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From the Department of Internal Medicine, Keio University School of Medicine (Director: Professor I. Mikata, M.D.)

Experimental Bacterial Endocarditis with Special Reference to Clinical Aspects

By

ITTAKU MIKATA

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During the past ten years, my colleague and I have particularly studied septic diseases, especially subacute bacterial endocarditis, clinically, pathologically, bacteriologically as well as experimentally.

In this report, I intend to discuss pathogenic factors of bacterial endocarditis as indicated by the findings of our animal experiments, and then to show the correlation between the features found in experimental endocarditis and those in human endocarditis.

PATHOGENESIS OF BACTERIAL ENDOCARDITIS

a) Experiments to find the most suitable method—

In order to produce experimental endocarditis, some factors must be added to bacterial infection, such as damaging the heart valve. As shown in

TABLE I EXPERIMENTAL ENDOCARDITIS, OUTLINE OF METHODS AND RESULTS OF OUR EXPERIMENTS

No.	Method	Animal	Incidence of endocarditis %	Factors likely participating in producing endocarditis	
1.	Phosphorus emulsion (intraperitoneal) + Streptococcus sanguis, Non-vitamin C diet,	Rabbit	4	25.0	Damages of the reticuloendothelial system and the liver, General malaise
2.	Cardiotonic + Str. sanguis	Rabbit	6	16.7	Vitamins C deficiency
3.	Organ phosphatide + Str. sanguis	<u>Rabbit 185</u>	<u>56.8</u>	Promotion of blood coagulability, Allergic factor	
4.	Exposure to altitude (low air pressure, rare oxygen) + Str. sanguis	Rat	5	40.0	Cardiac overload, Anoxia in the tissue, Erythrocytosis, Thrombocytosis
5.	Anastomosis of the abdominal aorta and the inferior vena cava	<u>Dog 6</u>	<u>83.5</u>	Alteration of the blood current, Cardiac overload, Inflow of a thrombus, Anoxia, Stress by a surgical operation	
6.	Digitamin + Str. sanguis	Rabbit	12	16.7	Accelerated cardiac action, a kind of hyperoxia?
7.	Exhaustion by compulsory exercises + Str. sanguis	Rabbit	4	50.0	Cardiac overload, hypoxemia
8.	Bloodletting + Str. sanguis	Rabbit	7	14.3	Cardiac overload, hypoxemia
9.	Anti-rabbit duck-serum + Str. sanguis	<u>Rabbit 104</u>	77.9	Allergic factor, Action of cytotoxin	
10.	Horse serum + Str. sanguis	Rabbit	12	8.3	Allergic factor
11a.	Str. sanguis alone	Rabbit	10	10.0	Bacterial virulence and affinity to the animal
b.	"	Dog	5	42.3	
		Rat	9		
12.	Raising in oxygen chamber + Str. sanguis	Rat	16	100.0	?

Table I, we tried twelve methods to produce endocarditis and used 376 rabbits, 33 dogs, 168 rats and 580 mice in all of our experiments. As a causative microorganism, we used a strain of Streptococcus sanguis, a subtype of streptococcus viridans group, isolated from a patient with subacute bacterial endocarditis. Among these twelve methods in Table I, No. 3 (using organ-phosphatide), No. 9 (using anti-rabbit-heart duck-serum) and No. 5 and No. 7 (overloading on the heart) yielded comparatively successful results in producing endocarditis. The incidence was 56.8 %, 77.9 %, 83.5 %, 50 % respectively. From these results we feel that the factors playing leading parts in producing endocarditis are: 1) increase in work load on the heart, 2) change in the blood components, 3) virulence of the causative microorganism, 4) susceptibility of the animal to it, 5) allergic factor and so forth. Other factors can not be neglected, however, these factors have very complicated relations between each other as shown in Figure 1.

Fig. 1 Relationship between bacterial endocarditis and various factors.

