ABSTRACT

PHYSICAL AND CHEMICAL RESPONSES OF

MAMMARY GLAND TO ENDOTOXIN

By

Bhola Nath Gupta

A total of 70 primiparous guinea pigs (40 for histopathologic examination and determination of nucleic acid content and 30 for selected enzyme studies) were used to study the physical and chemical effects of an endotoxin on the mammary gland. The guinea pigs were allowed to nurse 2 pups and were inoculated 6 days after whelping.

Endotoxin (0.5 mg.) obtained from *Escherichia coli* (026:B6) was injected into each mammary gland via the teat canal. Guinea pigs were euthanatized at 3, 6, 12 or 24 hours after inoculation. Histopathologic and biochemic (nucleic acid) alterations in the mammary glands due to endotoxin treatment were studied. Clinical and hematologic findings in endotoxin-induced mastitis in guinea pigs were correlated with coliform mastitis in cows.

Twenty-four hours after endotoxin inoculation, glutamic oxalacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase (ALP), acid phosphatase (ACP), lactic dehydrogenase (LDH), isocitric dehydrogenase (ICD), and creatine phosphokinase (CPK) levels in serum samples were determined. Mammary tissue enzyme levels for ALP, LDH, and succinic dehydrogenase (SDH) were also studied histochemically.

Inflammatory changes in the sinus papillaris and lactiferous sinuses were evident as early as 3 hours after inoculation of either endotoxin or physiologic saline solution (PSS). There was apparently little effect on mammary parenchymatous tissue until 6 hours. Massive infiltration of neutrophils into the alveoli was noticed 12 hours following endotoxin inoculation. However, slight inflammatory changes were also noted in PSS treated glands. Cloudy swelling, hyaline droplet degeneration of the alveolar epithelial cells and thick proteinaceous material enmeshed with neutrophils and cellular debris in the alveoli and lactiferous ducts were the most significant changes observed in the gland. There was moderate enlargement of the inguinal lymph nodes associated with hyperplasia and congestion. Marked leukopenia was observed in mastitic guinea pigs which was primarily due to neutropenia and lymphopenia. Signs of toxemia such as fever, increased heart rate, muscular tremors, depression, aphagia, and adipsia were observed. Grossly, there was (a) no evidence of galactopoiesis and (b) subcutaneous tissue around the mammary gland appeared hyperemic.

Intramammary inoculation of endotoxin or PSS had little effect on the mammary deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) concentration of lactating guinea pigs euthanatized 3 or 6 hours after inoculation. Mammary DNA increased 12 and 24 hours after endotoxin treatment coincident with migration of leukocytes in the glandular parenchyma. There was also a slight increase in mammary DNA following PSS treatment. There was indication that endotoxin, but not PSS, was responsible for a decrease in mammary cell RNA of about the same magnitude as the increase in whole-gland RNA resulting from leukocytic infiltration. This view was supported by a marked decrease in the RNA/DNA ratio after endotoxin treatment.

Degenerative changes in the mammary gland and probably in other body tissues accompanying endotoxin inoculation characteristically elevated the serum levels of GOT and GPT. The serum levels of ALP and ACP were found to be significantly decreased due to endotoxin treatment. The apparent increased activity of ALP in the mammary gland was thought to be due to massive infiltration of leukocytes.

It was concluded that endotoxin and PSS had initial irritating effects on the mammary gland. The inflammatory response was of much greater magnitude in the endotoxin-treated guinea pigs than in those receiving PSS. The effect of endotoxin was primarily on alveolar epithelial cytoplasm causing alteration and coagulation of native protein and a subsequent lowering of the milk-synthesizing capacity of the epithelial cells. Once all or part of the cytoplasm is coagulated, that part of the cell is no longer biologically active and becomes foreign material which is represented as hyaline granules in the epithelial cells or in the lumina of the alveoli.

Most of the clinical, hematologic, biochemic, gross, and microscopic changes in endotoxin-induced mastitis in guinea pigs appeared to be similar to those in coliform mastitis in the cow.

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INTRODUCTION AND OBJECTIVES

The word mastitis (inflammation of the udder) is derived from the Greek word *mastos*, which means mammary gland, and the suffix "itis", meaning inflammation. The corresponding Latin word for mammary gland is *mamma*, from which the word mammitis arises. Mastitis may occur in any mammalian species but is observed most frequently in dairy cows. Mastitis continues to be one of the most prevalent diseases of dairy cattle and is responsible for economic losses to the dairyman amounting to huge sums annually. The disease frequently occurs before maximal milk production is attained. Thus, it is one of the most frequent reasons for culling cows from the herd.

Such agents as bacteria, mycoplasma, viruses, fungi, algae (*Prototheca*), and trauma (mechanical, thermal and chemical) may contribute to the genesis of mastitis. Damage to the mammary epithelial cells by these causative agents and their metabolic products initiates the inflammatory response. It is currently believed that a combination of several factors rather than a single contributing factor may be required to induce clinical mastitis.

For many years, mastitis research has centered around control of the disease and the role of management practices. While these aspects are important, many questions have gone unanswered and insufficient knowledge has been gained on some of the more basic factors. Consequently, little is known about the influence of adverse physiologic and environmental changes on resistance to mastitis.

The research described herein was undertaken in order to study such basic areas as the pathologic and biochemic alterations of mammary gland due to endotoxin. Primiparous lactating guinea pigs were used in this experiment. Clinical, hematologic, macroscopic and microscopic findings, and the quantitation of nucleic acid and selected enzymes are reported for guinea pigs in which mastitis was induced by injecting endotoxin into the gland.

REVIEW OF THE LITERATURE

History

The disease, mastitis in cattle, has been known for more than one and one-half centuries. Because of its reputed contagiousness, public health significance, definite widespread character, and responsibility for economic losses, a wealth of literature has been written on the subject. For the sake of historical importance of this disease, the following paragraphs have been taken from *Bovine Mastitis*, written by Munch-Peterson (1938).

> "According to Leblanc (1901), Bardy de Brassac (1814) was the first to write on the subject. In 1816 Morier published a monograph on the engorgement of the gland; Vatel (1828) mentioned mastitis in his work on pathology, and Hutrel d'Arboval devoted a chapter to this subject. Then followed the observations of Duplenne in 1831, Dillon (1835), Lecoq and de Roche Lubin (1835) and Rainard (1845). Gelle, Lafosse, Roll, Cruzel and especially Saint Cyr, all devoted a considerable space in their works to this important subject.
>
> "A considerable amount of attention was also given to this disease in Switzerland e.g. Brennwald (1848), Gattiker (1848), Hürlimann (1848-9), Hess (1849), Gerlach (1854), Rast (1854), Zangger (1854), Eberhardt (1856), and Zurn (1877) dealt with the problem at great length. Franck (1876) was able to transmit the disease artificially by injection of a foul liquid mass or excretions from the diseased quarters into the teat canal of healthy quarters.
>
> "Nocard and Mollereau (1885), Bang (1889) and Kitt (1891) seem to have been the first to obtain definite evidence that mastitis of cattle was of microbial origin and it was their work which provided the starting point for modern scientific investigations of the disease."

It has been known for a number of years that *Escherichia coli* or some very closely related organism may cause mastitis. The first indication of this condition appears to be the work of Lucet (1889), who obtained nongelatin-liquefying, gram-negative bacilli from 12 cases of mastitis. One of the earliest recorded determinations of the bacterial content of milk was made by Schulz in 1892. He reported that the foremilk of cows contained large numbers of bacteria and the numbers decreased as the milking progressed. Ward (1898) reported persistence of bacteria in the milk ducts of the cow's udder. Since then, several researchers have investigated the bacterial content of cows' milk.

Coliform Group of Organisms

The coliform group of organisms includes a number of species of *Escherichia, Aerobacter,* and *Klebsiella* and the paracolon bacilli (Smith *et al.*, 1960; Schalm *et al.*, 1971). Certain members of this group, e.g., *Escherichia coli, Aerobacter aerogenes, Klebsiella pneumoniae, Escherichia freundii, Aerobacter cloacae*, and paracolon bacteria, have been found to cause mastitis in cows. Coliform bacteria inhabit the normal intestinal tract soon after birth. *Escherichia coli* occurs in the intestinal tract in much larger numbers than *A. aerogenes*.

Natural infection occurs commonly via the teat canal and rarely if ever is blood borne (Smith and Jones, 1966). A hematogenous route of infection has frequently been postulated in coliform mastitis because of its coexistence with puerperal uterine infection (Jubb and Kennedy, 1963). The infection is usually confined to 1 or 2 quarters, and the hindquarters are most frequently affected.

Infection appears in cows of all ages, and in all stages of lactation, although clinical manifestations are more frequent during the early

stages of lactation. The secretion in a majority of cases becomes a watery, perhaps yellow, fluid containing flakes and milk clots. In some cases, the milk may remain apparently normal during the 1st few days following appearance of clinical signs (Rowlands, 1939).

The presence of *E. coli* in the bovine udder has been reported on numerous occasions. In many instances the organism was associated with fulminating mastitis. Coliform organisms responsible for acute mastitis differ from ordinary strains by possessing a distinct capsule (Plastridge, 1958). Systemic signs of the disease are due to absorption of endotoxin rather than bacteremia (Schalm *et al.*, 1971).

During acute infection, the cow may appear normal at one milking and be very sick by the following milking. Acute inflammation is characterized by heat, pain, and edematous swelling of the udder together with discolored fibrinous clots in the milk. Signs of toxemia such as fever, increased heart rate, muscular tremors, depression, incoordination, aphagia, and adipsia are also observed (Plastridge, 1958; Schalm *et al.*, 1971). Some of the acutely affected cows may show complications of paraplegia.

Infection of the udder with coliform bacteria capable of causing mastitis not only results in poorer quality of milk but also leads to injury of the secretory tissue and hence to appreciable damage to the udder (Brown *et al.*, 1965). The extent of the lesions produced in the udder is dependent upon the virulence of the organism, and the severity and duration of the attack.

Escherichia coli

Escherichia coli was first isolated by Escherich in 1885 from the feces of infants but was more completely described in 1886 (Merchant

and Packer, 1967). Since then, the organism has been found in the intestinal tract of practically all vertebrates. This organism has occasionally been described as *Bacillus coli* or *Bacterium coli*.

Organisms which are placed in the genus *Escherichia* are widely distributed in nature and are commonly found in the intestinal tract of healthy people and animals. The bacilli are aerobic and facultatively anaerobic. They grow readily in 24 hours on all the usual laboratory media at temperatures ranging from 20 to 40 C. Their synthesizing powers are so well developed that the bacilli will grow in a medium consisting of inorganic salts, an ammonium salt, and glucose (Smith *et al.*, 1960). It is a lactose fermenting bacillus.

The organism is a short rod, 0.5 μ by 1.0 to 3.0 μ, varying from coccoid bipolar shapes to long filamentous forms. The organism usually occurs singly but short chains are not uncommon, does not produce spores, is usually motile with peritrichous flagella, but some strains do not possess flagella. The organism is readily stained and is gram negative (Breed *et al.*, 1967).

Endotoxin

Many gram-negative bacteria have been found to contain in their cell walls complex antigens composed of carbohydrate, lipid and protein or polypeptide-like material. Bacterial endotoxins are a protein-lipopolysaccharide complex (Braude, 1964). Synonyms for this complex are boivin or O antigens because these substances were first isolated by Boivin (Kabat and Mayer, 1964). The term, endotoxin, originated when it was traditionally believed that this material was an internal constituent of gram-negative bacteria. Autolysis or disruption of the cell wall is necessary for the release of the endotoxin complex. It has also been

demonstrated, however, that they can be released from the cell wall of intact bacteria. Endotoxins are not actively secreted, as are exotoxins, but are present in amounts dependent on the number of gram-negative bacteria.

Endotoxins are extractable from numerous unrelated species of bacteria by similar methods. They appear to be similar in chemical structure and properties when obtained in isolated form (Thomas, 1954). All endotoxins produce a syndrome of stereotyped physiologic and pathologic reactions in experimental animals. The effects of different endotoxins are qualitatively indistinguishable although different preparations may differ in potency.

Coliform Mastitis in Cows

Spontaneous Cases

There are numerous reports of spontaneous cases of coliform mastitis in the veterinary literature. The first indication of this condition appears to be the report of Lucet in 1889. Mattick and Williams (1925) and Malcolm (1933) have shown, however, that milk produced and handled under clean conditions contains a few coliform organisms. Chalmers (1934) studied the source of coliform organisms in the milk. He stated that the organisms might gain entrance to the milk either from the outside of the udder or from an intermittent infection through the teat canal.

Smith and Henderson (1934) described a case of acute mastitis caused by *E. coli* in a Jersey cow showing severe signs of systemic disturbances. There was a loss of appetite, cessation of rumination, constipation and elevation of body temperature (107 F.). The left rear quarter was swollen, hard, hot and painful. The infection remained localized in 1 quarter, but milk from all quarters showed increased numbers of

leukocytes. The milk secretion almost ceased within a few days. *Escherichia coli*, isolated from the milk, was pathogenic to guinea pigs. One milliliter of 18-hour broth culture was inoculated subcutaneously into 1 guinea pig and the other received 1 ml. intraperitoneally. Both guinea pigs died after 16 hours. Inoculation of infected milk samples into 2 more guinea pigs in a similar manner resulted in sickness, but both guinea pigs recovered by the 3rd day.

Gwatkin *et al.* (1938) has given a detailed description of 10 cases of coliform mastitis identified among 286 mastitic cows. Six animals died following infection and 4 recovered. Six of the 10 cases occurred several months after calving. They suggested that signs arising from this type of infection in recently freshened cows might be mistaken for milk fever and that the udder should be examined for the presence of acute mastitis in such conditions. Santagostino (1938) reported that acute bovine mastitis caused by coliform organisms always commenced with high fever, accompanied by an almost entire cessation of milk secretion. The secretion from affected glands was yellow in color, rich in leukocytes, and sometimes contained red clumps. He considered that although the disease was infectious, being frequently transmitted by milkers, it might be initiated by digestive disturbances or other predisposing causes.

Describing the relationship between coliform organisms and the bovine udder, Rowlands (1939) divided the subject into 3 separate headings: (a) coliform organisms associated with mastitis; (b) contamination of milk with coliform organisms following injury to the udder; and (c) coliform organisms in milk from apparently normal udders. He mentioned that certain cows might be capable of harboring bacteria in the mammary gland and that the milk could contain these bacteria for a considerable period of time. In such instances, there was a complete absence of

clinical signs without any noticeable alteration in the character of the milk. He further stated that clinical examination failed to reveal any difference between infected and noninfected quarters. He suggested bacteriologic examination of milk from individual cows to trace the source of coliform organisms in the bulk milk.

Kriisa (1939) gave an account of the clinical signs of mastitis in several herds during a 10-year period. Out of 723 cases, 217 (30%) were associated with coliform organisms. These were characterized by sudden onset, serum-like secretion from the mammary glands and frequent occurrence of paresis. An outbreak of acute mastitis with toxemia in 11 of a herd of 33 dairy cows during a period of 10 days has been reported by Burkhardt *et al.* (1943). The isolation of *Aerobacter aerogenes* from milk samples of 10 cows and no microorganisms from 1 cow established the etiologic agent.

