

EXPERIMENTAL STUDY ON THE PATHOGENESIS OF POLYMYOSITIS AND POLYOSTEOMYELITIS, WITH SPECIAL REFERENCE TO THE ROLE OF THE ALTERED STAPHYLOCOCCAL TISSUE-AFFINITIES INDUCED BY ADAPTATION TO THE ENVIRONMENTS WHERE THEY DEVELOPED, AND TO THE ROLE OF THE CHEMICAL CONSTITUENTS OF BONE-MARROW

by

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INTRODUCTION

Clinical experience shows that metastasis of acute suppurative myositis is limited to skeletal muscle, while metastasis of acute infectious osteomyelitis is limited to bone-marrow. Since both diseases are caused by hematogenous staphylococcal infection, it is theoretically possible that one could be a sequela of the other; but such case has never been reported, and it is still unknown why the two diseases do not occur concurrently. Some authors have suggested that these diseases are caused by the "myo-strain" or the "osteo-strain" of staphylococci, which have a certain affinity for skeletal muscle or bone-marrow. Recently some scholars have even advocated a "pluralism of pyogenic staphylococcus", demonstrating that these two strains have separate origins. The purpose of this treatise is to answer the following questions by experimental research: Are the origins of these strains really different? Or do they only seem different because of their adaptation to their environment? In short, is the monism well-founded?

THE METHOD OF SUBCULTURING THE F. D. A. 209-P STRAIN OF STAPHYLOCOCCUS AUREUS IN AN ATTEMPT TO ADAPT IT TO THE ENVIRONMENT OF SKELETAL MUSCLE OR BONE-MARROW OF RABBIT, AND THE ENZYMOLOGICAL ALTERATIONS IN THESE "ADAPTED STRAINS".

It is generally admitted that the nature of the metabolism of micro-organisms is determined by their enzyme structure. Since all adaptation phenomena of micro-organisms are associated with changes in their metabolism, the change of enzyme structure is said to occur in various ways depending on their external and internal environment, and this change is believed to be inherited in some instances; the variant strains of micro-organisms are, therefore, believed to develop through hereditary changes in their enzyme structures.

If this is true, is there any enzymological difference between the "osteo-strain" and "myo-strain" and other common strains of staphylococci? Or is it possible to artificially supply the standard strain with a affinity for bone or muscle, by training

the former ? To clear up this point, the following experiment was performed.

CHAPTER 1. THE METHOD OF SUBCULTURING THE F. D. A. 209-P STRAIN OF STAPHYLOCOCCUS AUREUS IN AN ATTEMPT TO ADAPT IT TO THE ENVIRONMENT OF SKELETAL MUSCLE OR BONE-MARROW OF RABBIT.

In order to cultivate and develop staphylococci, and to adapt them to the environment of the skeletal muscle or bone-marrow of rabbit, two methods are available: (1) to provoke inflammation in the skeletal muscle or bone-marrow of living rabbits; (2) to culture staphylococci in media containing the tissue extract of fresh skeletal muscle or bone-marrow. Although the former method is considered to be most desirable, it has the serious defect of making it impossible to estimate the influence of various other factors (such as the change of pH in the inflammatory lesion) on the staphylococci. Therefore, I used the latter method.

1. *Materials and Methods.*

(1) Broth agar media containing fresh muscle or bone-marrow extract of rabbits. Fresh skeletal muscle and bone-marrow were taken from an adult rabbit by surgical operation, and to each of these were added the same weight of sea-sand and four times the weight of physiological saline solution. These mixtures were ground in a mortar until they became homogeneous, and 0.5 cc of clear liquid obtained by centrifugation was added to 4.5 cc of three per cent broth agar media at 48°C. The solutions were gradually cooled in ice water. These procedures were performed aseptically. Another method was also tried: aseptically filtered tissue extract was added to broth agar media at the same rate as before. These media containing tissue extract were kept in a refrigerator. Before using these media, non-contamination was ascertained by keeping them at 37°C for 24 hours. The broth used in this study consists of 5.0 g of Polypeptone (TAKEDA), 5.0 g of meat infusion (Kyokuto), 1.5 g of NaCl and 500 cc of distilled water. The pH of this broth was neutralized by adding N/10 NaOH solution. Agar powder was added to this broth. This was sterilized in an autoclave, at 2 kg/cm² pressure. The pH of the tissue-containing media was 7.0, though the tissue extract itself was slightly alkaline.

(2) Standard strain of staphylococcus aureus. Staphylococcus aureus F. D. A. 209-P, kept in the Microbiological Department of Kyoto University Medical School, was employed in this experiment.

(3) Method. Staphylococcus aureus F. D. A. 209-P was successively subcultured on media containing tissue extract every 24 hours at 37°C. This strain was subcultured 70 times in the muscle-extract-containing media, and 40 times in the media containing bone-marrow extract. The strain trained in the muscle-extract-containing media was called THE MUSCLE-ADAPTED STRAIN, and the strain trained in the media containing bone-marrow extract was called THE BONE-MARROW-ADAPTED STRAIN. In this experiment the media containing bone-marrow extract were always produced by the second method described in (1). But in producing the muscle-extract-containing media, the first method was employed

for the first forty subcultures, and for the subsequent subcultures the second method was employed. During this experiment, the colonies were frequently isolated and were examined to determine the absence of contamination.

2. *Results.*

Little difference could be found between these adapted strains and the control strains, in shape, colour and size of colonies. Being forced to abandon the experiment during the summer, we left these cultures at room-temperature for two or three months. When they were again subcultured in the autumn, the muscle-adapted strain formed plentiful colonies on the muscle-extract-containing media, but very scanty colonies on other media; the bone-marrow-adapted strain formed plentiful colonies on the media containing bone-marrow extract, but very scanty colonies on other media.

3. *Discussion.*

This experiment of adding fresh muscle or bone-marrow extracts to the broth agar media was successful. Our special attention is called to the fact that the adapted strains kept through the summer formed plentiful colonies on the very media where they had been trained.

CHAPTER 2. THE EFFECT OF MUSCLE EXTRACT ON THE GROWTH OF STAPHYLOCOCCUS.

It was experimentally proved by Tria and Wakayama that the rare occurrence of myositis could be ascribed to a bactericidal effect of fresh muscle extract upon staphylococcus; that is, to the existence of bactericidin, which is said to be destroyed by heating for two hours at 60°C. In order to confirm this fact, the following supplemental experiment was performed.

1. *Materials and Methods.*

(1) Muscle extract. The filtering method, described in chapter 1, was used in preparing this extract.

(2) Tested strains of staphylococcus aureus.

a. Control strain.

b. Muscle-adapted strain.

c. Myositis strain. This strain was isolated from the pus of a myositis case in our clinic.

(3) Broth media. The broth was prepared as described in chapter 1, and repeatedly filtered till it became quite clear. 4.5 cc of which was placed in test-tubes and autoclaved at 2 kg/cm². In order to prevent turbidity, the tops of these test-tubes were plugged with gauze-covered cotton stoppers.

(4) Method. 1.0 cc, 0.5 cc and 0.25 cc of fresh muscle extracts were added to 4.5 cc of broth media aseptically. The quantity of the homogeneously suspended living cells in physiological saline solution was measured by Professor Torikata's precipitometers, and was adjusted to contain 0.0028 cc of living cells per 10 cc. These homogeneous suspensions of each strain were added to broth media containing fresh muscle extract, and to ordinary broth media, and incubated for 24 hours at 37°C. The quantity of living cells in these cultures was measured according to

electrocolorimetric turbidity. In measuring them, therefore, the cultures had to be well shaken so as to homogenize the living cells. The increase in turbidity due to added muscle extracts was subtracted from the total turbidity. The method employed here in measuring the quantity of living cells in clear broth media is based on the principle that the turbidity of broth media is proportionate to the quantity of living cells as measured by Professor Torikata's precipitometers.

2. Results (Table 1).

In ordinary broth media, the muscle-adapted strain and the control strain showed just the same amount of growth, and the myositis strain grew a little vigorously. In broth media containing 1.0 cc of muscle extract, the growth of the myositis strain was markedly greater than that of the other strains, and the muscle-adapted strain was next. When 0.5cc of muscle extract was added to 4.5 cc of broth media, the growth of the myositis strain and the muscle-adapted strain was much accelerated as compared with their growth in ordinary broth media, while that of the control strain was inhibited. In broth media containing 0.25 cc of muscle extract, the growth of all tested strains was promoted.

Table 1. The effect of muscle extract on the growth of staphylococcus.

