isolated extensor digitorum communis muscle from chicks on day 2 after infection. Naproxen treatment significantly reduced net protein degradation and PGE_2 production in infected chicks to levels seen in muscles of healthy controls. Quantitatively and qualitatively similer results were seen in isolated rat epitrochlearis muscle.

PROSTAGLANDIN-DEPENDANT MUSCLE WASTING DURING SYSTEMIC INFECTION IN THE BROILER CHICK (*Gallus domesticus*) AND LABORATORY RAT (*Rattus norvegicus*)

INTRODUCTION

A marked whole body wasting and protein loss from skeletal muscle are characteristics of infection and various pathological states. Infectious disease leads to increased muscle breakdown, urinary nitrogen loss and detectable losses of body protein (Beisel, 1984; Garlick et al., 1980). The relative importance of changes in muscle protein synthesis and degradation in muscle wasting during infection have been addressed by several authors (Fern et al.,1985; Rennie, 1985; Baracos, 1985). Many hormones and factors have been thought to be involved in the regulation of muscle protein metabolism during infection, including insulin, glucagon, growth hormone, thyroid hormone, glucocorticoids (Beisel, 1984), and more recently, prostaglandins (Baracos et al.,1983; Fagan & Goldberg, 1985).

Prostaglandins (PG) are cyclopentane derivatives formed from polyunsaturated fatty acids and released by most animal cell types in response to a variety of physiological and pathological stimuli (Samuelsson et al., 1978). Recently, the possibility that PG might be important in the regulation of muscle protein turnover in normal or pathological conditions has come to light. Skeletal muscles in humans and rats can produce metabolites of arachidonic acid including PGI_2, TXB_2, PGE_2 and $PGF_{2\alpha}$ (Nowak et al., 1983; Reeds et al., 1985; Rodemann and Goldberg, 1982). When added to muscles *in vitro*, PGE_2 stimulated muscle protein degradation, and $PGF_{2\alpha}$

activated protein synthesis (Rodemann and Goldberg, 1982; Reeds and Palmer, 1984). For example, when rat muscles were treated with PGE_2, protein synthesis was not affected, but protein degradation increased about 22% (Rodemann and Goldberg, 1982). By contrast, $PGF_{2\alpha}$ had no effect on protein degradation but increase protein synthesis (Rodemann and Goldberg, 1982; Reeds et al., 1985).

More recently, several authors have attemped to clarify the role of PG in regulation of muscle protein turnover in vivo. For example, injection of live *E. coli* into rats caused fever, and increased both PGE_2 production and protein degradation in skeletal muscle (Fagan and Goldberg, 1985). When the rats were pre-treated with indomethacin, an inhibitor of prostaglandin production, the increased protein degradation and prostaglandin production due to infection were inhibited (Fagan and Goldberg, 1985). Indomethacin has also been reported to prevent muscle protein loss and impaired contractile force due to *S. Pneunmoniae* infection (Ruff and Secrist, 1984). These studies suggested that the rapid onset of muscle wasting that take place during infection *in vivo* is signalled by a prostaglandin-dependent mechanism (Fagan and Goldberg, 1985; Ruff and Secrist, 1984), although it remains unclear as to which stimuli are responsible for enhanced PGE_2 release. However, the role of PGE_2 in signalling accelerated muscle proteolysis is controversial. Treatment of incubated muscles from septic rats with indomethacin did not change the rate of protein degradation, although PGE_2 production was inhibited (Hasselgren et al., 1985; 1988). Futher evidence is needed to confirm whether PGE_2 is important in regulation of muscle proteolysis. Finally, the regulatory function of PGE_2 production on

protein turnover in pathological processes have been studied in rats. However, no work has been reported in other species. Thus the objective of this study was to test for a prostaglandin-dependant mechanism in muscle wasting during systemic infection in the rapidly growing broiler chick (*Gallus domesticus*) and in the rat (*Rattus norvegicus*), using experimental *E. coli* infection previously developed in our laboratory (Tian & Baracos, 1988, in preparation).

Materials and Methods

Experimental design:

The study was designed to determine the effects of naproxen (6-methoxy-α-methyl-2-napthalene acetic acid) treatment on the growth of the whole body and skeletal muscle, muscle protein accumulation, net muscle protein degradation and PGE_2 production in experimental *Escherichia coli (E. coli)* infection. Chicks and rats were randomly assigned to four treatment groups respectively, *Control*, *Infected*, *Naproxen Control*, *Naproxen Infected*, such that the mean initial body weight and SEM of each group were similar to each other. One day before injection of *E. coli*, naproxen administration was initiated by addition to the drinking water. The dose was based on 1.6 mg / 100 g body weight / day. Preliminary experiments showed this was a minimum effective dose to block fever induced by the injection of *E. coli* endotoxin (Baracos, V., unpublished observations).

Animals and diet:

Day-old male broiler chicks of the Hubbard strain which had been vaccinated for Marek's disease were obtained from Co-op Hatchery (Edmonton, Alberta). The chicks were housed (14-20/pen) in electrically heated batteries (33-35^{o}) with raised wire-mesh floors and continuous lighting. The chicks were fed *ad libitum* a broiler starter diet based on wheat and soybean meal containing 2795 kcal metabolizable energy / kg and 23.3% crude protein (Appendix I). Nine week old male Sprage Dawley rats were housed in individual wire cages

and maintained on Wayne Laboratory chow and 12 h light:dark cycle. Feed and water were provided *ad libitum* throughout the period of study. The total numbers of birds and rats were more than that of studied because of expectant mortalyty. Feed intake was measured on a per pen basis (chicks) or individual basis (rats) and body weights were measured individually daily. Eight birds or seven rats from each treatment group were sacrificed on day 2 after the injection and muscles were dissected: extensor digitorum communis (EDC) and sartorius (SAR) muscles from chicks and extensor digitorum logus (EDL) and soleus (SOL) muscles from rats. Muscles were weighed and stored frozen at - 30°C until analyzed.

Experimental infection:

Chicks (8-day old) and rats (ten week old) were given systemic infection by intraperitoneal (i.p.) injection of *E. coli* (Tian & Baracos, 1988, in preparation). The cells used were of a single strain which was obtained from an outbreak of coliform septicemia in turkeys. Cell stocks were stored frozen in sheep blood at -70°C. A single i.p. injection containing 3 x 10^8 (chicks) or 8.1 x 10^8 (rats) colony forming units was given to each infected subject in a volume of less than 1.0 ml. The injection was given when birds were at ~ 100 g and rats were at ~ 250 g body weight, so that both species received 3 x 10^9 cells / kg liveweight. Control subjects were sham injected with sterile phosphate buffered, non-pyrogenic saline.

Analysis of protein

Frozen muscles were thawed and dissolved with 1.0 N NaOH at 55°C

for 4-12 hours. Muscle protein content was measured by a modified Bradford Method (Bradford, 1976), according to the changes suggested by Read and Northcote (1981). Bovine serum albumin (BSA) was used as protein standard in the assay.

