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DELMONT LABORATORIES, INC.
BIOLOGICAL SPECIALTIES
P. O. BOX AA, SWARTHMORE, PENNSYLVANIA 19081, U.S.A.

February 7, 1978

Miss Jennie C. Peterson
Hearing Clerk (HFC-20)
Food and Drug Administration
Room 4-65
5600 Fishers Lane
Rockville, Maryland 30857

Re: Notice of Opportunity for Hearing on Intent to Revoke Certain U.S. Licenses for Bacterial Vaccines and Bacterial Antigens (Docket No. 77N-0091)

Dear Miss Peterson:

On December 16, 1977, Delmont Laboratories, Inc., submitted a written appearance and request for hearing in response to the notice of opportunity for hearing in the above-referenced proceeding. Delmont manufactures Staphage Lysate (SPL), Type I and Types I and III for Staphylococcal Disease (bacterial antigen made from staphylococcus), under License No. 299. This letter is accompanied by data, information, and analyses in support of Delmont's request for a hearing, in accordance with the terms of the notice and the applicable regulations. This submission incorporates by reference all data and information contained in Delmont's submission to the advisory review panel on the safety, effectiveness, and labeling of bacterial vaccines and bacterial antigens with no U.S. standard of potency, in response to the Food and Drug Administration's notice of August 18,

1972 (37 Fed. Reg. 16690). For convenience, documents from that submission to which special reference is made are also included in this submission.

Although Delmont is submitting data in response to the notice of opportunity for hearing issued on December 9, 1977, the Company disputes the validity of that notice and the procedure under which it was issued. On November 8, 1977, FDA issued a notice of proposed rulemaking to classify bacterial vaccines and antigens on the basis of the report of the Advisory Panel. That notice proposed to classify Delmont's Staphage Lysate products in Category IIIB, so that the products would be taken off the market pending completion of studies to determine their safety and effectiveness. Delmont submitted comments on that proposal urging that its products be classified in Category IIIA, which would permit them to remain on the market pending completion of studies. Delmont's comments were accompanied by substantial new data concerning the safety and effectiveness of the products that supported a favorable risk-benefit assessment for inclusion in Category IIIA. FDA has issued no final order responding to Delmont's comments and issuing final classification regulations.

In these circumstances, issuance of a notice of opportunity for hearing on intent to revoke Delmont's license is premature and unlawful for two reasons. First, under the scheme established for FDA's biologics review by 21 C.F.R. § 506.25, initiation of proceedings to revoke biologics licenses for products covered by the review is based on the final classification assigned under the review. Although FDA has proposed to classify Delmont's products in Category IIIB, no final regulations have been issued. Presumably, Delmont's submission may convince the agency to place Staphage Lysate in Category IIIA in the final classification. If so, revocation proceedings will not be in order. Initiation of license revocation proceedings prior to completion of the classification rulemaking proceeding thus prejudges a question to be determined by the final rules and unlawfully denies Delmont an opportunity for meaningful comment on the proposed regulations in accordance with the requirements of 21 C.F.R. § 601.25 and the Administrative Procedure Act.

Second, initiation of license revocation proceedings at this time is also inconsistent with the agency's general regulations governing license revocations. Under the provisions of 21 C.F.R. § 601.5, FDA may initiate proceedings to revoke a biologics license on the grounds that the licensed

product is not safe and effective for all its intended uses or is misbranded with respect to any such use. But in the absence of evidence supporting license suspension under 21 C.F.R. § 601.6 or of "willfulness" within the terms of section 601.5, FDA must provide "a reasonable period for the licensee to demonstrate or achieve compliance" with the biologics licensing requirements before issuing a notice of opportunity for hearing. In the context of the biologics review, this provision requires, at the very least, that the licensee be given an opportunity to request that its product be classified in Category IIIA and that FDA evaluate the data in support of that request before a notice of opportunity for hearing is issued.

Delmont further notes that additional information and data relating to studies contained in this submission are being obtained in response to a request from officials of the Bureau of Biologics with whom the Company met on February 2, 1977. Those data relate to reports of animal safety studies conducted by the Fujizoki Pharmaceutical Co., Ltd., which are included in this submission.

Further, this submission includes the protocol of a clinical study of Staphage Lysate by John S. Silva, M.D. that

is being conducted at the Veterans Administration Hospital in Biloxi, Mississippi. A similar protocol has been submitted to the Keesler Air Force Base Medical Center Research Committee. This protocol is submitted in accordance with the provisions of 21 C.F.R. § 314.200(c)(2).

Respectfully submitted,

Charles E. Lincoln

Charles E. Lincoln
President

I. SAFETY DATA

A. ANIMAL SAFETY DATA

S - 27

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 lyzed 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 2 3	7.45 7.46 7.45	Meets the requirements of the test
6111462	1 2 3	7.42 7.43 7.43	-do-
6111463	1 2 3	7.44 7.42 7.46	-do-

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 2 3	98 gr. 96 89	111 gr. 109 104	Not recognized	Meets the requirements of the test
6111462	1 2 3	99 gr. 98 90	114 112 103	-do-	-do-
6111463	1 2 3	100 gr. 98 96	116 109 105	-do-	-do-

* 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 2 3	6 3 3	2.7°C 1.2 1.2	Meets the requirements of the test
6111462	1 2 3	3 3 3	1.2 1.0 1.2	-do-
6511463	1 2 3	3 3 3	1.2 1.1 1.2	-do-

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.

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

Anaphylatic 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 bubles	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.

FUJIZOKI PHARMACEUTICAL CO.,LTD.

International Division
5th Flr. Sun light Bldg.,
29-1, Toyotama-kita 5-chome,
Nerima-ku, Tokyo 176, Japan
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, Swarthnore,
Pennysylvania 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 Gratia 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 luces 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.)

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Nerima-ku, Tokyo 176, Japan
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

CONFIDENTIAL

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 Teratologenicity 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 FUJINO[1], Yuji SUGISAKI[1], Junko NAKAGAWA[1],
Masana KOMATSU[1]
and
Hachihiko HIRAYAMA[2]

Resarch Department Lab. I Fujizoki Pharmaceutical Co., Ltd.
6-7, Shimoochiai 4-chome, Shinjuku-ku, Tokyo[1]

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

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 20.0 ml/kg of SPL and 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
Resarch 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.

LD_{50} of SPL were as follows:

Species	Sex	Administration route (ml/kg)			
		p.o.	s.c.	i.p.	i.v.
Mouse	Male	100<	100<	100<	100<
	Female	100<	100<	100<	100<
Rat	Male	50<	50<	70<	50<
	Female	50<	50<	70<	50<

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 recieved 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 histomorpholagic 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.

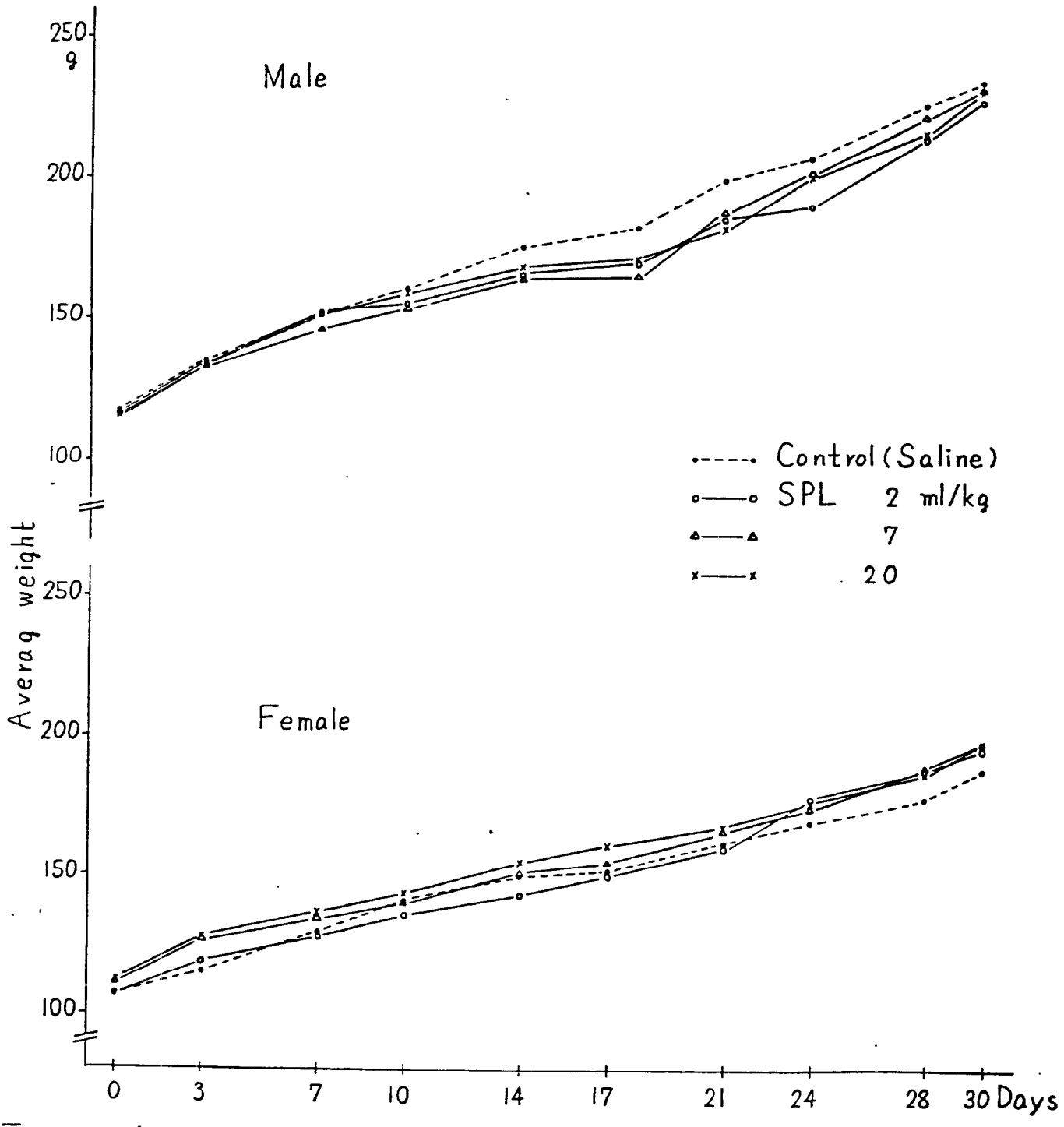

Fig. 1 Average body weights in male and female rats treated with SPI (s.c) for 30 days

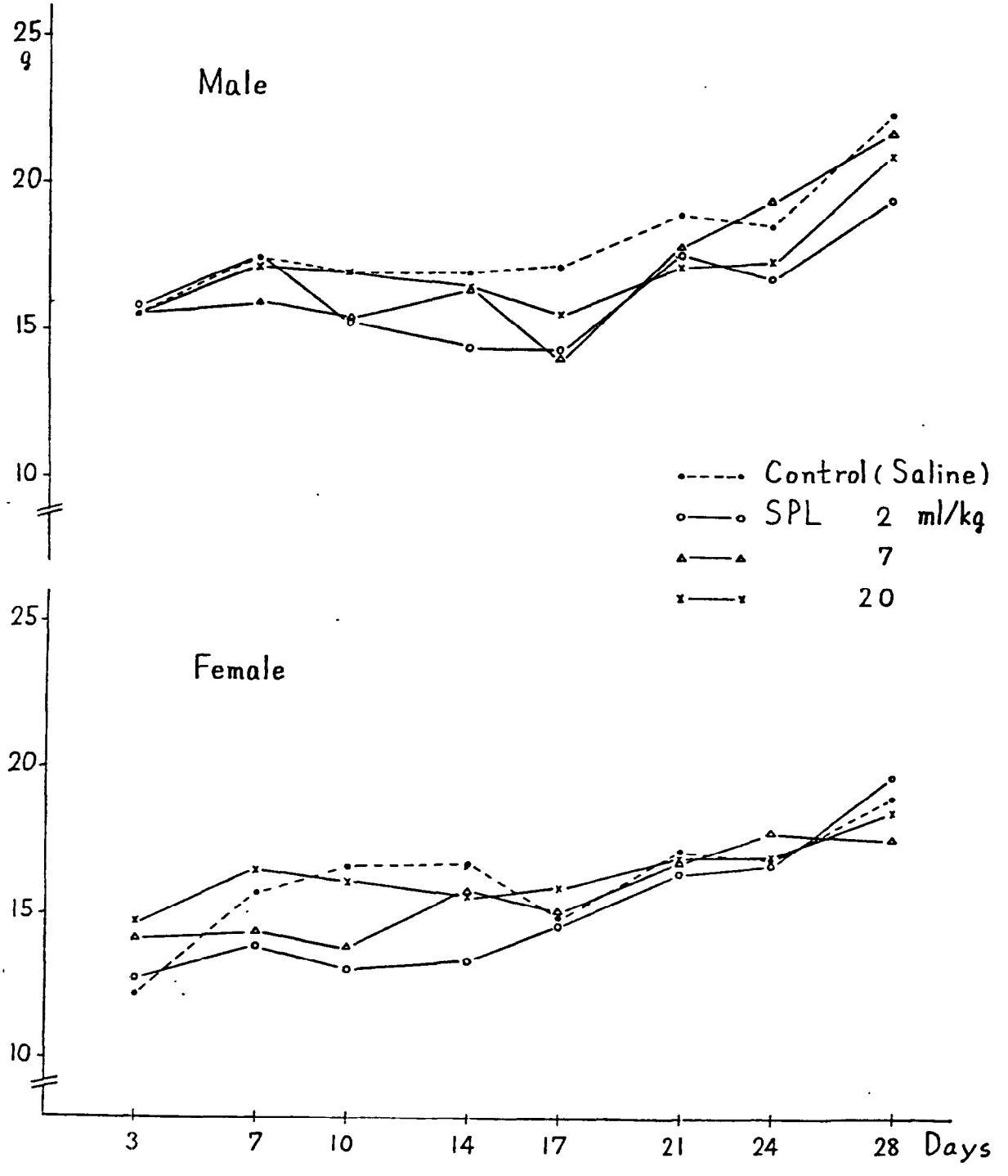

Fig. 2 Food consumptions in male and female rats treated with SPL (s.c) for 30 days

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	SPL 7 ml/kg	SPL 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
Seminal vesicle (g)	M	0.67 ± 0.24	0.82 ± 0.41	0.60 ± 0.45	0.54 ± 0.31
Ovaries (mg)	F	73.5 ± 13.6	67.8 ± 12.0	68.9 ± 7.9	69.3 ± 12.7
Uterus (g)	F	0.34 ± 0.08	0.34 ± 0.05	0.32 ± 0.05	0.37 ± 0.08

M: Male F: Female

*: $P < 0.05$

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	SPL 7 ml/kg	SPL 20 ml/kg
Brain	M	0.76 ± 0.09	0.80 ± 0.16	0.78 ± 0.10	0.80 ± 0.10
(g)	F	0.88 ± 0.06	0.84 ± 0.05	0.83 ± 0.07	0.82 ± 0.05
Lung	M	1.06 ± 1.05	0.99 ± 0.41	0.92 ± 0.50	0.88 ± 0.30
(g)	F	0.82 ± 0.31	0.79 ± 0.26	0.82 ± 0.18	0.68 ± 0.19
Thymus	M	0.29 ± 0.04	0.29 ± 0.05	0.31 ± 0.03	0.26 ± 0.06
(g)	F	0.28 ± 0.04	0.29 ± 0.03	0.28 ± 0.04	0.25 ± 0.00
Heart	M	0.41 ± 0.06	0.44 ± 0.03	0.43 ± 0.04	0.42 ± 0.00
(g)	F	0.39 ± 0.00	0.36 ± 0.00	0.38 ± 0.03	0.38 ± 0.03
Liver	M	5.07 ± 0.47	4.63 ± 0.44	4.79 ± 0.30	4.68 ± 0.35
(g)	F	5.26 ± 0.51	4.89 ± 0.88	4.86 ± 0.39	4.82 ± 0.50
Kidneys	M	0.95 ± 0.16	0.99 ± 0.10	1.05 ± 0.18	1.08 ± 0.14
(g)	F	0.96 ± 0.09	0.89 ± 0.05	0.88 ± 0.04*	0.85 ± 0.10*
Spleen	M	0.30 ± 0.05	0.27 ± 0.04	0.31 ± 0.04	0.32 ± 0.07
(g)	F	0.34 ± 0.03	0.32 ± 0.11	0.27 ± 0.00	0.30 ± 0.03
Adrenals	M	25.4 ± 4.8	24.7 ± 5.6	23.8 ± 4.2	23.7 ± 4.0
(mg)	F	32.8 ± 2.9	27.6 ± 2.4***	29.2 ± 4.9	29.3 ± 3.5*
Thyroid	M	15.3 ± 3.2	17.0 ± 2.4	16.7 ± 3.1	16.1 ± 2.6
(mg)	F	17.6 ± 2.7	16.5 ± 3.0	16.6 ± 2.5	16.9 ± 2.3
Hypophysis	M	5.5 ± 2.4	5.5 ± 2.3	5.1 ± 2.0	5.0 ± 1.2
(mg)	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
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

* : $P < 0.05$ *** : $P < 0.001$

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

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

M: Male F: Female

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

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

		Sex	Control (Saline)	SPL 2 ml/kg	SPL 7 ml/kg	SPL 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 ($\times 10^4$)		M	639 ± 137	778 ± 205	820 ± 132*	784 ± 81*
		F	702 ± 134	767 ± 97	720 ± 157	807 ± 83
Leucocytes ($\times 10^2$)		M	88 ± 35	112 ± 39	87 ± 46	70 ± 15
		F	95 ± 21	107 ± 31	93 ± 17	111 ± 20
Hemogramm %	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

*: $p < 0.05$ **: $p < 0.01$

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

Teratologenicity Study of SPL in Rats and Rabbits

Hachihiko HIRAYAMA

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

Summary

Teratologenicity study of SPL for staphylococcal disease manufactured by Delmont Laboratories Inc. was carried out in rats and rabbits. SPL was intraperitonially 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 teratologenic effect on both animals.

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 rfsorped 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	6	20	18	14
(Mean litter size)	(6)	(6.3)	(6.0)	(6.5)
No. of dead or resorped fetuses	1	4	0	2
(Fetal mortality)*	(14.3)	(16.6)	(0)	(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

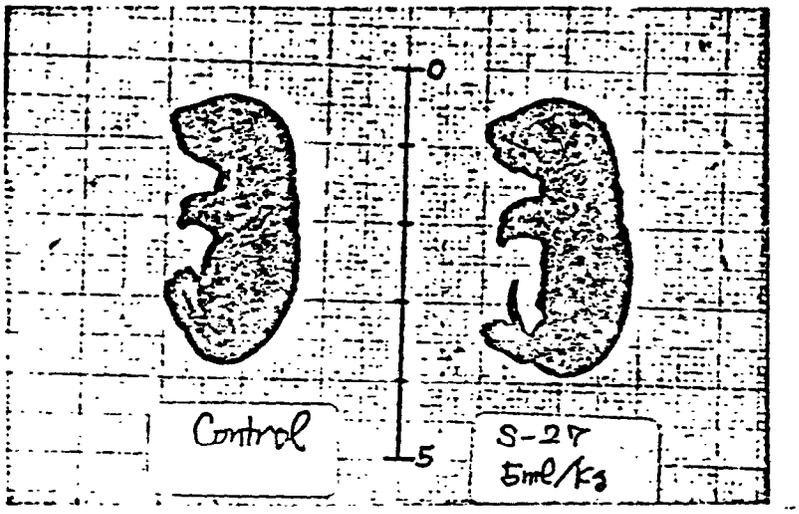

PLATE I-1 Left: A fetus from the pregnant rat. Right: A fetus from the pregnant rat treated intraperitoneally with 5ml/kg of S-27.

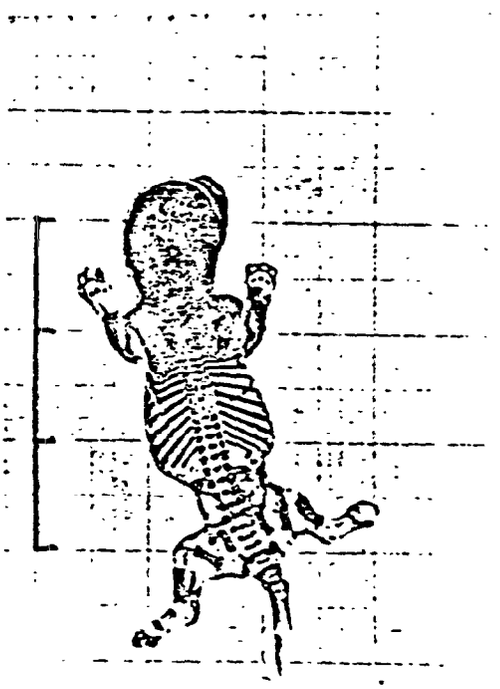

PLATE I-2 A normal skeleton of rat fetus in saline control group.

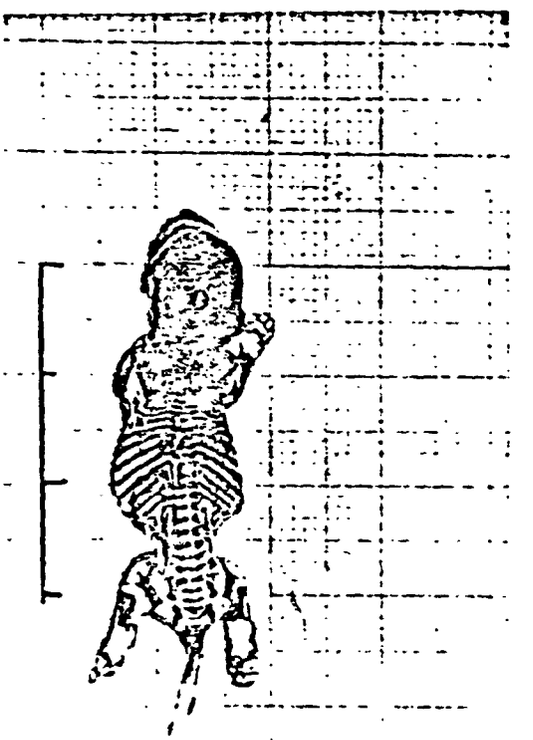

PLATE I-3 A normal skeleton of rat fetus from the pregnant rat treated intraperitoneally with 5ml/kg of S-27.

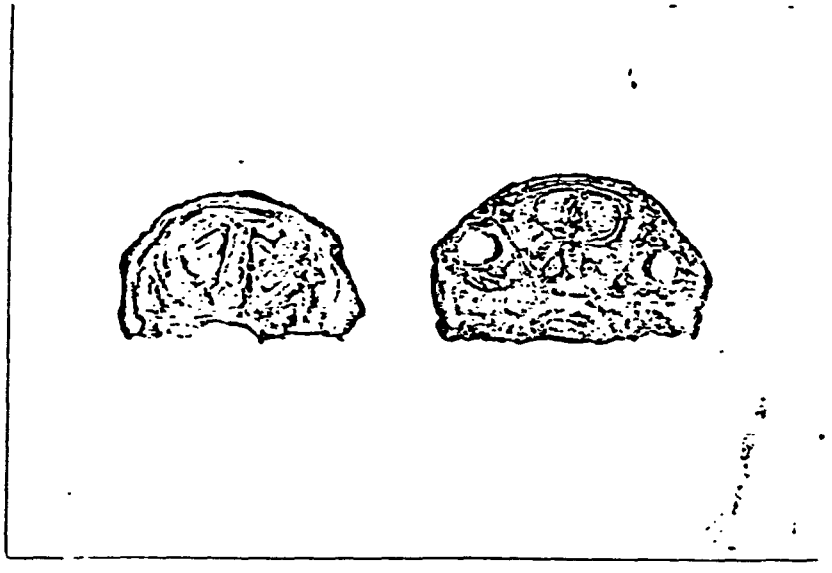

PLATE I-4 Palate and nasal cavities of control eat fetus; showing normally in the Wilson's section.

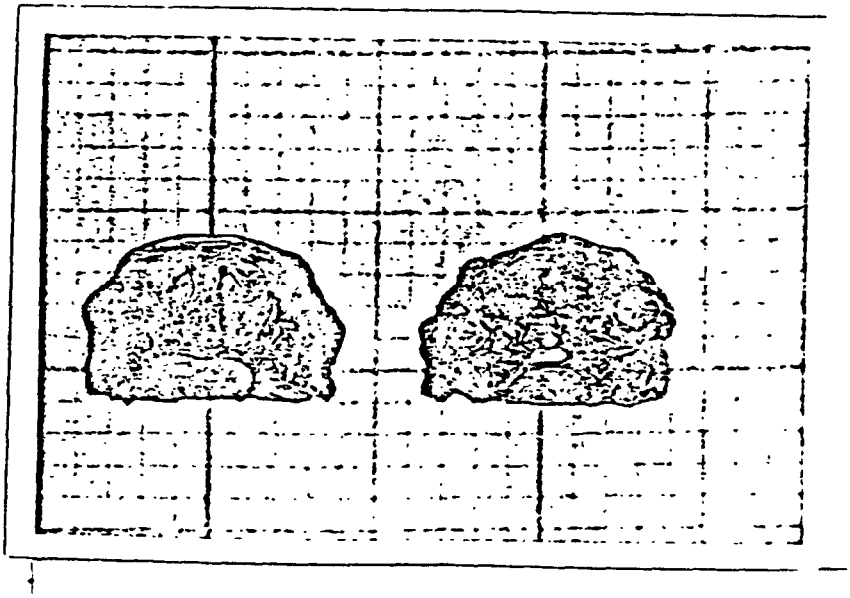

PLATE I-5 Palate, eyeballs and olfactory bulbs of fetus from the pregnant rat treated intraperitoneally with 5ml/kg of S-27; showing normally in the Wilson's section.