Seventy-nine cases of coliform mastitis in a mixed herd of 120 Guernsey and Holstein cows were reported by Murphy and Hanson in 1943. A majority of manifestations of infection (48 cases) was observed during active lactation while 31 cases occurred during the dry period shortly before calving. Of those cases occurring during lactation, 11 were categorized as peracute, 17 as acute or subacute and 20 as chronic. None of them was fatal. Feces were considered to be the main source of infection. The authors were of the opinion that coliform mastitis was far more prevalent than would be expected from available recorded information.

Bratlie (1948) found coliform mastitis in 26 cows with a total of 31 affected quarters. Severe signs of disease in only 4 cows were manifested by swelling and tenderness of the affected quarters and abnormal secretion. In more acutely affected animals, the milk secretion

was serous, yellow in color, fibrinopurulent and became jelly-like after a few hours. There was a rise in body temperature and anorexia. Only 3 out of 31 quarters affected became nonfunctional.

Schalm and Woods (1952), while investigating coliform mastitis in a large dairy herd, classified the disease in 4 different forms: (a) latent infection, (b) chronic infection, (c) acute local infection and (d) acute systemic infection which was accompanied by a rise in body temperature to 103 to 108 F. Generally, slight to marked signs of toxemia, anorexia, dehydration, rapid loss of weight and complete cessation of secretory activity in all quarters of the udder were observed. Such cases, when left untreated, often terminated in rapid deterioration and death.

Baier *et al.* (1954) considered that coliform mastitis could not be regarded as a purely local disorder. They suggested that a disturbance in the microflora of the intestine could lead to septicemia and hence, under circumstances favoring the udder as a predilection site for infection, coliform mastitis may result. They further suggested that the probability and degree of inflammation depended upon the tissue resistance of the affected animal and the virulence of the bacteria or their ability to produce toxins.

Aehnelt and Krause (1955) reported that coliform mastitis was most common in postparturient cows during the early postpartum period, especially in winter. In a majority of cases, the inflammatory reaction was of an acute parenchymatous type. Twenty-five cases of *Escherichia coli* mastitis were reported by Bellani (1956). Most of the cows had severe clinical signs of mastitis and 1 was complicated by paraplegia. A similar account of an acute mastitis complicated by paraplegia was recorded by Borghi (1957).

Redaelli (1957) classified bovine coliform mastitis as *benign* or *malignant* based on his observations of 50 acute cases of mastitis. The *benign* type was characterized by changes which affected only the mammary gland and without any gross changes in the milk. Recovery followed in a few days. The *malignant* type was characterized by fever, diarrhea, paraplegia, and marked gross changes in the secretion. He stated that in such cases, the prognoses were always unfavorable because of tissue damage and loss of secreting capacity of the mammary gland. In the latter form, he observed that the causal organisms had different serologic and biologic characteristics, correlated with severity and extent of the lesions.

Circumstances favoring the appearance and spread of coliform mastitis were also investigated by Kudelka and Holec (1960). They discussed the influence of unsatisfactory sanitary conditions such as poor drainage, damp bedding, improperly ventilated barns, as well as effects of machine milking, puerperal infections and digestive disturbances.

The incidence of clinical mastitis due to the coliform group of bacteria has been described in detail by Radostitis (1961). Of 978 cases of mastitis treated during a 3-year period, 80 (8.2%) were due to coliform bacteria. Of these, 56 (70.0%) were of the peracute type and 11 cows died in spite of treatment. He stated that coliform mastitis was difficult to diagnose by clinical means and treatment of such cows was often unsuccessful even with exhaustive therapeutic measures. Sojka (1965) has presented an excellent review of coliform mastitis and its therapeutic measures.

In a survey of bovine udder infections, 32 isolates out of 70 cultures of aerobic gram-negative rods were found to be *E. coli* (McDonald *et al.*, 1970). Most of these isolates (84.3%) were recovered from rear

quarters and during lactation. These findings were in agreement with the report of Kalich (1958). In this investigation, 69% of the posterior quarters were affected whereas only 31% involved the anterior quarters. The higher incidence in posterior quarters was also reported by Gwatkin *et al.* (1938) and Kudelka and Holec (1960).

Other reports in which coliform bacteria were associated with mammary gland infections were published by Steiger (1904), Jones (1918), Carpenter (1925), Lamont (1925), Hardenbergh and Schlotthauer (1927), Minett *et al.* (1929), Lesbouyries *et al.* (1933), Crossley (1946), Pounden (1947), Laigret and Leblois (1950), Johnson *et al.* (1951), Abd-el-Malek and Gibson (1952), Barnes (1954), Thomas (1955), Easterbrooks and Plastridge (1956), Hinze (1956), White (1957), Johnson and Siddique (1965), McDonald and Packer (1968), Pospisil and Salajka (1968), and Salajka (1968).

Experimental Mastitis

McEwen and Samuel (1946), in a preliminary trial, studied the contamination of teat canals following dipping the teat in a bacterial culture medium. Teats were exposed to a 24-hour culture of *E. coli* and then the cows were milked either with machine or hands. They recovered *E. coli* from a high proportion of the inner orifice of the teat canal. It was concluded that a single exposure of the outside of the teats was sufficient to cause contamination (infection) of the teat canal. They also discussed the significance of their findings in relation to the experimental production of bacterial mastitis in the cow.

The concept that mastitis caused by coliform bacteria is an acute endotoxemia has been postulated for many years (Pounden, 1947). Coliform organisms can acidify milk to the point where it becomes irritating to udder tissues. They seldom produce necrosis but are notorious for

endotoxin production (Westphal *et al.*, 1964). To mimic spontaneous coliform mastitis in cows, several specific experiments have been performed using either viable organisms or endotoxin. The agents to induce acute mastitis with systemic disturbances were introduced into the mammary glands via the teat canal (Schalm *et al.*, 1971).

Bortree *et al.* (1962) and Carroll *et al.* (1963) investigated the distribution of whey proteins during the early acute phase of experimental coliform mastitis. There was evidence of increased permeability of the capillaries in the mammary gland as denoted by increased pH, chlorides, serum albumin, and immune globulin in the milk. They suggested that whey protein analysis might be useful in studying physiologic responses in experimentally induced mastitis under controlled conditions.

Endotoxin extracted from *Aerobacter aerogenes* was inoculated at levels of 0.2 to 20.0 mg. into lactating mammary glands of cows (Carroll *et al.*, 1964). Maximal local response with marked edematous swelling in each instance was observed within 2 hours after inoculation. A marked breakdown in vascular permeability was in evidence, with transudation of high concentrations of serum albumin into the milk and a temporary hypoproteinemia. Leukocytic counts peaked in the milk within 6 hours, accompanied by leukopenia and a left shift. Elevations in rectal temperature (to 106 to 107 F.) occurred by 7 hours. The systemic response resembled that following injections of endotoxin into other animals. A twofold rise in blood glucose was observed within 5 hours following intramammary inoculation of endotoxin. They concluded that systemic signs of toxemia observed in peracute coliform mastitis were due to the intramammary lysis of the organisms followed by release and absorption of endotoxin.

Schalm *et al.* (1964) investigated the pathogenesis of experimental coliform mastitis in normal bovine lactating glands. Approximately 50

viable *A. aerogenes* organisms were introduced into the mammary gland of a cow on each of 4 consecutive days. The organisms rapidly multiplied until they stimulated the formation of an inflammatory exudate. The count of *A. aerogenes* fell precipitously as soon as there was evidence of leukocytic infiltration into the gland. At the time of apparent maximal death of the test organism, signs of toxemia were observed. It was presumed that the toxemia was due to the release of endotoxin from destroyed bacterial cells. The signs of toxemia subsided when further multiplication of the organisms remained suppressed. The inflammatory reaction appeared to play the primary role in bringing about recovery. They reported that usually the gland was cleared of the organism in 4 to 9 days. Thereafter, there was a rapid return to normal function.

In another experiment, Schalm *et al.* (1964) also investigated the relationship of size of inoculum to rapidity of development of mastitis. They reported that, except for a delay of onset of mastitis, a small inoculum of *A. aerogenes* was equally as effective as larger numbers of organisms in producing mastitis of a similar degree of severity. They also studied the events following inoculation of *A. aerogenes* into a normal quarter and another quarter having a mild inflammatory reaction (Schalm *et al.*, 1964a). The pre-existing mild inflammatory reaction was due to the presence of *Streptococcus uberis*. The quarter previously infected with *Str. uberis* inhibited the multiplication of *A. aerogenes* introduced at a level of 500,000 organisms. The opposite normal quarter responded peracutely to an identical inoculum. Similar results were obtained in quarters with pre-existing sterile inflammatory changes compared with normal quarters.

Schalm *et al.* (1964b, 1967) investigated the effects of humoral and cellular phases of acute inflammation, and leukocyte barrier to

A. aerogenes in the bovine mammary gland. Phagocytosis of *A. aerogenes* by leukocytes from bovine milk has also been studied *in vitro* by Jain and Jasper (1967).

Coliform Mastitis in Other Animals

Adler (1951) reported an outbreak of acute postparturient mastitis in swine caused by *A. aerogenes*. The disease developed very rapidly with acute systemic disturbances. Affected sows were often unable to rise. The mammary glands were hot, swollen and firm and there was purple discoloration of the skin. Grossly, on the cut surface the mammary glands were mottled grayish and softened, with foul-smelling central area of necrosis. Microscopically, there were congestion and necrosis with acute inflammatory changes. There were marked vacuolation and exfoliation of alveolar epithelial cells.

An outbreak of caprine mastitis caused by *Klebsiella pneumoniae* was reported by Adinarayanan and Singh (1968). The outbreak of mastitis occurred in a self-contained purebred stock of Jamunapari goats. Quarter samples collected from 2 acute cases of bilateral mastitis revealed the presence of gram-negative rods with the characteristics of *Klebsiella pneumoniae*. Milk samples were also examined from 15 goats and similar organisms were isolated from 4 goats. Herak *et al.* (1961) published details on the occurrence and treatment of *Klebsiella* mastitis in 5 cows and 1 goat. Severe parenchymatous mastitis was found in all animals. In spite of treatment, the mammary gland of the goat became necrotic and one-half of the udder sloughed off after a month.

Escherichia coli in Mastitis and Enteritis

One hundred thirty-six strains of coliform organisms obtained from bovine mastitis were examined biochemically by Fey (1953) and 113 strains proved to be *Escherichia coli*. The *Escherichia coli* (O-groups) found in infantile enteritis could also be demonstrated in cows. Strain 026:B6, used in our investigation, was also found in bovine mastitis. By environmental investigation, Strain 026:B6 was isolated from the feces of the mother and 3 children of the farmer's family without having caused illness. After successful treatment of the bovine mastitis, the organisms disappeared from the human feces. He has also mentioned the isolation of colibacteria of Type 026:B6 from several cases of infantile diarrhea.

During the course of a survey, coliform organisms isolated from udder secretions were tested (Rees, 1958). Forty-six different strains were examined serologically. Four strains were identified as similar serologic type to those found in calves with white scours. Serologic identities were confirmed by agglutinin cross-absorption tests with the corresponding type strains. He mentioned that those serotypes of coliform bacteria usually associated with white scours in calves seemed to occur most frequently in the bovine coliform flora and this might be the most probable source of infection in calves.

Mastitis in Small Laboratory Animals

According to Smith and Jones (1964), mastitis may occur in any mammalian species. A literature search has not revealed reports on spontaneous coliform mastitis in small laboratory animals. However, coliform organisms were isolated from 2 out of 125 milk samples examined from multiparous guinea pigs (Gupta *et al.*, 1971). Other microorganisms were also isolated (total 51.2% positive) from the milk samples examined.

Tissues from 398 mammary glands of 199 apparently healthy guinea pigs were also examined, but were found to be negative for the presence of coliform organisms (Gupta *et al.*, 1971a).

There are a few reports of spontaneous and experimental mastitis due to different agents in small laboratory animals. Infection of uterus and mammary gland was reported in 1 guinea pig (Hasselberg and Loeb, 1937), but an attempt was not made to isolate the causative agent. There were abscess formation, infiltration of lymphocytes, and increased amounts of connective tissue in the mammary gland.

Mammary glands of 76 mice were examined during postsecretory involution (Williams and Patnode, 1948). Of these mice, 11 had different degrees of inflammation. *Pasteurella sp.* was isolated from 1 of the affected glands, and subsequent injection of this organism near the nipple area produced mastitis throughout the gland. Preissecker (1958) reported that rodent pups nurse most of the time before they are able to feed on solid food. He observed inflammation of mammary glands resulting from minor trauma accompanying the act of suckling. In guinea pigs, especially, minor trauma might lead to parenchymatous mastitis, with purulent exudate and abscess formation (Cohrs *et al.*, 1958). The affected guinea pigs appear sick without any mortality. Mastitis has also been produced experimentally in rats by inoculating a pure culture of *Staphylococcus aureus.*[*]

Said (1969) studied the biochemical and histologic alterations in experimentally induced coliform mastitis in rabbits. Unfortunately, nonpregnant and nonlactating female rabbits of nondescript breed were used

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[*]Paape, M. J., and Tucker, H. A., Michigan State University, East Lansing, Mich.: personal communication, 1969.

in this experiment. *Escherichia coli* and endotoxin were inoculated separately into the mammary glands of rabbits through the teat canal. Histologic changes were apparently identical in mammary glands inoculated with either *E. coli* or endotoxin. There were desquamation and necrosis of epithelial cells along with acute to subacute inflammatory changes.

Mastitis in 2 guinea pigs was reported recently (Gupta *et al.*, 1970). High leukocyte counts on milk samples were indicative of mammary irritation. Microscopically, there were wide variations in the inflammatory processes characterized by acute to subacute inflammatory changes in the lobules of the mammary glands. An organism with the characteristics of α-hemolytic *Streptococcus* was isolated from milk and mammary tissue samples.

Staphylococcal mastitis has also been induced experimentally in guinea pigs (Gupta *et al.*, 1971b). Gradient numbers of *Staph. aureus* were inoculated into the mammary glands of guinea pigs. Acute to subacute inflammatory changes were observed in inoculated mammary glands after 2 or 4 days of infection. The causative agent (*Staph. aureus*) was isolated from 85 and 50% of the inoculated glands after 2 and 4 days, respectively. The normal architecture of the infected mammary glands was completely obliterated due to massive infiltration of leukocytes. There was necrosis of alveolar epithelial cells and subsequent proliferation of fibroblasts replaced the glandular parenchyma 4 days after infection.

Nucleic Acid Content of the Mammary Gland

Various quantitative methods have been developed in the course of studies of normal and experimentally induced changes in the structure of the mammary gland. Deoxyribonucleic acid (DNA) content, a measure

of cell numbers, and ribonucleic acid (RNA) content, an estimate of protein synthesis, were first applied to mammary tissue by Kirkham and Turner (1953). In most normal somatic tissues, the average amount of DNA per cell is essentially constant. It is similarly possible to use the increase of total DNA in a given tissue as a measure of cell numbers (Brown, 1953). These changes in DNA content have been used to demonstrate the periods of cellular proliferation or as a measure of cell numbers of a given tissue (Tucker and Reece, 1962; Munford, 1963, 1963a).

Use of deoxyribonucleic acid as a reference for expressing changes in tissue composition has been discussed in an excellent review of the nucleic acids, purines, and pyrimidines (Brown, 1953). A further review of anatomical and biochemical changes in the mammary gland with particular reference to quantitative methods of assessing mammary development has also been presented by Munford (1964). The periods of increased protein synthesis and cellular enlargement have been measured by the mammary nucleic acid content (Paape and Tucker, 1969). Changes in the levels of DNA, and RNA and other biochemical components in the mammary gland at different stages of development and function have been studied in a number of species (Greenbaum and Slater, 1957; Lewin, 1957; Brookreson and Turner, 1959; Griffith and Turner, 1959, 1961; Denamur, 1961; Tucker and Reece, 1962, 1963, 1963a, 1963b, 1963c; Nicoll and Tucker, 1965; Sinha and Tucker, 1966).