Measurement of net protein degradation and PGE_2 production

On day two after the infection, 7 to 8 chick from each treatment group were dissected. From each dissected bird, a extensor digitorum communis muscles (EDC) were taken with tendons intact, and incubated in 3 ml Krebs-Ringer bicarbonate medium containing glucose (15 mm) and insulin (0.01 U/ml). Muscles were incubated for 30 min at 36°C, then transferred to fresh medium and incubated for another 3 h. Net protein degradation was determined by measuring the amount of tyrosine released into the medium as described previously (Baracos et al., 1983). PGE_2 production was estimated by radioimmunoassy of the the medium after the 3 h incubation period as previously described (Baracos et al., 1983). The cross reactivity of the anti-PGE_2-antibody (ICN Immunbiologicals) with PGE_1 is 150%, but with $PGF_{2\alpha}$ less than 2%. The effective range of the assay was from 12.5-875 pg/sample.

Statistics

Results are expressed as means ± standard error of the mean of eight birds or seven rats. The results were analyzed with one way analysis of variance.

Results

Whole body and muscle growth

Healthy young broiler chicks and naproxen treated control chicks showed rapid rates of growth during the experimental period. After injection of *E. coli*, the growth of infected chicks continued at a low rate, such that the body weight of infected birds was less than that of controls by 8.6% (NS) on day 1 and 16.7% ($P < 0.05$) on day 2 after injection (Fig. 3.1). Rat grew (26%) more slowly than chicks (40%) in two days and *E. coli* injection significantly decreased the body weight in rats by 9.2% on day 1 and 10.2% ($P < 0.05$) on day 2 after injection (Fig. 3.2). Naproxen alone had no effect on body weight of rats or chicks. The body weight of infected chicks or rats treated with naproxen was intermediate between those of infected and saline-injected control subjects, such that the means for the naproxen treated infected subjects were not significantly different from either (Fig. 3.1; 3.2).

As shown in Table 3.1, the weight of 2 muscles in the chicks were depressed on day 2 after injection. The weights EDC and SAR muscle of infected birds were depressed by 22.6% and 29.3% ($P < 0.05$) respectively (Table 3.1), when body weight had fallen by 17%. In the rats, *E. coli* injection decreased muscle weight by 11.2% (NS) in SOL muscle and 10% ($P < 0.05$) in EDL muscle (Table 3.1); these changes were similar to the fall in whole body weight. Naproxen alone had no effect on muscle weight, however naproxen treated infected subjects had muscle weights which were intermediate between control and infected subjects (NS).

Protein content

Table 3.2 depicts the protein content of the same muscles shown in Table 3.1. In addition to the absolute weight, the total protein content and percent protein of the muscles were significantly lower than those of saline-injected controls in both infected chicks and rats. The changes in total protein contents seen in muscles of infected birds and rats were more dramatic than the changes in muscle weight (Table 3.2; 3.3). The total protein contents were depressed by *E. coli* injection by 37.2% in chick EDC muscle, 53.8% in chick SAR muscle, 46.4% in rat EDL muscle and 23.7% in rat SOL muscle. This was seen to result from a significant reduction in muscle protein content / g of tissue, which was significantly depressed ($P < 0.05$, n=7) in all muscles tested (EDC -18%, SAR -36%, SOL -13%, EDL -40%). Naproxen alone had no significant effect on total muscle protein or protein content / g wet weight. However, administration of naproxen to infected rats or chicks, in all cases significantly increased total muscle protein towards values seen in healthy controls. In chick EDC and SAR muscle, the loss of protein due to infection was inhibited by 41% and 93%, respectively. In rat EDL and SOL muscle, the response to infection was inhibited by 57% and 71%, repectively. In general a greater degree of inhibition was seen in the slow twitch muscle rat SOL and chick SAR, than in the fast twitch EDC and EDL muscles.

PGE_2 production and net protein degradation

Table 3.4 describes net protein degradation and PGE_2 production in isolated chick EDC muscle. Infected birds showed increased rate of net protein degradation by 44.8% and PGE_2 production by 148%

($P < 0.05$) on day 2 after injection (Table 3.3). Naproxen alone had no significant effect on either PGE_2 production or net protein degradation. However, naproxen treatment significantly inhibited PGE_2 production and net protein degradation in muscles from infected chicks, to values seen in healthy controls (Table 3.3).

Feed intake

Feed intake of control birds and rats was constant during 3 days of the study. Feed intake of infected and naproxen treated infected birds was depressed by 36% and 29% on day 1 and more severely depressed by 42% and 33% on day 2 after injection, respectively (Fig. 3.3). In rats, feed intake of infected and naproxen treated infected subjects was severely depressed by 64% and 62% respectively ($P < 0.05$) on day 1 and increased on day 2 after infection. On day 2 after infection, the feed intake of infected and naproxen treated rats was still 48% and 53% ($P < 0.05$) lower than control values, respectively (Fig. 3.4). Naproxen pretreatment did not have effect on feed intake in both control and infected subjects.

Discussion

The present study showed that naproxen administered orally, inhibited PGE_2 production by skeletal muscle. When naproxen was administered to rats or chicks, prior to systemic infection with *E. coli*, muscle protein loss was decreased by 41-93% ($P < 0.05$). The results support the suggestion that prostaglandins might be important in regulation of muscle protein turnover (Baracos et al., 1983; Goldberg et al., 1984; Rodemann and Goldberg, 1982) and identify their regulatory function not only in mammals but also in the domestic chicken.

In the present study, responses of chicks and rats to infection were quite similar with reduction of feed intake, accompanied by a slowing of growth in the rapidly growing chicks, and growth failure with body weight loss in the rats. Muscles from both species lost weight and especially protein in response to infection, although the size of this response varied with species and muscle type. For example, total protein in the fast twitch, rat EDL fell by 46.4% in response to infection, compared to slow twitch soleus (-23.7%). By contrast, in the chicks, the response of slow twitch, SAR (-53.8%) and fast twitch EDC muscles (-37%) were more similar. The data for the rat, showing a smaller response in soleus agree with work by other authors that this muscle resists atrophy during catabolic states (Goodlad et al., 1981). The regulation of protein turnover may differ in different types of skeletal muscle, and varying responsiveness to hormones and other substance (Frayn and Maycock, 1979; Angeras & Hasselgren, 1985; Hasselgren et al., 1986). Differences observed between chicks and rats, which in this study were given an identical

dose of bacteria on a per body weight basis, may be due to species difference, or to the different ages and growth tates at the time of infection.

It is noteworthy that PGE_2 production by mg of muscles of infected chicks was elevated by 148% ($P < 0.05$). The administration of naproxen reduced this enhanced PGE_2 production to levels seen in healthy controls. Under conditions of naproxen pretreatment, inhibition of PGE_2 release was associated with a significant reduction in muscle protein loss due to infection. The increased net protein degradation observed in incubated muscles was also abolished by naproxen pretreatment. This agrees with data of Fagan and Goldberg (1985) who treated rats with indomethacin prior to injection of *E. coli* or *E. coli* endotoxin, and observed decreased PGE_2 production and net protein degradation in rat muscle *in vitro*. Ruff and Secrist (1984) similarly treated rats with indomethacin prior to injection of *S. pneumoniae*, and prevented the loss of muscle protein and contractile force following infection. At concentration used in these experiments, naproxen along, up to 3 day administration, had no effect on body weight, muscle weight, muscle protein content, or net muscle protein degradation.