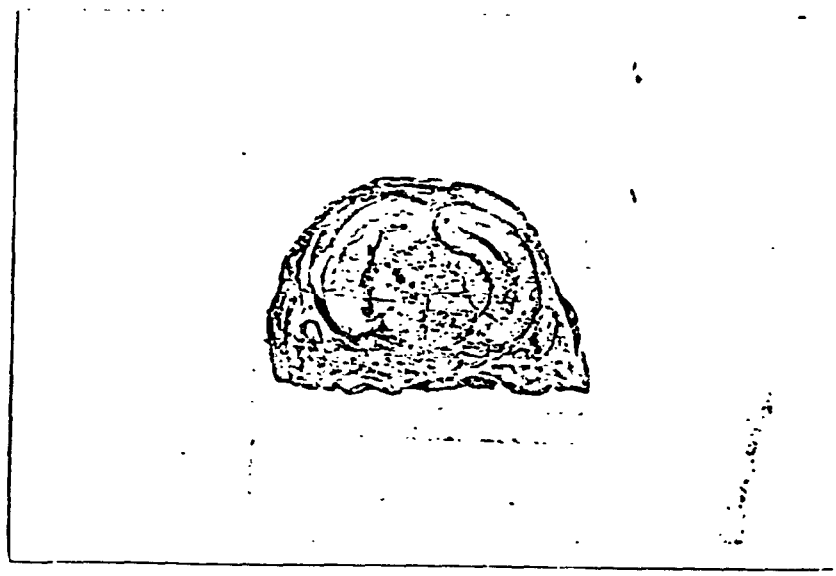

PLATE I-6 Skull and brain in control fetus; showing normally in the Wilson's section.

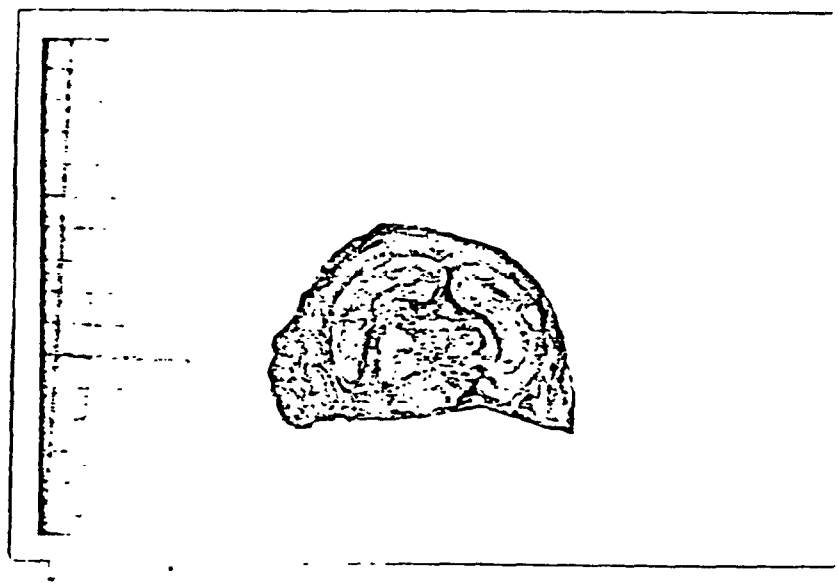

PLATE I-7 Skull and brain of fetus from the pregnant rat treated intraperitoneally with 5ml/kg of S-27.

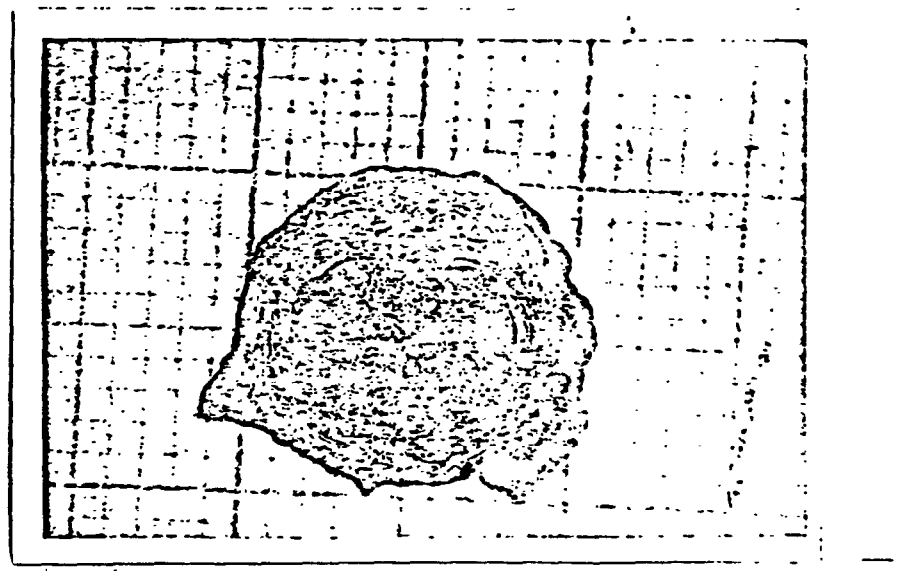

PLATE I-8 Cardiac ventricle and lung section of control fetus; showing normally in Wilson's section.

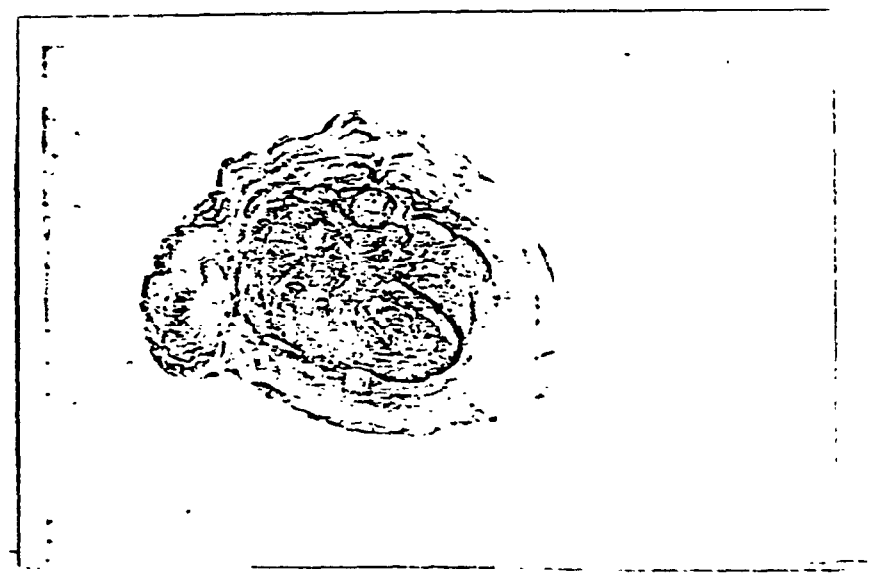

PLATE I-9 Cardiac ventricle, lung and heart of fetus from the pregnant rat treated intraperitoneally with 5ml/kg of S-27; showing normally in Wilson's section.

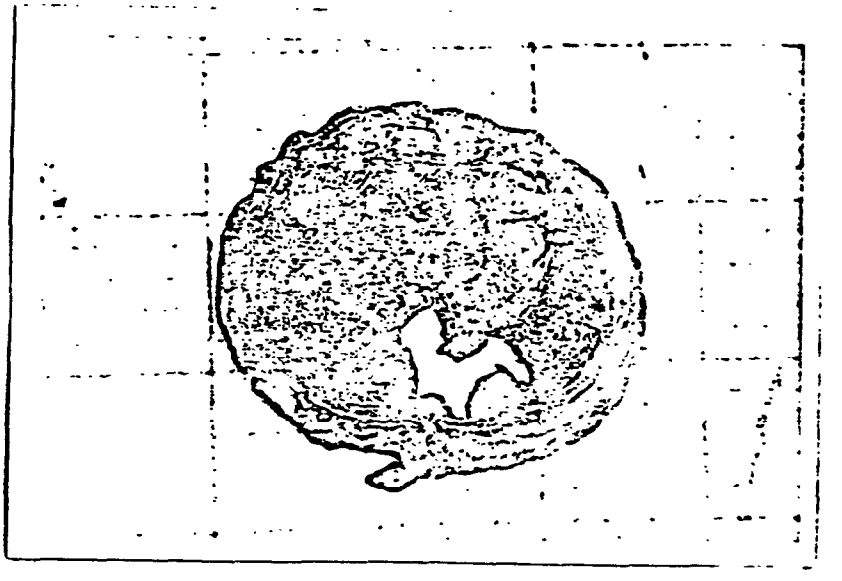

PLATE I-10 Liver ~~, kidney~~ and stomach of control fetus; showing normally in the Wilson's section.

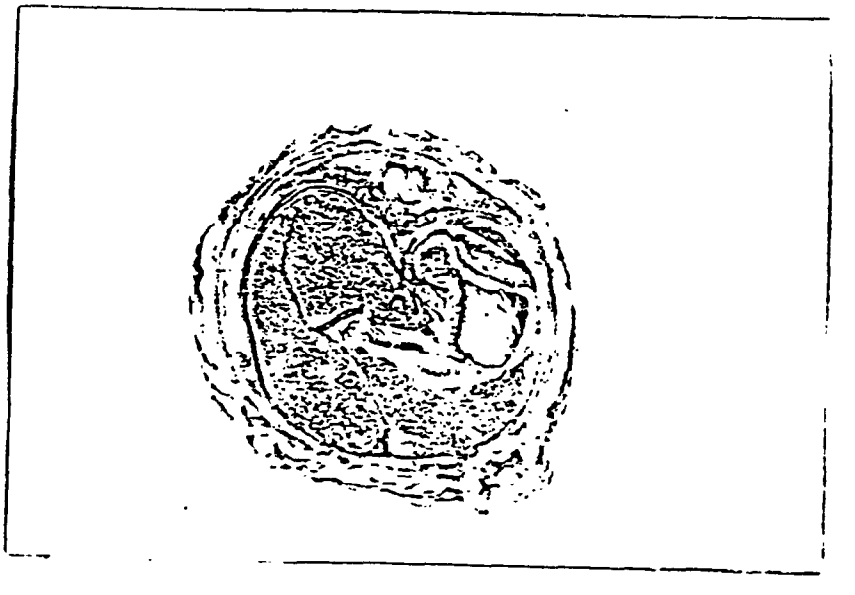

PLATE I-11 Liver and stomach of fetus from the pregnant rat treated intraperitoneally with 5ml/kg of S-27.

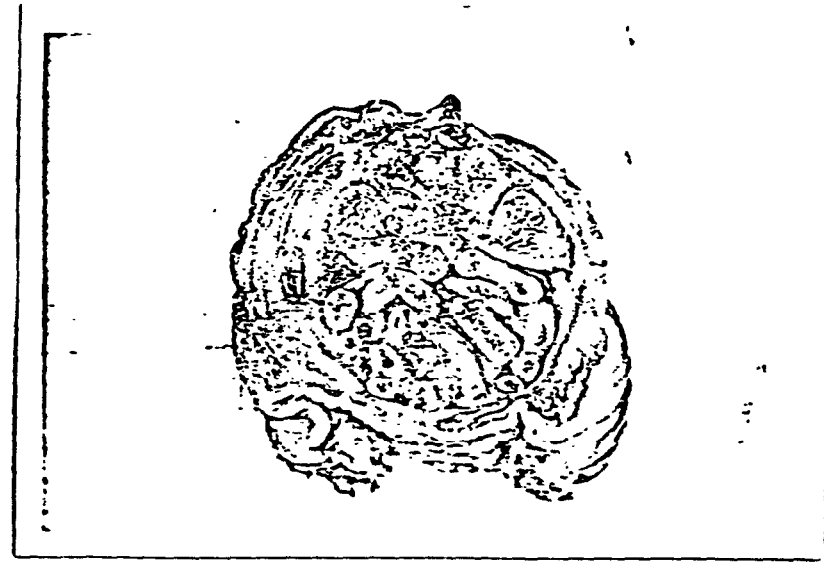

PLATE I-12 Kidney, intestine and spleen of control fetus; showing normally in the Wilson's section

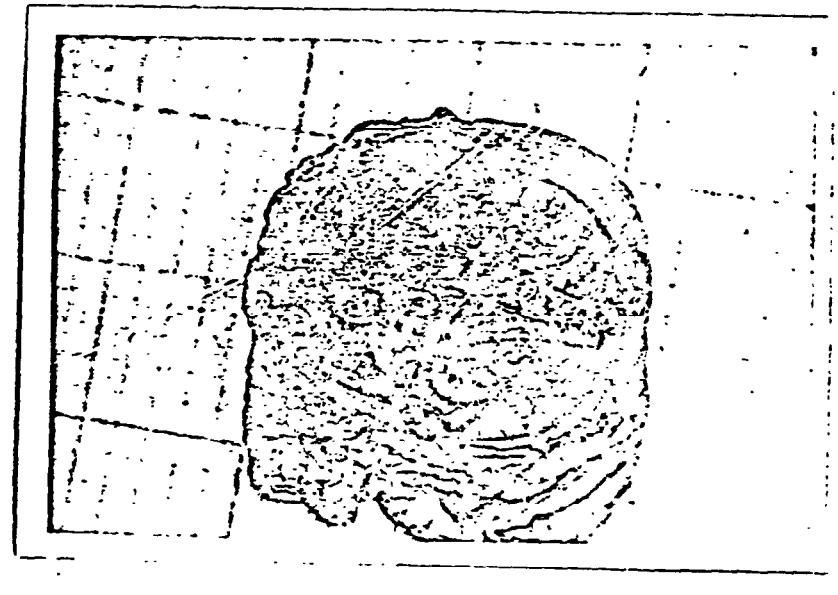

PLATE I-13 Kidney, testis and urinary bladder of control fetus; showing normally in the Wilson's section.

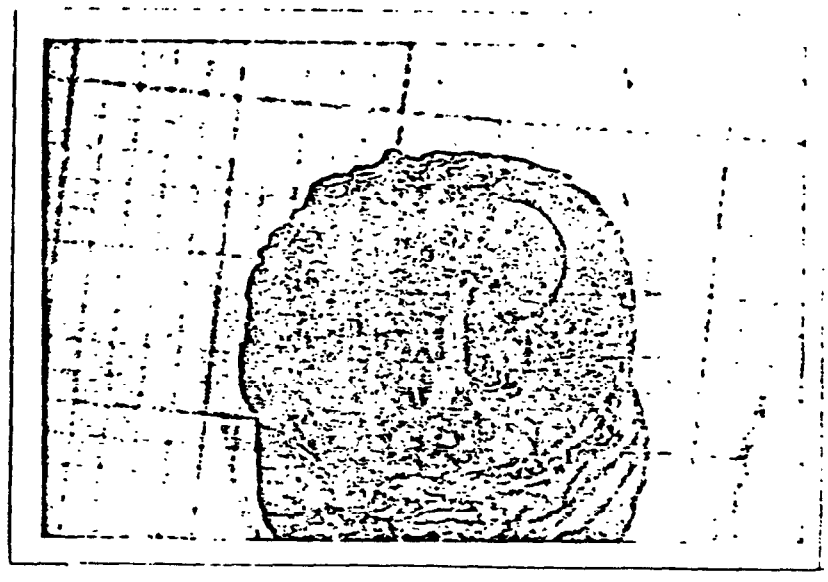

PLATE I-12 Kidney, intestine and spleen of control rabbit fetus; showing normally in the Wilson's section.

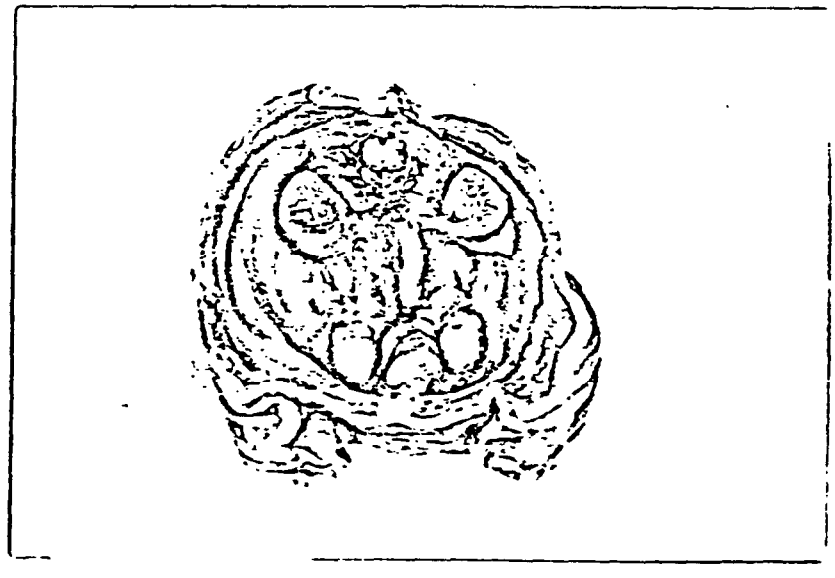

PLATE I-14 Kidney, testis and urinary bladder of fetus from the pregnant rat treated intra-peritneally with 5ml/kg of S-27; showing normally in the Wilson's section.

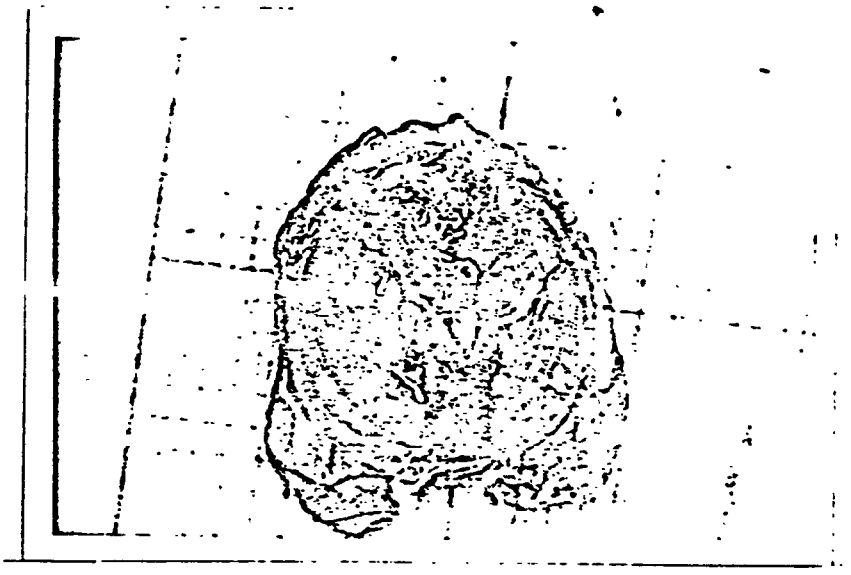

PLATE I-15 Kidney, ovary and uterus of control fetus; showing normally in the Wilson's section.

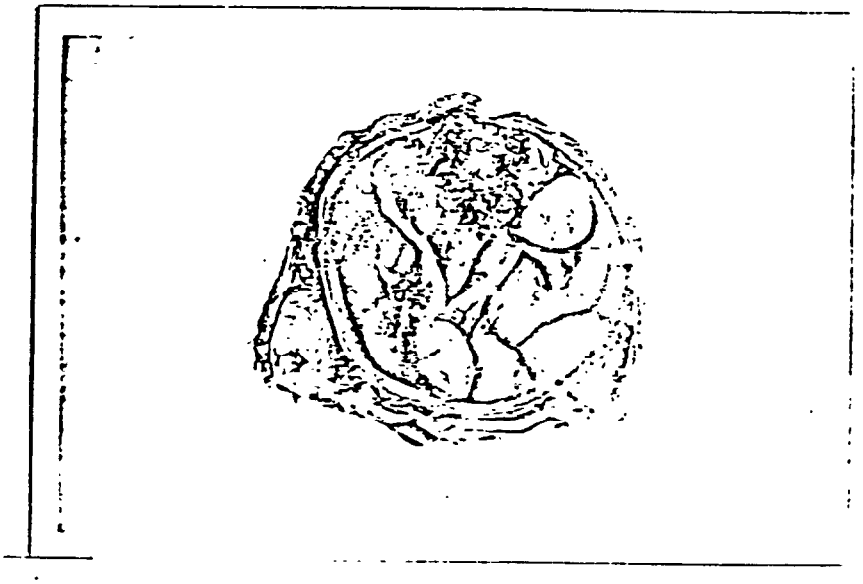

PLATE I-16 Kidney, ovary and uterus of fetus from the pregnant rat treated intraperitoneally with 5ml/kg of S-27; showing normally in the Wilson's section.

PHOTO-II

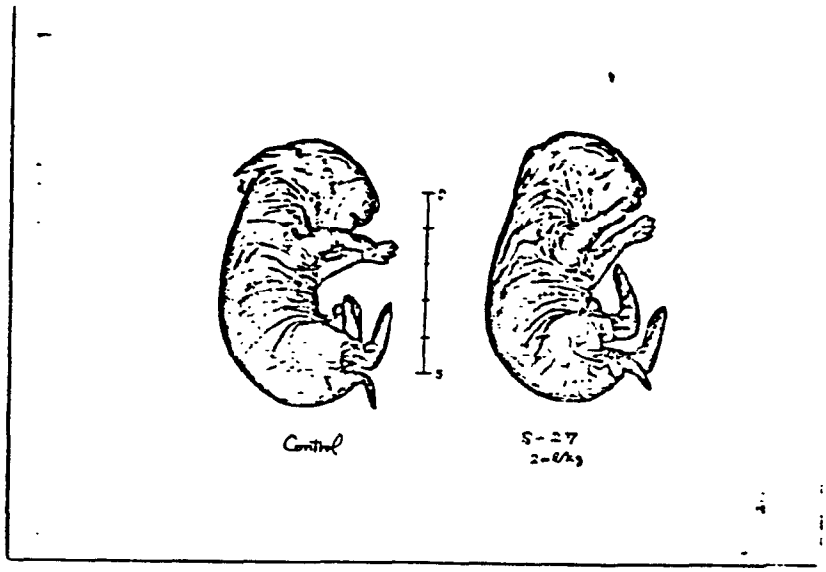

PLATE II-1 Left: A fetus from the pregnant rabbit. Right: A fetus from the pregnant rabbit treated intraperitoneally with 5ml/kg of S-27.

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

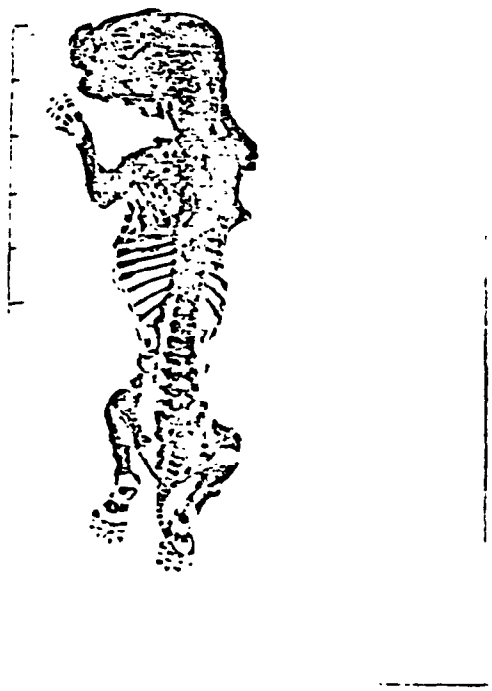

PLATE II-3 A normal skeleton of fetus from the pregnant rabbit treated intraperitoneally with 5ml/kg of S-27.

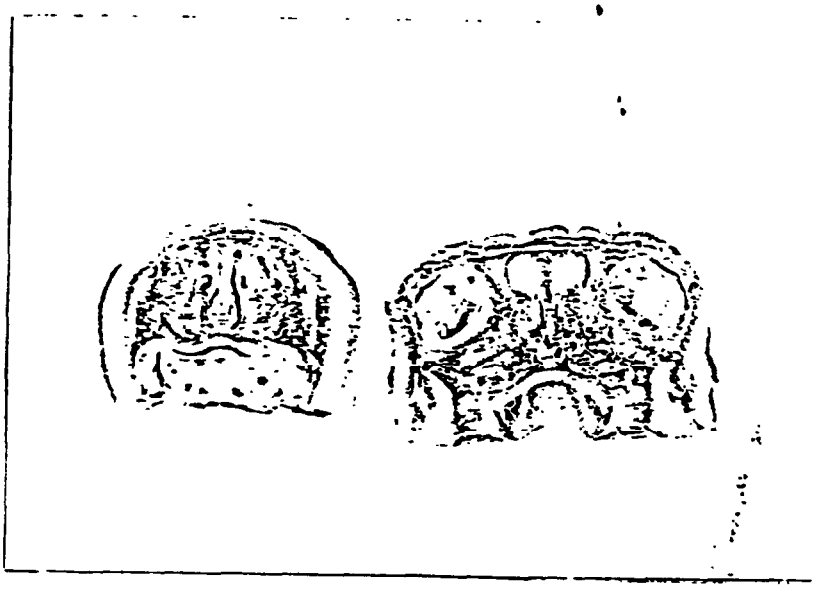

PLATE II-4 Palate and nasal cavities of control rabbit fetus; showing normally in the Wilson's section.