A particularly interesting and curious fact was that all the rats exposed to high concentration of oxygen at low, as well as at normal, pressure developed a definite endocarditis. This is completely contrary to the view by Highman & Altland and others who regarded altitude or low pressure and deficient oxygen as a promoting factor of endocarditis. Further investigation would clear up the

oxygen's effect on producing an endocarditis in rats.

b) *Experiments on reinfection—*

A rabbit with experimental endocarditis which had been produced by injections of *Streptococcus sanguis* and of bovine endocardial phosphatide was reinfected with the same strain after a course of over one month under treatment with penicillin. It was found dead on the next day. Autopsy revealed the following findings: a vegetation on the pulmonic ostium almost blocking the ostium; miliary white plaques of organized verruca in the right chamber wall (histologically, small healed fibrous foci); a remarkable dilatation of the right ventricle; strong evidences of congestion in the liver, spleen and kidneys; acute splenitis; pulmonary abscess and interstitial pneumonia; a cellular infiltration and necrosis in the adrenal cortex; and heavy bacteremia. The changes in the heart were complex. On the one hand, white plaques of completely organized verruca healed by penicillin therapy were seen; but on the other hand, a fresh thrombus containing many colonies of streptococcus was seen in the vegetation. This is the same as our experiences in clinical cases where both healing processes and fresh active lesions are noted in the same heart in a patient dying after treatment with antibiotics. We are, therefore, convinced that changes quite similar to those of human bacterial endocarditis could be obtained experimentally by using rabbits.

From the data obtained by the above two experiments, factors chiefly participating independently or dependently upon each other in development of endocarditis can be summarized as follows:

$$\text{Bacterial Infection} + \left\{ \begin{array}{l} 1. \text{ Overload on the heart} \\ 2. \text{ Changes in substances in the blood} \\ \quad (\text{Coagulation-promoting substance,} \\ \quad \text{Bacterial toxin, etc.}) \\ 3. \text{ Allergic factor} \\ 4. \text{ Previously damaged endocardium} \\ 5. \text{ ? Effect of Oxygen} \end{array} \right\} + \left\{ \begin{array}{l} \text{Conditions on the side of the animal} \\ (\text{Susceptibility, Affinity, etc.}) \end{array} \right.$$

HISTOLOGICAL LESIONS OF EXPERIMENTAL BACTERIAL ENDOCARDITIS

a) *Survey of the literatures—*

Various views have been set forth by many investigators on the histological lesions in the development of bacterial endocarditis, and Table 2

shows the representative ones, which can be divided into two categories: 1) lodging of a thrombus from the blood stream onto the endocardial cells previously damaged by a certain causes may be the starting-point of the initial lesion of endocarditis; 2) and the initial lesion is already existent in the valvular tissue when a thrombus lodges there.

TABLE II VIEWS ON HISTOLOGICAL FIGURES OF BACTERIAL ENDOCARDITIS

	Attaching importance to	Advocated by	In
1. View that regards a minute thrombus stuck to the endothel as initial lesion	① Endocardial damage	Rosenbach	1878
		Kaufmann	1922
	② Phagocytosis of endothelial cells	Nabeshima	1932
		Kinsella	1938
		Dietrich	1926
2. View that initial lesion occurs in the valvular tissue	③ Reactivity of the subendothelial layer	Klinge	1929
		Siegmund	1933
	④ Newly formed blood vessels in the valve	Apitz, Iahibashi	1933
		Beisch & Boehmig	1950
		Gross & Fried	1937

b) *Our view from the experiments—*

After histological studies of the rabbits and rats with experimental bacterial endocarditis, killed day by day with progression, we have developed a schema showing different figures from early stages toward completion of the disease. (Fig. 2)

Within 48 hours after injection of *Str. sanguis*, as a result of the bacteremia, a slight valvulitis with hypertrophy of the endothelial cells is noticed (B. in Fig. 2). Subsequently, as an initial lesion of endocarditis, a minute platelet thrombus on the endothelium (C.), a proliferation of histiocytic cells localized in the subendothelial layer (I.), a change called Boehmig's serous inflammation (K.) or a slight diffuse valvulitis especially in rats (G.) develops. In four or five days with later progression of each change, a thrombus formed on the surface of the valve increases in size, and some colonies of the streptococcus become visible in it. Then, the lamina

elastica is damaged by active proliferation of fibrocytes and cellular infiltration, and thus a bacterial endocarditis is completed.

