EXPERIMENTAL STUDY ON THE PATHOGENESIS OF POLYMYOSITIS (ANALYTICAL INQUIRY INTO THE SIGNIFICANCE OF MYOSIN-ATPASE SYSTEM AND ATP CONCERNED WITH BACTERIAL AFFINITY FOR SKELETAL MUSCLE)

by

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Received for publication Jan. 16, 1961

INTRODUCTION

Acute suppurative myositis is a disease of frequent occurrence in Japan and Scandinavian countries. In 10 or 20% of cases of this diseases skeletal muscles are successively involved, and the disease finally develops into polymyositis. A number of theories have been advanced on the pathogenesis of this disease; some of them attach importance to the condition of the infected individual, while others lay stress on the infecting microbe. Those who advance the former kind of theories include Ueno and Furukawa (1949) who first asserted that local hypersensitivity due to myoglobin was responsible for the occurrence of the disease, and Shiraha and Iwakiri (1955) who, further developing the above theory, attributed the disease to the diminution of local resistance resulting from local hypersensitivity due to myotoxin. Ozawa (1927), too, noting the prevalence of beriberi and this disease in Japan, demonstrated that vitamin B_1 deficiency is responsible for its occurrence. On the other hand, Martinotti (1898), one of those who makes much of the condition on the part of the infecting microbe, considered myositis to be caused by a strain of staphylococcus with strong affinity for muscle tissue, namely, Staphylococcus polymyositicus, while Ishihara (1955), stating that the causative organisms of myositis and osteomyelitis possessed strong, genetically-fixed tissue-affinity, and moreover characteristic chemical properties, tried to explain their tissue-affinity from the standpoint of pluralism.

According to recent genetic biochemical studies, the metabolic process and enzyme system of the bacterium are not permanently stable but undergo adaptive changes under the influences of environmental factors, and it is possible for these adaptive changes to obtain genetic fixation. Based on this new view, Masaki, a member of our laboratory, subcultured staphylococci of one and the same strain in different media, namely in broth agar media added with a rabbit muscle extract, or with a rabbit bone-marrow extract, and succeeded in obtaining the staphylococci whose enzymochemical properties were similar to those of the so-called myo- or osteo-strain. He further produced typical myositis and osteomyelitis in rabbits,

using these subcultured staphylococci. MASAKI thus showed the rationality of explaining the bacterial tissue-affinity monistically from the standpoint of enzyme adaptation.

Concluding that myositis was due to vitamin B_1 deficiency, FUJIWARA (1959), also a member of our laboratory, further enzymochemically investigated the correlationship between the chemical environment of tissue and the formation of adaptive enzymes in the causative organisms, while DOGURA and MAEDA (1959), also associates in our laboratory, pointed out that activation by the muscle extract of bacterial enzyme activity stood in correlationship with its stimulation of bacterial growth. According to MAEDA, bacterial growth is stimulated by undialyzable and thermolabile components of muscle extract, and as for the smooth muscle extract, it does not possess such a growth-stimulating action. Clinically, too, it is known that polymyositis never originates in smooth or heart muscles, and so it is appropriate to seek one of the etiologic factor of myositis in the chemical environment of the skeletal muscle.

With regard to differences in chemical composition between the skeletal and the smooth muscles, the myosin B of the latter is poorer in actin quantity than that of the former, and its ATPase reaction is weaker. The number of ATP bonds per unit of protein in particular is markedly smaller in smooth muscle myosin than in skeletal.

The undialyzable and thermolabile component of the muscle extract mostly consists of myosin, but it is presumptious to say that because of this fact only, the myositis and the muscle-adapted strain produce adaptive enzymes concerned with myosin-ATP metabolism; and that their growth is accelerated, their enzymatic activity activated by myosin-ATPase system and ATP contained in the muscle extract. Yet it is quite possible to infer from experimental results obtained by DOGURA that either myosin-ATPase system or ATP may play some important role in the growth of the myostrain, namely, a strain of staphylococcus with affinity for skeletal muscles. The following experiments were conducted to confirm the above inference.

PART I. EXPERIMENTAL CULTURE WITH THE VIEW OF ADAPTING STAPHYLOCOCCUS AUREUS, F. D. A. 209-P STRAIN, TO THE MYOSIN AND ATP OF RABBIT SKELETAL MUSCLE

The muscle protein consists of 35% water-soluble protein and 65% neutralsalt-soluble protein. Myosin, which was first extracted as glutinous globulinoid protein, and named as such by KÜHNE in 1868, occupies about two-thirds of the latter. Myosin first attracted attention as contractile protein constituting myofibril, and in 1939 ENGELHARDT and LJUBIMORA discovered that it possessed ATPase activity. It has a molecular weight of 8.4×10^5, and is very long and thin in shape, being $30\text{Å} \times 2{,}000\text{Å}$ in size. It differs little in amino acid composition from other common proteins. The muscle-adapted staphylococci obtained by MASAKI, DOGURA and

MAEDA seem to have been adapted to myosin. As myosin has a large molecular weight, however, most of it is absorbed into the SEITZ-E. K. bacterial filter during the process of sterilization, and as a result, only a small amount of it remains in the muscle extract. In the present investigation, therefore, we tried to obtain myosin as pure as possible in the form of crystals. The myosin crystals obtained were then added to culture media in a high concentration. The staphylococci which had attained a high myosin adaptation in these high-concentration media were bacteriologically and enzymochemically compared with the muscle-adapted and the myositis strain.

Table 1 Amino acid component of myosin

Amino acid	Nitrogen %	g %
Cystine/2	0.72	1.03
Aspartic acid	7.12	11.40
Threonine	3.44	4.31
Serine	3.44	4.81
Glutamic acid	13.00	22.80
Proline	1.84	2.53
Glycine	3.27	2.92
Alanine	6.53	6.94
Valine	3.52	4.92
Methionine	1.84	3.28
Isoleucine	3.52	5.50
Leucine	6.62	10.35
Tyrosine	1.51	3.25
Phenylalanine	2.26	4.46
Histidine	3.76	2.32
Lysine	14.25	12.40
Arginine	13.80	7.13
Tryptophan	0.66	0.80
Amid NH_3	7.2	
Total	98.30	111.32

(Kominz et al)

CHAPTER 1. MYOSIN FOR EXPERIMENTAL USE

(1) Preparation of Myosin

The rabbit was rapidly bled to death by severing A. carotis. After removal of viscera, skeletal muscles alone were washed twice with cold water, and placed in ice. Two hundred grams of muscle was cut out, and triturated in a homogenizer for several minutes. The triturated muscle was then washed twice with a 0.05 M KCl solution to remove the ATP, soaked for 10 minutes in a mixed solution of 0.6 M KI and 0.06 M sodium thiosulfate, and the myosin was extracted. A myosin solution obtained after removal of muscle residue was diluted with distilled water. When the KI concentration of this solution was thus reduced to 0.04 M, myosin separated itself as white precipitate at an isoelectric point. The above process was repeated several times for purification of myosin. Filtration and washing were carried out at a low temperature to prevent lowering of enzymatic activity, and denaturation of protein. Most of all the amino acids and salts were further removed by a 24-hour-long dialysis in cold water. Finally refrigerative desiccation was employed to obtain pure myosin crystals.

As myosin is very glutinous, denaturalizes easily, has a large molecular weight and is mostly absorbed into filter paper during the process of filter sterilization, it takes a long time to get an adequate amount of myosin. In the present investi-

gation, therefore, heat sterilization was employed to prevent denaturation. When myosin was heated in the form of weak alkaline solution, there was no heat coagulation, and sterilized myosin was obtained whose isoelectric point remained practically unchanged.

(2) Myosin and ATPase

At present almost no doubt is entertained about the identity of myosin with ATPase. DOGURA experimentally showed that the extract of skeletal muscles which contained myosin in a large quantity had also a strong ATPase activity; and that the contrary was true of the smooth muscle. According to DOGURA the muscle extract accelerates the ATPase activity of bacteria, and though it still remains undecided whether this accelerating action has any relationship with myosin or not, it is interesting to note that the staphylococci which fall under the category of the myostrain have their ATPase activity promoted, as soon as they come in contact with the extract of skeletal muscles. The ATPase activity is a prerequisite to the synthesis of bacterial protein.

(3) Isoelectric Precipitation of Myosin

Myosin extracted by using a 0.6 M KI solution precipitates as white crystals when gradually diluted. The isoelectric precipitation occurs at pH 6.5 with a KI concentration of 0.04 M. The incidence of isoelectric precipitation, expressed as function of pH and variation in salt concentration, is shown in Fig. 1.

