

THE EFFECT OF THE ANTIBIOTICS ON THE BLOOD FIGURES (1)

—The Effects of Streptomycin on the Number of
Leucocytes and their Ability of the Locomotion—

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姜永善 趙完圭 : 抗生物質이 血液像에 미치는 影響 (第 1 報)

—Streptomycin 이 白血球數와 그 運動能에 미치는 影響—

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INTRODUCTION

Since the antibiotic substances were discovered, they have greatly contributed many advantages to us. It is said that the antibiotic substances which have been discovered are nontoxically chemotherapeutic materials.

After 1944, when Streptomycin was introduced as one of the antibiotic substances by SCHATZ, BUGIE and WAKSMAN¹⁾(1945), its pharmacological, biochemical and therapeutical investigations were made in every way. We took up the reports on the blood figures in many works, as following.

MOLITOR et al.²⁾ (1945) using cats and rabbits for the material, investigated the variation of their blood pressures. MACHT³⁾ (1947) made up his work on the blood clotting. On acute toxicity, MOLITOR⁶⁾ (1946) also reported how the chemical effects the blood figures using monkeys for material. FELDMAN⁷⁾ (1945) and KORNEGAY⁸⁾ (1946) had counted the red, white blood cells, and ANDERSON⁹⁾ (1945) researched the blood figures of man daily treated with Streptomycin.

All of them said that Streptomycin does not act as a toxic substance, and does not make a remarkable variation on the blood figures. On the other, BROWNLEE and BUSHBY¹⁷⁾ (1943) reported that the leucocytes treated with Streptomycin were killed within an hour.

Judging from the above studies, it can be said that Streptomycin does not act toxically but is one of the safest chemicals for use as a chemotherapeutic agent.

But we performed this investigation as a certain change on the leucocytes was expected.

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EXPERIMENT I. THE CHANGE OF NUMBER OF LEUCOCYTES BY THE DOSES
OF STREPTOMYCIN

1) Forewords

Though Streptomycin was introduced as a safe and harmless chemotherapeutic reagent, BROWNLEE and BUSHBY¹⁷⁾ (1943) reported harmfulness to leucocytes at the influence of streptomycin. Therefore, we established this investigation to determine whether or not the number of leucocytes are variable by the action of Streptomycin.

2) Material and Methods

The white mice ranging 13-25 gr. in body weight were employed as material throughout this investigation. Each one of the experimental plots consisted with 5-10 mice. 0.2 c.c. of 0.1 mg., 2mg., 5mg., and 10mg. of streptomycin were dissolved in 5% Dextrose solution and injected intramuscularly.

Normal count of leucocytes in each mice was observed before treatment with Streptomycin. Spencer's haemocytometer was employed and the blood from the tail venous vessel was diluted and stained with Turk's reagent. In each case the blood was diluted with the reagent in a 1:20 dilution.

After the exact dilution, the pipette was shaken sufficiently to get an even distribution of the blood corpuscles. After filling the slide chamber with proper amounts of diluted blood, counting under a microscope was made. The number of treated material and amounts of Streptomycin in each case are shown in Table I.

Table I.

Plot	No. of Subjects	Doses (mg)	Remarks
1	5		5% Dextrose (Control)
2	7	0.1	
3	7	1	
4	6	2	
5	10	5	

3) Results

Table-II.

