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00N-1219

SUPI
VOL3

DELMONT LABORATORIES, INC.
BIOLOGICAL SPECIALTIES

P. O. BOX AA, SWARTHMORE, PENNSYLVANIA 19081, U.S.A.

March 31, 1978

Miss Jennie C. Peterson
Hearing Clerk (HFC-20)
Food and Drug Administration
Room 4-65
5600 Fishers Lane
Rockville, Maryland 20857

Re: Docket No. 77N-0091 -- Bacterial
Vaccines and Bacterial Antigens
With No U.S. Standard of Potency

Dear Miss Peterson:

On January 8, 1978, Delmont Laboratories, Inc., submitted comments on the Food and Drug Administration's proposal to amend the biologics regulations in response to the report of the Advisory Panel on Bacterial Vaccines and Bacterial Antigens with No U.S. Standard of Potency (42 Fed. Reg. 58266 (Nov. 8, 1977)). Those comments were accompanied by reports of toxicological studies of Delmont's product Staphage Lysate (SPL) for Staphylococcal Disease, conducted by the Fujizoki Pharmaceutical Co., Ltd., of Tokyo, Japan. On February 2, 1978, representatives of Delmont met with representatives of the Bureau of Biologics to discuss Delmont's comments and the data submitted in support of them. At that meeting, Bureau officials requested further information concerning the numbers of animals involved in the Fujizoki studies and the identity and composition of the SPL materials used in the studies. Delmont requested that information from Fujizoki and now transmits it to the agency as a supplement to its January 8, 1978, comments. The following documents are enclosed:

1. A list of questions sent to Fujizoki concerning the numbers of animals and lots of SPL used in various studies, together with Fujizoki's reply. Based on the lot numbers supplied by Fujizoki, Delmont has developed protocols of the SPL lots used in the Japanese studies, and those are attached.

2. Copies of the reports submitted on January 8 which have been annotated to incorporate the information provided by Fujizoki in response to Delmont's questions. Those reports include:

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a. Acute and subacute toxicity tests of SPL, a chronic toxicity test of SPL in rats, and a teratogenicity test in rats, which accompanied a November 29, 1977, letter from Fujizoki to Delmont. (Exhibit 1 of Delmont's January 8, 1978, submission.)

b. A report on the effect of SPL on the development of skin lesions in mice after inoculation with herpes simplex virus, which accompanied a December 1, 1977 letter from Dr. Kenji Takeya of Kyushu University to Delmont Laboratories. (Exhibit 2 of Delmont's January 8, 1978, submission.)

c. Various reports that accompanied a December 28, 1977, letter from Fujizoki to Delmont. (Exhibit 3 of Delmont's January 8, 1978 submission.)

In addition, we are enclosing three new reports not previously available:

1. A report of subacute and chronic toxicity tests of SPL in rabbits, by Hachihiko Hirayama and Haruo Ohkuma of Fujizoki Pharmaceutical Co., Ltd.

2. A report of a double blind, placebo controlled trial for evaluation of SPL as a treatment for warts, dated March 7, 1978.

3. An assay for specific and nonspecific host resistance enhancing activity of Staphage Lysate submitted to Delmont by Mason Research Institute of Worcester, Massachusetts, on February 14, 1978.

Respectfully submitted,

Charles E. Lincoln
Charles E. Lincoln
President 

SW

CC: John Robbins, M.D. (HFB-500) (w/encls.)
Robert P. Brady, Esq. (GCF-1) (w/o encls.)

Questions concerning numbers of animals and lots of SPL used.

1. In the paper by Fujino, Sugisaki, Nakagawa & Komatsu entitled Chronic, acute & subacute Toxicity Tests of SPL - what is number of rats and mice, both male and female used in Figures 1 and 2; Tables 1,2,3,4,5.
2. In paper by Dr. Takeya entitled Effect of SPL on development of skin lesions in mice after inoculation with Herpes simplex virus, how many mice per group? (There seems to be 3 per group)

Ask clarification of Figs. 1 - 5.

3. In paper by Dr. Takeya entitled Chemotactic accumulation of macrophages in the peritoneal cavity after inoculation of SPL and their antitumor activity.

Table 1) How many mice per group?
Table 2)

4. (a) In "Summary of results of tests conducted at Fuji-Toki Pharmaceutical Research Division" under Dr. Daichia Watanabe's direction, how many test animals (species, sex) were used in the Histamine test?

(b) In the paper by Azuma, Tokuda and Shibata entitled Immunopotentiator activity of Staphaga Lysate (Mudd), how many "subjects" or rabbits and mice were used?

Our Replies to your Questions
concerning
Numbers of animals and lots of SPL used

1. (A) Numbers of animals and lots of SPL used in Acute Toxicity Test

MICE

Route	p.o.										s.c.	i.p.	i.v.	Total
	Male					Female								
Sex														
Dose ml/kg	12.5	25.0	50.0	75.0	100.0	12.5	25.0	50.0	75.0	100.0				
No. of animals	10	10	10	10	10	10	10	10	10	10	-do-	-do-	-do-	400
Lot No.	4040193, 5021004, 5071411										Total of SPL: 300 ml			

RATS

The same method as in the above case of mice was adopted.

3 groups each for male and female
8 rats per group, 4 routes
Total: 320 rats

SPL Lot Nos. 4040193, 5021004, 5071411
Total Volume of SPL used: 1000 ml (approx.)

(B) Numbers of animal and lot of SPL used in Subacute Toxicity Test

RATS

1 group each for male and female
20 rats per group, S. C.
Total: 40 rats

SPL Lot No. 5071411
Total Volume of SPL used: 4000 ml (approx.)

(C) Numbers of animals and lots of SPL used in Chronic Toxicity Test

RATS

This report we've not yet received from Japan.

1 group of male
20 rats per group, S. C.
Total: 80 rats

SPL Lot Nos. 6011225, 6011229, 6011231, 6011234
Total Volume of SPL used: 10,000 ml (approx.)

- ✓ 2. Fig. 1, group of 18Z(-), SPL(-) 6 mice
Fig. 2, group of 18Z(-), SPL(+) 8 mice
Fig. 3, group of 18Z(+), SPL(-) 8 mice
Fig. 4, group of 18Z(+), SPL(+) 8 mice
Fig. 5, Average scores of mice in each group of the aboves (Fig. 1~Fig. 4)

SPL Lot No. 6012639 Volume: 25 ml (approx.)

- ✓ 3. Table 1. 6 mice per group. 4 groups.
Total 24 mice
Table 2. 6 mice per group. 4 groups.
Total 24 mice

SPL Lot No. 6090755 Volume: 120 ml (approx.)

- ✓ 4. (a) Matured normal cat (male and female)

3 cats were involved in each lot. total: 9 cats (male & female)

Lot Nos. 6090755, 6111462, 6111463 Total of SPL used: 1 ml (approx.)

(b) detail missing

PROBABLE SPL LOTS USED IN JAPANESE STUDIES

SPL #	TYPE I DATA	TYPE III DATA	FINAL TITER
4040195	4041286 Bot. #3 PFU 1.1×10^9 CFU 174 m/ml pH 7.35	4040177 Bot. #3 PFU 1.18×10^9 CFU 159 m/ml pH 7.49	$.24 \times 10^9$
4061293	4061286 Bot. #2 PFU 1.4×10^9 CFU 150 m/ml pH 7.46	4093089 Bot #1 PFU 1.03×10^9 CFU 127 m/ml pH 7.65	$.52 \times 10^9$
5021004	5031700 Bot. #1 PFU 1.03×10^9 CFU 157 m/ml pH 7.2	5021099 Bot. #3 PFU 1.3×10^9 CFU 162 m/ml pH 7.47	$.69 \times 10^9$
5071411	5072808 Bot #2 PFU $.63 \times 10^9$ CFU 154 m/ml pH 7.31	5071407 Bot. #1 PFU $.97 \times 10^9$ CFU 152 m/ml pH 7.43	$.55 \times 10^9$
6011225	6011218 Bot. #3 PFU 1.04×10^9 CFU 172 m/ml pH 7.30	6012619 Bot. #3 PFU $.72 \times 10^9$ CFU 169 m/ml pH 7.32	$.4 \times 10^9$
6011229	6011218 Bot #4 PFU $.9 \times 10^9$ CFU 164 m/ml pH 7.30	6012619 Bot #4 PFU 1.04×10^9 CFU 169 m/ml pH 7.30	$.48 \times 10^9$
6041231	6041226 Bot #3 PFU $.7 \times 10^9$ CFU 157 m/ml pH 7.51	6042627 Bot. #2 PFU $.8 \times 10^9$ CFU 149 m/ml pH 7.41	$.45 \times 10^9$
6041234	6041226 Bot. #4 PFU $.7 \times 10^9$ CFU 149 m/ml pH 7.51	6042627 Bot. #1 PFU 1.0×10^9 CFU 153 m/ml pH 7.41	$.4 \times 10^9$

SPL #	TYPE I DATA	TYPE III DATA	FINAL TITER
6042639	6091638 Bot. #2 PFU 1.0×10^9 CFU 135 m/ml pH 7.4	6042627 Bot. #4 PFU 1.7×10^9 CFU 176 m/ml pH 7.42	$.4 \times 10^9$
6090755	6111452 Bot #2 PFU 1.07×10^9 CFU 149 m/ml pH 7.5	6090741 Bot #5 PFU 1.3×10^9 CFU 143 m/ml pH 7.43	$.93 \times 10^9$
6111462	6111454 Bot #1 PFU 1.53×10^9 CFU 148 m/ml pH 7.58	6111951 Bot. #3 PFU 1.5×10^9 CFU 157 m/ml pH 7.45	$.5 \times 10^9$
611463	6111454 Bot #4 PFU 1.34×10^9 CFU 143 m/ml pH 7.55	6111951 Bot #1 PFU 1.70×10^9 CFU 137 m/ml pH 7.45	$.73 \times 10^9$
7040856	7040872 #2 PFU 1.79×10^9 CFU 196 m/ml pH 7.1	7052777 Bot #2 PFU $.64 \times 10^9$ CFU 145 m/ml pH 7.2	$.55 \times 10^9$
7021451	7021464 Bot #2 PFU $.9 \times 10^9$ CFU 189 m/ml pH 7.15	7021464 #4 PFU 1.48×10^9 CFU 165 m/ml pH 7.2	7033071 Bot #2 PFU 1.43×10^9 CFU 203 m/ml pH 7.1
			7033071 #3 PFU 1.40×10^9 CFU 187 m/ml 7.025
7033085	7040872 Bot #3 PFU 1.63×10^9 CFU 215 m/ml pH 7.15	7040872 #4 1.70×10^9 136 m/ml 7.1	7052777 Bot #1 $.60 \times 10^9$ 111 m/ml 7.2
			7033071 #4 1.45×10^9 201 m/ml 7.05
			$.53 \times 10^9$

 FUJIZOKI PHARMACEUTICAL CO., LTD.

International Division

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Tokyo, November 29, 1977

Mr. Charles E. Lincoln, President
DELMONT LABORATORIES, INC.
P.O. Box AA, Swarthmore,
Pennsylvania 19081
U. S. A.

Dear Charlie:

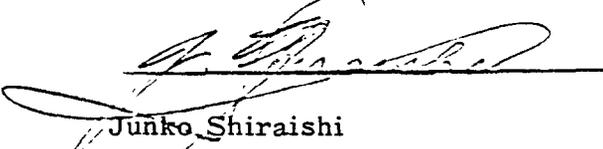
We have heard the information of SPL from Dr. Aoki.

Enclosed please find the copies of "Acute and Subacute
Toxicity Tests of SPL, Chronic Toxicity Test of SPL
in rats and Teratogenicity Study of SPL in Rats.

Thanking you for your kind cooperation, we remain

Sincerely yours,

FUJIZOKI PHARMACEUTICAL CO., LTD


Junko Shiraishi

International Division

Chronic Toxicity Test of SPL in rats

Ryuichi FUJINO¹⁾, Yuji SUGISAKI¹⁾, Junko NAKAGAWA¹⁾,
Masana KOMATSU¹⁾
and
Hachihiko HIRAYAMA²⁾

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Resarch Department Lab. II Fujizoki Pharmaceutical Co., Ltd.
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Summary

Chronic toxicity of SPL for staphylococcal disease manufactured by Delmont Laboratories Inc. was investigated in comparison with sterile saline as a control group for 182 days in Wistar rats.

Rats were given once a day at a subcutaneous dose of 0.8, 4.0 and ^{5 ml}20.0 ml/kg of SPL and ^{2.5 ml}20.0 ml/kg of sterile saline, respectively.

They were observed daily for appearance, behavior and survival: body weights were mesured periodically. All animals were sacrificed and examined at necropsy for abnormalities, and subsequently for histomorphologic alterations. Heamotological, biochemical and urinary examinations performed respectively.

No remarkable changes of every observations were observed in all groups treated with SPL and control group.

Acute and Subacute Toxicity Tests of SPL

Ryuichi FUJINO, Yuji SUGISAKI, Junko NAKAGAWA

and

Masana KOMATSU

Research Department Lab. I

Fujizoki Pharmaceutical Co., Ltd.

6-7, Shimoochiai 4-chome,

Shinjuku-ku, Tokyo

Acute and Subacute toxicities of SPL for staphylococcal disease manufactured by Delmont Laboratories Inc. were studied in mice and rats.

Acute Toxicity Test

SPL Lots used.

LD₅₀ of SPL were as follows:

4040193

5021004

5071411

Species	Sex	Administration route				(ml/kg)
		p.o.	s.c.	i.p.	i.v.	
Mouse (Total SPL 300 ml)	Male	100<	100<	100<	100<	10 mice / group
	Female	100<	100<	100<	100<	" " "
Rat (Total SPL 1200 ml)	Male	50<	50<	70<	50<	5 rats / group
	Female	50<	50<	70<	50<	" " "

} for each route

The median lethal doses for every administration routes in mice and rats were more than technically applicable maximum doses. In subacute toxicity test for 30 days, rats were given once a day subcutaneous dose of 2.0, 7.0 and 20.0 ml/kg, respectively. The control group received sterile saline at 20.0 ml/kg. They were observed daily for appearance, behavior and survival; body weights were measured periodically. All animals were sacrificed and examined at necropsy for abnormalities, and subsequently for histomorphologic alterations. Hematological, biochemical and urinary examinations performed respectively.

No remarkable changes of every observations were observed
in all groups treated with SPL and control group.

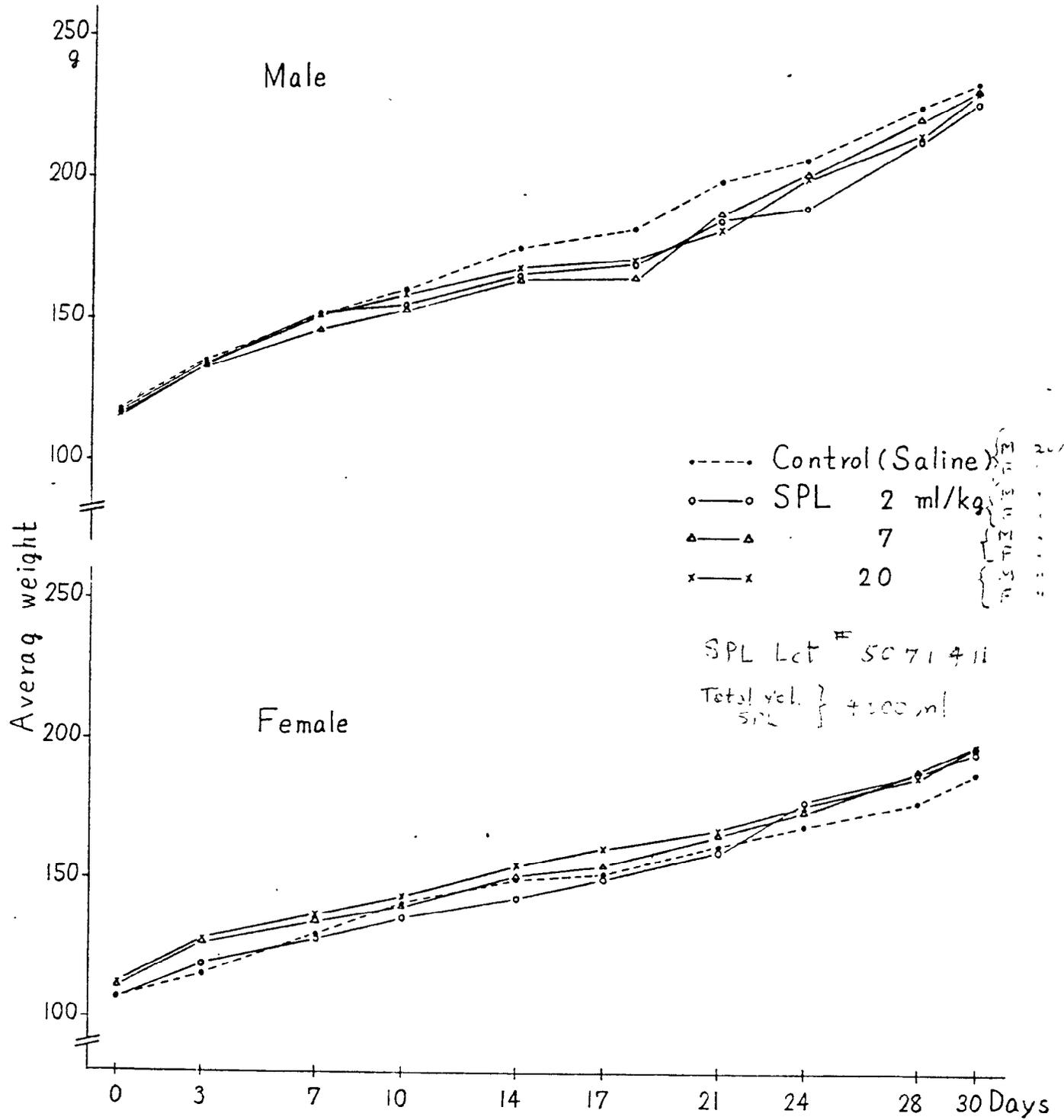


Fig. 1 Average body weights in male and female rats treated with SPL (s.c) for 30 days

Subacute Toxicity Test

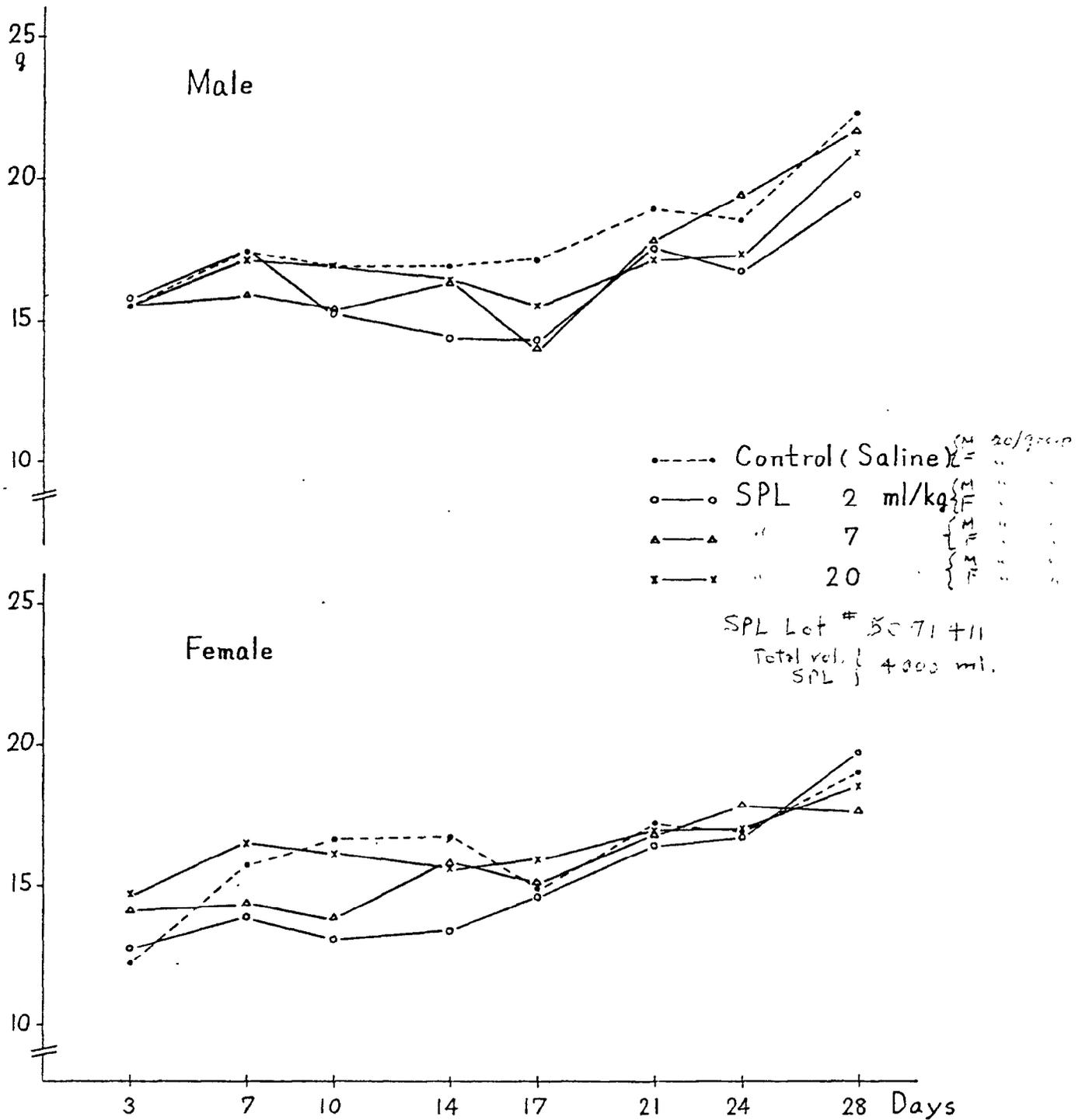


Fig. 2 Food consumptions in male and female rats treated with SPL (s.c) for 30 days

Table 1 Average wet weight of various organs in rats treated with SPL (s.c.) for 30 days (Mean ± S.D.)

Tissue	Sex	Control (Saline)	SPL		
			2 ml/kg	7 ml/kg	20 ml/kg
Body weight (g)	M	234 ± 34	227 ± 45	232 ± 43	231 ± 31
	F	188 ± 13	195 ± 17	198 ± 19	198 ± 21
Brain (g)	M	1.75 ± 0.11	1.77 ± 0.09	1.78 ± 0.19	1.81 ± 0.10
	F	1.64 ± 0.05	1.62 ± 0.07	1.62 ± 0.04	1.62 ± 0.09
Lung (g)	M	2.24 ± 1.58	2.13 ± 0.63	1.96 ± 0.62	1.96 ± 0.44
	F	1.51 ± 0.46	1.55 ± 0.51	1.60 ± 0.26	1.32 ± 0.25
Thymus (g)	M	0.69 ± 0.16	0.65 ± 0.17	0.71 ± 0.17	0.61 ± 0.18
	F	0.52 ± 0.09	0.57 ± 0.09	0.55 ± 0.10	0.50 ± 0.06
Heart (g)	M	0.95 ± 0.13	0.99 ± 0.18	0.98 ± 0.23	0.96 ± 0.12
	F	0.74 ± 0.04	0.71 ± 0.04	0.74 ± 0.08	0.75 ± 0.08
Liver (g)	M	11.81 ± 1.76	10.65 ± 2.76	11.05 ± 1.93	10.77 ± 1.44
	F	9.91 ± 1.32	9.52 ± 1.64	9.62 ± 1.23	9.53 ± 1.35
Kidneys (g)	M	2.20 ± 0.39	2.26 ± 0.55	2.45 ± 0.70	2.49 ± 0.34
	F	1.79 ± 0.12	1.73 ± 0.19	1.74 ± 0.15	1.67 ± 0.20
Spleen (g)	M	0.69 ± 0.08	0.61 ± 0.20	0.70 ± 0.15	0.74 ± 0.17
	F	0.64 ± 0.09	0.62 ± 0.20	0.54 ± 0.05*	0.60 ± 0.09
Adrenals (mg)	M	58.4 ± 8.2	54.7 ± 10.3	54.1 ± 8.2	53.8 ± 4.9
	F	61.5 ± 6.0	53.9 ± 6.6*	56.7 ± 10.1	58.0 ± 9.3
Thyroid (mg)	M	35.9 ± 9.5	38.8 ± 10.2	38.2 ± 8.5	37.1 ± 7.2
	F	33.2 ± 6.0	32.0 ± 5.5	32.6 ± 4.0	33.1 ± 3.1
Hypophysis (mg)	M	12.3 ± 4.7	12.5 ± 4.9	11.4 ± 3.8	11.3 ± 2.3
	F	13.3 ± 3.5	13.1 ± 4.0	13.4 ± 2.6	11.1 ± 3.9
Testes (g)	M	2.32 ± 0.44	2.21 ± 0.32	2.29 ± 0.26	2.24 ± 0.30
	F	0.67 ± 0.24	0.82 ± 0.41	0.60 ± 0.45	0.54 ± 0.31
Ovaries (mg)	M	73.5 ± 13.6	67.8 ± 12.0	68.9 ± 7.9	69.3 ± 12.7
	F	0.34 ± 0.08	0.34 ± 0.05	0.32 ± 0.05	0.37 ± 0.08

M: Male F: Female M.F. 20 each M.F. 20 each M.F. 20 each M.F. 20 each
 *: p < 0.05 SPL Lot # 5071+11

Table 2 Average wet weight of various organs (/100g body weight) in rats treated with SPL (s.c.) for 30 days (Mean ± S.D.)