Fig. 1 Isoelectric precipitation of myosin

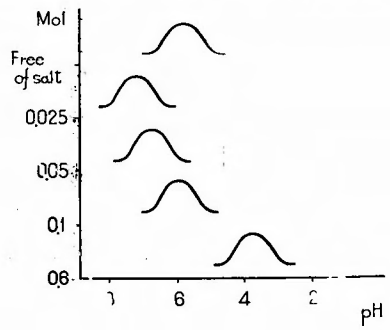


Table 2 Synthetic medium

KH_2PO_4	4.5g	$FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$	14.5mg
$MgSO_4 \cdot 7H_2O$	20mg	Glucose	2.25g
Thiamine	5mg	Nicotinic acid	5mg

L-Tryptophan	10mg		
L-Cystine	24mg		
Arginine-HCl	1900mg		
L-Proline	410mg		
Glycine	370mg		
DL-Alanine	59mg		
DL-Phenylalanine	41mg	Casamino acid	10g
DL-Valine	78mg	Tryptophan	20mg
L-Aspartic acid	98mg	Cystine	40mg
L-Glutamic acid	98mg	Alanine	50mg
L-Histidine-HCl	57mg		
L-Oxyproline	87mg		
L-Leucine	87mg		
L-Lysine-HCl	55mg		
DL-Methionine	37mg		
L-Tyrosine	45mg		

Aq. dest. 1,000 cc pH 7

Surgalla's medium	Casamino acid semi-synthetic medium

CHAPTER 2. GRADUAL REPLACEMENT OF AMINO ACIDS IN THE CULTURE MEDIUM WITH MYOSIN, AND SUBCULTURE OF STAPHYLOCOCCUS AUREUS, F. D. A. 209-P STRAIN IN THIS MEDIUM FOR THE PRODUCTION OF MYOSIN-ADAPTED STRAIN

(1) Materials

i) Amino Acid Synthetic Medium. SURGALLA's staphylococcus synthetic medium (1947) was used for subculture. Its ingredients are shown in Table 2.

ii) Myosin-containing Amino Acid Synthetic Medium. Myosin crystals produced after the methods described in CHAPTER 1 was dissolved in water, and made into a 2% solution with pH 7. This solution was added aseptically to the above-mentioned medium from which amino acids had gradually been removed. The amount of myosin added was increased properly from generation to generation (Table 3). Its final amount was 5 cc. Amino acid synthetic medium was used as control.

Table 3 Subculturing

Generation	Component of synthetic medium		
	Amino acid (cc)	Salt, vitamin etc (cc)	Myosin (2%) (cc)
1～ 5	2.5	2.5	0.5
6～10	2.5	2.5	1.0
11～15	2.0	2.5	1.0
16～20	2.0	2.5	1.5
21～25	1.5	2.5	1.3
26～30	1.5	2.5	1.5
31～35	1.0	2.5	1.5
36～40	0.5	2.5	1.0
41～45	0.5	2.5	1.5
46～50	0.5	2.5	2.0

iii) Standard Strain of Staphylococcus Aureus. Staphylococcus aureus, F. D. A. 209-P strain, kept in the Department of Microbiology, Kyoto University Medical School, was used.

(2) Subculture

As mentioned above, using synthetic media containing myosin specially prepared for each number, and control amino acid synthetic media, staphylococci coming from one and the same strain of F. D. A. 209-P were serially subcultured 50 times, each time at 37°C for 24 hours. To detect saprophytic contamination as early as possible, a loopful of culture was taken out with each number, and recultured in the broth agar medium for the observation of colonies. Stained preparations were also made and examined. The strain trained in myosin-containing media was designated as myosin-adapted strain, and the strain trained in control media as control strain.

CHAPTER 3. PRODUCTION OF ATP-ADAPTED STRAIN

The muscle extract contains a much greater quantity of ATP than it does myosin. That is, the skeletal muscle contains 250 mg of ATP per one hundred grams, but hardly any quantity of myosin. And so, when one wishes to speak on the ATPase activities of various strains, one must first ascertain the ATPase activity of the strain trained in ATP-containing media. F. D. A. 209-P strain of staphylococcus aureus subcultured 50 times in amino acid synthetic media containing ATP at the rate of 0.05% was designated as ATP-adapted strain.

PART II. THE EFFECTS OF MYOSIN AND ATP ON THE GROWTH OF ADAPTED, MYOSITIS, AND CONTROL STRAINS

As BAINBRIDGE (1911) pointed out, such heterotrophic bacteria as the staphylococcus cannot grow on myosin alone, even when adapted to it. It was attempted in PART II to ascertain whether or not myosin and ATP would accelerate the growth of adapted strains, the production of which had been described in PART I, and further how these adapted strains differed from the formerly obtained muscle-adapted and bone-marrow-adapted strains.

CHAPTER 1. GROWTH CURVES OF ADAPTED STRAINS

(1) Materials

i) Media. (a) Crude-myosin-containing medium (not subjected to dialysis, and contains a large quantity of salt and amino acid). (b) Medium containing purified myosin at the rate of 2%. (c) Myosin medium added with salt, vitamin, and sugar (the same quantities of salts, vitamins, and sugar contained in this medium as in the synthetic medium). (d) Myosin medium added with a small amount of amino acid, and with the same quantities of salts, vitamins and sugar as contained in the above medium (The quantity of amino acids in this medium is one tenth of that in the common synthetic medium, while the quantities of salts, vitamins and sugar are the same as those in the latter). (e) Synthetic medium containing myosin at the rate of 2%. (f) Amino acid synthetic medium. (g) ATP-containing synthetic medium (0.5g, 1g, and 2g of ATP, and 2g, 5g, and 10g of sugar respectively contained in 1,000 cc of this kind of media).

ii) Staphylococci for Experimental Use. (a) Control strain. (b) Muscle-adapted or bone-marrow-adapted strain. (c) Myosin-adapted strain. (d) ATP-adapted strain. (e) Mother strain. (f) Myositis or osteomyelitis strain.

(2) Methods

Staphylococci of each strain were inoculated into each medium in 0.05 mg/cc quantities. At certain intervals a part of the culture was taken out, and subjected to nephelometry, using a spectrophotometer whose wave length was adjusted to 660mμ. The turbidity thus measured was converted into cell quantity by the help of a previously prepared standard curve chart for turbidity and cell quantity. Professor TORIKATA's precipitometer was used for the measurement of cell quantity

in preparing the standard curve chart, and the turbidity due to myosin was deducted from the total value of turbidity.

For aerobic culture ERLENMEYER's flask with capacity for 200 cc was used, and shaken at proper intervals; and for relative anaerobic culture a wide-mouth test tube was used; and in the case of absolute anaerobic culture, liquid paraffin was placed on a liquid medium.

(3) Results

In the crude-myosin-containing medium the muscle-adapted strain showed an excellent growth, compared with the control strain (Fig. 2). In the purified-myosin-containing medium hardly any coccal growth was observable, and there was no variation in growth with strains (Fig. 3). Coccal growth was also weak in the medium added with salts, vitamins and sugar. But when a small amount of amino acids was added to the medium, the myosin-adapted strain began to show a rapid, active growth in contrast with the control strain whose growth was moderate (Fig.

Fig. 2 Growth curves of each strain of staphylococci in the media containing crude myosin

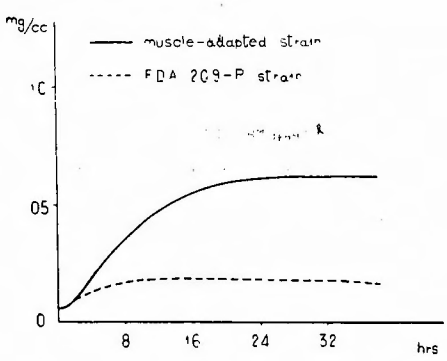


Fig. 3 Growth curves of each strain in the media containing purified myosin

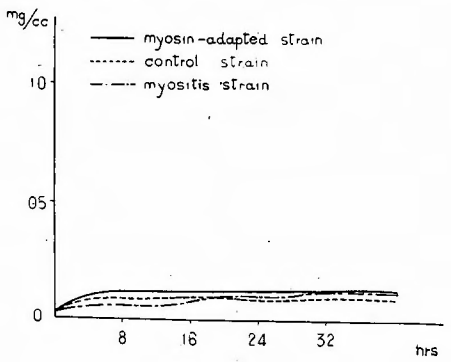


Fig. 4 Growth curves of each strain in the media containing myosin, salt, vitamin, glucose and 1/10 amount of amino acid