Plot		No. of Subj.	Before Treat	6 hrs after	12 hrs after	24 hrs after	48 hrs after	Remarks
I. (as a control)	M. V.	5	10,720	10,640	11,280	14,480	11,360	*t=0.03
	E. R.		7,600-16,320	4,120-17,000	8,240-15,280	11,080~	9,160-13,200	
	S. D.		3,308	4,874				
	S. E.		1,479	*2,179		17,520		
	ρ							
II	M. V.	7	12,966	6,554	10,211	13,211	13,024	*t=2.75 p<5%
	E. R.		7,560-20,200	3,360-13,520	6,080-16,040	8,640-23,800	8,280-17,040	
	S. D.		4,502	4,068				
	S. E.		1,702	1,537				
	ρ			0				
R. C.	120.95%	* 61.94%	90.52%	91.23%	114.64%			
III	M. V.	7	13,069	6,480	7,893	10,730	13,173	*t=3.89 p<1%
	E. R.		7,560-18,240	4,520-8,800	3,840-9,040	9,360-11,760	10,320~	
	S. D.		4,254	1,316			15,200	
	S. E.		1,608	497				
	ρ			0				
R. C.	121.91%	* 60.90%	65.54%	74.10%	115.95%			
IV	M. V.	6	2,067	9,287	17,147	15,360	15,544	*t=2.58 p<1%
	E. R.		7,320-17,760	4,240-14,320	10,720~	8,240-17,670	8,280~	
	S. D.		4,704	2,676	20,840		19,800	
	S. E.		1,920	1,092				
	ρ			0.89				
R. C.	112.56%	* 37.28%	152.01%	106.07%	136.83%			
V	M. V.	10	12,096	8,228	9,728	12,603	13,456	*t=3.79 p<1%
	E. R.		7,520-20,680	3,360-15,320	4,040-20,880	4,360-18,480	6,680-19,840	
	S. D.		4,047	1,196				
	S. E.		1,280	361				
	ρ			0.79				
R. C.	112.83%	* 77.33%	86.24%	87.03%	118.49%			
Average			12,266	7,755	10,841	12,903	13,730	

M. V. — Mean Value, E. R. — Extreme Range, S. D. — Standard Deviation, S. E. — Standard Error of the Mean, S. — Coefficient of Variation, R. C. — Rate with Control, P. — Probability, *—t-test

The number of leucocytes in each experimental plots treated with Streptomycin was remarkably decreased far below the normal range in 6 hours, but recovery to normal number was obtained in 12 hours. In 24 hours, the number of leucocytes was normal again.

EXPERIMENT II. THE CHANGE OF NUMBER OF LEUCOCYTES BY HOURS AFTER TREATED WITH STREPTOMYCIN

1) Forewords

KORNEGAY¹⁷⁾(1945) reported that Streptomycin concentration in the blood vessel was at its maximum value within 2-3 hours after injection of mice with Streptomycin. This second experiment was done to clarify the results of foregoing experiment(Exp. I). The number of leucocytes was counted at the intervals of each period in foregoing experiment.

2) Material and Methods

Same materials and methods as Exp. I were employed throughout this investigation. In first experimental plot, eight materials were divided into two groups, one of them was counted at 1 hr., 3 hrs., 9 hrs., 13 hrs. after 10 mg/0.2 c.c. of Streptomycin was injected. The other was counted after 2 hrs., 9 hrs., and 13 hrs. respectively. In the second experimental plot, nothing was injected to the 5 materials and counting was done at every 6 hours, through 24 hours.

3) Results

The results of the first experimental plot are shown in Table III.

Table-III

Group		No. of subj.	Before treat.	1hr. after	2hrs. after	3hrs. after	9hrs. after	13hrs. after
A	M. V.	4	11,580	5,350		3,200	10,870	11,733
	E. R.		8,000-13,640	3,800-6,900		2,800-3,500	9,120-11,880	9,800-13,200
B	M. V.	4	12,990		6,960		12,463	13,840
	E. R.		6,600-19,480		3,400-11,320		3,680-18,240	15,840-18,200

Table-IV.

	No. of Subj.	1st count.	6hrs. after	12hrs. after	18hrs. after	24hrs. after
M. V.	5	13,632	12,040	13,740	12,400	11,230
		8,080-21,080	8,920-15,280	11,120-16,720	9,120-15,080	8,360-14,800

M. V. - Mean Value.

E. R. - Extreme range.

M. V. - Mean Value, E. R. - Extreme range.

The result of second experimental plot is shown in table IV.