Naito (1958, 1958a) investigated the quantitative relationships between biochemical and structural alterations in the mammary glands of guinea pigs. The growth of guinea pig mammary glands was also measured using total weight, nitrogen content, DNA and RNA/DNA ratios as parameters of cellular changes (Heytler, 1956; Smith, 1956; Nelson *et al.*, 1962).

Mitoses in Lactating Mammary Gland

Loeb and Hesselberg (1917) and Kuramitsu and Loeb (1921) reported that mitotic activity ceased as soon as intense lactation commenced in guinea pig mammary glands. Cole (1933) did not find any evidence of mitosis in the mouse 7 days after parturition. Maeder (1922) and Weatherford (1929) rarely found mitoses during lactation whereas Jeffers (1935) occasionally observed mitoses in rats. During lactation in cows, Altman (1945) rarely noticed any mitoses in the mammary gland. Reece and Warbritton (1953) observed mitoses in less than 1% of the secretory cells on the 5th day of lactation in the rat. On the basis of these observations, Reece (1956) and Meites (1961) concluded that the increases in milk production during lactation were largely due to an increase in the secretory rate rather than further growth of the mammary gland.

Serum and Tissue Enzymes

There is a wide distribution of glutamic oxalacetic transaminase (GOT) in many body tissues whereas glutamic pyruvic transaminase (GPT) is found in high concentration in the liver (Benjamin, 1965). Elevations in the serum GOT and GPT levels have been found in various diseases involving tissue necrosis (Kuttler and Marble, 1958; Cornelius *et al.*, 1959).

Roussel and Stallcup (1966) determined the effect of season and age on GOT and GPT values in blood serum samples from Holstein-Friesian bulls. There was a significant increase in the GOT level in blood serum in the summer group compared with the winter group. However, differences between the 2 seasons were not observed in the GPT activity of blood serum. Crist *et al.* (1967) reported higher transaminase activity during May through September than for October through April.

Boots *et al.* (1969, 1970) investigated the effect of environmental temperature on plasma GOT and GPT in lactating cattle. The activities of these 2 transaminases were measured in plasma samples taken monthly from Holstein cows throughout 1 lactation and the corresponding gestation period. Both transaminases were positively and linearly affected by the stress of ambient temperature. They suggested that physiologic and environmental factors affecting transaminase activity must be considered in analyzing enzyme data.

The activity of alkaline phosphatase (ALP) in the mammary gland (Dempsey *et al.*, 1947) and its distribution in various other tissues (Bourne and MacKinnon, 1943) has been described. Levels of ALP in the mammary glands of rabbits inoculated with *E. coli* or endotoxin have been studied (Said, 1969). Alkaline phosphatase activity was most pronounced in the nuclei and cytoplasm of epithelial cells of ducts. There was a progressive increase in enzyme activity 24 hours after inoculation and it was found to be maximal on the 6th and 7th days. Enzyme activity was noted in capillaries and medium-sized blood vessels. Intense enzyme activity was also observed in the interstitial connective tissue. Kitchen *et al.* (1970) studied the distribution and activity of ALP, acid phosphatase (ACP) and various other enzymes in the major components of bovine milk. The range of activities found for the enzymes studied were also compared with some of the enzymes in mastitic milk.

Munford (1963) and Baldwin and Milligan (1966) investigated the changes in the concentrations of several enzymes associated with the initiation and maintenance of lactation in rats and mice. Serum phosphatase values were determined in normal cows and ewes and the findings were considered of little diagnostic value due to very wide variations (Allcroft and Folly, 1941; Garner, 1952). Serum lactic dehydrogenase

(LDH) and the distribution of electrophoretically distinct isoenzymes of LDH in normal cattle have been determined (Prasse, 1969).

Sheahan and Gaafar (1970) studied the histologic and histochemical changes in cutaneous lesions of experimentally induced and naturally occurring canine demodicidosis. Marked activities for dehydrogenases and ALP were found in dermal lesions. Uzoukwu and Sleight (1970) investigated the influence of sodium nitrite toxicosis on tissue LDH and succinic dehydrogenase (SDH) in guinea pigs. The tissue changes were compared with the effects of hypoxia produced in guinea pigs kept in a sealed chamber containing a mixture of air and nitrogen. In nitrite toxicosis, activities of LDH and SDH in various tissues were found to be decreased.

MATERIALS AND METHODS

Experimental Animals

Primiparous lactating guinea pigs (*Cavia porcellus*) were used as the experimental animal in this study. The selection of guinea pigs was based on the following reasons: (1) they were easy to obtain and to standardize without introducing significant variables; (2) their small size, minimal cost and maintenance permitted the use of many animals for the collection of meaningful data; (3) they were uniformly susceptible to endotoxin; (4) coliform organisms are found rarely in the guinea pig (Crecelius and Rettger, 1941); and (5) preliminary work indicated that data collected from them might be applied to cows and other lactating animals.

Care and Maintenance of Experimental Animals

Healthy guinea pigs were obtained from local guinea pig breeders. Four to five virgin females and a mature male guinea pig were housed together in solid-bottomed box cages (74 x 40 cm.) in which extra bedding* was provided. When physical signs of pregnancy were observed, the number of guinea pigs was reduced to 3 to 4 in each cage. After whelping, the sow and her pups were cohabited in individual cages under controlled temperature (22 $\pm$ 1 C.) and lighting (8 hours fluorescent light daily).

*Bio-Bed, Zen-Ely, P. O. Box 28, Ely, Minn.

The diet consisted of commercially available guinea pig ration* and water *ad libitum*. The guinea pigs were allowed to nurse 2 pups after whelping.

Endotoxin

The endotoxin** (lipopolysaccharide) was obtained from a commercial source, where it was produced from *Escherichia coli* (026:B6) by a phenolic extraction method as described by Westphal *et al.* (1952). The inoculum was prepared by dissolving 10 mg. of endotoxin in 10 ml. (1.0 mg./ml.) of sterile physiologic saline solution (PSS). The inoculum was stored in a freezer (-20 C.).

Animal Inoculation

Six days after parturition, the guinea pigs were anesthetized with ether*** using a closed chamber (Figure 1). The inguinal region was clipped with a fine clipper and scrubbed with 70% ethanol (Figure 2). Using a tuberculin syringe and a 27-gauge needle, each mammary gland of 20 guinea pigs was infused through the teat canal (Figure 3) with 0.5 ml. of the inoculum (0.5 mg. of endotoxin diluted in 0.5 ml. of sterile PSS, a total of 1.0 mg. of endotoxin per guinea pig). Twenty guinea pigs in the control groups received 0.5 ml. of sterile PSS (0.85% NaCl) into each gland administered in the same manner. Five guinea pigs from each treated (receiving endotoxin) and control (receiving PSS only) group were necropsied at 3, 6, 12, and 24 hours after inoculation (Table 1).

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*Purina Guinea Pig Chow, Ralston Purina Company, St. Louis, Mo.

**Difco Laboratories, Detroit, Mich.

***Ether for anesthesia, Mallinckrodt Chemical Works, St. Louis, Mo.

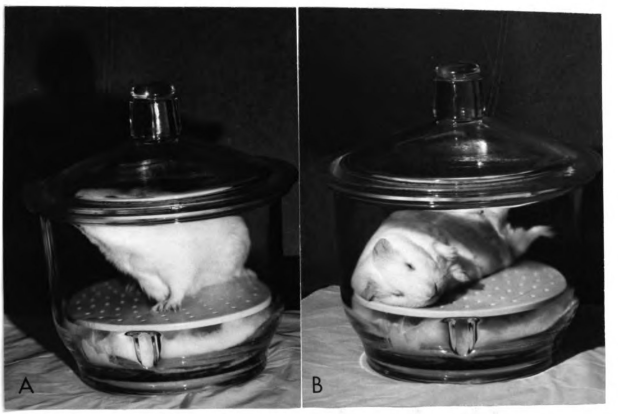

Figure 1. Anesthetization of guinea pig (A) with ether using a closed chamber (desiccator). Absorbent cotton soaked with ether was placed under the porcelain platform before putting the guinea pig in the desiccator. Anesthetized guinea pig (B).

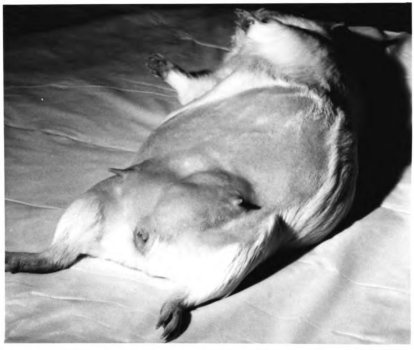

Figure 2. Inguinal and ventral abdominal areas of each guinea pig were clipped and scrubbed with ethanol after anesthetization and before inoculation.

Figure 3. Inoculation of endotoxin into the mammary gland through the teat canal of anesthetized guinea pig.

Table 1. Experimental protocol to study the effect of endotoxin on mammary glands of guinea pigs

Group No.	No. of guinea pigs/group	Treatment	Time of necropsy following intramammary infusion (hours)
1	5	Endotoxin	3
2	5	Endotoxin	6
3	5	Endotoxin	12
4	5	Endotoxin	24
5	5	PSS	3
6	5	PSS	6
7	5	PSS	12
8	5	PSS	24

Clinical Observations

Rectal temperatures were taken every 3 hours. Guinea pigs that were not to be necropsied until 12 or 24 hours after inoculation were returned to cages where they remained with their pups. Body weights were taken at the time of inoculation (Table 2). Guinea pigs were also weighed at the time of euthanasia (only those that were inoculated 24 hours earlier). Since there were only 10 guinea pigs (5 in each treated and control group) for the 24-hours necropsy (Table 1), 92 observations related to body weight differences in 24 hours were included from other similar experiments. Euthanasia, just prior to necropsy, was accomplished by intracardial injections of pentobarbital sodium solution.*

Hematologic Procedures

Immediately prior to euthanasia, blood samples for hematologic procedures and sugar determination were collected by cardiac puncture using an 18-gauge, 3.8-cm. needle and placed in vials containing 0.05 ml. of a 7.5% aqueous solution of tripotassium ethylenediaminetetraacetate (EDTA).** Hemoglobin (Hb.) content was determined by the cyanmethemoglobin method; packed cell volume (PCV) was determined by using microhematocrit tubes. Total leukocyte counts were made with an electronic cell counter.*** Differential (%) leukocytic counts were made by observing 100 cells in a blood smear stained with Wright's stain.† Serum from separate samples of clotted blood was used for determination of sugar

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*Toxital, Jensen-Salsbery Laboratories, Kansas City, Mo.

**B-D Vacutainer, Becton, Dickinson and Company, Rutherford, N.J.

***Coulter Counter, Model A, Coulter Electronics, Inc., Hialeah, Fla.

†National Aniline Division, Allied Chemical Corporation, New York, N.Y.

Table 2. Body weights (gm.) of guinea pigs at the time of inoculation

Group No.	Guinea pig No. 1	2	3	4	5	Mean[a]
1	810	635	760	700	865	754 ± 40
2	750	730	700	700	775	731 ± 14
3	675	690	615	575	630	637 ± 21
4	535	483	617	850	625	622 ± 63
5	665	690	655	650	775	687 ± 23
6	775	660	725	675	890	745 ± 41
7	760	710	700	725	660	711 ± 16
8	863	641	724	835	669	746 ± 44

[a]Values are means ± standard error.

content measured as total reducing substances (Folin and Wu, 1919).

Gross Pathologic and Histopathologic Procedures

Immediately after euthanasia, bilateral tissue samples from the mammary gland, inguinal lymph node, teat and the skin covering the gland were fixed in 10% buffered neutral formalin. Each teat was trimmed transversely into 3 segments of approximately equal size. Selected mammary tissue samples were also fixed in Heidenhain's Susa solution (Davenport, 1961). All specimens were paraffin*-embedded, sectioned 6 μ thick, and stained with Harris' hematoxylin and eosin Y (H & E) stain. Selected specimens were stained with oil red O and also subjected to the periodic acid-Schiff (PAS) reaction, employing Schiff's leucofuchsin and counterstained with Harris' hematoxylin stain. Staining techniques were followed according to the procedures as outlined in the Armed Forces Institute of Pathology's *Manual of Histologic and Special Staining Technics* (1960).

Mammary Nucleic Acid Analyses

Immediately after euthanasia, the mammary glands were removed, trimmed of extraparenchymal connective and adipose tissues, and weighed (Table 3). One representative tissue sample from each mammary gland (2 samples per guinea pig) was individually weighed, placed in 0.25 M sucrose solution, and stored at -50 C. until analyzed for nucleic acid content (Tucker, 1964). Before analyses, the mammary gland tissue samples were thawed and kept ice cold (1 to 2 C.) and then homogenized** after adding

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*Paraplast, Scientific Products, Evanston, Ill.

**Sorvall Omni-Mixer Homogenizer, Ivan Sorvall, Inc., Norwalk, Conn.

Table 3. Total weights (gm.) of both mammary glands of experimental guinea pigs

Group No.	Treatment	Guinea pig No. 1	2	3	4	5	Mean[a]
1	Endotoxin	19.1	18.6	25.8	23.4	27.3	22.8 ± 1.7
2	Endotoxin	28.1	22.4	18.0	22.2	22.8	22.7 ± 1.6
3	Endotoxin	14.5	13.9	19.0	16.7	15.0	15.8 ± 0.9
4	Endotoxin	16.7	13.3	11.3	22.7	15.0	15.8 ± 1.9
5	PSS	15.5	13.2	17.5	17.5	23.2	17.4 ± 1.6
6	PSS	24.1	20.7	26.3	24.6	17.8	22.7 ± 1.5
7	PSS	17.0	19.6	16.0	18.2	14.9	17.1 ± 0.8
8	PSS	16.7	12.5	12.0	19.8	17.0	15.6 ± 1.5

[a]Values are means ± standard error.

cold distilled water. Four milliliters of the homogenate were used to extract the nucleic acid. The extracts were analyzed with a spectrophotometer* reading the optical densities at wave lengths of 268 mμ for deoxyribonucleic acid (DNA) and 670 mμ for ribonucleic acid (RNA).

Serum Enzymes

Thirty primiparous lactating guinea pigs, in addition to the 40 assigned to the histopathologic portion of this study, were used to study the effect of endotoxin on serum enzymes. Housing and maintenance were as previously described. These guinea pigs were divided into 3 groups of 10 each (Table 4). Six days after parturition, the first 2 groups were anesthetized with ether before intramammary inoculation. Each mammary gland of the 1st group of guinea pigs was infused via the teat canal with 0.5 mg. of endotoxin diluted with 0.5 ml. of PSS. Guinea pigs in the 2nd group received only 0.5 ml. PSS each administered in the same manner. Guinea pigs in the 3rd group did not receive any treatment and were included as normal nursing females for uninoculated controls.