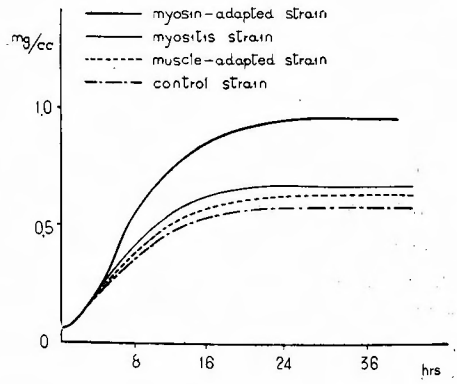
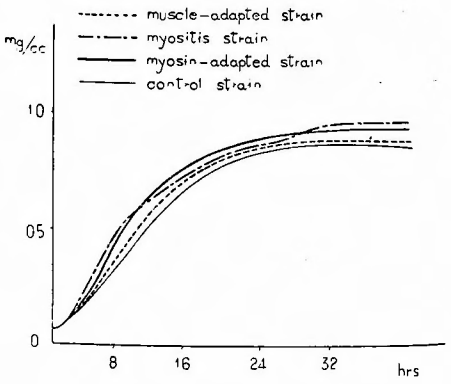


Fig. 5 Growth curves of each strain in the synthetic media containing myosin at the rate of 2% (aerobic condition)



4). Under an aerobic condition each strain grew vigorously in the synthetic medium containing myosin at the rate of 2%, and there was little difference in growth among the strains (Fig. 5). Under a relative anaerobic condition the myosin-adapted strain alone suffered little from growth-inhibiting influences, and the degree of growth differed with strains (Fig. 6). Under an absolute anaerobic condition each strain showed a very feeble growth. When the strains were cultured in the amino acid synthetic media, no marked differences were noted in the growth curves of the strains.

Fig. 6 Growth curves of each strain in the synthetic media containing myosin at the rate of 2% (relative anaerobic condition)

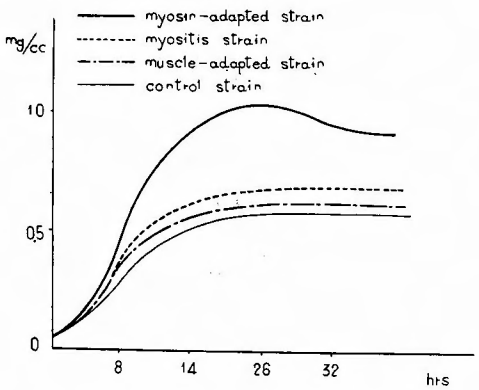
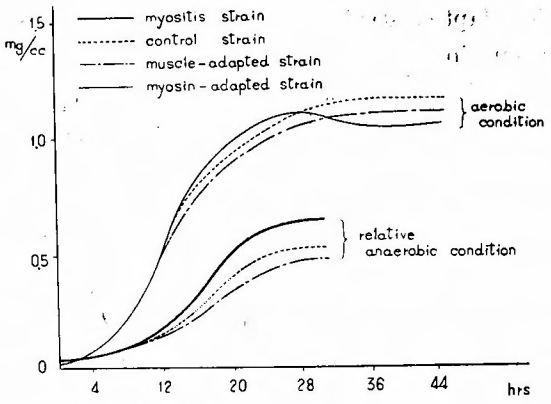


Fig. 7 Growth curves of each strain in the synthetic media



In the present investigation the coccal growth was generally excellent, and coccal quantity obtained was twice as much as that in MAEDA's experiments. This was probably due to the better aerobic condition achieved in the present investigation (Fig. 7).

As to the effects of ATP on staphylococci, MASAKI noted that it rather markedly prompted growth, when added to the medium in 0.1% concentration. But in the present investigation the addition of ATP to the synthetic medium did not seem to exert accelerating effects on coccal growth, even when glucose was added in increased quantities.

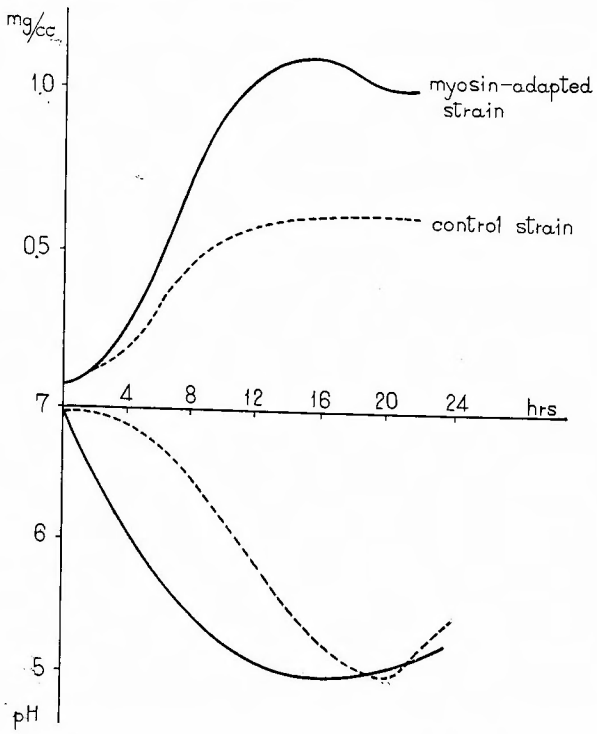
(4) Summary

The myosin of rabbit skeletal muscle promoted staphylococcal growth, and especially exerted marked accelerating effects on the growth of the myosin-adapted strain. This was perhaps because the myosin-adapted strain, in comparison with the muscle-adapted, had attained a higher degree of adaptation to myosin which was a principal protein constituent of skeletal muscles. Moreover, the myosin-adapted strain suffered less from the growth-inhibiting influence of a relative anaerobic condition than the others. The presence of myosin in the medium was noted to have been favorable for staphylococcal growth. This is perhaps because the enzyme system of protein metabolism which is not so easily liable to damage even under an anaerobic condition was induced as a result of adaptation.

CHAPTER 2. TURBIDITY OF THE MEDIUM AND CHANGES OF pH

DAVIS & STEPHENSON (1941) studies the changes of pH during the fermentation process of glucose, using Cl. acetobutylicum, and published a detailed report concerning the variations of pH with bacterial growth. For the first 12 hours bacterial growth runs nearly parallel to the lowering of pH, and after that the rise of pH is noted owing to the formation of aceton and butanol in the medium. The formation of these acids is said to be due to the adjustment of pH on the part of the bacterium whose metabolic process suffers changes in the medium. In the myosin-containing medium, too, the increase of turbidity of the medium accompanying staphylococcal growth was in parallel to the lowering of the pH in the medium for the first 12 hours. This lowering of pH was especially marked with the myosin-adapted strain (Fig. 8). This is considered to be one indication of the growth-promoting action of myosin.

Fig. 8 Growth curves and changes of pH in the myosin-containing media (relative anaerobic condition)



CHAPTER 3. MICROSCOPIC OBSERVATION OF ATTRACTION OF STAPHYLOCOCCI TO MYOSIN IN THE CASE OF PRECIPITATION OF MYOSIN ACCOMPANYING STAPHYLOCOCCAL GROWTH AND LOWERING OF PH IN THE MYOSIN-CONTAINING SYNTHETIC MEDIUM

Materials and Methods

Each strain was inoculated into the myosin-containing synthetic medium. and 12 hours later 0.02 cc of the liquid culture was taken, and dropped on a slide, taking care to make a circle of an equal diameter. The culture was then dried, fixed, stained and microscopically examined. The same procedure was repeated with another medium of the same kind, but this time the pH of the medium was lowered, not by staphylococcal growth, but by the addition of a N/10 HCl solution to the same level as that of the above mentioned medium. When the precipitation of myosin occurred, 0.02 cc of the liquid culture was taken from this medium as control material.

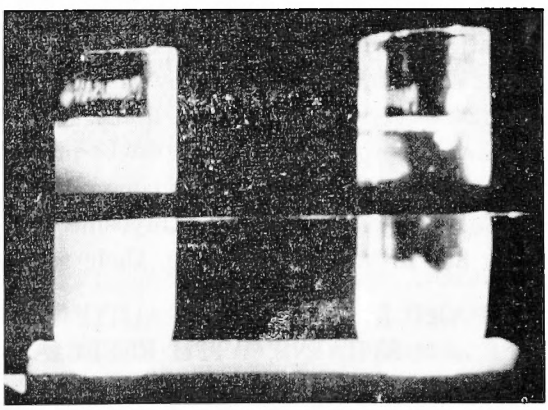
Results and Summary

Staphylococci of each strain, which had multiplied in the synthetic media containing myosin, were observed to be present in myosin in great numbers (Fig. 9). When control material was observed, however, staphylococci were found evenly distributed over the entire microscopic field, and there was noted no dense aggregation in myosin (Fig. 10). It is clear from the foregoing results that this attraction of staphylococci to myosin, in the case of precipitation of myosin accompanying staphylococcal growth and lowering of pH in the synthetic medium containing myosin is not induced by a mere physical force of absorption. It is reasonable to suppose that this attraction is due to staphylococcal chemotropism to myosin. In this experiment, however, difference in the degree of chemotropism among strains were not investigated; they will be studied in the following chapter.