As is shown in experimental results, decrease in number of leucocytes was followed rapidly within one hour after injection. This condition continued for about three hours. After this condition, recovery of the number of leucocytes to its normal range was attained after nine hours.

EXPERIMENT III. THE EFFECT OF STREPTOMYCIN ON THE ABILITY OF LEUCOCYTIC LOCOMOTION

1) Forewords

The effect of Streptomycin on the number of leucocytes can be clearly seen from the results of first and second experiment. Therefore, we furthermore observed the effect of Streptomycin on the ability of the leucocytic locomotion to search out the reason why the

number of leucocytes was decreased as described above.

2 Material and Methods,

White mice ranging from 13-20 gr. in body were employed as material in this investigation. One drop of blood from the tail venous vessel was mixed with dihydrostreptomycin directly on the slide glass. 5% Dextrose was used as a solvent was dropped on the slide glass, when the one drop of blood from the tail venous vessel was attached to the cover glass, by putting the cover glass on the slide the diffusion of blood through the medium was soon attained. Observation was done when the blood corpuscles rested motionless about 15 minutes after this manipulation.

In this observation, slide glass was maintained within the incubating box and kept at $38^{\circ} \pm 2^{\circ}C$, then one of the most motile neutrophile was chosen trace, using a sight apparatus. By doing this manipulation for three minutes, the distance was attainable in getting the velocity of leucocytes in each case. Two or three neutrophiles on a slide were traced and changed to another and soon. One experimental plot consisted with 15 neutrophiles. The observation was done under the power of 1500 using oil immersion method.

Treating plots employed are as follows.

- 1) control (nothing treated)
- 2) 1 mg. (in 0.05 c.c of 5% Dextrose)
- 3) 2.5mg. (" ")
- 4) 5mg. (in 0.05c.c of 5% Dextrose)
- 5) 6.5mg. (" ")
- 6) 10mg. (" ")

3) Results.

The results of this investigation are shown in Table V.

Table-V Averages of 15 Subjects

	$38^{\circ}C \pm 2^{\circ}C$					
	Normal (μ)	1mg. (μ)	2.5mg. (μ)	5mg. (μ)	6.5mg. (μ)	
M. V.	15.42	7.58	3.66	1.24	0.54	M. V. Mean Value
E. R.	8.58- 24.04	1.23- 14.71	1.67- 8.47	0- 2.38	0- 3.04	E. R. Extreme Range
S. D.	4.74	4.60	1.84	0.94	0.62*	S. D. Standard Deviation
S. E.	1.22	1.19	0.48	0.64	0.16	S. E. Standard Error of the Mean
		t=4.75	t=3.06	t=4.38	t=3.4	t.....t-test
		p<1%	p<1%	p<1%	p<1%	p.....probability

Discussing from Table V, we can ascertain the marked decrease of locomotion ability of the leucocytes as one increases of Streptomycin. When more than 10 mg. of Streptomycin was used, not only was the locomotion of leucocytes absent but also Brownian movement of the granules in the leucocytes ceased within 15 minutes. In the experimental plot treated with 6.5mg. of Streptomycin, that standard deviation is larger than mean value is due to the fact that eight out of fifteen neutrophiles were motionless.

DISCUSSION

Streptomycin has been known as a safe chemical of no toxicity. MOLITOR² (1946) re-

ported that blood pressure of a cat was slightly depressed when treated with this chemical and in the case of rabbit, same phenomenon was detectable as that treated with Histamine. Moreover, treating with 120-375mg./kg. intramuscularly, resulted in a most severe influence of the heart and eventually death occurred. MACHT⁵(1947) stated that Streptomycin reveals thrombolytic effect on blood coagulation nothing concerning the change of number of leucocytes at a single dose of Streptomycin. But there are many reports on the change of blood figures after repeated doses of Streptomycin.