Quantity of muscle extract added to 4.5cc of broth media (cc)	Strain	Myositis strain	Muscle-adapted strain	Control strain
1.0		1.80	0.80	0.25
0.5		3.20	2.47	2.07
0.25		3.49	3.24	2.46
0.0		2.80	2.25	2.25

(mg/cc)

3. Discussion.

The activating effect of muscle extract on the growth of the muscle-adapted strain was already observed in chapter I, with respect to comparatively old cultures which had been kept for several months. In this experiment, the activating effects of muscle extract on the growth of the myositis strain and muscle-adapted strain was observed using fresh cultures. The results seem to show the induction of a new strain whose growth becomes vigorous when muscle extract is added, when staphylococcus aureus F. D. A. 209-P is subcultured on media containing muscle extract. Although no definite relationship could be substantiated, muscle extract itself is considered to have a certain bactericidal effect on the growth of staphylococcus.

CHAPTER 3. ON THE PEPTONASE ACTIVITIES OF THE ADAPTED STRAINS AND CONTROL STRAINS.

Imaizumi has already reported in detail on the peptonase activity of staphylococcus. The following experiment was performed according to his method.

1. Materials and Methods.

(1) Enzyme preparation. Dried cells prepared by the following method were employed. Colonies of staphylococcus were gathered and washed with physiological

saline solution. The sediment made by centrifuging these suspensions was mixed with four times as much pure acetone, and the mixture was filtered through a funnel, and dried by adding ether. These procedures had to be done within three minutes, lest the enzyme should be destroyed. The dried cells were kept in a H_2SO_4 desiccator, and, before actually using these, glycerin-water (glycerin 1 + distilled water 1) was added to these dried cells. This mixture was forcibly ground in a mortar for thirty minutes.

(2) Substrates. Peptone-buffer solution (WITTE, TERUUCHI) was employed. The solution was adjusted to the desired pH by adding N/10 NaOH solution.

(3) Buffer solution. SPRENSSEN's phosphate buffer solution was employed.

(4) The muscle and bone-marrow extracts. These were made by the filtering method.

(5) Strains tested :

- a. Control strain.
- b. The muscle-adapted strain.
- c. Staphylococcus aureus isolated from the pus of a child with acute osteomyelitis in our clinic.
- d. Staphylococcus aureus isolated from the pus of a myositis case in our clinic.
- e. Staphylococcus aureus isolated from the pus of a chronic osteomyelitis case in our clinic.

(6) Method. Test mixture: 2 cc of 4% enzyme solution was mixed with 16 cc of peptone-buffer solution. The final concentration of peptone was 1.5%. The mixture was incubated for 24 hours at 37°C under toluene. The increased acidity in 5.0 cc of the digested solution was measured by the method of formoltitration with N/10 NaOH solution. The increased acidity in an enzyme buffer mixture without substrate, which was incubated under the same conditions, was reduced from that of the test mixture.

2. Results.

(1) pH-optimum (Table 2). The pH-optimum of the hydrolysis of peptone by the dried cells of the control strain was pH 7.5.

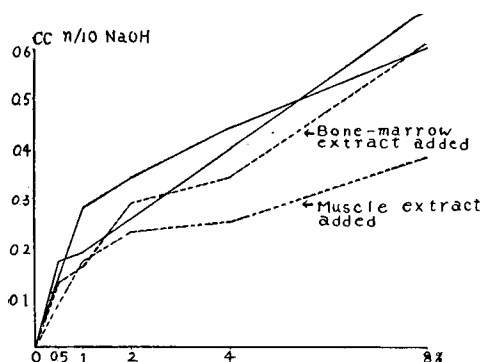
Table 2. pH-optimum.

pH	6.0	6.5	7.0	7.5	8.0
Increased acidity. cc (N/10 NaOH)	0.21	0.22	0.57	0.96	0.57

(2) Peptonase activities in various concentrations of enzyme preparation (Table 3, Figure 1).

(3) The influences of muscle extract and bone-marrow extract on the enzymatic hydrolysis of peptone by the dried cells (Tables 4, 5, 6, 7). Reaction mixture: 2 cc of enzyme preparation, 18 cc of substrate-buffer solution (final concentration of peptone was 1.5%, pH 7.5) and the muscle extract (0.1 cc). Mixtures as control : glycerinwater in place of the enzyme preparation, buffer solution in place of the peptone-buffer solution, physiological saline solution in place of the muscle extract.

Figure 1. Peptonase activities of the control strain in various concentrations of enzyme preparation.



All these mixtures were incubated at 37° C and tested as above-mentioned.

The fresh muscle extract itself showed no peptonase activity; and it inhibited the enzymatic activity of the dried cells of the control strain. The fresh muscle extract prepared with distilled water inhibited the enzymatic activity of the dried cells of the control strain. The relation between the inhibitory effect on the enzymatic activity of the dried cells of the control strain and the quantity of the muscle extract could not be determined.

Table 3. Peptonase activities of the control strain in various concentrations of enzyme preparation.

Concentration (%)	Increased acidity. cc (N/10 NaOH)		
	Control	Muscle extract added (0.1cc)	Bone-marrow extract added (0.1cc)
8	0.60	0.38	
4	0.44	0.25	
2	0.34	0.23	
1	0.28	0.17	
8	0.68		0.61
4	0.40		0.34
2	0.34		0.29
1	0.19		0.16
0.5	0.17		0.13

Table 4. The inhibitory effect of muscle extract on the peptonase activity of the control strain.

	Increased acidity. cc (N/10 NaOH)		
	Teruuchi	Teruuchi	Witte
Spontaneous hydrolysis of peptone	0.0	0.02	0.02
Hydrolysis of peptone by muscle extract.	0.10		0.23
Hydrolysis of peptone by muscle extract prepared with distilled water.	0.10		0.23
Hydrolysis of peptone by enzyme preparation.	0.77	0.82	1.59
Muscle extract added.	0.72	0.35	1.53
Muscle extract prepared with distilled water added.	0.70		1.59
Substrate	Teruuchi	Teruuchi	Witte

Every muscle extracts prepared by the following methods showed inhibitory effects: (i) Muscle extract prepared by aseptic procedure using physiological saline

solution; (ii) Muscle extract prepared by the filtering method, immediately after bleeding a rabbit to death; (iii) Muscle extract prepared by the filtering method, using the muscle of a rabbit removed 24 hours after bleeding to death. On the other hand, the muscle extracts prepared by the following methods showed no inhibitory effect on the enzymatic activity of dried cells of the control strain:

(i) The muscle extract prepared by the filtering method, using stiffened muscle of a rabbit; (ii) The muscle extract heated for five minutes in boiling

Table 5. The effects of muscle extracts in various quantities on the peptonase activity of the control strain.

Quantity of muscle extract	Increased acidity. cc (N/10 NaOH)
Control (0)	0.69
0.05cc	0.36
0.10cc	0.55
0.20cc	0.58
0.40cc	0.47

water. It was also observed that rabbit serum inhibited the enzymatic activity of dried cells of the control strain, but the serum diluted by adding physiological saline solution or distilled water had no influence on the enzymatic activity of dried cells of the control strain.

Table 6. The effects of muscle extracts prepared by various methods.

Kinds of added muscle extracts	Increased acidity. cc (N/10 NaOH)
Control	0.74
Muscle extract prepared by surgical procedure	0.58
Muscle extract prepared from a rabbit killed by bleeding	0.52
Muscle extract prepared from a rabbit left for 24 hours after death	0.65
Muscle extract prepared from stiffened muscle	0.78
Boiled muscle extract	0.73
Serum	0.58
Control II	0.78

Table 7. The effects of muscle extract and serum on the peptonase activity of control strain.

	Increased acidity. cc (N/10 NaOH)
Control	0.38
Muscle extract added (0.1cc)	0.27
Hemolytic serum added (0.1cc)	0.37
Clear serum added (0.1cc)	0.36

(4) The peptonase activities of the muscle-adapted strain and the control strain (Tables 8 and 9). No special differences could be observed between the peptonase activities of these two strains. When 0.1 cc of muscle extract was added to both

of them, however, the peptonase activity of the dried cells of the control strain was markedly inhibited, while that of the muscle-adapted strain was slightly inhibited. The inhibitory rate was denoted as follows; Inhibitory rate: I. R.

$$\text{I. R.} = \frac{A - B}{A} \times 100\%$$

A : Increased acidity when no muscle extract was added.
B : Increased acidity when muscle extract was added.

Table 8. The peptonase activities of the muscle-adapted strain and control strain.

Number of subcultures on muscle-extract-containing media		Increased acidity. cc (N/10 NaOH)			
		17	29	37	46
Adapted strain	control	0.83	0.88	0.77	1.16
	muscle extract added	0.74	0.82	0.67	1.07
	inhibitory rate (%)	10.8	6.8	13.0	7.7
Control strain	control	0.82	0.87	0.93	1.00
	muscle extract added	0.47	0.59	0.76	0.84
	inhibitory rate (%)	42.7	32.1	18.3	16.0

Table 9. The peptonase activities of the muscle-adapted strain and control strain.