The results of this study and those cited above do not agree with those of Hasselgren and coworkers (Hasselgren et al, 1985; 1988). In their experiments, indomethacin, administered *in vitro* (Hasselgren et al, 1985; 1988), partially inhibited PGE_2 production but did not significantly reduce accelerated protein degradation in muscles of septic rats. Several possible explanations arise for this discrepancy with the present results. The addition of indomethacin to incubation

medium (3×10^{-6} M) decreased PGE_2 production weakly, by only 20% in controls and by 50% in muscles of septic rats (Hasselgren *et al.*, 1985). This is in contrast to our observations (Baracos et al., 1983) and those of Rodemann and Goldberg(1982) where indomethacin abolished PGE_2 production *in vitro* at low concentrations (2.8×10^{-6} M). Since indomethacin is extremely insoluble, one possibility is that in the studies of Hasselgren et al. (1985; 1988) this agent was not solubilized at an effective concentration. Secondly, if acutely increased PGE_2 levels signal the activation of protein degradation, such that, PGE_2 production is important in the initiation of accelerated protein degradation but not in its maintenance, then indomethacin treatment *in vitro* would not necessarily change the rate of protein degradation even if it inhibits PGE_2 production. Futhermore, if inhibition of PGE_2 synthesis may influence protein degradation after a lag time of several hours, then the changes of the rate of protein degradation due to indomethacin treatment would not be seen during 2 hours of incubation period. A further complicating factor is that cycloheximide (0.5×10^{-3} M) was used in the *in vitro* muscle incubations by Hasselgren et al (1985; 1988) to inhibit protein synthesis; this allows determination of protein degradation. This agent has been found to inhibit muscle PGE_2 production (McKinley and Turinsky, 1986). Since 70% inhibition of PGE_2 production can be demonstrated with 1×10^{-6} M cycloheximide (McKinley and Turinsky, 1986), it seems unlikely that Hasselgren et al. would have been able to observe PG-dependent muscle protein catabolism in their system. The same authors also treated rats with indomethacin (3 mg / kg) *in vivo*, 45 min before induction of

sepsis and again after 3 hours (Hasselgren et al., 1985). Since they did not report whether indomethacin had achieved an effective concentration *in vivo*, these results are difficult to interpret.

The involvement of prostaglandins in regulation of muscle protein turnover requires further study. In related work (Strelkov *et al.* submited for publication), we have been able to observe a PG-dependent component of cancer-induced muscle wasting, by pretreatment with naproxen. However, it is impossible to give the medicine to human and animals before they are infected because it is very difficult to know when they will be infected. Thus, a question is raised if the protein degradation would be stopped after it had already started. If so, it is useful to use medicine to control the muscle weight and protein loss during infection. Controlled studies compairing the administration of naproxen at different times relative to infection, will allow clarification of PG role in maintenance of elevated rates of protein breakdown.

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Table 3.1

EFFECT OF NAPROXEN TREATMENT ON MUSCLE WEIGHT AFTER *E. coli* INFECTION IN CHICKS AND RATS

	MUSCLE WEIGHT (mg)	
TREATMENT	CHICK EDC	CHICK SARTORIUS
CONTROL	23.0 ± 0.6^{a}	359.1 ± 16.0^{a}
INFECTED	17.8 ± 0.8^{b}	253.9 ± 7.1^{b}
NAPROXEN CONTROL	24.6 ± 1.0^{a}	356.8 ± 16.9^{a}
INFECTED +NAPROXEN	20.3 ± 1.3^{ab}	313.4 ± 14.9^{ab}
	RAT SOLEUS	RAT EDL
CONTROL	75.6 ± 2.67^{ad}	100.9 ± 2.83^{a}
INFECTED	67.1 ± 2.93^{ab}	90.8 ± 2.74^{b}
NAPROXEN CONTROL	77.5 ± 2.26^{cd}	106.4 ± 1.28^{a}
INFECTED +NAPROXEN	69.0 ± 1.88^{ad}	96.7 ± 2.50^{ab}

Values are means ± SEM of 8 birds or 7 rats. Means in a column not sharing a common supercript are significantly different, $P < 0.05$.

Subjects were injected with *E. coli* (3×10^{9} colony forming units / kg body weight) or saline. Naproxen was given continuously in the drinking water on the day prior to injection. Extensor digitorum communis (EDC), Sartorius, Soleus and extensor digitorum longus (EDL) were dissected 2 days after infection.

Table 3.2.

EFFECT OF NAPROXEN TREATMENT ON MUSCLE PROTEIN CONTENT IN E. coli INFECTED CHICKS AND RATS

	CHICK MUSCLE TOTAL PROTEIN (mg)	
TREATMENT	EDC MUSCLE	SARTORIUS MUSCLE
CONTROL	2.58±0.09[a]	20.51±0.97[a]
INFECTED	1.62±0.07[b]	9.46±0.61[b]
NAPROXEN CONTROL	2.53±0.09[a]	21.13±2.11[a]
INFECTED +NAPROXEN	2.01±0.11[b]	19.72±1.59[a]

	RAT MUSCLE TOTAL PROTEIN (mg)	
	EDL MUSCLE	SOLEUS MUSCLE
CONTROL	6.74±0.57[a]	5.23±0.12[a]
INFECTED	3.61±0.11[b]	3.99±0.08[b]
NAPROXEN CONTROL	6.55±0.09[a]	5.22±0.15[a]
INFECTED +NAPROXEN	5.38±0.18[a]	4.87±0.22[a]

Values are means ± SEM of 7-8 subjects. Means in a row not sharing a commom supercript are significantly different at $P < 0.05$.

Subjects were injected with *E. coli* (3×10^9 colony forming units / kg body weight) or saline. Naproxen was given continuously in the drinking water on the day prior to injection. Dissection was carried out at day 2 after the infection. Muscles from subjects in Table 1 were analyzed for protein.

Table 3.3.

EEFECT OF NAPROXEN TREATMENT ON NET PROTEIN DEGRADATION AND PGE_2 PRODUCTION IN CHICK EDC MUSCLE DURING E.coli INFECTION

TREATMENT	PROSTAGLANDIN E_2 PRODUCTION (PGE_2 pg/mg tissue/3h)	NET PROTEIN BALANCE (Tyr nmol/mg tissue/3h)
CONTROL	55.62± 6.8[a]	0.29±0.02[a]
INFECTED	138.00±20.4[b]	0.42±0.04[b]
NAPROXEN CONTROL	71.61± 6.9[a]	0.28±0.03[a]
INFECTED +NAPROXEN	41.61± 5.9[a]	0.33±0.03[a]

Values are means ± SEM of 7-8 muscles. Means not sharing a common supercript letter are significantly different, $P < 0.05$.

Muscles were incubated in 3.0 ml Krebs-Ringer bicarbonate medium containing 15 mM glucose and 0.01 U/ ml insulin for 3 hours.