Tissue	Sex	Control (Saline)	SPL		
			2 ml/kg	7 ml/kg	20 ml/kg
Brain (g)	M	0.76 ± 0.09	0.80 ± 0.16	0.78 ± 0.10	0.80 ± 0.10
	F	0.88 ± 0.06	0.84 ± 0.05	0.83 ± 0.07	0.82 ± 0.05
Lung (g)	M	1.06 ± 1.05	0.99 ± 0.41	0.92 ± 0.50	0.88 ± 0.30
	F	0.82 ± 0.31	0.79 ± 0.26	0.82 ± 0.18	0.68 ± 0.19
Thymus (g)	M	0.29 ± 0.04	0.29 ± 0.05	0.31 ± 0.03	0.26 ± 0.06
	F	0.28 ± 0.04	0.29 ± 0.03	0.28 ± 0.04	0.25 ± 0.00
Heart (g)	M	0.41 ± 0.06	0.44 ± 0.03	0.43 ± 0.04	0.42 ± 0.00
	F	0.39 ± 0.00	0.36 ± 0.00	0.38 ± 0.03	0.38 ± 0.03
Liver (g)	M	5.07 ± 0.47	4.63 ± 0.44	4.79 ± 0.30	4.68 ± 0.35
	F	5.26 ± 0.51	4.89 ± 0.88	4.86 ± 0.39	4.82 ± 0.50
Kidneys (g)	M	0.95 ± 0.16	0.99 ± 0.10	1.05 ± 0.18	1.08 ± 0.14
	F	0.96 ± 0.09	0.89 ± 0.05	0.88 ± 0.04*	0.85 ± 0.10*
Spleen (g)	M	0.30 ± 0.05	0.27 ± 0.04	0.31 ± 0.04	0.32 ± 0.07
	F	0.34 ± 0.03	0.32 ± 0.11	0.27 ± 0.00	0.30 ± 0.03
Adrenals (mg)	M	25.4 ± 4.8	24.7 ± 5.6	23.8 ± 4.2	23.7 ± 4.0
	F	32.8 ± 2.9	27.6 ± 2.4***	29.2 ± 4.9	29.3 ± 3.5*
Thyroid (mg)	M	15.3 ± 3.2	17.0 ± 2.4	16.7 ± 3.1	16.1 ± 2.6
	F	17.6 ± 2.7	16.5 ± 3.0	16.6 ± 2.5	16.9 ± 2.3
Hypophysis (mg)	M	5.5 ± 2.4	5.5 ± 2.3	5.1 ± 2.0	5.0 ± 1.2
	F	7.1 ± 1.8	6.7 ± 1.9	6.8 ± 1.4	5.5 ± 2.0
Testes (g)	M	0.99 ± 0.12	1.00 ± 0.18	1.01 ± 0.13	0.97 ± 0.10
	F				
Seminal vesicle (g)	M	0.28 ± 0.08	0.34 ± 0.15	0.24 ± 0.14	0.23 ± 0.10
Ovaries (mg)	F	39.1 ± 6.6	34.7 ± 5.8	34.9 ± 3.2	35.0 ± 4.9
Uterus (g)	F	0.18 ± 0.03	0.17 ± 0.00	0.16 ± 0.00	0.20 ± 0.04

M: Male F: Female M.F. 20 each M.F. 20 each M.F. 20 each M.F. 20 each

*: P < 0.05

***: P < 0.001

SPL Lot # 5071411

Table 3 Biochemical examination on serum in rats treated with SPL(s.c.) for 30 days (Mean \pm S.D.)

	Sex	Control- (Saline)	SPL		
			2 ml/kg	7 ml/kg	20 ml/kg
Total bilirubin (mg/dl)	M	0.2 \pm 0.0	0.2 \pm 0.0	0.2 \pm 0.0	0.2 \pm 0.0
	F	0.2 \pm 0.0	0.2 \pm 0.0	0.2 \pm 0.0	0.2 \pm 0.0
Thymol turbidity test (Maclagan unit)	M	0.4 \pm 0.1	0.4 \pm 0.2	0.4 \pm 0.1	0.4 \pm 0.2
	F	0.3 \pm 0.1	0.3 \pm 0.1	0.3 \pm 0.1	0.3 \pm 0.1
Alkaline phosphatase (Kind-King unit)	M	20.2 \pm 9.6	18.7 \pm 3.5	19.8 \pm 5.7	17.6 \pm 1.8
	F	12.7 \pm 2.7	13.4 \pm 2.9	12.2 \pm 2.1	12.3 \pm 2.0
S-GOT (Karmen unit)	M	180 \pm 51	172 \pm 40	171 \pm 21	165 \pm 22
	F	140 \pm 17	170 \pm 55	158 \pm 23	135 \pm 18
S-GPT (Karmen unit)	M	44 \pm 12	48 \pm 10	43 \pm 5	40 \pm 8
	F	49 \pm 15	44 \pm 15	51 \pm 19	54 \pm 20
Total protein (g/dl)	M	6.3 \pm 0.3	6.2 \pm 0.4	6.2 \pm 0.4	6.2 \pm 0.3
	F	6.6 \pm 0.4	6.4 \pm 0.2	6.3 \pm 0.3	6.6 \pm 0.3
A/G ratio	M	0.9 \pm 0.1	0.9 \pm 0.1	1.0 \pm 0.1	0.9 \pm 0.1
	F	0.9 \pm 0.1	0.9 \pm 0.1	1.0 \pm 0.1	0.9 \pm 0.1
Albumin (g/dl)	M	3.0 \pm 0.1	3.0 \pm 0.2	3.0 \pm 0.1	3.0 \pm 0.1
	F	3.1 \pm 0.1	3.1 \pm 0.2	3.1 \pm 0.1	3.2 \pm 0.1
Total cholesterol (mg/dl)	M	51 \pm 9	50 \pm 15	55 \pm 5	55 \pm 10
	F	49 \pm 5	45 \pm 10	43 \pm 5	48 \pm 5
Blood urea nitrogen (mg/dl)	M	23 \pm 3	21 \pm 5	23 \pm 4	23 \pm 3
	F	22 \pm 4	21 \pm 4	21 \pm 2	20 \pm 2
Creatinine (mg/dl)	M	0.7 \pm 0.0	0.7 \pm 0.1	0.6 \pm 0.0	0.7 \pm 0.1
	F	0.7 \pm 0.1	0.7 \pm 0.1	0.7 \pm 0.1	0.7 \pm 0.0
Sodium mEq/l	M	141 \pm 1	145 \pm 10	148 \pm 18	145 \pm 4 *
	F	144 \pm 8	142 \pm 1	141 \pm 2	140 \pm 2
Potassium mEq/l	M	5.9 \pm 0.9	5.9 \pm 0.7	6.3 \pm 0.3	6.2 \pm 0.5
	F	5.7 \pm 0.4	5.5 \pm 0.5	5.3 \pm 0.3 *	5.0 \pm 0.4 ***
Blood sugar mg/dl	M	172 \pm 26	175 \pm 12	149 \pm 18 *	159 \pm 19
	F	158 \pm 16	168 \pm 17	165 \pm 19	171 \pm 15

M: Male F: Female

* : p < 0.05 *** : p < 0.001

M.F. 20 each

M.F. 20 each

M.F. 20 each

M.F. 20 each

SPL Lot # 5071411

Subacute Toxicity Test

Table 4. Hematological findings in rats treated with SPL (s.c.) for 30 days (Mean ± S.D.)

	Sex	Control (Saline)	SPL			
			2 ml/kg	7 ml/kg	20 ml/kg	
Hemoglobin (g/dl)	M	15.6 ± 0.7	16.6 ± 1.0	15.4 ± 0.6	15.2 ± 0.9	
	F	15.3 ± 1.2	15.8 ± 0.8	15.8 ± 0.9	15.3 ± 0.6	
Hematocrit (%)	M	45.8 ± 2.7	47.6 ± 5.5	45.7 ± 2.4	45.9 ± 3.3	
	F	46.1 ± 2.9	45.0 ± 2.0	45.5 ± 1.7	45.0 ± 1.9	
Erythrocytes (x 10 ⁴)	M	639 ± 137	778 ± 205	820 ± 132*	784 ± 81*	
	F	702 ± 134	767 ± 97	720 ± 157	807 ± 83	
Leucocytes (x 10 ³)	M	88 ± 35	112 ± 39	87 ± 46	70 ± 15	
	F	95 ± 21	107 ± 31	93 ± 17	111 ± 20	
Hemogram %	Baso.	M	0	0	0	0
		F	0	0	0	0
	Eosin.	M	0.3 ± 0.3	0.7 ± 0.4*	0.8 ± 0.4**	0.7 ± 0.4
		F	1.3 ± 0.6	1.2 ± 0.4	1.4 ± 1.0	1.5 ± 1.0
	Neut.	M	22.5 ± 12.8	23.3 ± 10.6	18.1 ± 7.3	21.0 ± 8.5
		F	20.8 ± 6.7	13.2 ± 5.1*	20.2 ± 5.3	18.4 ± 6.4
	Lymph.	M	74.1 ± 12.2	73.2 ± 10.3	78.0 ± 8.2	75.1 ± 8.4
		F	74.7 ± 7.0	81.3 ± 5.6	75.8 ± 5.4	76.3 ± 7.9
	Mono.	M	3.0 ± 0.8	2.8 ± 1.4	3.1 ± 1.3	3.2 ± 1.1
		F	3.2 ± 1.1	4.3 ± 2.3	2.6 ± 1.4	3.8 ± 1.8

M: Male F: Female M.F. 20 Each M.F. 20 Each M.F. 20 Each M.F. 20 Each
 *: p < 0.05 **: p < 0.01
 SPL Lot # 5071 + 11

Table 5. Urinalysis in rats treated with SPL (s.c.) for 30 days

		Control (Saline)		SPL					
				2 ml/kg		7 ml/kg		20 ml/kg	
		M /9	F /10	M /9	F /9	M /8	F /10	M /8	F /9
pH	6	0	8	1	4	0	6	1	7
	7	7	2	8	5	8	4	7	2
	8	2	0	0	0	0	0	0	0
Protein	-	1	0	0	0	0	0	0	0
	±	6	9	8	9	5	10	8	9
	+	0	1	0	0	0	0	0	0
	++	2	0	1	0	3	0	0	0
Glucose	-	9	10	9	9	7	10	8	9
	±	0	0	0	0	1	0	0	0
Ketons	-	9	10	9	9	8	9	8	9
	±	0	0	0	0	0	1	0	0
Occult blood	-	9	7	6	7	6	7	6	6
	±	0	2	3	2	2	3	2	3
	+	0	1	0	0	0	0	0	0

M: Male F: Female

SPL Lot # 5071411

Teratogenicity Study of SPL in Rats
and Rabbits

Hachihiko HIRAYAMA

Research Department Lab. II
Fujizoki Pharmaceutical Co., Ltd.
9-14, Nishiki 2-chome,
Nerima-ku, Tokyo

Summary

Teratogenicity study of SPL for staphylococcal disease manufactured by Delmont Laboratories Inc. was carried out in rats and rabbits. SPL was intraperitoneally given to pregnant rats for 11 days from day 6 to day 16 of gestation at dose levels of 0.02, 0.5 and 5 ml/kg/day and to pregnant rabbits for 13 days from day 6 to day 18 of gestation at dose levels of 0.02, 0.2 and 2.0 ml/kg/day.

SPL had no teratogenic effect on both animals.

(Lot) S-27 { Lot # 6111483
 { " 7021481
 { " 7033085

Table 1 Teratogenic effects of S - 27 against rat fetuses

	Control	S - 27 (ml/kg/day x 11 ; i.p.)		
		0.05	0.5	5.0
No. of dams	2	3	5	4
Total No. of implantation	25	29	48	38
No. of survival fetuses	24	28	44	38
(Mean litter size)	(12.0)	(9.3)	(8.8)	(9.5)
Dead or resorped fetuses	1	1	4	0
(Fetal mortality ; %)*	4.0	3.4	8.3	0
Sex ratio (♂/♀)	12/12	17/11	24/20	20/18
Mean fetal body weight (g)(♂)	3.51	3.51	3.39	3.45
(♀)	3.11	3.28	3.06	3.29
External anomalies	0	0	0	0
(%)**	(0)	(0)	(0)	(0)
Skeletal anomalies	0	0	0	0
(%)**	(0)	(0)	(0)	(0)
Visceral anomalies	0	0	0	0
(%)**	(0)	(0)	(0)	(0)
Growth retardation***	1	1	6	0
(%)**	(4.2)	(3.6)	(13.6)	(0)

* : No. of dead or resorped fetuses/ No. of total implantation x 100 (%)

** : No. of abnormal fetuses/ No. of fetuses examined x 100 (%)

*** : Body weight <3.0 g.

Table 2 Teratogenic effects of S - 27 against rabbit fetuses

	Control	S - 27 (ml/kg/day x 13 ; i.p.)		
		0.02	0.2	2.0
No. of dams	1	3	3	2
Total No. of implantation	7	24	18	16
No. of survival fetuses (Mean litter size)	6 (6)	20 (6.3)	18 (6.0)	14 (6.5)
No. of dead or resorped fetuses (Fetal mortality)*	1 (14.3)	4 (16.6)	0 (0)	2 (12.5)
Mean fetal body weight (g)	53.1	54.1	53.2	50.7
External anomalies (%)*	0 (0)	0 (0)	0 (0)	0 (0)
Skeletal anomalies (%)**	0 (0)	0 (0)	0 (0)	0 (0)
Visceral anomalies (%)	0 (0)	0 (0)	0 (0)	0 (0)

* : No. of dead or resorped fetuses/ No. of total implantation x100 (%).

** : No. of abnormal fetuses/ No. of fetuses examined x 100 (%).

PHOTO-I

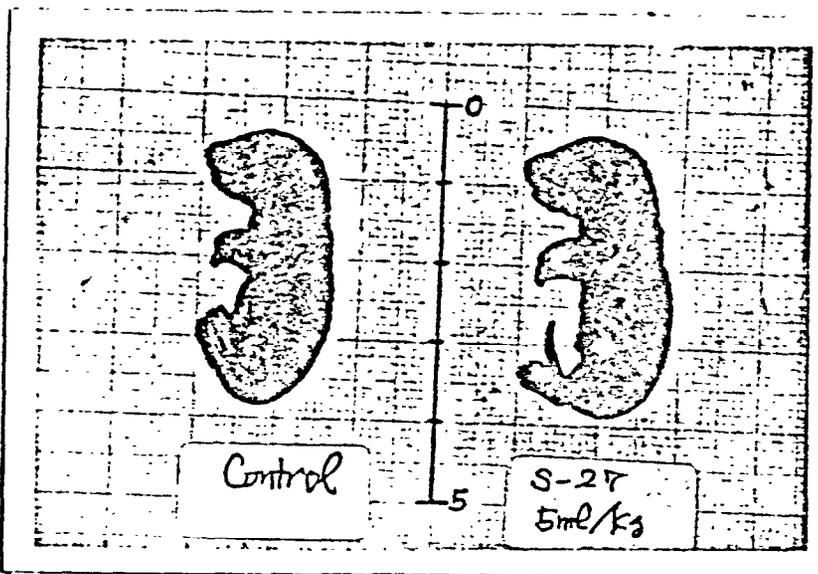


PLATE I-1 Left: A fetus from the pregnant rat. Right: A fetus from the pregnant rat treated intraperitoneally with 5ml/kg of S-27.

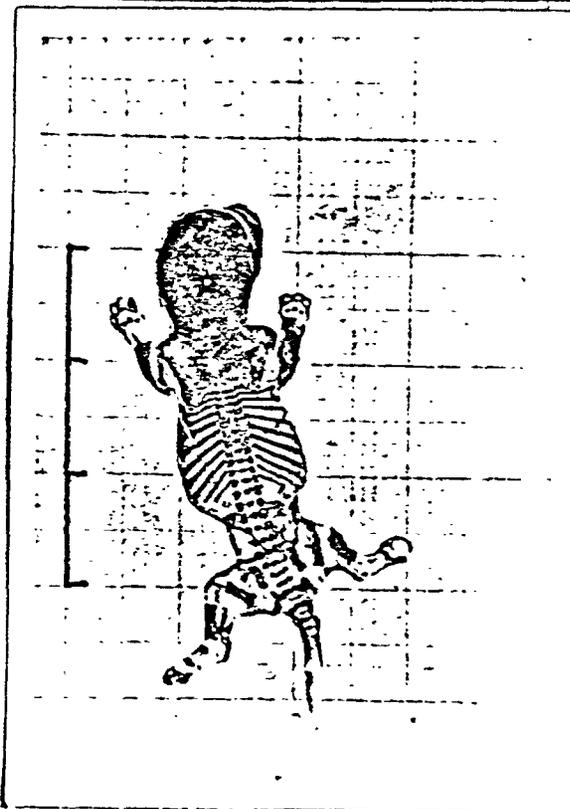


PLATE I-2 A normal skeleton of rat fetus in saline control group.

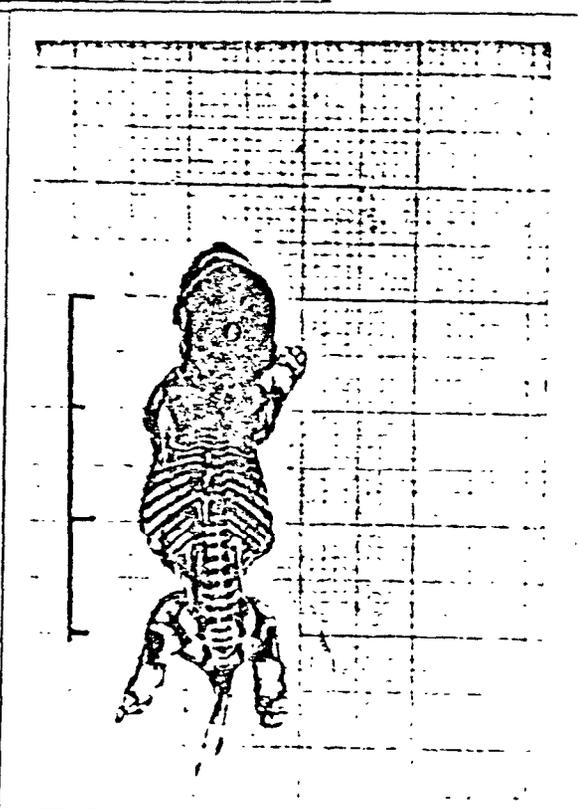


PLATE I-3 A normal skeleton of rat fetus from the pregnant rat treated intraperitoneally with 5ml/kg of S-27.

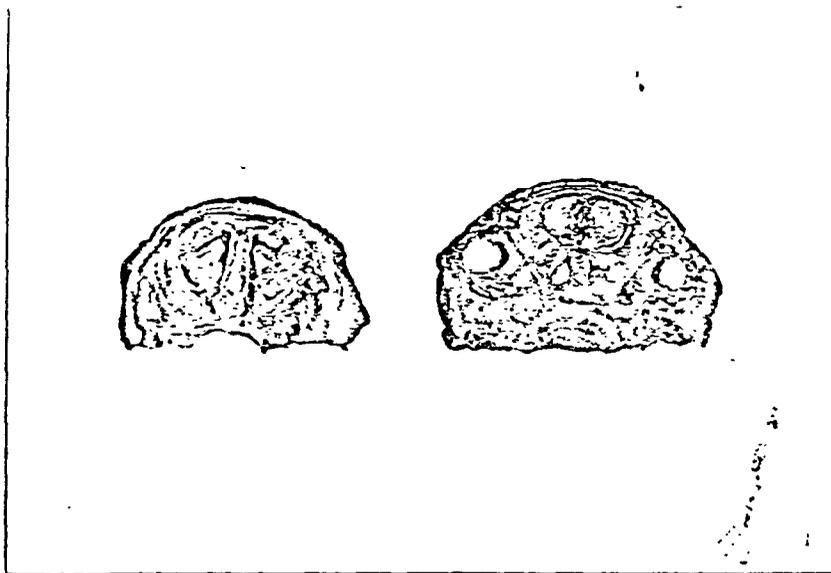


PLATE I-4 Palate and nasal cavities of control rat fetus; showing normally in the Wilson's section.

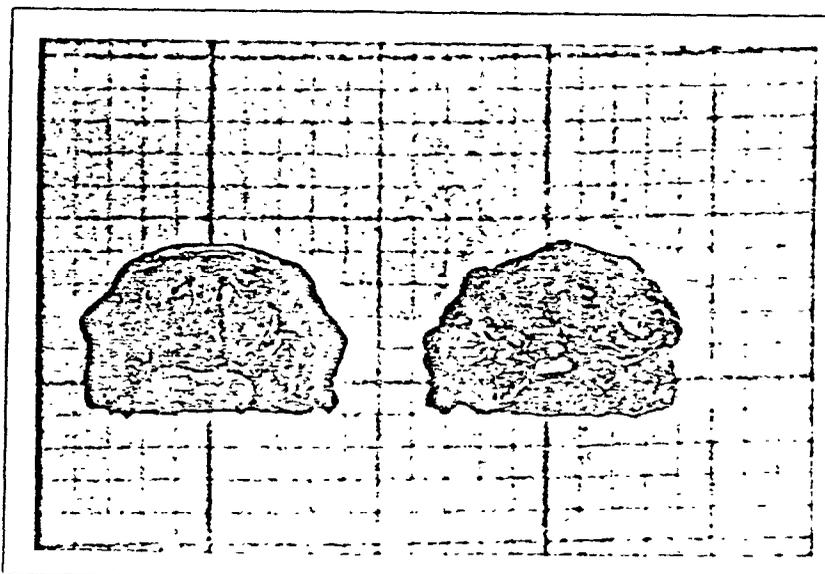


PLATE I-5 Palate, eyeballs and olfactory bulbs of fetus from the pregnant rat treated intraperitoneally with 5ml/kg of S-27; showing normally in the Wilson's section.

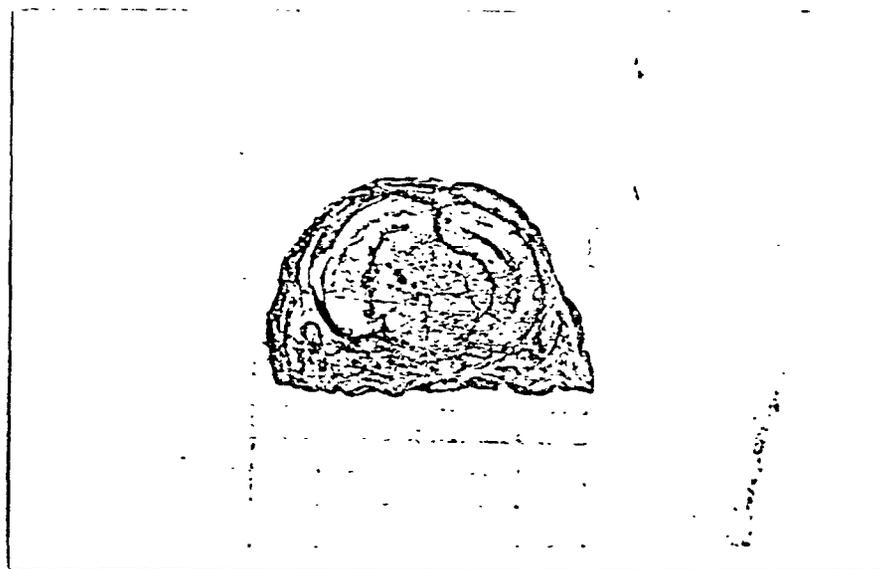


PLATE I-6 Skull and brain in control fetus; showing normally in the Wilson's section.