On the 7th postpartum day (24 hours after intramammary infusion), blood samples (12 to 20 ml.) were collected from all guinea pigs by cardiac puncture using an 18-gauge, 3.8-cm. needle. Subsequently, the surviving guinea pigs were euthanatized with pentobarbital sodium solution administered intracardially. Serum samples from clotted blood were used for the following enzyme determinations:

1. Glutamic oxalacetic transaminase (GOT)
2. Glutamic pyruvic transaminase (GPT)
3. Alkaline phosphatase (ALP)

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*Spectrophotometer, Model DB, Beckman Instruments, Inc., Fullerton, Calif.

Table 4. Experimental protocol for determination of enzymes in sera and mammary tissues of guinea pigs

Group No.	No. of guinea pigs	Body wt. (gm.)[a]	Treatments
1	10	751 ± 28	Anesthesia and endotoxin
2	10	718 ± 43	Anesthesia and PSS
3	10	813 ± 20	None (no anesthesia or inoculation)

[a]Values are means ± standard error.

4. Acid phosphatase (ACP)

5. Lactic dehydrogenase (LDH)

6. Isocitric dehydrogenase (ICD)

7. Creatine phosphokinase (CPK)

The levels of serum enzymes were determined according to methods described by Sommer (1954) for ALP and ACP, Reitman and Frankel (1957) for GOT and GPT, Cabaud and Wróblewski (1958) for LDH, and Sigma (1967) for ICD and CPK.

Tissue Enzymes

Specimens from mammary glands from the aforementioned guinea pigs (first 2 groups, Table 4) were frozen immediately following euthanasia, using a mixture of solid carbon dioxide and acetone for histochemical examination. The frozen tissues were sectioned 10 μ thick. The mammary tissue ALP was determined according to the method described (Burstone, 1959) using α-naphthyl acid phosphate* as the substrate coupled with azoene fast blue RR salt.* The levels of LDH and succinic dehydrogenase (SDH) were determined by using sodium lactate as the substrate coupled with nitro blue tetrazolium (NBT) according to the technic of Barka and Anderson (1963).

Analyses of Data

The statistical significance of treatment differences was determined by the F test (Snedecor, 1956; Steel and Torrie, 1960).

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*Sigma Chemical Company, St. Louis, Mo.

RESULTS

Clinical Findings

Recovery from ether anesthesia was uneventful. After 12 hours, the teat and skin covering the mammary glands of those guinea pigs given endotoxin became hyperemic and the glands were firm and edematous. These glands were devoid of milk. General appearance included arching of the back and erection of body hair. Signs of toxemia such as increased heart and respiratory rates, central nervous system depression, and muscular tremors were also observed. The body temperature of endotoxin treated guinea pigs (40.2 to 40.4 C.) was increased ($P < 0.05$) over those receiving only PSS (39.8 to 39.9 C.), 6 hours after inoculation (Figure 4).

There was a loss in body weight of 6.6% in endotoxin-treated guinea pigs after 24 hours whereas PSS-treated guinea pigs lost only 1.4% in 24 hours (Table 5). The difference was highly significant ($P < 0.005$).

Hematologic Findings

Hemoglobin values remained unchanged in all groups of guinea pigs (Table 6). A slight hemoconcentration (high PCV) was noted in the PSS-treated guinea pigs at 12 hours and in the endotoxin-treated guinea pigs at 24 hours (Table 7). In comparison with PSS-treated groups at 3 or 24 hours, there was a significant ($P < 0.05$, and $P < 0.005$, respectively) decrease in the number of total leukocytes in circulating blood of endotoxin-treated guinea pigs (Table 8). Absolute values for total neutrophils and lymphocytes were similarly depressed (Tables 9, 10, and 11).

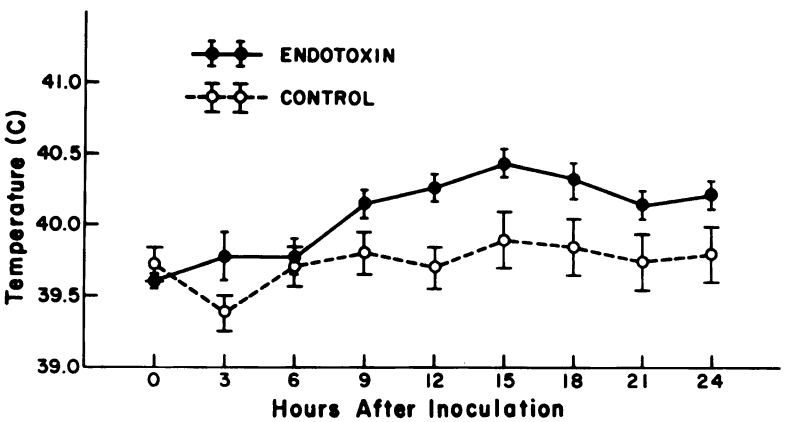

Figure 4. Change in body temperature due to intramammary inoculation of endotoxin in guinea pigs. Note the increase in temperature (solid line) 6 hours after endotoxin inoculation.

Table 5. Effect of endotoxin on the body weight (gm.) of guinea pigs after 24 hours

Total No. of guinea pigs observed[a]	Treatment	Average initial body weight	Average final body weight (24 hours later)	Average difference	Loss of body weight (%)	Remarks
51	Endotoxin	727.6 ± 15.2	680.2 ± 15.0	47.4	6.6 ± 0.4	Significant ($P < 0.005$) decrease compared with PSS-treated guinea pigs
51	PSS	700.8 ± 17.3	691.1 ± 16.9	9.7	1.4 ± 0.4	

[a]Initially, there were only 10 guinea pigs (5 in each group) for the 24-hour observation. To make the data more meaningful, 92 observations (46 in each group) from similar experiments not included in this thesis research were added to this part of the experiment. Thus, the effect of endotoxin on body weight losses was computed by observing a total of 51 inoculated and 51 control guinea pigs.

Table 6. Hemoglobin concentration (gm./100 ml.) in blood of experimental guinea pigs

Group No.	Treatment	Time (hours)	Guinea pig No. 1	2	3	4	5	Mean[a]
1	Endotoxin	3	9.2	12.5	12.4	11.9	11.5	11.5 ± 0.6
2	Endotoxin	6	12.1	10.3	11.2	10.3	11.2	11.0 ± 0.3
3	Endotoxin	12	9.1	11.8	9.4	11.4	9.8	10.3 ± 0.5
4	Endotoxin	24	11.6	12.4	11.9	10.9	10.7	11.5 ± 0.3
5	PSS	3	12.1	10.4	11.1	10.7	11.8	11.2 ± 0.3
6	PSS	6	10.3	13.6	10.0	10.7	9.6	10.8 ± 0.7
7	PSS	12	10.7	11.0	10.7	11.9	12.3	11.3 ± 0.3
8	PSS	24	11.0	9.5	10.9	11.3	11.3	10.8 ± 0.3

[a]Values are means ± standard error.

Table 7. Percent of erythrocytes (packed cell volume) in blood of experimental guinea pigs

Group No.	Treatment	Time (hours)	Guinea pig No. 1	2	3	4	5	Mean[a]
1	Endotoxin	3	34.5	45.5	46.0	42.5	41.0	41.9 ± 2.1
2	Endotoxin	6	42.0	41.0	39.5	41.5	39.0	40.6 ± 0.6
3	Endotoxin	12	36.0	40.0	34.0	40.0	36.0	37.2[b] ± 1.2
4	Endotoxin	24	41.2	44.0	40.5	40.5	40.0	41.2[c] ± 0.7
5	PSS	3	43.0	40.0	41.0	38.5	40.0	40.5 ± 0.7
6	PSS	6	41.5	46.0	37.0	39.0	37.0	40.1 ± 1.7
7	PSS	12	43.0	42.7	39.4	42.2	42.6	42.0 ± 0.7
8	PSS	24	39.5	37.0	39.0	40.5	39.5	39.1 ± 0.6

[a]Values are means ± standard error.

[b]Significantly ($P < 0.01$) decreased compared with the matching control group No. 6.

[c]Significantly ($P < 0.05$) increased compared with the matching control group No. 8.

Table 8. Effect of endotoxin on number of circulating leukocytes in blood of guinea pigs (total number/cmm.)

Group No.	Treatment	Time (hours)	Guinea pig No. 1	2	3	4	5	Mean[a]
1	Endotoxin	3	3,708	3,800	5,100	2,400	5,300	4,062[b] ± 528
2	Endotoxin	6	5,600	5,000	3,900	4,000	7,800	5,260 ± 710
3	Endotoxin	12	3,500	3,267	7,800	4,950	4,606	4,825 ± 809
4	Endotoxin	24	4,150	2,800	4,000	4,200	3,400	3,710[c] ± 268
5	PSS	3	8,300	13,916	15,700	8,100	5,600	10,323 ± 1,913
6	PSS	6	6,600	7,000	6,700	7,700	5,826	6,765 ± 303
7	PSS	12	6,280	9,850	3,550	7,000	6,300	6,596 ± 1,005
8	PSS	24	7,040	4,998	10,662	7,900	7,750	7,670 ± 910

[a]Values are means ± standard error.

[b]Significantly ($P < 0.01$) decreased compared with the matching control group No. 5.

[c]Significantly ($P < 0.005$) decreased compared with the matching control group No. 8.

Table 9. Effect of endotoxin on circulating total neutrophils (absolute number/cmm.)

Group No.	Treatment	Time (hours)	Guinea pig No. 1	2	3	4	5	Mean[a]
1	Endotoxin	3	1,372	1,520	4,233	624	1,431	1,836[b] ± 620
2	Endotoxin	6	4,256	1,500	2,262	1,520	5,382	2,984 ± 782
3	Endotoxin	12	560	1,666	4,914	2,723	1,935	2,360 ± 726
4	Endotoxin	24	913	448	840	630	782	723[c] ± 83
5	PSS	3	3,071	7,932	8,321	2,511	4,312	5,229 ± 1,220
6	PSS	6	2,046	3,570	2,479	2,926	1,864	2,577 ± 309
7	PSS	12	2,889	2,364	1,243	980	2,394	1,974 ± 367
8	PSS	24	2,816	1,949	3,945	2,528	4,340	3,116 ± 446

[a] Values are means ± standard error.

[b] Significantly ($P < 0.05$) decreased compared with the matching control group No. 5.

[c] Significantly ($P < 0.005$) decreased compared with the matching control group No. 8.

Table 10. Effect of endotoxin on circulating segmented neutrophils (absolute number/cmm.)

Group No.	Treatment	Time (hours)	Guinea pig No. 1	2	3	4	5	Mean[a]
1	Endotoxin	3	1,075	1,178	3,366	552	848	1,404[b] ± 502
2	Endotoxin	6	3,808	1,450	2,141	1,480	4,914	2,759 ± 688
3	Endotoxin	12	525	1,360	4,368	1,980	1,290	1,905 ± 658
4	Endotoxin	24	664	392	680	420	442	520[c] ± 62
5	PSS	3	2,988	7,793	8,164	2,430	4,200	5,115 ± 1,205
6	PSS	6	2,046	3,500	2,345	2,849	1,864	2,521 ± 296
7	PSS	12	2,763	2,364	994	980	2,331	1,886 ± 375
8	PSS	24	2,816	1,649	3,838	2,449	4,030	2,956 ± 442

[a]Values are means ± standard error.

[b]Significantly ($P < 0.01$) decreased compared with the matching control group No. 5.

[c]Significantly ($P < 0.005$) decreased compared with the matching control group No. 8.

Table 11. Effect of endotoxin on circulating lymphocytes (absolute number/cmm.)

Group No.	Treatment	Time (hours)	Guinea pig No. 1	2	3	4	5	Mean[a]
1	Endotoxin	3	2,225	2,166	816	1,680	3,604	2,098[b] ± 453
2	Endotoxin	6	1,064	3,000	1,404	2,400	2,262	2,026[c] ± 350
3	Endotoxin	12	2,870	1,632	2,730	2,079	2,579	2,378 ± 229
4	Endotoxin	24	2,988	2,240	3,000	3,276	2,516	2,804[d] ± 187
5	PSS	3	4,897	5,427	7,222	5,346	952	4,769 ± 1,034
6	PSS	6	4,422	3,360	4,154	4,466	3,729	4,026 ± 212
7	PSS	12	3,014	7,289	2,130	5,600	3,465	4,300 ± 940
8	PSS	24	4,083	2,799	6,184	4,977	3,333	4,275 ± 602

[a]Values are means ± standard error.

[b]Significantly ($P < 0.05$) decreased compared with the matching control group No. 5.

[c]Significantly ($P < 0.005$) decreased compared with the matching control group No. 6.

[d]Significantly ($P < 0.05$) decreased compared with the matching control group No. 8.

However, there was a marked increase in nonsegmented neutrophils in endotoxin-treated guinea pigs (Table 12). Some immature neutrophils with toxic changes (bluish cytoplasm) were seen 24 hours following endotoxin inoculation. Numbers of basophils in endotoxin-treated guinea pigs increased significantly ($P < 0.005$) at the 3-hour determination (Table 13). Absolute values for eosinophils remained unchanged (Table 14). There was a significant ($P < 0.005$) depression of monocytes 3 and 6 hours after endotoxin treatment (Table 15). Total blood sugar levels (245.2 mg./100 ml.) were significantly ($P < 0.05$) higher in 6-hour endotoxin-treated animals as compared with PSS-treated guinea pigs (170.3 mg./100 ml.). There was no significant difference in blood sugar levels at 3-, 12-, or 24-hour postinoculation samples (Table 16).

Gross Pathologic Findings

There were no significant macroscopic changes in mammary glands of guinea pigs 3 or 6 hours after either endotoxin or PSS administration. However, there was a remarkable difference in the gross appearance of mammary tissues after 12 and 24 hours for endotoxin-treated glands as compared with those receiving PSS. The skin covering the mammary gland was hyperemic and the glandular tissues were devoid of milk. The subcutaneous tissues were edematous and congested. Inguinal lymph nodes were moderately enlarged and congested.

Histopathologic Findings

Histopathologic features of mammary glands, teats, adjoining skin and inguinal lymph nodes from different guinea pigs in the same group were variable. There was also variation in degenerative and inflammatory changes in different areas of the same tissue.

Table 12. Effect of endotoxin on circulating nonsegmented (stab) neutrophils (absolute number/cmm.)

Group No.	Treatment	Time (hours)	Guinea pig No. 1	2	3	4	5	Mean[a]
1	Endotoxin	3	297	342	867	72	583	432 ± 136
2	Endotoxin	6	448	50	117	40	468	225 ± 96
3	Endotoxin	12	35	306	546	743	645	455 ± 128
4	Endotoxin	24	249	56	160	210	340	203 ± 47
5	PSS	3	83	139	157	81	112	114 ± 15
6	PSS	6	0	70	134	77	0	56 ± 25
7	PSS	12	126	0	249	0	63	88 ± 47
8	PSS	24	0	300	107	79	311	159 ± 62

[a]Values are means ± standard error.

Table 13. Effect of endotoxin on circulating basophils (absolute number/cmm.)

Group No.	Treatment	Time (hours)	Guinea pig No. 1	2	3	4	5	Mean[a]
1	Endotoxin	3	37	0	51	48	53	38[b] ± 15
2	Endotoxin	6	0	0	117	0	0	23 ± 23
3	Endotoxin	12	35	0	0	0	0	7 ± 7
4	Endotoxin	24	42	0	40	0	0	16 ± 10
5	PSS	3	0	0	0	0	0	0 ± 0
6	PSS	6	0	0	0	0	0	0 ± 0
7	PSS	12	0	0	107	210	252	114 ± 52
8	PSS	24	0	0	0	0	0	0 ± 0

[a]Values are means ± standard error.