CHAPTER 4. QUANTITATIVE INVESTIGATION OF ATTRACTION OF STAPHYLOCOCCI TO MYOSIN IN THE CASE OF PRECIPITATION OF MYOSIN ACCOMPANYING STAPHYLOCOCCAL GROWTH AND LOWERING OF PH

The staphylococci which have grown and multiplied in the precipitated myosin are more easily sedimented than those floating in the liquid medium; and so it is possible to separate them from each other by proper centrifugation in order to examine the effects of precipitated myosin on the unequal distribution of staphylococci in the medium (Fig. 11).

Fig. 11 Staphylococci which have grown and multiplied in the precipitated myosin are more easily sedimented (right tube) than those floating in the liquid medium (left tube)



Materials and Methods

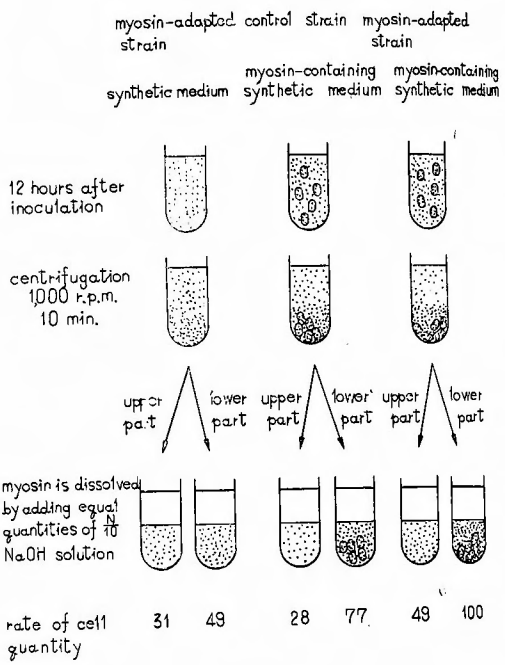
Adapted strains were cultured respectively on the amino acid, and the myosin-containing synthetic media. And 8, 12 and 24 hours later, part of the culture was taken out, and centrifuged at 1,000 r. p. m. for 10 minutes to sediment chiefly those cocci

which had multiplied in myosin. The medium was thus divided into two equal parts. These lower and upper parts were then added to equal quantities of N/10 NaOH solution. Myosin was thus dissolved, and the turbidity due to staphylococci alone was obtained. The degree of turbidity was measured with a photoelectric spectrophotometer, and converted into cell quantity.

Results and Summary

Twelve hours after inoculation the coccal distribution was found to differ with strains, that is, the myosin-adapted strain showed a higher degree of distribution in myosin fraction than the control in the myosin-containing synthetic medium (Fig. 12).

Fig. 12 Distribution rate of each strain in the synthetic media containing myosin



CHAPTER 5. DISCUSSION

Masaki noted for the first time that the muscle extract exerted promoting effects on staphylococcal growth, and somewhile later Maeda reported that the smooth muscle extract possessed no growth promoting action. Further, Dogura instituted a quantitative comparison between the ATPase of the skeletal muscle and that of the smooth muscle, and showed that ATPase existed in a much greater quantity in the former muscle than in the latter. On the other hand, there is practically no doubt about the identity of ATPase with myosin. Csápo (1950) reported that myosin was contained twice as much in the skeletal muscle than in the smooth. In the present investigation, the effects of myosin on staphylococcal growth was investigated, and it was discovered that myosin favored the growth of each strain, especially that of the myostrain. It was further ascertained that ATP was of little account as a growth-promoting factor; and that the adaptive enzymes were not easily damaged even under an anaerobic condition. Somewhile ago Dogura and Maeda proved that the growth-promoting action came from the undialyzable and thermolabile colloidal component of the muscle extract, and supposed that this colloidal component might be myosin. The results obtained in the present investigation are in accord with this, their supposition.

PART III. PROTEASE ACTIVITIES OF ADAPTED, AND MYOSITIS STRAINS, WITH ESPECIAL REFERENCE TO THEIR MYOSIN-HYDROLASE AND ATP-ASE ACTIVITIES

Myosin accelerates the growth of each strain, especially that of the myosin-adapted strain. From this fact it is reasonably considered that staphylococci develop adaptive enzymes which can make use of myosin as a protein source. With this in mind, the protease activities of these strains were studied, and compared, paying special attention to the activity of myosin-hydrolase which is an adaptive enzyme.

The growth and multiplication of bacteria depend on their uptake of peptide and amino acids produced by the resolving action of protease, their ability to synthesize protein, and their acquisition of energy for protein synthesis. Under such circumstances the study of this metabolic process assumes considerable importance.

According to Suda (1949), such energy-rich phosphate bonds as ATP are involved in the bacterial metabolic process of protein synthesis; high energy is freed from ATP in the presence of ATPase, and facilitates intra-cellular uptake of amino acids, and the synthesis of protein. Ochoa schematized this process of uptake of amino acids, as shown in the figure.

1. $\text{ATP} + \text{Amino acid} \xrightarrow[\text{pH 5 Enzyme I}]{} \text{Adenyl-amino acid} + \text{Pyro-phosphoric acid}$

2. $\text{S-RNA} + \text{Adenyl-amino acid} \xrightarrow[\text{pH 5 Enzyme II}]{} \text{Amino acid-S-RNA} + \text{AMP}$

3. $\text{Amino acid-S-RNA} \xrightarrow[\text{microsome-ribo-nucleo-proteins}]{\text{polymerization}} \text{Polypeptid} + (\text{S-RNA})n \xrightarrow[\text{stereonize}]{} \text{Protein}$

In short, bacterial protease and ATPase are considered to be enzymes engaged in protein synthesis, namely, that enzymes are indispensable for bacterial growth and multiplication. This consideration motivated the following experiments.

CHAPTER 1. PROTEASE ACTIVITIES OF ADAPTED AND MYOSITIS STRAINS, WITH ESPECIAL REFERENCE TO THEIR MYOSIN-HYDROLASE ACTIVITIES

Masaki reported that staphylococci aurei, especially those of the muscle-adapted strain, possessed a high protease activity. But is it possible that the strain adapted to myosin alone likewise possesses a high protease activity? Ishigami and Kataoka gave detailed reports on protease activity for peptone and casein, but nothing is yet known about the enzyme system for myosin. The following experiments were carried out to clarify this matter.

Materials and Methods

i) Enzyme Materials. Dried Cell Powder: Strains trained in synthetic and myosin-containing media were further cultured for multiplication in the same media at 37°C for 18 hours. Formerly the agar medium was used for bacterial multiplication, but this time the synthetic medium was used in order to maintain the

adaptive enzymes of the adapted strain in as good a condition as possible. These liquid media were then centrifuged, and from the thus collected staphylococci, myosin was washed away with a 0.6 M KI solution. After this procedure one volume of cells was treated three times with four volumes of aceton, and twice with three volumes of ether. The cells were then dried in a H_2SO_4 desiccator. The dried powder thus produced was suspended at the rate of 4-8% in glycerin-water (1 : 1), and this suspension was used as an enzyme preparation.

ii) Substrates. Peptone, casein and myosin were respectively dissolved in buffer solution at the rates of 0.5, 1.0 and 2%. The pH of solution was adjusted by properly adding a NaOH solution.

iii) Buffer solution. m/15 KH_2PO_4-m/15Na_2HPO_4 ; SØRENSEN's phosphate buffer solution was used.

iv) Strains for Experimental Use. (a) F. D. A. 209-P mother strain. (b) muscle- or bone-marrow-adapted strain. (c) myosin-adapted strain. (d) ATP adapted strain. (e) Control strain. (f) myositis strain ; from our clinic.

v) Methods. 1 cc of enzyme preparation was added to 19 cc of substrate buffer solution. And 0.5 cc of toluol was placed on this mixed solution of 20 cc. The container was corked tightly, and incubated at 37 C for 24 hours. The control solutions were prepared by using glycerin-water in place of enzyme preparation, and buffer solution in place of buffer substrate solution.

Immediately after the making of reaction solution, and 24 hours after incubation at 37°C, 5 cc of solution was taken from each container, and 1 cc of a neutralizing formalin solution was added to it. Its acidity was measured after SØRENSEN's method of formol titration. A 0.1% phenolphthalein solution was used as indicator. The increased acidity obtained by deducting a control value from a total value of acidity was considered due to enzymatic activity.