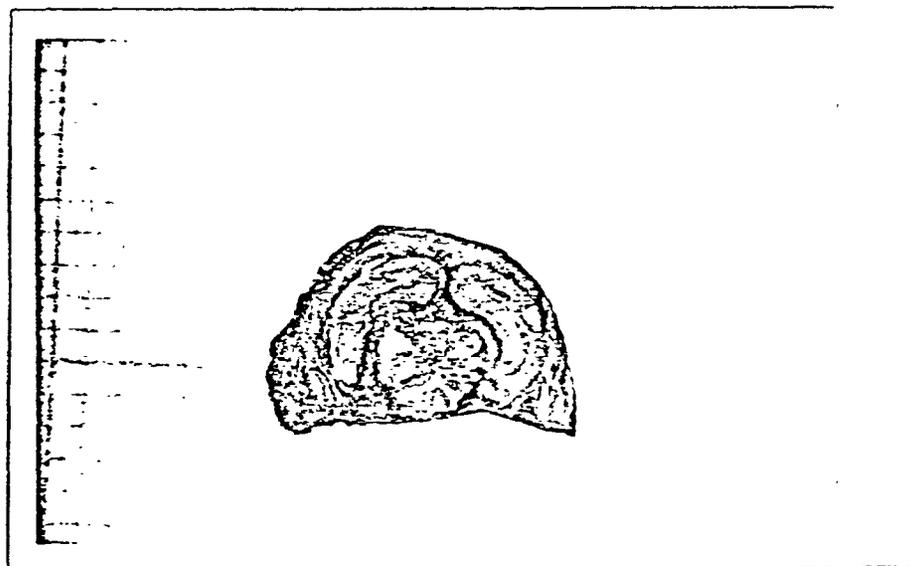


PLATE I-7 Skull and brain of fetus from the pregnant rat treated intraperitoneally with 5ml/kg of S-27.

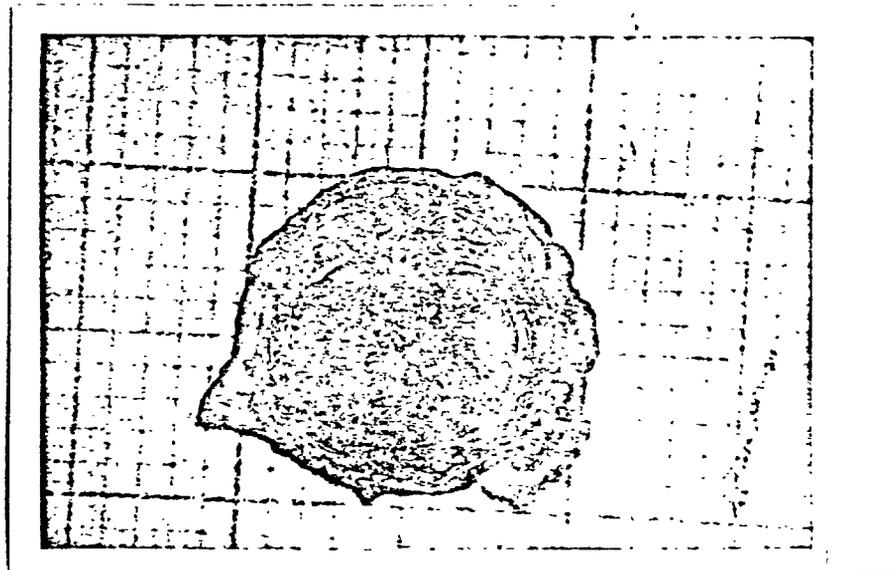


PLATE I-8 - Cardiac ventricle
and lung section of control fetus;
showing normally in Wilson's
section.

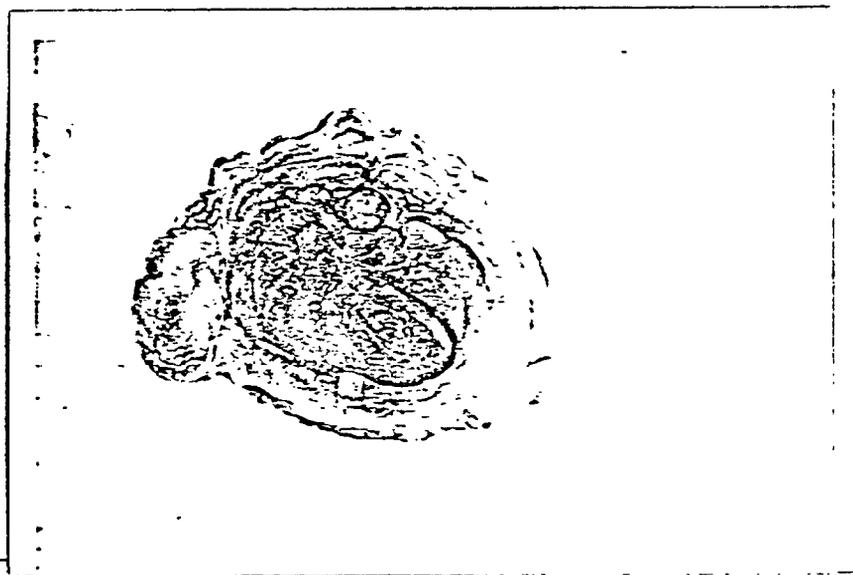


PLATE I-9 Cardiac ventricle,
lung and heart of fetus from the
pregnant rat treated intraperito-
neally with 5ml/kg of S-27; show-
ing normally in Wilson's section.

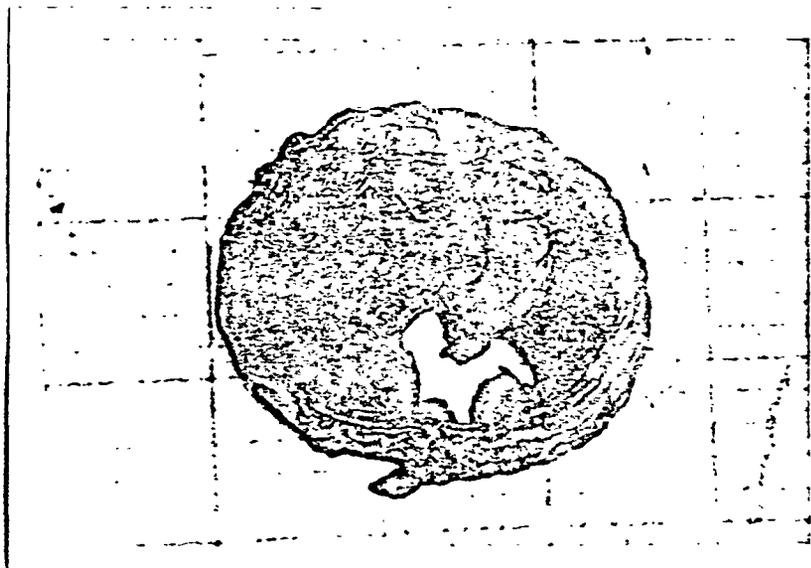


PLATE I-10 Liver, ~~kidney~~ and stomach of control fetus; showing normally in the Wilson's section.

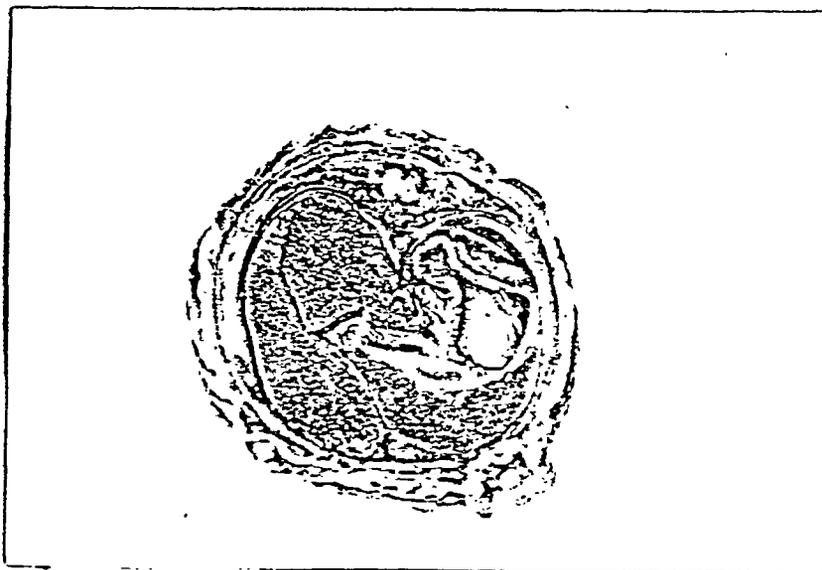


PLATE I-11 Liver and stomach of fetus from the pregnant rat treated intraperitoneally with 5ml/kg of S-27.

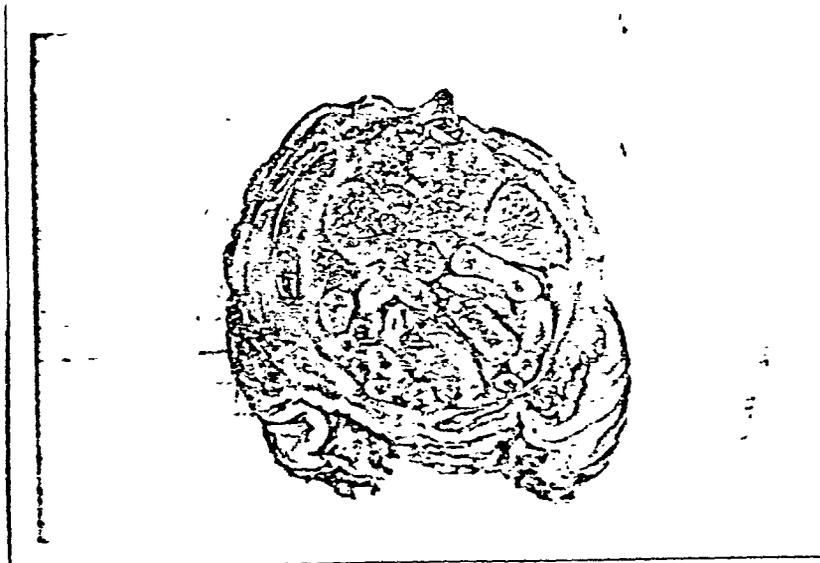


PLATE I-12 Kidney, intestine
and spleen of control fetus;
showing normally in the Wilson's
section

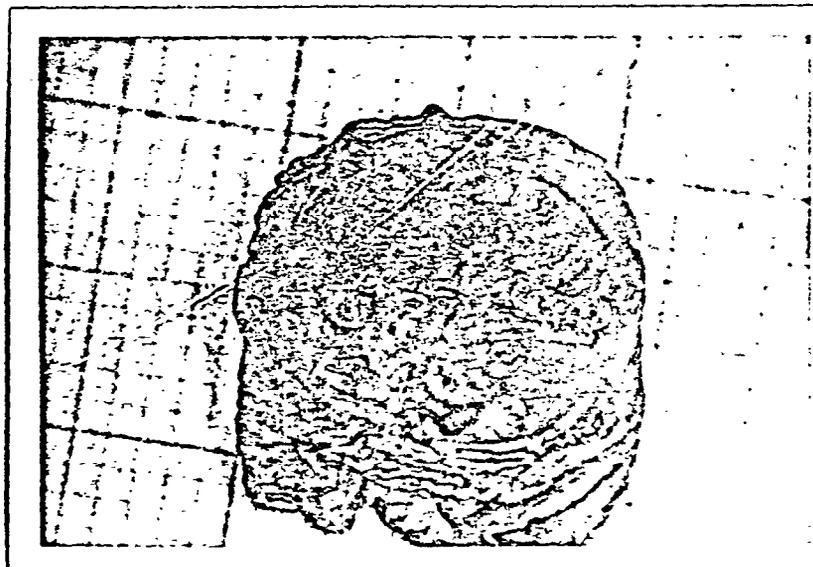


PLATE I-13 Kidney, testis and
urinary bladder of control fetus;
showing normally in the Wilson's
section.

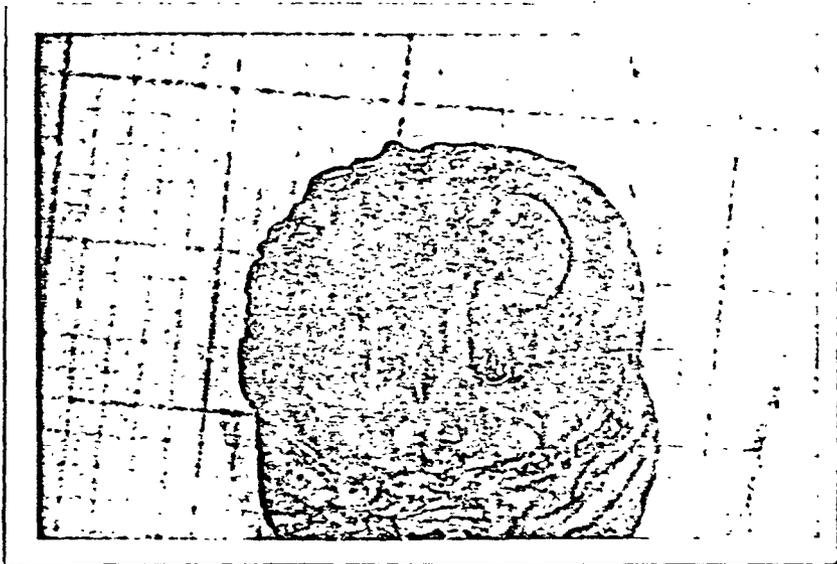


PLATE I-12 Kidney, intestine
and spleen of control rabbit
fetus; showing normally in the
Wilson's section.

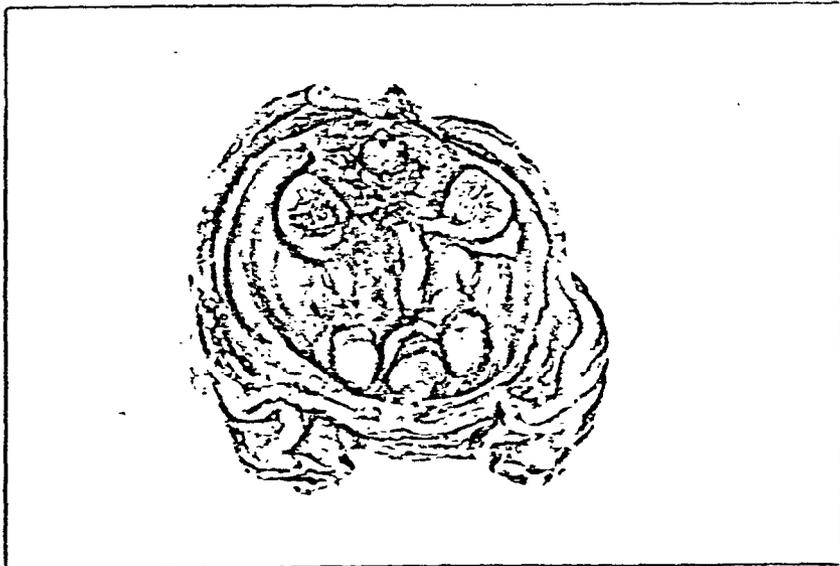


PLATE I-14 Kidney, testis and
urinary bladder of fetus from
the pregnant rat treated intra-
peritoneally with 5ml/kg of S-27;
showing normally in the Wilson's
section.

PHOTO-II

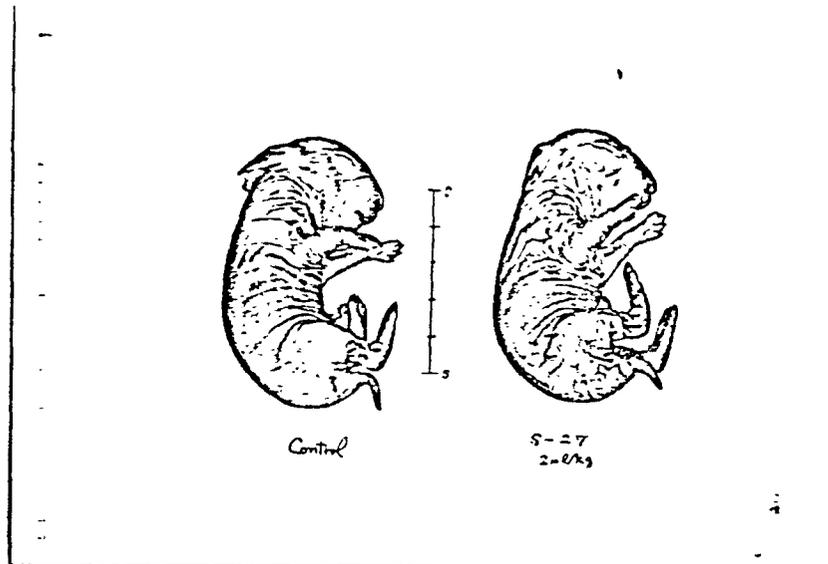


PLATE II-1 Left: A fetus from the pregnant rabbit. Right: A fetus from the pregnant rabbit treated intraperitoneally with 5ml/kg of S-27.

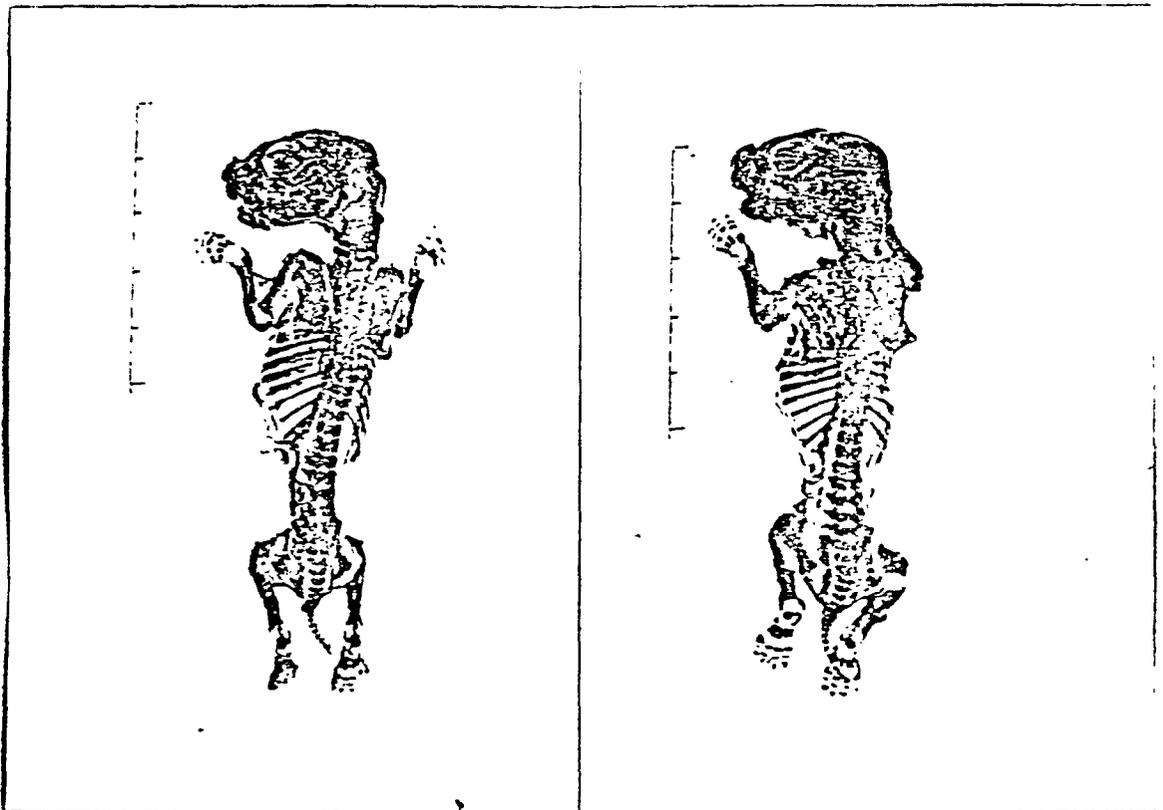


PLATE II-2 A normal skeleton of rabbit fetus in saline control group.

PLATE II-3 A normal skeleton of fetus from the pregnant rabbit treated intraperitoneally with 5ml/kg of S-27.

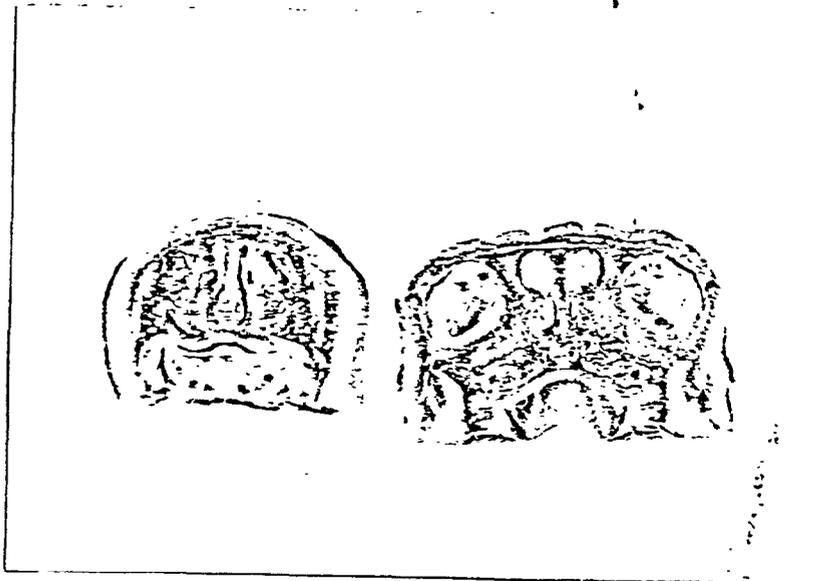


PLATE II-4 Palate and nasal cavities of control rabbit fetus; showing normally in the Wilson's section.

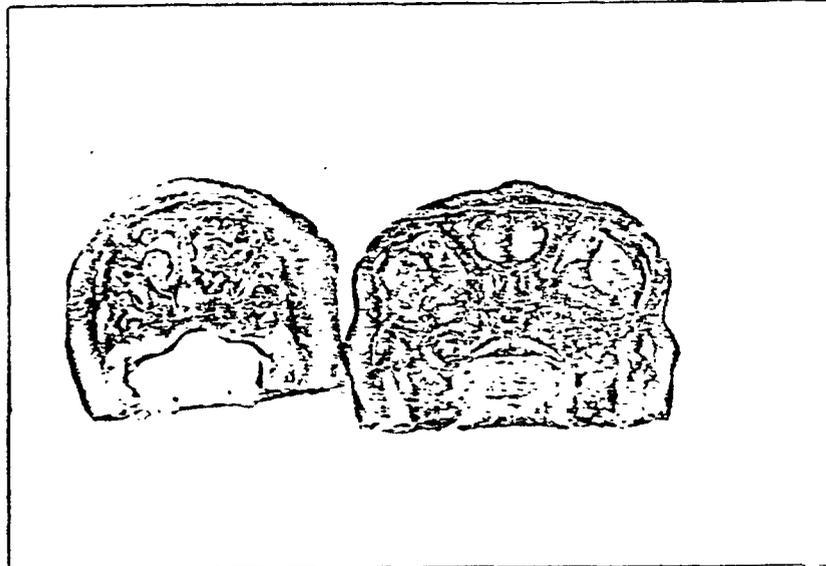


PLATE II-5 Palate, eyeballs and olfactory bulbs of fetus from the pregnant rabbit treated intraperitoneally with 5ml/kg of S-27; showing normally in the Wilson's section.



PLATE II-6 Skull and brain in control rabbit fetus; showing normally in the Wilson's section.

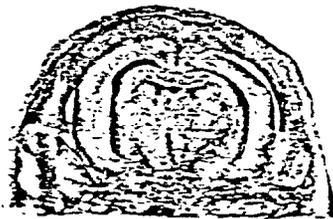


PLATE II-7 Skull and brain of fetus from the pregnant rabbit treated intraperitoneally with 5ml/kg of S-27.

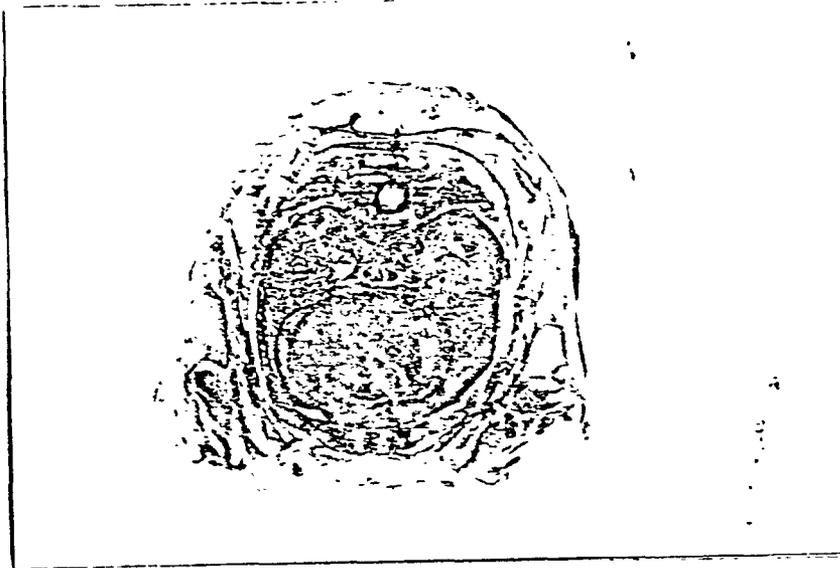


PLATE II-8 Cardiac ventricle
and lung section of control rabbit
fetus; showing normally in
Wilson's section.