Figure 3.1.

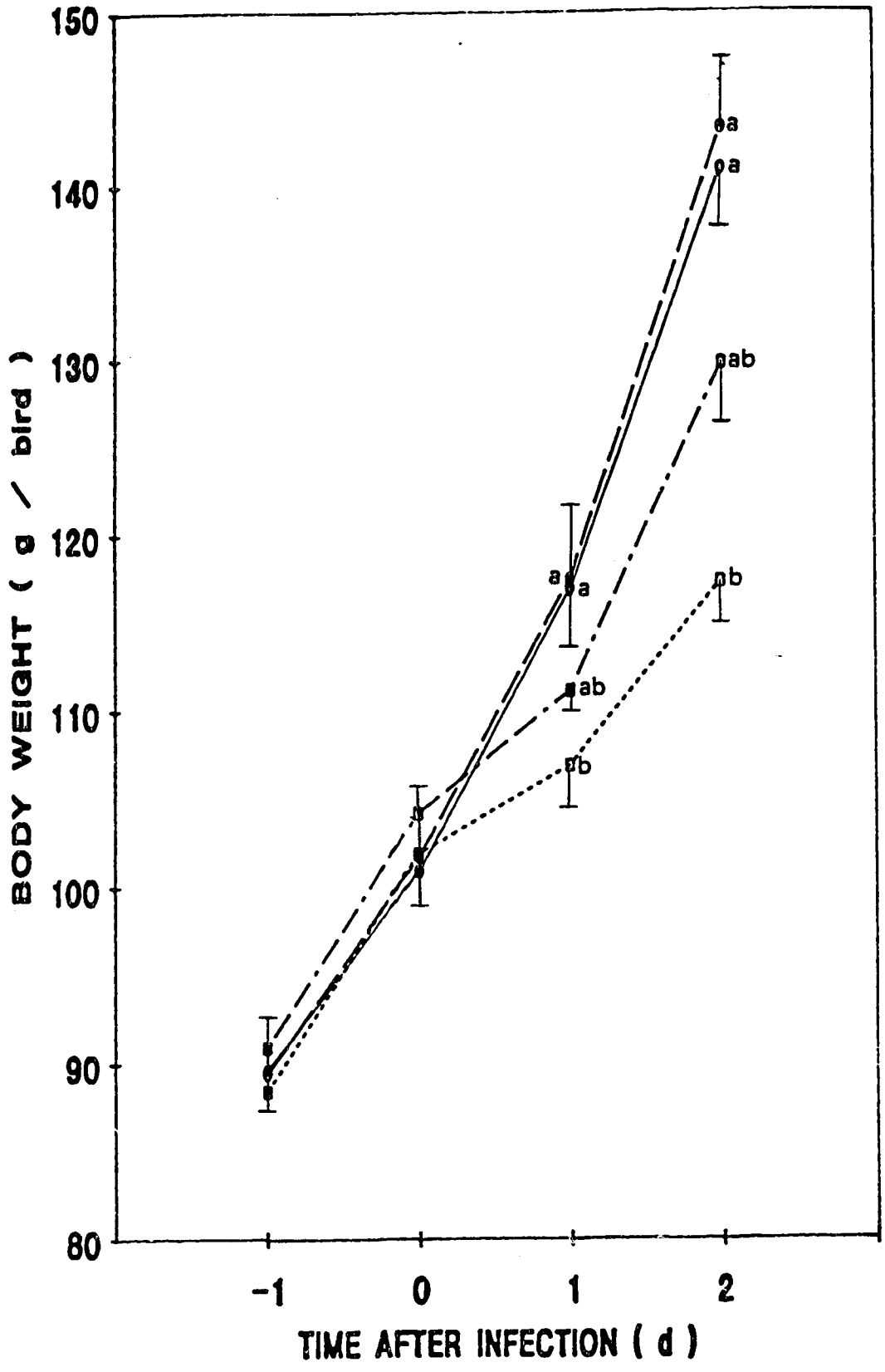

150
140
130
120
110
100
90
80
BODY WEIGHT (g / bird)
-1
0
1
2
TIME AFTER INFECTION (d)
a
a
ab
a
a
ab
b
b

Figure 3.2.