Results

(1) Optimum pH. When myosin was used as substrate, the highest increase of acidity was obtained at pH 7.5 (Fig. 13). As in KATAOKA's experiments, pH 7.5 was used as optimum for casein and peptone.

(2) Concentration of Substrate. Each substrate solution contained a substrate at the rate of 2% (Fig. 14).

Fig. 13 pH-optimum

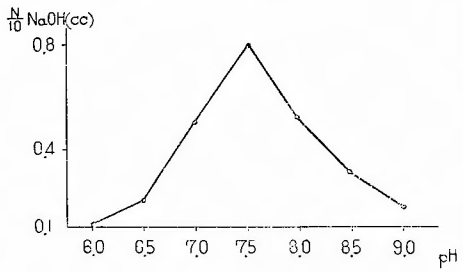
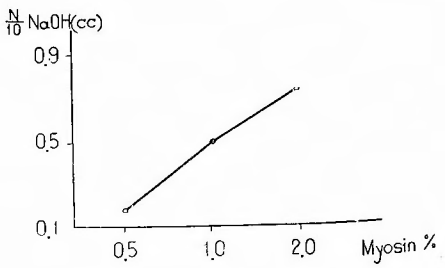
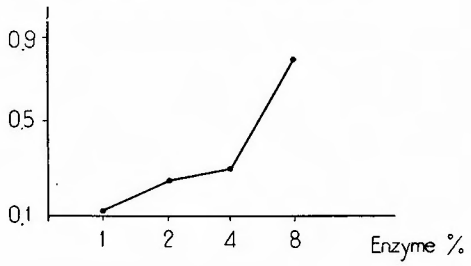


Fig. 14 Protease activities in various concentrations of substrate



(3) Concentration of Enzyme. When casein and peptone were substrates, a 4% enzyme solution was used, but as the increase of acidity was small in the case of myosin, the concentration of enzyme was raised to 8% (Fig. 15).

Fig. 15 Protease activities in various concentrations of enzyme



4) Protease Activities of Experimental Strains. The degree of enzymatic activity did not vary noticeably with strains when peptone and casein were used as substrates, but when myosin was used, the myosin-adapted strain showed the largest increase of enzymatic activity (Table 4). The enzymatic activity of the myositis strain was augmented to a higher degree than that of the muscle-adapted strain. Similar to the control strain, the ATP-adapted strain showed no increased protease activity.

Table 4 Protease activity of each strain

Strain \ Substrate	Increased acidity cc N/10 NaOH		
	Myosin	Casein	Peptone
Muscle-adapted strain	0.68	0.52	0.57
Bone-marrow-adapted strain	0.72	0.56	0.52
Myositis strain	0.80	0.58	0.81
Myosin-adapted strain	0.81	0.61	0.75
Control strain	0.70	0.58	0.68
F. D. A. 209-P strain	0.72	0.61	0.70
ATP-adapted strain	0.72	0.62	
Control strain	0.68	0.62	

Summary

By foregoing experiments, the enzyme system which can make use of myosin as protein source was clarified. And moreover it was clarified that myosin-adapted and myositis strain, as strain with affinity for skeletal muscles, showed a marked increase of myosin-hydrolase activity in comparison with the other strains.

CHAPTER 2. ATP-ASE ACTIVITIES OF ADAPTED STRAINS

Materials and Methods

i) Enzyme Solution. The procedures described in CHAPTER 1 were followed to prepare the enzyme solution. DOGURA used a suspension of living cells, but dried cell powder treated with aceton and ether is as good an enzyme material as living cells: with it the enzymatic activity is easily measured, if it is sufficiently used, and moreover, it has the advantage of making possible an exact quantitative determination of cells. The enzyme solution was prepared at low temperature, and in as short a time as possible to prevent the destruction of enzymes.

ii) Substrate Solution. ATP-Na, when put to experimental use, was solved

in a buffer solution in an adequate concentration.

iii) Buffer Solution. m/10 Veronal-N/10 HCl (MICHAELIS) buffer solution was used.

iv) Strains for Experimental Use. The same strains were used as in CHAPTER 1.

v) Methods. The method of DU-BOIS POTTER was used. The reaction solution was made by adding together 1 cc of enzyme solution, 1 cc of substrate solution, and 8 cc of buffer solution. The composition of the control solution was: 1 cc of substrate solution + 9 cc of buffer solution and 1 cc of enzyme solution + 9 cc of buffer solution.

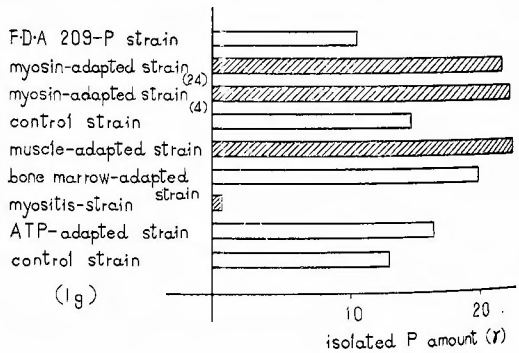
The solution were placed in ice either immediately after being prepared, or after being left in a water bath at a proper temperature for a proper period of time, and then 1 cc of solution was taken from each of the solutions, and deproteinized with a 5% trichloracetic acid solution. Free phosphorus contained in the supernatant was colored, using FISKE-SUBBAROW's method, and its quantity was measured with a photoelectric colorimeter. The balance left after the deduction of the control value from the increased quantity of inorganic phosphorus was designated as enzymolytic value of phosphorus.

In the preliminary experiment the optimum pH was ascertained to be 9.0, the optimum temperature 37°C, reaction time 5-15 minutes, and a proper substrate concentration 0.1%.

Results and Summary

The muscle-adapted strain showed the highest ATPase activity, though by a narrow margin. Then came the myosin-adapted, the bone-marrow-adapted, and the control strains in the order mentioned. As for the myositis strain, it showed a remarkably low ATPase activity. The increase of this activity of the ATP-adapted strain was very little, but in comparison with that of the control strain it may be called a relative increase (Fig. 16).

Fig. 16 ATPase activity of each strain



It is considered from the foregoing results that the increase of ATPase activity preceding the protein metabolism is a secondary phenomenon of adaptation in protein metabolism, but not due to enzymatic adaptation to ATP contained in the medium. Thus considered, the increased ATPase activity on the part of the myosin-adapted strain acquires an interesting significance.

PART IV. EXPERIMENTAL PRODUCTION OF MYOSITIS IN RABBITS, AND THE HALTING RATE OF P^{32}-LABELED STAPHYLOCOCCI

CHAPTER 1. EXPERIMENTAL PRODUCTION OF MYOSITIS WITH USE OF VARIOUS STRAINS

Staphylococci of the mother strain and the myosin-adapted strain were injected into the extensor thigh muscle of the rabbit to ascertain the minimum doses of these strains required to produce muscle abscess. KATAOKA and FUJIWARA, after trying experimentally to produce polymyositis by intravascular injections of staphylococci, stated that both the halting rate in muscles and the incidence rate of polymyositis were low; and that the development of disease depend on the suitability of the muscular chemical environment to the post-halting growth of staphylococci. Alterations in the muscular chemical composition resulting from denervation of N. ischiadicus, and the effects of denervation on the incidence of polymyositis are dealt with in the following PART V.

(1) Materials

i) Experimental Animals. Rabbits weighing 700-1,000 g were used.

ii) Experimental Strains. Various generations of the mother and the myosin-adapted strains.

(2) Methods.

0.5, 1.1, 1.5, 1.8, and 2.2 mg of staphylococci of each strain were respectively suspended in 1 cc of distilled water, and infected into the extensor thigh muscle of the rabbit. The muscle was incised 48 hours later to see whether myositis or abscess had developed.

Table 5 Production of experimental myositis by intramuscular injection of each strain

No.	Weight (g)	Strain	Quantity (mg)	Findings
53	800	My-a-s	2.2	Muscle abscess
51	980	My-a-s	1.8	Muscle abscess
45	750	C-s	1.5	Myositis
46	800	My-a-s	1.5	Myositis
47	820	My-a-s	1.5	Myositis
50	650	C-s	1.5	Myositis
24	1700	My-a-s	1.1	Nil
72	720	My-a-s	1.1	Myositis
73	780	C-s	1.1	Nil
74	780	C-s	1.1	Nil
85	780	My-a-s	1.1	Myositis
86	910	My-a-s	1.1	Myositis
87	910	My-a-s	1.1	Myositis
88	840	My-a-s	1.1	Nil
82	760	C-s	1.1	Nil
83	830	C-s	1.1	Nil
84	860	C-s	1.1	Nil
48	900	My-a-s	0.5	Nil
52	820	My-a-s	0.5	Nil

My-a-s Myosin-adapted strain, C-s Control strain

Results and Summary

Staphylococci of each strain invariably caused myositis, when used in 2.2-1.5 mg quantities (Table 5). It was histologically noted that the degree of inflammation rose in parallel with the increase of cell quantity injected. And each strain produced the same picture of inflammation. When injected in 1.1 mg quantities, the adapted strain, in comparison with the control, gave a markedly higher incidence rate of myositis, and so this quantity was assumed as the minimum causative dose. The minimum dose of the control strain was about 1.5 mg.