[b]Significantly ($P < 0.005$) increased compared with the matching control group No. 5.

Table 14. Effect of endotoxin on circulating eosinophils (absolute numbers/cmm.)

Group No.	Treatment	Time (hours)	Guinea pig No. 1	2	3	4	5	Mean[a]
1	Endotoxin	3	0	114	0	48	212	75 ± 40
2	Endotoxin	6	280	500	117	80	156	227 ± 76
3	Endotoxin	12	35	68	78	50	0	46 ± 14
4	Endotoxin	24	0	0	0	0	0	0 ± 0
5	PSS	3	249	278	0	81	56	133 ± 85
6	PSS	6	66	0	67	231	175	108 ± 42
7	PSS	12	188	99	36	70	63	91 ± 26
8	PSS	24	70	0	107	237	0	83 ± 44

[a]Values are means ± standard error.

Table 15. Effect of endotoxin on circulating monocytes (absolute numbers/cmm.)

Group No.	Treatment	Time (hours)	Guinea pig No. 1	2	3	4	5	Mean[a]
1	Endotoxin	3	74	0	0	0	0	15[b] ± 15
2	Endotoxin	6	0	0	0	0	0	0[b] ± 0
3	Endotoxin	12	0	33	156	99	92	76 ± 27
4	Endotoxin	24	208	112	120	294	102	167 ± 37
5	PSS	3	83	278	157	162	280	192 ± 38
6	PSS	6	66	70	0	77	58	54 ± 14
7	PSS	12	126	99	36	140	126	105 ± 19
8	PSS	24	70	250	427	158	78	197 ± 66

[a]Values are means ± standard error.

[b]Significantly ($P < 0.005$) decreased compared with the matching control groups No. 5 and 6.

Table 16. Values for total blood sugar (mg./100 ml.) of experimental guinea pigs

Group No.	Treatment	Time (hours)	Guinea pig No. 1	2	3	4	5	Mean[a]
1	Endotoxin	3	195.0	107.9	262.3	182.0	188.0	187.0 ± 24.5
2	Endotoxin	6	187.1	199.4	356.2	255.6	227.5	245.2[b] ± 30.2
3	Endotoxin	12	116.5	188.6	146.1	150.6	206.7	161.7 ± 19.9
4	Endotoxin	24	221.5	248.6	185.5	208.4	277.8	228.4 ± 16.0
5	PSS	3	156.8	173.0	232.6	155.4	146.2	172.8 ± 15.6
6	PSS	6	171.4	161.8	174.2	177.0	167.2	170.3 ± 2.7
7	PSS	12	143.9	186.8	157.3	161.9	164.2	162.8 ± 6.9
8	PSS	24	191.0	218.0	252.8	187.1	175.6	204.9 ± 13.8

[a]Values are means ± standard error.

[b]Significantly ($P < 0.05$) increased compared with the matching control group No. 6.

Three Hours After Inoculation

No significant degenerative changes were noticed in the glandular tissues of either endotoxin- or PSS-treated guinea pigs 3 hours after inoculation (Figures 5 and 6). Occasionally, a few neutrophils were observed in capillaries, smaller blood vessels and interalveolar stroma. The alveoli and lactiferous ducts were apparently normal. The secretory activity of glandular parenchyma did not appear to be affected. However, endotoxin-treated glands were slightly congested compared with PSS-treated glands. Microscopic changes in the teats of both groups were those of an acute inflammation but the inflammatory changes were much more pronounced in endotoxin-treated teats. There was margination of neutrophils along the walls of blood vessels in the teat (Figure 7). Neutrophils had also migrated into the perivascular connective tissues (Figure 8). The lamina propria of the sinus papillaris in teats was edematous and contained inflammatory cells, primarily neutrophils (Figure 9). The lactiferous sinuses contained inflammatory exudate (Figure 10). Germinal centers of lymphoid follicles of inguinal lymph nodes were more prominent in endotoxin-treated guinea pigs.

Six Hours After Inoculation

The endotoxin-treated mammary glands started to show some clotting and mineralization of secretion in the lumina of alveoli and alveolar ducts which appeared homogeneous, hyalinized and slightly bluish in color (Figure 11). Occasionally, the milk clot was surrounded by serofibrinous material. The clotting of milk varied from one lobule to another as well as from one alveolus to another in the same lobule. Capillaries were distended and the glandular parenchyma was moderately congested. The occasional presence of neutrophils was still limited to smaller blood vessels,

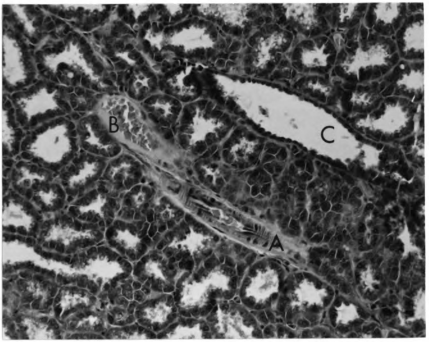

Figure 5. Normal mammary gland of a guinea pig 3 hours after PSS inoculation. Artery (A), vein (B), and alveolar duct (C). H & E stain. x 190.

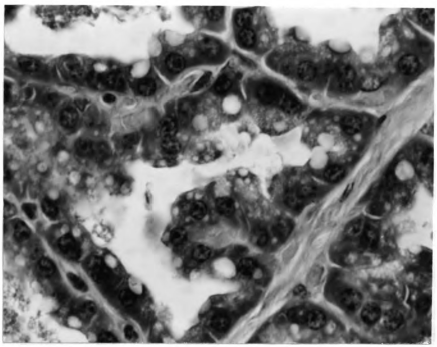

Figure 6. Alveoli of the mammary gland 3 hours after endotoxin treatment. H & E stain. x 760

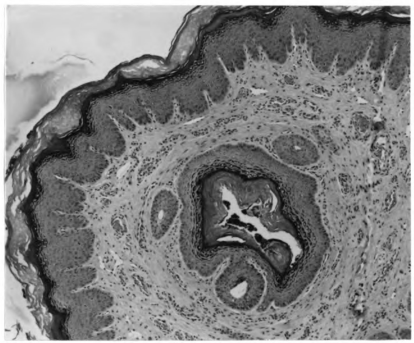

Figure 7. Cross section of teat (lower third) 3 hours after endotoxin treatment. Note congestion in the subcutaneous tissues. H & E stain. x 75.

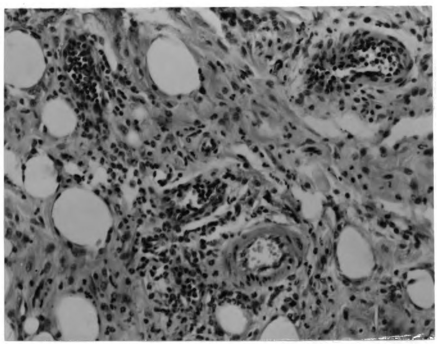

Figure 8. Infiltration of neutrophils into the connective tissues of teat 3 hours after endotoxin treatment. H & E stain. x 190.

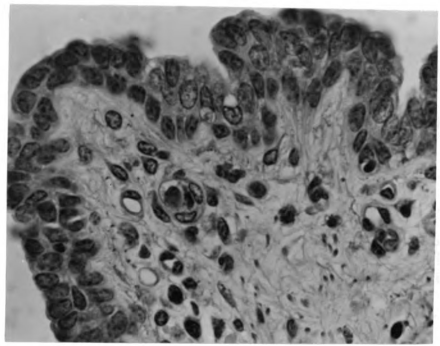

Figure 9. Edema in the lamina propria of sinus papillaris 3 hours after endotoxin treatment. H & E stain. x 760.

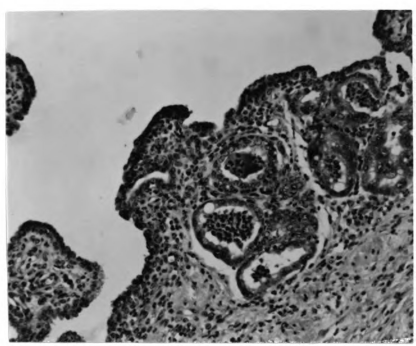

Figure 10. Inflammatory exudate in lactiferous sinuses 3 hours after endotoxin treatment. H & E stain. x 190.

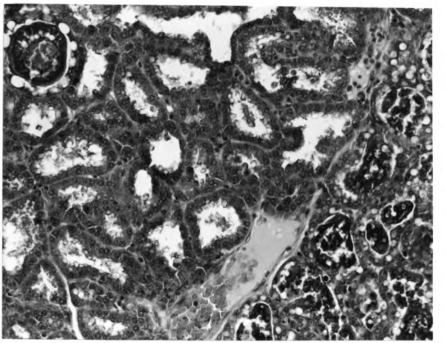

Figure 11. Clotted milk in the alveoli 6 hours after endotoxin treatment. H & E stain. x 190.

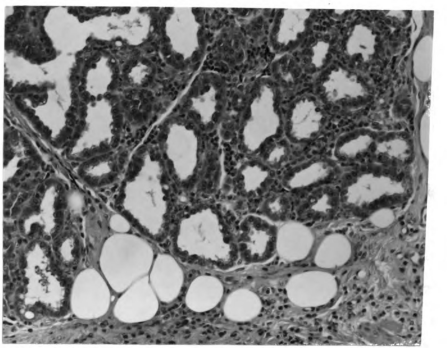

Figure 12. Infiltration of neutrophils into the interalveolar tissues 6 hours after PSS treatment. H & E stain. x 190.

capillaries and interalveolar stroma. The alveolar epithelial cells were cuboidal to tall columnar with circular empty spaces of different diameters. These spaces were probably occupied by fat globules denoting active secretory function. The histopathologic changes in PSS-treated glands were also characterized by the presence of neutrophils in capillaries and interalveolar spaces (Figure 12). The changes in teats (Figures 13 and 14) of endotoxin- and PSS-treated animals were similar to those found in guinea pigs euthanatized at 3 hours. Lymphatic follicles of inguineal lymph nodes were very prominent.

Twelve Hours After Inoculation

The inflammatory changes in the mammary glands of guinea pigs treated with endotoxin were pronounced. There was moderate to massive infiltration of neutrophils into the alveoli (Figures 15 and 16), interalveolar spaces and interlobular ducts (Figure 17). The mammary glands were markedly congested and the interlobular spaces were edematous. The interlobular connective tissue septa were infiltrated with inflammatory cells, primarily neutrophils (Figure 18). The secretory activity of the glandular parenchyma did not seem to be completely disrupted as evidenced by the presence of milk in lumina of the alveoli, although some of the milk had clotted (Figure 19) and possessed laminated rings with leukocytes or fat globules in the center. A few lactiferous ducts contained red blood cells intermixed with milk (Figure 20). The epithelial cells of the alveoli contained numerous round empty spaces, previously occupied by fat globules (oil red O stain). These spaces were located either side by side in the alveolar epithelial cytoplasm or they had coalesced resulting in bigger vacuoles. In affected areas, the epithelial cytoplasm appeared granular and contained some pinkish hyaline granules

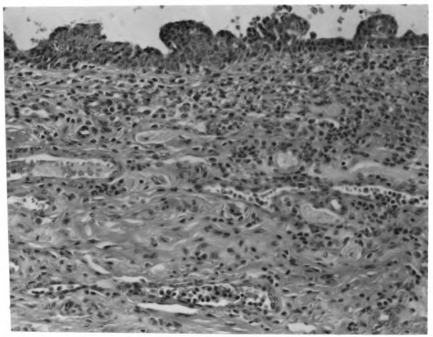

Figure 13. Longitudinal section of teat 6 hours after PSS treatment. Note the inflammatory cells. H & E stain. x 190.

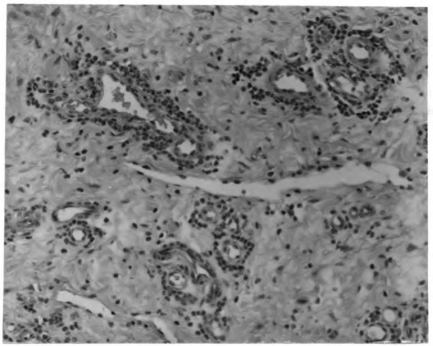

Figure 14. Connective tissue of teat 6 hours after PSS treatment. Note the perivascular infiltration of neutrophils. H & E stain. x 190.

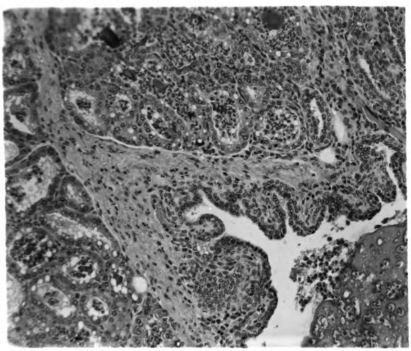

Figure 15. Infiltration of neutrophils into the alveoli and lactiferous ducts 12 hours after endotoxin treatment. H & E stain. x 165.

Figure 16. Higher magnification of Figure 15. Note the infiltration of leukocytes into the alveoli (top center). Lactiferous ducts (A) also contain leukocytes (arrows) enmeshed in clotted milk (B). H & E stain. x 360.

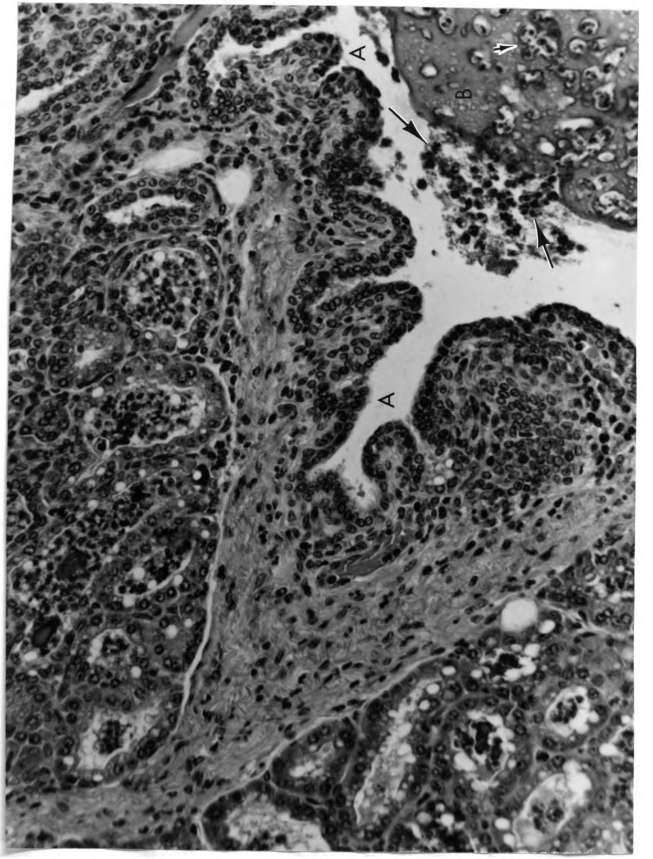

Figure 16

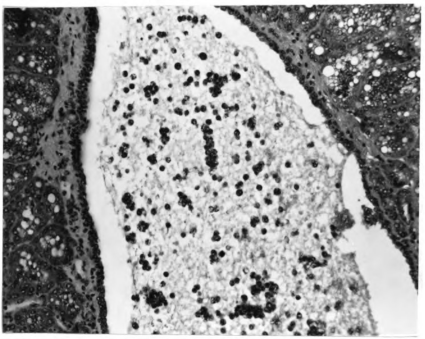

Figure 17. Infiltration of leukocytes into the lactiferous duct and its lamina propria 12 hours after endotoxin treatment. H & E stain. x 165.