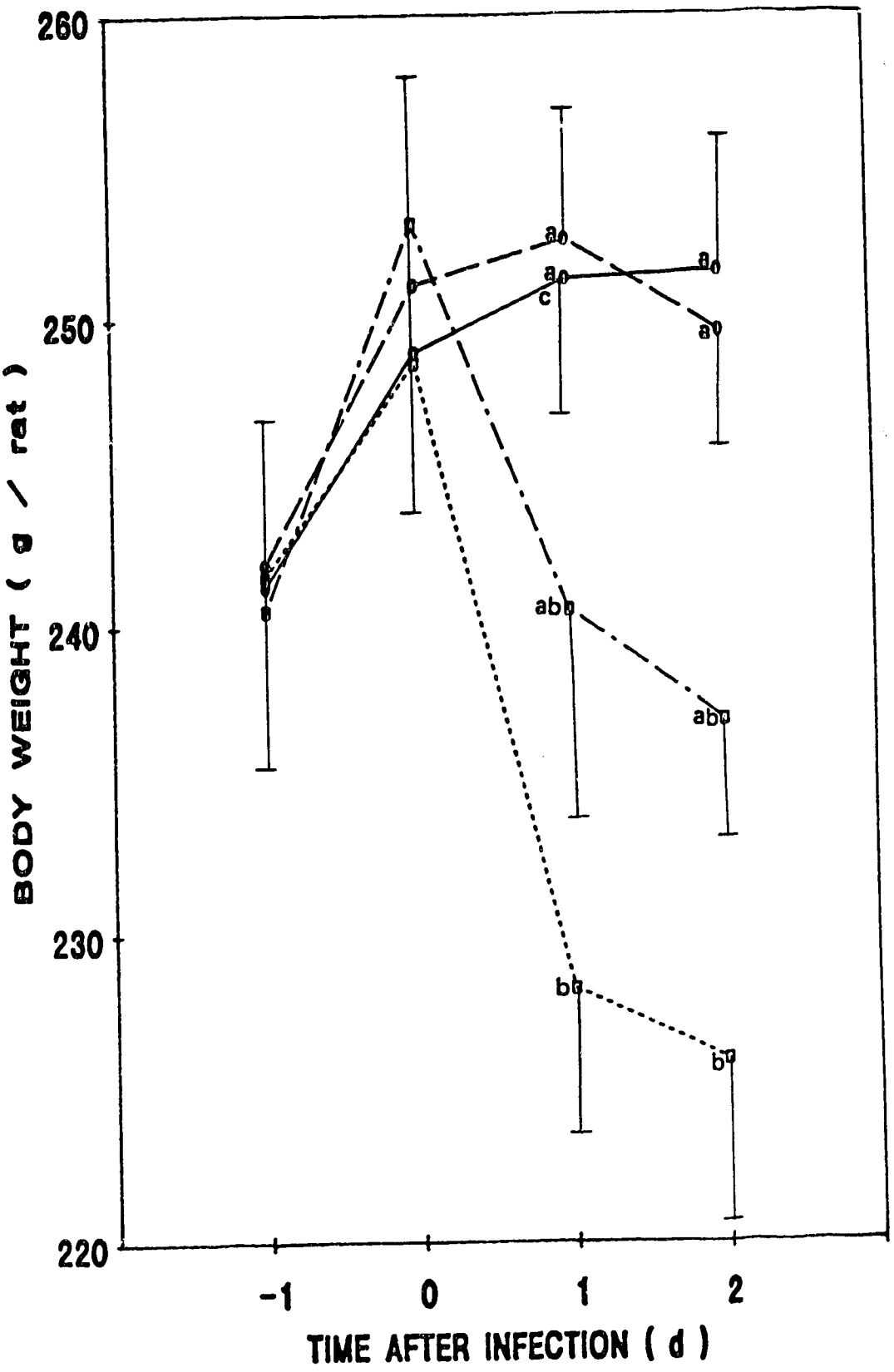

260
250
240
230
220
BODY WEIGHT (g / rat)
-1
0
1
2
TIME AFTER INFECTION (d)
a
a
a
c
a
a
ab
ab
b
b

Figure 3.3.

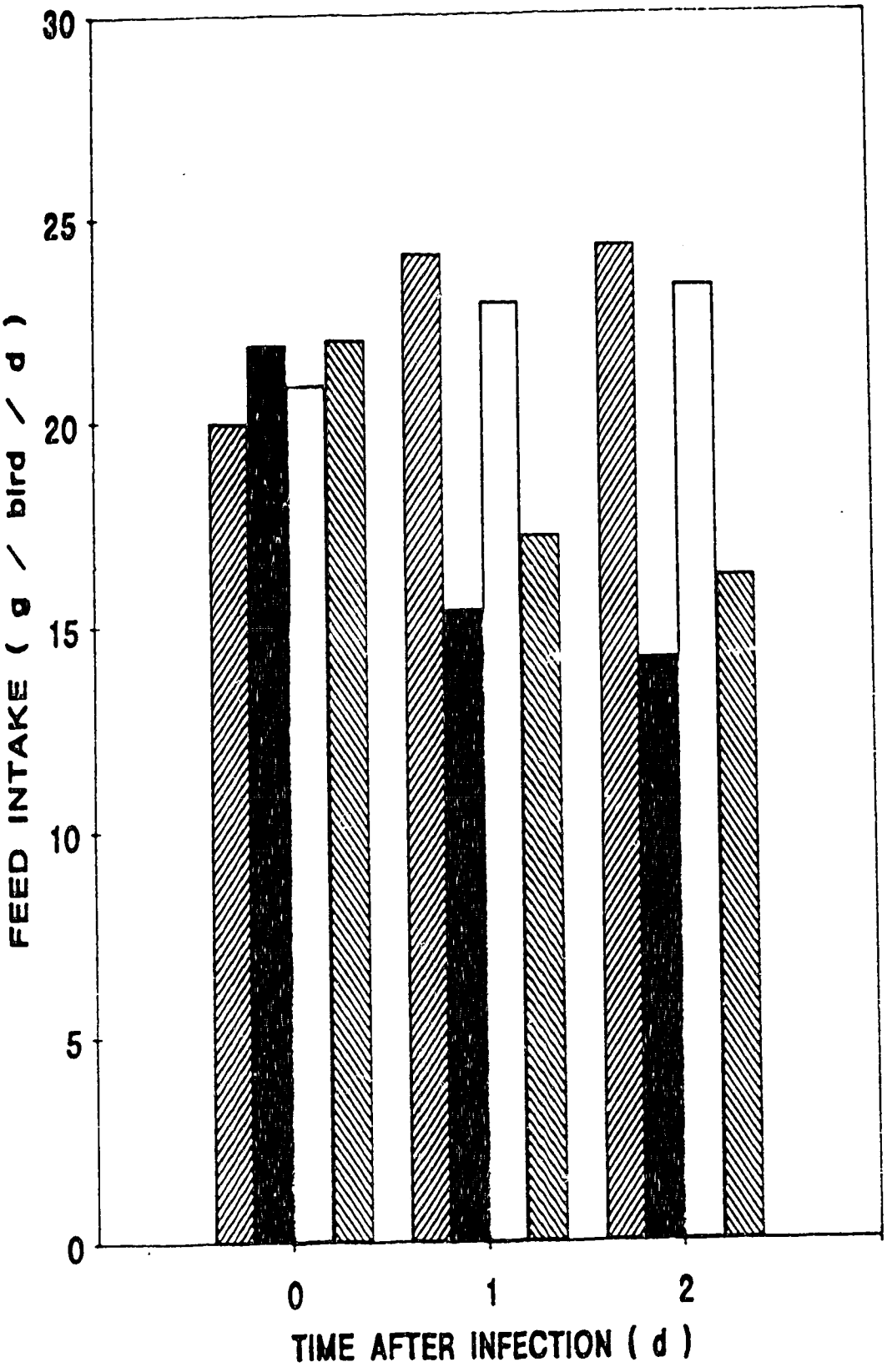

30
25
20
15
10
5
0
FEED INTAKE (g / bird / d)
0
1
2
TIME AFTER INFECTION (d)

Figure 3.4.