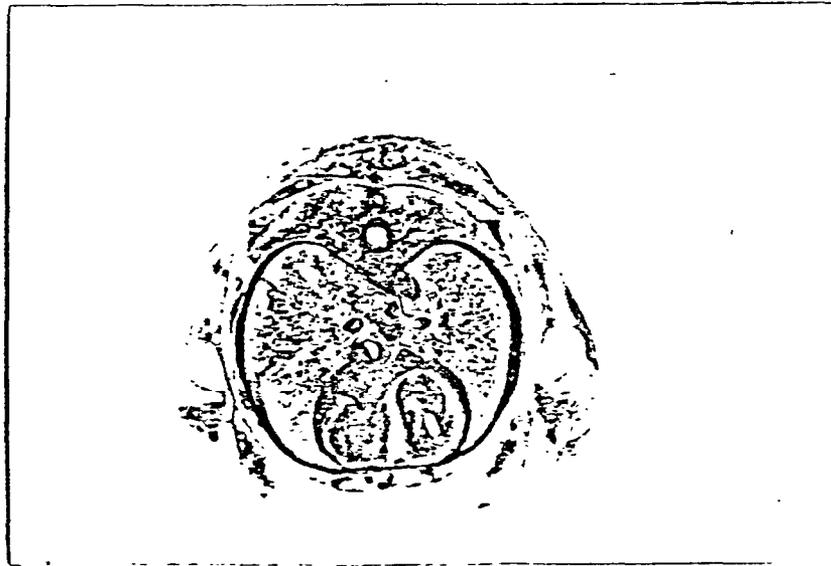


PLATE II-9 Cardiac ventricle,
lung and heart of fetus from
the pregnant rabbit treated
intraperitoneally with 5ml/kg
of S-27; showing normally in
Wilson's section.



PLATE II-10 Liver, kidney and stomach of control rabbit fetus; showing normally in the Wilson's section.

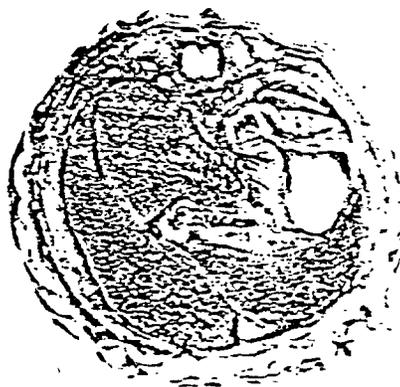


PLATE II-11 Liver and stomach of fetus from the pregnant rabbit treated intraperitoneally with 5ml/kg of S-27.



PLATE II-13 Kidney, testis and urinary bladder of control rabbit fetus; showing normally in the Wilson's section.

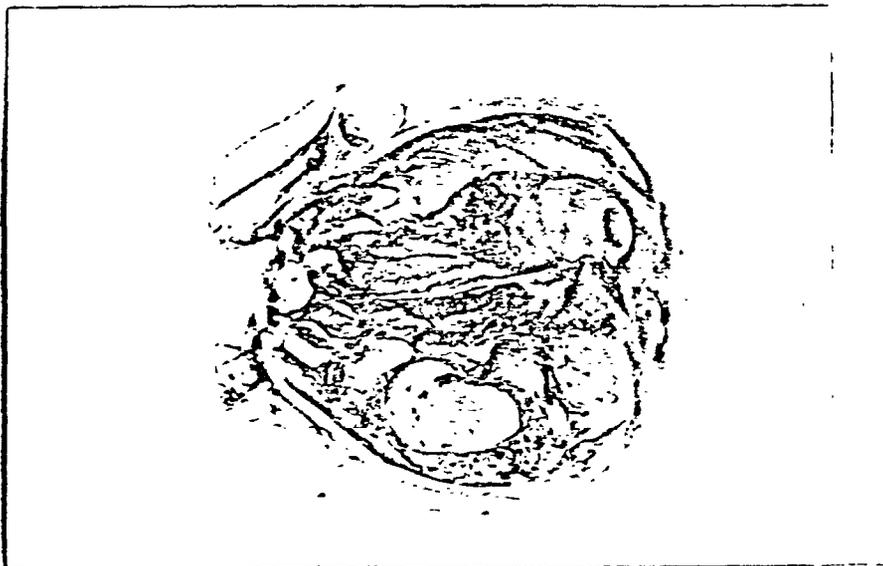


PLATE II-14 Kidney, testis and urinary bladder of fetus from the pregnant rabbit treated intraperitoneally with 5ml/kg of S-27; showing normally in the Wilson's section.

DEPARTMENT OF MICROBIOLOG.
SCHOOL OF MEDICINE, KYUSHU UNIVERSITY
FUKUOKA, 812 JAPAN

December 1, 1977

Mr. Charles Lincoln
Delmont Laboratories, Inc.
P.O. Box AA, Swathmore
Pennsylvania 19081, U. S. A.

Dear Mr. Lincoln:

On behalf of the letter from Mrs. Emily H. Mudd of November 16, I am writing you in the hope that our data on the SPL obtained by our laboratory would be of some help for breaking the situation.

Last three years several members of our laboratory have engaged intensively in the research of SPL. Firstly, in order to test the effect of SPL, staphylococcus-sensitized mice were challenged with bacteria, fungi and viruses such as Listeria, Candida and herpes simplex virus, in combination with SPL treatment. However, the effect of staphylococcus itself was so great that the effect of SPL could not be detected. Therefore, considering that the SPL does activate macrophages provided the recipients were previously sensitized by staphylococcus (this is the situation in which the SPL is used in humans), we recently made the following experiments. Mice sensitized by Staphylococcus aureus 18Z strain were left for two months. These mice were found to be as susceptible to herpes simplex virus infection as untreated mice. After SPL treatment, however, these mice became resistant to herpes simplex virus infection (see attached report). The same kinds of experiments are under way by using bacteria and fungi.

Secondly, quantitation of the potency of SPL has also been of our interests. Since the principle of the effect of SPL is thought to be the activation of macrophages, we tried to find the sensitive system in which macrophages were used. We found that the effect of SPL could be quantitated by using suppressive effect of macrophages against murine ascitic tumor cells. As can be seen in attached report, good dose response was obtained between the SPL and its suppressive effect against ascitic tumor cells. This observation, in turn, will provide the support for the idea that the SPL is really a potent activator for macrophages.

DEPARTMENT OF MICROBIOLOG.
SCHOOL OF MEDICINE, KYUSHU UNIVERSITY
FUKUOKA, 812 JAPAN

- 2 -

I really hope this letter and the attached reports
would be helpful for you.

Sincerely yours,



Kenji Takeya, M.D.
Professor of Bacteriology
President of Kyushu University

cc: Dr. Emily H. Mudd
Fujizoki Company

DEPARTMENT OF MICROBIOLO.
SCHOOL OF MEDICINE, KYUSHU UNIVERSITY
FUKUOKA, 812 JAPAN

EFFECT OF SPL ON THE DEVELOPMENT OF SKIN LESION IN
MICE AFTER INOCULATION WITH HERPES SIMPLEX VIRUS

Development of herpetic skin lesions produced in the midflank of mice after challenge with herpes simplex virus has been used as a model of skin response to the virus. In case of ICR mice, a vesicle appears at the site of injection 4 to 5 days after infection. Soon it changes to eruptive lesion remaining at the injected locus. By the 7th day a zoster-form lesion of eruption and necrosis develops on the inoculated side of the flank. Around this time the mice may die with involvement of the central nervous system.

With the use of this disease model, effect of SPL on the development of skin lesions in mice has been studied and the results obtained indicate that the SPL treatment has protective effect against herpes simplex virus inoculation in staphylococcus-sensitized mice.

Materials and Methods

Sensitization with staphylococcus: Staphylococcus aureus, strain 18Z, cultured in broth for 18 hr at 37C, was washed 3 times by centrifugation with phosphate buffered saline (PBS) and finally suspended in PBS to give 10^9 viable counts per ml. The suspension in 0.1 ml volume was inoculated intramuscularly once a week in one of the four extremities changing each time. For control studies PBS was inoculated instead of bacterial

suspension. Mice were used two months after the final injection of staphylococcus suspension.

SPL (staphylococcal phage lysate): SPL was supplied by Delmont Laboratories, Inc. A volume of 0.1 ml was inoculated intraperitoneally once a day. The treatment started two months after the last inoculation of staphylococcus-sensitization and continued for 14 days. For the control studies broth was inoculated in place of SPL.

Virus and challenge: Herpes simplex virus type 1, strain Hayashida, was used for challenge infection. The strain was isolated from a patient with active labial herpetic lesions in Vero cell cultures. After removing the hair manually over the midflank of ICR mice under a light anesthesia, a volume of 0.05 ml of the virus was injected intradermally by a 26 gauge needle. The virus challenge was done at 7th day of SPL (or broth for control) treatment.

Scoring the development of lesions: Development of the skin lesion was scored as follows; local vesicle 1, local eruption 3, local eruption with necrosis 4, scattered zoster-form lesion 6, continuous zoster-form eruption 8, continuous zoster-form eruption with severe necrosis 10.

Results

Susceptibility to herpes simplex virus of normal ICR mice (18Z-, SPL $\bar{+}$) is shown in Fig. 1. A half of mice

developed severe zoster-form necrotic lesions. SPL-treatment without staphylococcus-sensitization (18Z-,SPL+) also had almost no effect, i.e., 5 out of 8 mice developed zoster-form lesions (Fig. 2). While the 18Z-sensitized mice (18Z+,SPL-) had a tendency of slightly resistant to herpes simplex virus infection without SPL treatment (Fig. 3), resistance of staphylococcus-sensitized and SPL-treated mice (18Z+,SPL+) had a strong tendency of resistance to herpes simplex virus infection (Fig. 4 and 5). Mean scores of lesions for each group are shown in Fig. 5.

Summary

SPL-treatment of ICR mice previously sensitized with Staphylococcus aureus was found to have protective effect against herpes simplex virus inoculation.

Legend for figures

Figs. 1-4. Effect of SPL on the development of herpetic skin lesions in staphylococcus-sensitized mice. ICR mice were inoculated subcutaneously with Staphylococcus aureus, strain 18Z, 8 times with weekly intervals. Two months after the last inoculation of 18Z strain, the mice were inoculated with SPL with daily intervals for 14 days via intraperitoneal route. Seventh day after the first inoculation of SPL, mice were challenged intradermally with Hayashida strain of herpes simplex virus type 1. Development of local lesions and zoster-form lesion was scored thereafter. Control includes (1) without 18Z, without SPL treatment, (2) with 18Z, without SPL treatment, and (3) without 18Z, with SPL treatment.

Fig. 5. Mean scores of the herpetic skin lesions.

Fig 1 Group of 18Z (-), SPL (-) : 6 mice
2 " " " (-) " (+) : 8 "
3 " " " (+) " (-) : 8 "
4 " " " (+) " (+) : 8 "
5 Average scores of mice in each group of the above
(Figs 1-4)†
SPL Lot # 6042639
Vol used. approx. 25 ml.

Fig. 1

18Z(-), SPL(-)

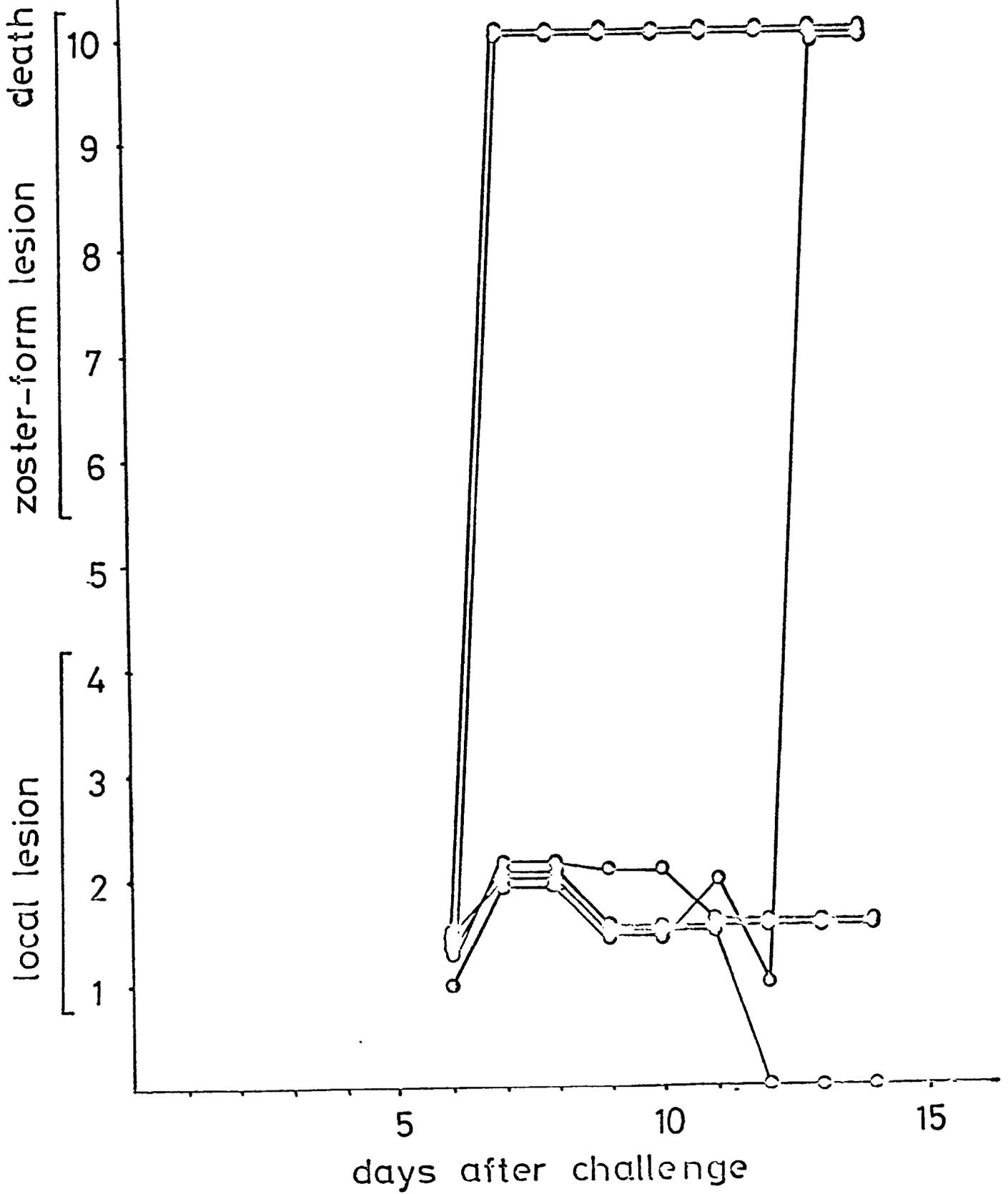


Fig. 2

18Z(-), SPL(+)

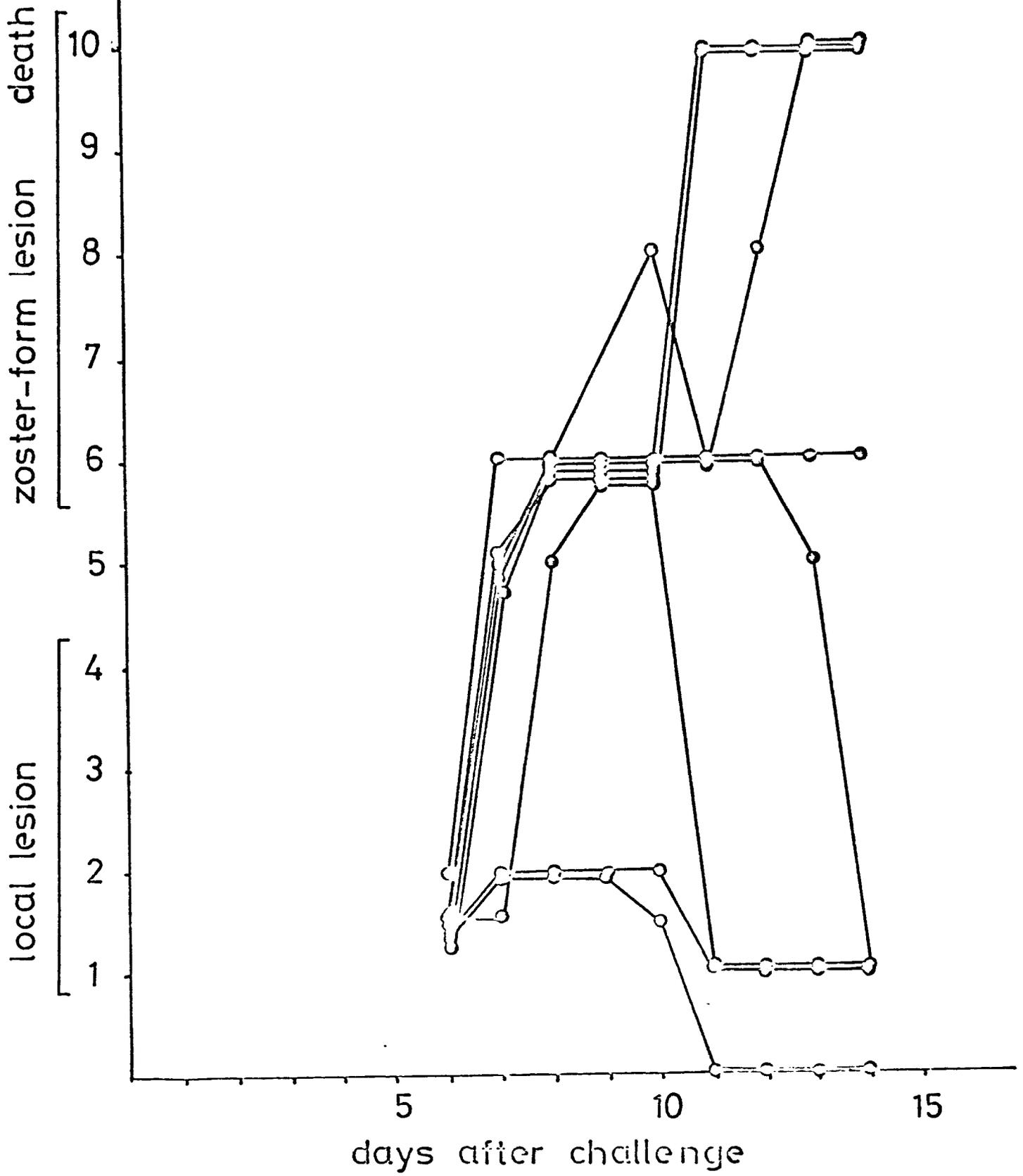
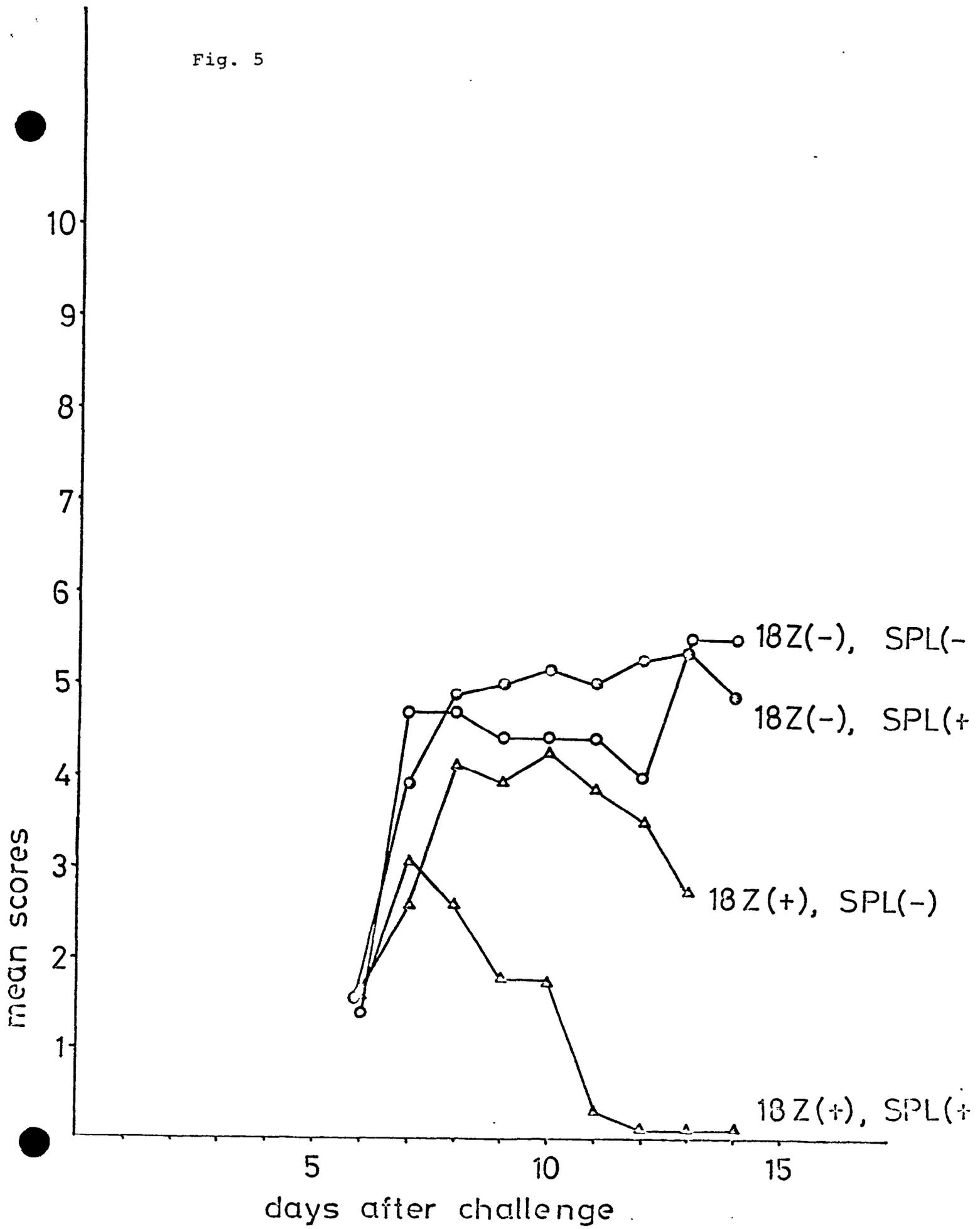


Fig. 5



DEPARTMENT OF MICROBIOLOGY
SCHOOL OF MEDICINE, KYUSHU UNIVERSITY
FUKUOKA, 812 JAPAN

CHEMOTACTIC ACCUMULATION OF MACROPHAGES IN THE
PERITONEAL CAVITY AFTER INOCULATION OF SPL AND
THEIR ANTITUMOR ACTIVITY

SPL has been presumed to activate macrophages and enhance the resistance to bacterial and fungal infections. Especially, SPL appears to exhibit such an effect on infections, when it is applied locally to the infected areas. Therefore, SPL may be able to accumulate macrophages locally and to activate them to give enhanced ability to kill microorganisms. The experiments were designed to analyze such abilities separately in mice.

Outbred ddY mice were used as hosts. Mice were injected subcutaneously with 1×10^8 viable organisms of Staphylococcus aureus (18Z) once a week for 4 or 8 weeks for the pre-treatment. For the measurement of chemotactic activity for macrophages, 0.1 ml of SPL was inoculated into the peritoneal cavity and peritoneal cells were counted at various intervals. For the assay of antitumor activity, 1×10^6 viable cells of Sarcoma 180, an ascites tumor, were inoculated intraperitoneally and various amounts of SPL were inoculated intraperitoneally every day for 7 days after tumor inoculation. Volumes of ascites or total packed volumes of tumor cells were assessed on day 10.

Mice were pretreated with 18Z for 8 weeks and 0.1 ml of SPL was injected intraperitoneally 48 hr after the last inoculation of 18Z. Peritoneal cells were counted before the inoculation of SPL and 4, 24 and 48 hr after the inoculation. When SPL was injected into normal controls,

the number of peritoneal cells increased slightly at 24 and 48 hr. When SPL was inoculated into mice pretreated with 18Z, the number of peritoneal cells increased rapidly and strikingly from 4 to 24 hr. (Fig.1)

Mice were pretreated with 18Z for 4 or 8 weeks and inoculated with tumor cells after the interval of 6 weeks. SPL was inoculated every day for 7 days from the day of tumor inoculation. When 1.0 ml of SPL was inoculated, tumor growth was inhibited completely in both groups (Tables 1 and 2). Definite effects were obtained with 0.3 ml of SPL in both groups. Suppressive effects were weak or negligible, when 0.1 ml of SPL was injected.

Summary SPL exhibited chemotactic activity for macrophages and antitumor activity on sarcoma 180 in mice pretreated with Staphylococcus aureus strain 18Z.

Fig. 1. Peritoneal cell number after intraperitoneal administration of SPL (0.1 ml) in normal and 18Z-pretreated mice. (mean of 5 \pm S.D.)