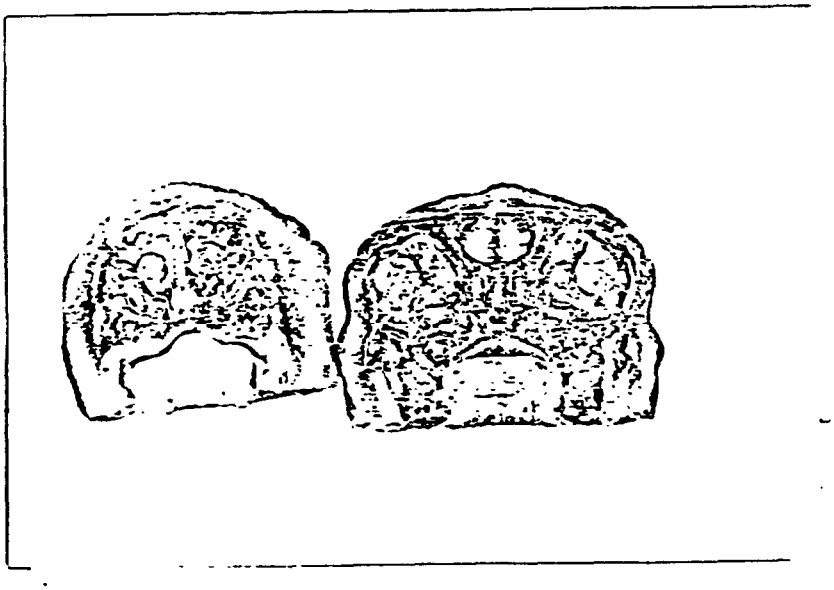

PLATE II-5 Palate, eyeballs and olfactory bulbs of fetus from the pregnant rabbit treated intraperitoneally with 5ml/kg of S-27; showing normally in the Wilson's section.

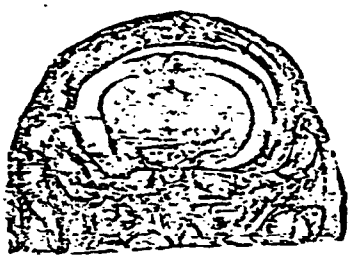

PLATE II-6 Skull and brain in control rabbit fetus; showing normally in the Wilson's section.

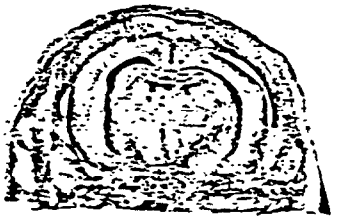

PLATE II-7 Skull and brain of fetus from the pregnant rabbit treated intraperitoneally with 5ml/kg of S-27.

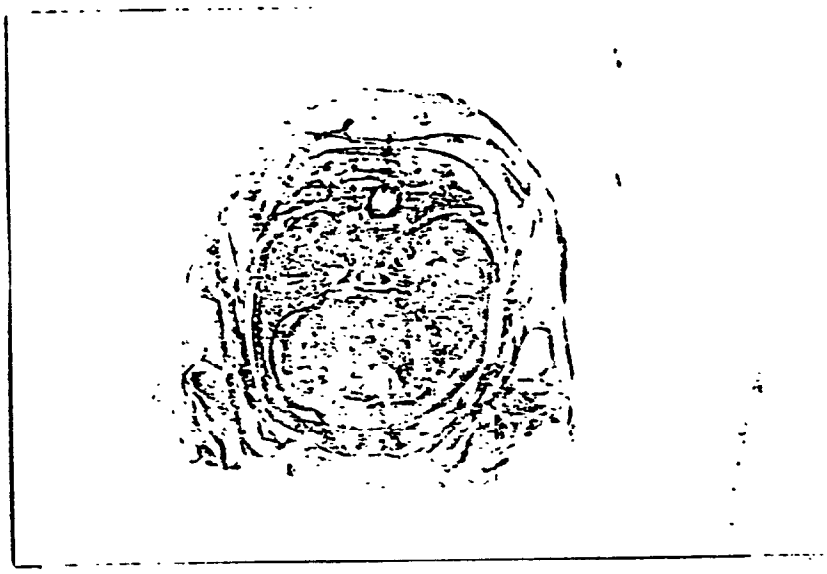

PLATE II-8 Cardiac ventricle and lung section of control rabbit fetus; showing normally in Wilson's section.

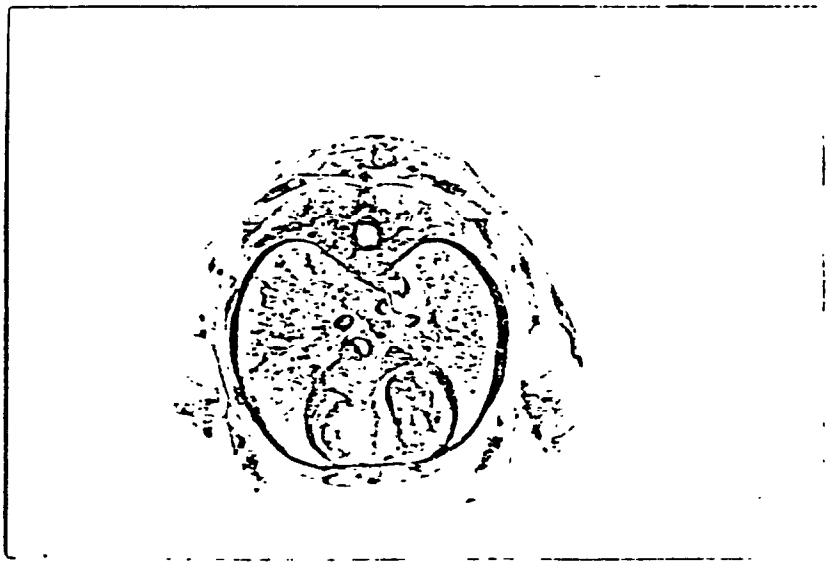

PLATE II-9 Cardiac ventricle, lung and heart of fetus from the pregnant rabbit treated intraperitoneally with 5ml/kg of S-27; showing normally in Wilson's section.

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

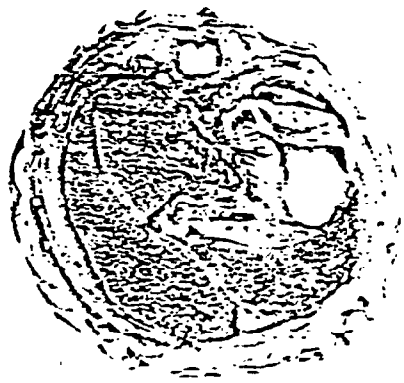

PLATE II-11 Liver and stomach of fetus from the pregnant rabbit treated intraperitoneally with 5ml/kg of S-27.

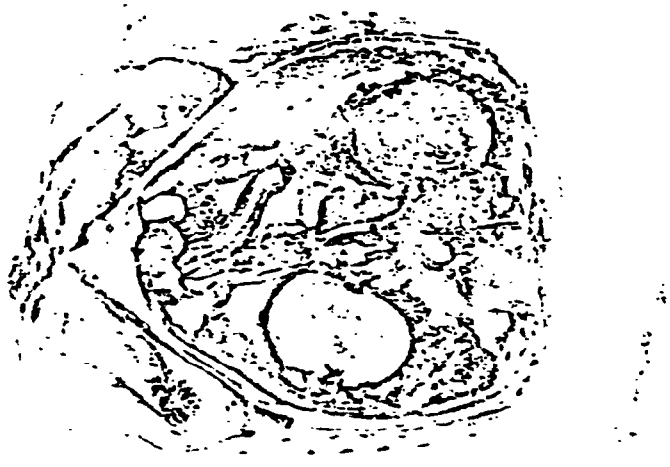

PLATE II-13 Kidney, testis and urinary bladder of control rabbit fetus; showing normally in the Wilson's section.

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.

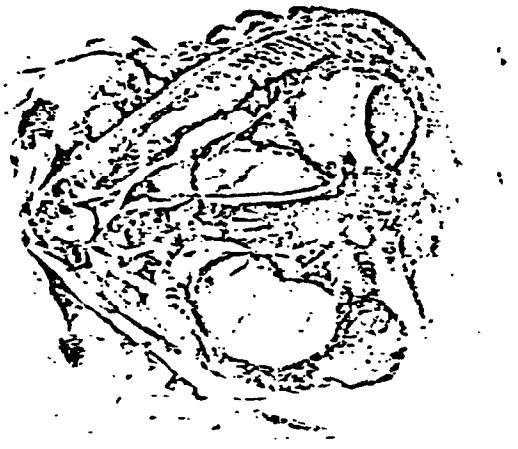

PLATE II-15 Kidney, ovary and uterus of control rabbit fetus; showing normally in the Wilson's section.

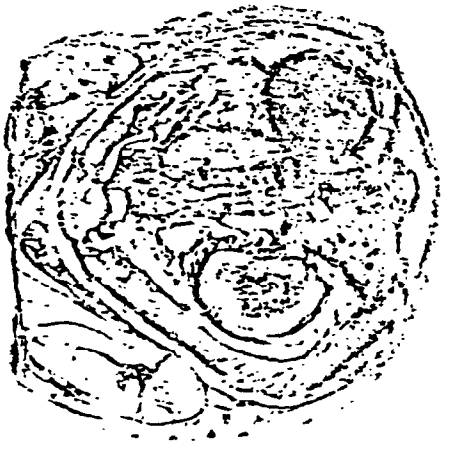

PLATE II-16 Kidney, ovary and uterus of fetus from the pregnant rabbit treated intraperitoneally with 5ml/kg of S-27; showing normally in the Wilson's section.

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 CO_2 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-C_3H 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 $\overline{C}$ SRBC	PFC/Culture	
		Treatment $\overline{C}$ -	Anti-Thy+C +
-	-	128 ± 40	156 ± 4
-	+	400 ± 16	N.D.
10	+	1260 ± 28	192
1	+	600 ± 24	216 ± 32
0.1	+	456	164 ± 20

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 $1x10^8$ viable organisms of Staphylococcus aureus (18Z) once a week for 4 or 8 weeks for the pre-treatment. For the mesurement 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, $1x10^6$ 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 assesed 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 administeration of SPL (0.1 ml) in normal and 18Z-pretreated mice. (mean of 5 ± S.D.)

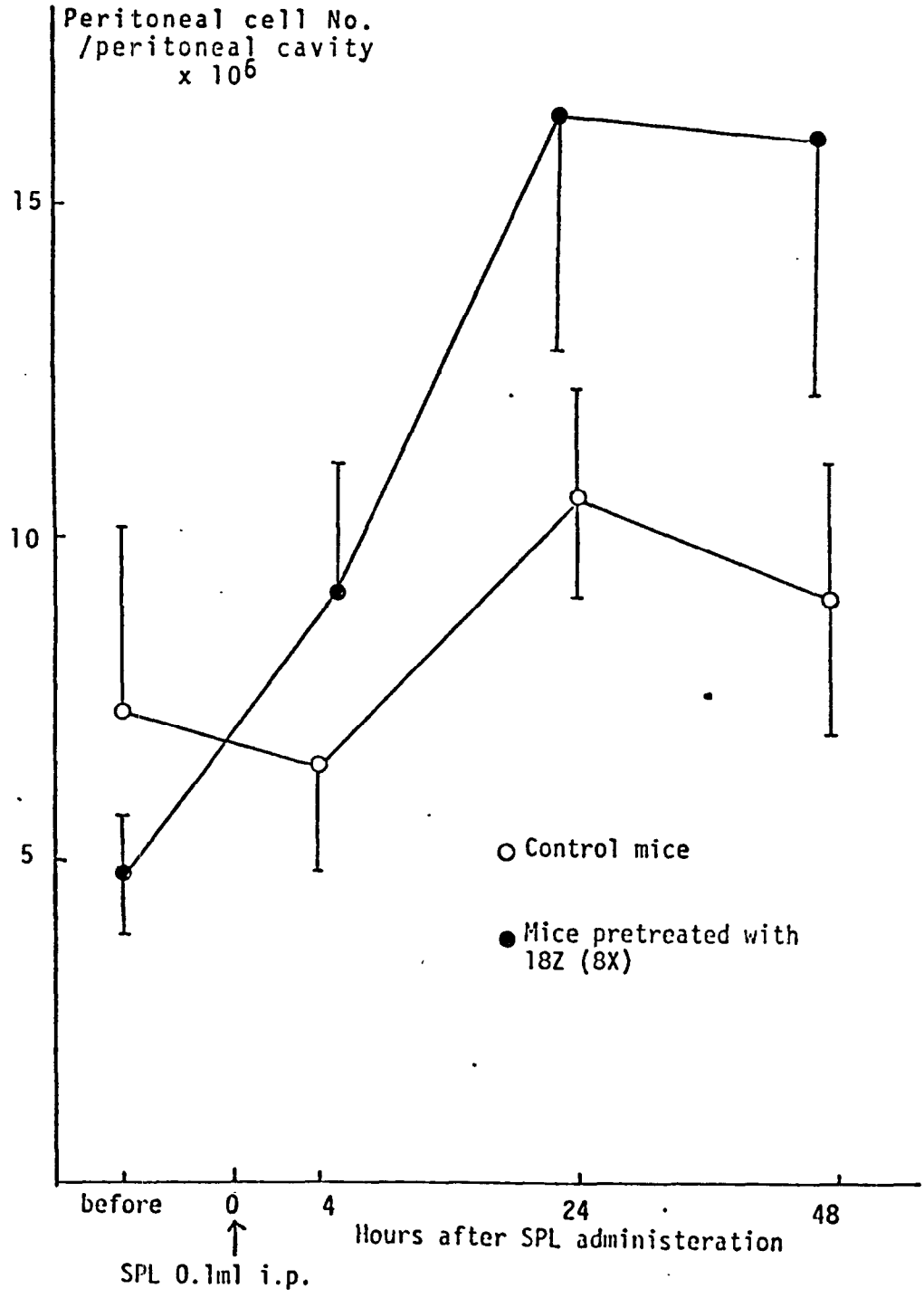

Table 1. Antitumor activity of SPL against Sarcoma 180 in ddY mice.

S.aureus (18Z)	Interval	Sarcoma 180 implantation (Cell No./body)	SPL (ml/body)	Volume of ascitic fluid	Total packed cell volume	Cell growth (T/C)	Activity
	weeks	i.p.	i.p.	ml	ml	%	
			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	-

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

S.aureus (18Z)	Interval	Sarcoma 180 implantation (Cell No./body)	SPL (ml/body)	Volume of ascitic fluid	Total packed cell volume	Cell growth (T/C)	Activity
	weeks	i.p.	i.p.	ml	ml	%	
			1.00	0	-	-	+++
$1x10^8$/body/week s.c. inj. x 8	6	$1x10^6$	0.30	0.75	0.27	33.8	++
			0.10	1.92	0.64	80.0	±
			0	2.30	0.80	100	-

B. HUMAN SAFETY DATA

PROFESSOR:
R. DOUGLAS THORNES, M.A., M.D., PH.D.

TELEPHONE DUBLIN 774967.

Royal College of Surgeons in Ireland,
Department of Experimental Medicine
Colman K. Byrnes Research Centre,
St. Laurence's Hospital,
North Brunswick St., Dublin 7.

6TH January, 1976

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

Dear Mr. Lincoln,

Thank you for your letter of the 20 December, 1976 regarding nebulizers.

In discussion with various members of our clinical staff we have come to the conclusion that nasal spray is of little value in getting substances into the lungs. This appears to be best accomplished by the oral route, the patient instructed to enhale rapidly so that some at least of the spray gets down into the bronchi.

You will by now have received a letter from me requesting further supplies of SPL and we look forward to receiving same. In the meantime I send you the results to date of our comparison of C.Parvum and Staphage on the absolute lymphocyte counts of patients. It is the absolute lymphocyte count that I feel is important and it should be above 1,500 per c.mm before any immunochemotherapy can be effective.

Looking forward to hearing from you. All the best for 1977.

Yours sincerely,

Douglas Thornes.

ENC.

C. PARVUM

'LIVE LYNCH	29.7.76	1.9.76	START 24.9.76	10.11.76	8.12.76					
LYMPH COUNT.	1431	1176	2112	2369	372					
'ARION FLYNN	13.10.76	START 10.11.76	1.12.76							
LYMPH COUNT	912	1098	399							
'GT. McDONALD	11.8.76	8.9.76	START 6.10.76	3.11.76	17.11.76	29.11.76	1.12.76	STOP 8.12.76		
LYMPH. COUNT	720	616	1276	463	672	1455	2451	2020		
TRICK CROTTY	7.9.76	21.9.76	START 7.10.76	14.10.76	21.10.76	28.10.76	4.11.76	STOP 11.11.76	23.11.76	
LYMPH COUNT	4331	5494	4399	3404	4032	3403	4557	3472	2964	

STAPHAGE

BRUNA BASINI	30.8.76	0.1 15.11.76	0.2 25.11.76					
LYMPH COUNT	1600	1080	2208					
ANNREE STANLEY	21.7.76	7.9.76	0.1 6.10.76	0.2 13.10.76	0.3 20.10.76	0.4 27.10.76		
LYMPH COUNT	1422	3074				4625		
GILLIAN FLEMING	10.6.76	20.9.76	0.1 15.11.76	0.2 24.11.76	1.12.76	0.3 8.12.76		
LYMPH COUNT	734	1520	1188	2448	1443	2968		
EDDIE LANLESS	1.6.76	0.1 13.10.76	0.2 21.10.76	0.3 27.10.76				
LYMPH COUNT	1092	1935	2780	2016				
NANCY LANLOR	18.8.76	17.11.76	0.1 24.11.76	0.2 1.12.76	0.3 8.12.76			
LYMPH COUNT	1134	1140		2444	2806			
PATRICK MOORE	1.11.76	0.1 8.11.76	0.2 17.11.76	7.12.76				
LYMPH COUNT	1230	1,127	1333	1364				

PROFESSOR:
R. DOUGLAS THORNES, M.A., M.D., PH.D.

TELEPHONE DUBLIN 721482

Royal College of Surgeons in Ireland.
Department of Experimental Medicine,
Colman K. Byrnes Research Centre,
St. Laurence's Hospital,
North Brunswick St., Dublin 7.

12th January, 1978

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

JAN 18 1978

Dear Mr. Lincoln,

Thank you for the continuing supply of Staphage. I hope we will require more very soon as we have now set up a protocol for recurrent operable melanoma. It is our intention to treat patients with Staphage 2 ml given intranasally weekly and 1 ml intradermally weekly for one month and then monthly for one year.

We have had problems with inhalation of Staphage. Various forms of vaporisers have been used. We now think that intranasal drops (provided the patient inhales very rapidly and exhales through the mouth) ~~that~~ a considerable amount of Staphage gets into the lungs and the remainder trickles down the back of the throat and so is the equivalent of oral medication. By using 2 mls we should ensure reasonable dosage. In addition to this the extra millilitre given intradermally in a minimum of five sites is about as much as we can expect the patients to tolerate.

One of the troubles of immunotherapy is the problem of attendance and follow-up at Outpatients. I understand that some centres, in fact, treat patients twice weekly. Most of our patients find it difficult to attend weekly and rapidly desire to come only once every month. I would very much appreciate your ideas on this dosage schedule. It is essential to establish a practical and workable programme.

Looking forward to hearing from you.

Kind regards,

Yours sincerely,

Douglas Thornes.

STAPHAGE REPORT

PATIENT'S NAME BRUNA BASINI DIAGNOSIS RECURRENT INFECTIONS

STATE OF DISEASE STATIC

DOSE OF STAPHAGE 1 ml in 5 sites I.D. weekly

CLINICAL RESPONSE TO STAPHAGE EXCELLENT

	DATE	HB.	WBC	ALC
Start	15.11.76	12.6	4.5	1080
Stop	25.11.76	13.0	6.9	2208

COMMENT

Dramatic subjective improvement. Patient subsequently developed no further infections.

STAPHAGE REPORT

PATIENT'S NAME FLORENCE CAMPBELL DIAGNOSIS CARCINOMA OF THE BREAST STAGE I ANERGY

STATE OF DISEASE IN REMISSION

DOSE OF STAPHAGE Initially 1 ml in 5 sites intradermally followed by oral 1 ml weekly

CLINICAL RESPONSE TO STAPHAGE Not been evaluated.

	DATE	HB.	WBC	ALC
Start	22.3.77	12.7	4.3	1118
	26. 4.77	13.0	7.1	2201
	20.7. 77	12.0	4.4	616
Oral	12.10.77	12.3	5.5	1320
	19.10.77			
	26.10.77			
	3.11.77			

COMMENT

This patient has a low absolute lymphocyte count response to Staphage therapy. Use here prophylactics against recurrence.

STAPHAGE REPORT

PATIENT'S NAME NORA O'DEA DIAGNOSIS LYMPHOSARCOMA

STATE OF DISEASE ADVANCED AND NOT RESPONDING TO THERAPY

DOSE OF STAPHAGE 1 ml in 5 sites I.D. weekly

CLINICAL RESPONSE TO STAPHAGE: FURTHER EXACERBATION

	DATE	HB.	WBC	ALC
Start	9. 2.77	14.5	5.6	1848
	16.3.77	14.8	4.7	1833
	20.4.77	14.1	4.3	1634
Stop	25.5.77	14.2	3.5	1015

COMMENT

Possible enhancement of tumour growth. Patient, however, subsequently responded again to chemotherapy.

STAPHAGE REPORT

PATIENT'S NAME GWEN BORROWES DIAGNOSIS RECURRENT INFECTIONS, ANERGY

STATE OF DISEASE STATIC

DOSE OF STAPHAGE 1 ml in 5 sites I.D. weekly

CLINICAL RESPONSE TO STAPHAGE: Nil

	DATE	HB.	WBC	ALC
Start	8. 2.77	13.8	5.1	2601
	1. 3.77	14.1	4.4	2456
	16.3.77	14.8	5.1	3519
Stop	13.4.77	14.9	4.6	2852

COMMENT

No apparent effect of Staphage

STAPHAGE REPORT

PATIENT'S NAME MAIREAD MUCKIAN DIAGNOSIS HODGKIN'S DISEASE, STAGE IVB.

STATE OF DISEASE

DOSE OF STAPHAGE 1 ml in 5 sites I.D. weekly

CLINICAL RESPONSE TO STAPHAGE: Exacerbation of disease was not responding to chemotherapy. Staphage started and patient subsequently responded

	DATE	HB.	WBC	ALC
Start	22.12.76	14.1	13.0	
	19. 1.77	12.2	5.4	864
	16. 2.77	13.4	6.4	2560
Stop	13.4.77	14.4	4.5	585

COMMENT

Response to chemotherapy following course of Staphage

STAPHAGE REPORT

PATIENT'S NAME ALICE MOHAN DIAGNOSIS CARCINOMA OF THE BREAST, STAGEIV

STATE OF DISEASE ADVANCED

DOSE OF STAPHAGE 1 ml in 5 sites I.D. weekly

CLINICAL RESPONSE TO STAPHAGE NIL. Subsequent response to chemotherapy.

	DATE	HB.	WBC	ALC
Start	6. 4.77	13.0	5.0	1150
	4. 5.77	14.0	2.6	
	11. 5.77	13.6	3.6	648
Stop	18. 5.77	13.2	4.1	656

COMMENT

No obvious response to Staphage but patient subsequently responded to chemotherapy. Prior to Staphage failed to respond. Treatment was stopped because of a rash.

STAPHAGE REPORT

PATIENT'S NAME ANTHONY HEANEY DIAGNOSIS MELANOMA

STATE OF DISEASE DISSEMINATED LUNG SECONDARIES, SKIN SECONDARIES

DOSE OF STAPHAGE 1 ml in 5 sites I.D. weekly later combined with inhalation of 1 ml w

CLINICAL RESPONSE TO STAPHAGE: DRAMATIC WITH COMPLETE REGRESSION OF SKIN SECONDARIES. LUNG SECONDARIES SLOWLY DECREASING

		DATE	HB.	WBC	ALC
	Start	9.3.77	17.3	7.2	3999
		18.4.77			
		4.5.77	18.1	8.0	3520
		18.5.77			
	Stop	25.5.77	16.9	9.2	5612
Oral	Start	1.6.77			
		22.6.77	16.3	7.7	2079
		29.6.77			
		13.7.77			
		10.8.77	15.8	7.6	2280
Oral + I.D.		7.9.77	16.5	6.9	
		21.9.77			
		28.9.77			
		5.10.77	16.1	8.1	2754

COMMENT

This patient was maintained on Levamisole and Warfarin. He developed exacerbation which responded to the introduction of Staphage. Treatment of Staphage continuing by inhalation only.