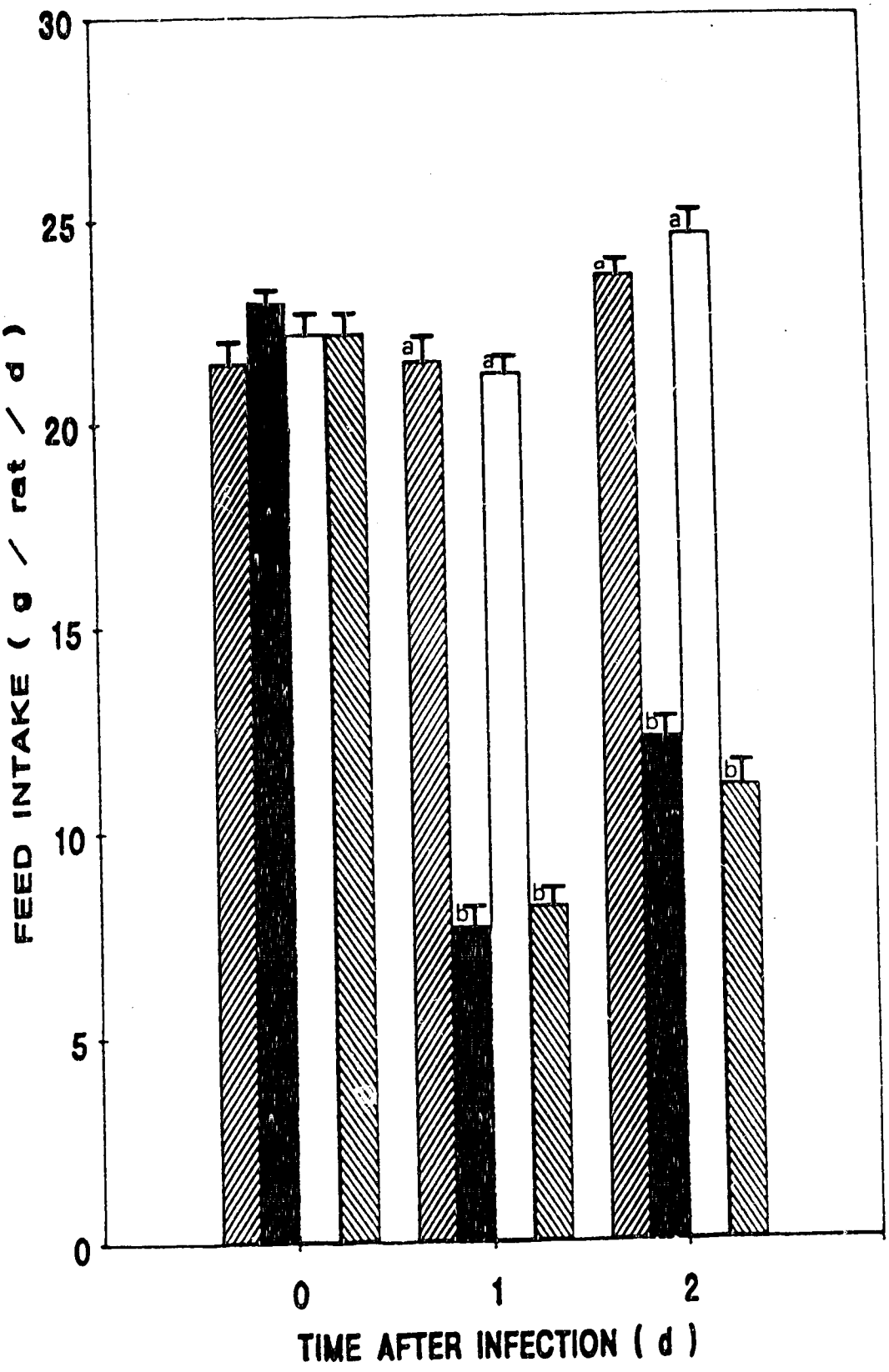

30
25
20
15
10
5
0
FEED INTAKE (g / rat / d)
a
a
a
a
b
b
b
b
0
1
2
TIME AFTER INFECTION (d)

Figure 3.1: Effect of naproxen treatment on body weight in chicks infected with *E. coli*.

Values are means ± SEM of 8 birds. Means in the same day not sharing a common letter are significantly different, $P < 0.05$. Control birds were injected with 1.0 ml sterile phosphate buffered saline and infected birds were injected 1.0 ml containing 3×10^8 . coli (i.p.) at day 8 *ex ovo*. Naproxen was given in the drinking water at the day before the injection.

o——o Control; o– – –o Naproxen control;

□—·—□ Infected; □·····□ Naproxen Infected.

Figure 3.2: Effect of naproxen treatment on body weight in rats infected with *E. coli*.

Values are means ± SEM of 7 rats. Means in the same day not sharing a common letter are significantly different, $P < 0.05$. Control rats were injected with 0.3 ml sterile phosphate buffered saline and infected rats were injected with 0.3 ml *E. coli* containing 8.1×10^8 cells (i.p.). Naproxen was given in the drinking water at the day before the injection.

o——o Control; o– – –o Naproxen control;

□—·—□ Infected; □··· ·□ Naproxen infected.

Figure 3.3: Effect of naproxen treatment on feed intake during *E. coli* infection in chicks.

Broiler chicks were housed in battery cages 10-22 birds/pen. *Ad Libitum* feed intake for each group was determined daily. Chicks were fed broiler starter ration which contains 23.3% CP and 2795 Kcal

ME/kg. The number given represent average value of a group (pen) birds. Naproxen was given in drink water at the day before the injection.

Figure 3.4: Effect of naproxen treatment on feed intake during *E.coli* infection in rats.

Rats were housed in individual cages. *Ad libitum* feed intake for each rat was determined daily. Values are means ± SEM of 7-11 rats. Means in a day not sharing a common letter are significantly different, $P < 0.05$.

IV. GENERAL DISCUSSION

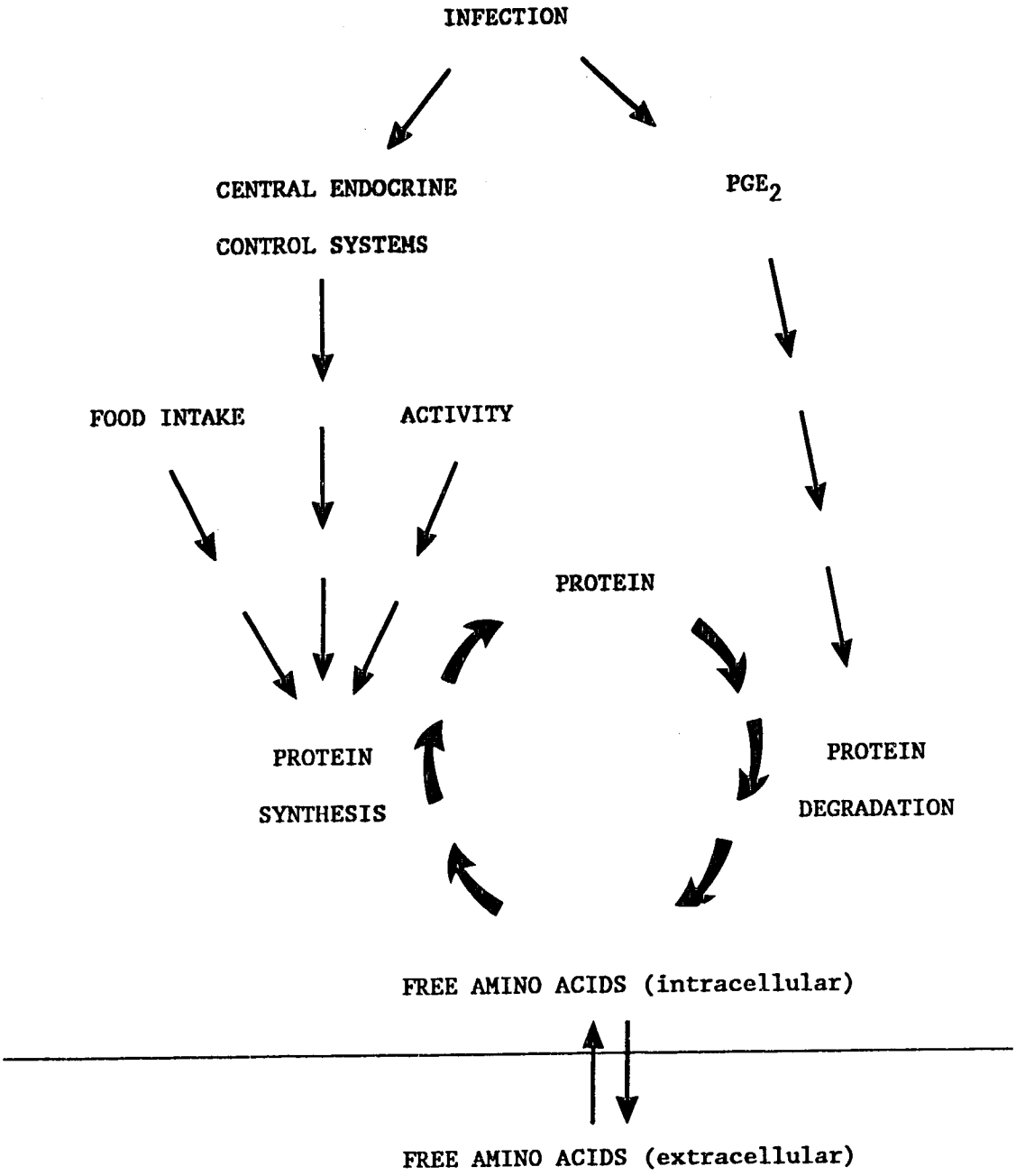
A. Prostaglandin-dependant muscle wasting in infection