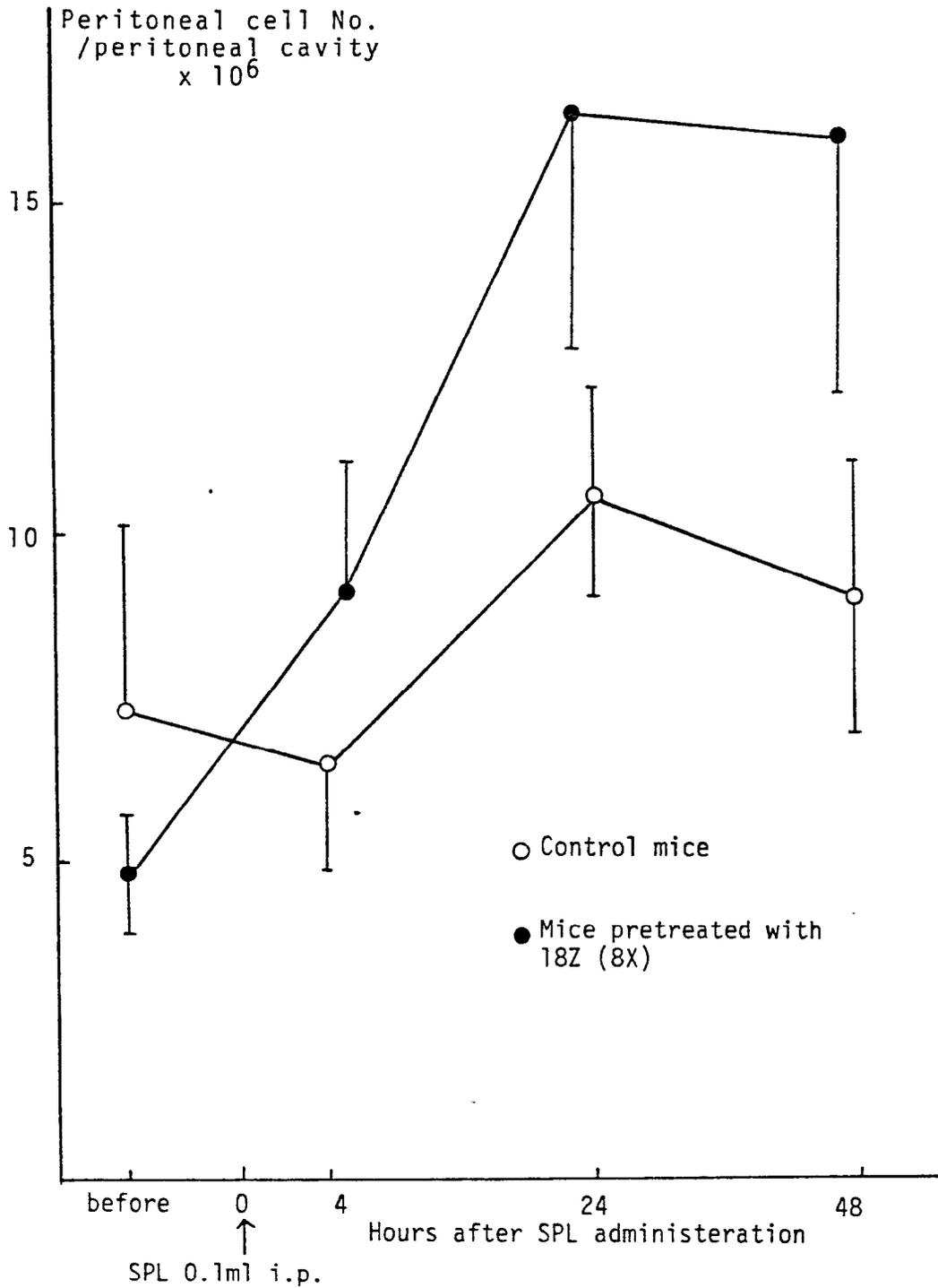


Table 1. Antitumor activity of SPL against Sarcoma 180 in ddY mice.

<u>S.aureus</u> (18Z)	Interval	Sarcoma 180 implantation (Cell No./body)	SPL (ml/body)	Volume of ascitic fluid ml	Total packed cell volume ml	Cell growth (T/C) %	Activity
	weeks	i.p.	i.p.				
			1.00	0	-	-	+++
1x10 ⁸ /body/week s.c. inj. x4	6	1x10 ⁶	0.30	0.55	0.12	16.9	++
			0.10	1.08	0.35	49.3	+
			0	1.98	0.71	100	-

6 mice/group : 4 groups (Total: 24 mice)

SPL Lot # 6090755 (vol. 120 ml approx.)

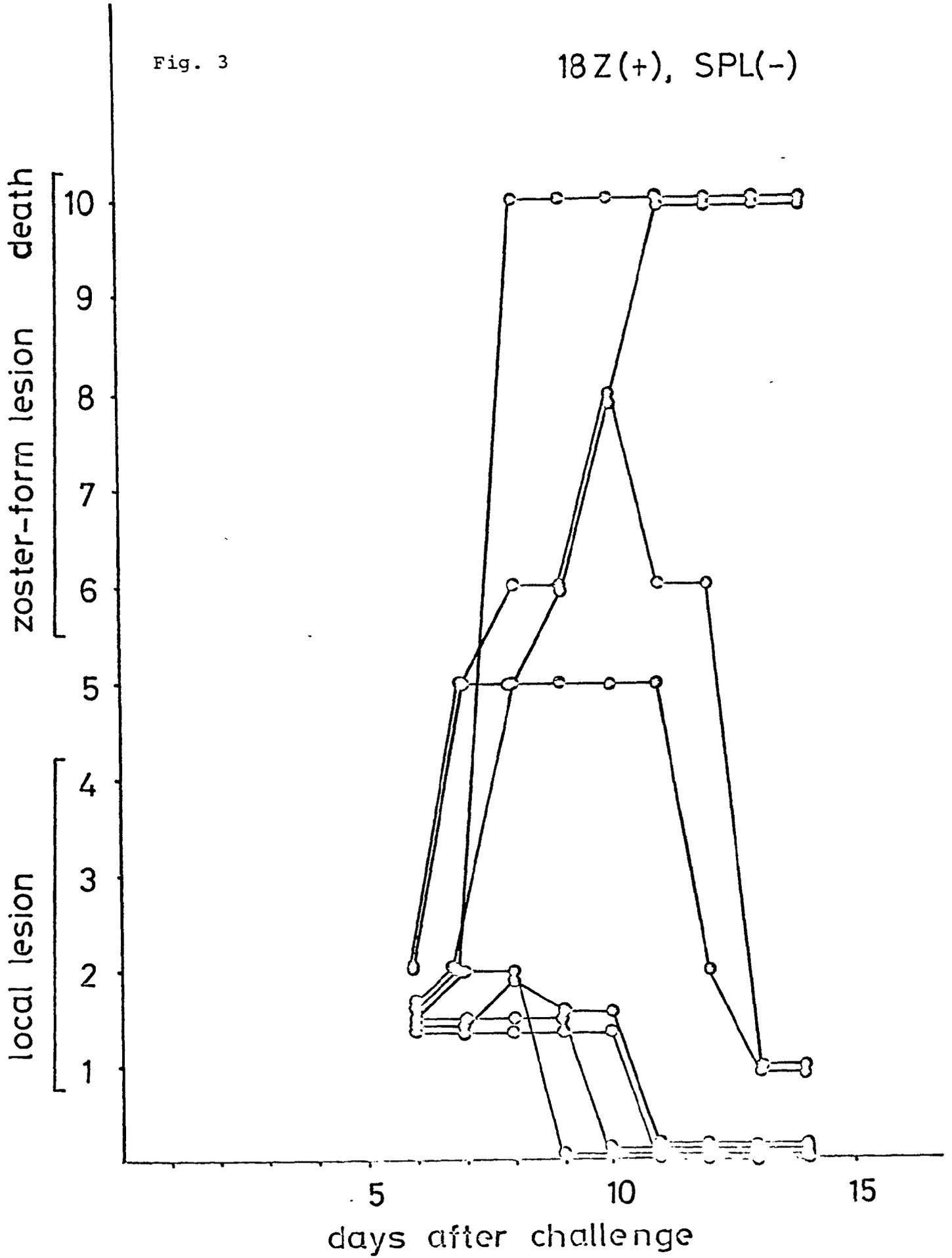
Table 2. Antitumor activity of SPL against Sarcoma 180 in ddY mice.

<u>S.aureus</u>	(18Z)	Interval	Sarcoma 180 implantation (Cell No./body)	SPL (ml/body)	Volume of ascitic fluid ml	Total packed cell volume ml	Cell growth (T/C) %	Activity
		weeks	i.p.	i.p.				
				1.00	0	-	-	+++
1×10^8 /body/week s.c. inj. x 8		6	1×10^6	0.30	0.75	0.27	33.8	++
				0.10	1.92	0.64	80.0	±
				0	2.30	0.80	100	-

6 mice per group = 4 groups (total. 24 mice)
SPL Lot " 6090755 (vol 120 ml approx.)

Fig. 3

18 Z(+), SPL(-)



 FUJIZOKI PHARMACEUTICAL CO., LTD.

International Division

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Phones: (03) 994-9361 (6 lines)
Telex: J28612
Cable: Fujizokireagent Tokyo

Head Office & Tokyo Plant

6-7, Shimoochiai 4-chome,
Shinjuku-ku, Tokyo 161, Japan
Phones: (03) 952-1391

Tokyo, Dec. 28, 1977

Mr. Charles E. Lincoln, President
DELMONT LABORATORIES, INC.
P.O.Box AA, Swarthmore,
Pennsylvania 19081,
U.S.A.

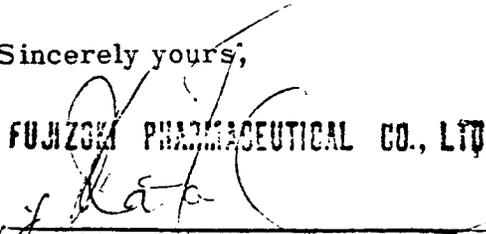
Dear Charlie:

For the purpose of presenting to forthcoming review by FDA, we herewith send you copies of the following datas and results of assessment.

1. Assessments and Studies of SPL
2. "S-27" Summary of Results of Tests Conducted at Fujizoki Pharmaceutical Research Division
3. Chemotactic Accumulation of Macrophages in the Peritoneal Cavity after Inoculation of SPL and Their Antitumor Activity.
4. Susceptibility of Staphylococcus aureus Clinical Isolates to Gratiaria Bacteriophage.
5. Influence of Staphage Lysates (SPL) on Immune Responses In Vitro. presented to The Fourteenth General Assembly The Central Regional Chapter of The Bacteriological Society of Japan
6. Immunopotentiator Activity of Staphage Lysate
7. Immunochemotherapy for Infections----- ←
With particular reference to Staphage Lysate.

We hope these datas will be helpful to you, and wish you a Happy New Year.

Sincerely yours,


FUJIZOKI PHARMACEUTICAL CO., LTD

Yozo Matsubara, manager,
International Division

Assessments and Studies of SPL

I. Proposed Specifications, Tests and Assays (submitted to the Ministry of Public Welfare, June 9, 1977)

As stated in the attached paper, Summary of Results of Tests Conducted at Fuji-Zoki Pharmaceutical Research Division, dated May 27, 1977.

Supplementary Notes:

Staining.--- When examined microscopically with the gram stain, neither any gram-positive bacteria nor any gram-negative organisms are demonstrable in the product.

Sterility.--- When examined by the sterility tests, the product is (1) bacteria-free and (2) fungus-free.

Absence of abnormal Toxicity. --- Inject 5ml of SPL intraperitoneally into each of two guinea pigs weighing about 350gm and observe the animals for seven days after the injection: the animals show no discernible signs or symptoms of toxicity.

Histamine and Histamine-Like Substances. ---

Glass Containers. --- Glass containers for injection of SPL and (1) clear, colorless and free of any visible bubbles and (2) meet the requirements of the test for alkaline dissolution.

Insoluble Impurities. --- When examined with the naked eye holding the sample in a brightness of 1000 lucas approx. directly below a white light source, the product is clear and contains no readily detectable, insoluble impurities.

II. Macrophage Chemotactic Test. (Mitsuyama, M., Miyake, T., Nomoto, K. and Taketani, K. from the Department of Bacteriology, Kyushu University School of Medicine) Confidential

Abstract:

Procedure of Test: Two groups of ten about 4-week-old ICR mice each were used. One group received single s.c. doses of 1×10^8 Staph. aureus 18Z cells weekly for a period of eight weeks for sensitization while the other group was kept untreated over the same period. Eighteen days after the final injection, the animals in these two groups were injected i.p. with 0.1ml of SPL and intraperitoneal macrophages of each animal were counted at 4, 24 and 48 hours after the eliciting injection.

Results: The animals given the eliciting dose of SPL after sensitization with Staph. aureus 18Z cells showed statistically significantly higher intraperitoneal macrophage counts, as compared to the control

group of normal untreated mice. The finding indicate that an eliciting i.p. dose of SPL gave rise to a significant intraperitoneal accumulation of macrophages in mice sensitized with Staph. aureus.

III. Test for Phage Activity. (Shigeno, N., Mitsuma, T. and Kojima, K. from the Junior College of Medical Technology and Nursing, affiliated with Niigata University. The 1977 Symposium on Staphylococci, Sept. 3, 1977, Okayama)

See the attached paper.

IV. Influence on Immune Responses In Vitro. (Mitsuma, T., Shigeno, N., Kojima, K. and Tanaka, Y. from the Junior College of Medical Technology and Nursing, affiliated with Niigata University, and from the Santo Hospital. The 14th Gen. Assembly of the Central Regional Chapter of the Jap. Soc. Bacteriol., June, 1977, Gifu; and the 41st East Japan Joint Meeting of the Jap. Soc. Dermatol., Sept. 24, 1977, Tokyo)

See the attached paper.

V. Immunopotentiator Activity. (Azuma, C., Tokuda, Y. and Shibata, T. from the Dept. of Dermatology, Tokyo College of Medicine. The 25th Gen. Assembly of the Jap. Soc. Chemotherapy, June 1977, Gifu, and the 41st East Japan Joint Meeting of the Jap. Soc. Dermatol., Sept. 24, 1977, Tokyo)

See the attached paper.

VI. Clinical Report. (Tsuda, S. and Minami, K. from the Dept. of Dermatol., Kurume University School of Medicine. MINOPHAGEN MEDICAL REVIEW, 21(5), 53-56, 1976)

See the attached paper.

VII. Controlled Double-Blind Trials. (To be concluded by the end of December 1977)

Subjects: Patients with multiple viral verrucosis.

Control drug: Broth (beef heart infusion broth) employed for the preparation of SPL.

Participating institutions: Tokyo Univ. (Dept. of Dermatol.), Kyushu Univ. (Dept. of Dermatol.), Defense Forces Univ. (Dept. of Dermatol.), Niigata Univ. (Dept. of Dermatol.), Ehime Univ. (Dept. of Pharmacol.), Tokyo Coll. Med. (Dept. of Dermatol.), Kurume Univ. (Dept. of Dermatol.) and Kansai Med. Coll. (Dept. of Dermatol.)

SUMMARY OF RESULTS OF TESTS CONDUCTED AT FUJI-ZOKI
PHARMACEUTICAL RESEARCH DIVISION

Institution: Division of Pharmaceutical Research, Fuji-
Zoki Pharmaceutical Company, Ltd., Tokyo

Chief Investigator-in-Charge: Daiichi Watanabe,

Period of Testing: From April 12, 1977, till May 31, 1977

Laboratory Conditions of Testing: Room temperature,
20 - 24°C; relative humidity, 55-65%.

Test Samples: Lot Nos. 6090755, 6111462 and 6111463.

Nature and Description:

S-27 is a clear, colorless or slightly yellowish-brown liquid containing the filtrate from liquid culture of Staphylococcus aureus cells lysed by specific bacteriophage.

Results

Lot No.	Description	Evaluation
6090755	This is a clear, colorless or slightly yellowish-brown liquid	Meets the requirements of the test
6111462	-do-	-do-
6111463	-do-	-do-

Hydrogen Ion Concentration:

Procedure of Test: The pH of S-27 was determined potentiometrically as directed in the Determination of Hydrogen Ion Concentration under General Test Procedures, the Biological Products Standards.

Results

Lot No.	Test No.	pH	Evaluation
6090755	1	7.45	Meets the requirements of the test
	2	7.46	
	3	7.45	
6111462	1	7.42	-do-
	2	7.43	
	3	7.43	
6111463	1	7.44	-do-
	2	7.42	
	3	7.46	

Staining:

Procedure of Test: The test was performed as directed in the Staining Test under General Test Procedures, the Biological Products Standards.

Results: All three Lots proved to meet the requirements of the staining test in all three repeated runs.

Sterility:

Procedure of Test: As directed in the Tests for Sterility (1) and (2) under General Test Procedures, the Biological Products Standards.

Results: Each Lot proved to meet the requirements of the tests for sterility in all three repeated runs.

Absence of Abnormal Toxicity:

Procedure of Test: As directed in the Test to Rule Out Abnormal Toxicity (1) under General Test Procedures, the Biological Products Standards.

Results: Each Lot proved to meet the requirements of the test to rule out abnormal toxicity (1) in all three repeated runs.

Weight Loss in Mice:

Procedure of Test: Inject 0.5ml of the product intraperitoneally into each of not less than five mice at approximately 5 weeks of age, and keep the mice under observation for 5 days after the injection: at the end of the 5-day observation the sum of the body weights of all mice exceeds that recorded on the day of injection, and the animals show no discernible symptoms of toxicity during and at the end of this period.

Results

Lot No.	Test No.	Total weight * on injection	Total weight 5 days later	Abnormality	Evaluation
6090755	1	98 gr.	111 gr.	Not recognized	Meets the requirements of the test
	2	96	109		
	3	89	104		
6111462	1	99 gr.	114	-do-	-do-
	2	98	112		
	3	90	103		
6111463	1	100 gr.	116	-do-	-do-
	2	98	109		
	3	96	105		

* Total weight of 5 mice

Pyrogen:

Procedure of Test: As directed in the Pyrogen Test under General Test Procedures, the Biological Products Standards, but using doses of 1.0ml of each test sample per kg of body weight of animals.

Results

Lot No.	Test No.	Total of animals involved	Total temperature of pyrogenetic animals	Evaluation
6090755	1	6	2.7°C	Meets the requirements of the test
	2	3	1.2	
	3	3	1.2	
6111462	1	3	1.2	-do-
	2	3	1.0	
	3	3	1.2	
6511463	1	3	1.2	-do-
	2	3	1.1	
	3	3	1.2	

Histamine and Histamine-Like Substances:

Procedure of Test: As directed in the Tests for Histamine under General Test Procedures, the Japanese Antibiotics Standards, but using doses of 0.02ml of test sample per kg of body weight of animals. 3 cats were involved for each lot of SPL (Total 9 cats male and female)
SPL Lot # 6090755, 6111462, 6511463.

Results: Each Lot proved to meet the requirements of the test for histamine in all three repeated runs.

Anaphylactic Shock:

Procedure of Test: Select four guinea pigs each weighing between 350 and 500gm approx. Inject each animal intraperitoneally with 0.02ml of the test

sample q. 48 hours in a total of three doses for sensitization. Two and three weeks after the final sensitizing dose test the animals for anaphylactic shock by single intravenous injection of 0.2ml of the same test sample, using two animals each: the animals show no discernible signs or symptoms of shock.

Results: Each Lot was found to meet the requirements of the test for anaphylactic shock in all three repeated runs.

Glass Containers:

Procedure of Test: As directed in the Test of Glass Containers for Injection under General Test Procedures, the Japanese Pharmacopeia.

Results:

Lot No.	Test No.	(1)	(3) Procedure I*		Evaluation
			Quantity of Sample Taken	Titer	
6090755	1	Clear, colorless and no bubbles	5.0010(g)	0.06(ml)	Meets the requirements of the test
	2	- " -	5.0006	0.05	
	3	- " -	5.0005	0.05	
6111462	1	- " -	5.0005	0.05	"
	2	- " -	5.0008	0.05	
	3	- " -	5.0005	0.05	
6111463	1	- " -	5.0005	0.06	"
	2	- " -	5.0006	0.05	
	3	- " -	5.0005	0.05	

* Factor for 0.02N sulfuric acid = 1.009

Insoluble Impurities:

Procedure of Test: As directed in the Item (11) under Injections, the General Notices, J.P.

Results: Each Lot proved to meet the requirements under Injections (11) in all three repeated runs.

Assay:

Carry out the assay by the phage plaque-forming unit (PFU) counting technique on plates of Trypticase soy agar (TSA) seeded with Staphylococcus aureus.

Materials: Use Trypticase soy broth (TSB) as the diluent for the test sample S-27, and a 3-hour TSB culture (31°C) of Staphylococcus aureus strain 3A as the reference organism.

Assay Results: Each ml of the test sample contains not less than 5×10^7 and not more than 5×10^8 PFU of staphylococcal bacteriophage.

DEPARTMENT OF MICROBIOLOGY
SCHOOL OF MEDICINE, KYUSHU UNIVERSITY
FUKUOKA, 812 JAPAN

CHEMOTACTIC ACCUMULATION OF MACROPHAGES IN THE
PERITONEAL CAVITY AFTER INOCULATION OF SPL AND
THEIR ANTITUMOR ACTIVITY

SPL has been presumed to activate macrophages and enhance the resistance to bacterial and fungal infections. Especially, SPL appears to exhibit such an effect on infections, when it is applied locally to the infected areas. Therefore, SPL may be able to accumulate macrophages locally and to activate them to give enhanced ability to kill microorganisms. The experiments were designed to analyze such abilities separately in mice.

Outbred ddY mice were used as hosts. Mice were injected subcutaneously with 1×10^8 viable organisms of Staphylococcus aureus (18Z) once a week for 4 or 8 weeks for the pre-treatment. For the measurement of chemotactic activity for macrophages, 0.1 ml of SPL was inoculated into the peritoneal cavity and peritoneal cells were counted at various intervals. For the assay of antitumor activity, 1×10^6 viable cells of Sarcoma 180, an ascites tumor, were inoculated intraperitoneally and various amounts of SPL were inoculated intraperitoneally every day for 7 days after tumor inoculation. Volumes of ascites or total packed volumes of tumor cells were assessed on day 10.

Mice were pretreated with 18Z for 8 weeks and 0.1 ml of SPL was injected intraperitoneally 48 hr after the last inoculation of 18Z. Peritoneal cells were counted before the inoculation of SPL and 4, 24 and 48 hr after the inoculation. When SPL was injected into normal controls,

the number of peritoneal cells increased slightly at 24 and 48 hr. When SPL was inoculated into mice pretreated with 18Z, the number of peritoneal cells increased rapidly and strikingly from 4 to 24 hr. (Fig.1)

Mice were pretreated with 18Z for 4 or 8 weeks and inoculated with tumor cells after the interval of 6 weeks. SPL was inoculated every day for 7 days from the day of tumor inoculation. When 1.0 ml of SPL was inoculated, tumor growth was inhibited completely in both groups (Tables 1 and 2). Definite effects were obtained with 0.3 ml of SPL in both groups. Suppressive effects were weak or negligible, when 0.1 ml of SPL was injected.

Summary SPL exhibited chemotactic activity for macrophages and antitumor activity on sarcoma 180 in mice pretreated with Staphylococcus aureus strain 18Z.

Fig. 1. Peritoneal cell number after intraperitoneal administration of SPL (0.1 ml) in normal and 18Z-pretreated mice. (mean of $5 \pm$ S.D.)