STAPHAGE REPORT

PATIENT'S NAME CARMEL MCQUAILE DIAGNOSIS CARCINOMA OF BREAST STAGE IV

STATE OF DISEASE ADVANCED

DOSE OF STAPHAGE 1 ml in 5 sites I.D. weekly

CLINICAL RESPONSE TO STAPHAGE EQUIVOCAL

	DATE	HB.	WBC	ALC
Start	22. 1.77	13.1	3.4	
	9. 2.77	12.6	3.3	1054
	16. 2.77	12.8	7.7	3080
	9. 3.77	12.8	5.3	1802
	4. 5.77	11.4	3.8	646
Stop	18. 5.77	12.3	5.3	1378

COMMENT

The effect of Staphage was not maintained after initial apparent improvement in the absolute lymphocyte count. This patient subsequently responded well to Hyperthermia and further chemotherapy

STAPHAGE REPORT

PATIENT'S NAME JEAN FAGAN DIAGNOSIS CARCINOMA OF THE BREAST STAGE IV

STATE OF DISEASE Advanced

DOSE OF STAPHAGE 1 ml in 5 sites Intradermally weekly.

CLINICAL RESPONSE TO STAPHAGE: NIL

	DATE	HB.	WBC	ALC
START	12. 1.77	12.6	7.4	3182
	9. 2.77	12.6	7.4	3182
	8. 3.77	11.4	7.0	1960
	5.4.77	12.3	8.3	3237
	3. 5.77	11.6	6.9	1311
STOP	17. 5.77	12.7	8.8	2352

COMMENT

Staphage may have helped to keep disease static. Wellbeing of patient was improved considerably. Unfortunately rash developed. This patient was subsequently treated with Thymosin and severe rash developed.

STAPHAGE REPORT

PATIENT'S NAME J.TERRITT DIAGNOSIS LYMPHOSARCOMA

STATE OF DISEASE ADVANCED

DOSE OF STAPHAGE 1 ml in 5 sites I.D.weekly

CLINICAL RESPONSE TO STAPHAGE: Further exacerbation

	DATE	HB.	WBC	ALC
START	19. 1.77	14.7	7.3	
STOP	9. 2.77	13.8	5.5	1155

COMMENT

Staphage in this case did not produce any benefit and may have increased the growth rate.

STAPHAGE REPORT

PATIENT'S NAME FRANCIS GUNN DIAGNOSIS CHRONIC MONONUCLEOSIS

STATE OF DISEASE CHRONIC

DOSE OF STAPHAGE 1 ml in 5 sites I.D. weekly

CLINICAL RESPONSE TO STAPHAGE NIL

	DATE	HB.	WBC	ALC
START	7. 2.77		4.8	3121
	30.3.77	16.6	6.3	3,150
	12. 4.77	12.6	5.3	2,173
STOP	3. 5.77	14.2	5.6	2,576

COMMENT

After two months patient developed rash which subsided when Staphage stopped.

STAPHAGE REPORT

PATIENT'S NAME ELIZABETH MAXWELL DIAGNOSIS HODGKIN'SDISEASE

STATE OF DISEASE CHRONIC

DOSE OF STAPHAGE 1 ml in 5 sites intradermally weekly

CLINICAL RESPONSE TO STAPHAGE NIL

	DATE	HB.	WBC	ALC	
START	11. 1.77	12.6	4.4	1496	start
	8. 2.77	12.9	6.4	2112	
STOP	12. 4.77	12.2	5.4	1512	

COMMENT

No demonstrably subjective effect. Increase in absolute lymphocyte count.

STAPHAGE REPORT

PATIENT'S NAME MRS. ANREE STANLEY DIAGNOSIS TOXOPLASMOSIS WITH ANERGY

STATE OF DISEASE STATIC

DOSE OF STAPHAGE 1 ml in 5 sites I.D. weekly

CLINICAL RESPONSE TO STAPHAGE NIL

	DATE	HB.	WBC	ALC	
START	15.2.77	14.2	9.5	3,800	start
	23. 3.77	15.0	9.7	2,910	
STOP	25. 4.77	15.4	6.3	2078	stop

COMMENT

No effect.

STAPHAGE REPORT

PATIENT'S NAME GILLIAN FLEMING DIAGNOSIS ANERGY, COLLAGEN DISEASE

STATE OF DISEASE STATIC

DOSE OF STAPHAGE 1 ml in 5 sites I.D. weekly

CLINICAL RESPONSE TO STAPHAGE MARKED SUBJECTIVE IMPROVEMENT

	DATE	HB.	WBC	ALC
START	15.11.76	14.6	3.3	1118
	24.11.76	13.8	4.8	2448
	1.12.76	14.2	3.9	1443
	12. 1.77	14.0	4.5	2430
	2. 2.77	13.2	5.7	2850
	29.3.77	12.9	5.3	2279
STOP	9. 5.77	14.2	5.0	2350

COMMENT

An excellent subjective result after Levamisole and proteolytic enzymes had failed to produce any permanent result.

STAPHAGE REPORT

PATIENT'S NAME NANCY LAWLOR DIAGNOSIS CARCINOMA OF BREAST,STAGE IV

STATE OF DISEASE Very advanced

DOSE OF STAPHAGE 1 ml in 5 sites I.B. weekly

CLINICAL RESPONSE TO STAPHAGE Not assessable. No immediate improvement

	DATE	HB.	WBC	ALC	
START	17.11.76	14.3	3.00	1140	start
	1.12.76	14.9	9.2	2444	
STOP	8.12.76	13.0	6.1	2806	stop

COMMENT

Rise in absolute lymphocyte count. Otherwise not assessable.

STAPHAGE REPORT

PATIENT'S NAME PATRICK MOORE DIAGNOSIS HODGKIN'S DISEASE, STAGE IVb

STATE OF DISEASE Exacerbation following MOPP chemotherapy

DOSE OF STAPHAGE 1 ml in 5 sites intradermally weekly

CLINICAL RESPONSE TO STAPHAGE Further exacerbation of disease followed by response to chemotherapy after one week. Staphage only

	DATE	HB.	WBC	ALC	
START	8.11.76	16.0	4.9	1127	start
STOP	17.11.76	14.5	4.3	1333	stopped
START	27. 7.77	13.6	7.3	1022	Started, 1 ml in 5 sites intradermally given weekly 4 weeks. Increased to 1 ml intradermally; 1 ml by inhalation for six weeks. Clinical response good, disease stabilised. Respons. to Coumarin therapy
	4. 7.77				
	11. 8.77				
	18. 8.77	13.5	7.3	584	
	25. 8.77	14.0	7.5	300	
	31. 8.77				
	7. 9.77	13.6	6.4	1344	
	15. 9.77	13.7	5.8	1508	
	5.10.77	13.4	6.6	1392	
	19.10.77				
STOP	26.10.77	14.1	7.2	1800	

COMMENT.

Apparent exacerbation from first course but subsequent response to chemotherapy. One year later following three courses of MOPP response stabilised.

STAPHAGE REPORT

PATIENT'S NAME EDWARD LAWLESS DIAGNOSIS HODGKIN'S DISEASE STAGE IV

STATE OF DISEASE Relapse following chemotherapy

DOSE OF STAPHAGE 1 ml in 5 sites weekly

CLINICAL RESPONSE TO STAPHAGE: Patient again responded to chemotherapy but it could be stopped on the 6.11.76

	DATE	HB.	WBC	ALC	
START	1. 9.76	14.4	5.7	1938	Start
	13.10.76	14.1	3.5		
	21.10.76	13.6	4.1	2780	
STOP	27.10.76	12.8	4.8	2016	Stopped

COMMENT

Patient with advanced disease responded again to chemotherapy (Staphage therapy)

RECEIVED FEB 1 1978

CLEVELAND CLINIC

THE CLINIC CENTER • 9500 EUCLID AVENUE, CLEVELAND, OHIO 44106, U.S.A. • 216/444-2200 • CABLE CLEVCLINIC CLV.
DEPARTMENT OF COLON AND RECTAL SURGERY
Victor W. Fazio, M.D.
David G. Jagelman, M.D.
Rupert B. Turnbull, Jr., M.D.
Frank L. Weakley, M.D.

To Whom It may concern

The Colon-Rectal Surgery Department of the Cleveland Clinic has recently completed a study of 56 Crohn's patients evaluating the efficacy of Staphage Lysate therapy in patients with persistent complications of Crohn's disease refractory to others modes of patient management. Presently we are in the process of analysis the immunological data and correlating it with the clinical response. The study design is outlined in the attached protocal. The final report will be presented in symposium form in June at the Cleveland Clinic.

Sincerely,

Patricia J. Blair

Patricia J. Blair, M.D.
Research Fellow
Colon-Rectal Surgery

PJB/es

RESUME

Name: Patricia Joan Blair
Address: 12000 Fairhill Road, Cleveland Ohio, 44120
Date of Birth: June 19, 1945
Age: 32
Marital Status: Single Place of Birth: Kearns, Utah

Career Goals: Colon-Rectal Surgeon with Research Interests in Tumor Immunology and Computer Application to Medical Problems.

Thesis: Correlation of Electronic Structure and Carcinogenic Activity of 3,4 Benzopyrene and 1,2; 5,6 Benz(a)anthracene.

Education Background:

School	Location	Dates of Attendance	Degree
Rolla High School	Rolla, Mo.	1959-1963	1963
University of Missouri-Rolla	Rolla, Mo. " "	1963-1965 1967-1969	B.S. Chem. Minor Physics

Interests - Math, Science
Phi Kappa Phi-Honor Society; Tau Beta Pi, (Engineering Honor Society); Society of Women Engineers and Scientists; American Chemical Society; Chemical Society of London; Dean's List; Curator's Scholarship.

University of California at Los Angeles	1965-66	None

Interests - Curator's Scholar; Dean's List

University of Missouri Rolla	1969-1970	MS. Theoretical Chemistry Minor Physics

Interests - Dean's List; American Physics Society

University of Missouri School of Medicine Columbia, Missouri	1971-1975	M.D.

Interests - March of Dimes Scholar; American Business Women's Scholarship.

New York University School of Medicine	1975-1976	Internship in general surg
United Kingdom International Scholar	6/76-1/77	Research in Asp of Inflammatory Bowel Disease w Dr. John Goligh Leeds, England

Work Experience:

University of Missouri Rolla	1963-1965 1967-1969	Research Assistant Teaching Assistant
University of California at Los Angeles	1966-67	Laboratory Technician Kidney Transplantion Tissue Typing
William O'Neil and Company (stock broker)	1965-66	Computer Analyst and Programmer
University of Missouri Rolla	1969-70	Graduate Research and Teaching Assistant
University of Missouri School of Medicine Columbia	1971(summer) 1972 (June)	Biochemical Res. Asst. Information Science Research Assistant
New York University School of Medicine	1975-76	Assistant Instructor General Surgery

References:

Dr. Donald A. Bestiel, Assistant Professor, Department of Chemistry (Thesis Advisor) University of Missouri, Rolla, Missouri 65401.
Dr. Dudley Thompson, Dean and former Chairman, Department of Chemistr University of Missouri, Rolla, Missouri 65401.
Dr. Paul Teraski, Professor, Department of Surgery UCLA, Westwood, California,
Dr. Ed Deland, Assistant Professor, Department of Thoracic Surgery UCLA, Department of Surgery and RAND consultant, Westwood, California.
Dr. Donald Lindberg, Chairman Department of Information Science, University of Missouri-School of Medicine Columbia, Missouri 65201.
Dr. Mitchel Rosenholtz, Assistant Dean of Students, University of Missouri School of Medicine, Columbia, Missouri 65201
Dr. Frank Cole Spencer, Chairman Department of Surgery, New York University, New York, N.Y. 10016
Dr. Joseph Ransonhoff, Chairman Department of Neurosurgery, New York University, N.Y., N.Y. 10016.
Dr. Rupert Turnbull, Senior Surgeon, Department of Colon-Rectal Surgery, Cleveland Clinic Foundation

Meetings Attended and Papers Presented:

1. American Chemical Society, Midwestern Section, St. Louis, Missouri, Oct. 1969, "Correlation of Electronic Structure and Nuclear Magnetic Resonance of Mono-and di-substituted Fluorobenzenes".

Cleveland Clinic Foundation Department of Colon-Rectal Surgery	1/77-Present	Research Fellow
Case Western Reserve School of Medicine Department of Biometry (Applications of Computers in Medicine)	1/77-Present	National Library of Medicine Research Fello

Meetings Attended and Papers Presented: (Cont'd.)

2. American Chemical Society-Midwestern Section, Columbia Missouri, May 1970, "Correlation of Electronic Structure and Carcenogenic Activity of 3,4 Benz(o)pyrene".

3. Medical Student Research Honor's Program University of Missouri School of Medicine, May 1971, Columbia, Missouri, "Electronic Structure Interaction of 3,4 Benz(o) Pyrene and Isolated Nucleic Acids".

4. Theorectical Biophysics Conference, New Orleans, Louisiana, May, 1972, "Electronic Structure of Helical DNA and Theoretical Interaction with 3,4 Benz(o) Pyrene"

5. American Society of Clinical Laboratory Technicians, St. Louis, Missouri, June 1974, "Use of Anion Gap in Detection of Lab Errors".

6. American Association of Liver Diseases (Slide Workshop), Chicago, Illinois, Oct. 1974, "Liver Manifestation of Alpha, Antitrypsin Deficiency.

7. Medical Student Research Honor's Program, Columbia, Missouri, May 1975, "Computer Model of Fluid and Electolyte Balance in the critically ill patients."

8. Nutritional Care of the Criticially Ill, Glasgow, Scotland November 1976," Interaction of Hyperalimentation and the immune system of the criticall ill surgery patient."

9. Participate in the "Inflammatory bowel Workshop", Tarrytown New York, October 1977, "Patient Evaluation using longitudinal immunoprofile data"

10. Second Annual New Mexico-Australia Immunology Symposium, "HLA types in Crohn's Disease associated with anklosing Spondylitis", January 1978

A TRIAL OF STAPHAGE LYSATE

AS ADJUNCTIVE THERAPY

IN

PATIENTS WITH CROHN'S DISEASE

Prepared by: Patricia J. Blair, M.D.
Research Fellow
Department of
Colon and Rectal Surgery

Under the
direction of: Rupert B. Turnbull, Jr., M.D., C.M., F.A.C.S.
Senior Surgeon
Department of Colon and Rectal Surgery
Cleveland Clinic Foundation
Cleveland, Ohio

TABLE OF CONTENTS

INTRODUCTION . . . . . 1 - 2

Over-all Objectives. . . . . 3 - 4

Staphage Lysate:
Preparation . . . . . 5 - 5A
Legality of Usage . . . . . 6 - 7

Study Design . . . . . 8 - 10

Immunologic Profile. . . . . 10 - 12

Fat Absorption Profile 13

Documentation of Disease: Extent and Severity . . . . . 14 - 16

Follow up. 17

Treatment Program. . . . . 17 - 18

Monitor of Toxicity Reaction 18

Data Analysis. . . . . 19 - 20

Personnel Involved in Project . . . . . 21 - 22

Bibliography . . . . . 23 - 25

APPENDIX (TABLE I) Summary of Immunologic Aspect of Crohn's Disease, as per Inflammatory Bowel Disease, Kirsner and Shorter.

Package Insert

Criteria of Diagnosis of Crohn's Disease from the National Cooperative Crohn's Disease Study

Immunoprofile (Sample Sheet)

Basic Data (Summary Sheet)

Patient Evaluation (Sample Sheet)

* * * *

INTRODUCTION

Patients with the complications of Crohn's disease unresponsive to the traditional forms of therapy including hyperalimentation, surgery, medical management with Azulfidine and Prednisone, and ACTH, become disabled and unable to continue in their previous professions and to enjoy a satisfactory quality of life. Currently, there is little available to this group, often very young individual's expect a life of prolonged pain and suffering. Thus these unmanageable complications, although not life-threatening, presents major problems to the patient.

A detailed discussion of the voluminous literature on the immunologic aspects of Crohn's disease and inflammatory bowel disease is beyond the scope of this paper and has been recently reviewed in a comprehensive, excellent manner by Kirsner and Shorter.[1] A brief summary table of major results are present in Table I of the appendix.

The Cleveland Clinic Foundation has a uniquely large Crohn's patient population which manifest a wide variety of the complications associated with Crohn's disease. Many of the immunologic studies on Crohn's disease have been incomplete and productive of confusing data, because of small patient population and lack of clearly defined criteria for establishing diagnosis of Crohn's. The Cleveland Clinic Foundation is ideal for a comprehensive study of the immunologic status of Crohn's patients because

of (1) high volume of Crohn's patients with each of the complications to be studied, (2) experienced personnel familiar with Crohn's disease in the Departments of Colon and Rectal Surgery, Gastroenterology, Pathology, Immunopathology, Radiology, Pediatrics, and Research Lipid Chemistry. Thus it seems appropriate to attempt a definitive study of the immunology of Crohn's disease at this institution utilizing our unique facilities, patient population, and scientific expertise.

OVERALL OBJECTIVES:

This report deals mainly with the first of a two-phase project concerning the immunology of Crohn's disease. Phase I is a pilot trial of staphage lysate as an adjunctive form of therapy for the complications of Crohn's disease. The three-fold purpose of this pilot trial is to:

(1) Define the types of complications (i.e. patient groups) responsive to staphage lysate therapy.

(2) Define the changes in the immunoprofile which reflect the clinical changes in the patient.

(3) Design a controlled clinical trial based on the results of the pilot study randomizing staphage lysate and Azulfidine-Prednisone in active, acute cases, and randomizing staphage lysate and Azulfidine-Prednisone, and placebo in active, nonacute cases.

This trial will be designed with the help of Dr. H. B. Houser, Chairman of the Department of Biometry, Case-Western Reserve, and will be so designed that the data will be comparable to the adjunctive therapy trial recently concluded by the National Crohn's Cooperative Study Group.

Phase II of the project will involve a three-fold assessment of the immune status of the surgical Crohn's patient — (1) preoperatively and postoperatively by using the immunoprofile and data analysis technique developed in Phase I of this project, (2) intraoperatively will be determined by immune studies (T and B cell count, mitogen screens, et cetera) on blood entering a disease segment of bowel (via the superior mesenteric artery) and leaving the diseased segment of bowel (via the inferior mesenteric vein) and by conducting T and B cell count and mitogen screens on the involved

lymph nodes and, (3) finally a controlled clinical trial will be designed focusing on those complications of Crohn's disease which responsed best to immune therapy during Phase I . Further elaborations of Phase II of this project will be presented in additional reports.

Staphage Lysate was selected as the immune stimulator for evaluation based on its proven success in a select group of Crohn's patients with unhealed posterior wounds and enterocutaneous fistulas. Each of these patients were treated by Dr. Dale Rank and each had received prior conventional therapy without response.[2]

The comprehensive immunoprofile has been devised with the assistance of Dr. Georges Mathe[3] and Dr. S. Deodhar [4] for the purpose of monitoring the use of staphage lysate as a immune stimulator.

The following sections present the background of staphage lysate and the study design of this project.

STAPHAGE LYSATE - PREPARATION:

Staphage lysate (SPL) is prepared by lysing parent cultures of S. aureus of the Sterotypes I and III of the International Types of Cowan with a staphlococcus bacteriophage of the Gratia strain. To maintain maximum immunologic potency, no preservation is added. Each lot is tested for safety and sterility according to the U. S. Public Health Service requirements for biologic products. The regent lots are partially standardized on the basis of the bacterial and phage particle count. According to Baker, each milliter of the regent lot of the staphage lysate contains ten billion staphlococcus bacteriophage particles and the lysate of approximately two billion Staphylococcus aureus cells.

CLINICAL USE:

The use of SPL provides safe therapy against ubiquitous staphylococcal infection occurring in many disease in addition to providing a nonspecific type of therapy which stimulates lymphoproliferative responses in both T and B cell subpopulation present in human peripheral blood. It is further demonstrated that laboratory animals hypersensitized to Staphlococcus and treated specifically with SPL are nonspecifically protected against challenge with vaccinia virus influenza virus[5] and virulent field strains of E. Coli.[6] In the human situation, results of treatment with SPL of the staphylococcal hypersensitive subjects suffering recurrent herpes virus and aphthous ulcers were reported as successful and often dramatic.[7] All cases had been essentially treatment failure with other therapy. These findings support the interpretation that SPL in staphylococcal hypersensitive subjects acts as an immunopotentiator of nonspecific cell-mediated resistance.

The use of staphage lysate in America has been primarily in the realm of the allergists, since it was initially developed as a treatment for staphylococcal infection. Baker,[8] Smith,[9] Mills,[10] and Mudd,[11] found it useful in treatment of chronic bronchial asthma, allergy, carbuncle, furuncle and abscesses. Mudd, et al.[12] reported successful treatment of recurrent herpes virus and aphthous ulcers.

The recent work of J. H. Dean and R. B. Herberman[13] of the National Cancer Institute, support the interpretation that staphage lysate acts as an immunopotentiator of nonspecific cell-mediated resistance.

LEGALITY OF USAGE

Staphage lysate was licensed by the FDA in 1955, License No. 299 for use orally, nasally, and by injection. Dr. Jack Robbins, Chairman of Biological Products Section of the FDA, states, "Staphage lysate, like the other medicationslicensed prior to 1963, do not have definite indications for therapy listed in the original granted license, because this was not part of the procedure prior to 1963." (14)

All medication licensed prior to 1963 remain licensed under a "grandfather clause" and may be used without regard to initial usage. However, Dr. Robbins also suggested that if desirable, a Investigational Drug number could be granted for the controlled trial. And at the completion of the trial period, a new 1977 license number can be applied for specifically indicating the applicability of staphage lysate in inflammatory bowel disease.

In the interest of satisfying, any and all possible objections that might arise in the future regarding the use of staphage lysate in Crohn's disease, the Investigations Drug number applications is in process.

MECHANIZATION OF ACTION

In an effort to understand the mechanisms of action, J. H. Dean and R. B. Herberman of the National Cancer Institute have demonstrated that staphage lysate stimulates lymphoproliferative responses in both T and B cell subpopulations in human peripheral blood and in cord blood. These findings support the interpretation that stphage lysate acts as an immuno-potentiator of nonspecific cell-mediated resistance. (13)

Professor R. Thornes of the Department of Experimental Medicine of Byrnes Research Center, Dublin, Ireland is in the process of designing a

study that would include staphage lysate in multi-drug treatment program in combination with C. Parvum and levamisole for use in patients with metastatic cancer, because of its usefulness in their initial pilot studies, as previously mentioned.[15]

As previously mentioned, staphage lysate has been selected for evaluation in the treatment of complications of Crohn's disease, based on its proven success in a select group of Crohn's patients with unhealed posterior wound and enterocutaneous fistulas refractive to medical and surgical management.

STUDY DESIGN

The subject of this investigation will be several of the complications of Crohn's disease which are currently, inadequate, when treated with conventional medical and surgical management. While surgical intervention is the short-term answer, it provides long-range problems, because of the recurrent nature of many of their problems making a short bowel syndrome inevitable in patients consistently requiring revisions in order to maintain a satisfactory life-style.

CRITERIA FOR INCLUSION INTO THE STUDY ARE:

(1) Diagnosis of Crohn's disease.

(2) Presence of one or more of the following complications listed below.

(3) Markedly depressed, immunoprofile.

1. Diagnosis of Crohn's disease:

(a) Small intestine

(b) Colon

(c) Small intestine and colon. The criteria for making the diagnosis is that criteria suggested by the National Crohn's Cooperative Study, as listed in the appendix.

2. Complications to be considered are:

(1) Diarrhea with fluid and electrolyte imbalance.

(a) High ileostomy output.

(b) Consistent - greater than 2,500 a day for more than three months.

(c) Intermittent - such that patient requires three or more yearly hospitalizations for fluid management.

(d) Greater than six liquid stools.

(2) Residual disease:

(a) Ileostomy - prestomal recurrence.

(b) Ileostomy - disease residual at the time of surgery to avoid creation of a short bowel.

(c) Ileostomy - stomal ulcerations with contact bleeding causing massive hemorrhage at least one time.

(3) Fistula - enterocutaneous:

(a) Abdominal, usually from the site of the original anastomosis to abdominal skin.

(b) Perianal

(4) Spondylitis

(5) Arthritis

(6) Unhealed posterior wounds, status postproctocolectomy and split-thickness skin graft.

(7) Primary diagnosis cases in which Azulfidine and Prednisone and/or ACTH have not been successful and surgery is indicated because of symptoms, but not because of acuteness of disease.

(8) Recurrent disease with symptoms in less than one year, not responsive to medical management.

(9) Crohn's of stomach and/or duodenum with pyloric and gastro-jejunostomy contracture.