Infectious diseases, which are frequently accompanied by a marked loss of body weight and body protein, and generalized wasting of skeletal mascle, are an important source of loss of production in animal agriculture. Infection has long been known as major stimulus to the organism. Infection calls forth defensive responses which sustain the chemical reactions of life. Many metabolic alterations which occur during infectious illness or inflammation are similar and regardless of the stimuli that provoke the response. The present study found significant body weight, especially muscle weight and protein loss due to infection. The protein loss was due in large part, to decreased protein synthesis. The increased muscle PGE_2 production during infection was related to the increased net protein loss. Naproxen pretreatment significantly inhibited protein loss, PGE_2 production, as well as increased net protein degradation. These results agree with the suggestions that PGE_2 may play an important role in regulation of protein turnover.

B. Role of PGE_2 in increased net protein degradation

Many studies have found that, during infection, muscle protein degradation increased significantly, (Klasing and Austic, 1984a; Fagan and Goldberg, 1985), which was related with increased PGE_2 production (Fagan and Goldberg, 1985). PGE_2 have been suggested to signal protein degradation and it is related to accelerated protein

degradation during infection. However, the present study did not find increased muscle protein degradation although PGE_2 production and net protein degradation were significantly increased during infection. This might possibly be due to: 1). different stage of infection has different regulation of protein turnover although the overall effect of negative nitrogen balance stays the same; 2). the proteolysis may have increased dramatically right after infection, thereafter protein synthesis decreased instead of accelerated proteolysis to maintain the calabolic state. In the stage of infection of the present study, 2 days after infection, protein degradation decreased while protein synthesis also decreased significantly. The increased PGE_2 production may have caused higher rate of protein degradation than that would otherwise appear without PGE_2 stimulation. Naproxen inhibited PGE_2 production, and consequently may have lowed rate of protein degradation. The situation may be illustrated as follows:



Infection results in a variety of mediators which affect protein turnover in muscles, such that the rate of protein synthesis is less than the rate of protein degradation and net catabolism occurs. PGE_2 may influence protein degradation, resulting in a higher rate, than would otherwise appear. This would explain the inhibition of free amino acid release by naproxen.

C. Should PG-dependent muscle wasting be inhibited therapeutically ?

This question naturally arises from this work and it maybe considered in the context of the role of prostaglandins in infection in general, and the possible adaptive aspects of muscle wasting during infection.

i. Role of PG in infection

Prostaglandins (PG) are local hormones with an important role in the regulations of diverse cellular functions and interactions. They are derivatives of the polyunsaturated fatty acids. PGE_2 is produced in many tissues including skeletal muscle. The short half-life of PGE_2 limits its activity to the site of production. PGE synthesis in a given tissue is initiated by a stimulus in that tissue, and the PGE_2 then modifies further response to that stimulus. Body infection is recognized by mononuclear phagocytes (blood monocytes and fixed tissue macrophages). Activated mononuclear phagocytes produce factors which may modulate the metabolism of protein, coordinately with primary host defense activities. PGE_2 is one of the products of activated mononuclear phagocytes.

The different prostaglandins have different, sometimes opposite efffects. Of all the arachidonic acid metabolites, only PGE has shown to have a clear role in the regulations of cellular and humoral immune responses (Goodwin and Ceuppens, 1983). Several *in vivo* and *in vitro* studies have confirmed that PGE_2 acts as a feedback inhibitor of celluar immune responses and these response can be enhanced in vivo as well as in vitro by the administration of cyclooxyglnase inhibitors (Goodwin and Ceuppens, 1983). PGE_2

inhibits T cell, but not B-cell mitogenesis. However, the role of PGE_2 in immune regulations is It is clear that under certain experimental conditions PGE_2 can deliver activating signals. For example, PGE_2 induces immature thymocytes to differentiate into mature T cells (Bach et al., 1975). In multiple sclerosis patients, an increased production of PGE_2 by the monocytes causes increased adherence of lymphocytes to measles-infected cells (Dore-Duffy and Zurier, 1979). Therefore, PGE_2 should be considered a regulator rather than a universal suppresor for T cell reactivity. PGE_2 has also been found to have dual effects (inhibition or activation) of B-cells, natural killer cells (NK), and macrophages. PGE_2 can both enhance and block NK activation by interferon. PGE_2 may play a role in regulating macrophage function based on the level of activation of the macrophage (McCarthy and Zwilling, 1981). Thus, PGE_2 concentration, activation and differentiation state of the target cell, and duration of PGE-target-cell interaction all seem to be important factors whose complete roles have not been fully delineated.

ii. Role of muscle wasting in infection

One purpose of muscle protein degradation during infection may be to provide a source of metabolizable energy and free amino acids for synthesis of proteins involved in host defence. Metabolic rate may rise 10 to 20% in mild infection, and up to 50% in severe infection (Baracos, 1985; Long, 1977; Wilmore and Kinney, 1981). In extreme cases where infection is complicated by multiple trauma or burns, metabolic rates may be up to double the predicted normal value (Baracos, 1985; Wilmore and Kinney, 1981). Since the catabolism

resulting from infection is generally accompanied by anorexia, muscle serves as an important store of nutrients. In the infected host, muscles act as a source of energy and gluconeogenic precursors. Increased skeletal muscle catabolism is accompanied with increased amino acid release which was also demonstrated in the present study.

It has also been known that the catabolism of muscle protein would appear to be coupled to a number of anabolic processes related to host defense, including activation and proliferation of phagocytes and lymphocytes and antibody synthesis. Host defense systems are dependent on the synthesis of new proteins, such as the hepatic acute phase proteins (Beisel, 1984). The creation of new, or additional varieties of highly specialized proteins would appear to be an important element of host metabolic response to infection. It has been sugested that infection-related alteration in plasma amino acids are the result of an elevated rate of flux of amino acid from muscle to liver and a subsequent increase in a utilization for synthesis of serum protein (Wannemacher, et al., 1974). Evidence of increased protein synthesis in liver tissue was found following malaria infection in rats (Fern et al, 1985), and following *E. coli* infection in chicks (Klasing and Austic, 1984b). In addition to a general increase in hepatic protein synthesis, a variety of proteins which are not ordinarily produced in large quantities are synthesized within the liver. These include the acute phase reactants (fibrinogen, haptolobin, serum amyloid A, C-reactive protein, a_2-macrofetoprotein), and proteins involved in the function of the kinin, complement and coagulation system. The synthesis of these proteins, whose functions are related to immunocompetance, maintenance of organ function and healing, could

utilize amino acids derived from muscle protein.