Here we can estimate the time of bacterial infection into the valve, since we could first find Gram-positive microorganisms in the platelet thrombus of (D.) and fibrinoid substance of (J.), and not

Fig 2 Schema of histological figures in developing bacterial endocarditis, worked out from our experimental data; A: normal valve, B: swelling and edema of the endothel, swollen fibrocytes, diffuse valvulitis, C: sticking of a minute thrombus, D: bacterial infection into the thrombus and cellular infiltration in it, E: formation of vegetation and ulceration, enlargement of the infected thrombus and enforced cellular infiltration, G: diffuse valvulitis, H: sticking of a thrombus, I: proliferation of histiocytes in the subendothelial layer, J: productive verruca and fibrinoid substance, K: serous inflammation (Boehmig), L: fibrinous inflammation (Boehmig).

(Illustration of Fig. 2, continued from the previous paper)

1-endothel-cell, 2-the lamina propria, 3-the lamina elastica, 4-lymphocyte, 5-leukocyte, 6-fibrocyte, 7-platelet thrombus, 8-colony of the causative microorganisms, 9-cellular infiltration, 10-degenerated nucleus of fibrocyte, 11-fibrinoid substance, 12-destroyed elastic fibers, 13-proliferation of histiocytes, 14-hypertrophy of fibrocytes, 15-newly formed blood vessel, 16-organized thrombus.

in the preceding stages. The process from (B.) to (F.) coincides with the first view that regards a thrombus stuck to the endothelium as an initial lesion, and the process from (I.) to (F.) with the second view that regards a tissue reaction in the subendothelial layer as an initial lesion. The one from (K.) to (F.) is consistent with Boehmig's theory which belongs to the latter view.

It is very important that we could find these four different types in early changes produced by the same method of experiment. Now we can understand that each of the former investigators put forward their own view by observing only one of these types.

In all cases, these initial lesions always occur outside the elastica and the basal membrane, and upon these lesions a thrombus containing bacterial colonies is formed. Then inflammatory reaction from inside of the valve presses on the elastica, resulting in its destruction. Thereafter there is only one mode to completion of endocarditis.

Though there is no relation between types of an initial lesion or early changes and a method employed to produce an endocarditis, those factors such as cardiac distress, change in blood components,

allergic factor etc. are likely to have some influence on the endothelial cells and the subendothelial tissue of the valve to foster an onset of an initial lesion then a completion of endocarditis.

(Several sides corresponding to each figure of the schema were projected when lectured at the Annual Convention.)

EFFECTS OF ADRENOCORTICAL HORMONS, ESPECIALLY OF CORTISONE UPON DEVELOPMENT AND TREATMENT OF EXPERIMENTAL ENDOCARDITIS

a) Effects upon development of the disease—

Experiments were performed by methods No. 3 and No. 9 (s. Tab. I). In the latter method,

TABLE III DOSIS AND DURATION OF CORTISONE ACETATE INJECTIONS IN EACH GROUP

Method	Group	No. of rabbit	Day	1	2	3	4	5	6	7	8	9	10	11	12	Daily dose of cortisone
No. 9	A	20														1.0 - 10.0 mg
	B	12														1.0 - 12.5 mg
	C	16														1.0 - 12.5 mg
	D	10														control
No. 3	E	14														2.5 - 50.0 mg
	F	8														control

□ : cortisone injection, √ : injection of Str. sanguis and antiserum/phosphatide

0.4ml of anti-rabbit-endocardial duck-serum and 0.5ml of living *Str. sanguis* suspension were given intravenously daily four or five days to 58 rabbits which were divided into four groups according to the modes of cortisone administration. (Table III).