3. Immunologic Profile: Sample sheets for the immune profile, as included in the appendix.

Following documentation of disease location and severity, a broad immunoprofile will be performed to evaluate the current immune status of the patient. This will include:

(1) In vivo test — reactions to recall antigens.

(a) Protein purified derivative (PPD)

(b) Candida albicans

(c) Mumps

(d) Streptokinase-streptodornase

(e) Trichophyton

(f) ST-EP from CCF Allergy Dept. (made from enteric cultures).

(g) ST-RP from CCF Allergy Dept. (made from respiratory cultures).

(h) Staphage lysate diluted 1:10

(i) Staphage lysate diluted 1:100

The first four of these skin tests are those commonly used to assess memory, recognition, and inflammatory responses. It has been shown by Al Sarraf, et al.[15] that 90-100 percent of the population will respond to at least one of three antigens, if the three antigens are from the first four listed above.

The staphage lysate in two dilutions serve as any initial skin test to determine possible allergic reactions to the drug (although none has ever been reported in its 22 years of use).

Reactions to primary antigen, such as DNCB, are useful in investigation of sensitization and inflammatory responses; but once a positive reaction has been attained, the test cannot be repeated. It is necessary to re-evaluate our patients at various stages, and thus this test was not deemed useful.

(2) The in vitro tests include:

(a) Routine complete blood count with differential, lymphocyte, immunoblast monocytes being of great importance.

(b) T and B lymphocytes cell count.

<u>In vitro tests</u>:

(1) Immunological and cytochemical counts with cell surface markers
T-lymphocytes (spontaneous rosettes with sheep red blood cells)
B-lymphocytes (membrane immunoglobulins).

(2) Function of mononuclear cells -
Cell-mediate immunity, lymphocyte transformation by mitogen, phytohemagglutinin (PHA), Poke weed mitogen (PWM), concanavalin A (Con A).

Quantitative immunoglobulins:

IgG

IgA

IgM

Total protein

Serum albumin

C_3

C_4

Carotene

TRANSFERRIN

Serum protein electrophoresis:

The complete immunoprofile will be performed prior to beginning treatment. The delayed skin tests will be repeated at three-month intervals. The remainder of the immunoprofile will be repeated after treatment begins at intervals of three days, two weeks, two months, four months, and then on a six-months' basis. The profile will also be repeated at anytime the patient has a significant clinical change.

The patient should be off steroids for a minimum of two weeks prior to the initial immunoprofile. An exception is made in the case of patients' refractory to medical management and who are surgical candidates, if the SPL therapy fails. The immunoprofile will be performed, staphage lysate treatment begun and steroids tapered, as per consultation with the Gastro-enterology Department. It is noted that these patients' constitute a separate

group and that they can be observed only clinically, and declared as treatment failure requiring surgery. In these cases the immunoprofile correlation will not be valid for the initial value.

Absorprtion profile for diarrhea cases:

72-Fecal fat

Lipoprotein electrophoresis

Quantitative lipoprotein

Vitamin A absorption test

D-xylose absorption test

DOCUMENTATION OF DISEASE

Extent and Severity

(1) Diarrhea with fluid and electrolyte imbalance:

(a) High ileostomy output consistent with -

(1) Daily output record for one month preceding admission, medication records indicating amount of Lomotil and laudanum, patient has been receiving, daily.

(2) 72-Hour fecal fat

(3) Fasting lipoprotein electrophoresis

(4) Quantitative lipoprotein

(5) Cholesterol and triglycerides

(6) Ileostomy injection and UGI and SBFT

(7) Visualization via use of gastroscopy into ileostomy with pictures and biopsies.

(b) Intermittent -

(1) Daily output record for one month preceding admission, medication records indicating amount of Lomotil and laudanum, a patient has been receiving, daily. All hospital admission records including intake and output charts for past year.

(2) Absorption studies -

(a) 72-Hour fecal fat

(b) Fasting lipoprotein electrophoresis

(c) Quantitative lipoprotein

(d) Cholesterol and triglycerides

(e) Barium enema, UGI with SBFT

(f) Colonoscopy with multiple sequential biopsies and photographs.

(3) Residual disease -

(a-c) Ileostomy injection, UGI with SBFT, if stoma large enough for visualization with gastroscopy and sequential biopsies and pictures.

(d) Ileocolic anastomosis, barium enema, colonoscopy with sequential pictures and biopsies.

(4) Fistula - enterocutaneous -

(a) Abdominal with ileostomy, fistulogram, stomal injection. UGI with SBFT photography documentation of amount of drainage -- high output to have stoma bag in place with accurate measurement. Low output to have record of number of pads and amount of daily saturation.

(b) Perianal - barium enema, if colon intake otherwise, photographs and probe to determine length.

(5) Spondylitis - x-rays of thoracic, lumbar and sacral spine, HLA-W27, RA factor in addition to routine immunological profile. Work up for residual disease; if ileostomy: stomal injection; otherwise, barium enema, orthopedic and rheumatic consultations.

(6) Arthritis - x-ray of involved joint and contralateral joint, HLA-W27 and RA factor with rheumatology consult.

(7) Unhealed posterior wound, photographs, number of daily pads, and degree of saturation; probe of deep unhealed pockets to determine presence and depth.

(8) Primary disease not responsive to medical management. To be placed on protocol prior to surgery in patients whose symptoms are not critical. Previous GI work up would be adequate. Colonoscopy, when it is not a danger to the patient, with sequential pictures and biopsies.

(9) Recurrent disease with symptoms in less than one year, not responsive to medical therapy. Barium enema, UGI with SBFT, colonoscopy with sequential biopsies and pictures.

(10) Crohn's of stomach and/or duodenum with pyloric contracture, gastric retention with or without gastrojejunostomy with contracture. Gastroscopy with biopsies and pictures, UGI with SBFT and gastric emptying time.

FOLLOW UP:

Activity of disease in each individual category will be followed, daily, while the patient is in the hospital. At the time of discharge, the patient will be given a daily summary sheet and asked to record the daily observations pertinent to the type of complications the patient has. Samples of the summary sheet are provided in the appendix. These summary sheets will be reviewed every two weeks with the physicin-in-charge of the patient either by office visit or by telephone contact, after the summary sheets have been received in the mail.

At two month and six month intervals the patient will have repeat studies listed under disease documentation to allow evaluation of disease extent and activity at that time.

Immunoprofile with the exception of delayed hypersensitivity will be repated at two, four, and eight week intervals.

TREATMENT PROGRAM:

Initial staphage lysate skin test is performed at the time of delayed hypersensitivity testing. After completion of the immune profile the patient receives:

(1) Subcutaneous injection in hip and contralateral, arm, 0.05 ml. every second day until clinical and immunological response over initial value occurs; then injections are spaced one day further apart until they are once a week. At four weeks, check if the patient is asymptomatic, and immune profile stable. The injection interval will be lengthened to once every other week.

Criteria for improved immunoprofile are:

(a) Greater than 15 percent rise of T or B cells over initial value.

(b) Absolute number of lymphocytes greater than 15 percent above initial value.

(2) Nasal spray, 0.3 ml., given as microaerosol mist, daily, until clinical symptoms improve and immunological (10 percent lymphs or 10 percent T and B cells) over initial value, then spray goes to twice a week with re-evaluation at two weeks. If patient is asymptomatic and immunoprofile is stable, re-evaluation in four weeks' time.

MONITOR OF TOXICITY REACTIONS:

During the 22 years of usage of staphage lysate, sixty million doses of the medication have been administered. The only untoward reaction reported and contained in the FDA drug file of Delmont Laboratories are:

(1) "The limit of tolerance is the maximum quantity of SPL that can be given to a person without producing signs of a general reaction of a vaccine-like nature, i.e., malaise, increase in temperature or chills."(*) This reaction subsides in 18-24 hours after stopping medication.

(2) Local reaction of redness and mild pruritis at sites of injections.

During this study, all questionable flu-like reactions are considered a dry reaction and so listed in separate research notebook specifically for this purpose and reported to the FDA, Delmont Laboratories, and in the patients' charts.

(*) See packaging insert.

DATA ANALYSIS:

The data will be analyzed in four phases by Dr. H. B. Houser, Chairman of Biometry at Case-Western Reserve University.

(1) The pre-staphage lysate treated patient will have his immuno-profile compared with the normal values and any other treatment groups for which the immunoprofile values are available.

(2) The sequential values of the profile will be studied for each individual and then the two groups pre and post-stimulation will be compared.

(3) The clinical course of the patient will be correlated with the individuals sequential immunoprofile.

(4) The biostatical methods of forecasting will be used to aid in determining the program.

Mathematical models of the immune system of each of the individual patients will be constructed and with the use of the model and biostatical forecasting, the necessary and sufficient data base for long-term immune follow up of the patient will be determined. Essentially, this involves having several complete immunoprofiles of the same individual and then predicting what the next complete profile will be based on with only partial data. Initially, this exercise will be done when all the data is available so that the predicted results can be compared with the actual laboratory determination and the model refined. This is a very important concept, because many of the patients will return to small communities to be followed by family physicians who have limited access to immunopathology laboratories. With the use of the model, it may be possible to base adjustments in treatment on two or three of the laboratory parameters rather than all of the parameters initially needed.

Mathematical modeling and forecasting are sophisticated techniques and usually require large data bases. Therefore, it may be easier to attempt this type of analysis when a controlled clinical trial begins.

In conclusion: the immunological aspects of inflammatory bowel disease has fascinated investigators for years. However, no concrete help with the management of patient complications has been forthcoming from the realm of immunology. This study is only the first phase of an effort to obtain some clues into the relationship of Crohn's disease and immuno-deficiency, immunotherapy, and hopefully, into immunorestoration and eventually immunoprophylaxis. If the staphage lysate treatment of Crohn's disease continues to be as promising as the initial cases, then it will be imperative that a controlled clinical trial be conducted. The trial would be designed with the help of Case-Western Reserve University Department of Epidemiology and Department of Information Science, Biometry and would all be in comparison with the recently completed Cooperative Crohn's Disease Study.

"A theory is only sound if it smooths the way, not to tranquility, but to greater effort." GIDE

PERSONNEL INVOLVED IN THE PROJECT:

Clinical: Rupert B. Turnbull, Jr., M.D., C.M., F.A.C.S.
Senior Surgeon, Department of Colon and Rectal Surgery,
Cleveland Clinic Foundation, Cleveland, Ohio

Trish Blair, M.D., M.S.
Research Fellow, Department of Colon and Rectal Surgery,
Cleveland Clinic Foundation, Cleveland, Ohio

Joan Kerr, R.N.
Enterostomal Therapy Department
Cleveland Clinic Foundation, Cleveland, Ohio

Pathology: William A. Hawk, M.D.
Review of surgical slides and colonoscopy biopsies.

Immunopathology: Sharad Deodhar, M.D., Ph.D.
Head, Immunopathology Laboratory
Assists with immunoprofiles of patients

Marcia Heller, M.S.
Tissue culture technician
Works with mitogen screen techniques and
developing staphage lysate

Rafael Valenzuela, M.D.
Tissue culture laboratory
T and B cell evaluation

Dr. Bernice Schacter
Histocompatibility Laboratory

Allergy: Richard R. Evans, M.D.
Department of Allergy
Cleveland Clinic Foundation
Delayed hypersensitivity tests

Gastroenterology: Ben Sullivan, M.D. - performs colonoscopy
Michael Sivak, M.D.- " "

FDA Consultant: Jack Robbins, M.D.
Director, Biological Products Section, Federal Drug
Administration, Washington, D.C.

Data Analyses: Dr. H. B. Houser, M.D., Ph.D., Chairman, Dept. of Biometry,
Case-Western Reserve University
Dr. Robin Lake, Ph.D., Information Scientist, Asst. Professor,
Dept. of Biometry, Case-Western Reserve University

Data Analyses - continued:

Dr. Lewis Wolfenson, Ph.D., Systems Analyst,
Asst. Professor, Department of Biometry,
Case-Western Reserve University
Dr. Judah Rosenblatt, Ph.D., Experimental Design
Consultant and Biostatistics, Prof. in the Department of
Biometry, Case-Western Reserve University

b/s

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3. Georges Mathe, M.D., Professor of Oncology,
University of Paris Sud,
Institut de Cancérologie et d'Immunogénétique,
Unité Fred Siguier, Hopital Paul Brousse,
and Division of Hematology, Institut Gustave Roussy,
Villejuif, France
(Personal visit)

4. Sharad Deodhar, M.D., Ph.D.
Head, Department of Immunopathology
Cleveland Clinic Foundation
Research Division
Cleveland, Ohio

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TABLE I

(1)

Brief summary table of major studies of the immunologic aspects of Crohn's disease, as per Kirsner and Shorter.

I	Coincident Autoimmune Disease with Crohn's.		Hammer, B. et al., "Disease Associated with Ulcerative Colitis and Crohn's Disease" Gut 9:17-21, 1968
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TABLE I - continued

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BACTERIAL ANTIGEN

STAPHAGE LYSATES (SPL)* — *Staphylococcus aureus*, [illegible] Types [illegible]

for

STAPHYLOCOCCAL INFECTIONS

RATIONALE

Clinical benefits following vaccination with *Staphylococcus aureus* or its [illegible] components have long been attributed to the suppose[illegible] calling up of antibacterial anti-bodies. However, modern clinical and laboratory work does not support this interpretation. [illegible]

Benefit from staphylococcal vaccines and equivalent staphylococcal products is now coming to be understood as resulting from favorable effect upon the state of cellular sensitivity to Staphylococcus. [illegible]

Cellular hypersensitivity to *Staphylococcus aureus* which is exhibited by a large proportion of human subjects is markedly reduced by repeated SPL treatments. Favorable clinical results are reported in these studies [illegible] which, however, are still uncontrolled. A concomitant though not necessarily causally related increased capability of the macrophages to inactivate staphylococci, has also been demonstrated in the laboratory. [illegible]

An additional feature of SPL is the fact that the active Gratia Bacteriophage contained in this preparation is indeed polyvalent as demonstrated by its ability to lyse in vitro [illegible] a significant proportion of field strains of staphylococcus, in particular the notorious hospital strains. The ability of an appropriate bacteriophage to lyse staphylococci in vivo has been demonstrated in experimental animals under certain conditions by Barte[illegible] et al. [illegible]

THE PREPARATIONS

Staphage Lysates are prepared by [illegible] of parent cultures of S. aureus Serologic Types I & III [illegible] with a polyvalent staphylococcus bacteriophage. After ultrafiltration the lysates contain antigenic fractions of S. aureus and active bacteriophage.

In contrast to antibiotics, Staphage Lysate selectively acts on pathogenic staphylococci, yet spares the normal bacterial flora and there is no recorded [illegible] [illegible] [illegible] [illegible] [illegible] resistant staphylococci. The use of SPL provides rational therapy against ubiquitous staphylococcal infection occurring in many diseases. [illegible]

Staphage Lysates are staphylococcal vaccines containing bacteriophage, and the heat labile and heat stable antigenic fractions plus the extracellular and intracellular enzymes of S. aureus and culture medium ingredients (sodium chloride, beef heart infusion, Bacto-[illegible]).

SPL is standardized on the basis of bacterial cell content before lysis and phage activity. Each milliliter contains [illegible] million colony forming units of *Staphylococcus aureus* [illegible] million staphylococcus bacteriophage plaque forming units.

To maintain maximum antigenic potency and phage activity no preservative is added to SPL. Each lot is tested for safety and sterility according to U. S. Public Health Service requirements for biologic products.

ADMINISTRATION and DOSAGE

Staphage Lysates are used by the methods which best suit the particular condition under treatment: (a) subcutaneous injection; (b) intranasal aerosol inhalation, nose drops or instillation by displacement technique; (c) irrigation or topical application; (d) oral dosage and combinations of these routes as indicated alone or in [illegible]

The severity or urgency of the infection and the response of the patient should be the guiding factors determining the proper dosage schedule. Injections and aerosols are administered in gradual increments to the limit of tolerance which is highly individualized. (see PRECAUTIONS)

The [illegible] tolerance is the maximum quantity of SPL that can be given [illegible] producing [illegible] general reaction [illegible] [illegible] malaise [illegible] increase in temperature [illegible] Full maintenance dosage [illegible] [illegible] ranges [illegible] aerosol inhalation [illegible]

The initial dose of Staphage Lysate should be small to observe the individual response, then increases are made according to urgency and tolerance. Local reactions of redness and mild pruritus may occur within six hours, subside in [illegible] hours and are followed by a [illegible] [illegible] The dosage schedule outlined is for adults. Children take about one half this dose by injection and by aerosol. Infants are best treated with nose drops, sprays or topical applications.

SUBCUTANEOUS INJECTION

A separate, heat-sterilized, or disposable syringe and small bore needle should be used for each patient to prevent transmission of homologous serum hepatitis and other infectious agents from one person to another.

Acute Staphylococcal Infections: the initial dose of Staphage Lysates Types I & III varies from [illegible] [illegible] administered subcutaneously in deltoid region. Subsequent injections are given in alternate arms avoiding a previous site. According to therapeutic urgency and to tolerance, increments of [illegible] are made at [illegible] intervals until the patient's maximum tolerance is reached which is usually [illegible] [illegible] This dose is continued until resolution [illegible] [illegible] gradually [illegible] interval between treatments [illegible] by the patient's response [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] then proceed with [illegible] dose and increments are made at [illegible]

Subacute and Chronic Staph[illegible] [illegible] [illegible] SPL Types I & III should be [illegible] to [illegible] with the interval between [illegible] [illegible] [illegible] [illegible] increments of [illegible] are made at succeeding [illegible] [illegible] maximum [illegible] [illegible] maintenance dosage is continued [illegible] [illegible] [illegible] progress.

To prevent relapse, [illegible] recommended that booster therapy with inoculations, aerosols or nose drops be given at two to four week intervals at the physician's discretion for an indefinite period.

Intranasal Aerosol Inhalation

Aerosols or nose drops of Staphage Lysate are the classic method to induce a specific local and systemic immunity to staphylococci by [illegible] and rapid absorption of the antigen in the anterior nares, the main reservoir of pathogenic staphylococci. [illegible] intranasal aerosol inhalation [illegible] direct access to the sinuses, throat and bronchial tubes. Combined with subcutaneous injections better clinical results are obtained. A nebulizer with nasal tips is used, attached by rubber tubing having a hand valve to an air or oxygen supply with regulating valve. An output of air flow of five liters per minute is desirable. A measured dose of SPL is nebulized by closing the hand valve on inspiration, then the breath is held a few seconds and exhaled through the mouth, avoiding hyperventilation. Patients without allergies [illegible] dose of Staphage Lysates Types I & III [illegible] adding sufficient sterile water to make [illegible] for each administration. Subsequent inhalations at one to three day intervals may be increased by [illegible] SPL according to urgency and tolerance to a maximum of [illegible]

Patients with certain allergic cases [illegible] asthma, hay fever, chronic pulmonary disease and multiple allergies should be treated by inhalation of SPL [illegible] [illegible] [illegible] To determine sensi[illegible] [illegible] [illegible] be [illegible] with [illegible] SPL [illegible] and [illegible] extreme caution [illegible] [illegible] dose of SPL [illegible]

*Trade Mark

crements of [illegible] to 0.2 ml. are made at three to four day intervals, according to tolerance, to a usual maximum of 1.5 ml. by aerosol inhalation. For better serologic coverage SPL Types I & III may be given by subcutaneous injection and orally without aftereffects. See PRECAUTIONS.

Maintenance dosage is continued until control of the illness is well established, then monthly booster therapy helps to maintain progress and aid in the prevention of reinfection.

Nasal Drop Instillation with SPL

A rapidly expanding use of Staphage Lysates is the daily administration of nose drops at home between visits. The main focus of dissemination of S. aureus is from the anterior nares; therefore, local treatment is rational and effective. Patients with allergies should be started on SPL Type I only until a therapeutic plateau is reached, then Types I & III may be cautiously tried. Reactions are rare with this mode of administration.

Sterile dropper-applicator bottles of 5 ml. capacity are recommended (available at any allergy pharmaceutical company). To 2 or 3 ml. of sterile water for injection in the sterile bottle, add 1 ml. of the desired type of SPL. Because the lysates contain no preservative, strict aseptic technique must be adhered to in order not to contaminate the solution.

Instructions for Use of Nose Drops. One or two drops in each nostril, one to three times a day. Have some other person instill the medicine or use a hand mirror, as the dropper must not touch the nose or anything else. [illegible] with the chin up so that drops enter the upper part of the nose. [illegible] and swallow. Do NOT use the solution if it becomes cloudy. Store in refrigerator.

Oral Usage

The specific therapy of staphylococcal enterocolitis should include an oral dose of [illegible] to 2 ml. of Staphage Lysate Type I in water one to three times daily. Codein sulfate 15 to 30 mg by mouth is advised after each dose [illegible] to allay spasm and diarrhea. For systemic action SPL Types I & III by injection or nose drops [illegible] once the oral disease.

Topical Application

[illegible] with frequent usage of Staphage Lysates, accessible lesions of the skin, mucous membranes including eye and ear infections, burns, sinus tracts and ulcers may be treated with sprays, drops, packs or irrigations of SPL Types I & III as often as indicated.

PRECAUTIONS

This product should not be used if cloudy. This would indicate contamination.

Staphage Lysates in the 10 ml. vial should be used only for aerosols, nose drops, oral and topical application, not for subcutaneous injection.

In common with all antigens employed to stimulate the production of antibodies that are protective in the event of subsequent disease, SPL presents the remote potential of host sensitization to staphylococcal or bovine protein. No instances of anaphylactic shock have been observed in many thousands of injections or aerosols but the physician must bear this possibility in mind, be prepared to deal with such an emergency by having at hand epinephrine, intravenous antihistamines and corticosteroids.

Prior to the administration of Staphage Lysates by aerosol inhalation, a history must be obtained from the patient or parent to disclose any allergic tendencies. If positive, start with SPL Type I alone with an interval of 24 hours to observe [illegible] tolerance, then proceed accordingly.

Patients with bronchial asthma, pulmonary fibrosis and emphysema, bronchiectasis and multiple allergies are most likely to have reactions to SPL Type III. During an acute asthmatic episode [illegible] should not be used by inhalation but [illegible] and nose drops may be safely given in small doses. Precede the use of SPL Type [illegible] in those cases that have respiratory distress with [illegible] [illegible] [illegible] [illegible] advisable [illegible] See

Intranasal Aerosol Inhalation, paragraph 2.

There are no contraindications to the administration of Staphage Lysates when given according to directions.

GENERAL INSTRUCTIONS

Staphage Lysates do not contain a preservative. Therefore sterile technique is essential. [illegible] syringes and needles should be autoclaved or boiled for at least fifteen minutes. When a parenteral dose of SPL is withdrawn from the 1 ml. ampule, the remainder must be utilized immediately as aerosol, nose drops, orally or topically depending upon the condition under treatment. The importance of nasal administration is stressed because the nasal mucosa frequently harbors pathogenic staphylococci, often the identical strain isolated from infections in other parts of the body.

When using the 10 ml. vial, the rubber cap should be wiped with 70% alcohol and lightly flamed before withdrawal of the solution. Do not use the contents of any vial that becomes cloudy or turbid due to contamination. Staphage Lysates must be refrigerated at 5° C. (? or ± 3° C.).

Nebulizers should be rinsed under warm water after use, then sterilized without disassembling by submersion in boiling water for at least fifteen minutes. The addition of alcohol or other glassware detergent aids cleansing but should be followed by a thorough rinsing.

AVAILABLE

Staphage Lysates SPL are supplied laboratory fresh in:

1 ml. ampules, boxes of 10, for subcutaneous injection and aerosol use.

10 ml. multiple dose vials for aerosol, nose drops, oral and topical administration.

SPL - Serologic Type I - white label, 10 ml. vials [illegible].

SPL - Serologic Types I & III in equal parts [illegible].

Nebulizer outfits with [illegible] [illegible] valve and nasal tube for use with air compressor or oxygen supply.

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PATIENT ELIGIBLE FROM NATIONAL COOPERATIVE CROHN'S STUDY.

A. Patients eligible for the study may have Crohn's Disease of the colon, or of the small intestine, or of both colon and small intestine. For the purposes of this study, a patient has Crohn's Disease of the colon if any one or more of the following criteria are present:

1) Inflammatory disease of the colon with associated extensive small bowel involvement that has the characteristic radiologic features of Crohn's Disease or extends more than 18 inches proximal to the ileocecal valve. This criterion is intended to exclude patients with "backwash ileitis" of ulcerative colitis.

2) Inflammatory disease of the colon, the appearance of which on barium x-ray examination or biopsy is similar to classic regional enteritis. If in the mind of the investigator there is significant doubt as to whether the colon disease is Crohn's Disease or ulcerative colitis the patient should be excluded. The radiologic appearance of regional enteritis of the colon is described by Marshak (6,7).

3) Inflammatory disease of the colon which is segmental, i.e., it spares the rectum. Such rectal sparing must be documented by a normal rectal biopsy.

4) Inflammatory disease of the colon with associated entero-enteric or other fistula or fistulas.

B. Confirming and supporting the diagnosis of Crohn's Disease of the colon but not sufficient for the diagnosis are the following criteria:

1) Typical appearance of regional enteritis of the colon at operation, or colonoscopy.