Substrate		Increased acidity. cc (N/10 NaOH)			
		Peptone (Witte)	Peptone (Teruuchi)	Casein	Gelatine
Adapted strain	control	0.62	0.57	0.45	
	muscle extract added	0.58	0.50	0.35	
	inhibitory rate (%)	6.4	12.3	15.5	
Control strain	control	1.27	0.97	0.61	0.35
	muscle extract added	1.08	0.72	0.45	0.22
	inhibitory rate (%)	15.0	25.8	26.2	37.1

The increased acidity caused by hydrolysis of casein and gelatine was measured in the same way as we measured peptonase. The caseinase and gelatinase activities of the muscle-adapted strain and control strain are illustrated in Table 9. (5) The enzymatic activities of the dried cells of the tested strain c, d and e (Table 10). By adding 0.1 cc of muscle extract, the peptonase activity of the tested strain d

Table 10. Peptonase activities of the myositis strain and osteomyelitis strain.

Strain	Increased acidity and inhibitory rate				
	Control	Muscle extract added	Inhibitory rate	Bone-marrow extract added	Inhibitory rate
F. D. A. 209-P strain	1.16	1.06	9.4%	1.04	11.3%
Myositis strain	1.15	1.21	-5.6%	—	—
Child-osteomyelitis strain	1.36	1.20	13.3%	1.31	3.9%
Osteomyelitis strain	1.18	1.06	11.3%	1.26	-6.4%

was inhibited less than that of the muscle-adapted strain. The peptonase activity of the tested strains c and e were activated by adding 0.1 cc of bone-marrow extract, though these were inhibited by adding the same amount of muscle extract. As to the peptonase itself, the tested strain c showed the most marked activity, as compared with the other strains.

3. Discussion.

The inhibitory effect of muscle extract on the peptonase activity of staphylococcus seems to have a connection with the results of the experiment described in chapter 2. The serum was diluted, as it contained several times as much soluble protein as muscle extract refractometrically measured. It is interesting to note, in connection with the results of chapter 1 and 2, that there is a difference between the inhibitory effects of muscle extract on the muscle-adapted strain and control strain, and that muscle extract or bone-marrow extract activates the peptonase activity of muscle-and bone-marrow-adapted strains respectively.

CHAPTER 4. THE PHOSPHATASE ACTIVITIES OF THE ADAPTED STRAINS AND CONTROL STRAINS.

Some authors have indicated it should be prescribed by the very intense phosphatase activity of prostate cancer cells that the metastasis of them is often confined exclusively to the bone. Are there any similar facts to be observed in regard to osteomyelitis? It has been stated by Boivin and Mesrobeau that staphylococcus aureus possesses two phosphatase systems which exhibit optimal activity at pH 7 and 10. This conclusion was based on studies involving the autolytic liberation of free phosphate from the complex esters of the cells. This experiment was modelled on the joint study of Heard and Wynne, who had investigated the phosphatase activity of *Clostridium acetobutylicum*.

1. Materials and Methods.

(1) Enzyme preparation. A buffered suspension of living cells was employed. The quantity of living cells was measured by Professor TORIKATA's precipitometers, and this suspension was used immediately after preparation.

(2) Substrates. The glycerophosphates used were "sodium glycerophosphate" prepared by the Merck Co. and the Tokyo Kasei Co.; the latter was proven to be a β -ester, but whether the former was an α -ester or a β -ester was uncertain.

(3) Buffer solution. M/10-glycocoll-N/10 NaOH buffer solution was used.

(4) $MgSO_4$ solution. $MgSO_4$ was dissolved in distilled water.

(5) Tested strains of staphylococcus aureus.

a. Control strain.

b. Bone-marrow-adapted strain.

c. Muscle-adapted strain.

d. Tested strain d. This strain was isolated from the pus of a case with calcaneal osteomyelitis.

e. Tested strain e. This strain was isolated from the bone-marrow of a rabbit that died within 24 hours after the intravenous injection of a culture of the tested strain c described in chapter 3.

f. MgSO_4 -trained strain and control strain. The solution of MgSO_4 was diluted by 3% broth agar, and the final concentration of MgSO_4 was 0.05 mol. This mixture was divided into test-tubes, and autoclaved at 2 kg/cm^2 . The staphylococcus aureus F. D. A. 209-P was successively subcultured twenty times on those media, and this strain was named " MgSO_4 -TRAINED STRAIN". The control strain was subcultured on 3% broth agar media. To get the enzyme preparation of this strain, it was cultivated on both broth and broth agar media, each of which contained 0.1 mol of MgSO_4 .

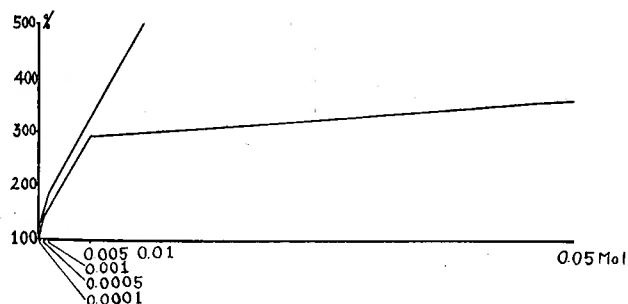
(6) Method. The reaction mixture was as follows; 1.0 or 2.0 cc of buffered suspension of living cells, 5.0 cc of substrate solution, 5.0 cc of buffer solution and 0.5 cc of toluene. In the control mixture buffer solution was used in place of a buffered suspension of living cells, and in place of the substrate solution distilled water was used. All mixtures were shaken in test-tubes and incubated at 37°C . Before and after incubation, 1.0 cc of each mixture was removed and added to 9 cc of trichloroacetic acid solution, which was filtered, and 3.0 cc of each filtrate was removed with a pipette. The phosphorus values of the filtrates were measured by FISKE-SUBBAROW's method using an electrocolorimeter. Phosphorus values were expressed either as mg% of isolated inorganic phosphorus per mg. of living cells, or as mg% of isolated inorganic phosphorus in 1.0 cc of the reaction mixture. The phosphorus values of the control mixtures were lower than those of the reaction mixtures.

2. Results.

(1) The pH-optimum and the relation of reaction velocity to substrate concentration. All attempts to measure the optimal pH of the hydrolysis of glycerophosphate by living cells having failed, the following experiments were performed at pH 10, according to Boivin and Mesrobian's study. No definite relation could be found between the reaction velocity and the substrate concentration. While the above experiment was being repeated, however, it was found that a dense solution (over 5%) of glycerophosphate could not be fermented by 150 mg. of living cells—the quantity usually employed in this experiment; and 2.5% glycerophosphate solution proved to be the most suitable.

(2) The effect of magnesium ions (Table 11, Figure 2). The MgSO_4 solution was added to the reaction mixtures, and the final concentrations of MgSO_4 in these

Figure 2. The effect of magnesium ions on the alkaline phosphatase activity of the control strain.



mixtures are shown in Table 11. The mixtures were incubated for 24 hours at 37°C , and the increased amount of inorganic phosphorus was measured. The activating effect of magnesium ions on the phosphatase activity of living cells prepared from the control strain is shown in Table 11.

Table 11. The effect of magnesium ions on the alkaline phosphatase activity of control strain.

Enzyme preparation	Concentration of substrate	Concentration of Mg ions	Isolated P in 24 hours (mg%)	Activation rate (%)
Aceton-ether-dried cells	8%	0	3.7	100
		0.01 mol	18.5	500
		0.001 mol	6.95	187
		0.0001 mol	4.75	128
Living cells	2.5%	0	0.6	100
		0.05 mol	2.15	358
		0.005 mol	1.75	291
		0.0005 mol	0.85	141

Figure 3. The alkaline phosphatase activities of the $MgSO_4$ -trained and the control strain.

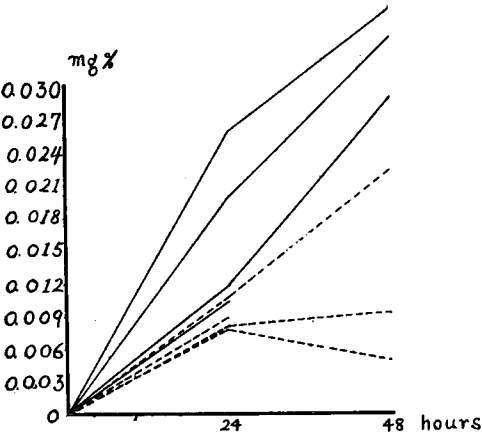


Table 12. The alkaline phosphatase activities of $MgSO_4$ -trained strain and control strain.

Strain	Isolated P per mg. of living cells			
	$MgSO_4$ -trained strain		Control strain	
Hours incubated	24	48	24	48
	0.010	—	0.009	—
	0.012	0.029	0.008	0.009
	0.026	0.038	0.011	0.023
	0.020	0.034	0.009	0.005

strain (Table 14, Figure 5). No special differences could be perceived between them.

(6) The activating effects of magnesium ions on the alkaline phosphatase activities of various strains (Table 15). The rate of activation was denoted as follows :

(3) The differences between alkaline phosphatase activities of the control strain and the $MgSO_4$ -trained strain (Table 12, Figure 3). The $MgSO_4$ -trained strain showed marked alkaline phosphatase activity as compared with the control strain.