Each group, except for the control group, was further divided according to the doses of cortisone. In another method, cortisone was given for eleven days, beginning 2 days before the first injection of phosphatide and *Str. sanguis*. Ten mg of rabbit brain phosphatide and 0.7ml of *Str. sanguis* suspension were injected 4 times every other day.

Fig-3 Incidence of produced endocarditis (open bars) and mortality (solid bars) in each group.

The results obtained were as shown in Figure 3. Development of an endocarditis seemed to be prevented by 2.5mg pro die of cortisone in groups B and C and by 5.0mg in group E. Mortality rate was lower in 6.25mg cortisone of group B, in all of group C and in 2.5mg and 5mg of group E. From this it may be assumed that in some doses, cortisone may be effective to prevent the disease, though the amount is not fixed but depends on experimental method. I think, it is also noteworthy that a preventive effect was noted in groups B, C and E where cortisone injection was begun preceding the inducing injections, and that mortality rate was low in groups C and E in which the duration of cortisone injections was longer.

b) Effects upon therapeutic response—

A method used in this experiment was almost the same as in the previous ones. Phosphatide and *Str. sanguis* were injected into rabbits, and from the day following the last injection, cortisone acetate was given daily, 5mg in group A and B, 25mg in group C for ten days, and treatment with

300,000 units of procain penicillin was given for five days in group B, C and D. (Table IV)

TABLE IV EFFECT OF CORTISONE UPON TREATMENT WITH PENICILLIN

Group	No. of rabbit	Day	1	2	3	4	5	6	7	8	9	10	Dosis of cortisone ^a days	Dosis of penicillin ^b
A	5	↓	□	□	□	□	□	□	□	□	□	□	5mg x 10	-
B	5	↓	□	□	□	□	□	□	□	□	□	□	5mg x 10	300,000x5
C	5	↓	□	□	□	□	□	□	□	□	□	□	25mg x 10	300,000x5
D	5	↓	□	□	□	□	□	□	□	□	□	□	-	300,000x5
E	5	↓	□	□	□	□	□	□	□	□	□	□	-	-

□ : cortisone injection, ↓ : the last injection of *Str. sanguis* and phosphatide

TABLE V THERAPEUTIC RESPONSES BY PENICILLIN AND CORTISONE IN EACH GROUP

Group	Average survival days	Death rate %	Temperature			Body-weight			Appetite		
			P ^a	T ^b	A ^c	P	T	A	P	T	A
A	14	33	△	△	△	→	→	→	→	→	→
B	10	60	△	△	△	→	→	→	→	→	→
C	12	100	△	△	△	→	→	→	→	→	→
D	9	65	△	△	△	→	→	→	→	→	→
E	2	75	△	△	△	→	→	→	→	→	→

^a P: During preliminary injections to induce endocarditis, i.e. before treatment
^b T: During treatment
^c A: After treatment

Table V shows the average survival days of the animals, mortality and some symptoms such as temperature, weight and appetite before, during and after treatment with penicillin and cortisone with comparison between each group. In Group B sufficient amount of penicillin and an adequate dose of cortisone were used for treatment and yielded better results than Group D which was treated with penicillin alone. Group C where a surplus amount of cortisone was given with penicillin therapy produced the worst results, 100% mortality.

As to the effects of cortisone we can summarize as follows:

1. An adequate amount of cortisone may prevent development of experimental endocarditis.
2. Cortisone must be given before the inducing procedures.
3. Administration of cortisone for an adequate period may reduce mortality.
4. Proper doses of cortisone combined with penicillin therapy may prolong survival days.
5. Excessive doses of cortisone may increase mortality.
6. Treatment with a moderate amount of cortisone without penicillin may improve the condition for some time.