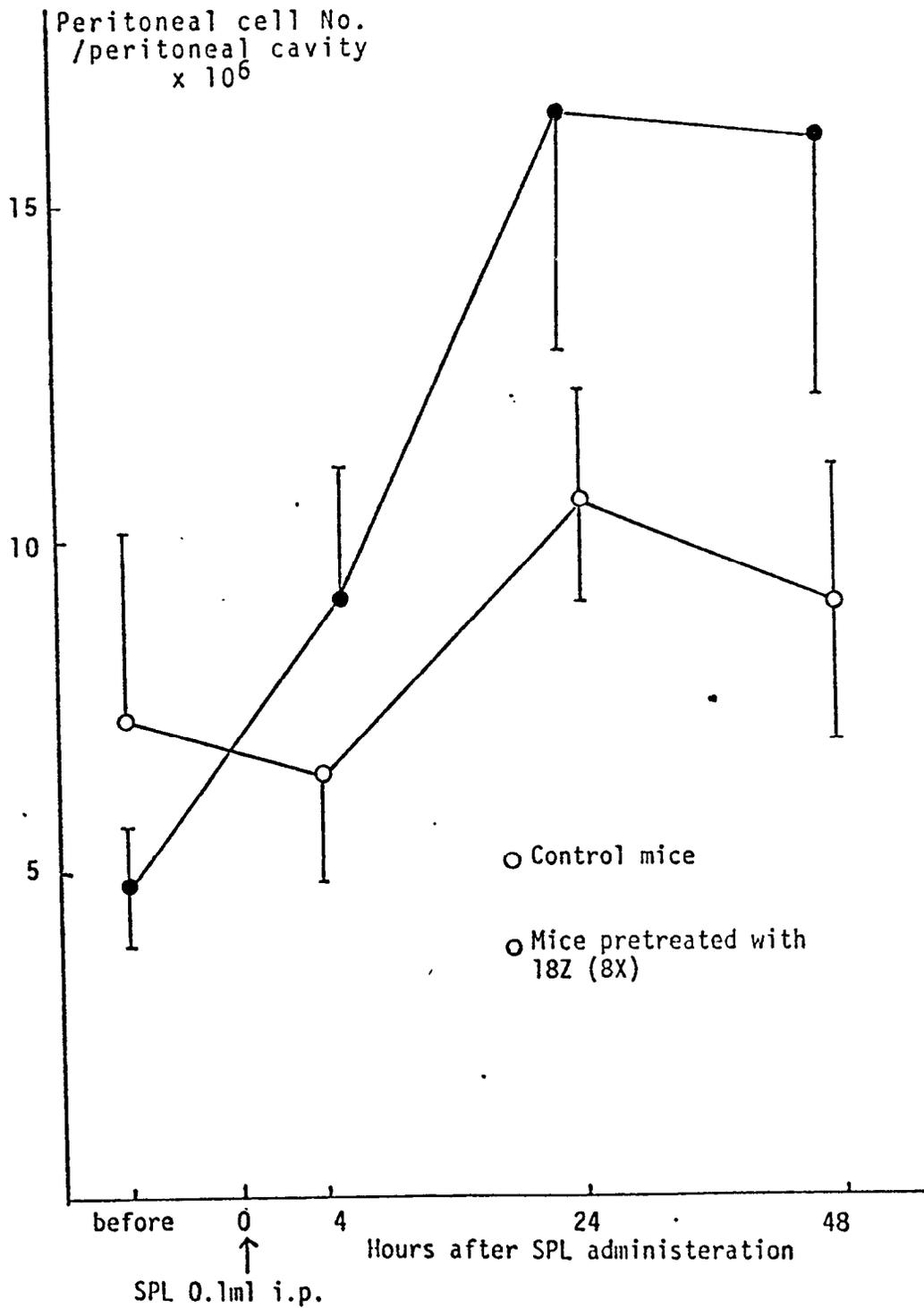


Table 1. Antitumor activity of SPL against Sarcoma 180 in ddY mice.

<u>S.aureus</u> (18Z)	Interval	Sarcoma 180 implantation (Cell No./body)	SPL (ml/body)	Volume of ascitic fluid ml	Total packed cell volume ml	Cell growth (T/C) %	Activity
	weeks	i.p.	i.p.				
			1.00	0	-	-	+++
1×10^8 /body/week s.c. inj. x4	6	1×10^6	0.30	0.55	0.12	16.9	++
			0.10	1.08	0.35	49.3	+
			0	1.98	0.71	100	-

6 mice/group - 7 groups (Total: 42 mice)

SPL Lot # 20890755 (Vol. 120 ml approx)

Table 2. Antitumor activity of SPL against Sarcoma 180 in ddY mice.

<u>S.aureus</u>	(18Z)	Interval	Sarcoma 180 implantation (Cell No./body)	SPL (ml/body)	Volume of ascitic fluid ml	Total packed cell volume ml	Cell growth (T/C) %	Activity
		weeks	i.p.	i.p.				
				1.00	0	-	-	+++
				0.30	0.75	0.27	33.8	++
1×10^8 /body/week s.c. inj. x 8		6	1×10^6	0.10	1.92	0.64	80.0	±
				0	2.30	0.80	100	-

6 mice/group 4 groups (Total 24 mice)

SPL Lot # 6090755 (Vol. 120 ml approx.)

Susceptibility of Staphylococcus aureus
Clinical Isolates to Gratia Bacteriophage

Shigeno, N., Mitsuma, T. and Kojima, K.

Junir College of Medical Technology and
Nursing affiliated with Niigata University

Summary:

(1) Of a total of 466 Staphylococcus aureus isolates from various clinical specimens studied, 201 strains (45.1%) were found susceptible to SPL.

(2) Isolates from the otorrhea, in particular, were very frequently susceptible to the phage (43 out of 54 strains, or 79.6%).

(3) Isolates from the sputum showed a relatively low rate of susceptibility, 41 out of 118 strains or 34.7%.

(4) Organisms isolated from the nasopharynx were almost as susceptible as those from the pus, the rates being 78/181 (43.1%) and 29/68 (42.6%), respectively.

References:

- 1) Gratia, A., Proc, Soc, Exp, Biol. Med. 18, 217 (1921).
- 2) Larkum, N.W., J. Inf. Dis. 45, 34 (1929).
- 3) Smith, P. B. and Mudd. S., Proc. Soc. Exp. Biol. Med. 134, 225 (1970).

Table 1. Susceptibility to SPL of Staph. aureus
clinical isolates.

	Naso- pharynx	Suptum	Otor- rhea	Pus	Urine	Other	Total No. Strains	
Susceptible	78 (43.1)	41 (34.7)	43 (79.6)	29 (42.6)	7 (41.2)	3 (37.5)	201 (45.1)	
Degree of susceptibi- lity	+++	53 (29.3)	26 (22.0)	22 (40.7)	16 (23.5)	5 (29.4)	2 (25.0)	124 (27.8)
	++	13 (7.2)	6 (5.1)	10 (18.5)	5 (7.4)	2 (11.8)	0	36 (8.1)
	+	12 (6.6)	9 (7.6)	11 (20.4)	8 (11.8)	0	1 (12.5)	41 (9.2)
Insusceptible	103 (56.9)	77 (65.3)	11 (20.4)	39 (57.4)	10 (58.8)	5 (62.5)	245 (54.9)	
Total No. Strains	181 (100)	118 (100)	54 (100)	68 (100)	17 (100)	8 (100)	446 (100)	

* Figures represent the numbers of isolates, and those in parentheses the corresponding percentages.

The Fourteenth General Assembly
THE CENTRAL REGIONAL CHAPTER OF THE
BACTERIOLOGICAL SOCIETY OF JAPAN

Abstracts of Presentations

Director, Central Chapter: Dr. Sadao Miyamura, professor,
Niigata University School of Medicine

Charman of the Assembly: Dr. Wataru Kondo, professor,
Niigata University School of Dentistry

Session: From 14:25 to 17:45, Oct. 29 (Sat.), 1977
From 09:00 to 14:00, Oct. 30 (Sun.), 1977

Place: Lecture Hall, Niigata University School of
Medicine, 757 Ichibancho, Asahimachidori,
Niigata City

14. Influence of Staphage Lysates (SPL) on Immune Responses In Vitro

Mitsuma, T,* Shigeno, N.,* Kojima, K.* and Tanaka, M.** (* Junior College of Medical Technology and Nursing affiliated with Niigata University and ** Santo Hospital)

Objective:

Staphage Lysates (Delmont Laboratories Inc., U.S.A.) (SPL) is the whole product from lysis of Staphylococcus aureus cells by specific bacteriophages and, therefore, not only holds the active phage in it but also contains bacterial cellular components as well as the constituents of culture medium. It has initially been introduced for use as a therapeutic agent against Staphylococcus aureus infection and, recently, has been acquiring importance on account of its non-specific immunostimulant property. This report describes the results of an in vitro study we conducted to investigate the effect of SPL on antibody production.

Materials and Methods:

The study was carried out using DK1 mice and sheep erythrocytes (SRBC) as antigen. Normal mouse splenic cells (2×10^7 cells per tube) were cultured with

SRBC in Marbrook tubes in a CO₂ incubator at 37°C. After four days of incubation, the cultures were examined for numbers of antibody-producing cells by the plaque-forming cell (PFC) counting according to the Mishell-Dutton method. Immediately prior to incubation various concentrations of SPL were added to the cultures to assess their effect on the PFC assay in comparison with SPL-free controls.

Results and Discussion:

A significant increase in the number of antibody-producing cells was evident in cultures containing SPL; the cultures to which SPL had been added at a final concentration of 10% showed an approximately three-fold increase of PFC (1260±28/tube), compared to the SPL-free control (PFC: 400±16/tube). With the decrease in the concentration of SPL added to the culture, the PFC diminished progressively to approach the control level.

An additional set of experiments was performed in the same manner and with the same reagents as in the foregoing experiment but using, in place of the normal mouse splenic cells, a splenic cell fraction except T cells eliminated by treating normal mouse spleen cells with anti-C₃H brain rabbit (anti-Thy) serum and complement. There was no appreciable effect of SPL on these cells; the cultures containing SPL showed essentially the same PFC

counts as the SPL control. The finding does not suggest that SPL has the ability to act directly upon antibody-producing cells to facilitate their nonspecific division.

Further studies to clarify the mechanisms of action of SPL, particularly in these respects, are in progress.

TABLE Effect of SPL on the in vitro Anti-SRBC response of mouse spleen cells

Concentration of SPL (%)	In vitro Immunization \bar{C} SRBC	PFC/Culture	
		Treatment \bar{C} -	Anti-Thy+C +
-	-	128 \pm 40	156 \pm 4
-	+	400 \pm 16	N.D.
10	+	1260 \pm 28	192
1	+	600 \pm 24	216 \pm 32
0.1	+	456	164 \pm 20

221. Immunopotiator Activity of Staphage Lysate
(Mudd)

Azuma, C., Tokuda, Y. and Shibata, T.
Department of Dermatology, Tokyo
College of Medicine, Tokyo

Staphage lysate (Mudd), referred hereinafter to as SPL, is a staphylococcal phage and its extensive studies of Prof. Mudd and his associates have demonstrated enhancement of resistance to infections in animals treated with this preparation. The underlying immunologic mechanism, nevertheless, is not clearly known as yet. This presentation summarizes the results of our recent study leading us to conclude that SPL has the property of acting as an immunopotentiator.

1) A series of patients with collagen diseases and other immune deficiency syndrome received subcutaneous doses of 10^7 to 10^8 SPL, each course consisting of ten doses injected q. 48 hours. There was clinical evidence of significantly increased defensive capacity against infection in these cases. Enhanced immune responsiveness was also observed in the PPD skin test.

2) In the treated series of patients, increase in bactericidal activity of neutrophils appeared to

parallel the enhancement of cutaneous response to PPD.

These findings indicate potentiation of the function of peripheral neutrophil leukocytes by SPL.

3) Experiments were performed to assess the effect of SPL in enhancing the resisting power of the host against infection, compared to various other antigenic sensitization in terms of minimal pus-forming doses, with the results summarized in the table shown below. As can be seen, a greater degree of increase in the host's resistance to infections was obtained by sensitization with live staphylococci than by that with SPL alone.

Minimal Pus-forming Doses (72h - 96h)

	Antigen Staph. aur.		Bacterial counts			
	Imm. R	Del. R	2800x10 ⁴	1400x10 ⁴	700x10 ⁴	350x10 ⁴
Staph. sensitization	(-)	(++)	?	derma- titis	derma- titis	derma- titis
Staphage lysate (Mudd)	(++)	(-)	+++	+++	+	±
Staph. infection with croton oil dermatitis			++	+ ~ ±	-	-
BCG sensitization	(-)	(-)	++	++	+	±
DNCB sensitization	(-)	(-)	++	++	+	±
Cont.	(-)	(-)	++	++	+	±

* Sensitization with live cells

4) Significant enhancement of the resistibility to infections was obtained by inoculation with SPL in rabbits and mice previously sensitized with live bacterial cells (Staph. aureus strain 209P). Furthermore, peritoneal macrophages from these animals showed increased bactericidal activity in vitro.

The data indicate that SPL acts as an immunopotentiator on the lymphocyte-macrophage-neutrophil system.

The 25th General Assembly of the Japanese Society of Chemotherapy, June 1977.

Immunochemotherapy for Infections -----
With Particular Reference to Staphage Lysate

Shingo Tsuda and Kikuo Minami
Department of Dermatology, Kurume University
School of Medicine, Kurume, Fukuoka Prefecture

Apart from the present subject immune deviation, I would like to add some comment on treatment of such severe, intractable staphylococcal infections of the skin. It will be concerned with staphage lysate, or SPL, supplied from Dr. Taketani of the Department of Bacteriology, Kyushu University, for clinical trials. The preparation is the product from lysis of Staphylococcus aureus by staphylococcus bacteriophage and, consequently, totally represents the antigenic components of the organism as illustrated in Table 7. In the United States, it has been clinically tried in more than 3,000 cases by Mudd and coworkers and reported to have proven effective in chronic refractory cases of staphylococcal infections. Through the clinical experience with SPL in these over 3,000 patients it has been ascertained that SPL is non-irritant and non-toxic and has no sensitizing effect in man. A pattern of erythematous reactions of considerable

interest has been observed in the skin test with SPL performed on patients with staphylococcal infections (Fig. 13). That is, in patients receiving intradermal injections of SPL (0.1ml) at weekly intervals, both the immediate and delayed local reactions were pronounced after the first intradermal injection and, thereafter, the local erythematous reaction diminished progressively in intensity and at the same time there occurred a progressive symptomatic improvement. A similar phenomenon has also been observed at our clinic.

We performed clinical trials of SPL in the cases shown in Table 8. The patients were treated with SPL alone and each patient was assessed as to degree of clinical improvement to evaluate effectiveness of the medication. Most of the patients studied had chronic intractable staphylococcal infections while the case material also included some patients with viral diseases. The treatment was considerably effective although no conclusive statement can be made here because of the relatively small series studied.

I would like to briefly describe two of these cases. A 62-year-old male had lesions of roentgen ulcer with secondary infection in the right dorsum pedis and interdigital regions, with so pronounced local edematous swelling as to cause difficulty in walking. The patient had been

treated elsewhere with antibiotics and other medicaments over the past few years, and, as the previous treatment failed to produce any significant improvement, he was begun on SPL. Figure 14 is a photograph of the affected area of the right foot taken on the first examination at our clinic.

Figure 15 shows the exanthems of the same area about two months after the start of SPL therapy, by which time he received a total dose of 9ml of the drug. The patient became completely relieved of edema and swelling and also considerably relieved from difficulty in walking.

The cutaneous lesion about 6 months of SLP therapy with a total dose of 16.5ml is shown in Figure 16. By this period the secondary infection had subsided almost completely and the patient became capable of walking as usual.

Another male patient, aged 63 years, was initially treated with oral and parenteral antibiotics along with ointments containing antibiotics since Staphylococcus epidermidis was isolated from pustules (Fig. 17). As no trend to improvement was noted in a few weeks of the antibiotic therapy re-examination was made and cultures disclosed Candida parakrusei and Trichophyton rubrun; the case was diagnosed as sycosis parasitaria.

Figure 18 is photograph of the same case, showing

the region two months after the first examination. The patient received SPL in subcutaneous doses of 0.5ml and intradermal doses of 0.6ml and a topical antitrichophytic preparation. Whilst the mechanisms whereby SPL produces such clinical improvement as yet are not clear, Mudd et al. have inferred that the administration of SPL elicits delayed hypersensitivity which has been previously induced to Staphylococcus aureus, thereby leading to activation of macrophages with consequent specific or nonspecific clinical effects.

From the analysis of the host's immune responses to staphylococci, it would seem rational to speculate that the disease state of severe intractable infection represents a condition which may be referred to as immune deviations. It is considered to be of profound significance that the phenomenon was observed not in such relatively rare diseases as leprosy and leishmaniasis but in staphylococcal infections of the skin which are commonly encountered. It has long been questioned whether cell-mediated immunity might have any significance in the host's defense mechanism against staphylococcal infection, but the results of the present analysis seem to reconfirm its importance. It follows that, in treating the host with intractable infection consequent to deviations of immune

response, chemotherapy alone does not suffice but immuno-chemotherapy for the infection should be undertaken as in immunochemotherapy for cancer.

The author is gratefully indebted to Prof. Takeya, Department of Bacteriology, Kyushu University School of Medicine, for the generous supply of SPL preparation. Acknowledgement is also made to Dr. Nomoto, assistant professor of medical bacteriology, Kyushu University, for his constant interest and guidance in this investigation.

References

- 1) Turk, J.L. and Bryceson, A.D.M.: Immunological phenomena in leprosy and related disease: Advances in Immunol., 13, 209-266, 1971.
- 2) Nomoto, K.: Recognition of antigens: Tampakushitsu-Kakusan-Koso (Protein, Nucleic Acids and Enzymes), 18, 168-177, 1973.
- 3) Nomoto, K., Yamada, H., Mashiba, H. and Takeya, K.: Immune response against hamster erythrocytes in the low responder mouse strains. VII. Differentiation of T lymphocytes into effector cells in delayed hypersensitivity and helper cells in antibody production: Japan, J. Microbiol., 1, 127-133, 1974.

- 4) Salmon, G.G., Jr., and Symonds, M.: Staphage lysate therapy in chronic staphylococcal infections: J. Medical. Society of New Jersey., 60, 188-193, 1963.
- 5) Mudd, S., Taubler, J.H. and Baker, A.: Delayed-type hypersensitivity to Staphylococcus aureus in human subjects: J. Reticuloend. Society, 8, 493-498, 1970.

Table 7. Staphage Lysate (SPL) is the Complete Representation of the Antigenic Components of Staphylococcus aureus.

-
- SPL contains the metabolites of the Staphylococci.
 - SPL contains both the heat stable and heat labile antigenic fractions, plus the intra- and extra-cellular enzymes of the Staphylococci.
 - SPL contains the solubilized products of the cell wall and the protein contents of the lysed Staphylococci.
 - SPL contains the active bacteriophage which produced the lysis of the Staphylococci.
 - SPL is a laboratory-fresh product, wholly free of preservatives or other denaturants.

Table 8

Nature of infection	Clinical results			
	Total	Excellent	Greatly improved	Unimproved
Folliculitis	2	1	1	
Furuncle	3	1	2	
Furunculosis	2		1	1
Ulcer due to radiation + Secondary infection	1	1		
Acne pustulosa	3	1	2	
Sycosis parasitaria	1	1		
Herpes zoster	6	3	2	1
Pustulosis palmo-plantaris	2			2

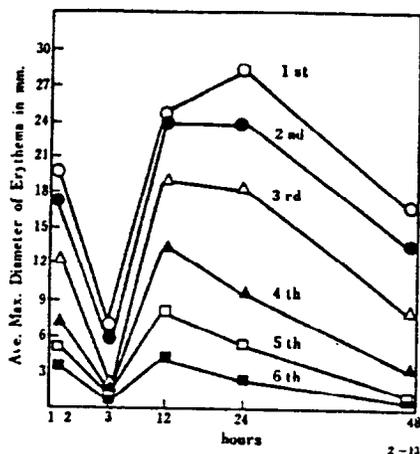


Fig. 13

Erythematous reactions over a 2-day period in patients receiving six intradermal injections of SPL (0.1 ml) at weekly intervals.
(Mudd, S. : J. Reticuloend. Soc., 8, 1970)



Fig. 16



Fig. 17

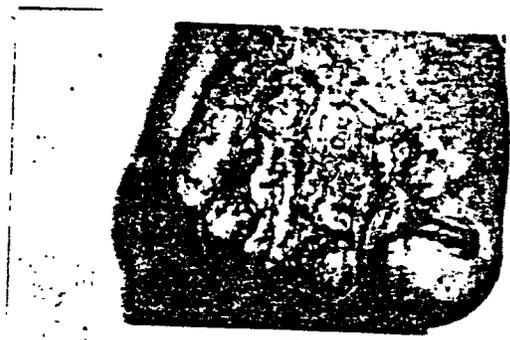


Fig. 14



Fig. 18

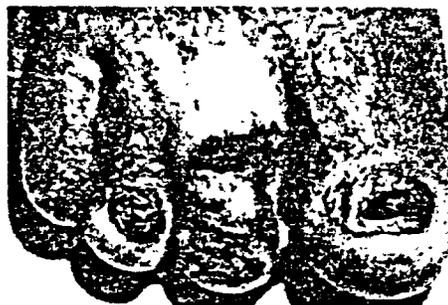


Fig. 15

Fig. 15

Subacute and Chronic Toxicity Tests
of S-27* in rabbits

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and

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Summary

Subacute and Chronic toxicities of S-27 for staphylococcal disease manufactured by Delmont Laboratories Inc. (Swarthmore, Pa.19081) have been investigated in comparison with sterile saline as a control group for 1 month and 3 months on the long term toxicity in rabbits. Rabbits were given every other day at a subcutaneous dose of 0.01, 0.1 and 1.0 ml/kg of S-27 and 0.5 ml/kg of sterile saline, respectively.

In the both tests, 6 rabbits per group were employed (male = 3, female = 3).

They were observed daily for appearance, behavior and survival: body weights were measured periodically. All animals were sacrificed and examined at necropsy for abnormalities, and subsequently for histopathological findings. Hematological, biochemical and urinary examinations were performed respectively.

No remarkable changes of every observations were observed in all groups treated with S-27 and control group.

* S-27 is the secret name assigned by Fujizoki pharmaceutical Co., Ltd. to SPL for staphylococcal disease manufactured by Delmont Laboratories Inc. (Swarthmore, Pa. 19081)

SPL lot Nos. used in the study: Lot No. 6111463
" 7021481
" 7033085

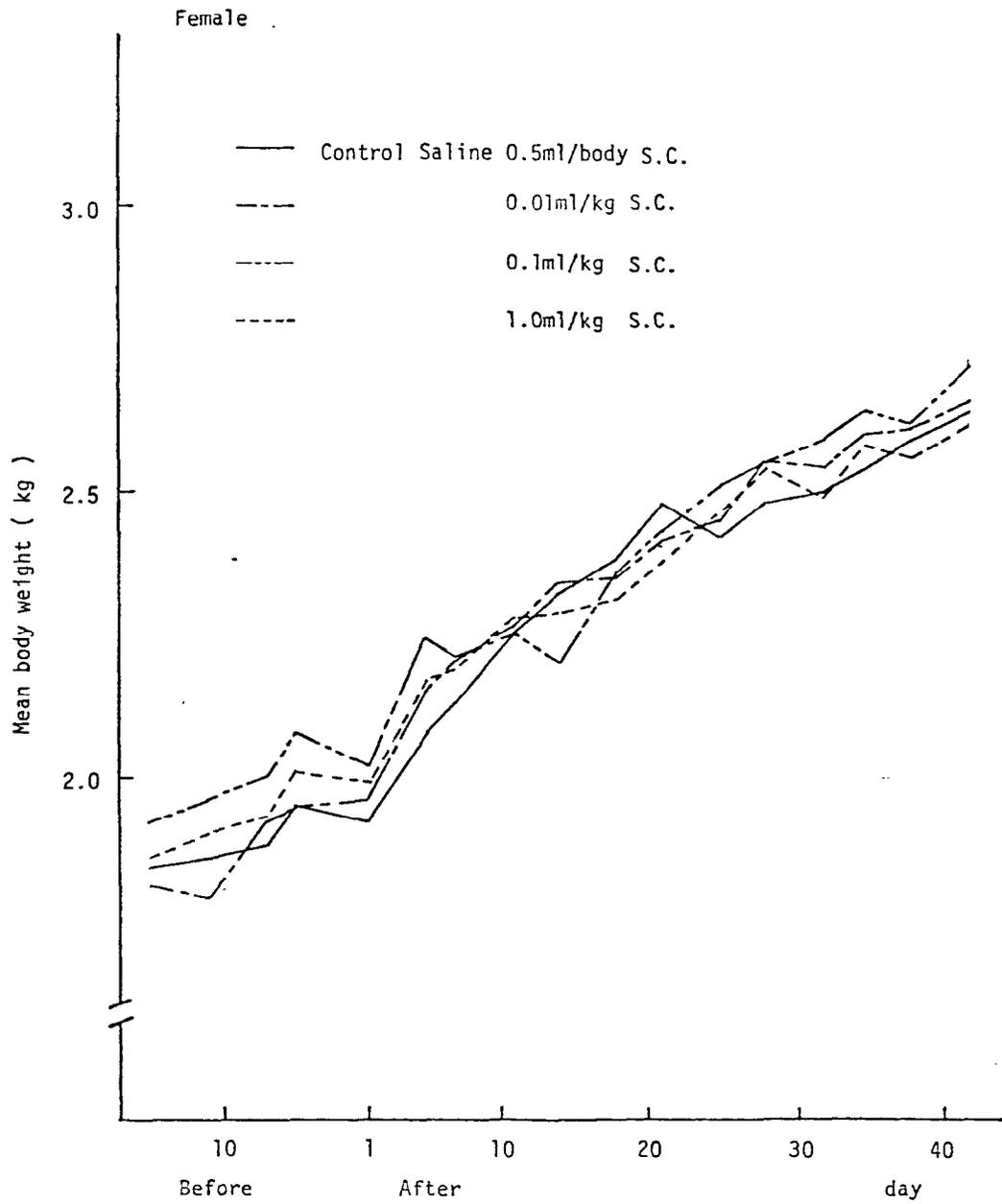


Fig. 1 Body weight gains of female rabbits administered with S-27 subcutaneously for 1 month.