SMITH, MCCLOSKEY⁴(1945), FELLMAN and HINSHAW⁵(1945) observed no toxic effect upon guinea pig treated 50,000-100,000 u./kg. of Streptomycin for ten days continuously. MOLITOR⁶(1946) also observed no effect upon blood figures of monkey dosed daily with 25mg./kg. FELLMAN et al.⁷(1945) and KORNEGAY et al.⁸(1946) reported no visible effect upon the leucocytes count was observed. ANDERSON, JEWELL⁹(1945), MADIGAN et al.¹⁰(1947) and FARRINGTON¹¹ reported no effect on the blood figure of man dosed daily with Streptomycin. Though KEEFER¹²(1945) only reported 10% increase of eosinophiles of seven out of sixteen men who had treated 3 gr. daily for 120 days.

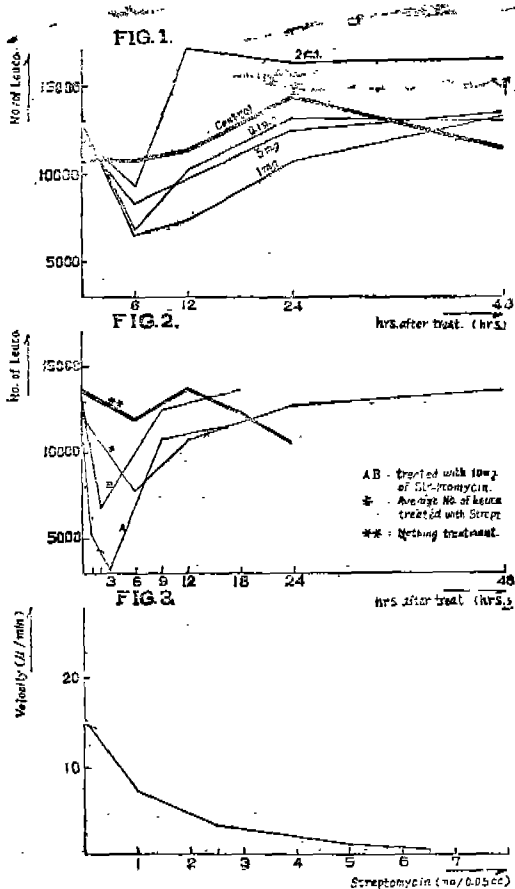
LEPAGE and CAMFBELL¹³(1946) reported that a fatal dose of Streptomycin to mouse were amounts of 18,000-40,000 units and after 40,000 units were given 100% of the mice were dead from asphyxiation.

Judging from the past investigations, it has been known that Streptomycin has no effect upon the blood corpuscle counts within the limit of fatal doses. But in accordance with our experimental data (as is shown in Table I, Table II, Fig.1 and Fig.2), we state that remarkable decreases in number of leucocytes were detectable in every experimental plots after six hours of treatment.

But on this account, no externally abnormal conditions were recognizable and at least in nine hours after treatment, there was a normal condition in which the number of leucocytes was recovered.

In view of this fact, it can be expected that moderate amount of streptomycin shows no toxic effect on living thing. On the contrary, we can assume the fact that the number of leucocytes was decreased far below the normal range transiently by the action of streptomycin.

In Experiment I, phenomena in which the numbers of leucocytes were decreased, almost the same degree in all experimental plots were observed.



The number of leucocytes in each plots were decreased having no relation to their doses of Streptomycin as shown in Table II. Fig. 1 Normal condition, in which the number of leucocytes was recovered, was attained in nine hours gradually, and then recovering was done completely from that time on.

KORNEGAY⁴⁾(1946) reported streptomycin kept their maximum concentration in the blood serum in 10-15 minutes after injection of 5,000-10,000 u./kg. of Streptomycin was done intramuscularly to a mouse and that gradual decreasing of concentration of streptomycin followed from that time on, and all of the streptomycin was discharged completely in 2-3 hours after injection.

ZINTEL et al.¹⁵⁾(1945) also reported that the maximum concentration of Streptomycin in the blood of man was attained in one hour and almost complete of this was done in 24 hours.