EXPERIMENTS ON THERAPEUTIC EFFECTS

a) With a single use of antibiotic—

Leucomycin, penicillin and streptomycin were

independently tested in the treatment of experimentally induced bacterial endocarditis of rabbits. Improvement of the general symptoms was noted by the daily administration of 300,000 units of penicillin, or of over 125 mg of streptomycin. Most of the rabbits treated with penicillin showed negative blood or organ cultures. Mortality rate was the lowest in the streptomycin group (over 125 mg). The survival period was shortest in the penicillin 100,000 unit group. It was prolonged in the leucomycin and the streptomycin groups. (Table IV)

TABLE VI THERAPEUTIC EFFECTS OF LEUCOMYCIN, PENICILLIN AND STREPTOMYCIN IN SINGLE USE

Antibiotic used daily dose for	Number of rabbits	Improvement of symptoms *	Positive culture, blood/organ	Death rate	Average survival days
Leucomycin 100-200 mg 10days	7		47	43	19
Penicillin 100,000 u. 5days	4		5	75	6
300,000 u. 5days	16		8.5	56	12
Streptomycin less than 100mg 10days	8		75	50	19
over 125 mg 10days	12		35.4	0	22

* White parts show proportion of the cases where improvement of symptoms was noticed.

Histological lesions of every group were investigated from the standpoint of therapeutic effects, especially studied from the point of disappearance of bacteria in the thrombus (by Gram stain), organization and fibrosis in the basal and peripheral parts of the thrombus (Hematoxylin-Eosine and Mallory's stain), regeneration of endothelial cells on the surface of the thrombus and cellular infiltration in the tissue, decreasing in leucocytes and increasing in small round cells.

Judging from these findings, those animals treated with over 125 mg of streptomycin showed well-developed organization and fibrosis of the thrombus and almost complete disappearance of the bacteria in the vegetation. Treatment with less than 100 mg of streptomycin was ineffective, and penicillin was slightly effective. Leucomycin's effect was indefinite.

b) *Treatment of endocarditis caused by streptomycin-resistant strain of Streptococcus sanguis—*

Streptomycin resistant strain of *Str. sanguis* was used to cause an experimental bacterial endocarditis. As shown in Table VII, endocarditis caused by it did not respond well to streptomycin

treatment, this fact was also proved by histological studies. Sensitivity of the organism isolated from a diseased rabbit one month after onset of endocarditis and treatment was examined and it was shown to be as resistant as the original strain.

TABLE VII BACTERIAL ENDOCARDITIS CAUSED BY STREPTOMYCIN-RESISTANT STRAIN/SENSITIVE STRAIN, TREATED WITH 125-250 mg OF STREPTOMYCIN FOR 10 DAYS

Caused by	Number of rabbits	Improvement of symptoms *	Positive culture, blood/organ	Death rate	Average survival days
Streptomycin Sensitive strain (25 /cc)	12		35.4 %	0	22
Resistant strain (5000 /cc)	18		66.2 %	33 %	2

* White parts show proportion of the cases where improvement of symptoms was noted.

The results obtained in this series of experiments are summarized as follows:

1. The streptomycin resistant strain can cause an endocarditis in rabbit similar to the sensitive ones.
2. Virulence of the resistant strain is stronger than that of the sensitive strains.
3. Endocarditis by the resistant strain does not respond to streptomycin.
4. Resistance did not change in vivo after one month.

RECONSIDERATION ON ANTIBIOTIC THERAPY

In spite of larger doses of antibiotic than that usually used clinically, therapeutic effect in the above experiments was not always satisfactory. In order to explain this observation, some experiments were made.

a) *Experiment using Streptococcus mitis—*

In all of the above experiments we used a strain of *Str. sanguis* that has been reported to cause a hardly-curable endocarditis in man, but here a strain of *Str. mitis*, a common cause of human endocarditis, was employed. Only one rabbit out of 8 infected with *Str. mitis* developed endocarditis. It was well cured by streptomycin.