2) Associated perineal fistulas or persistent large anal fissures or ulcers.

C. For the purpose of this study a patient has Crohn's disease of the small intestine if any of the following criteria is present:

1) Chronic inflammatory disease of the small intestine with typical radiologic appearance on barium x-ray examination (7).

2) Chronic inflammatory disease of the small intestine with typical histologic appearance on full thickness biopsy of a small bowel lesion.

3) Typical gross appearance of regional enteritis of the small intestine, visualized at operation and confirmed by the finding of a non-caseating granuloma in a mesenteric lymph node.

D. Confirming and supporting the diagnosis of Crohn's disease of the small intestine, but not sufficient alone for the diagnosis are the following criteria:

1) Typical gross appearance of regional enteritis of the small intestine visualized at operation or endoscopically but not confirmed by tissue examination.

2) Associated perineal fistulas or persistent large anal fissures or ulcers.

In evaluating patients for the study by these criteria, investigators will exercise particular care in excluding patients with tuberculous enteritis, histoplasmosis or other fungal disease of the bowel, ischemic colitis, typical chronic ulcerative colitis and diverticular disease.

E. Specifically excluded from the study are the following categories of patients:

1) Patients with diagnosis of Acute Regional Enteritis made at the time of laparotomy for acute abdominal pain with or without tissue being obtained. A patient with Acute Regional Enteritis is defined as follows: A patient with no previous history of small intestinal or colonic disease who at laparotomy for acute abdominal pain has no evidence of chronic inflammatory changes of the small or large bowel but an acutely inflamed small intestine. Subsequent postoperative x-rays of the small bowel and colon are normal.

2) Patients with inflammatory disease involving the rectum and/or peri-rectal areas only.

3) Patients who may require surgery in the near future as defined by the following criteria:

a) The appearance of "toxic megacolon" with evident colonic atony and dilatation, and signs of peritoneal irritation on physical examination.

b) Active gastrointestinal bleeding requiring transfusion at a rate greater than 500 ml/week to maintain hematocrit constant or avoid hypotension.

c) Evidence of non-localized peritoneal soilage by bowel content.

d) Intestinal obstruction expected to require imminent surgical relief.

e) Intraabdominal abscess or perirectal abscess requiring surgical drainage.

NAME _____

CLINIC NO. _____

TELEPHONE NO. _____

Immunoprofile

Pre and Post Stimulation

Quantity of T and B Cells:	Absolute	Ratio
Number of B Cells		
Number of T Cells		
Surfaces IgG		

Function of T and B Cells:

Transformation to Mitogens:	cpm	ratio	cpm	ratio	cpm	ratio
Control						
a. Pokeweed						
b. Phytohemagglutinin						
c. Concanavalin A						
d. Staphage Lysate						

WBC Counting 300 Cells:

PMN _____

Lymphocytes _____

Eosinophils _____

Monocytes _____

Basophiles _____

Sedimentation Rate _____

Immunoglobulins Quantitative:

IgG _____

IgM _____

IgA _____

Serum Protein Electrophoresis: Gamma

Beta

Alpha 2

Alpha 1

Albumin

TOTAL PROTEIN

C_3

C_4

	CM	B	preBeta
Lipoprotein Electrophoresis			
Quantitative Lipoproteins			
Fecal Fat			
Vitamin 'A' Absorption: PRE			
POST			
Total Protein			
Serum Albumin			
Carotene			
Transferrin			

Skin Tests:

	24-HOURS	48-HOURS
PPD		
Mumps		
Trichophyton		
Monilia Albicans		
Varidase		
Staphage Lysate		
Stock E.P. 1-5		
Stock R.P. 1-5		

BASIC DATA Review Number 1 2 3

Colitis or Crohn's ?	4
First Attack or Relapse ?	5
Sex	6
Year of Presentation	7 8
Age at Onset	9
Mode of Onset	10
Precipitating Factor/s	11
Occupation	12
Treatment Prior to Presentation	13
Duration Disease at Presentation	14
Associated Diseases	15
Pregnancy	16
	17

SURGICAL FOLLOW UP

Med. Rev. No. [1 2 3] Op. Sgn. [4] Age @ 1st Op. [5] Age at review [6]

	1st op.	2nd op.	3rd op.	4th op.
Duration of disease				
Interval - prev. op.				
Prev. treatment				
Urgency / Indications				
Operative findings				
Extent of disease				
Operation performed				
Pathology				
Wounds / Postop. stay				
Early postop. comps.				
Early postop. comps.				
Time hosp. death				

Follow up from 1st op.		General health	
Recurrence site		Medical treatment	
Recurrence time		Wounds	
Yrs. free symptoms		Ileostomy	
Yrs. + symptoms due rec		Ileostomy function	
Yrs. + symptoms - other cause		Weight change	
		Bowel action	
Late complications		Persistent disease	
Late postop. death-time		Occupation / Social	
		Sex function	
		Overall assessment	
		Colitis or Crohn's	
		Card number	

MEDICAL FOLLOW UP

Realtime year									
Yr. of follow up	FRA	1	2	3	4	5	6	7	8
Pattern disease	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Extent disease	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Course disease	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Local comps.	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Systemic comps.	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Treatment	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Outcome	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

CROHN'S STUDY

INSTRUCTIONS: Complete this form each day before going to bed. Your answers should describe the preceding 24 hours.

DATE: _____

MONTH: _____

	Mon.	Tues.	Wed.	Thurs.	Fri.	Sat.	Sun.
1. Number of liquid or very soft stools.							
2. Back pain: 0 = None 1 = Mild 2 = Moderate 3 = Severe							
3. Abdominal pain: 0 = None 1 = Mild 2 = Moderate 3 = Severe							
4. General well-being: 0 = Generally well. 1 = Slightly under par. 2 = Very poor. 3 = Terrible							
5. Medication: a. Lomotil b. Laudanum Number of pills consumed in 24 hours.							
EXAMPLE: a. (6) Lomotil indicates quantity taken orally and/or injections.							
a. Lomotil							
b. Laudanum							
c. Prednisone							
d. Azulfidine							
e. Sulfasalazine							
f. Percocan							
g. Codeine							
h. Percocan							

THE JOURNAL OF IMMUNOLOGY
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Vol. 115, No. 4, October 1975
Printed in U.S.A.

IN VITRO HUMAN REACTIVITY TO STAPHYLOCOCCAL PHAGE LYSATE[1]

J. H. DEAN, J. S. SILVA, J. L. McCOY, S. P. CHAN, J. J. BAKER, C. LEONARD, AND R. B. HERBERMAN

From the Department of Immunology, Litton Bionetics, Inc., Kensington, Md. 20795, and the Medical Center, Keesler Air Force Base, Biloxi, Miss. 39531, and the Laboratory of Immunodiagnosis, National Cancer Institute, Bethesda, Md. 20014

The cell-mediated reactivity of normal individuals to staphylococcal phage lysate (SPL) were tested in vitro in the lymphocyte stimulation (LS) and leukocyte migration inhibition (LMI) assays. There were 95% positive responses in LS (stimulation ratio ≥ 3 with $p < 0.01$) and 67% positive responses in LMI (migration index ≤ 0.80). Enriched subpopulations of T and B lymphocytes were prepared with rosette formation and density gradient centrifugation. SPL stimulated lymphoproliferative responses in both T and B cell subpopulations whereas phytohemagglutinin (PHA) stimulated only the T cell subpopulation. Cord blood leukocytes were tested in the LS assay and 41% gave positive responses to SPL, 81% to PHA, and 17% with SLO. SPL appears to be a useful reagent for the in vitro study of cell-mediated reactivity, and may provide somewhat different information from that obtained with other mitogens or antigens.

Since Nowell (1) first described the morphologic transformation and proliferation of lymphocytes from normal subjects induced by the plant lectin phytohemagglutinin (PHA)[2] the literature has been replete with numerous reports of other substances inducing lymphoproliferative responses. Pearlman, *et al.* (2) first described the *in vitro* induction of lymphocyte blastogenesis by a soluble antigen derived from the tubercule bacillus (PPD) and demonstrated that this response was limited to individuals demonstrating delayed cutaneous reactivity to PPD. In addition, crude soluble products of streptococcus (3) and staphylococcus culture filtrates (4) have been shown to induce proliferation of human peripheral blood leukocytes. It has never been clearly elucidated whether the lymphoproliferative responses associated with the bacterial products of staphylococcal and streptococcal cultures act as nonspecific lymphocyte mitogens in a manner similar to PHA or act as specific antigens requiring prior sensitization of normal subjects' lymphocytes.

Recently, some workers have demonstrated that 94% of normal human subjects had positive delayed-type cutaneous reactivity to a phage lysate of *Staphylococcus aureus* (SPL) (5) and have proposed an important role of cell-mediated immunity (CMI) in resistance to staphylococci (6). In limited studies, SPL produced clinical improvement in some patients with furunculosis, pustular acne, pyodermia, eczema, bronchial asthma, upper respiratory infections, and staphylococcus enterocolitis, after topical and parenteral treatment (5, 7, 8). In animal models involving induction of immunity by repeated injections of low virulence *S. aureus* followed by challenge with SPL, Shayegani and associates observed increased staphylococcidal activity of immune leukocytes (9), and increased protection of mice to vaccinia or influenza virus challenge (10, 11).

This present study was undertaken to evaluate the use of SPL in the *in vitro* assessment of cell-mediated reactivity. The ability of normal human peripheral blood lymphocytes from adults and from cord blood to respond to SPL was examined in a lymphocyte stimulation assay. In addition, the kinetics of this response and the responding cell types were partially characterized. SPL was also tested in the leukocyte migration inhibition (LMI) assay.

MATERIALS AND METHODS

Reactants tested. The staphylococcal antigen preparation (SPL) used in these studies was commercially obtained and was prepared by lysing cultures of virulent, coagulase-positive *Staphylococcus aureus* by infection with the Gratia strain of staphylococcus bacteriophage. The SPL used in these studies was prepared from *S. aureus* of the serotypes I and III of the international type cultures of Cowan. Each lot of this reagent is partially standardized on the basis of bacterial and phage particle count. Each milliliter of reagent contains the bacteriophage lysate of approximately two billion *S. aureus* cells along with approximately ten million staphylococcus bacteriophage particles (7). An important feature of this reagent for *in vitro* assays is that no preservative has been added. The protein concentration of each lot of SPL used was determined by the method of Lowry *et al.* (12). SPL was stored at 4°C until used in the *in vitro* assays described below.

Streptolysin (type O) (SLO) was obtained from Hyland Laboratories, (Costa Mesa, Calif.) and purified PHA was obtained from Burroughs Wellcome (Triangle Park, N. C.).

Stimulation of human peripheral blood leukocytes. Heparinized whole blood was obtained from normal adult donors who had no history of chronic staphylococcal disease. Peripheral blood mononuclear leukocytes (PBL) were isolated by the Ficoll-Hypaque (FH) flotation method of Böyum (13) with a commercially available FH solution (Bionetics Laboratory Products, Kensington, Md.). Lymphocytes from this source were resuspended, after appropriate washing, at 2×10^6

[1] Submitted for publication February 25, 1975.

This work was supported in part by United States Public Health Service Contracts NIH-NCI-72-227 and NIH-NIDR-72-2448 from the National Institutes of Health, Bethesda, Md. 20014.

[2] Abbreviations used in this paper: PHA, phytohemagglutinin; PPD, purified protein derivative; SPL, phage lysate of *Staphylococcus aureus*; CMI, cell-mediated immunity; LMI, leukocyte migration inhibition; SLO, streptolysin (type O); PBL, peripheral blood mononuclear leukocytes; FH, Ficoll-Hypaque; ^{3}H-TdR, tritiated thymidine; SR, stimulation ratio; E, erythrocytes; MI, migration index; LIF, leukocyte migration inhibition factor.

cells/ml in medium RPMI 1640 (Grand Island Biological Co., Grand Island, N. Y.), containing 25 mM HEPES buffer, 1% glutamine, 50 μg/ml gentamicin (Microbiological Associates, Bethesda, Md.) and 10% fresh frozen human AB serum (heat-inactivated 56°C for 30 min). The lymphocytes were tested for proliferative reactivity to SPL and PHA with a microculture (200 μl) lymphocyte transformation assay. One hundred microliters of the cell suspension were added in quadruplicate to wells of a Falcon Microtest II plate (Falcon Plastics, Oxnard, Calif.) and varying dosages of SPL and PHA were added to these wells in 100-μl volumes diluted in complete medium. All microcultures were pulse labeled for 8 hr before harvest with 1 μCi of tritiated thymidine (^{3}H-TdR) (specific activity = 6.7 Ci/mM; New England Nuclear, Boston, Mass.) after varying days in culture. Cells from each microculture were harvested on a multiple-sample harvestor precipitator (Otto Hiller Co., Madison, Wis.) onto strips of fiberglass scintillation paper (Reeves Angel Co., Englewood Cliffs, N. J.), washed with 5% cold trichloroacetic acid, and transferred to glass scintillation vials containing 5 ml of a toluene-based scintillation cocktail (LSC, Yorktown Research, New Hyde Park, N. Y.).

Vials were counted on a Packard Model 2450 scintillation counter for 10 minutes or to an error of 1% or less. Data were calculated by computer using the Welch modification of the Student *t*-test to determine significance. Data were expressed as the stimulation ratio (SR) which is the ratio of mean cpm in experimental cultures to the mean cpm in control cultures of PBL alone. PBL cultures with an SR ≥ 3 were considered positive and were all significantly different from control cultures at $p < 0.01$.

Stimulation of cord blood leukocytes. The response of cord blood leukocytes to SPL was assessed in one of our laboratories (S.C. and J.B.) by a previously described macroculture lymphocyte stimulation method routinely employed in that laboratory to detect bacterial antigen recognition (14). Because of high levels of ^{3}H-TdR incorporation into FH-isolated leukocytes, cord blood mononuclear cells were prepared by 1 × G sedimentation followed by nylon wool filtration and then reconstituted at a concentration of 5×10^5 cells/ml in medium RPMI-1640 supplemented with 20% autologous plasma and 50 μg/ml gentamicin. One-milliliter volume of this cell suspension was cultured in triplicate in 1-dram vials. Control cultures received 0.1 ml medium, while test cultures received 0.1 ml of an appropriate concentration of antigens or mitogen in medium. One microcurie of ^{3}H-TdR was incubated with each culture for 4½ hr before harvest. Antigen-treated cultures were labeled and terminated on the 7th day. Cultures containing PHA were likewise terminated for convenience at the same time the antigen cultures were terminated (day 7), which accounts for the lower levels of PHA reactivity observed. Cell suspensions were harvested onto fiberglass paper strips that were assayed for ^{3}H-TdR incorporation as described above. A stimulation ratio of ≥ 3, representing significant stimulation at $p < 0.01$, was considered positive.

Preparation of lymphocyte subpopulations. The ability of SPL to trigger lymphoproliferation in T- and B-lymphocyte-enriched subpopulations was studied by rosette separation methods, as described (15, Dean, J. H., Silva, J. S., McCoy, J. L., Cannon, G. B., and Herberman, R. B., submitted to J. Immunol.). Briefly, sheep erythrocytes (E) rosettes were formed by the method of Jondal *et al.* (16) at 4°C for 1 hr and then separated from non-rosetting subpopulations on FH gradients. This step was repeated and the E-rosetting subpopulations were pooled. The E-depleted subpopulation, enriched for B lymphocytes and monocytes, were also studied. Each subpopulation was monitored for surface immunoglobulin (Ig) staining by using fluorescein-labeled goat anti-human IgM and goat anti-human IgG (Hyland Laboratories, Costa Mesa, Calif.) and esterase staining activity. Unseparated PBL contained 24 ± 3% Ig staining cells; the E-rosetting subpopulation contained ≤ 5% Ig-staining cells and < 1.0% esterase staining; and the non-E-rosetting subpopulation contained 74% Ig staining cells and 26 ± 2% esterase staining cells. Unseparated PBL, E-rosetted enriched (T), and E-depleted (B cells and monocytes) cells were cultured as described above with appropriate concentrations of SPL or PHA and the proliferative response of the PBL and each subpopulation was assayed by ^{3}H-TdR incorporation at the appropriate time period.

LMI assay. The reactivity of normal donor leukocytes to SPL was studied by a direct LMI assay previously described (17). Briefly, various concentrations of SPL or medium were added in a 0.5-ml volume to 2×10^7 leukocytes suspended in McCoy's 5a medium (Grand Island Biological Co.) supplemented with 10% fetal calf serum, penicillin, and streptomycin. This mixture was incubated at 37°C for 45 min in a humidified 5% CO_2 atmosphere. Sterile Micropets (Clay Adams and Co., Parsippany, N. J.) were filled with 25 μl of cell suspension, one end was closed with sealing clay (Seal-Ease Capillary Tube Sealer, Clay Adams and Co.), and centrifuged at 200 × G for 10 min. Each capillary was quickly cut with a vial-file just below the cell-liquid interface, placed into Lexy culture chambers (Mini-Lab Co., Quebec, Canada) and 1 ml of complete media was added. Experimental cultures contained 0.1 ml of the appropriate concentration of SPL. All tests were performed in quadruplicate.

Cover slips were pressed into place, being careful to leave no air bubbles. All migration chambers were incubated on a level shelf at 37°C for 24 hr in a humidified 5% CO_2 incubator. After incubation, the areas of migration were projected onto paper with a Zeiss microscope (20 ×), were traced, and then measured by planimetry. The migration index (MI) for each test group was calculated by the following formula:

$$MI = \frac{\text{Mean migration area in presence of antigen}}{\text{Mean migration area in the absence of antigen}}$$

Replicate areas of migration were almost always within 10% of each other and were more frequently within a 2 to 3% range. Cord bloods were not studied in this assay because of the insufficient volumes of blood available.

RESULTS

Dose-response kinetics. The dose-response kinetics was determined for each lot of SPL by culturing varying concentrations of PBL from three individuals. Representative data from such studies are presented in Figure 1. The concentration of SPL in each vial that was found to give optimum proliferative response was used in all LS studies employing that vial and was found not to vary significantly in all vials of that particular lot. The optimum dosage of SPL giving maximum proliferation in the microculture LS assays ranged between 30 and 65 μg of protein well and in most LS studies 35 μg of SPL/well was employed. Undiluted SPL was found to contain approximately 7 mg/ml of protein.

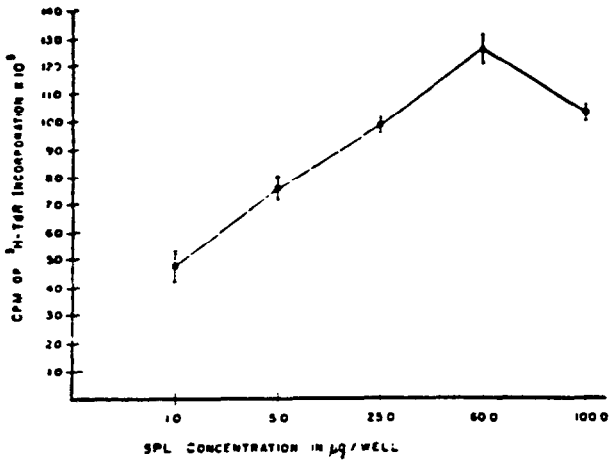

Figure 1. Dose response of human peripheral blood leukocyte cultures to varying concentrations of staphylococcal antigens (SPL). Symbol (●) represents mean cpm of ³H-TdR incorporation in PBL cultures from three normal donors [±mean standard error (S.E.)] terminated after 7 days in culture.

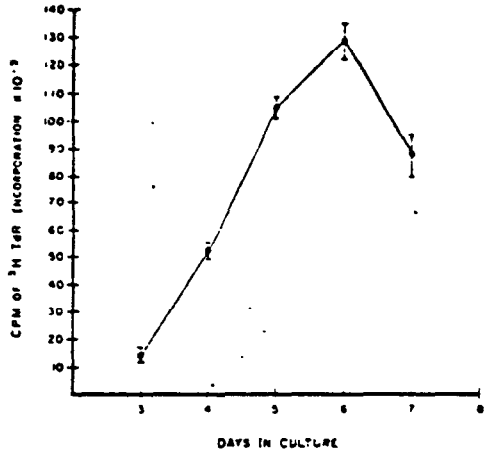

Figure 2. Kinetics of lymphoproliferative response in cultures of human peripheral blood leukocytes induced by staphylococcal antigens (SPL). Symbol (●) represents mean cpm of ³H-TdR incorporated into DNA of peripheral blood leukocytes from three donors (±S.E.).

Kinetics of lymphoproliferation to SPL. The kinetics of the lymphoproliferative response to SPL was also examined with PBL from several donors to determine if this response follows a delayed time course similar to that seen with other bacterial products. The results of these studies are presented in Figure 2. The lymphoproliferative response to SPL was maximum on day 6, although strong reactivity was also observed on both days 5 and 7. This was in contrast to the maximum proliferative response observed with PHA, which under these same microculture conditions peaked on day 4.

Lymphoproliferative response of adult human PBL. PBL obtained from 82 healthy adult donors were cultured with SPL, PHA, or medium alone. The PHA cultures and their respective controls were pulsed and harvested on day 4. The cultures containing appropriate concentrations of SPL and their respective controls were pulsed and harvested on day 6 because of the results obtained in the kinetics experiments. The results of these lymphocyte proliferation assays are shown in Table I. Ninety-five per cent of the donors' leukocytes cultured with SPL demonstrated a lymphoproliferative response. The mean level of ³H-TdR incorporation into DNA in leukocytes cultured with SPL was 78,759 cpm and the mean SR was 127. All of these same donor leukocytes demonstrated a positive lymphoproliferative response to PHA, giving a mean cpm of 97,686 and a mean of SR of 271. Three of the four donors with a negative response to SPL (mean SR of 1.4) had normal levels of PHA reactivity, with a mean SR of 112 while the fourth normal donor had borderline low PHA reactivity with a mean cpm of 2552 and an SR of 17.

Lymphoproliferative response of cord blood leukocytes. Leukocytes from 57 cord bloods were also examined in a microculture lymphocyte stimulation assay with appropriate concentrations of SPL, SLO, or PHA (Table II). All cultures were pulsed, labeled with ³H-TdR, and terminated on day 7. Of the 46 cord blood samples cultured with SPL, 19 (41%) demonstrated significant ³H-TdR incorporation with a mean SR of 13.8. A lesser number of these same cord blood samples (17%) responded with lymphoproliferation to SLO with a similar mean SR (19.3). Eighty-one per cent (46 of 57) of the cord blood samples demonstrated significant proliferation in the presence of PHA. The PHA values (SR) obtained were lower than routinely obtained possibly because the cultures were terminated for convenience at the same time the bacterial antigen cultures were terminated on the 7th day.

Responses of PBL subpopulations to SPL. In order to define the lymphocyte subpopulations undergoing a proliferative response to SPL, adult human PBL were separated by E-rosette formation into T and B cell-enriched subpopulations. Unseparated PBL and enriched subpopulations were cultured with SPL (45 μg/well), PHA (0.1 μg/well), or medium alone.

TABLE I

Lymphoproliferative response of human peripheral blood lymphocytes to staphylococcal phage lysate and phytohemagglutinin

Stimulant[a]	Positive Responses[b] (No. Positive/Total Tests)	Mean SR[c] of Responders	Mean cpm (±S.E.) of Responders
SPL	95 (78/82)	127.3 (±14)	78,759 (±3610)
PHA	100 (82/82)	271.4 (±37)	97,686 (±4843)

[a] SPL was used at 35 μg/well, and PHA at 0.5 μg/well.

[b] Stimulation ratio ≥3.0 was considered positive and was significantly different from control values, $p < 0.01$.

[c] Mean stimulation ratio of responding blood samples relative to medium control (± mean standard error).

TABLE II

Stimulation of nylon wool purified cord blood lymphocytes with staphylococcal phage lysate, streptolysin-O, and phytohemagglutinin

Treatment	% Responders (P/T)[a]	Mean SR[b] of Responders	Mean cpm[c] of Responders
SPL	41% (19/46)	13.8	35,722
SLO	17% (9/52)	19.3	10,590
PHA	81% (46/47)	15.3	5,713

[a] Percentage of positive lymphoproliferative responses observed in cord blood samples treated with above reactants relative to non-treated medium control cultures (positive reactions/number of bloods tested). Stimulation ratio ≥3.0 was considered positive when significantly different from control values at $p < 0.01$.

[b] Mean stimulation ratio (SR) of responding cord blood samples relative to medium control.

[c] Mean counts per minute (cpm) of responding cord blood samples.

The results of one representative experiment is shown in Figure 3. In response to SPL, the non-E rosetting cells had a level of incorporation similar to the unseparated PBL and the E-rosetted cells had an increased level of stimulation. In contrast, the non-E-rosetting cells had very little response to PHA. SPL clearly triggered lymphocyte proliferation in both T and non-T cell subpopulations in contrast to PHA which, by the same separation procedures, triggered primarily T cells (Dean, J. H., Silva, J. S., McCoy, J. L., Cannon, G. B., and Herberman, R. B., submitted to J. Immunol.).