(4) The differences between alkaline phosphatase activities of the muscle-adapted strain and the control strain (Table 13, Figure 4). No special differences could be perceived between them.

(5) The differences between the alkaline phosphatase activities of the bone-marrow-adapted strain and the control

Table 13. The alkaline phosphatase activities of muscle-adapted strain and control strain.

Strain	Isolated P per mg. of living cells			
	Muscle-adapted strain		Control strain	
Hours incubated	24	48	24	48
	0.007	—	0.008	—
	0.012	0.022	0.011	0.022
	0.005	0.019	0.007	0.019

Figure 4. The alkaline phosphatase activities of the muscle-adapted strain and the control strain.

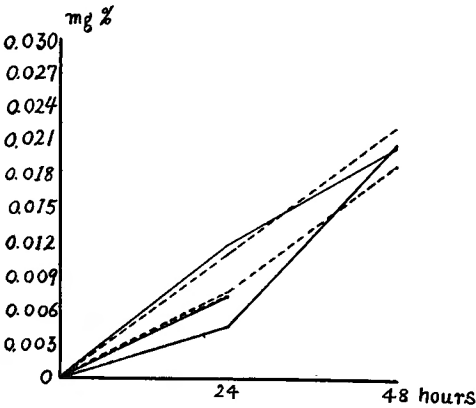


Figure 5. The alkaline phosphatase activities of the bone-marrow-adapted strain and the control strain.

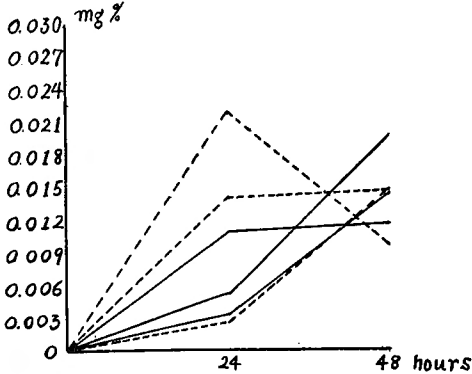


Table 14. The alkaline phosphatase activities of bone-marrow-adapted strain and control strain.

Strain	Isolated P per mg. of living cells			
	Bone-marrow-adapted strain		Control strain	
Hours incubated	24	48	24	48
	0.011	0.012	0.014	0.014
	0.003	0.014	0.022	0.009
	0.005	0.020	0.003	0.014

Table 15. The activating effect of magnesium ions on the alkaline phosphatase activity of each strain.

		MgSO ₄ -trained strain	Muscle-adapted strain	Bone-marrow-adapted strain
Isolated P per mg. of living cells	control	0.029	0.014	0.042
	Mg ⁺⁺ added	0.021	0.015	0.038
Activation rate (%)		139	94	111
Concentration of Mg ions (mol)		0.05	0.05	0.1

Activation rate = $\frac{A' - B'}{A'} \times 100 \%$ A': P increase when Mg⁺⁺ was added.
B': P increase when Mg⁺⁺ was not added.

The rate of activation for each strain is illustrated in Table 15.

(7) The alkaline phosphatase activities of bone-marrow extract, and the inhibitory effects on it in adding the bone-marrow-adapted strain and the control strain (Table 16, Figure 6). In order to investigate the phosphatase activity of bone-marrow extract, the following reaction mixtures were prepared: 1.0 cc of bone-

Figure 6. The alkaline phosphatase activities of bone-marrow extract, and the inhibitory effects on it in adding the bone-marrow-adapted strain and the control strain.

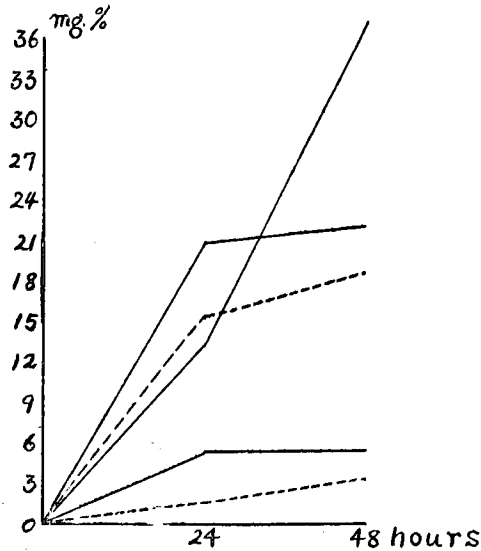


Table 16. The alkaline phosphatase activity of bone-marrow extract, and the inhibitory effects on it in adding the bone-marrow-adapted strain and the control strain.

Enzyme sources	Isolated P (mg%)
Bone-marrow extract 1.0cc	37.55
Bone-marrow extract 1.0cc + bone-marrow-adapted strain 146mg	18.60
Bone-marrow extract 1.0cc + control strain 146mg	22.35
Bone-marrow-adapted strain 146mg	3.60
Control strain 146mg	5.05

phosphatase activity to isolate inorganic phosphoric acid from "sodium glycerophosphate" at pH 10, and that this phosphatase is activated by magnesium ions in the same way as the phosphatase of other animals and plants. Toluene had no effect on this phosphatase activity. The phosphatase is said to exhibit no potential activity in living cells or in micro-organisms, but is always in a repressed state.

marrow extract as enzyme source, 5.0 cc of 2.5 % substrate solution, 5.0 cc of buffer solution and 0.5 cc of toluene. The amount of isolated P was measured. The bone-marrow extract showed very vigorous phosphatase activity at pH 10. When mixtures containing both 1.0 cc of bone-marrow extract and staphylococcal suspensions were employed as enzyme sources, their phosphatase activity was weaker than that of bone-marrow extract, but far stronger than that of staphylococci.

(8) The alkaline phosphatase activities of the osteomyelitis strains (Table 17, Figure 7). These two tested strains showed less alkaline phosphatase activity than the control strain.

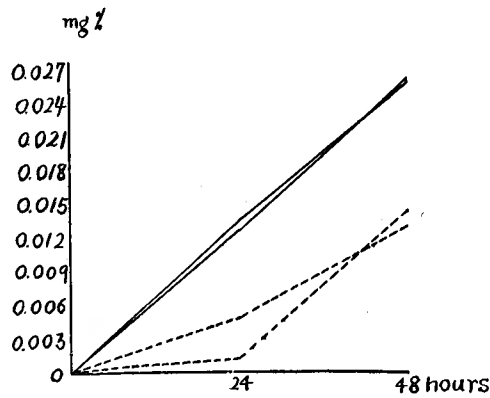
3. Discussion.

In this chapter, it has been proved that staphylococcus aureus has an alkaline

Table 17. The alkaline phosphatase activities of the osteomyelitis strains and F.D.A. 209-P strain.

Strain	Isolated P (mg%)	
	24	48
F. D. A. 209-P strain	0.013	0.026
Child-osteomyelitis strain	0.001	0.014
F. D. A. 209-P strain	0.013	0.025
Calcaneal-osteomyelitis strain	0.005	0.013

Figure 7. The alkaline phosphatase activities of the osteomyelitis strains.



Therefore, the fact that the activating rates of magnesium ions for the phosphatase activity of each “adapted strain” was different, and that the difference between the phosphatase activities of the bone-marrow-adapted strain and control strain was less conspicuous than the difference between those of the mixtures containing bone-marrow extract and the bone-marrow-adapted strain or control strain, is presumed to be the manifestation of some significant changes in the enzymatic structure of “adapted strains”.

The enhanced phosphatase activity in the bone-marrow-adapted strain activated by magnesium ions makes it possible to presume that the bone-marrow-adapted strain has to do with the formation of osteo-strain; but the result of this experiment (8) showed that the osteo-strain revealed weaker phosphatase activity. This problem awaits our future study. The phosphatase activity of bone-marrow was strongly inhibited by adding bone-marrow-adapted strain, while the addition of a control strain revealed weaker inhibition of phosphatase activity. This leads us to consider that the apoenzyme of phosphatase of the bone-marrow-adapted strain should be more similar to that of the bone-marrow extract, and therefore, tend to combine more easily with the coenzyme of phosphatase of the bone-marrow extract, than the counterpart of the control strain.

THE PRODUCTION OF EXPERIMENTAL OSTEOMYELITIS AND MYOSITIS IN RABBITS, AND AN INVESTIGATION INTO THE TISSUE-AFFINITIES OF “ADAPTED STRAINS”.

The bone-marrow-adapted and muscle-adapted strains were artificially produced in vitro, and it was proved that they have peculiar enzymological characteristics. Then, is there any relation between these characteristics of the adapted strains and the tissue-affinities of the so-called osteo-strain or myo-strain? To ascertain this, the following experiments were performed in vivo.

CHAPTER 5. PRODUCTION OF EXPERIMENTAL MYOSITIS IN RABBITS WITH THE ADAPTED STRAIN AND A CONTROL STRAIN.