CHAPTER 2. INVESTIGATION OF TISSUE-AFFINITY WITH USE OF P^{32}-LABELED STAPHYLOCOCCI

KATAOKA and FUJIWARA injected proper quantities of P^{32}-labeled staphylococci of different strains into the vein to investigate their halting rates in various organs, and noted that this rate was very low for muscle. In the present investigation, therefore, staphylococci were directly injected into muscle. And the remaining rate as well as the halting rate were studied, using different strains.

Materials

i) Rabbits weighing about 900 g were used as experimental animals.

ii) 5 mc of P^{32} salt.

iii) Casamino Acid Semi-Synthetic Medium.

Methods

0.5 mc of P^{32} was added to 100 cc of the semi-synthetic medium to replace part of the phosphate taken into the staphylococcus with P^{32}.

Staphylococci of adapted strains were cultured at 37°C for 24 hours, and then centrifuged. The collected cocci were washed with distilled water. After centrifugation was repeated three times, the supernatant was ascertained to contain no radioactivity. 1.5 mg of organisms was then suspended in 1 cc of distilled water Twenty-four and 48 hours after the injection of suspension, all the extensor muscle were excised. 1 gram of muscle taken from the site of injection, and 9 g from other parts were homogenized together. And then each 1 g of them was burnt to ashes in an electric furnace to measure its radioactivity with a GEIGER counter.

Results and Summary

i) Growth Curve. The addition of P^{32} to the medium had no effect on staphylococcal growth. GEIGER counts obtained from the same quantity of cells of each strain were nearly the same.

ii) No effects were exerted on the protease activity of staphylococci.

iii) In the case of intra-muscular injection, the injected cocci spread more rapidly than had been expected. The cell quantity at the site of injection was reduced to 1/6, 24 hours later, and 48 hours later to 1/12.

Nearly similar results were obtained with each strain, but the mean value of the halting rate was somewhat higher with the myosin-adapted strain, and with regard to 1 g of muscle taken from the site of injection, the myositis strain showed a little higher halting rate (Table 6). As intense myositis developed in all the

Table 6 Intramuscular injection of P^{32}-labeled each strain (c. p. m.)

	Time hrs	A (c. p. m.)	B (c. p. m.)	Mean value (c. p. m.)	Halting rate (24 hrs) Remaining rate (48 hrs)
Myosin-adapted strain	24	33	18	19.5	10.0
	48	4	4	4	20.0
Muscle-adapted strain	24	33	12	14.1	7.3
	48	5	3	3.2	22.0
Bone marrow -adapted strain	24	30	12	13.8	7.1
	48	4	3	3.1	22.0
Control strain	24	30	11	12.9	6.7
	48	5	2	2.3	17.0
Myositis strain	24	36	14	16.2	8.4
	48	5	2	2.3	14.0
P^{32} salt	24	15	8	8.7	4.5
	48	3	0	0	0

A : 1 g of muscle taken from the site of injection
B : 1 g of homogenized muscle (9 g) taken from other parts

cases, the relation between the halting and the incidence rate could not be fully investigated. Staphylococci of the myositis strain showed a tendency for local aggregation. As for GEIGER counts, they greatly decreased 48 hours later, and no noticeable differences were noted in counts among strains. The higher halting rate and the smaller minimum causative dose of the myosin-adapted, and the myositis strains may be taken as an indication of their affinity for skeletal muscle. The question of turnover is involved in the remaining rate. GEIGER counts obtained 48 hours later were nearly natural counts. Such being the case, the remaining rate did not significantly differ among strains.

PART V. EXPERIMENTAL PRODUCTION OF MYOSITIS IN THE RABBIT WITH DENERVATED N. ISCHIADICUS

The experiments of PART IV dealt with the condition on the part of the infecting microbe which was conducive to the development of myositis, but it was therein noted that the chemical environmental factors of muscle, too, played a significant role in the causation of disease. In the following experiments, therefore, muscular atrophy and other changes of muscular composition were produced in the experimental animal by severing N. Ischiadicus. The effects of these altered conditions on the development of disease were studied, together with the muscle-affinity of adapted strains.

CHAPTER 1. ATROPHY OF M. GASTROCNEMIUS DUE TO THE SEVERANCE OF N. ISCHIADICUS

Rabbits weighing about 1 kg were used as experimental animals. The sciatic nerve on the right side was excised about 1 cm long at the level of the trochanter

Fig. 17 Muscle atrophy after denervation

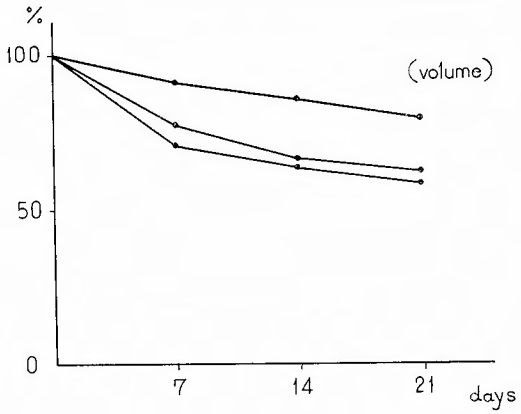
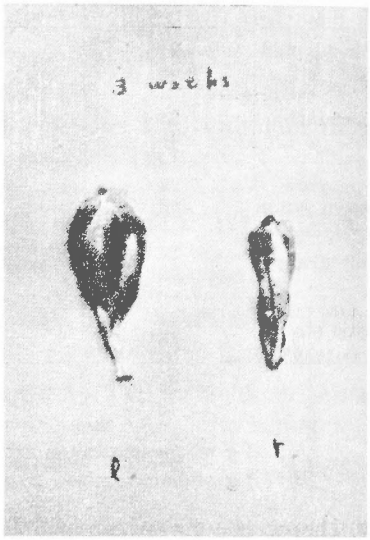


Fig. 18 Muscle atrophy after denervation
right : 3 weeks after denervation
left : without denervation



major. The control animal was subjected to surgical treatments of the same degree, but caution was exercised not to hurt its sciatic nerve.

The measurement of M. gastrocnemius was taken 1,2 and 3 weeks after the above operative procedure to investigate the progress of atrophy. Results are shown in Fig. 17 and 18. NAKAHARA, TOWER and GRODIUS had also obtained nearly the same results.

Muscular atrophy histologically produced thinning of muscle fibers and increase of interstitial connective tissue, but neutral fat remained unchanged. Chemical changes were rather conspicuous, comprising, among others, decrease of myosin amount, increase of ATPase activity (noted only in the first week after denervation), increase of oxygen consumption, increase and accumulation of creatinine and lactic acid, and increase of muscle protease activity. On the other hand, such physical factors as pH, temperature, motion and blood circulation were not influenced so much. It is particularly noteworthy that the reduction in amount of myosin was accompanied by the increase of ATPase activity.

CHAPTER 2. INTRA-MUSCULAR AND INTRA-VENOUS INJECTIONS OF ADAPTED STRAINS

Experimental animals prepared in the preceding experiment were used, and staphylococci of the myosin-adapted, and the control strain were injected. After injection the rabbits were fixed so that the right and left lower extremities might have an equal amount of motion.