Therefore, we can say, it is not due to the ineffectiveness of the antibiotics, but due to the resistance of *Str. sanguis* used to produce the endocarditis that prevented cure in our experimental endocarditis.

b) *Therapeutic effect judging from size of vegetation—*

Judging from the size of the vegetations seen in the valve, the rabbits which had a vegetation

of bean size showed 100% mortality, positive blood or organ cultures and evidences of venous congestion such as hepatomegaly and pleural or peritoneal fluid, regardless to kind or dosage of drugs. On the other hand, the rabbits with miliary or smaller vegetations showed a very low death-rate, few positive cultures and no evidence of congestion.

From this, it will be obvious that we can not expect satisfactory effectiveness of antibiotics if a vegetation has already grown beyond a certain size. We know how difficult it is to eradicate bacteria in a vegetations since positive cultures were obtained from large vegetations, while cultures of the heart blood were negative.

TABLE VIII HEMODYNAMICS OF A DOG WITH EXPERIMENTAL ENDOCARDITIS STUDIED BY CARDIAC CATHETERIZATION

BLOOD PRESSURE (mm Hg)	Right Ventricle	Before	15 / -5 : 3	↑
		After	21 / -4 : 4	
	Pulmonary Artery	Before	12 / -7 : 2	↑
		After	20 / -5 : 11	
	So-called Pulmonary Capillary	Before	0 / -12 : -7	
		After	-----	
	Femoral Artery	Before	160 / 115 : 130	↑
		After	220 / 100 : 145	
CARDIAC OUTPUT (l/min.)		Before	1.94	↓
		After	1.15	

Table 8 shows the hemodynamics of a dog with experimentally produced endocarditis investigated by cardiac catheterization. This dog had a large vegetation on the aortic and mitral valves. Hypertension in the right ventricle, pulmonary artery and femoral artery and decrease in cardiac output etc. were noticed. These data well coincide with the congestion that appeared in the rabbits with bean-size vegetation above mentioned.

DISCUSSION ESPECIALLY IN REFERENCE TO CLINICAL ASPECTS

From our experiments above mentioned, we have many important evidences from which the pathogenesis or pathological (histological) processes of human endocarditis can be analogized. We could damage the valve of rabbit beforehand and succeeded in producing endocarditis upon it. Therefore, we believe that the methods we tested suc-

cessfully using rabbits can be available for further investigation of pathogenesis or other problems.

At autopsy we have found that there were mixtures of a healing process and a fresh active focus in experimental endocarditis treated with antibiotics, and this is just the same as we often experience in human cases.

As to use of adrenocortical hormone for treatment of subacute bacterial endocarditis, many problems still remain unsolved. We can not decide conclusively without cautious consideration. The only thing we can say now from our experiments is that a certain amount of cortisone may be effective for prevention of the disease and when used combined with antibiotics. Subacute bacterial endocarditis has both new and old lesions at the same time as mentioned; and therefore, the amount and duration, if cortisone therapy is attempted, must be well considered in preventive and therapeutic senses.

Streptococcus sanguis, a subtype of *Str. viridans*, has already been known as an annoying causative agent of subacute bacterial endocarditis, and it was proved also by experiment. Therefore, it is very important to detect a subtype of *Str. viridans* as early as possible, and the precipitation-reaction we reported before is quite helpful for it. It is our future responsibility to solve the problem why endocarditis caused by *Str. sanguis* is so difficult to cure and to find a satisfactory therapeutic method and regimen for it.

When a vegetation became larger than a given size, all of the animals could not be cured, regardless of chemotherapeutics employed. This seems to mean that some effective treatment is absolutely necessary in the early stages of subacute bacterial endocarditis that causes a destruction and deformity of the valve without fail, in order to prevent the formation of large verruca. The animals which manifested signs or symptoms of congestive heart failure, including the dog examined by catheterization, died after a protracted course of the disease under chemotherapy. This phenomenon coincides with that in human cases. We have previously stressed that prevention and treatment against cardiac failure are as important as antibiotic therapy against infection in treatment of subacute bacterial endocarditis.