Young rabbits, fed for several days with okara (bean refuse) and grass, were used. Each strain was densely suspended in distilled water.

Experiment 1. Production of experimental myositis in rabbits by intramuscular injection of each strain (Table 18). The dense suspension of each strain was injected into the extensor thigh muscles of rabbits, which were examined by autopsy or incision after two or three days.

Table 18. Production of experimental myositis by intramuscular injection of each strain.

No.	Weight (g)	Strain	Quantity (mg)	Findings
24	480	m-a-s	1.5	Muscle abscess
33	700	m-a-s	1.5	Muscle abscess
40	500	b-m-a-s	1.5	Muscle abscess
44	500	b-m-a-s	1.5	Muscle abscess
45	500	c-s	1.5	Muscle abscess
34	650	m-a-s	0.8	Nil
35	450	m-a-s	0.4	Nil
46	500	c-s	1.5	Muscle abscess

m-a-s : muscle-adapted strain, c-s : control strain, b-m-a-s : bone-marrow-adapted strain

Experiment 2. Production of experimental myositis in rabbits by intravenous injection of each strain (Table 19). Bacterial suspensions containing 3.0 mg. or 4.5 mg. of staphylococci were injected into the ear-vein of rabbits. The results are illustrated in Table 19.

Table 19. Production of experimental myositis by intravenous injection of each strain.

No.	Weight (g)	Strain	Quantity (mg)	Findings
19	800	m-a-s	4.5	Myositis, Arthritis
32	500	m-a-s	3.0	Myositis, Arthritis
39	450	b-m-a-s	3.0	Nil
42	600	c-s	3.0	Nil

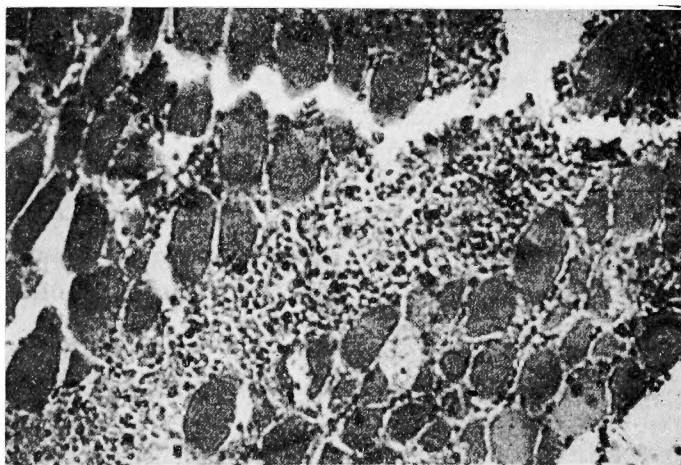
Table 20. Production of experimental myositis by intra-arterial injection of each strain.

No.	Weight (g)	Previous management of muscle	Strain	Quantity (mg)	Findings
30	500	MgSO ₄ sol. (2.5mol, 0.5cc) was injected	m-a-s	1.5	Multiple muscle abscesses
41	700	ditto	c-s	1.5	Nil
38	600	ditto	b-m-a-s	1.5	Nil
43	750	ditto	—	—	Nil
26	600	—	m-a-s	3.0	Nil
27	450	—	c-s	1.5	Nil

Experiment 3. Production of experimental myositis in rabbits by intra-arterial injection of each strain (Table 20). Bacterial suspensions containing 1.5 mg. of each strain were injected into the femoral arteries of rabbits, immediately after the injection of 0.5 cc of 2.5 mol MgSO_4 solution into the extensors of the thighs. Rabbit 30 showed serious multiple muscle abscesses in the left thigh, where 0.5 cc of 2.5 mol MgSO_4 solution had been injected. And the other rabbits showed no changes macroscopically.

(§) Addition. Histological findings of the muscle abscess (Figure 8). As in

Figure 8. Histological findings of the muscle abscess. Inflammatory cell infiltrations are observed among the muscle fibers.



SCRIBA's description, inflammatory cell infiltrations were observed among the muscle fibers.

Discussion.

The muscle-adapted strain inoculated hematogenously showed special affinity for skeletal muscles of rabbits as compared with the other tested strains. Martinotti isolated a strain of staphylococcus from the liver, kidney and spleen of a fatal case of senile decay at autopsy. He found that rabbits always developed myositis following intravenous injection of this strain. Therefore he named this strain "staphylococcus polymyositicus". Osawa produced experimentally multiple muscle abscesses in rabbits by intravenous injection of staphylococci isolated from the pus of myositis cases. A recent study of Ishihara shows that the strains of staphylococcus isolated from the pus of myositis cases more readily produce myositis in rabbits than other strains. The special tissue-affinity of muscle-adapted strains indicates the same characteristics in both the muscle-adapted strain and the myo-strain.

CHAPTER 6. PRODUCTION OF EXPERIMENTAL OSTEOMYELITIS IN RABBITS AND AN INVESTIGATION INTO THE TISSUE-AFFINITIES OF "ADAPTED STRAINS".

Acute infectious osteomyelitis usually arises following a hematogenous infection of staphylococci. As to etiology of osteomyelitis, Lexer attached importance to the physiological anatomical peculiarity of the epiphysis, and this is still considered important. Thompson stated that there were three important factors for the experimental production of osteomyelitis in rabbits, i) use of young rabbits, ii) use of the osteo-strain, iii) arrangement of experimental condition in such a way as to bring about a relatively prolonged type of infection. Hirayama inquired into

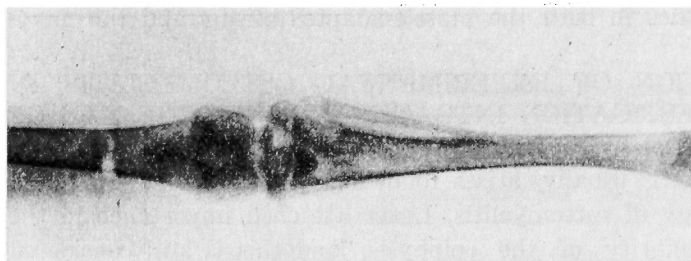
the fate of bacteria in bone-marrow and found that staphylococci carried to the bone-marrow hematogenously, (i. e. through a trophic artery, branches of arteries through Havers's canals, or metaphyseal arteries), could grow only in the venous sinus corresponding to the line of ossification. Shioda obtained the same results in his experimental study of osteomyelitis. These facts indicate that the local chemical state of the bone-marrow at the line of ossification, must be very significant for the further development of staphylococci which cause osteomyelitis. Ossification begins with the formation of bone-stroma and is completed by the deposition of inorganic substances, mainly such as calcium salts. The inorganic matter of bone is said to be as follows, calcium phosphate 85.0%, calcium carbonate 1.5%, alkaline salt 2.0%, magnesium phosphate 1.5%, calcium fluorinate 0.3%, and calcium chloride 0.2%. The deposition of calcium salts is said to be caused by the phosphatase in the line of ossification which hydrolyzes phosphoric esters in the blood and isolates phosphoric acid. This phosphoric acid then combines with calcium and grows as an initial germinal crystal, and calcium phosphate or carbonate deposits around this crystal. Bone, especially growing bone, is rich in phosphomonoesterases which are activated by magnesium ions, and skeletal muscle has little or none of that. However, it is stated that the skeletal muscle contains a large amount of adenylypyrophosphatase which is inhibited by magnesium ions. And phosphomonoesterases are especially plentiful in the line of ossification of growing bone which is often the site of beginning osteomyelitis.

In order to produce a chemical milieu where staphylococci can develop suitably, magnesium salt diluted so as to cause no lesions was injected into the medullary cavity. Then these rabbits were injected hematogenously with the bone-marrow-adapted strain whose phosphatase activity is enhanced by magnesium ions or bone-marrow extract.

Materials and Methods.

(1) Rabbits. Young rabbits about 50 days old, weighing 500 g. were used after

Figure 9. Roentgenogram of rabbit 56, taken on the 6th day after injection. Periosteal ossification caused by subperiosteal pus-accumulation is observed.



feeding for several days with okara and grass.

(2) Management of bone-marrow before infection with staphylococci. The skin of the inner surface of a rabbit's leg was incised and a mandrel-needle was inserted through the fascia into the bone-marrow, and $MgSO_4$

solution or bacterial suspension was slowly injected. The incised wound was then sutured.

(3) Diagnosis of osteomyelitis. Although local swelling, lameness, leucocytosis and rectal temperatures were examined, roentgenograms taken 12-15 days after

Figure 10. Roentgenogram of rabbit 16, taken on the 13th day after injection. Sequester-formation is observed.