Since Streptomycin is absorbed rapidly into the blood the number of leucocytes must be greatly decreased within six hours rather than after six hours as shown in Experiment II (Table III, Fig. 2). In view of the results of these Experiment I and Experiment II, it was apparent that the decrease in number of leucocytes is caused by the direct action of Streptomycin to leucocytes.

Similarly, since it soon became evident that Streptomycin had a considerable effect on leucocytes, Experiment III was made to reveal a possible general relation between the number of leucocytes and Streptomycin itself was tested on the slide glass extracted from a blood vessel and mixed with Streptomycin directly.

COMMANDON et al.⁶⁾(1913), JOLLY⁹⁾(1913), SUGIYAMA¹⁰⁾(1925), PHILIPSBORN¹¹⁾(1923, 30) and WATANABE¹²⁾(1931, 32) observed the motility of leucocytes and determined the velocity of them.

It was apparent from the Table V, Fig. III the more doses of Streptomycin given to leucocytes, the less motile the leucocytes became. SCHÖNBACH and CHANDLER⁸⁾(1947) published that phagocytosis of polymorphonuclear leucocytes was decreased when 500u./ml. of streptomycin was treated to leucocytes. BROWNLEE and BUSHBY⁷⁾(1948) also reported that leucocytes treated with 1,000 u./ml. of Streptomycin were killed within 30 minutes.

In view of the results of Experiment III, when 10 mg. of Streptomycin dissolved in 0.05 c.c of 5% Dextrose was mixed with one drop of blood, leucocytes were dead within 15 minutes. But leucocytes similarly treated with less than 5 mg. of streptomycin were kept alive at least 30 minutes showing motilities related with proportional to the doses of streptomycin.

There remained one other point of investigation in the survival time of leucocytes treated with variable doses of streptomycin for future studies.

SUMMARY

1. Number of leucocytes was decreased transiently when less than fatal doses of Streptomycin were treated to mice.
2. Number of leucocytes in its minimum value was attained within six hours after treatment with Streptomycin and its value was as low as 1/4 that of normal one.
3. The motile ability of leucocytes was diminished as the doses of Streptomycin increa-

sed.

4. Leucocytes were killed rapidly after dosed more than a certain limit of streptomycin.
5. There were not apparent evidence that close relation existed between the decrease in number of leucocytes and the inactiveness of motile ability of leucocytes.
6. In order to clarify the causes of decrease in number of leucocytes, must expect in future studies in which special methods will be employed.

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摘 要

Streptomycin은 아직까지 가장 動物體에 安全한 抗生物質로 알려져 있으나, BROWNLEE 와 BUSHBY (1948)에 의하면 白血球가 Streptomycin 處理에 의해 죽는다고 하였다. 本人等은 Streptomycin을 處理한 흰쥐의 白血球數의 變化와 白血球 自體의 運動能에 미치는 影響을 調査하였다. 致死를 이르기까지 하는 最低量 以內인 10mg, 5mg, 2mg, 1mg, 0.5mg을 각각 흰쥐에 注射한 후 一定한 時間까지의 白血球數의 變化를 보면 處理후 1時間에 서부터 6時間 사이에 白血球數가 急激히 減少하며 正常數인 12,000에서 最下 3,000 臺까지 내려 간다. 減少하는 程度는 處理量의 多少에 關係없이 大概 同一하게 일어나는 것이다. 減少하는 原因을 追求하고자 쥐의 尾部로부터 採血하여 이에 Streptomycin을 直接 接觸시켜 白血球의 運動能을 調査하였다. Streptomycin의 量에 比例하여 白血球의 運動速度는 低下한다. 一定量을 超過할 때는 白血球는 短時間내에 죽는다. 處理量의 多少를 不問하고 一定한 程度로 數의 減少가 일어나는 것과는 달리 白血球의 運動能은 量에 따라 많은 變化를 가져온다. 이것으로 보아 白血球數의 減少와 運動能의 低下와는 特別한 關係가 없는 것으로 보며 白血球數 減少의 原因에 대해서는 此後 實驗에서 밝혀야 할 것이다.