Human LMI assays with SPL. LMI assays were performed with human PBL samples using varying concentrations of SPL. These results are summarized in Table III. Eight of 12 individuals (67%) in LMI tests demonstrated positive migration inhibition with undiluted SPL. Reactivity decreased as the concentration of SPL decreased. There were four individuals who were tested in repeated experiments. Three gave positive reactions repeatedly, whereas the fourth was borderline positive once and negative when the test was repeated. Cord blood leukocytes were not studied by the LMI assay because of insufficient blood volumes. The subpopulation responsible for the production of leukocyte migration inhibition factor (LMIF) is currently under study in our laboratory with an indirect LMIF assay.

TABLE III

Inhibition of normal donor leukocyte migration by SPL. *

Normal Donor	Migration Index (MI) to SPL at Various Dilutions		
	Undiluted	1/2	1/4
L. J. (Exp. 1)	0.80	0.79	0.96
L. J. (Exp. 2)	0.86	0.84	1.09
G. F. (Exp. 1)	0.75	0.91	0.99
G. F. (Exp. 2)	0.80	0.97	
L. L. (Exp. 1)	0.68	0.86	
L. L. (Exp. 2)	0.71	0.95	1.05
R. W. (Exp. 1)	0.62	0.73	0.91
R. W. (Exp. 2)	0.52	0.79	0.91
M. K.	0.44	0.97	
J. R.	0.97	1.25	
K. N.	0.86	1.06	0.99
K. B.	0.84	0.89	1.01
J. M.	0.74	0.82	0.98
C. K.	0.99	0.99	1.00
B. M.	0.44	1.00	
A. J.	0.41	0.71	

* $\frac{\text{Total positives}^{**}}{\text{Total no. tests}}$ = 11/16 (69%) 4/16 (25%) 0/10 (0%).

** Reactions were considered positive if M.I. was ≤ 0.80. Statistical evaluation of the results demonstrated that $p < 0.01$ when the M.I. was ≤ 0.80.

DISCUSSION

Antigens or mitogens which produce strong stimulation of lymphocytes of most normal individuals have been useful in the assessment of cellular immune competence. Decreased or undetectable stimulation of lymphocytes by mitogens has been found in many cancer patients, mainly those with advanced disease (18, 19) and in patients with some forms of immunodeficiency disease (20, 21). The results of the present study indicate that SPL may be a useful addition to the reagents used to measure functional reactivity of human lymphocytes. Its lack of toxicity *in vitro* is a clear advantage over some other bacterial preparations (17). In addition, it produced high levels of stimulation with lymphocytes from most normal adults tested. Preliminary studies from our laboratory show that some cancer patients and other patients with immunodeficiencies have little or no reactivity to SPL. SPL may be a particularly good supplement to PHA for such studies, since it appears to measure reactivity of a different or additional subpopulation of lymphocytes than that stimulated by PHA. When PHA and SPL were tested in parallel with rosette-separated lymphocyte subpopulations, a major and consistent difference was seen. As previously reported (Dean, J. H., Silva, J. S., McCoy, J. L., Cannon, G. B., and Herberman, R. B., submitted to J. Immunol.), PHA triggered proliferation primarily in the E-rosetting subpopulation. In contrast, SPL was able to stimulate the fraction depleted of E-rosette-forming cells, strongly, as well as the fraction enriched for E-rosette-forming cells. The agent may therefore be quite useful in studying reactivity of human non-T cells. It is not yet clear that the non-T cell responding to SPL is a B-cell, since the fraction studied also contained monocytes and null cells. However, it is likely that B cells are involved, since T and B cells are the only cell types which have been demonstrated to be stimulated by mitogens or antigens (22, 23). A strong stimulant for human B cells is needed since endotoxin, which triggers high levels of proliferation in the mouse B lymphocyte component (24), in our hands, produces only weak and unreliable stimulation of human lymphocytes. We are presently attempting to fractionate SPL to determine if a separate component can stimulate non-T cells, selectively.

The very high levels of stimulation produced by SPL and

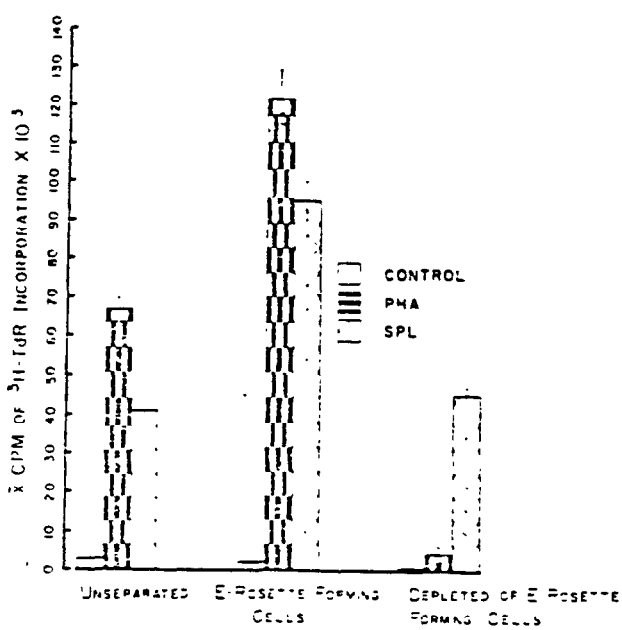

Figure 3. Lymphoproliferative response of human peripheral blood leukocyte subpopulations to PHA and SPL. Populations represented are unseparated PBL, T cell enriched by E rosetting, and E depleted, enriched for E-lymphocytes, monocytes and null cells. Bars represent cpm of ^{3}H-TdR incorporation by E-rosette subpopulation in medium alone, PHA, and SPL. Optimal mitogenic concentrations of PHA (0.1 μg per well) and SPL (45 μg per well) were employed. PHA cultures from the same experiments were terminated on the 4th day. SPL cultures were terminated on the 7th day, and values represented for the medium control represent an average of the 4th and 7th day values.

the almost universal reactivity to it by normal adults, raises the question of whether it is acting as a mitogen or as a specific antigen. There are several pieces of evidence which suggest that SPL is recognized as an antigen in lymphocyte stimulation. Although there was a very high incidence of reactivity to SPL, a few normal adults with no evidence for cellular immune deficiency were non-reactive. Each of the four SPL non-reactors did react to PHA: all but one had normal levels of PHA reactivity. One might expect a high frequency of reactivity to SPL since *S. aureus* is almost ubiquitous (6) and infections with *S. aureus* are very common, beginning with colonization of the umbilical stump of the newborn, followed by various clinical and subclinical infections. In addition, many individuals are carriers of *S. aureus* or have infections which are often chronic. In spite of this exposure, less than 5% of normal adults had detectable antibodies to *S. aureus* by counter immunoelectrophoresis and gel diffusion (25), although delayed hypersensitivity reactions to SPL was observed in 94% of adults tested (5). Our observation of inhibition by SPL of migration of the leukocytes of many, but not all, normal adults is also consistent with frequent cellular immune reactivity to SPL in humans. Lymphocytes from normal mice were not stimulated by this material (Dean, J. H., unpublished data), although specific cell-mediated reactivity to SPL was induced in mice by deliberate immunization with *S. aureus* (26, 27).

Other investigators have also observed frequent stimulation of human lymphocytes by materials derived from *S. aureus* (28). Taranta (29) looked for reactivity with culture filtrates of 131 strains of Gram-positive and Gram-negative bacteria and found it restricted to only *S. aureus* and a limited number of streptococcal strains. Comparable filtrates from *S. albus* failed to produce lymphocyte stimulation.

An additional observation which was consistent with the antigenic activity of SPL in lymphocyte stimulation was the time course of response. Maximum proliferation was not seen until 6 days, similar to that observation for tuberculin and other bacterial antigens, and different from the earlier peak seen with mitogens in the microculture assay (29).

The evidence for nonspecific mitogenic action of SPL is the very high levels of stimulation, and the reactivity of many cord blood lymphocytes. Greater than 100-fold stimulation has been mainly associated with mitogens like PHA. However, some bacterial antigens have been found to produce high levels of stimulation (30). It is also generally assumed that newborn humans have little or no cell-mediated immune reactivity, and that only 7S antibodies are transferred from the mother. However, if SPL were simply acting as a mitogen it would be surprising that more than half of the samples were non-reactive, whereas a much higher incidence of reactivity was induced by PHA. It is known that the human fetus develops cellular immune competence some time before birth (31) and it is possible that some fetuses are sensitized by transplacental passage of bacterial antigens or by some mechanism, as was previously demonstrated in germ-free piglets (32).

To resolve the question more clearly of whether SPL acts as a mitogen or antigen in lymphocyte stimulation it will be necessary to perform some prospective studies in negative reactors and look for conversion in association with staphylococcal infections. It will also be helpful to correlate directly the presence of lymphocyte proliferative activity with other assays of immunity to staphylococcal antigens.

Acknowledgments. The authors wish to thank Dr. Stuart Mudd for his comments and interest in this work and Miss Kathy Murphy for the preparation of this manuscript.

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II. EFFECTIVENESS DATA

A. CONTROLLED STUDIES

This section contains the protocol for a clinical study of Staphage Lysate being conducted by John S. Silva, M.D., at the Veterans Administration Hospital in Biloxi, Mississippi. It is submitted in accordance with 21 C.F.R. § 314.200(c)(2).

19 Jan 78

Dear Mr. Lincoln:

The enclosed protocol has been accepted for clinical trial by the Veterans Administration Hospital in Biloxi, Ms. We are presently attempting to define the clinical efficacy of staphage lysate in the prevention of wound infections in surgical patients who are identified as high risk. We have tested over one hundred patients without complication. Our incidence of reactivity is 82 percent. While the data is certainly preliminary, it would appear that reactivity to SPL may be useful for identifying immunodepressed surgical patients. We have too few patients treated to be able to make any statement concerning prevention of infections.

In addition, we are in the process of submitting a similar protocol to Keesler Air Force Base Medical Center Research Committee, and anticipate within 12-18- months to have sufficient number of patients to determine if SPL will be efficacious.

Sincerely yours,

JOHN S. SILVA, MD.
Principal Investigator

STATEMENT OF STUDY

1. Background

Infection is the major cause of morbidity and mortality following surgery or trauma. In 1967, an estimated 1.4 million patients had post-operative wound infections (1) with a total cost of nearly 23 billion dollars (2). The rate of infection can be approximated $I_r = D.V/HR$, where D = dose of bacteria with a virulence V and HR represents the host resistance. Factors which increase D increase I_r as noted in clean case infection rate = 5.1% but in clean-contaminated and dirty cases the rates are 10.8 and 22% respectively (3). Most attempts to reduce infection rates have been directed towards lowering the dose of bacteria.

While D is certainly important in the development of wound infections in the final analysis, it is deficient host mechanisms which fail to eliminate invading microorganisms. A number of acquired defects of HR hav been noted. Impaired phagocytosis and intracellular killing of bacteria have been reported in septic and burn patients (4-6). Of particular importance is the series of preoperative assessment of host resistance by MacLean (7-9). By simple skin tests to recall antigens (PPD, Varidase, mumps, candida and trichophyton) they were able to identify patients with impairment of HR. Patients who did not respond to more than one antigen had 20% septic episodes and 33% mortality, compared to normals with less than 5% incidence of sepsis.

2. Problem:

Having a method for the identification of patients who have increased risk for sepsis and death, what can be done? Howard succinctly answers, "....nonspecific adjuvants (of host resistance) would be even more effecti in the prevention or treatment of infection in patients rendered immunolog gically deficient by surgery or trauma." (6)

Method of study:

After informed consent surgery patients will be skin tested preoperatively with recall antigens as previously described (7). Patients who do not react to two or more antigens will be randomized into treatment or control groups by the serial sealed envelope method. Skin testing will be repeated 2nd-4th postoperative day and weekly thereafter.

Patients selected to receive the therapy regimen will have a test dose of 1:100 SPL,0.1cc intradermally, to assess allergic reactions. If there are none, the patient will receive 0.1cc SPL intradermally followed by 0.3cc via aerosol on day one and 1.0cc SPL orally on day two. The protocol will start on the day prior to surgery and be repeated on the second and fifth postoperative days. Infection surveillance will be carried out by the principal investigator with tabulation of wound infections, septic complications and nosocomial infections in both control and treatment groups. Data will be analyzed with Chi square statistic using a 2 x 2 square and the Yates continuity correction.

Pertinent work on this problem by others:

An exhaustive review of the literature failed to reveal other studie designed to potentiate host resistance and reduce septic complications.

In animals sensitized to S. aureus, staphage lysate augmented phagocytosis and intracellular killing of both S. aureus and E. coli (10,11). In addition, SPL also protected mice against otherwise fatal doses of vaccina and influenza viruses (12, 13). Clinically, SPL has been used successfully in the treatment and prevention of chronic staphylococcal infections (14) abcesses (15) and asthma (16). Over 20,000,000 doses of SPL have been administered without serious allergic reactions. No documented anaphylactic reaction has occurred.

Pertinent work in this field by the principal investigator:

Staphage lysate was found to stimulate immunodepressed cancer patients (17) as well as stimulate both T-and B-lymphocytes (18). It is currently under investigation by the principal investigator as a measure of the immunocompetence of cancer patients and has been approved for use as a skin test reagent. We are now attempting to characterize the ability of SPL to stimulate *in vitro* the movement of neutrophils and monocytes. To our knowledge, this property has not been reported in any other substance and may be casually related to the immunopotentiating ability of SPL.

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1975

JOHN S. SILVA, M.D.

INTERESTS

Surgical Oncology

Tumor Immunology Research

EDUCATION

B.S., Chemistry, Providence College, 1968, magna cum laude.

M.D., George Washington University, 1974.

BACKGROUND

1975 - present	Resident, General Surgery, Keesler Medical Center.
1974 - 1975	Intern, General Surgery, Keesler Medical Center.
1972 - 1974	Medical student, George Washington University.
1968 - 1972	Research Assistant, Department of Biological Sciences, Brown University.
1970 - 1974	Participant, Medical Education Program for Reserve Officers, United States Air Force.
1968 - 1968	Participant, National Institutes of Health Honor Science Program, Providence College.

EXPERIENCE

Engaged in research defining pathways of sugar phosphates in erythrocytes.

Engaged in research purifying purine salvage pathway enzymes and studies of interaction with antipurine chemotherapeutic agents.

Engaged in research developing a lymphocyte stimulation assay and use of this assay in evaluation of immune status of cancer patients.

Engaged in research defining histocompatibility antigens.

Experienced in the assay of metabolic intermediates of enzymatic or thin layer, paper and high pressure liquid chromatography techniques.

Experienced in isolation and purification of enzymes.

Experienced in preparation of human tumor cell extracts.

MEMBERSHIPS

Alpha Omega Alpha

American Society of Microbiologists

Delta Epsilon Sigma

PUBLICATIONS

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B. OTHER EFFICACY DATA

1. Studies in Humans

Preprint from
The Journal of The Medical Society of New Jersey
May 1963, Vol. 60, 180-193

GEORGE G. SALMON, JR., M.D.
Short Hills

MARGARET SYMONDS, M.B., B.S.
New Vernon

Staphage Lysate Therapy in Chronic Staphylococcal Infections

Exceptionally good results are here reported in the use of a specially prepared staphylococcic antigen for the control of chronic staphylococcic infections.

STAPHYLOCOCCAL infections continue to be prevalent. An ever-increasing number of reports of staphylococcal disease appear in the scientific, medical, and (perhaps more significantly) the lay press. Elek[1] states, "In fact, by sheer weight of numbers and distribution, staphylococci are the most widespread of all human pathogens." These infections are difficult to treat because of the frequent emergence of antibiotic-resistant strains, particularly in hospital patients. It is now noted that antibiotic-resistant strains are increasing in the general population. This is the basis of the present-day alarm. As each new antibiotic is developed, its use is followed remorselessly by the appearance of insensitive strains[2] of staphylococcus. It is of greatest importance to find out the factors governing host susceptibility and resistance to staphylococcal colonization and infection. Host resistance is of prime importance in preventing infection with these ubiquitous organisms.[3] All means by which this resistance can be increased must be welcome additions to our armamentarium.

Over the course of the last nine years, the senior author has increasingly used in his pediatric and pediatric allergy practice a staphylococcal antigen prepared by the lysis of virulent staphylococcal cultures. It is the purpose of this paper to present the results of this therapy to date.

CASE MATERIAL

THE cases forming the subject of this study are grouped here according to the location of the staphylococcal infection or according to the anatomic diagnosis as follows: furunculosis; pustular acne; pyodermia; eczema; bronchial asthma (intrinsic or bacterial); staphylococcal intestinal involvement and upper respiratory infection (U.R.I.). Regardless of whether the patient had one or more localized areas of infection, or a more generalized infection, colonization of the host with staphylococcus pyogenes was the basic condition.

Each case of staphylococcal infection included in this study was either selected from a regular pediatric practice or was a member of the family of a pediatric patient and was treated at some time between January 1954 and June 1961. Antibiotics were usually tried before the start of staphylococcus bacteriophage lysate therapy.* Most patients received

courses of tetracycline, penicillin, chloromycetin, and a sulfa derivative. Very few did not receive three of these drugs in separate series prior to SPL* treatment.

FOLLOW-UP

THE cases presented here in summary were all available for follow-up. Effort was made to locate the physician of each patient who had moved away or changed doctors. These physicians were asked for brief summaries of the patient's condition. Patients thus reported were included in this study.

BACTERIOPHAGE LYSATE

STAPHYLOCOCCUS bacteriophage lysate† is prepared from broth cultures of virulent strains of coagulase-positive staphyloccocus aureus, belonging to serologic Type I and Type III, which have been lysed by staphylococcal bacteriophage. These strains were originally obtained from human clinical lesions. The SPL* consists of these lysates together with live staphylococcal bacteriophage. No preservative is added. Bacterial sterility is obtained by the use of excess bacteriophage and filtration, and maintained by strict aseptic technic. The product contains lysed staphylococcus cell wall material, staphylococcus intracellular substances rendered soluble by the lytic process, the metabolites and enzymes elaborated by the staphylococcus, culture media ingredients in which the staphylococci are grown and living staphylococcal bacteriophage. Thus, this product contains a full representation of the antigenic components of the staphylococcus without any preservative which may denature the proteins.[4]

ADMINISTRATION

ONCE the nature of the infection is established, the patient is given a series of SPL* treatments. From 1954 to July 1959 all doses were given intranasally. Children less than two years old were given an instillation applied directly to the nasal mucosa by medicine dropper daily by the parent at home. Adults and children over two years old received nasal instillation of SPL* by aerosol apparatus,† fitted with sinus adaptors.† Treatments were given one to three times each week for five or six weeks unless improvement was noted earlier. Following this course, if symptoms recurred, two or three further treatments were given.

In July 1959 work was completed that indicated that SPL* was safe for injection. When the product was licensed by the National Institutes of Health, the work was reviewed by the authors, and new subjects were given SPL intradermally or subcutaneously in graduated doses. These cases are also included in this study.

DOSAGE SCHEDULE

THE dosage schedule was arranged to produce the desired result without causing excessive systemic or focal reactions.

SPL-I* is usually given alone at first. The initial dose is 0.05 or 0.1 cubic centimeters and successive doses are double the previous dose unless excessive reaction is noted. When a full cubic centimeter is reached, SPL-I and SPL-III are started at 0.1 cubic centimeter doses and volume increased as with the SPL-I alone. Frequency varies from every other day to weekly until the full dose is reached. Thereafter, monthly intervals are usually maintained. However, if an acute inflammatory process occurs while the prolonged interval is maintained (and if that process is thought to be due to staphylococcic infection) two or three doses are often given at two-day intervals.

No anaphylactic reactions have been reported by or to the manufacturers.

CASES TREATED

FORTY-EIGHT patients in our series had *furuncles and abscesses* due to staphylococcus (Group 1). Their ages ranged from one week

*Hereafter SPL is used for Staphylococcus bacteriophage lysate therapy.

†Material was produced and marketed by Delmont Laboratories, Inc., Swarthmore, Pa. The aerosol apparatus was a Vapo-frin® Nebulizer. The sinus adaptor was a DeVilbiss® Tr-40.

Group	of the Infection Nature	Cases Treated	No. Recoveries	%	No. Improvements	%	No. Failures or Relapses	%
1	Furunculosis	48	48	100	0	0	0	0
2	Pustular acne	14	11	79	3	21	0	0
3	Pyodermia	24	24	100	0	0	0	0
4	Eczema	60	52	86	7	11	1	2
5	Bronchial asthma	34	30	88	2	6	2	6
6	Respiratory infection	409	304	76	98	23	7	2
7	Intestinal disorders	18	17	94	0	0	1	6
	Total	607	486	80	110	18	11	2

to 62 years. The one-week-old infant's mother was known to be chronically infected by staphylococcus. This new-born child (who developed a breast abscess) was started on SPL* therapy as soon as fluctuation was determined. Most of the patients in this group had been given antibiotics or sulfa drugs before starting SPL.* Those who developed boils while on SPL therapy for other staphylococcal conditions were not given other treatment. These lesions usually cleared within a few days.

Duration of symptoms before the start of treatment varied from nineteen years to one day. Twenty-eight were three months or less in duration; ten between three months and one year; five between one and five years; and five between five and 20 years. Twenty-four cases cleared after from one day to six weeks of treatment. Sixteen cleared after six weeks to six months of treatment. Four cleared after six months to one year. And the remaining four were symptom-free after they had been given treatment for periods up to 19 months.

In the 48 cases, there was not one single failure. All the patients have remained free of of boils for a follow-up period of seven months to four years.

Fourteen patients who had *pustular acne* were treated (Group 2). The first four patients who had acne and who received SPL therapy* were being treated for chronic recurrent sinusitis. They had flare-ups of chronic acne during the treatment. When these flare-ups subsided, the skin of each patient was free of pustules or of reddened indurated areas. It was apparent they had recovered from the acne. This encouraged us to treat ten other patients who had severe acne. Each patient had previously undergone extensive treatments with other agents. They were discouraged people. Of this group, seven recovered completely and three showed improvement. Of these three, one had received severe x-ray burns of the involved area. Her acne was much better, but she has required more frequent injections to boost her immunity than did any other patient in the practice reported.

Twenty-four cases of persistent *pyodermia with purulent weeping infected eczema were treated*. (Group 3). These varied in duration of symptoms preceding SPL* treatment from three months to nineteen years. Each patient was given SPL for from two weeks to six months. In this group there were no failures: each patient recovered. No localized inflammations, and no areas of purulent eczema remained. One who had lesions for 19 months before treatment, experienced nausea, depression, and dizziness after seven treatments. These symptoms were so severe that she left our care. However, her skin cleared two weeks after stopping the treatment and has been clear 18 months, ever since her last treatment.

Sixty cases of *eczema* were treated with SPL therapy (Group 4). In some, the eczema became severe at the time of acute infections. In others, the eczema was thought to be due to bacterial sensitivity because of repeated improvement on antibiotic therapy. Some in this group were being treated for other manifestations of their staphylococcal infections. Duration of symptoms before the start of treatment varied from one day to fourteen years.

Fifty-two cases are free of eczema and have remained free during the follow-up period which varies from four years to seven months. Seven of the eight remaining patients are im-

proved. One is an adult who had dry, cracking eczema of the hands for 14 years. She was given SPL* therapy for nine months and has shown marked improvement, and has maintained this improvement for two years. One has had treatment for brief periods at irregular intervals for eight years; his lesions recur, and he returns for one or two treatments that help him enough so that he seeks our aid at each return of symptoms. Another, an infant, had a generalized weeping eczema and improved, but left our care after eight weeks; it is reported by others that there are two large areas of persistent dry, scaly eczema. While this patient was obviously improved, it is the question whether time or other factors may be credited for her improvement, as well as this therapy. Four are considered improved, but not cured. The sixtieth patient in this group illustrated a difficult problem that has occurred during the use of SPL therapy. This was a 15-year-old girl with eczema of the face, trunk, and antecubital fossae, as well as in the popliteal spaces; this has been severe for 13 years since she was one year of age. Therapy was started in this case with very small doses intranasally. However, she became acutely ill on starting treatment. Her lesions became worse (as is expected), but she also experienced abdominal pain and elected to discontinue treatment. These reactions confirmed to us the etiologic role of staphylococcus. Had smaller doses been given, milder side effects would not have discouraged her and she would have tolerated continuation of therapy. However, the rare severe reaction does not seem to justify starting all patients on minute doses.

Thirty-four patients were treated for chronic recurrent *bronchial asthma* (Group 5). These usually had fever with each asthmatic attack which was not seasonal. Antibiotics seemed to increase the effectiveness of conventional asthma therapy, thus indicating (together with the almost invariable appearance of coagulase positive staphylococcus in throat and nose cultures) the bacterial etiology. The duration of symptoms before the start of SPL therapy ranged from one month to 21 years. Average duration was three years. However, the mode duration was considerably shorter.

The interval from the first treatment to the recovery varied as in other types of diseases caused by staphylococcus and treated with SPL.* Recoveries, when they did occur, took place in from a few days to 20 months.