On the other hand, severe muscle wasting during infection could result in death of infected subject which causes loss of production of animal agriculture. Therefore, it is considered important to reduce the loss of agricultural production due to infection.

iii. Inhibition of PG production

If it could be established that muscle wasting could be inhibited by inhibition of PG synthesis, without danger to the host, then 2 ways could be used to achieve this.

a) PG synthesis inhibitor

The present study showed that naproxen treatment significantly inhibited weight and protein loss, PGE_2 production and net protein degradation in muscle during infection. The results suggest that PGE_2 have 2 possible roles in regulation of protein turnover, that is: 1) initiate the catabolism, 2) maintain the catabolism state, or both.

In addition to signalling protein degradation, PGE_2 has an effect on active T-cell and B-cell immune function although it suppreses immunological cells as mentioned above. Thus, it turns clear that the level of PGE_2 would be important during infection. The ideal way is to control PGE_2 production at a certain level during infection so that it plays a positive role in immune function, and results in a certain degree of protein degradation to satisfy the requirement of amino acids by liver and immune functions for defense but do not cause severe proteolysis which could result in death of infected subjects. Further studies would be required since little work

has been done in this area. To control the level of protein degradation during infection, prostaglandin synthesis inhibitors such as naproxen, aspirin, indomethacin could be used to control the level of PGE_2 production
and different time point of infection should be selected for the administration of the medicine.

b). Dietary modification

Recent studies have suggested that dietary modification of fatty acid would change prostaglandin production. For example, high dietary polyunsaturated fatty acids of the omega-6 fatty acid results in high PGE_2 production which supresses immunological and may facilitate tumor cell metastasis and proliferation. The injection of omega-3 fatty acids significantly inhibited the synthesis of PGE_2 (Karmali et al., 1984). In animal studies, dietary fish oil, which contains high levels of omega-3 fatty acids, decreased the formation of PGE_2 and retarded the growth of tumor cells (Karmali et al., 1983). Therefore, dietary modification could also be employed to control the level of PGE_2 production during infection.

Overall, to determine whether PG are responsible for signalling or maintenance of increased muscle protein degradation, the effects of different time points of naproxen treatment during infection would be of interest. Since dietary fatty acids are known to affect PG synthesis, the effects of different dietary fatty acid on PGE_2 level and protein turnover during infection would also be of some interest.

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APPENDIX I.

Farm Broiler Starter with Canola Meal (0-4 weeks)

INGREDIENTS	PERCENTAGE
Ground wheat (13% protein)	54.9
Stabilized fat	3
Soybean meal (46% protein)	30.5
Canola meal (36% protein)	5
Ground limestone	2
Biofos	1.8
Iodized salt	0.35
Farm broiler micromix-87	2
DL Methionine	0.15
Selenium premix	0.05
Biotin premix	0.2
Coccideostat (Amprol)	0.05
Calculated Analyses	
Metabolized Energy (kcal / kg)	2795
Protein (%)	23.3

APPENDIX II.

Farm Broiler Finisher with Canola Meal (4-6 weeks)

INGREDIENTS	PERCENTAGE
Ground wheat (13% protein)	64.95
Stabilized fat	3
Soybean meal (46% protein)	21
Canola meal (36% protein)	5
Ground limestone	1.8
Biofos (15% Ca-21% P)	1.6
Iodized salt	0.35
Farm broiler micromix-87	2
DL Methionine	0.1
Biotin premix	0.2
Calculated Analyses	
Metabolized Energy (kcal / kg)	2863
Protein (%)	20.2

APPENDIX III.

SPECIFIC RADIOACTIVITY OF FREE PHE

TISSUE	TIME AFTER INJECTION (min)	SPECIFIC RADIOACTIVITY (DPM/nMOL) CONTROL	INFECTED
SARTORIUS MUSCLE	10	1560±195	1726±235
	25	2118± 56	2158±156
	40	1739± 53	1821± 71
HEART	10	1589±101	1328±209
	25	1848± 63	1895±127
	40	1596± 70	1584± 54
LIVER	10	2301±235	2154±117
	25	2081±117	1953±111
	40	1568± 96	1688± 98

Valeus are means ± SEM of 7 to 8 birds.

In preliminary experiment, chicks were given a sigle i. p. injection of sterile solution containing phe (150 mM) and [U-^{3}H]-phe (200 uCi/ml; 1.0 ml per 100 body weight). The specific radioactivity of phe in intracellular pool were determined on day 2 after infection.

APPENDIX IV.

SPECIFIC RADIOACTIVITY OF FREE PHE

TISSUE	TIME AFTER INJECTION (min)	SPECIFIC RADIOACTIVITY (DPM/nMOL) CONTROL	INFECTED
SARTORIUS MUSCLE	10	1400±85	1339±96
	30	1247±73	1457±98
HEART	10	1291±44	1316±121
	30	1104±38	1247±78
LIVER	10	1199±49	1157±26
	30	939 ±45	1015±72

Valeus are means ± SEM of 7 to 8 birds.

In the experiment, chicks were given a sigle i. p. injection of sterile solution containing phe (150 mM) and [U-^{3}H]-phe (150 uCi/ml; 1.0 ml per 100 body weight). The specific radioactivity of phe in intracellular pool were determined on day 2 after infection.

APPENDIX V.

EFFECT OF *E. coli* INFECTION ON BODY WEIGHT GAIN

TIME AFTER INFECTION	BODY WEIGHT GAIN (g / d)		RALATIVE BODY WEIGHT GAIN (%)	
	CONTROL	INFECTED	CONTROL	INFECTED
0	14.8±1.5	15.7±1.3	17.6±1.3	18.8±1.36
0-2	19.3±0.8	9.7±1.8*	17.2±0.6	8.5±1.5*
2-4	21.6±1.1	15.8±1.8*	14.2±0.3	11.6±1.0*
4-6	24.8±1.8	19.9±1.6	12.7±0.8	12.0±0.8
6-10	31.7±2.0	29.7±2.0	13.0±0.7	14.5±0.6
10-15	45.0±1.7	37.7±2.6*	12.2±0.5	11.6±0.6
15-20	50.5±2.1	44.9±3.0	8.6±0.5	9.0±0.7
20-25	69.2±7.6	55.2±4.1	8.3±1.2	8.0±0.5
25-30	57.6±6.3	58.1±2.5	4.9±0.5	5.8±0.2

Values are means ± SEM of 8-10 birds. *P < 0.03 significantly different from control.

Control birds were injected with 1.0 ml sterile phosphate buffered saline and infected birds were injected with 1.0 ml 1.0×10^9 *E. coli* (i.p), at day 8 *ex ovo*.