Results

Intra-muscular Injection. It was hard to cause myositis with the minimum

Table 7 Production of experimental myositis by intra-muscular injection of each strain

One week after denervation

No.	Weight (g)	Strain	Quantity (mg)	Side	Findings
1	985	C-s	1.5	r	Nil
				l	Myositis
2	1010	C-s	1.8	r	Myositis
				l	Myositis
3	1000	My-a-s	1.1	r	Nil
				l	Myositis
4	990	My-a-s	1.5	r	Myositis
				l	Myositis

Two weeks after denervation

No.	Weight (g)	Strain	Quantity (mg)	Side	Findings
5	1015	C-s	1.5	r	Myositis
				l	Myositis
6	1010	C-s	1.8	r	Myositis
				l	Myositis
7	980	My-a-s	1.1	r	Nil
				l	Myositis
8	1000	My-a-s	1.5	r	Myositis
				l	Myositis

C-s Control strain, My-a-s Myosin-adapted strain

Table 8 Production of experimental myositis by intra-venous injection of each strain

One week after denervation

No.	Weight (g)	Strain	Quantity (mg)	Abscess					
				Liver	Kidney	Lung	Arth.	Mm. r	of leg l
1	1000	C-s	4	+			+		
2	1000	C-s	4.5	++			++		+
3	980	C-s	5	++			++	+	+
4	900	C-s	6	+	+		+		
5	1000	My-a-s	4	+					+
6	1000	My-a-s	4.5	+++	+		++		+
7	1100	My-a-s	5	+++	+		++		+
8	1000	My-a-s	6	+			++	+	

Two weeks after denervation

No.	Weight (g)	Strain	Quantity (mg)	Abscess					
				Liver	Kidney	Lung	Arth	Mm. r	of leg l
1	1000	C-s	4	+			+		
2	1000	C-s	4.5	+++			++		+
3	980	C-s	5	++			++	+	+
4	900	C-s	6	+	+		+		
5	1000	My-a-s	4	+					+
6	1000	My-a-s	4.5	+++	+		++		+
7	1100	My-a-s	5	+++	+		++		+
8	1000	My-a-s	6	+			++	+	

C-s Control strain, My-a-s Myosin-adapted strain

causative dose determined in PART IV, especially in the side of denervation (Table 7).

Intra-venous Injection. In the first week after denervation it was likewise difficult to cause myositis in the denervated side, and no significant difference was noted on this point between adapted and control strains (Table 8).

CHAPTER 3. COMPARISON OF INCIDENCE RATES DUE TO INTRA-ARTERIAL INJECTIONS OF ADAPTED AND CONTROL STRAINS

Experimental animals prepared in CHAPTER 1 were used. A proper quantity of staphylococci of each strain was suspended in 1 cc of a sterile isotonic saline solution, and injected precisely into the exposed A. femoralis. An incision was made in the muscle 48 hours later to ascertain the development of myositis.

Results

Injection of 4 mg-2.5 mg of cells into both Aa. femorales killed the experimental animal in 4-12 hours. No findings of polymyositis were macroscopically obtained from such cases. Likewise no development of polymyositis was noted when 4.0 mg cells were injected in one A. femoralis. Results obtained when 2.5-1.5 mg of cells were injected into both Aa. femorales are shown in the Table 9 and 10. As shown in the table, the minimum causative dose was capable of causing hematogenous infection. No proportional relationship was established between cell quantity

Table 9 Production of experimental myositis by intra-arterial injection of each strain

One week after denervation

No.	Weight (g)	Strain	Quantity (mg)	Side	Findings (Site / Volume of abscess mm³)
42	1015	My-a-s	2.5	r	Nil
				l	Nil
21	1000	My-a-s	2.2	r	Nil
				l	Myositis (M. gastroc.)
14	1020	My-a-s	2.0	r	Nil
				l	Myositis (M. gastroc.)
22	985	My-a-s	1.8	r	Nil
				l	Myositis (M. gastroc.)
8	1000	My-a-s	1.5	r	Nil
				l	Nil

Two weeks after denervation

42	1010	My-a-s	2.5	r	M. a. (M. biceps fem. 300)
				l	Nil
29	1120	My-a-s	2.2	r	M. a. (M. tib. ant. 48 M. gast. 300)
				l	Myositis (M. tib. ant.)
35	983	My-a-s	2.0	r	Myositis (M. tib. ant)
				l	M. a. (M. tib. 24 M. gast. 464 M. bicep 315)
28	960	My-a-s	1.8	r	M. a. (M. gastroc. 200)
				l	M. a. (M. gastroc. 600)
36	1008	My-a-s	1.5	r	Nil
				l	Nil

M. a. Muscle abscess, My-a-s Myosin-adapted-strain

Table 10 Production of experimental myositis by intra-arterial injection of each strain

One week after denervation

No.	Weight (g)	Strain	Quantity (mg)	Side	Findings (Site / Volume of abscess mm³)
125	980	C-s	2.5	r	Nil
				1	Nil dead after 2 hrs
141	1010	C-s	2.2	r	M. a. (M. tib. ant. 60)
				1	M. a. (M. tib. ant. 60)
129	1002	C-s	2.0	r	Nil
				1	M. a. (M. tib. ant. 27)
102	975	C-s	1.8	r	Nil
				1	M. a. (M. gast. 36 M. tib. 180)
108	1063	C-s	1.5	r	Nil
				1	Nil

Two weeks after denervation

117	1020	C-s	2.5	r	M. a. (M. gastroc. 300)
				1	M. a. (M. gastroc. 14)
145	1000	C-s	2.2	r	M. a. (M. gastroc. 112)
				1	M. a. (M. tib. ant. 60)
121	970	C-s	2.0	r	Nil
				1	M. a. (M. gastroc. 160)
101	995	C-s	1.8	r	Nil
				1	Nil
103	1010	C-s	1.5	r	Nil
				1	Nil

M. a. Muscle abscess, C-s Control strain

injected on the one hand, and the degree of inflammation and the size of abscess on the other. One week after denervation the minimum causative dose of the myosin-adapted strain was 1.8 mg in the non-denervated side, while in the denervated side, even 2.5 mg of cells were not enough to cause disease. Two weeks later the minimum causative dose was 1.8 mg in both sides, but in the denervated side the measurement of muscle abscess was smaller (Table 9) (Fig. 19) (Fig. 20). On the other hand, the minimum causative dose of the control strain one week later 1.8 mg in the non-denervated side, while 2.2 mg in the denervated side. Two weeks later it was 2.0 mg in the non-denervated side, and 2.2 mg in the denervated side (Table 10) (Fig. 21) (Fig. 22). On the whole, the minimum causative doses of the myosin-adapted strain were smaller than those of the control strain. It was especially noted that when the myosin-adapted strain was used, the incidence rate of disease was very low in the denervated side one week after denervation.

Summary

It was confirmed in the foregoing experiments that myositis was harder to cause in the denervated, atrophied muscle than in the normal. When the myosin-adapted strain was used one week after denervation, it was prominently demonst-

rated that atrophied muscle was less susceptible to myositis.

The decrease of myosin in the atrophied muscle goes on with the lapse of time, and ATPase activity shows a conspicuous increase one week after denervation. MASAKI once reported that ATP, when added to the bouillon, accelerated bacterial growth. In the present investigation, too, the increase of myosinase and ATPase activity of the adapted strain was noted. It is therefore considered that the low incidence of myositis in the atrophied muscle one week after denervation is chiefly due to the reduction in amount of myosin in muscle. The smooth muscle contains myosin and ATP in a much less quantity than does the skeletal, and its ATPase activity, too, is markedly lower. DOGURA and MAEDA experimentally proved that the activation of bacterial enzymes by the smooth muscle extract was of low potency. This experimentally-ascertained fact agrees with a clinical experience that the smooth muscle is least susceptible to myositis.

SUMMARY AND CONCLUSION

An experimental inquiry was made into the pathogenesis of polymyositis, and we tried to clarify the essential nature of the muscle-affinity of causative microbe by investigating the roles of the myosin-ATPase system, and ATP, bacterial growth-promoting factors in the muscle extract.

Do myosin and ATP really possess a growth-promoting action? If they do, how is myosin utilized by myositis and adapted strains? What are the behaviors of adaptive enzymes, protease and ATPase? How do adapted strains compare with a control strain in the ability to cause myositis? Do adapted strains show an adaptation phenomenon? Do they have tissue affinity? The present investigation was instituted to answer these questions. Results obtained are as follows.

(1) Though myosin is a principal protein constituent of muscle, the staphylococcus, one of heterotrophic bacteria, cannot grow on it alone. But myosin promotes the growth of myosin-adapted, muscle-adapted and myositis strains, when added to the amino acid medium. It is worthy of special notice that the myosin-adapted strain suffers less from the growth-inhibiting influence of a relative anaerobic condition than the control.

(2) In the myosin-containing medium, the lowering of pH occurs along with staphylococcal growth, and is accompanied by the precipitation of myosin. In such a case staphylococci of each strain, especially of the myosin-adapted strain show a tendency to unequal distribution. They are chiefly found in the myosin fraction.

(3) In the synthetic medium, ATP has no marked effects on staphylococcal growth. (MASAKI reported that ATP accelerated coccal growth in the bouillon.)

(4) The myosin-adapted, and the myositis strain show a marked increase of myosin-hydrolase activity, in comparison with the control, but no significant difference is noted between the ATP-adapted and the control strain.

(5) The muscle-adapted, and myosin-adapted strains show a high ATPase activity, but the ATPase activity of the ATP-adapted strain is only little higher than that of the control. This activity of the myositis strain is always low.