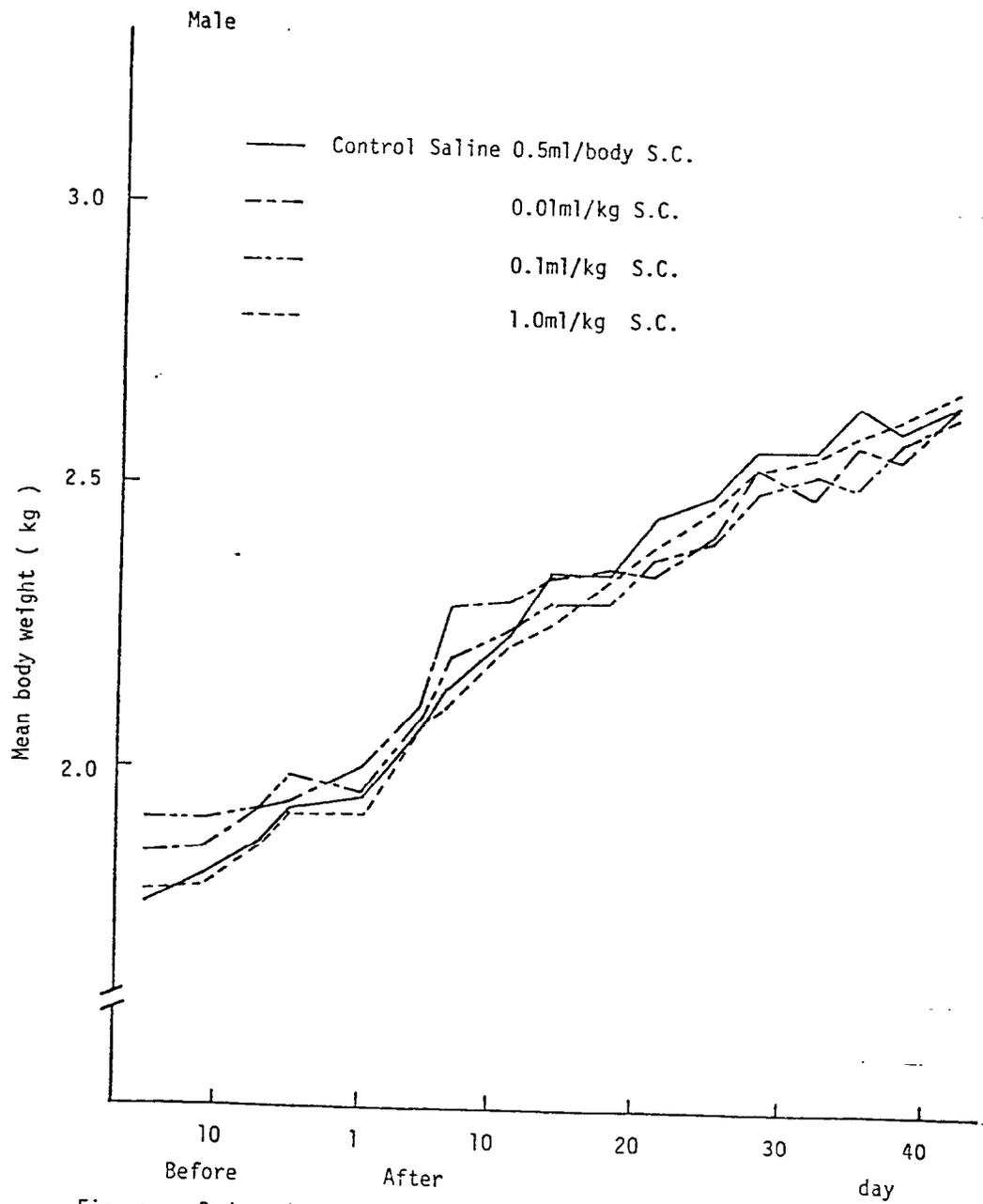


Fig. 2 Body weight gains of male rabbits administered with S-27 subcutaneously for 1 month.

Table 1 Hematological findings of male rabbits administered with S-27 subcutaneously for 1 month.

Dose (ml/kg/day)	Rabbit No.	R.B.C. ($\times 10^4/\text{mm}^3$)	W.B.C. ($\times 10^2/\text{mm}^3$)	Ht (%)	Hb [g/dl(%)]	Platelet ($\times 10^4/\text{mm}^3$)	Pth-time (Sec.)	Leucocyte differentiation (%)			
								Eos	Neut.	Lymph.	Mono.
Control	1	541	70	36.0	11.6 (72.5)	30.4	7.0	3	9	88	0
	2	614	134	36.5	12.4 (77.5)	31.0	8.5	2	8	89	1
	3	600	107	37.5	12.3 (76.9)	23.2	7.9	1	18	80	1
	Mean	585.0	103.7	36.7	12.1 (75.6)	28.2	7.8	2.0	11.7	85.7	0.7
0.01	1	557	98	36.0	10.8 (67.5)	22.6	7.5	1	10	89	0
	2	568	112	36.0	10.1 (63.1)	27.0	7.6	0	12	87	0
	3	594	137	39.0	10.9 (68.1)	27.2	8.6	0	14	86	0
	Mean	573.0	115.7	37.0	10.6 (66.3)	25.6	7.9	0.3	12.3	87.3	0
0.1	1	592	70	40.0	10.9 (68.1)	21.2	7.5	1	14	84	1
	2	639	92	40.0	12.0 (75.0)	24.0	7.5	0	28	72	0
	3	609	101	37.0	12.6 (78.8)	6.4	7.5	1	17	80	2
	Mean	613.3	87.7	39.0	11.8 (73.8)	18.1	7.5	0.7	19.7	78.7	1.0
1.0	1	594	67	41.0	13.5 (84.4)	13.2	7.8	0	9	89	2
	2	565	89	36.0	11.2 (70.0)	41.6	7.5	0	13	87	0
	3	597	109	36.0	12.0 (75.0)	15.6	8.1	1	10	89	0
	Mean	585.3	88.3	37.7	12.2 (76.3)	23.5	7.8	0.3	10.7	88.3	0.7

Table 2 Hematological findings of female rabbits administered with S-27 subcutaneously for 1 month

Dose (ml/kg/day)	Rabbit No.	R.B.C. ($\times 10^4/\text{mm}^3$)	W.B.C. ($\times 10^2/\text{mm}^3$)	Ht (%)	Hb [g/dl(%)]	Platelet ($\times 10^4/\text{mm}^3$)	Pth-time (Sec.)	Leucocyte differentiation (%)			
								Eos.	Neut.	Lymph.	Mono.
Control	1	619	91	39.0	13.1 (81.9)	19.2	7.7	0	17	82	1
	2	586	82	38.0	13.8 (86.3)	27.6	7.4	0	21	79	0
	3	513	94	40.0	12.3 (76.9)	16.2	6.7	0	10	89	1
	Mean	572.7	89.0	39.0	13.1 (81.9)	21.0	7.3	0	16.0	83.3	0.7
0.01	1	597	93	37.0	12.3 (76.5)	15.0	8.1	0	19	81	1
	2	589	62	39.0	12.7 (79.4)	33.8	7.3	0	20	79	1
	3	587	121	39.5	13.1 (81.9)	11.2	7.5	0	16	84	0
	Mean	591.0	92.0	38.5	12.7 (79.4)	20.0	7.8	0	18.3	81.3	0.3
0.1	1	592	105	36.0	13.0 (81.3)	44.6	7.7	1	17	82	0
	2	555	103	37.0	13.0 (81.3)	35.0	8.3	1	8	91	0
	3	614	125	41.0	13.3 (83.1)	20.6	7.5	1	19	79	1
	Mean	587.0	111.0	38.0	13.1 (81.9)	33.4	7.8	1.0	14.7	84.0	0.3
1.0	1	593	102	38.5	13.1 (81.9)	23.0	7.7	0	19	81	0
	2	600	71	38.0	13.2 (82.5)	31.2	7.5	0	12	88	0
	3	628	84	39.0	13.1 (81.9)	25.2	7.9	1	17	81	1
	Mean	607.0	85.7	38.5	13.1 (81.9)	26.5	7.7	0.3	16.0	83.3	0.3

Table 3 Organ weight of male rabbits administered with S - 27 subcutaneously for 1 month.

[Control]

Exp. No. of rabbit	1	2	3	Mean
Body weight(kg)	2.63	2.53	2.53	2.56
Cerebrum	6.810	6.910	6.362	6.694
Cerebellum	2.471	1.951	2.096	2.172
Pituitary grand	0.033	0.023	0.014	0.023
Thyroid	0.285	0.290	0.202	0.259
Thymus	3.001	4.102	3.209	3.437
Heart	6.404	5.691	5.294	5.796
Lung	16.432	9.816	9.047	11.735
Liver	77.889	89.107	59.259	75.418
Spleen	0.784	0.965	0.733	0.827
Stomach	29.807	31.684	30.213	30.568
Kidney -R.	6.703	4.301	6.155	5.720
-L.	6.565	6.521	5.949	6.345
Adrenal grand -R.	0.130	0.142	0.135	0.136
-L.	0.072	0.153	0.120	0.115
Testis -R.	1.654	1.627	1.970	1.750
-L.	1.715	1.547	1.957	1.740
Epididymis -R.	0.366	0.780	0.625	0.590
-L.	0.561	0.472	0.793	0.609
Prostate	0.361	0.482	0.623	0.489
Urinary bladder	3.297	3.669	3.266	3.388

Table 4 Cont'd.

[S-27 0.01 ml/kg]

Exp. No. of rabbit	1	2	3	Mean
Body weight (Kg)	2.67	2.70	2.60	2.66
Cerebrum	7.351	6.805	6.678	6.944
Cerebellum	2.398	1.993	2.099	2.164
Pituitary gland	0.045	0.030	0.026	0.034
Thyroid	0.420	0.220	0.212	0.284
Thymus	2.970	3.269	4.331	3.523
Heart	5.249	5.243	7.099	5.864
Lung	9.980	7.877	12.010	9.956
Liver	56.920	59.407	68.622	61.650
Spleen	1.110	0.629	0.687	0.809
Stomach	24.161	21.731	21.731	24.504
Kidney -R.	6.070	5.857	6.740	6.222
-L.	5.902	6.036	6.929	6.288
Adrenal gland -R.	0.088	0.104	0.155	0.116
-L.	0.075	0.132	0.144	0.117
Testis -R.	1.599	0.549	2.440	1.347
-L.	1.800	0.630	2.801	1.744
Epididymis -R.	0.850	0.312	0.735	0.632
-L.	0.569	0.339	0.900	0.603
Prostate	0.592	0.762	0.674	0.676
Urinary bladder	2.310	2.615	3.280	2.735

Table 5 Cont'd.

[S-27 0.1 ml/kg]

Exp. No. of rabbit	1	2	3	Mean
Body weight (Kg)	2.55	2.53	2.48	2.52
Cerebrum	7.288	6.990	7.216	7.165
Cerebellum	1.739	2.015	2.513	2.089
Pituitary gland	0.024	0.029	0.030	0.034
Thyroid	0.337	0.153	0.336	0.275
Thymus	4.859	1.649	5.544	5.017
Heart	4.579	6.696	6.676	5.983
Lung	10.009	11.054	10.556	10.540
Liver	67.250	60.021	62.242	63.171
Spleen	0.599	0.859	1.437	0.965
Stomach	23.759	28.940	31.004	27.901
Kidney -R.	5.463	6.054	6.403	5.973
-L.	5.671	6.342	6.556	6.190
Adrenal gland -R.	0.061	0.160	0.135	0.119
-L.	0.046	0.130	0.121	0.099
Testis -R.	1.683	2.587	1.671	1.980
-L.	1.599	2.922	1.757	2.093
Epididymis -R.	0.492	1.018	0.701	0.737
-L.	0.433	0.962	0.722	0.706
Prostate	0.430	0.396	0.622	0.483
Urinary bladder	5.166	4.149	3.725	4.347

Table 6 Cont'd.

[S-27 1.0 ml/kg]

Exp. No. of rabbit	1	2	3	Mean
Body weight (Kg)	2.58	2.30	2.50	2.47
Cerebrum	6.922	7.495	6.820	7.079
Cerebellum	1.879	2.722	1.724	2.108
Pituitary gland	0.025	0.042	0.023	0.030
Thyroid	0.206	0.260	0.203	0.223
Thymus	4.192	5.039	3.499	4.243
Heart	5.850	5.954	5.462	5.755
Lung	10.275	9.844	9.680	9.933
Liver	73.695	69.064	72.975	71.911
Spleen	0.673	2.511	1.498	1.561
Stomach	28.945	27.452	28.968	28.455
Kidney -R.	6.364	7.074	6.705	6.714
-L.	6.640	7.047	6.453	6.713
Adrenal gland -R.	0.107	0.112	0.145	0.121
-L.	0.106	0.082	0.136	0.108
Testis -R.	0.913	1.693	1.755	1.454
-L.	2.057	2.198	1.472	1.909
Epididymis -R.	0.549	0.910	0.721	0.727
-L.	0.747	0.816	0.700	0.754
Prostate	0.351	0.623	0.705	0.560
Urinary bladder	3.303	3.139	3.723	3.388

Table 7 Organ weight of female rabbits administered with
with S-27 subcutaneously for 1 month.

[Control]				
Exp. No. of rabbit	1	2	3	Mean
Body weight (Kg)	2.51	2.40	2.37	2.43
Cerebrum	6.421	6.809	7.043	6.757
Cerebellum	2.076	1.955	2.313	2.115
Pituitary grand	0.024	0.024	0.030	0.026
Thyroid	0.143	0.144	0.140	0.142
Thymus	2.227	2.306	2.741	2.425
Heart	4.536	4.810	5.153	4.833
Lung	6.867	8.488	8.604	7.986
Liver	54.715	59.543	63.267	59.175
Spleen	0.744	0.934	0.971	0.883
Stomach	24.813	25.846	23.614	24.758
Kidney -R.	5.737	6.384	6.170	6.097
-L.	5.547	6.152	5.968	5.889
Adrenal grand -R.	0.084	0.107	0.112	0.101
-L.	0.116	0.141	0.123	0.127
Ovary -R.	0.175	0.192	0.232	0.200
-L.	0.249	0.145	0.413	0.269
Uterus	1.970	1.237	1.204	1.470
Urinary bladder	2.824	3.406	2.814	3.015

Table 8 Cont'd.

[S-27 0.01ml/kg]

Exp. No. of rabbit	1	2	3	Mean
Body weight (Kg)	2.48	2.31	2.63	2.47
Cerebrum	6.960	6.918	7.206	7.028
Cerebellum	2.330	2.766	2.354	2.483
Pituitary gland	0.030	0.047	0.021	0.033
Thyroid	0.222	0.199	0.220	0.214
Thymus	2.946	2.814	6.064	3.908
Heart	5.479	6.376	6.304	6.053
Lung	4.837	8.875	9.701	8.982
Liver	68.575	61.133	70.745	66.817
Spleen	1.128	1.598	0.988	1.238
Stomach	26.895	27.342	24.097	26.010
Kidney -R.	5.696	6.985	6.372	6.351
-L.	5.885	7.068	6.032	6.328
Adrenal gland -R.	0.109	0.080	0.164	0.118
-L.	0.104	0.090	0.170	0.121
Ovary -R.	0.500	0.384	0.250	0.378
-L.	0.229	0.290	0.130	0.216
Uterus	1.851	1.374	1.220	1.482
Urinary bladder	3.820	2.705	3.243	3.273

Table 9 Cont'd.

[S-27 0.1ml/kg]				
Exp. No. of rabbit	1	2	3	Mean
Body weight (Kg)	2.61	2.43	2.67	2.57
Cerebrum	7.349	6.847	7.708	7.031
Cerebellum	1.934	2.162	2.267	2.121
Pituitary grand	0.029	0.030	0.027	0.029
Thyroid	0.020	0.241	0.275	0.179
Thymus	2.250	4.146	3.108	3.168
Heart	6.139	5.370	5.680	5.730
Lung	11.091	8.336	8.822	9.417
Liver	66.279	57.855	64.249	67.794
Spleen	0.907	1.052	1.080	1.013
Stomach	28.133	28.145	30.038	28.772
Kidney -R.	5.887	5.502	5.948	5.779
-L.	5.397	5.983	5.736	5.705
Adrenal grand -R.	0.145	0.075	0.120	0.113
-L.	0.132	0.136	0.131	0.133
Ovary -R.	0.221	0.376	0.121	0.239
-L.	0.351	0.354	0.135	0.280
Uterus	2.150	1.523	2.208	1.900
Urinary bladder	3.385	1.442	4.521	3.116

Table 10 Cont'd.

[S-27 1.0 ml/kg]

Exp. No. of rabbit	1	2	3	Mean
Body weight (Kg)	2.81	2.67	2.57	2.68
Cerebrum	7.310	6.640	7.051	7.000
Cerebellum	2.335	2.195	2.020	2.183
Pituitary gland	0.024	0.033	0.020	0.025
Thyroid	0.167	0.210	0.225	0.201
Thymus	3.173	4.795	3.936	3.968
Heart	6.174	5.330	5.532	5.679
Lung	11.810	8.487	7.947	9.415
Liver	66.423	60.994	63.020	63.479
Spleen	1.035	0.863	0.661	0.853
Stomach	35.126	28.839	29.000	30.988
Kidney -R.	7.037	5.746	5.967	6.250
-L.	7.504	5.951	6.357	6.604
Adrenal gland -R.	0.156	0.140	0.127	0.141
-L.	0.126	0.116	0.120	0.121
Ovary -R.	0.380	0.363	0.167	0.303
-L.	0.380	0.242	0.266	0.296
Uterus	1.618	1.621	1.063	1.434
Urinary bladder	2.890	4.483	3.663	3.679

Table 11 Urinary analysis of rabbits administered with S-27 subcutaneously for 1 month.

Sex	Dose (ml/kg/day)	Exp. No. of rabbit	pH	Occult blood	Protein (mg/dl)	Glucose (%)	Urobilinogen (Ehrlich unit/dl)	Bilirubin	Ketone body
Male	Cont.	1	8	-	Trace	-	1	-	-
		2	8	-	Trace	-	1	-	-
		3	8	-	Trace	-	1	-	-
	0.01	1	6	-	-	-	0.1	-	-
		2	6	-	-	-	0.1	-	-
		3	6	-	-	-	0.1	-	-
	0.1	1	6	-	-	-	0.1	-	-
		2	6	-	-	-	0.1	-	-
		3	5	-	-	-	0.1	-	-
	1.0	1	8	-	Trace	-	0.1	-	-
		2	6	-	Trace	-	0.1	-	-
		3	6	-	-	-	0.1	-	-
Female	Cont.	1	6	-	Trace	-	0.1	-	-
		2	6	-	Trace	-	0.1	-	-
		3	6	-	Trace	-	0.1	-	-
	0.01	1	5	-	-	-	0.1	-	-
		2	6	-	Trace	-	0.1	-	-
		3	6	-	-	-	0.1	-	-
	0.1	1	6	-	Trace	-	0.1	-	-
		2	6	-	Trace	-	0.1	-	-
		3	6	-	Trace	-	0.1	-	-
	1.0	1	7	-	-	-	1	+	-
		2	6	-	Trace	-	0.1	-	-
		3	5	-	-	-	0.1	-	-

Table 12 Blood biochemical findings in the rabbits treated subcutaneously with S-27 for 1 month

Dose (ml/kg)	T.P (g/dl)	A/G —	ch-E: (U/ml)	Al-p (mU/ml)	S-GOT (U)	S-GPT (U)	LDH (U)	CPK (mU/ml)	γ-GTP (mU/ml)	U-N (mg/dl)	T-lip. (mg/dl)	T-choL. (mg/dl)	Na (mEq/l)	K (mEq/l)	Cl (mEq/l)	Ca (mEq/l)
M 0.01	5.7	2.0	0.45	129	10	18	194	230	4	23.8	184	51	141	4.0	105	6.0
0.1	5.5	2.7	0.41	142	21	44	151	152	5	25.9	175	46	138	4.6	101	6.1
1.0	5.4	2.4	0.42	147	22	28	84	100	4	26.1	184	47	139	4.1	102	5.9
control	5.9	2.7	0.47	147	10	23	97	111	3	38.1	176	42	151	6.8	103	7.9
F 0.01	5.5	2.3	0.42	141	17	27	108	148	4	28.2	254	79	141	4.2	103	6.1
0.1	5.5	2.2	0.58	138	24	23	188	131	4	25.6	231	69	141	4.3	105	6.1
1.0	4.7	2.4	0.38	130	6	17	90	87	2	23.2	197	60	118	3.8	87	4.9
control	5.1	3.5	0.49	172	8	22	97	105	3	27.8	243	77	143	4.5	103	6.0

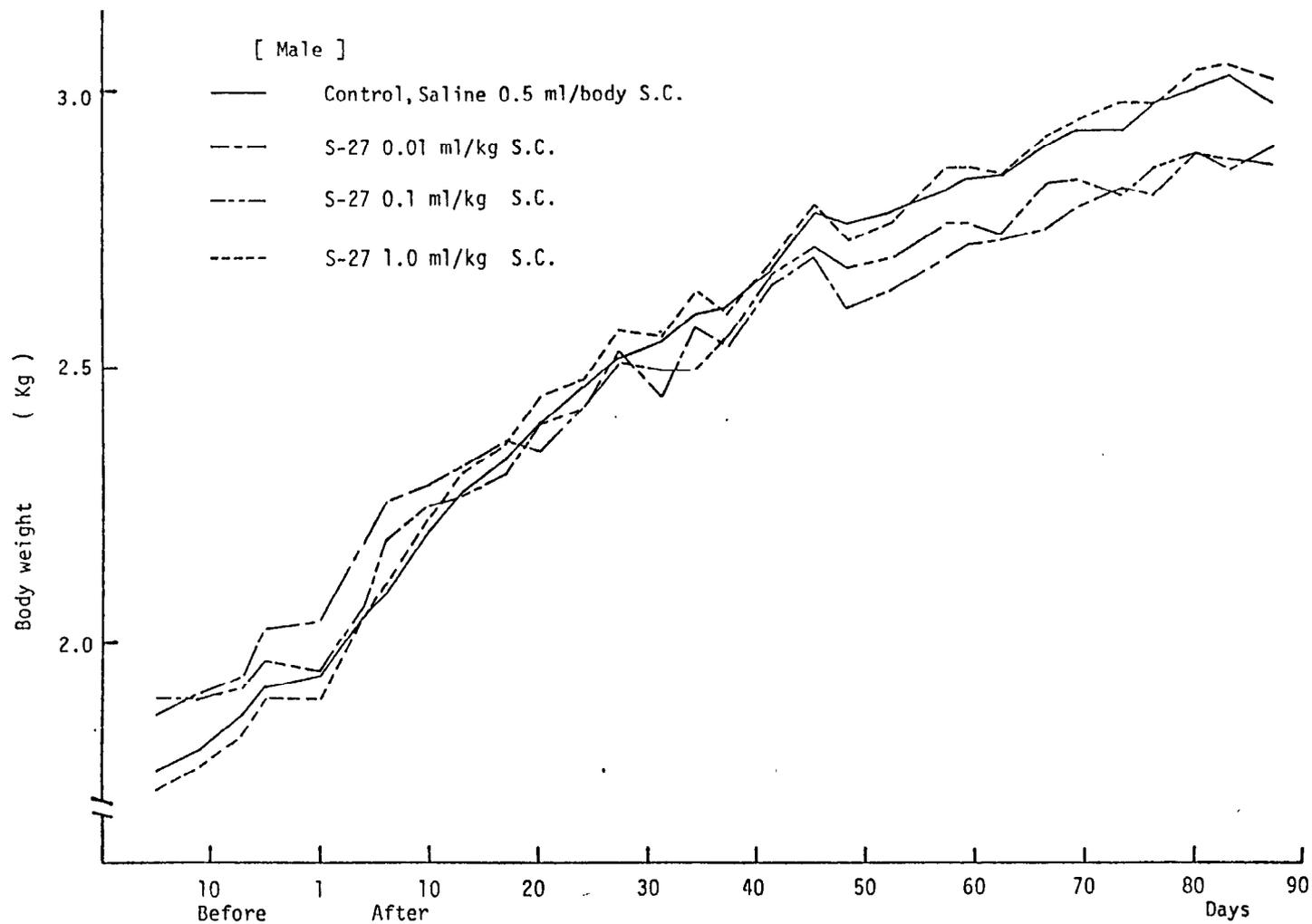


Fig. 1 Body weight gains of male rabbits administered with S-27 subcutaneously for 3 months.