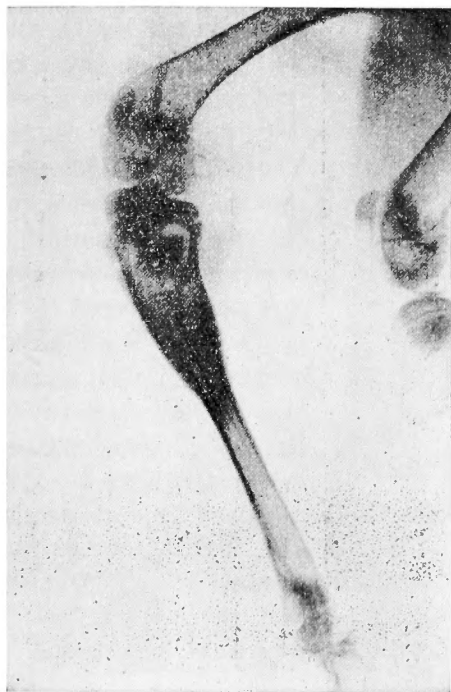
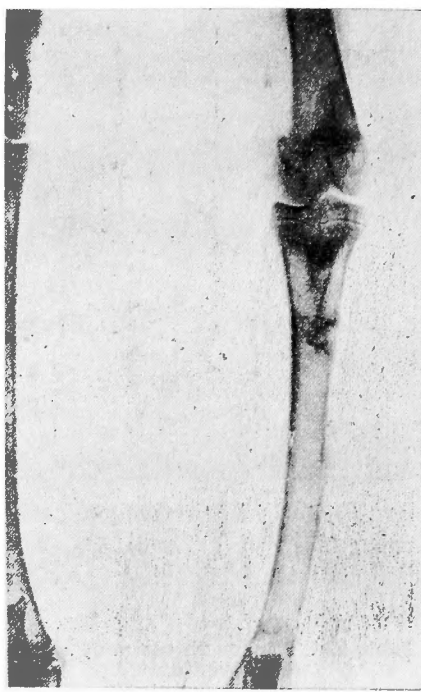


Figure 11. Roentgenogram of rabbit 16, taken on the 97th day after injection. Two sequesters are still observed.



injection were the most deciding factor in the diagnosis of osteomyelitis.

Experiment 1. Production of experimental osteomyelitis by hematogenous infections of staphylococcus (Table 21). The rabbits which had had no previous injection into the medullary cavities showed no changes. The rabbits which had had their medullary cavities injected with 0.5 cc of 2.5 mol MgSO_4 solution showed marked bone changes.

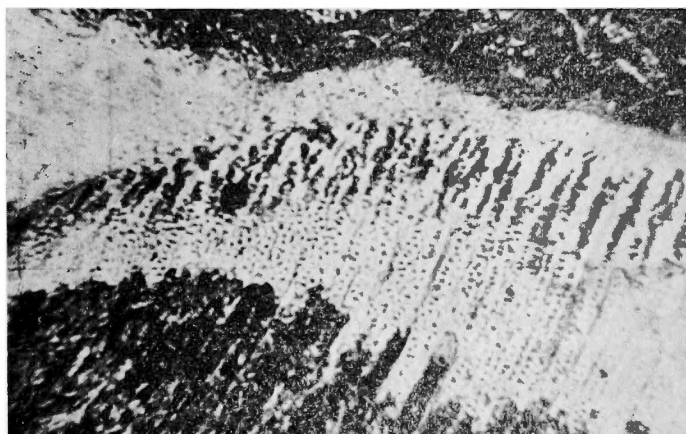
Table 21. Production of experimental osteomyelitis by hematogenous infection of each strain.

No.	Weight (g)	Previous management of bone-marrow	Strain	Quantity (mg)	Injection	Findings
39	450	—	b-m-a-s	3.0	intra-venous	Nil
36	400	—	b-m-a-s	1.5	intra-arterial	Nil
32	500	—	m-a-s	3.0	intra-venous	Nil
42	600	—	c-s	1.5	intra-venous	Nil
16	750	MgSO_4 sol. (2.5mol, 0.5cc) was injected	b-m-a-s	1.5	intra-arterial	Osteomyelitis
56	500	ditto	b-m-a-s	2.0	intra-arterial	Osteomyelitis
13	450	ditto	b-m-a-s	1.5	intra-venous	Osteomyelitis
28	500	ditto	m-a-s	1.5	intra-arterial	Osteomyelitis

Experiment 2. What kind of change occurred following the injection of MgSO_4 solution into medullary cavity? Rabbit 20, 23 and 49 were injected with 0.5 cc of 2.5 mol MgSO_4 solution into the medullary cavities of their tibias, and were roen-

tgenographed on the 13th day (20), 27th day (49), 38th day (20) and 55th day (49). They showed no bone changes, and there were no pathological changes in the histological specimens (Figure 12).

Figure 12. Ossification line of tibia of rabbit 23.
No special changes are observed.



Experiment 3. The concentration of $MgSO_4$ solution related to the occurrence of experimental osteomyelitis (Table 22). The most favorable condition of the bone-marrow for the development of experimental osteomyelitis can be considered to be produced by the injection of 2.5 mol $MgSO_4$ solution into the medullary cavity. Rabbit 11, 82 and 83 were injected with 3.0 mg.(11),

1.5 mg. (82, 83) respectively of the control strain into the regional femoral arteries, and then 0.5 cc of 1.5 mol-(83), 2.0 mol-(82) and 0.5 mol-(11) $MgSO_4$ solution were injected into their medullary cavities. Among them, only rabbit 82 showed bone changes.

Table 22. The concentration of $MgSO_4$ solution related to the occurrence of experimental osteomyelitis.

No.	Weight (g)	$MgSO_4$ solution	Strain	Quantity (mg)	Injection	Findings
31	580	2.5mol, 0.5cc	c-s	1.5	intra-arterial	Osteomyelitis
82	500	2.0mol, 0.5cc	c-s	1.5	intra-arterial	Osteomyelitis
83	500	1.5mol, 0.5cc	c-s	1.5	intra-arterial	Nil
11	600	0.5mol, 0.5cc	c-s	3.0	intra-arterial	Nil

Experiment 4. Production of experimental osteomyelitis in rabbits by intramedullar injection of each strain (Table 23). Rabbit 17 was injected with 3.0 mg. of bone-marrow-adapted strain into the medullary cavity, and developed osteomyelitis. The other rabbits were injected with the same amount of muscle-adapted strain or control strain and they showed no bone changes. Rabbit 14 was injected with both 3.0 mg. of bone-marrow-adapted strain and 0.5 cc of 2.5 mol $MgSO_4$ solution, and developed serious total necrosis of the tibia (Figure 13, 14, 15).

Table 23. Production of experimental osteomyelitis by intramedullar injection of each strain.

No.	Weight (g)	Strain	Quantity (mg)	Findings
17	550	b-m-a-s	3.0	Osteomyelitis
72	550	m-a-s	3.0	Nil
75	550	c-s	3.0	Nil
14	800	b-m-a-s 3.0 + $MgSO_4$ sol. (2.5mol, 0.5cc)		Total necrosis of tibia

Figure 13. Roentgenogram of rabbit 14, taken on the 12th day after injection. Marked callus-formation is observed.



Figure 15. Tibia of rabbit 14. Histological specimen.

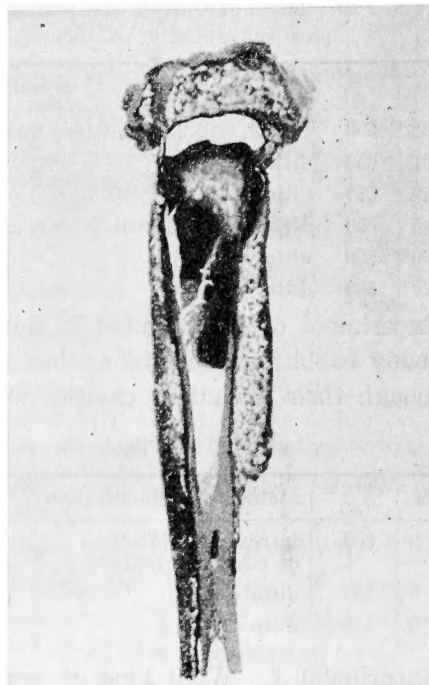


Figure 14. Ossification line of the tibia of rabbit 14. Severe inflammation was caused in this area.



Experiment 5. The differences in pathogenicity of each strain in the production of experimental osteomyelitis in rabbits (Table 24). The results of experiments 3 and 4 give us some suggestions in regard to the infecting power of each strain in the production of experimental osteomyelitis. Rabbit 48 was injected with bone-marrow-adapted strain after previous injection of 0.5 cc of 1.25 mol

MgSO₄ solution into the bone-marrow, and developed osteomyelitis. Other rabbits were injected with muscle-adapted strain or control strain and showed no bone changes. Still other rabbits received 2.5 mol MgSO₄ solution in their bone-marrow

and developed osteomyelitis.

Table 24. The difference in the pathogenicity of each strain in the production of experimental osteomyelitis.

