

Experimental Examination on the Mechanism of Abnormal Milk Secretion

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Mastitis is one of the principal diseases of dairy cattle. Numerous reports have been made on the bacteriological and pathological studies of mastitis. There are a few papers reporting that mastitis has been studied from the host side in pathological-physiology.

Generally, spontaneous infection disease, including bovine mastitis, does not always appear as clinical symptoms, if the host receives microorganism infection.

Nutritional and environment condition, autonomic nervous and hormonal control of host, and constitution and heredity appear to be related to the induced factor for the occurrence of mastitis.

In this paper, a few problems observed from the host side of abnormal milk secretion will be introduced.

Induced factors of abnormal milk secretion and milk quality of abnormal milk

In mastitic milk, the increase of such chemical composition as sodium, chlorine and calcium, the decrease of potassium and increase of serum protein in the milk are found. It is considered that the changes suggest the increase in capillary vascular permeability in the mammary gland, then when the abnormality of milk progressed, the leukocytes might emigrate and increase in the milk, and keratinized and apocrinized mammary glandular cells might be secreted in the milk as such solid particles as clots, shreds and flakes. Ac-

ordingly, it is considered that mastitic milk is a product by inflammatory response such as increased vascular permeability and the emigration of leukocytes in the mammary gland.

On the other hand, it is thought that the causes of abnormal milk secretion are the induced factors of inflammatory response of the mammary gland, but no mechanism about the occurrence of inflammatory response in the mammary gland has been made known yet.

The induced factors of abnormal milk secretion and milk quality of abnormal milk are shown in Table 1. Such factors as environment and feeding management, wound of udder, and subclinical diseases stimulate the autonomic nervous and endocrine system, and accelerate the secretion of such chemical mediators as catecholamine and acetylcholine and hormones.

It seems that since hormones and chemical mediators have an action to induce inflammation in the mammary gland in some process, abnormal milk is secreted, AND THAT the bacterial growth and the exhibition of toxicity of the microorganisms are found in some stages of occurrence of mastitis.

Action of hormones and inflammatory response on the mammary gland

Ungar¹⁰⁾ reported that growth hormone, mineral corticoid (DOC), thyrotropic hormone, and others have an action to activate plasmin and that in such organisms as

Table 1. Induced factors of abnormal milk secretion and milk quality of abnormal milk

Induced factors	Changes of milk
1. Environment management Atmospheric phenomena Equipment of cow shed Noise 2. Feed and feeding Undernutrition Overfeeding of concentrate Overfeeding of leguminous pasture	1. Permeability of vessels. Increase of Na, Cl, Ca, Mg, and serum protein in the milk 2. Leukocytes emigration 3. Apocrinization of epithelium 4. Decrease of casein, lactose, and citric acid
3. Latent diseases Reproductive disturbance Metabolic disturbance Digestive disturbance Chronic mastitis	
4. Hormonal unbalance Estrogen Adrenal cortex hormones Thyroid hormone	
5. Injury of udder and teat	

Staphylococcus and *Streptococcus*, some enzyme contained in the bacterial toxin participated in the production of an inflammation-inducing substance by the aid of plasmin contained in the blood plasma. It was presumed that the theory might participate in the mechanism of occurrence of mastitis.

Recently, on account of a rapid progress of the inflammatory chemistry, it has been confirmed that the occurrence of inflammation is performed by the chemical mediator called plasma kinin synthesized by the inflammatory enzymes. The plasma kinins such as bradykinin are characterized as nona-, deca-, and hendeca-peptides and synthesized by the destruction of α_2 -globulin in body fluids, and the action against inflammatory response of kinin is stronger and longer than that of histamine. Today, it is speculated that histamine is a primary mediator of inflammation and that inflammatory response is maintained by kinin which is a secondary mediator.

Inflammatory action of hormones in the mammary gland

Yoshida & Iizuka¹¹⁾ and Iizuka *et al.*³⁾ re-

ported from their experimental studies on the occurrence of bovine mastitis and the production of alcohol-test-positive milk that abnormal milk had been secreted even after the administration of estrogen, DOC and thyroxine. Then, Motoi & Iizuka⁸⁾ reported inflammatory action of adrenalin in the experimental rat mastitis. However, no mechanism about occurrence of inflammation by these hormones has been made known as yet with the exception of the action to kinin releasing enzymes.

Motoi & Iizuka⁸⁾ reported that the inflammatory response of the rat mammary gland revealed with the changes of chemical components in the mammary gland and the milk, such as an increase of glycogen, sodium, and chlorine contents and leukocytes count, and a decrease of K/Na ratio, that the strength of kinin-releasing enzymes activity seemed generally to be parallel to the intensity of inflammatory response in the mammary gland, and that the severity of inflammatory response in the mammary gland was more intense in the groups treated with estrogen and adrenalin than in the groups treated with any other hormones.