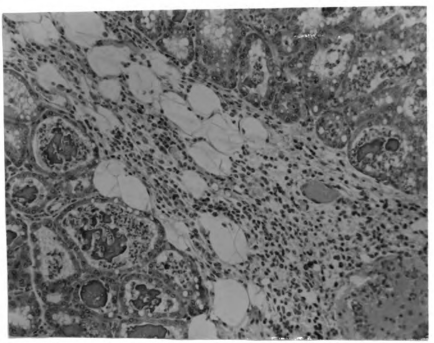

Figure 18. Inflammatory cells in the interlobular connective tissue septa 12 hours after endotoxin treatment. H & E stain. x 165.

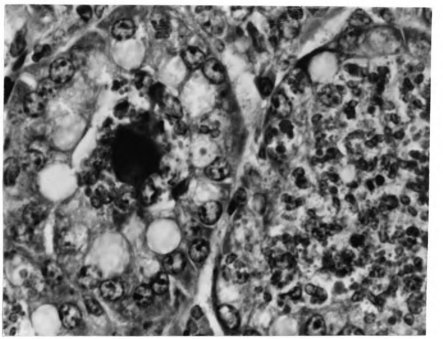

Figure 19. Degenerative and inflammatory changes in 2 alveoli 12 hours after endotoxin inoculation. H & E stain. x 760.

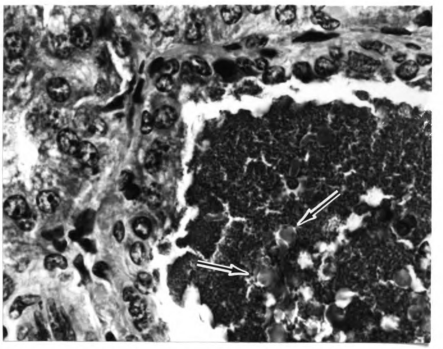

Figure 20. Red blood cells (arrows) intermixed with milk in a lactiferous duct. H & E stain. x 760.

(Figure 21). These hyaline bodies varied in size (2 to 10 μ in diameter) and were located intracellularly as well as in the alveolar lumina. Active phagocytosis of these granules by neutrophils was observed in the alveoli. Neutrophils laden with hyaline bodies were also seen in lactiferous ducts.

In teats, the endothelial cells of smaller blood vessels were markedly swollen (Figures 22 and 23) and the subendothelial spaces were edematous. There was infiltration of neutrophils into the intimal layer (tunica elastica interna) of smaller blood vessels. There was exudation of serous fluid around the blood vessels with massive infiltration of neutrophils into the surrounding connective tissues (Figure 24). The lumina of the sinus papillaris contained clotted milk enmeshed with neutrophils, cellular debris and fibrin. Breakdown of collagen fibers expressed by a fibrinoid reaction was observed in subcutaneous tissues surrounding the mammary gland. Adipose tissues and the connective tissue capsule around the mammary gland were congested and infiltrated with neutrophils.

There was marked variation in the microscopic structures of different inguinal lymph nodes of the same guinea pig. Some of the lymph nodes were apparently normal or, at most, had hyperplastic follicles, whereas others had marked inflammatory changes characterized by infiltration of neutrophils into the cortical areas around the follicles, marginal sinuses, capsule and pericapsular adipose tissue. In general, the lymph nodes were markedly congested.

Slight inflammatory changes were also noticed in PSS-treated mammary glands. This was characterized by infiltration of neutrophils into the alveoli, interalveolar spaces and capillaries (Figures 25 and 26). These changes were not as clearly evident as in endotoxin-treated glands. Occasionally, the glandular tissue contained islands of parenchyma

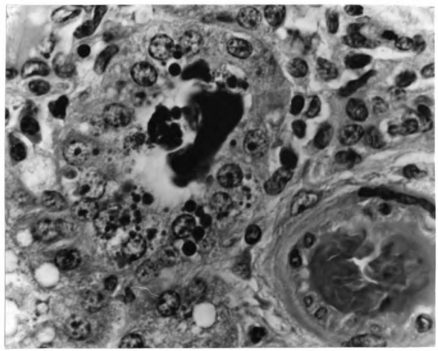

Figure 21. Hyaline droplet degeneration in epithelial cells of alveoli 12 hours after endotoxin treatment. H & E stain. x 760.

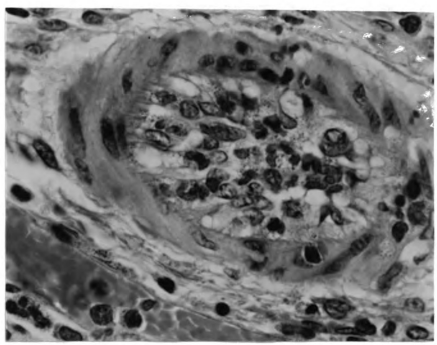

Figure 22. Swelling of endothelial cells of an artery 12 hours after endotoxin inoculation. H & E stain. x 760.

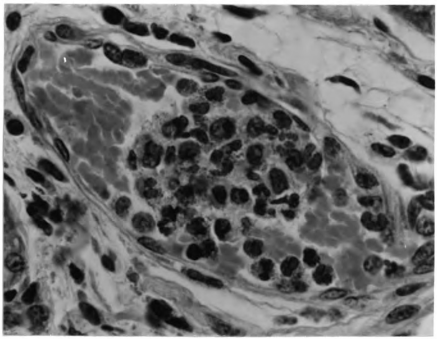

Figure 23. Swelling of endothelial cells and sludging of leukocytes in a vein 12 hours after endotoxin treatment. H & E stain. x 760.

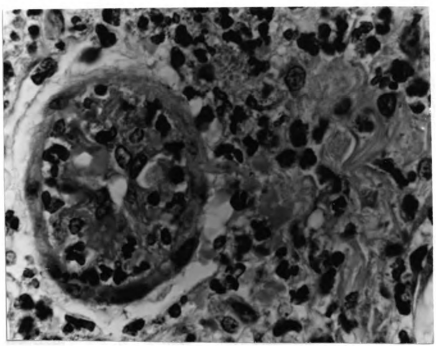

Figure 24. Neutrophils in and around the blood vessel of a teat 12 hours after endotoxin inoculation. H & E stain. x 760.

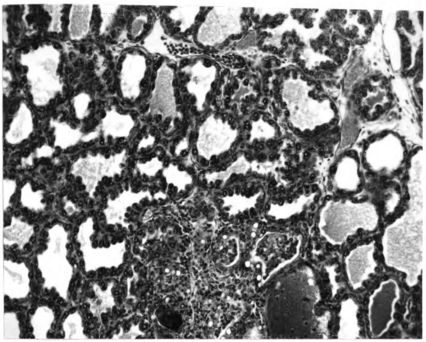

Figure 25. Leukocytes in alveoli and capillaries 12 hours after PSS treatment. H & E stain. x 165.

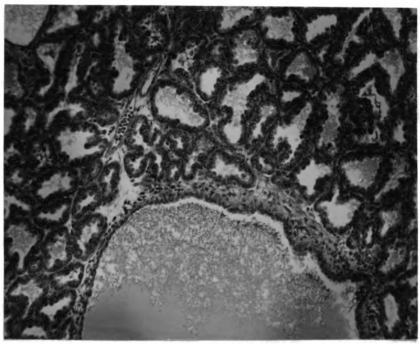

Figure 26. Infiltration of leukocytes into the lamina propria of lactiferous duct 12 hours after PSS treatment. H & E stain. x 165.

characterized by chronic inflammatory changes. These changes were observed in both endotoxin- and PSS-treated mammary glands and were considered to be normal (Trautmann and Fiebiger, 1952).

Twenty-Four Hours After Inoculation

There were marked degenerative and inflammatory changes in endotoxin-treated mammary glands 24 hours after inoculation. Most of the lobules were uniformly affected (Figure 27), although a few normal lobules were also evident. The affected lobules were characterized by the presence of massive numbers of neutrophils, cellular debris, clotted milk and fibrin clots in the alveoli and interalveolar ducts. Some of the alveoli appeared to be obliterated due to the massive influx of inflammatory cells (Figure 28). Interalveolar and interlobular spaces were edematous and infiltrated with neutrophils, lymphocytes, and plasma cells. The connective tissue capsule and adipose tissues covering the mammary gland were also edematous and markedly infiltrated with neutrophils (Figure 29). The alveolar epithelial cells were low cuboidal with distinct free borders towards the center. The borders were markedly thickened, forming a circle around the inflammatory exudate (Figure 30). Some of the epithelial cells were without nuclei and also contained vacuoles which were completely clear with sharp boundaries. Some of the nuclei were distinctly vesicular with margination of chromatin material.

Hyaline droplet degeneration was most prominent in the epithelial cells of the alveoli. This was characterized by the presence of numerous cytoplasmic acidophilic granules which were distinctly differentiated from the remainder of the cytoplasmic materials (Figure 31). The round, homogeneous, hyaline bodies measured approximately 2 to 10 μ in diameter (Figure 32). Extracellular pinkish or bluish hyaline bodies were also

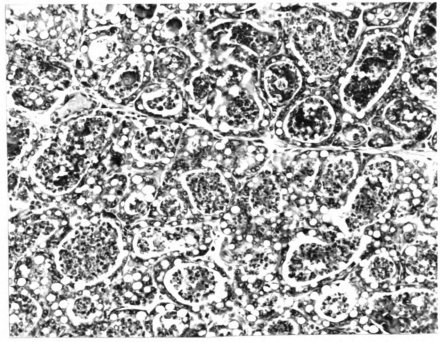

Figure 27. Acute mastitis 24 hours after endotoxin inoculation. H & E stain. x 190.

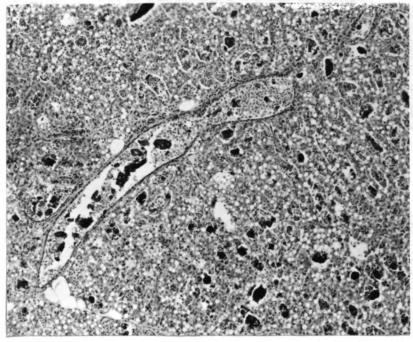

Figure 28. Massive influx of leukocytes in the mammary gland 24 hours after endotoxin inoculation. H & E stain. x 75.

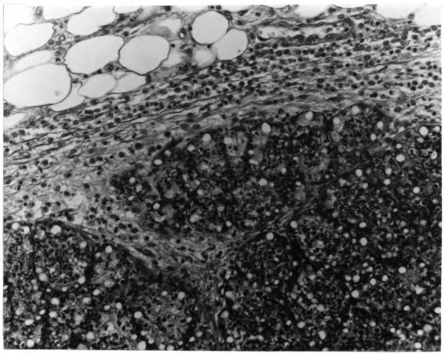

Figure 29. Connective tissue capsule, interalveolar stroma and alveoli infiltrated with inflammatory cells. H & E stain. x 190.

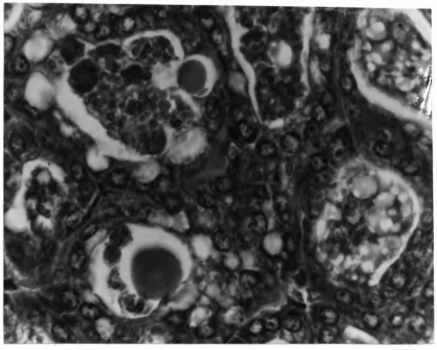

Figure 30. Thickening of the free borders of alveolar epithelial cells 24 hours after endotoxin treatment. H & E stain. x 760.

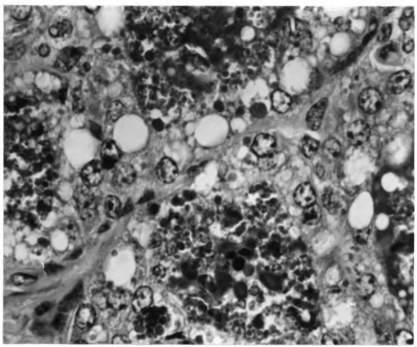

Figure 31. Hyaline granules in the alveoli of mammary gland 24 hours after endotoxin treatment. H & E stain. x 760.

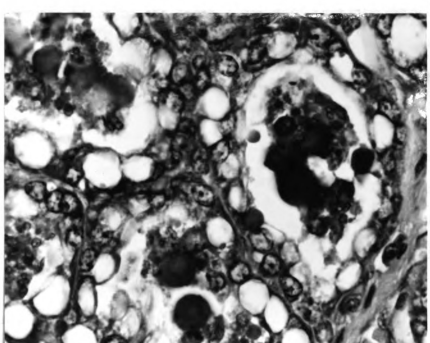

Figure 32. Larger and smaller hyaline bodies in endotoxin-treated mammary gland. H & E stain. x 760.

observed where epithelial cells were markedly disrupted. Similar but smaller hyaline bodies were phagocytized by neutrophils present in the alveoli (Figure 33). Hyaline bodies were also present in lactiferous ducts enmeshed with milk clot, fibrin and neutrophils (Figure 34). The milk clot present in alveoli and lactiferous ducts had taken a basophilic stain. Occasionally, the alveoli contained larger, rounded, homogeneous and concentrically laminated bodies staining zonally with a bluish tinge (Figure 35). These structures were similar to corpora amylacea found in bovine mammary glands.

There were numerous germinal centers in the inguinal lymph nodes. These were surrounded by wide zones of closely packed and darkly staining lymphocytes. Active proliferation of germinal centers was denoted by numerous mitotic figures (Figure 36). There were necrosis, hemorrhage and infiltration of neutrophils in the connective tissue capsule and pericapsular adipose tissues of inguinal lymph nodes (Figure 37). The medulla and the hilus of the lymph nodes were markedly congested.

The mammary glands of PSS-treated guinea pigs were similar to 12-hour PSS-treated glands, although there were fewer inflammatory cells.

Influence on Mammary Nucleic Acid Content

Intramammary inoculation of endotoxin or PSS had very little effect on mammary DNA or RNA concentration of lactating guinea pigs euthanatized 3 or 6 hours after inoculation. However, mammary DNA had increased significantly ($P < 0.01$) 12 and 24 hours after endotoxin inoculation (Table 17). There was also a slight increase in mammary DNA content following PSS treatment after 12 and 24 hours.

No significant ($P > 0.05$) difference in RNA concentration of endotoxin-treated mammary gland was found even after 24 hours (Table 18). Infusion

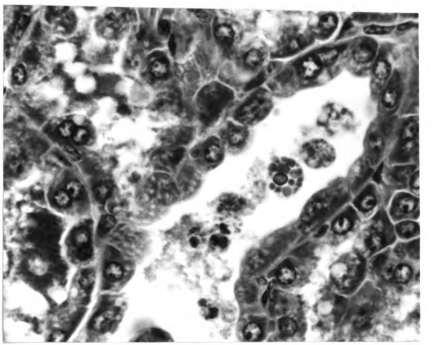

Figure 33. Hyaline granules phagocytized by neutrophils in the mammary gland of a guinea pig treated with endotoxin. H & E stain. x 760.