No.	Weight (g)	Previous management of bone-marrow	Strain	Quantity (mg)	Injection	Findings
52	600	MgSO ₄ sol.(2.5mol, 0.5cc) was injected	b-m-a-s	0.2	intra-arterial	Osteomyelitis
61	650	ditto	m-a-s	0.2	intra-arterial	Osteomyelitis
62	650	ditto	c-s	0.2	intra-arterial	Osteomyelitis
48	450	MgSO ₄ sol.(1.25mol, 0.5cc) was injected	b-m-a-s	1.5	intra-venous	Osteomyelitis
69	500	ditto	m-a-s	1.5	intra-venous	Nil
70	500	ditto	c-s	1.5	intra-venous	Nil

Experiment 6. Experiment in immunized rabbits (Table 25). As Table 25 shows, young rabbits immunized against staphylococcal infections showed no bone changes, though their medullary cavities were treated in the same way as rabbit 16 and 56.

Table 25. Experiment in immunized rabbits.

No.	Weight (g)	Method of immunization	Elapsed days	Previous management of bone-marrow	Strain	Quantity (mg)	Findings
8	600	intravenous injection of 0.3mg. of b-m-a-s	8	MgSO ₄ sol.(0.5mol, 0.5 cc) was injected.	b-m-a-s	5.0	Nil
9	550	ditto (0.2mg.)	8	ditto	c-s	5.0	Nil
10	550	ditto (1.5mg.)	8	ditto (2.5mol, 0.5cc)	b-m-a-s	4.6	Nil

Experiment 7. What kind of prerequisite for the production of osteomyelitis should be given by the intramedullary injection of MgSO₄ solution? (Table 26). Rabbit 79 was injected with 1.5 mg. of the control strain, after the injection of 0.5 cc of 2.5 mol NaCl solution into its medullary cavity and showed no sign of osteomyelitis in roentgenograms. Rabbit 21 was injected with 4.5 mg of control strain into the regional femoral artery immediately after crushing of the bone several times with a mandrel-needle. Rabbit 50, 53 and 55 received by injection into their femoral arteries (53, 55) or ear-vein (50), both 0.5 cc of 2.5 mol MgSO₄ solution (50, 53) or 1.25 mol MgSO₄ solution (55) and 2.3 mg. of bone-marrow-adapted strain; they showed no sign of osteomyelitis in roentgenograms.

Table 26. What kind of prerequisite for the production of osteomyelitis should be given by the intramedullary injection of MgSO₄ solution?

No.	Weight (g)	Previous management of bone-marrow	Strain	Quantity (mg)	Injection	Findings
79	550	NaCl sol.(2.5mol, 0.5cc) was injected	c-s	1.5	intra-arterial	Nil
50	630	—	b-m-a-s 2.3 + MgSO ₄ sol. (2.5 mol, 0.5cc)		intra-venous	Nil
53	600	—	b-m-a-s 1.0 + MgSO ₄ sol. (2.5 mol, 0.5cc)		intra-arterial	Nil
55	650	—	b-m-a-s 1.0 + MgSO ₄ sol. (2.5 mol, 0.5cc)		intra-arterial	Nil
21	500	crushing	c-s	4.5	intra-arterial	Nil

Experiment 8. Experiment with osteo-strains (Table 27). These results are illustrated in Table 27.

Table 27. Experiment with osteo-strains.

No.	Strain and Quantity	Injection	Course	Findings
1	Osteomyelitis strain in chapter 3. (c) 35 mg.	intra-venous	died in 12 hours	Small abscesses of liver and kidney
2	ditto 3.5 mg.	intra-venous	died in 20 hours	ditto
3	ditto 0.35 mg.	intra-venous	died in 48 hours	Bilateral hyperemia of inf. end of femur and upp. end of tibia
4	ditto 0.35 mg.	intra-venous	died in 24 hours	Nil
18	Osteomyelitis strain in chapter 4. (d) 0.046 mg.	intramedullar	died on 8th day	Arthritis of knee-joint

Discussion.

(1) Young rabbits were injected with staphylococcal suspensions hematogenously after injection of 2.5 mol $MgSO_4$ solution into the medullary cavities of their tibias, and showed typical signs of osteomyelitis in roentgenograms.

(2) The bone-marrow-adapted strain proved to have affinity for bone-marrow.

(3) The results of experiment 8 seem to accord with the views of Thompson, who proved experimentally the existence of an osteo-strain. It is of great importance in such experiments to avoid early lethal effects of the osteo-strain.

(4) Young rabbits were used as advocated by Lexer, but the results of experiment 6 should be explained by Shioda's study.

(5) The most interesting problem raised by these experiments is the mechanism whereby the intramedullar infusion of $MgSO_4$ solution urges the development of osteomyelitis in bone-marrow. Is a functional or organic locus minoris resistentiae caused by $MgSO_4$ solution? This question may be answered by the following facts: i) $MgSO_4$ solution injected into skeletal muscle where there is little or no phosphomonoesterase, could not cause myositis in similar conditions, except in special cases; ii) The paralyzing action of $MgSO_4$ solution disappears at once in the presence of calcium ions; iii) In these cases of experimental osteomyelitis in rabbits, the focus of inflammation is always in the epiphysis and the metaphysis, though $MgSO_4$ solution was injected into the diaphyseal regions; iv) Histological findings revealed no changes in bone-marrow after the injection of $MgSO_4$ solution. The contradiction of i) can be resolved perhaps by the differences in phosphomonoesterase activity between skeletal muscle and bone-marrow.

CHAPTER 7. SOME SUPPLEMENTARY EXPERIMENTS COMMENTING ON THE PLURALISM OF PYOGENIC STAPHYLOCOCCUS.

ISHIHARA et al have isolated the various strains of staphylococci from the lesions of osteomyelitis, myositis, furuncle, carbuncle, mastitis and so on. And they have made it clear that there were many distinct biochemical characters between these strains, such as the different pH-optimum of coagulase production and various modified growing states caused by adding phosphoric-, metaphosphoric and

pyruvic acid or A. T. P. to the broth media where they developed. Furthermore, these strains showed the different affinities for some tissues of rabbit, for example, the bone-marrow, skeletal muscle or skin. These biochemical characteristics were fixed after one year's subculturing. From these experimental results they concluded the pluralism of pyogenic staphylococcus.

On the contrary, as above-mentioned, we have shown that staphylococcus aureus F. D. A. 209-P can become a so-called osteo-strain or a myo-strain under the influence of environment.

To investigate whether these two conflicting results can be reconciled to each other or not, some supplementary experiments were performed.

Materials and Methods.

(1) Staphylococcus aureus strains.

a. Carbuncle strain. This strain was isolated from the lesion of carbuncle on the back of an old male admitted to our clinic.

b. Myositis strain. The same old male who had the large carbuncle on his back, later developed myositis of right thigh and then of the left thigh by hematogenous spread. This strain was isolated from the pus of the muscle abscess. So these strains, the carbuncle strain and the myositis strain, were of the same origin.

c. Osteomyelitis strain. This strain was the test strain d of chapter 4.

d. Osteomyelitis strain. This strain was the test strain c of chapter 3.

e. Muscle-adapted strain.

f. Bone-marrow-adapted strain.

g. Control strain.

h. MgSO_4 -trained strain.

(2) Chemical agents.

a. Meta-phosphoric acid (Nakarai Co.).

b. KH_2PO_4 , chemical pure (Katayama Co.).

c. Pyruvic acid (Nakarai Co.).

d. Phosphoric acid (Nakarai Co.).

e. Adenosine triphosphoric acid (Na_4) (Wako Co.).

(3) Broth media.

These media were prepared as described in chapter 2, and standardized according to the degree of turbidity.

(4) Blood plasma.

Citrated blood (1%) from a healthy adult male who had suffered no staphylococcal infection recently, was centrifuged and the clear liquid (plasma) was pipetted off.

Experiment 1. Influences of various chemical agents upon the staphylococcal growth in broth media (Table 28).

Meta-phosphoric acid and KH_2PO_4 were each dissolved in distilled water to make 10% solutions. Phosphoric acid and pyruvic acid were each diluted in broth (pH 7.0) as 10% solutions. A. T. P. was dissolved in broth (pH 7.0) to make a 1% solution. All these solutions were filtered through Seitz's sterilizing filter so

Table 28. Influences of various chemical agents upon the staphylococcal growth.