Kinin-releasing enzymes and kinin in the mammary gland

Kinin-releasing enzymes—kinin system in the mammary gland has been confirmed by Motoi & Iizuka.⁸⁾ It has been reported that kinin-releasing enzymes contained kallikrein, plasmin and trypsin. Each enzyme may be activated individually by the type of inflammation or inflammatory factors.

In fact, Motoi & Iizuka⁸⁾ reported that kallikrein was activated by bacterial endotoxin and hormones, and plasmin or trypsin is activated by bacterial exotoxin.

On the other hand, Leach⁷⁾ observed that kinin-like substance extended in the bovine milk, and Hauenaghel²⁾ *et al.* mentioned the effect of plasma kinins and kallikrein on milk ejection in the ewe.

Kinin-releasing enzymes of micro-organisms

It is thought that the inflammation by such bacteria as *Streptococcus bovis*, *Staphylococcus aureus* and *Escherichia coli* isolated from field materials of bovine mastitis, was induced by the enzymes in the bacterial toxin. These bac-

terial extracts were infused into the mammary gland, and the strength of the inflammatory response and the activity of kinin-releasing enzymes were compared 5 hours after the extract infusion.

The inflammatory response of the mammary gland by the extract of *Escherichia coli* was quite strong. The inflammatory response and the activity of kinin-releasing enzymes were less intense in the mammary gland infused alkali extract than in that infused water extract. This result is presumed to indicate that kinin-releasing enzymes might participate in the inflammatory response of the mammary gland induced by bacterial extracts.

Anti-inflammatory action of various drugs against non-specific mastitis

A decrease of milk yield is found in chronic mastitis too, not to mention in clinical or acute mastitis. No milk yield recovered without anti-inflammation and the revival of the function of the mammary glandular cell, if the pathogen in the mammary gland is sterilized by antibiotics. Here is a significance of anti-inflammation. It is apt to forget anti-inflammatory treatment, only the application of antibiotics.

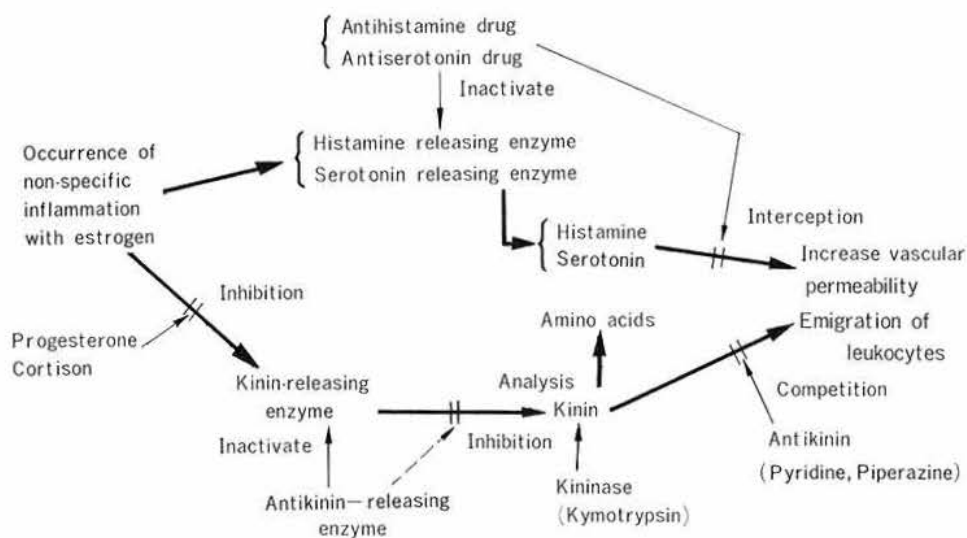


Fig. 1. Function of various antiphlogistics.

Generally, steroid hormone is used as anti-phlogistica. The application of a large quantity of steroid is undesirable because the lactating cow has a long pregnancy period. If the mechanism of occurrence of inflammation develops in the above-mentioned process, Fig. 1 shows the function of kinin-releasing enzymes, anti-inflammatory substances consisting mainly of kinin system. Some of the anti-phlogistica shown here have been applied to the disturbance of circulatory organs, non-specific inflammation, and allergic diseases in medical treatment. These antiphlogistica contain anti-kinin drugs as pyridine and piperazine derivative, then anti-histamine and anti-serotonin too.