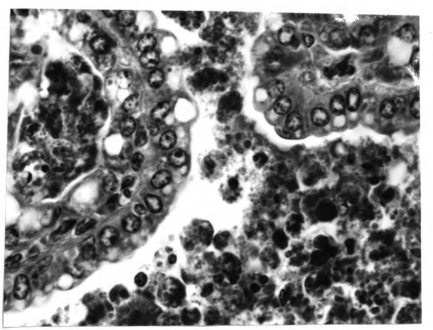

Figure 34. Hyaline granules phagocytized by neutrophils in the lactiferous ducts. H & E stain. x 760.

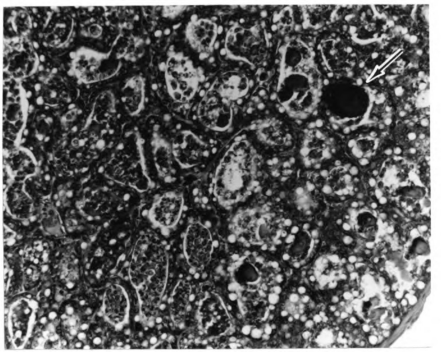

Figure 35. Corpora amylacea (arrow) and inflammatory exudate in the alveoli 24 hours after endotoxin treatment. H & E stain. x 190.

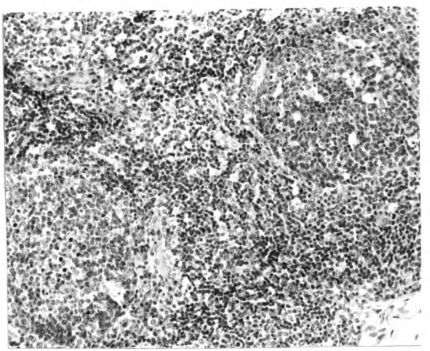

Figure 36. Active germinal centers in the lymphoid follicles of inguinal lymph node 24 hours after endotoxin treatment. H & E stain. x 190.

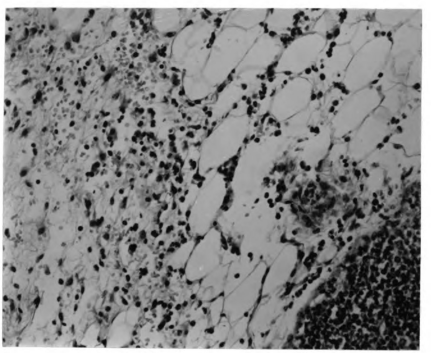

Figure 37. Necrosis, hemorrhage and infiltration of neutrophils in pericapsular adipose tissues of lymph node. H & E stain. x 190.

Table 17. Influence of endotoxin on DNA concentration (mg./gm.) of lactating mammary glands of guinea pigs

Group No.	Treatment	Time (hours)	Guinea pig No. and Mammary Glands										Mean[a]
			1 Lt.	1 Rt.	2 Lt.	2 Rt.	3 Lt.	3 Rt.	4 Lt.	4 Rt.	5 Lt.	5 Rt.	
1	Endotoxin	3	1.86	2.12	1.78	1.62	2.84	1.94	1.54	1.71	2.20	2.20	1.98 ± 0.12
2	Endotoxin	6	2.36	2.52	2.10	1.41	1.97	1.40	1.61	2.16	1.71	1.82	1.91 ± 0.12
3	Endotoxin	12	3.80	4.07	4.39	3.75	3.69	4.68	2.96	3.10	3.29	3.14	3.69[b] ± 0.18
4	Endotoxin	24	4.43	4.19	4.37	3.59	4.07	4.77	5.01	4.94	4.94	4.49	4.48[c] ± 0.14
5	PSS	3	1.86	1.68	1.75	1.37	2.29	1.54	2.20	2.40	1.94	1.78	1.88 ± 0.10
6	PSS	6	1.84	1.73	3.39	1.94	2.20	2.33	2.20	1.82	1.86	2.01	2.13 ± 0.15
7	PSS	12	3.54	3.00	2.48	3.69	2.16	3.05	3.10	2.69	3.66	3.21	3.06 ± 0.16
8	PSS	24	3.56	3.98	3.64	3.05	2.69	3.10	2.24	2.65	3.14	3.24	3.13 ± 0.16

[a]Values are means ± standard error.

[b]Significantly ($P < 0.01$) increased compared with the matching control group No. 7.

[c]Significantly ($P < 0.01$) increased compared with the matching control group No. 8.

Table 18. Influence of endotoxin on RNA concentration (mg./gm.) of lactating mammary glands of guinea pigs

Group No.	Treatment	Time (hours)	Guinea pig No. and Mammary Glands										Mean[a]
			1 Lt.	1 Rt.	2 Lt.	2 Rt.	3 Lt.	3 Rt.	4 Lt.	4 Rt.	5 Lt.	5 Rt.	
1	Endotoxin	3	7.35	9.88	8.14	8.63	16.05	8.88	6.65	13.17	8.53	7.89	9.52 ± 0.92
2	Endotoxin	6	9.64	10.51	12.77	6.74	8.30	6.95	7.51	8.30	7.98	11.88	9.06 ± 0.65
3	Endotoxin	12	10.34	11.13	13.17	11.32	8.05	7.98	6.50	7.73	7.04	7.04	9.03[b] ± 0.72
4	Endotoxin	24	6.74	10.23	6.05	8.79	6.29	8.63	9.30	9.71	8.96	8.96	8.37[c] ± 0.46
5	PSS	3	7.20	10.51	9.37	9.13	9.98	10.23	8.86	17.62	7.98	8.53	9.94 ± 0.91
6	PSS	6	7.04	10.69	9.98	9.81	8.30	9.81	12.38	9.54	10.34	8.46	9.64 ± 0.46
7	PSS	12	8.96	11.62	9.88	14.00	9.54	14.63	12.07	9.98	12.77	9.30	11.27 ± 0.65
8	PSS	24	15.06	10.16	13.79	9.03	9.47	11.69	12.38	14.00	14.85	13.46	12.37 ± 0.70

[a]Values are means ± standard error.

[b]Significantly ($P < 0.01$) decreased compared with the matching control group No. 7.

[c]Significantly ($P < 0.01$) decreased compared with the matching control group No. 8.

of PSS had little effect on mammary RNA concentration 3 or 6 hours after inoculation. However, RNA concentration of PSS treated glands had increased significantly ($P < 0.01$) after 12 or 24 hours (Table 18).

Relative to corresponding RNA/DNA ratio at 3 hours (Group 1) after endotoxin treatment, there was a significant ($P < 0.01$) decrease in RNA/DNA ratio 24 hours (Group 4) after endotoxin treatment (Table 19). There was also a decrease in RNA/DNA ratio 24 hours after PSS treatment (Group 8) compared to 3-hour (Group 5), but that decrease was not significant ($P > 0.05$).

Effect on Selected Enzymes

Serum Enzymes

Mean values for serum GOT and GPT in endotoxin-treated guinea pigs were markedly higher than the mean values for either PSS-treated or uninoculated control guinea pigs (Table 20). Values for ALP and ACP were significantly ($P < 0.005$) decreased in endotoxin-treated guinea pigs. Sera values for CPK appeared slightly reduced as compared to PSS-treated guinea pigs, but they were nearly identical to nontreated guinea pigs (Table 20). There were no significant differences in the levels of serum LDH and ICD.

Tissue Enzymes

Histochemically, there were marked variations in the activities of ALP, LDH and SDH in the alveolar epithelial cells from one lobule to another (Figures 38 and 39). However, the activity of ALP appeared to be slightly increased in endotoxin-treated mammary glands, which was observed near the free borders of alveolar epithelial cells (Figure 40). The neutrophils present in the lumina of alveoli, interalveolar spaces,

Table 19. Influence of endotoxin on RNA/DNA ratios of lactating mammary glands of guinea pigs

Group No.	Treatment	Time (hours)	Guinea pig No. and Mammary Glands										Mean[a]
			1 Lt.	1 Rt.	2 Lt.	2 Rt.	3 Lt.	3 Rt.	4 Lt.	4 Rt.	5 Lt.	5 Rt.	
1	Endotoxin	3	3.96	4.66	4.57	5.33	5.65	4.57	4.33	7.70	3.88	3.59	4.82 ± 0.31
2	Endotoxin	6	4.09	4.17	6.09	4.77	4.22	4.96	4.67	3.85	4.66	6.53	4.80 ± 0.28
3	Endotoxin	12	2.72	2.73	3.00	3.02	2.18	1.71	2.20	2.50	2.14	2.24	2.44 ± 0.13
4	Endotoxin	24	1.52	2.44	1.39	2.45	1.54	1.81	1.86	1.97	1.82	2.00	1.88[b] ± 0.11
5	PSS	3	3.88	6.27	5.36	6.68	4.35	6.66	4.03	7.35	4.10	4.78	5.34 ± 0.41
6	PSS	6	3.84	6.20	2.95	5.05	3.78	4.20	5.63	5.24	5.57	4.22	4.67 ± 0.32
7	PSS	12	2.53	3.87	3.98	3.79	4.42	4.80	3.90	3.71	3.49	2.90	3.73 ± 0.21
8	PSS	24	4.24	2.55	3.79	2.96	3.52	3.77	5.53	5.28	4.72	4.15	4.05 ± 0.30

[a]Values are means ± standard error.

[b]Significantly ($P < 0.01$) decreased compared with group No. 1.

Table 20. Effect of endotoxin on serum enzyme values for experimental guinea pigs

Group No.	Glutamic oxalacetic transaminase (Sigma-Frankel units/ml.)	Glutamic pyruvic transaminase (Sigma-Frankel units/ml.)	Alkaline phosphatase (Sigma units/ml.)	Acid phosphatase (Sigma units/ml.)	Lactic dehydrogenase (LDH units/ml.)	Isocitric dehydrogenase (Sigma units/ml.)	Creatine phosphokinase (Sigma units/ml.)
1	101.9 ± 37.1	56.2[a] ± 12.2	1.2[a] ± 0.1	1.5[a] ± 0.1	384.0 ± 82.9	845.8 ± 49.4	8.9 ± 2.9
2	51.4 ± 7.4	31.9 ± 2.0	2.0 ± 0.2	2.3 ± 0.2	351.5 ± 80.2	841.7 ± 27.7	14.7 ± 3.2
3	42.5 ± 3.2	28.5 ± 1.5	2.3 ± 0.2	1.5[a] ± 0.2	272.0 ± 41.3	825.3 ± 32.1	8.8 ± 1.4

[a]Significantly ($P < 0.005$) different than other values in the same column.

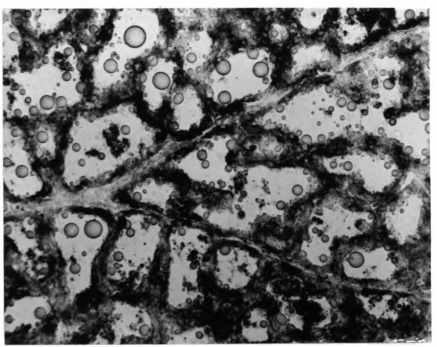

Figure 38. Mammary gland of guinea pig showing positive alkaline phosphatase activity in 3 different lobules. Note a few neutrophils in the lumina of alveoli. Substrate sodium naphthyl acid phosphate coupled with fast blue RR. x 190.

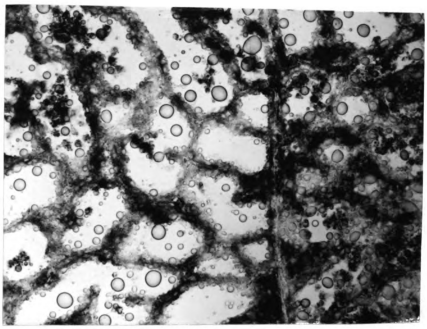

Figure 39. Alkaline phosphatase activity in the mammary gland of a guinea pig 24 hours after endotoxin treatment. Note the variations in the level of activity of the enzyme in 2 different lobules. Substrate sodium naphthyl acid phosphate coupled with fast blue RR. x 190.

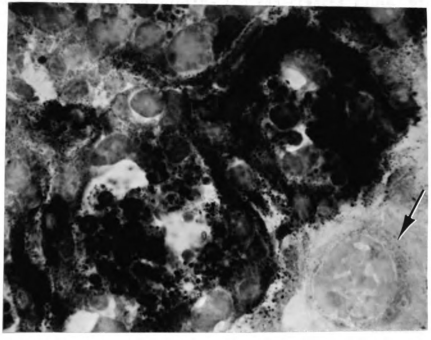

Figure 40. Intense reaction of alkaline phosphatase in the epithelial cells of alveoli and neutrophils in the lumina of mammary gland treated with endotoxin. Note little reaction in the wall of a blood vessel (arrow). Substrate sodium naphthyl acid phosphate coupled with fast blue RR. x 760.

and ducts also showed markedly intense ALP activity (Figure 41). Usually, the ALP activity was more intense in the lower 2/3 of the alveolar epithelial cells towards the basement membrane in PSS-treated mammary glands. The interalveolar stroma and fibrous connective and adipose tissues were almost negative for ALP activity compared with the mammary parenchymatous tissue having marked secretory function (Figure 42).

There was apparently no difference in the activities of LDH and SDH in the mammary glands of endotoxin- and PSS-treated guinea pigs (Figures 43 and 44). The formazan granules denoting the level of enzymes (LDH or SDH) were more numerous in epithelial cells of the alveoli than in the connective tissue septa of the mammary gland. Formazan granules were also noted consistently in or around the fat droplets (Figure 44).

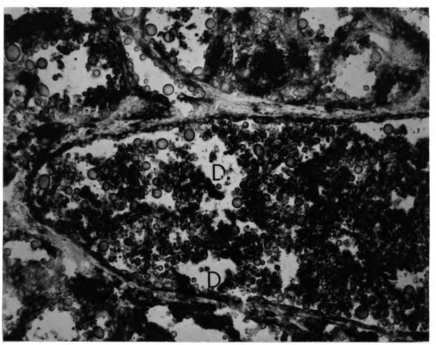

Figure 41. Lactiferous duct (D) and alveoli containing leukocytes and cellular debris showing intense reaction for alkaline phosphatase 24 hours after endotoxin treatment. Substrate sodium naphthyl acid phosphate coupled with fast blue RR. x 190.

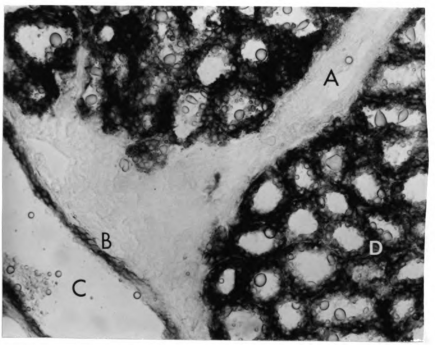

Figure 42. Mammary gland of guinea pig. Note the negative alkaline phosphatase reaction in interlobular septum (A) and subepithelial layer (B) of interlobular duct (C), compared with intense reaction in alveolar epithelial cells (D). Substrate sodium naphthyl acid phosphate coupled with fast blue RR. x 190.

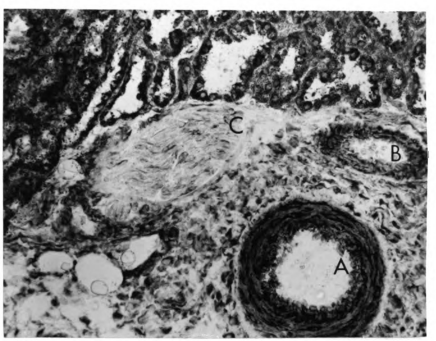

Figure 43. Strong reaction of lactic dehydrogenase in alveolar epithelial cells (top), tunica media of an artery (A) and vein (B). A faint reaction is present in nerve (C). Substrate sodium lactate coupled with NBT. x 190.