	KH ₂ PO ₄				Meta-phosphoric acid				Phosphoric acid				Pyruvic acid				A. T. P.											
	1	0.5	0.1	0.05	0	1	0.5	0.1	0.05	0	1	0.5	0.1	0.05	0	1	0.5	0.1	0.05	0	1	0.5	0.1	0.05	0.01	0.005	0	
%	1	0.5	0.1	0.05	0	1	0.5	0.1	0.05	0	1	0.5	0.1	0.05	0	1	0.5	0.1	0.05	0	1	0.5	0.1	0.05	0	0.005	0	0.50
Carbuncle strain	0.5	0.5	0.6	0.6	0.5	0	0.2	0.4	1.7	0.5	0.2	0.1	0.6	2.1	1.8	0.1	0.2	2.4	4.8	3.2	0.6	0.6	0.5	0.6	0.5	0.6	0.4	0.4
Myositis strain	1.2	1.3	0.8	0.6	0.7	0	0.1	0.8	1.7	1.0	0	0.2	0.3	0.7	1.5	0	0.1	1.8	3.1	2.7	1.3	0.8	0.8	0.7	0.6	0.6	0.6	0.6
Osteomyelitis strain											0	0.1	0.7	1.5	2.0	0	0	2.0	2.7	2.8	1.5	0.8	1.5	1.0	0.6	0.6	0.6	0.6
MgSO ₄ -trained strain	0.6	0.6	0.7	0.6	0.7	0	0	0.3	0.4	0.5	0.1	0.1	0.1	0.6	1.1	0.1	0.1	0.1	2.3	2.8	0.9	0.8	0.6	0.6	0.6	0.6	0.6	0.6
Muscle-adapted strain	0.7	0.5	0.6	0.6	0.6	0	0	0.7	0.7	0.6	0.1	0.1	0.1	0.6	1.2	0.1	0.1	0.1	2.1	2.1	0.8	0.5	0.6	0.6	0.6	0.6	0.6	0.6
Bone-marrow-adapted strain	0.3	0.3	0.9	0.4	0.6	0	0	1.5	1.6	0.6	0.1	0.1	0.1	0.5	0.8	0	0	0	1.7	1.9	0.6	0.5	0.6	0.6	0.6	0.6	0.6	0.6
Control strain	0.4	0.3	0.7	1.0	0.9	0	0	0.1	0.4	0.4	0.1	0.1	0.1	0.5	0.9	0	0.1	0	1.9	1.9	0.9	1.0	0.8	0.5	0.7	0.7	0.7	0.7
pH of media	6.0	6.2	6.4	6.6	7.0	3.4	4.2	6.0	6.2	7.0	2.0	2.4	3.2	6.0	7.0	2.4	3.0	4.6	6.0	6.8	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0

as to be aseptic, and diluted with broth to the desired concentration under aseptic conditions. The media containing chemical agents were incubated at 37°C for 24 hours to test for contamination.

To the media containing chemical agents, 0.1 cc of a homogeneous suspension of each tested strain was added. The number of living cells in these cultures was measured by the method described in chapter 2. While being measured the media must be shaken vigorously.

(1) Influence of KH₂PO₄ upon the staphylococcal growth. The addition of KH₂PO₄ (0.05, 0.1, 0.5, 1.0%) to broth media caused marked acceleration of the growth of the myositis strain, but the growth of the carbuncle strain, which had the same origin as the myositis strain, showed no change. The growth of the MgSO₄-trained strain, and muscle-adapted strain, was the same as that of the carbuncle strain, while the growth of the bone-marrow-adapted strain and the control strain was inhibited by the addition of KH₂PO₄.

(2) Influence of meta-phosphoric acid upon the staphylococcal growth. In broth media containing 0.05% meta-phosphoric acid the growth of the carbuncle strain, the myositis strain and the bone-marrow-adapted strain was accelerated by 170-340%, the growth of the muscle-adapted strain was slightly accelerated and the growth of the MgSO₄-trained strain was slightly inhibited. 0.1% meta-phosphoric acid greatly accelerated the growth of the bone-marrow-adapted strain, slightly

accelerated the growth of muscle-adapted strain, and inhibited the growth of all the other strains. 0.5% meta-phosphoric acid inhibited the growth of all tested strains except the control strain and the myositis strain. 1.0% meta-phosphoric acid inhibited the growth of all tested strains completely.

(3) Influence of phosphoric acid upon the staphylococcal growth. When 0.05% phosphoric acid was added to the broth media, the growth of the control strain was accelerated, but the growth of all other strains was inhibited. When phosphoric acid was inversed to 0.1%, the growth of all the strains was completely inhibited except for the carbuncle strain, the osteomyelitis strain and the myositis strain. At 0.5% or 1.0%, the growth of all tested strains was completely inhibited.

(4) Influence of pyruvic acid upon the staphylococcal growth. When 0.05% pyruvic acid was added to the broth media, the growth of the control strain was greatly accelerated, while the growth of the myositis strain was slightly accelerated; the growth of all the other strains except for the muscle-adapted strain and the control strain was inhibited. With 0.1% pyruvic acid, the growth of all tested strains except for the myositis strain and the osteomyelitis strain c was inhibited. At 0.5% or 1.0%, the growth of all tested strains was completely inhibited.

(5) Influence of A. T. P. upon the staphylococcal growth. When 0.005% A. T. P. was added to the broth media, the growth of the control strain, the myositis strain, and the osteomyelitis strain was slightly accelerated, while the growth of all the other strains showed no change. At 0.01%, the growth of the osteomyelitis strain c was greatly accelerated; the growth of the carbuncle strain and the control strain was slightly accelerated; the growth of the other strains except for the muscle-adapted-strain was not changed. At 0.05%, the growth of all tested

Table 29. Clotting times.

pH	Clotting time		
	6.0	7.0	8.0
Control strain	60	60	80
Muscle-adapted strain	45	35	60
Bone-marrow-adapted strain	150	45	65
MgSO ₄ -trained strain	90	40	45
Osteomyelitis strain	150	55	65
Osteomyelitis strain	80	40	60
Carbuncle strain	300	190	300
Myositis strain	200	140	160

minutes

strains except for the bone-marrow-adapted strain was accelerated. At 0.1%, the growth of the myositis strain and the osteomyelitis strain was greatly accelerated; the growth of all the other tested strains was slightly accelerated.

Experiment 2. On the coagulase production of the tested strains (Table 29).

It is said that staphylococcal coagulase is a characteristic of pathogenic strains. This experiment was modelled on Fisk's and Fisher's studies on the coagulase of staphylococcus. Each tested strain was cultivated at 37°C for 24 hours on broth media whose pH had been previously adjusted to 6.0, 7.0 and 8.0. Ten drops of the broth cultures were added by capillary pipettes to 0.5 cc of blood plasma that

had been diluted 1:5 in physiological solution. The clotting times were measured till the mixtures become jelly-like. The results are illustrated in Table 29.

Discussion.

Generally speaking these experimental results accord with those shown by ISHIHARA, i. e. the growth of the myositis strain was accelerated by the addition of meta-phosphoric acid, A. T. P., and phosphoric acid to the broth media, and the pH-optimum for coagulase production by the osteomyelitis strains is 8.3 to 7.7. These observations reveal a certain similarity concerned with biochemical characteristics between the osteomyelitis strain and the bone-marrow-adapted strain, and between the myositis strain and the muscle-adapted strain. Especially we should like to emphasize the fact that the carbuncle strain and the myositis strain were of the same origin, but they showed distinct altered biochemical characteristics; that is, when the carbuncle strain is carried to muscle hematogenously and cause myositis, its chemical characteristics are completely altered to adapt itself to a new environment, and it turns into myositis strain. It is very interesting to note that artificially induced muscle-adapted strain and bone-marrow-adapted strain showed certain similarities to the myositis strain or the osteomyelitis strain respectively, concerning biochemical characteristics. These similarities will perhaps be exactly explained by future study employing synthetic amino acid media.

CONCLUSION

The altered tissue-affinity of staphylococcus induced by the formation of adaptive enzymes can be expected to play an important role in the occurrence of acute suppurative polymyositis and acute infectious polyosteomyelitis. The questions concerning the so-called myo-strain and osteo-strain have been scientifically solved to some extent. Furthermore, in the pathogenesis of acute infectious osteomyelitis, importance is to be attached to the role of chemical environment of the bone-marrow favoring the growth of staphylococcus; in other words, it seems that Lexer's theory concerning the pathogenesis of osteomyelitis must be reconsidered.

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和 文 抄 録

多発性筋炎及び骨髄炎の発生原因に関する実験的研究, 特にブドウ球菌の組織環境に対する適応現象に基く組織 親和性の変化と骨髄の化学的環境の役割について

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多発性筋炎及び骨髄炎の発生原因において特に起炎ブドウ球菌の発育組織環境に対する親和性の変化を重視して実験的吟味を行い、次の結論に到達した。

黄色ブドウ球菌 F. D. A. 209-P 株を家兎横紋筋汁又は骨髄汁を含む培地上に継代培養して得た菌株について、(1) その酵素作用を追求し母菌株と比較した結果と、(2) 家兎に該菌株を接種してその組織親和性を検討した結果から、急性化膿性多発性筋炎及び急性感染性多発性骨髄炎の発生原因には、発育組織環境に対す

る適応酵素生成によつて誘導される起炎ブドウ球菌の組織親和性の変化が重要な意義を有することが認められた。

更に家兎における骨髄炎発生の実験結果から、急性感染性骨髄炎の発生原因には、ブドウ球菌の発育に好適な骨髄の化学的環境が甚しく重大な役割を演じており、従来骨髄炎発生に関して唱えられている Lexer の説は再考されるべきであることを強調するものである。