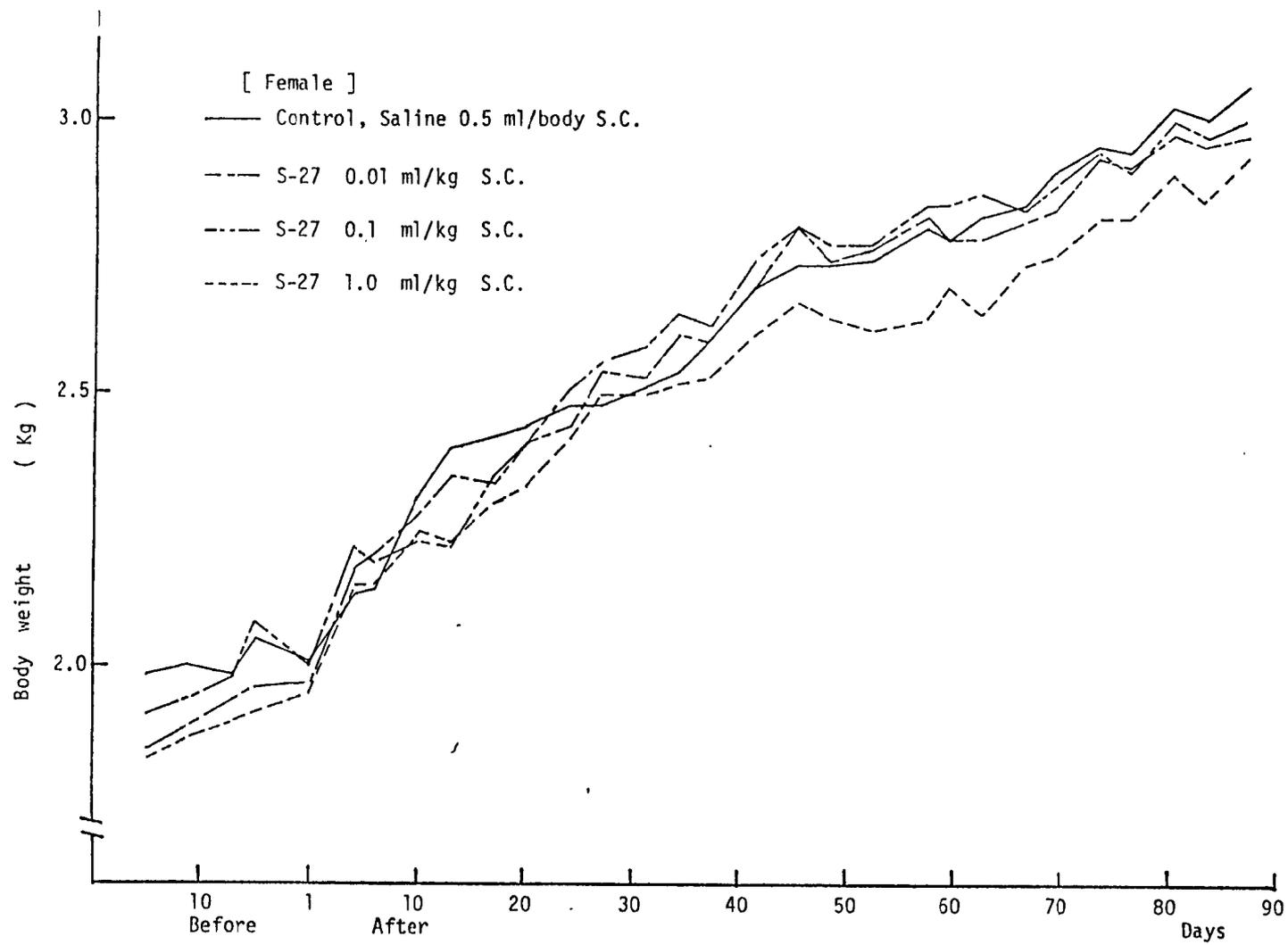


Fig. 2 Body weight gains of female rabbits administered with S-27 subcutaneously for 3 months.

Table 1 Hematological findings of male rabbits administered with S-27 subcutaneously for 3 months.

Dose (ml/kg/day)	Rabbit No.	R.B.C. ($\times 10^4/\text{mm}^3$)	W.B.C. ($\times 10^2/\text{mm}^3$)	Ht (%)	Hb [g/dl (%)]	Platelet ($\times 10^4/\text{mm}^3$)	Pth-time (Sec.)	Leucocyte differentiation (%)			
								Eos.	Neut.	Lymph.	Mono.
Control	1	616	113	45.0	15.4 (96.3)	15.0	8.6	0	19	80	1
	2	537	96	35.0	12.1 (75.6)	20.2	8.0	1	28	70	0
	3	519	86	36.5	12.7 (79.4)	34.6	7.9	1	12	83	4
	Mean	557.3	98.3	38.8	13.4 (83.8)	23.3	8.2	0.7	19.7	81.0	1.7
0.01	1	541	120	36.0	12.3 (76.9)	38.4	8.7	0	15	84	1
	2	607	119	42.0	14.2 (88.8)	21.4	9.6	2	32	63	3
	3	548	71	37.0	12.1 (75.6)	11.4	7.9	0	22	74	4
	Mean	565.3	103.3	38.3	12.9 (80.6)	23.7	8.7	0.7	23.0	73.7	2.7
0.1	1	545	84	37.5	12.3 (76.9)	41.0	8.9	0	27	73	0
	2	532	133	35.5	12.1 (75.6)	29.4	8.4	1	22	74	3
	3	550	97	36.0	12.7 (79.4)	36.6	8.4	1	14	82	3
	Mean	542.3	104.7	36.3	12.4 (77.5)	35.7	8.6	0.7	21.0	76.3	2.0
1.0	1	622	81	42.5	14.2 (88.8)	11.2	8.1	1	19	78	2
	2	552	91	36.0	12.6 (78.8)	16.4	8.7	0	28	70	2
	3	583	119	40.0	13.6 (85.0)	18.6	7.9	2	19	79	0
	Mean	585.7	97.0	39.5	13.5 (84.4)	15.4	8.6	1.0	22.0	82.3	1.3

Table 2 Hematological findings of female rabbits administered with S-27 subcutaneously for 3 months.

Dose (ml/kg/day)	Rabbit No.	R.B.C. ($\times 10^6/\text{mm}^3$)	W.B.C. ($\times 10^2/\text{mm}^3$)	Ht (%)	Hb [g/dl(%)]	Platelet ($\times 10^4/\text{mm}^3$)	Pth-time (Sec.)	Leucocyte differentiation (%)			
								Eos.	Neut.	Lymph.	Mono.
Control	1	515	115	34.0	11.2 (70.0)	22.0	8.1	1	22	75	2
	2	578	87	37.5	12.9 (80.6)	11.2	8.2	1	13	86	0
	3	534	95	35.5	12.2 (76.3)	22.0	7.9	1	29	68	2
	Mean	542.3	99.0	36.3	12.1 (75.6)	18.4	8.1	1.0	21.3	76.3	1.3
0.01	1	561	89	37.5	12.4 (77.5)	23.8	8.2	0	17	82	1
	2	561	120	37.0	12.6 (78.8)	16.6	8.3	0	26	74	0
	3	561	75	41.0	13.3 (83.1)	22.2	7.8	1	19	80	0
	Mean	561.0	94.7	38.5	12.8 (80.0)	20.8	8.1	0.7	20.1	78.7	0.3
0.1	1	544	99	38.5	12.8 (80.0)	28.4	8.1	0	28	72	0
	2	543	53	37.5	11.9 (74.4)	18.2	8.3	1	21	77	1
	3	554	69	37.0	12.3 (76.9)	13.2	8.4	0	13	85	2
	Mean	547.0	73.7	37.7	12.3 (76.9)	19.9	8.3	0.7	21.3	77.3	0.7
1.0	1	666	78	41.0	12.9 (80.6)	20.0	7.9	1	29	67	3
	2	568	87	36.0	12.3 (76.9)	22.2	8.5	0	22	75	3
	3	624	87	36.0	11.5 (71.9)	23.0	8.0	0	13	85	2
	Mean	619.3	84.0	37.7	12.2 (76.3)	21.7	8.1	0.3	21.3	75.7	2.7

Table 3 Organ weight of male rabbits administered with S-27 subcutaneously for 3 months.

[Control]				
Exp. No. of rabbit	1	2	3	Mean
Body weight (kg)	2.50	3.17	3.08	2.92
Cerebrum	7.470	7.381	7.369	7.406
Cerebellum	2.701	2.403	2.555	2.553
Pituitary gland	0.021	0.032	0.024	0.026
Thyroid	0.282	0.305	0.262	0.283
Thymus	4.305	4.055	3.725	4.028
Heart	7.989	6.834	6.754	7.192
Lung	16.274	9.641	10.180	12.032
Liver	51.125	59.022	59.915	56.688
Spleen	1.071	0.956	1.445	1.157
Stomach	22.921	29.947	27.405	26.758
Kidney -R.	6.637	5.771	5.999	6.136
-L.	6.617	5.952	6.537	6.369
Adrenal gland -R.	0.217	0.122	0.165	0.168
-L.	0.158	0.188	0.155	0.167
Testis -R.	3.280	2.414	3.070	2.922
-L.	3.511	2.464	3.103	3.026
Epididymis -R.	1.483	0.877	1.814	1.391
-L.	1.211	0.946	1.562	1.240
Prostate	0.762	0.542	0.523	0.609
Urinary bladder	4.377	5.276	4.666	4.773

Table 4 Cont'd.

[S-27 0.01 ml/kg]				
Exp. No. of rabbit	1	2	3	Mean
Body weight (kg)	2.76	2.91	2.79	2.82
Cerebrum	6.551	6.212	7.472	6.745
Cerebellum	1.562	2.359	2.630	2.184
Pituitary gland	0.010	0.012	0.022	0.015
Thyroid	0.222	0.235	0.263	0.240
Thymus	2.562	3.924	2.689	3.058
Heart	5.380	6.373	5.726	5.826
Lung	9.980	9.600	9.312	9.630
Liver	58.647	62.995	59.717	60.453
Spleen	1.120	0.821	0.968	0.970
Stomach	26.866	29.972	24.824	27.221
Kidney -R.	6.395	6.304	6.031	6.243
-L.	6.328	6.024	5.962	6.105
Adrenal gland -R.	0.136	0.158	0.132	0.142
-L.	0.104	0.153	0.150	0.135
Testis -R.	1.501	3.220	2.811	2.511
-L.	1.602	2.923	2.891	2.472
Epididymis -R.	0.492	1.351	1.050	0.868
-L.	0.644	1.182	1.192	1.006
Prostate	0.512	0.676	0.813	0.667
Urinary bladder	3.262	4.103	3.385	3.583

Table 5 Cont'd.

[S-27 0.1 ml/kg]				
Exp. No. of rabbit	1	2	3	Mean
Body weight (kg)	3.03	3.40	3.13	3.19
Cerebrum	6.858	6.538	7.364	6.920
Cerebellum	1.757	1.944	2.039	1.913
Pituitary gland	0.037	0.012	0.020	0.023
Thyroid	0.114	0.202	0.286	0.201
Thymus	2.133	3.180	2.774	2.696
Heart	8.060	6.927	5.537	6.841
Lung	11.231	11.294	10.756	11.094
Liver	65.490	76.148	65.571	69.070
Spleen	0.780	1.530	1.636	1.315
Stomach	29.136	35.230	31.212	31.859
Kidney -R.	6.787	5.731	6.214	6.244
-L.	6.735	6.557	6.189	6.494
Adrenal gland -R.	0.192	0.245	0.164	0.200
-L.	0.160	0.250	0.194	0.201
Testis -R.	3.289	2.950	2.013	2.751
-L.	3.242	3.017	1.863	2.707
Epididymis -R.	0.999	1.119	0.817	0.978
-L.	1.189	1.248	0.863	1.100
Prostate	0.743	0.663	0.546	0.650
Urinary bladder	5.730	5.866	4.270	5.283

Table 6 Cont'd.

[S-27 1.0 ml/kg]				
Exp. No. of rabbit	1	2	3	Mean
Body weight (kg)	2.84	2.81	3.18	2.94
Cerebrum	7.164	7.515	4.992	6.557
Cerebellum	2.086	2.720	2.154	2.320
Pituitary gland	0.019	0.026	0.035	0.027
Thyroid	0.274	0.240	0.292	0.268
Thymus	1.138	5.918	2.641	3.232
Heart	5.912	7.635	6.186	6.578
Lung	9.220	11.639	9.614	10.158
Liver	58.637	62.237	58.557	59.810
Spleen	0.742	1.031	0.935	0.902
Stomach	27.494	29.915	29.260	28.890
Kidney -R.	6.432	7.450	6.454	6.779
-L.	6.126	7.335	6.807	6.756
Adrenal gland -R.	0.082	0.173	0.138	0.131
-L.	0.110	0.184	0.154	0.149
Testis -R.	2.791	2.921	2.559	2.757
-L.	2.906	2.713	2.371	2.663
Epididymis -R.	0.917	1.320	0.954	1.063
-L.	0.856	1.468	1.130	1.151
Prostate	0.833	0.527	0.641	0.667
Urinary bladder	5.397	5.807	7.480	6.228

Table 7 Organ weight of female rabbits administered with S-27 subcutaneously for 3 months.

[Control]				
Exp. No. of rabbit	1	2	3	Mean
Body weight (kg)	2.99	3.00	3.12	3.04
Cerebrum	7.517	7.426	7.511	7.484
Cerebellum	2.301	2.554	2.349	2.401
Pituitary grand	0.029	0.032	0.028	0.030
Thyroid	0.260	0.343	0.303	0.302
Thymus	2.281	4.754	2.294	3.110
Heart	8.001	6.821	5.823	6.882
Lung	13.342	10.004	7.145	10.164
Liver	49.026	48.774	64.270	54.023
Spleen	0.785	0.851	0.887	0.841
Stomach	29.021	24.860	30.311	28.064
Kidney -R.	6.391	6.321	6.982	6.565
-L.	6.411	6.222	6.451	6.361
Adrenal grand -R.	0.141	0.189	0.143	0.158
-L.	0.144	0.155	0.120	0.140
Ovary -R.	0.862	0.788	0.800	0.817
-L.	0.636	0.921	0.812	0.790
Uterus	2.676	2.630	3.033	2.780
Urinary bladder	3.591	3.010	3.307	3.302

Table 8 Cont'd.

[S-27 0.01 ml/kg]				
Exp. No. of rabbit	1	2	3	Mean
Body weight (Kg)	3.52	2.91	2.96	3.13
Cerebrum	7.480	7.529	7.472	7.493
Cerebellum	2.141	2.088	2.267	2.165
Pituitary gland	0.024	0.030	0.022	0.025
Thyroid	0.261	0.152	0.256	0.223
Thymus	2.487	3.390	3.854	3.244
Heart	7.142	7.593	6.659	7.221
Lung	11.256	9.378	10.566	10.400
Liver	50.232	54.263	64.279	56.258
Spleen	0.861	0.893	1.098	0.950
Stomach	30.505	26.924	29.471	28.967
Kidney -R.	5.724	5.441	7.099	6.088
-L.	5.906	6.011	7.315	6.410
Adrenal gland -R.	0.126	0.170	0.144	0.147
-L.	0.168	0.155	0.176	0.166
Ovary -R.	0.683	0.462	0.724	0.623
-L.	0.786	0.672	0.579	0.679
Uterus	2.403	1.863	3.063	2.333
Urinary bladder	3.402	3.228	3.090	3.140

Table. 9 Cont'd.

[S-27 0.1 ml/kg]				
Exp. No. of rabbit	1	2	3	Mean
Body weight (kg)	2.98	2.86	3.20	3.01
Cerebrum	7.259	7.028	7.001	7.186
Cerebellum	1.983	1.631	2.515	2.043
Pituitary grand	0.054	0.027	0.023	0.035
Thyroid	0.321	0.249	0.263	0.278
Thymus	2.306	3.161	4.281	3.249
Heart	8.056	5.556	4.887	6.166
Lung	14.420	11.223	10.637	12.093
Liver	63.214	54.910	67.798	61.974
Spleen	2.263	0.834	0.658	1.252
Stomach	28.622	27.398	29.727	28.582
Kidney -R.	7.778	6.313	6.196	6.762
-L.	7.212	6.397	6.454	6.688
Adrenal grand -R.	0.169	0.256	0.114	0.180
-L.	0.118	0.248	0.104	0.156
Ovary -R.	0.644	0.832	0.826	0.767
-L.	0.890	0.771	0.974	0.879
Uterus	2.263	3.284	3.063	2.870
Urinary bladder	2.410	2.103	1.701	2.071

Table 10 Cont'd.

[S-27 1.0 ml/kg]				
Exp. No. of rabbit	1	2	3	Mean
Body weight (kg)	2.39	3.48	2.78	2.88
Cerebrum	7.401	7.982	7.398	7.593
Cerebellum	2.308	2.534	2.109	2.317
Pituitary gland	0.035	0.046	0.031	0.037
Thyroid	0.296	0.215	0.140	0.217
Thymus	1.968	1.537	2.293	2.932
Heart	7.194	6.598	5.057	6.283
Lung	12.807	9.798	8.768	10.458
Liver	54.800	65.481	50.877	57.052
Spleen	1.188	1.179	0.861	1.076
Stomach	23.473	40.171	23.201	28.948
Kidney -R.	7.012	6.591	4.999	6.201
-L.	6.721	6.762	4.998	6.160
Adrenal gland -R.	0.162	0.134	0.087	0.128
-L.	0.145	0.147	0.096	0.192
Ovary -R.	0.784	0.863	0.812	0.820
-L.	0.814	0.799	0.896	0.836
Uterus	2.006	3.959	3.005	2.990
Urinary bladder	2.735	2.636	2.951	2.774

Table 11 Urinary analysis of rabbits administered with S-27 subcutaneously for 3 months.

Sex	Dose (ml/kg)	Exp. No. of rabbit	pH	Occult blood	Protein (mg/dl)	Glucose (%)	Urobilinogen (Ehrlich unit/dl)	Bilirubin	Ketone body
Male	Cont.	1	5	-	Trace	-	0.1	-	-
		2	5	-	Trace	-	1	-	-
		3	5	-	Trace	-	0.1	-	-
	0.01	1	7	-	-	-	1	-	-
		2	7	-	Trace	-	0.1	-	-
		3	5	-	Trace	-	1	-	-
	0.1	1	6	-	Trace	-	1	-	-
		2	6	-	Trace	-	0.1	-	-
		3	6	-	-	-	0.1	-	-
	1.0	1	7	-	-	-	0.1	-	-
		2	6	-	-	-	0.1	-	-
		3	7	-	-	-	0.1	-	-
Female	Cont.	1	6	-	-	-	0.1	-	-
		2	6	-	-	-	0.1	-	-
		3	6	-	-	-	0.1	-	-
	0.01	1	5	-	-	-	0.1	-	-
		2	6	-	-	-	0.1	-	-
		3	6	-	-	-	0.1	-	-
	0.1	1	6	-	-	-	0.1	-	-
		2	6	-	-	-	0.1	-	-
		3	6	-	-	-	0.1	-	-
	1.0	1	6	-	-	-	0.1	-	-
		2	6	-	-	-	0.1	-	-
		3	6	-	-	-	0.1	-	-

Table 12 Blood biochemical findings of male rabbits administered with S-27 subcutaneously for 3 months.

Dose (ml/kg/day)	Rabbit No.	T-P (g/dl)	A/G	ch-E (U/ml)	Al-p (mU/ml)	s-GOT (Karmen)	s-GPT (Karmen)	LDH (Wróbleski)	CPK (mU/ml)	γ-GTP (mU/ml)	U-N (mg/dl)	T-Lip (mg/dl)	T-chof (mg/dl)	Na (mEq/l)	K (mEq/l)	Cl (mEq/l)	Ca (mEq/l)
Control	1	5.6	2.5	0.54	132	26	28	134	135	5	17.4	88	19	144	3.9	107	7.6
	2	6.0	2.5	0.46	124	14	16	72	106	4	23.7	128	33	143	4.4	104	6.6
	3	6.3	2.9	0.53	105	6	11	104	92	7	22.8	135	30	144	4.3	103	6.3
	Mean	6.0	2.6	0.51	120	15	18	103	111	5	21.3	117	27	144	4.2	105	6.8
0.01	1	5.2	3.2	0.48	99	36	26	93	94	3	22.3	100	24	139	4.7	99	6.2
	2	5.0	2.3	0.34	108	8	15	63	149	4	18.2	232	61	147	4.1	106	6.4
	3	5.4	3.9	0.49	112	24	40	37	65	4	17.2	103	20	142	3.6	105	6.4
	Mean	5.2	3.1	0.44	106	23	27	64	103	4	19.2	145	35	143	4.1	103	6.3
0.1	1	6.7	1.6	0.49	79	15	18	73	78	3	21.0	164	45	144	4.3	107	6.5
	2	6.4	1.7	0.50	84	29	34	88	99	7	32.9	146	34	151	4.0	106	6.5
	3	5.6	2.5	0.52	94	29	24	60	98	4	23.7	155	47	139	4.5	105	6.0
	Mean	6.2	1.9	0.50	86	24	25	74	92	5	25.9	155	42	145	4.3	106	6.3
1.0	1	5.6	1.9	0.49	95	13	16	144	170	4	23.7	147	32	139	3.6	99	6.3
	2	6.3	1.5	0.43	99	14	41	125	121	5	23.6	135	34	143	3.7	102	6.5
	3	5.7	2.3	0.53	120	25	34	95	154	5	20.9	128	28	144	3.7	104	6.5
	Mean	5.9	1.9	0.48	105	17	30	121	148	5	22.7	137	31	142	3.7	102	6.4

Table 13 Blood biochemical findings of female rabbits administered with S-27 subcutaneously for 3 months.

Dose (ml/kg/day)	Rabbit No.	T-P (g/dl)	A/G	ch-E (U/ml)	Al-p (mU/ml)	s-GOT (Karmen)	s-GPT (Karmen)	LDH (Wróbleski)	CPK (mU/ml)	r-GTP (mU/ml)	U-N (mg/dl)	T-Lip (mg/dl)	T-chole (mg/dl)	Na (mEq/l)	K (mEq/l)	Cl (mEq/l)	Ca (mEq/l)
Control	1	6.2	2.6	0.49	83	9	23	72	167	2	27.9	150	36	142	3.7	106	6.2
	2	6.0	1.8	0.52	85	11	20	376	372	3	21.3	230	78	144	3.5	105	6.4
	3	(5.0)	(3.3)	(0.51)	(138)	(6)	(10)	(42)	(48)	(2)	(180)	(230)	(60)	(111)	(4.5)	(81)	(5.5)
	Mean	(n=2) 6.1 (n=3) (5.7)	(2.7)	(0.45)	(102)	(9)	(18)	(163)	(196)	(2)	(224)	(203)	(58)	(132)	(3.9)	(99)	(6.0)
0.01	1	5.3	2.7	0.47	86	21	21	104	191	2	27.4	143	42	146	3.9	103	6.5
	2	5.7	3.0	0.65	118	6	11	112	55	4	23.9	176	50	141	4.0	105	6.4
	3	6.1	2.5	0.65	127	10	16	95	165	3	27.1	198	57	150	4.6	106	6.3
	Mean	5.7	2.7	0.59	110	12	16	104	137	3	26.1	172	50	146	4.2	105	6.4
0.1	1	5.6	1.3	0.49	50	16	8	498	522	2	25.1	326	69	144	3.8	104	6.0
	2	5.9	2.8	0.71	138	10	13	283	231	2	22.7	156	42	147	3.9	107	6.1
	3	5.8	2.8	0.52	93	58	22	111	84	4	24.2	141	38	146	3.5	108	6.3
	Mean	5.8	2.3	0.57	94	28	14	297	279	3	24.0	208	50	146	3.7	106	6.1
1.0	1	6.3	1.9	0.57	74	12	10	237	272	5	20.5	172	55	143	3.5	108	5.6
	2	6.0	2.9	0.65	102	8	15	107	110	2	27.4	205	68	146	4.0	109	6.6
	3	5.8	3.1	0.49	112	10	14	64	83	3	29.2	187	50	149	6.5	109	7.2
	Mean	6.0	2.6	0.57	96	10	13	136	155	3	25.7	188	58	146	4.7	109	6.5

March 7, 1978

Double Blind, Placebo Controlled Trial
for Evaluation of SPL in Wart

This trial is conducted by the committee for clinical evaluation of SPL prepared by Delmont Laboratories in Wart.

The controls received broth used to produce the SPL.

Both SPL and placebo are administered at 0.1 ml/injection into subcutaneous sequential injections at weekly interval.

Results : (Based on 117 patients)

Efficacy : SPL was clinically and statistically significantly better than placebo in improvement of wart.

	Status of Patients				
	cases	excellent	Good	No change	Dropouts
SPL	57	17	7	28	5
placebo	60	11	3	40	6

Safety : A few patients in the beginning of the trial experienced transient pruriginous and pain in both groups, but delayed type reactions (swelling and redness) observed were thought to be immunopharmacological activity of SPL.

Heamatological, biochemical and urinary examinations were performed before and after administration of SPL. No remarkable changes were observed in the both groups. It was also observed that no cross reaction of STS for syphilis test with SPL and placebo were demonstrated in both groups.

March 7, 1978

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