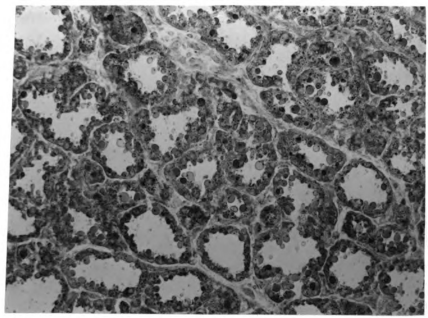

Figure 44. Strong reaction of succinic dehydrogenase in alveolar epithelial cells compared with weak reaction in interalveolar stroma and interlobular septum. Note the formazan granules in or around the fat droplets. Substrate sodium succinate coupled with NBT. x 190.

DISCUSSION

The present concept is that endotoxin is a form of liposaccharide complex which could be released on disruption of bacterial cells or the complex could be extracted from intact bacteria (Braude, 1964; Ribi *et al.*, 1964). Schalm (1965) reported that endotoxin is located on or in the cell wall of gram-negative bacteria, and is released upon autolysis. However, there is much difference of opinion among investigators as to what part of this macromolecule complex is responsible for the biologic activities. The endotoxins have a very wide spectrum of biologic activities, but not all of the activities of endotoxin are related to each other. The wide variety of recorded physiologic and pathologic effects on body tissues and different systems suggests that endotoxin is a generalized cellular poison (Kass *et al.*, 1964). One of the distinctive features of the endotoxins is their fast action (Braude, 1964).

Adipsia and aphagia resulting in concomitant body weight losses were among the most prominent signs of endotoxin treatment in mice as reported by Berry and Smithe (1959) and Turner (1964). Appetite loss was proved to be due to gastric stasis (inhibition of emptying capacity). Gastrointestinal tract motility was not measured in the present experiment, but there was significant ($P < 0.005$) decrease in body weight (6.6%) in endotoxin-treated guinea pigs after 24 hours. In PSS-treated guinea pigs, there was a loss of only 1.4% in 24 hours. The significant body weight loss in endotoxin-treated guinea pigs was probably due to anorexia as well as adipsia. Loss of appetite might be due to inhibition

of gastrointestinal tract motility similar to that observed in mice. Endotoxin also appeared to block completely the absorption or utilization of ingested food (Berry and Smithe, 1959). Although Turner (1964) reported a diarrhea in mice administered high doses of endotoxin, the feces from our guinea pigs always appeared to be normal. However, our dose of endotoxin for guinea pigs was comparatively smaller than used in mice (1.0 mg./guinea pig, mean body weight 686 $\pm$ 22 gm.). There might be some difference in susceptibility because of undefined species differences.

There are 2 routes (hematogenous and galactogenous) by which pathogenic organisms theoretically may gain access to mammary tissue. But all evidence indicates that entrance of the organism through the ductus papillaris (teat canal) accounts for most cases of mastitis (Smith and Jones, 1966). A hematogenous route of infection has frequently been postulated in coliform mastitis because of its coexistence with puerperal uterine infection (Jubb and Kennedy, 1963). Whatever may be the route of entry or types of organisms and agents involved, transient leukopenia is observed consistently in experimental and field cases of bovine mastitis during the first few days of infection (Cole and Easterbrooks, 1958; Theilen *et al.*, 1959; Schalm, 1965).

In the present study, there was a decrease in leukocytes of the peripheral blood 3 hours after endotoxin treatment (Table 8). There was a further decrease of leukocytes after 24 hours which was primarily accounted for by neutropenia (Tables 9 and 10) and lymphopenia (Table 11). Coincidentally, most hemograms showed an increase in the immature forms of neutrophils denoting a shift to the left (Table 12). The depression of leukocytes in guinea pigs having acute mastitis appears to be similar to the leukocytic response in mastitic cows during acute stages of

infection as reported by Cole and Easterbrooks (1958), Theilen *et al.* (1959), and Schalm (1965). Several factors, as explained by Carroll *et al.* (1964), enter into the development of leukopenia in mastitic animals: (a) the draining of peripheral leukocytes into the area of injury, and (b) effects produced by corticosteroids of stress on circulating lymphocytes. Braude (1964) reported that endotoxin enters the leukocytes and acts to drive them out of circulation.

Carroll *et al.* (1964) reported a twofold rise in blood glucose 5 hours following intramammary inoculation of endotoxin in a cow. A similar hyperglycemic reaction was observed in guinea pigs 6 hours after endotoxin administration (Table 16). This early hyperglycemia was attributed to gluconeogenesis as a consequence of the stress phenomenon causing mobilization of glycogen stores from the liver and other sites.

Endotoxin had a profound effect on the permeability of blood vessels in the mammary gland. This effect was observed as early as 3 hours after infection (the first observation) in teats and lactiferous sinuses where endotoxin was in direct contact with these tissues. The endothelial cells were markedly swollen and neutrophils were observed around capillaries and small blood vessels. The nuclei of the epithelial cells of the mammary gland did not appear to be affected as there was no evidence of pyknosis or karyorrhexis.

Hyaline degeneration is a heterogeneous group of tissue changes characterized by a translucent and homogeneous appearance and by acidophilia. This degeneration can be brought about by a variety of conditions which produce disturbances in protein metabolism (Runnells *et al.*, 1965). In such tissues as the cornea of the eye, stratum corneum of the skin and ovulation scars in the ovary, the presence of hyaline bodies has been considered a normal alteration. In other organs such as kidney and

mammary gland, the presence of hyaline droplets is considered an abnormal physicochemical change. Except for the acute inflammatory changes, hyaline droplet degeneration was the most prominent feature in endotoxin-treated mammary glands.

Histopathologic lesions indicated that endotoxin disrupted the epithelial cytoplasm, probably by degradation and coagulation of protein. The presence of hyaline granules in the epithelial cytoplasm as well as in the lumina of the alveoli and ducts was suggestive of its primary action on epithelial cytoplasm. Once all or part of the cytoplasm is coagulated, that part of the cell is no longer biologically active and becomes foreign material which is represented as hyaline granules or bodies in the epithelial cells or in the lumina of the alveoli. Since these changes were not observed in PSS-treated glands, it was concluded that these cytoplasmic alterations might lower the capacity for milk synthesis.

The presence of corpora amylacea is indicative of excessive desquamation of epithelial cells due to alterations in the protein structures of these cells (Runnells *et al.*, 1965). Desquamated cells or leukocytes form the central core of the corpora amylacea. By gradual and repeated precipitation of secretory materials, formation of fibrin and deposition of minerals, the whole mass becomes laminated (McFadyean, 1930; Morrill, 1938). The cellular exudates around the central cores in the alveoli become compressed, dehydrated and rolled into spherical masses. Corpora amylacea are so commonly observed in mammary glands and other tissues that they are seldom considered of any pathologic significance (Runnells *et al.*, 1965). Although corpora amylacea have never been reported in the mammary gland of guinea pigs, their occasional presence in endotoxin-treated glands and not in PSS-treated glands may be further indication of cellular alteration due to endotoxin treatment.

The elevation in DNA content reflects an increase in cell numbers which may result from stimulation of mitotic activity in the mammary gland or from infiltration of leukocytes and/or other migratory cells into the glandular parenchyma. The former possibility seems unlikely because mitotic figures are rare in mammary glands of lactating animals (Reece, 1956; Meites, 1961). There is, however, histopathologic evidence as reported by Emmel *et al.* (1926), that leukocytes emigrate into rat mammary tissue during lactation. Slater (1962) suggested that increased mammary DNA during early involution is partially due to leukocytic infiltration.

After 3 or 6 hours of intramammary inoculation of either endotoxin or PSS, there was very little change in the mammary DNA concentration of lactating guinea pigs. However, there was a significant increase of DNA concentration in mammary gland 12 or 24 hours after endotoxin treatment.

Microscopic examination of mammary tissue from our guinea pigs revealed no abnormal mitotic activity in the glandular parenchyma. Mammary tissues from guinea pigs euthanatized 3 or 6 hours after intramammary infusion of either endotoxin or PSS possessed very little inflammatory change. In mammary glands of guinea pigs killed 12 or 24 hours following endotoxin treatment, leukocytic infiltration was much more extensive as compared with PSS-treated gland. Therefore, histologic evidence obtained during the present investigation indicates that increased mammary DNA following either endotoxin or PSS treatment may be due, at least in part, to leukocytic infiltration and not to mitosis in the mammary gland.

Although endotoxin treatment resulted in increased mammary DNA concentration after 12 or 24 hours, the RNA concentration of these glands was not significantly different from that of guinea pigs killed 3 or 6 hours after treatment (Table 18). An increase in the concentration of

RNA would be the expected result of leukocytic infiltration of the mammary gland if the RNA content of the parenchymal cells remained unchanged. Indeed, PSS infusion increased mammary RNA concentrations after 12 or 24 hours concomitant with increases in DNA. These data suggest that intramammary infusion of endotoxin, but not PSS, resulted in decreased mammary cell RNA of about the same magnitude as the increase in whole gland RNA resulting from leukocytic infiltration. This view is further supported by a marked decrease in RNA per cell (RNA/DNA ratio) 24 hours after endotoxin treatment (Table 19). Relative to corresponding RNA/DNA ratios 3 hours after treatment, endotoxin treatment resulted in a 60.4% decrease ($P < 0.01$) in RNA/DNA ratio after 24 hours. In contrast, the RNA/DNA ratio 24 hours after PSS treatment was decreased 22.6%, and this decrease was not significant ($P > 0.05$). The slight dilution of mammary RNA following PSS treatment may possibly arise from a difference in RNA/DNA ratio of leukocytes and mammary parenchymal cells.

Endotoxin and PSS appear to have initial irritating effects on the mammary gland. The inflammatory response is of much greater magnitude in the endotoxin-treated guinea pigs than in those receiving PSS.

Enzymes serve many functions in the animal, and their production and subsequent activity in the blood are affected by many environmental and physiologic or pathologic factors (Roussel and Stallcup, 1966). The intrinsic cellular enzymes would probably never escape into the blood stream if the cells remained intact indefinitely. But the cells are injured in many ways leading to partial or complete destruction as a result of various physiologic and pathologic processes. With the disruption of the internal cellular structure and disintegration of the cell wall, many of the cellular enzymes leak into the interstitial fluid and find their way into the blood. The circulating plasma contains

appreciable quantities of these enzymes which have their origin in the cells of various tissues of the body (Hoffman, 1964).

This work must obviously be regarded as a preliminary step in the study of the effect of endotoxin on selected serum and mammary tissue enzymes. Although there are significant differences in the level of the activity of several enzymes (ALP, ACP, GOT, GPT, and CPK), no definite explanation has been given to elucidate the mechanism at the cellular level. It is of some importance that the effect of endotoxin on selected enzymes has been studied, but it becomes equally, if not more, important to find out the mechanisms of action which may prove an asset in clinical medicine.

There is a wide distribution of transaminase enzymes in various body tissues (Benjamin, 1965) and the enzyme levels have been found to be elevated in various diseases involving tissue necrosis (Kuttler and Marble, 1958; Cornelius *et al.*, 1959). The levels of transaminases were elevated during summer months compared to winter months (Roussel and Stallcup, 1966; Crist *et al.*, 1967; Boots *et al.*, 1969, 1970). Significant increases in the serum levels of GPT and GOT were also found in endotoxin-treated guinea pigs compared with those receiving PSS treatment. Whether these increases were due to tissue damage at the cellular level or to elevation in body temperature in endotoxin-treated guinea pigs are questions that were not answered by this work. Degenerative changes at the cellular level may or may not be recognized with light microscopy. However, there are indications of degenerative and inflammatory effects, inhibition of protein synthesis, and increase in body temperature, all of which followed endotoxin administration. Therefore, the transaminase may leak into the plasma in appreciable quantities due to degenerative changes of cellular walls. There is also a possibility of the cellular

membranes becoming more permeable (for the passage of transaminases) during a significant rise in body temperature.

Histochemical studies indicated that there was more activity of ALP in endotoxin-treated glands. This finding is in agreement with Said (1969). He reported that ALP activity was most pronounced in the nuclei and cytoplasm of epithelial cells of mammary glands of rabbits inoculated with either *E. coli* or endotoxin. The apparent increased activity of ALP in our experiment was probably due, at least in part, to a massive infiltration of leukocytes which are normally positive for ALP reaction.

The deposition of formazan granules marks the sites of intracellular enzyme activity and nonspecific reduction of tetrazolium salts does not occur to any significant extent (Barka and Anderson, 1963). Formazan granules were consistently observed in or around the fat droplets (Figure 44) indicating that the oxidative enzymes (dehydrogenases) are probably attached to fat droplets in the mammary gland.

SUMMARY AND CONCLUSIONS

Acute effects of an endotoxin from *Escherichia coli* were studied following injection into the mammary glands of guinea pigs. The inoculation of endotoxin, diluted in sterile physiologic saline solution (PSS) was made into the mammary gland via the teat canal.

Marked leukopenia, primarily due to neutropenia and lymphopenia, was observed in endotoxin-treated guinea pigs. A hyperglycemic reaction was found in guinea pigs 6 hours after endotoxin inoculation. Signs of toxemia such as increased heart rate, muscular tremors, depression, aphagia and adipsia were also observed.

Grossly, at the 12th and 24th postinoculation hours, there was no evidence of galactopoiesis, and hyperemia of the subcutaneous tissues around the mammary gland was seen. Inguinal lymph nodes were also found to be enlarged and congested.

Histopathologic changes in the mammary glands were those of acute inflammation and degeneration. Inflammatory changes in the sinus papillaris and lactiferous sinuses were evident as early as 3 hours after inoculation of either endotoxin or PSS. There was apparently little effect on mammary parenchymatous tissue until 6 hours. Marked infiltration of neutrophils into the alveoli was noticed 12 hours following endotoxin inoculation. However, slight inflammatory changes were also noted in PSS-treated glands. Cloudy swelling, hyaline droplet degeneration of the alveolar epithelial cells and the presence of a thick proteinaceous material enmeshed with neutrophils and cellular debris in the alveoli

and lactiferous ducts were the most significant microscopic findings in the gland.

An increased concentration of mammary deoxyribonucleic acid (DNA) resulted from leukocytic infiltration into the gland but failure to detect concurrent increases in the concentration of ribonucleic acid (RNA) suggested that endotoxin decreased parenchymal RNA thereby lowering the protein synthesizing capacity of the mammary gland. Although PSS also resulted in a slight inflammation after 12 hours, its effect on the RNA/DNA ratio was slight, indicating only minor influences on the capacity of the gland to synthesize protein.

Degenerative changes in the mammary gland and probably in other body tissues accompanying endotoxin inoculation characteristically elevated the serum levels of glutamic oxalacetic transaminase and glutamic pyruvic transaminase. The serum levels of alkaline phosphatase and acid phosphatase were found to be significantly decreased when endotoxin was given. The increased activity of alkaline phosphatase in the mammary gland was thought to be partly due to marked infiltration of leukocytes. No differences in the levels of lactic dehydrogenase and succinic dehydrogenase in the mammary glands of treated or control groups of guinea pigs were revealed by histochemical examination.

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