Fig. 9 Staphylococci of each strain, which have multiplied in the synthetic medium containing myosin, are observed to be present in myosin in great numbers (×700)

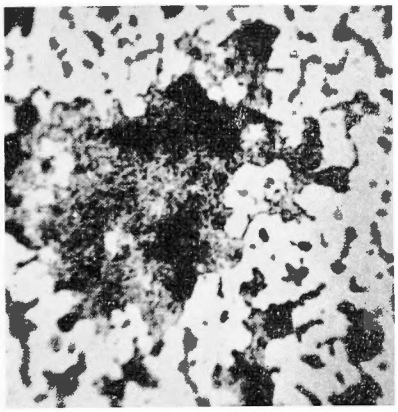


Fig. 10 Staphylococci are found evenly distributed, and there is noted on dense aggregation in myosin (×1000)

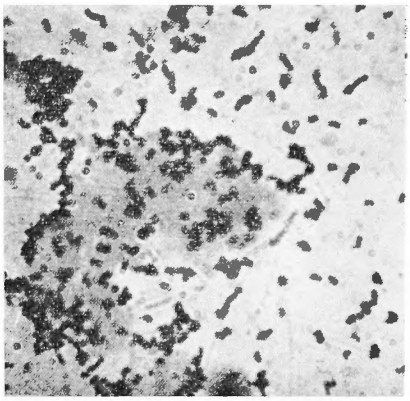


Fig. 19-22 Experimental production of myositis in rabbit with denervated N. ischiadicus by intra-arterial injection of staphylococci

Fig. 19 No. 21 in Table 9

Fig. 20 No. 35 in Table 9

Fig. 21 No. 102 in Table 10

Fig. 22 No. 121 in Table 10

From the fact that the increase of ATPase activity is small with ATP-adapted strain, but large with the myosin-adapted, it is considered that ATPase which is formed as adaptive enzyme, does not derive from ATP, a substrate in the medium, but from adaptation of the metabolic system to utilization of myosin as a protein source.

(6) Staphylococci were intramuscularly injected with a view to cause myositis and abscess, and it was found that the cell quantity required for the development of disease was smaller with the myosin-adapted strain than with the control.

(7) When P^{32}-labeled organisms were injected into muscle, the myosin-adapted, and the myositis strain showed a high halting rate.

(8) Muscular atrophy in the infected individual reduces the incidence rate of myositis and abscess, regardless of the mode of infection. Especially one week after denervation of N. Ischiadicus, the development of disease is markedly repressed. This tendency to repression is most conspicuous when the myosin-adapted strain is used. It is considered that the reduction in amount of myosin, and the increased ATPase activity due to muscular atrophy have much to do with this tendency.

The present author conducted investigations chiefly into the roles of myosin-ATPase system and ATP to solve the problems concerning the muscle-affinity of the staphylococcus, and the growth-stimulating effects of the muscle extract. He clarified the identity of myosin with MAEDA and DOGURA's undialyzable and thermolabile component of the muscle extract, and also showed the great significance attached to the utilizability of myosin as a protein source.

The enzymochemical adaptation on the part of the infecting microbe is, needless to say, of much importance, but nonetheless the condition on the part of the infected individual, too, is not to be ignored. Trauma, fatigue, degeneration and tissue chemical changes of the skeletal muscle may lead to denaturation of myosin-ATPase system and ATP, and as a result, chemical environment, favorable for the halt and growth of bacteria will be created. The present author wishes to point out the possibility that polymyositis may develop in this way.

The author wishes to thank Dr. KOICHI ISHIGAMI, the instructor of our clinic, for his many valuable suggestions and criticisms throughout the present investigation.

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多発性筋炎の成因に関する実験的研究，特に病原菌の横紋筋親和性に関係する筋ミオシン・ATPase 系および ATP の意義についての解析と検討

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最近の遺伝生化学的研究によると，細菌の代謝および酵素系は恒常的なものでなく，細菌のおかれた環境条件によつて適応変化し，しかもこの性質は遺伝的にも固定化されることが明らかとなつて来た．

この観点から，われわれの教室では同一菌株から出発したブドウ球菌を，家兎の横紋筋浸出液，骨髄浸出液を含む培地に継代培養して得られた各菌株が，それぞれ臨床的に患者からえられたいわゆる Myostrain または Osteostrain と呼ばれる菌株と酵素化学的に相似の特性を有するようになり，また動物実験においても，それらの菌株が家兎にそれぞれ定型的な筋炎或いは骨髄炎を惹起せしめうることを明らかにした．

さらに横紋筋浸出液の非透析性，非耐熱性成分が，細菌の発育促進作用と酵素能賦活作用を有することが明らかとなつたが，平滑筋浸出液には，このような発育促進作用がなかつた．

一方，臨床的に多発性筋炎は平滑筋や心筋には発症しないことが知られており，即ち横紋筋の化学的環境

に筋炎発症の一因を求めることができるものと考えられる.

横紋筋が平滑筋にくらべて化学的組成で異なるのは，平滑筋のミオシンBは横紋筋のそれよりActin量が少なく，またATPase反応も弱く，とくにまた単位蛋白量当りのATP結合点の数が平滑筋ミオシンでは横紋筋ミオシンよりも著しく少ないことである．筋汁中の非透析性，非耐熱性の膠質成分の大部分を占めるものがミオシンであるからといつて，ただちに筋汁適応菌および筋炎起炎菌がミオシン・ATP代謝について適応酵素を形成しており，筋汁中に含まれているミオシン・ATPase系またはATPによつて発育を促進され，酵素能を賦活されたとはいえないが，横紋筋親和性菌，すなわちMyostrainの発育に対してミオシン・ATPase系またはATPの果している役割はかなり重要であると推定できるので，これを実験的に検討した.

即ち，a) ミオシンおよびATPに菌発育促進が認められるかどうか，について細菌学的に合成培地を用い，菌発育による混濁度を測定した．b) 横紋筋浸出液，ミオシンおよびATPを含む培地に継代培養して得られた各適応菌および筋炎起炎菌において，ミオシンがいかに利用されているか．適応酵素として考えられるプロテアーゼ，ATPaseの態度を酵素化学的に検討した．c) 各ブドウ球菌あるいはP^{32}で標識した各菌を実験家兎に注入し，さらに坐骨神経を切断して筋萎縮を起さしめた家兎に各菌株を注入することによつて，実験的に筋炎を多発発症せしめ，適応現象，組織親和性が認められるか否かについて検討した.

その結果，

1) 筋肉の主要構造蛋白質であるミオシンだけでは，ブドウ球菌のようなheterotrophicの細菌は発育できないが，ミオシン加アミノ酸培地ではミオシン適応菌，筋汁適応菌，筋炎起炎菌などが，特に培地中のミオシンによつてその発育を促進された．比較的嫌気条件下においてミオシン適応菌の発育は対照菌の発育に比べて阻害度が低かつたことは注目に値いする.

2) ミオシン加液状合成培地において各菌株を発育せしめると，培地のpHが低下するにつれてミオシンが絮状沈澱として析出してくるが，この際各菌株とも，とくにミオシン適応菌などはその菌塊が培地の中でもとくにミオシン画分に偏在して分布する傾向を示した.

3) 合成培地中のATPが菌発育におよぼす影響は著明ではなかつた.

4) ミオシン適応菌，筋炎起炎菌のミオシン分解酵素能は亢進し，対照菌のそれらより高い値を示したが，ATP適応菌の値と対照菌の値との間には大差はなかつた.

5) ATPase能は筋汁適応菌，ミオシン適応菌において高く，ATP適応菌は対照菌よりやや高い値を示すにすぎず，筋炎起炎菌においては常に低い値を示すことが注目された．ATP適応菌のATPase能亢進は軽微で，むしろミオシン適応菌においてATPase能の亢進を認めたことから，適応酵素として成立するATPaseは培地中の基質としてのATPによるよりも，むしろ菌体がミオシンを蛋白源として利用する一連の代謝経路として成立した適応の結果であると考えられる.

6) 菌の筋肉内注入実験において，筋炎または筋膿瘍の発症に要する菌量はミオシン適応菌が対照菌より少なかつた.

7) P^{32}標識菌を筋肉内に注入した実験においてはミオシン適応菌，筋炎起炎菌が高い止着率を示した.

8) 被感染個体側の筋萎縮によつて，筋炎または筋膿瘍の発症率は菌がいずれの感染形式をとつても低下し，とくに坐骨神経切断後第1週には発症が抑制された．とくにミオシン適応菌においてこの傾向が強かつた．即ち筋萎縮に伴なうミオシン含量の減少とATPase活性の亢進が，この現象の成立に重要な意義をもつているものと考えられた.