Iizuka *et al.*^{4),6)} reported that the anti-inflammatory effects of the antiphlogistica were tested against non-specific mastitis induced by the administration of estrogen, judging from changes of the mammary gland, such as an inhibitory ability of increasing vascular permeability, histamine synthesis, leukocytes emigration, and an increase of glycogen.

From the results, it is found that there is an anti-inflammatory action in all the drugs, except in histamine. Accordingly, it is confirmed that the kinin-releasing enzymes – kinin system in the process of inflammation participates in the mechanism of abnormal milk secretion.

Bacterial growth action of estrogen in the mammary gland

There have been a number of cases of bovine mastitis complicated with ovarian cyst or endometritis in the field. In some bovine cases, mastitis has been induced by overfeeding of leguminous pasture grass containing estrogen-like substances. Iizuka *et al.*^{4),5),6)} and Yoshida & Iizuka¹¹⁾ reported from their experimental studies on the occurrence of mastitis by the administration of estrogen. From these results, it is considered that sex hormones are related to the secretion of abnormal milk.

The inflammatory actions of estrogen in the mastitis have been investigated by using the rat mammary gland.⁵⁾ Estradiol monobenzoate was injected subcutaneously to the rat at intervals of 3 hours consecutively, a single dose of *Streptococcus* was infused into the mammary gland after the administration of the secondary estrogen, and the relations to inflammatory response and bacterial growth were observed during their development period. The maximum number of bacteria in the estrogen-treated group reached the highest level 8 hours after the infusion of microorganisms and it was maintained during 24 hours.

The difference was found in the number of bacteria in the mammary gland between the estrogen-treated group and the control group treated with microorganisms alone. The inflammatory response was a little stronger in the mammary gland of the estrogen treated group than in that of the non-estrogen-treated group, but difference was hardly found between both groups. The reason is that the toxicity of the used microorganisms might be mild.

The multiplication of bacteria in the mammary gland treated with estrogen could be explained as follows: (1) estrogen has a stimulatory effect on the growth of bacteria (2) an inflammatory focus supplies a medium of bacterial growth and (3) a decline of defensive function accelerates the growth of bacteria.

About (1) described above, Frank and Pounden¹⁾ reported that estrogen and progesterone accelerated in vitro the growth of bacteria. On the other hand, San Clement and Mackenzie⁹⁾ observed that sex hormones inhibited the growth of bacteria.

Iizuka *et al.*⁵⁾ reported that three microorganisms, *Streptococcus fecalis*, *Staphylococcus aureus* and *Escherichia coli* obtained from udders affected with mastitis, were incubated in the presence of estradiol benzoate and diethylstilbestrol in concentration ranging between 0.01 $\mu\text{g/ml}$ and 10 $\mu\text{g/ml}$.

No stimulation against the growth of micro-

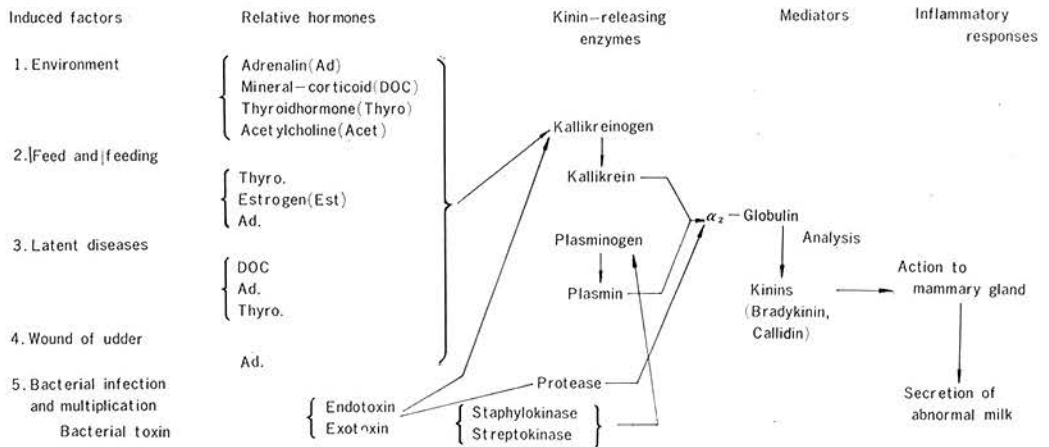


Fig. 2. A summarized scheme for the mechanism of abnormal milk secretion.

organisms was observed in any concentrations of both hormones. From this experiment, it is confirmed that the mechanism of the bacterial growth in the presence of estrogen might be explained from two other theories.

Consequently, these results and previous reports account for the scheme shown in Fig. 2 on the summary of mechanism of abnormal milk secretion.

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