Thirty patients have been observed to be symptom-free for a period from eight months to four years with no recurrences. These patients are considered as having recovered. Two patients are considered improved. One child (lost to follow-up after 13 months of treatment) was improved when last seen. A 14-year-old boy had asthmatic bronchitis and asthma since 15 months of age, and had asthma with each cold which recurred at intervals of a few weeks until the start of SPL* therapy. He now experiences mild expiratory wheezes with these colds which recur on the average only twice a year. These two patients are considered improved. Two patients, one child and one adult, showed no change and have continued to wheeze as they did before treatment.

Four hundred and nine patients who had *chronic upper respiratory Infections* were treated (Group 6). They are frequently designated as chronic sinusitis, chronic tonsillitis, chronic laryngitis, chronic bronchitis, chronic otitis media, as well as chronic upper respiratory infection. This arbitrary differentiation is based on criteria too personal and too flexible to be of much value; so they are described here together under the general heading 'U.R.I.' Four or more acute exacerbations in a six-month period was considered to characterize a "chronic" illness.

The nose and throat cultures grew coagulase positive staphylococcus persistently before SPL* therapy in almost all these patients. (Phage typing was not done.) After treatment, 304 of the 409 patients had only one or two illnesses each year, and no longer had persistent or chronic symptoms following each acute illness. These patients are considered as having recovered. Ninety-eight have had fewer attacks and were generally better between acute episodes. These are called "improved." Of the remaining seven patients (listed as unimproved because the symptoms persisted) three are worth noting. In one, staphylococcus was no longer recovered in nose and throat cultures after treatment; yet he failed to improve clinically. One continues to have "sinus

headaches" after treatment, but now these headaches occur during the pollen season only. In one case, our conclusions are unreliable, since he received cortisone for rheumatoid arthritis during the therapy. The effect of antigen therapy is uncertain when given concomitantly with steroids. The other four continued to have repeated febrile and upper respiratory infections with nasal discharge.

Eighteen patients who had *intestinal symptoms* were treated (Group 7). Symptoms varied from three weeks to 27 years' duration before start of SPL therapy.* Many were selected for SPL therapy because they had or gave a history of frequent staphylococcal infections, usually furuncles or abscesses, in the past; and had shown repeated, brief periods of improvement on antibiotic therapy. Some had positive staphylococcal nose, throat or skin cultures at the time of onset of therapy. The symptoms varied from three weeks to 27 years' duration before the start of therapy. Fourteen with the following diagnoses were treated; chronic diarrhea; mesenteric lymphadenitis; nonspecific intestinal allergies (these patients were being treated for other symptoms of staphylococcal infection). All these patients have remained symptom-free. These 14 patients are listed as having recovered.

Three adult patients with *ulcerative colitis* diagnosed elsewhere were treated. They all had positive cultures of staphylococci from the stool. These patients showed improvement. Two experienced recurrences of old staphylococcal infections of the skin during treatment; one of these infections was a severe acne that had been quiescent for years. This acne ran a short course and the patient rapidly showed marked improvement of both the acne and ulcerative colitis. This patient was one of the first four acne cases treated. The other case developed an acute cellulitis of the foot. The original foot infection had occurred 20 years previously and was then called a fungus infection. The cellulitis subsided with some difficulty, but the patient has remained free of both cellulitis and of colitis symptoms since the acute episode.

One child under ten years of age had ulcerative colitis. He showed some temporary improvement with this and the other therapies tried. He eventually went to surgery and died.

COMMENT

BURKY[5] experimented with rendering rabbits sensitive to low ragweed, by uniting the weak antigen with staphylococcal toxin achieving the hypersensitive state. He observed that "It is probable that substances with little or no antigenic activity will become antigenic when combined with or attached to the (staphylococcal) toxin radical . . . At this time it is not clear why staphylococcal toxin alters the antigenicity of ordinarily inert substances. However, the result reported here, and other experiments which have been done all point to the fact that it has the ability to increase antigenicity." This probably accounts for the fact that some asthmatics will have positive skin tests to a variety of inhalants, but become symptom-free on antistaphylococcal therapy without further desensitization to the inhalant antigens,[6] and also the occurrence of eczema and similar lesions during treatment. Prigal[7] in his discussion of asthma and infection underlines the *contagion* factor, and suggests that intrafamilial staphylococcal infection may be responsible for the 'inheritance' of asthma rather than a genetic mechanism.

The mode of action of SPL* in no way resembles that of antibiotics or chemotherapeutic drugs, nor even the direct "phage" action as tried by D'Herrelle and other early workers. It is a specific vaccine, requiring a period of time to bring about an immune response. As all the patients treated had been chronically sick with long standing staphylococcal infections, the initial dose, if given during an acute episode, did, in fact, often bring about an early response (in most instances within four to 12 hours). These situations may represent accelerated immune responses. In fact, all human subjects have previously been exposed to staphylococcal antigen.

UNTOWARD EFFECTS

A FREQUENT accompaniment of staphylococcal disease is hepatic enlargement with a soft, sometimes tender liver. This was frequently seen on selected patients and it was

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:

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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 susceptibility	+++	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.

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 neutrophile 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 dosis, 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 Dosis (72h - 96h)

	Antigen Staph. aur.		Bacterial counts			
	Imm. R	Del. R	2800×10^4	1400×10^4	700×10^4	350×10^4
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 Staphyococcus 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 elicites 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 immunochemotherapy 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

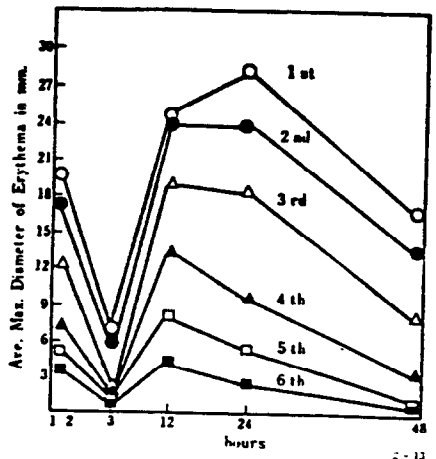

Fig.13 Erythematous reactions over a 2-day period in patients receiving six intradermal injections of SPL (0.1 ml) at weekly intervals.
(Mudd. S. : J. Reticuloend. Soc., 8, 1970)

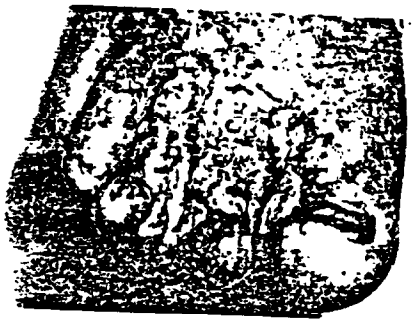

図 14

Fig. 14

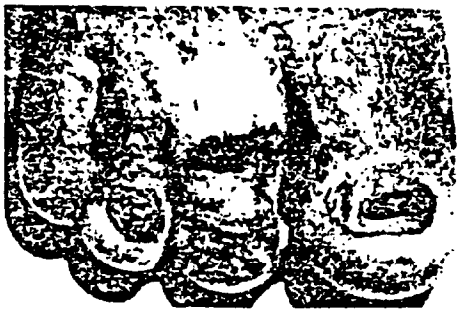

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Fig. 15

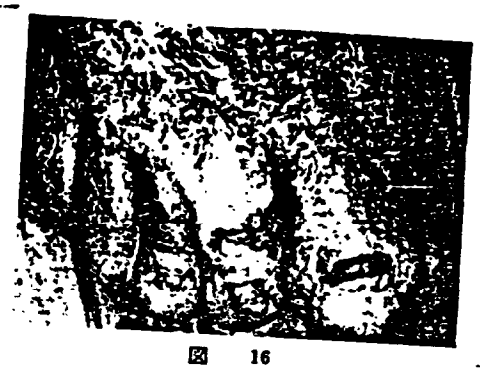

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Fig. 16

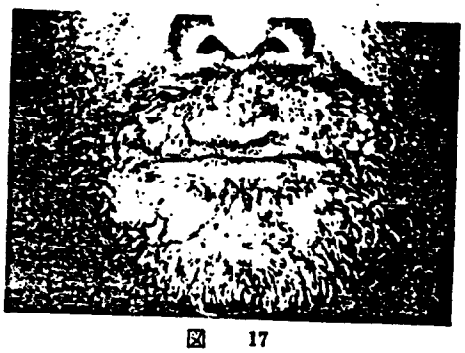


図 17

Fig. 17

図 18

Fig. 18

DALE C. RANK, M.D., F.A.C.S. — WILLIAM T. BOWERS, M.D., F.A.C.S., P.A.
JERRY W. BANE, M.D.
DIPLOMATES AMERICAN BOARD SURGERY
GENERAL SURGERY
801 W. RANDOL MILL ROAD
ARLINGTON, TEXAS
275-3300

September 29, 1977

OCT 7 1977

Mr. Charles G. Lincoln
DelMont Laboratories Inc.
Biological Specialities.
P.O. Box AA
Swarthmore, Pennsylvania 19081

Dear Mr. Lincoln:

Thank you for your helpful and informative letter of September 13, 1977.

The English, who actually did the key studies of viral (?) isolation in regional enteritis and transplantation to mice, are using Leveamisole. As I am in communication with them, I suggested to them that you would send a full packet of information on your excellent product to the primary physician involved in treatment there. (Dr. Donald Mitchell MRC Tuberculosis and Chest Diseases Unit: Brompton Hospital:- Fulham Road: London, England SW36HP.)

Our work with SPL is progressing beautifully.

You may be interested to know that I have a rather significant series now of advanced severe herpetic stomatatis essentually treated with SPL, many cases of recurrent lymphangitis (including post radical mastectomy cases), and several patients SPL in a program of chemoimmunotheraphy. So far, my greatest usage has been BCG and pharmacologic doses of Vitamin A, but I plan to use SPL more aggressively in the future.

As always, I am very appreciative for your help, and advice, and information.

Best of luck to you and your family in your worthwhile work.

Most sincerely yours,

Dale C. Rank, M.D., F.A.C.S.

DCR/db

801 W. [illegible] HILL RD.
ARLINGTON, TEXAS

Immune Stimulation Therapy for Inflammatory Disease of the Gut

(Study Nov., 1975 to Dec. 1977)

A. 15 patients

Crohn's 13

Mucosal ulcerative colitis 2

B. Indications for Rx

1.	Recurrent disease (post resection)	5
2.	Failure medical Rx	10
3.	Diffuse enterocolic disease	4
4.	Young age (21 years or younger)	2
5.	Morbid obesity (steroid avoidance	2
6.	Immune incompetence	1
7.	Anorectal sepsis	2
8.	Nutritional failure	5
9.	Mucosal ulcerative colitis more than 10 years	2

C. Method of Rx

1. Agent: Staphage Lysate (Delmont Laboratory, Swarthmore, Penna.)

2. Method of Rx.

a. Initial

Systemic Intradermal
Sub Q
Aerosol Nebulization

Frequency 1-2 x /week

Dosage Begin with 0.25 cc total dose. Gradually increased to 1.0 cc total dose.

b. Subsequent oral SPL

Frequency 1-3 x /week, depending on response to Rx

Dosage 1 cc.

3. Study

CBC, SMA_{16}, Immunoglobulin assay, protein electrophoresis, serum zinc

Recall skin antigens (mumps, Candida, trichophyton, tuberculin, streptokinase-steptodornase, SPL.

Complete GI study, including proctosigmoidoscopy ± coloscopy.

Results:

Depressed lymphocyte count	1
Skin anergy	2
weak response skin antigens	3
Low serum zinc	2
High IgA or IgM (Blocking antibodies?)	6

D. RESULTS

1. Objective healing or marked improvement	7/13
2. Control of diarrhea	10/11
3. Control of blood in stool	2/3
4. Nutritional or growth improvement	4/5
5. Relief of abdominal pain	4/5
6. Disappearance of abdominal mass	4/4
7. Relief of partial intestinal obstructions	5/6
8. Healing anorectal sepsis	2/2
9. Failure of Rx over 3 months	2/15
Remission regained	1/2

E. Complications

1. Flulike symptoms (transient)	1/15
2. Intolerance oral SPL	2/15
3. Sufficient to terminate Rx	0

DALE C. R[illegible], M.D.
801 W. [illegible]
ARLINGT[illegible] [illegible]LL RD.
XAS

Patient	Diagnosis/ Age onset	Surgery	Med Rx	Status on Rx	Immune Rx	Result & Status
Bengfort 21 WF 25843	Crohn's distal ileum & (L) colon to anus. Mass RLQ onset 16 yrs	0	Azulfadine & steroids 6 mos	Diarrhea 5-10/D 97 lbs Active Crohn's 20 cm Procto Zn normal (?) internal fistula	SPL systemic → oral 6 mos	1 BM/D 109 lbs Procto almost normal Good health
Aiken 50 WM 24162	Crohn's distal ileum (30 cm) Massive hemorrhage Skin anergy onset 40 yrs.	(R) ileo colectomy 12/75	0	Post op satisfactory. Procto normal	SPL systemic to oral 24 mos	Completely well 1/78
Lattimore 30 WF	Crohn's entire colon anal fistula 50% small bowel	(R) ileocolectomy 10/70 P.O.ACTH/ Azulfadine. Anal fistulectomy 5/73. Complete colectomy & resection 30 cm ileum with ileorectomy Oral steroids & Azulfadine p.o.		Constant pain Rx Diarrhea 5 x/D Severe pain Abd mass & partial obstruction 85 lbs 7/76	SPL systemic to oral 18 mos	BM's semiformed 2-3 x/D Pain better (Rare pain Rx) 85 lbs Clin much better employed Procto shows ileum to be nearly healed with (1) 3 cm patch of Croh at anastomosis
Stults 53 WF 20008	Sigmoid resection for diverticulitis (Perforating 9/73. Diarrhea 4-6 x Day. Incidental finding-- Crohn's patchy through small bowel No anergy		Steroids & Azulfadine x 4yrs. Gallstones & back pain onset	Diarrhea 4-5x/day Abd pain. Partial obstruction on several occasions Tender mass RLQ	Oral SPL x 6 wks	Immed. ↓ stools to 2 a day (partly forme Lomotil↓ from 8 2/D. Increased energy

DALE C. BANK D.
801 W. RANDOLPH
ARLINGTON, TEX

	Mucosal ulc Colitis (universal)					
Brown 46 WF 26256	Bloody diarrhea onset Age 36. 5-10 x/day No anergy skin	No resectional surgery	Azulfadine 7 yrs.	Very little change in course	SPL systemic to oral x 9 mos.	1-3 soft BM/day. Rare blood. Procto shows ↓ pus & blood but no major healing. Subjective improvement great. 12/77
Spiva 35 WM 23725	Crohn's colon, distal ileum, section onset age 31. No anergy	Total colectomy & ileostomy 9/75	No post op Rx	Severe ano-rectal sepsis (mucosal)	SPL systemic & oral 4 mos.	Rectal pain & dischi sharply ↓ 12/77
Morgan	(R) ileocolectomy 1970 for obstruction Crohn's ileum & ascending colon		Post op ACTH & Azulfadine 5 yrs	Diffuse Crohn's partially throughout small bowel & worse at ileocolic anastomosis pain	SPL systemic to oral 18 mos. Failure	Complete subjective improvement for 18 mos.
			Return of abn pain & diarrhea 8/77. Procto better. x-ray essen unchanged except no evidence of internal fistula on x-ray.		Resume SPL oral + whatever needed for anemia On azulfadine. Oral SPL 2 mos. Steroid for stress only. Zn	Subj/77 Improvement BM's essen normal Dec/77

DALE C. DANN, M.D.
801 W. RANDOL MILL RD.
ARLINGTON, TEXAS

	Crohn's disease. Rectum & ileum					
Thomas 43 WF 14792	Diarrhea episodic 1956-1968 (75 lb wgt loss 1956) Anorectal suppuration after hemorrhoidec-tomy 1968	No resectional surgery No resectional surgery	Steroid & sulfa 9 yrs	Persistent diarrhea. Partial obst with RLQ mass Dec 1976	SPI systemic x 13 mos.	2-3 partly formed BM's /d. Disappearance obst & RLQ mass 12/77
	No anergy					
Kuyawa 36 WF 11581	Diarrhea 8-10 x/day beginning age 35 Crohn's severe colon & rectum Recall antigens weakly +	No resectional surgery	sympt Rx only	Diarrhea 10 x/day Crohn's rectum & distal ileum	SPL systemic oral 10 mos	Normal BM's. Procto normal 12/77 X-ray ileum sl im-proved 12-77
Hughes 45 WF 17953	Bloody diarrhea 8-10 x/day beginning age 35. Crohn's severe colon & rectum Recall antigens weakly +	Small bowel fistula after hysterectomy 1972. No major surgery.	Steroids & Azulfadine 6 yrs.	Bloody diarrhea 10 x /day Rheumatoidarthri-tis	SPL systemic to oral 9 mos.	Normal BM's. Procto almost normal. Joints unchanged.
Waddell 48 WF 14811	Probable Crohn's of rectum 1975 - Severe diarrhea 18-20/D with anal fistula Anal surgery x 2 with no healing Skin anergy	anorectal surgery only		Diarrhea 8-12 x/D Chronic anal sepsis	SPL systemic 12 mos.	Complete remission 10/76
	Stopped SPL 9/76			Recurrence diarrhea 18-20/day Anorectal sepsis	SPL systemic resumed 11/76	Complete remission 12/77 On Rx 1 x /month.

DALE G. DAN M. D.
RANDOL MILL RD.
ARLINGTON, TEXAS

Gay 15 WF 26283	Crohn's rectum (Mid) Diarrhea, freq with blood, 3 yrs. Recurring infections, Anemia. No skin anergy.	No resectional surgery	Poor health at diagnosis	SPL Systemic to oral 9 mos.	Normal BM's, Wgt gain. Sl↑ hgb. Good health 12/77.
Eiland 27 W M 26104	Crohn's of ileum Age 19 yrs. No skin anergy	Resection ileum. 1969 (19 yrs age)	1976 Onset recur obst not helped with steroids & Azulfadine	SPL systemic to oral 3 mos. Gen health fine. Recurrent obst.	
	(R) ileocolectomy 5/77 Active disease (mild)			Cont SPL post op 6 mos.	Normal health Normal BM's 12/77
Randerson 61 WF 27344	Crohn's of ileum 5+ yrs. Procto abn Weak response to recall skin antigens	Small bowel resection 1975. No benefit.	Abd pain. BM's 10-12 x/day. Wgt 80 lbs. Steroids & Azulfadine with benefit 2 yrs.	SPL systemic & oral 6 mos. No benefit	No benefit. Plan intravenous or oral enteral elemental diet 2 weeks 1/78
North 43 WF 27170	Mucosal ulc colitis, universal 17 yrs (onset 26 yrs age) No skin anergy	No resectional surgery	Watery BM's 5-6 x /day Bloody 1 x/wk. Pain & tenderness LLQ Steroids & Azulfadine for 17 yrs.	SPL systemic & oral 6 mos No benefit	Mild & BM's to 3/day. Not as loose. No pain and tenderness LLQ first time in 5 yrs. Feels better.

DALE C. BANK, M. D
601 W. RANDOL MILL RD.
ARLINGTON, TEXAS

Immune Stimulation for Aphthous

(Herpetic) Stomatitis & Rhinitis

(Jan 1976 to Jan 1978)

A. 5 pts.

B. Indications

Continuous ulcerative disease of over 12 mos duration

C. Method of Rx

(Same as for the Gut)

D. Study

1. (Same as for the gut)
2. Results

Skin anergy	1/5
Weak response to skin recall antigen testing	1/5
Elevated IgA or IgM (block antibodies)	2/5

E. Results of Rx

Complete control of disease	4/5
Improvement 50%	1/5
Failures of Rx	0

F. Complications of Rx 0

DALE C. [illegible], M.D.
801 W. [illegible] MILL RD.
ARLINGTON, TEXAS

APHTHOUS STOMATITIS (HERPETIC)

Pt	Duration of disease	Locations	Prior Rx	SPL Rx	Results
›sher 1 WF 5903	15 yrs continuous ulceration Skin anergy	oral cavity	Vitamins Vaccinations antibiotics	systemic to oral x 12 mos (current)	Complete remission 12/77
chuman 2WF 1804	2-3 yrs. Chr nasal infection sinusitis, nasal ulcerations. No anergy	Nasal mm's & sinuses	Antibiotics Nasal & sinus surgery	systemic SPL 12 mos (current)	Complete remission 12/77
/ptak 5 WF 1129	4 yrs No anergy	Sinus infections Fever blister about mouth and buccal cavity. Almost continuous	Antibiotics Vitamins	SPL systemic to oral 6 mos (current)	No infections or herpetic ulcers 12/77
'illiamson 4 WF 4258	30+ years	oral cavity, tongue (continuous disease)	Antiobiotic Topical Rx Vitamins Vaccination	SPL systemic x 6 mos. Ended June, 1976	2 minor ulcers No disease 2 yrs. 12/77
›berts 4 WF 5349	3 yrs Weak recall antigen response	oral cavity & pharynx continuous disease	Antiobiotic Topical Rx Pain Rx	SPL systemic & oral 14 mos. Current	Improved but episodes of breakdown 10 mos. SPL with only small ulcer occasionally in past 4 mos. 12/77

2. Studies in Animals

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

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 MICROBIOLOGY
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 eruptic 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+) 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.

Fig. 2

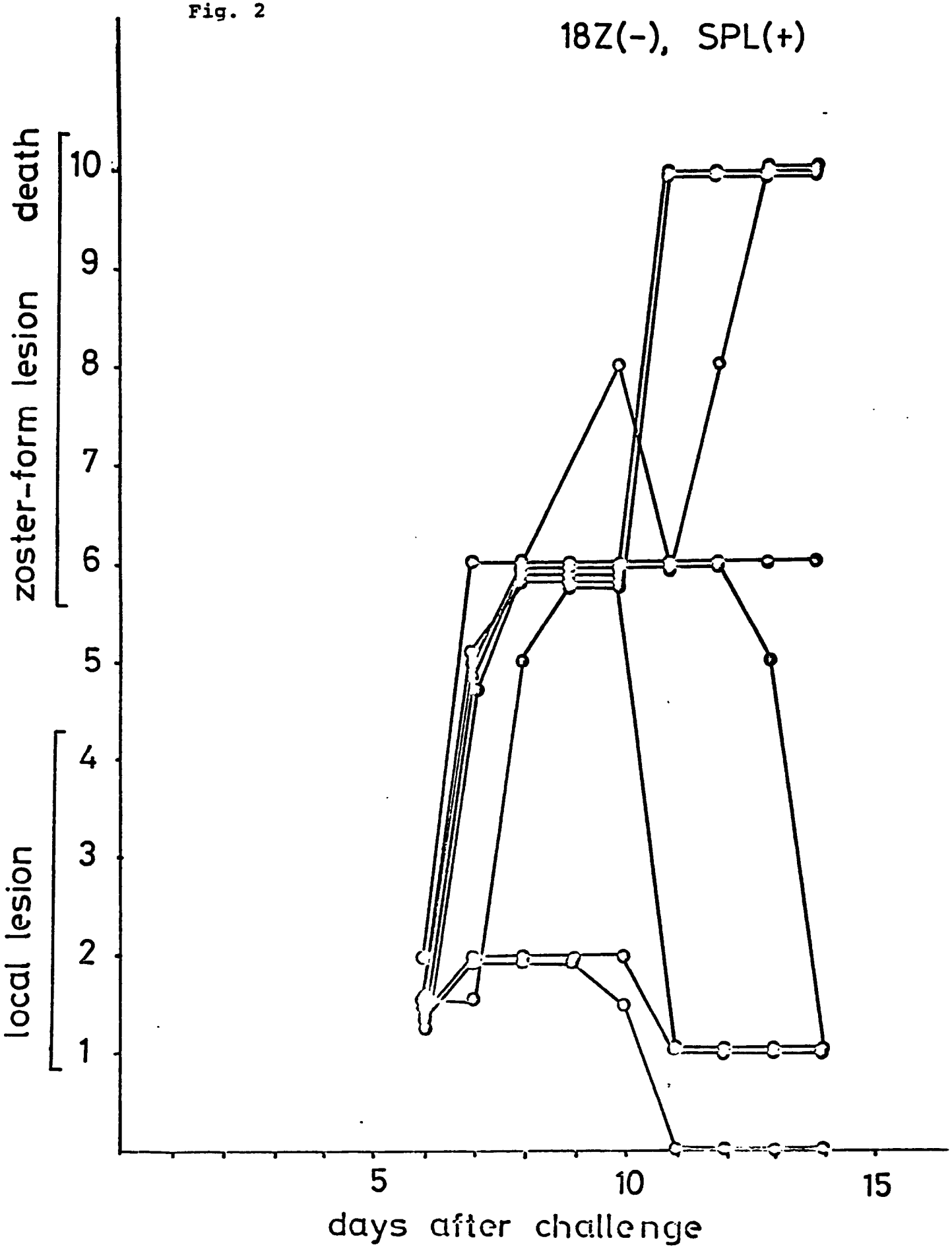

18Z(-), SPL(+)
death
zoster-form lesion
local lesion
10
9
8
7
6
5
4
3
2
1
5
10
15
days after challenge

Fig. 3

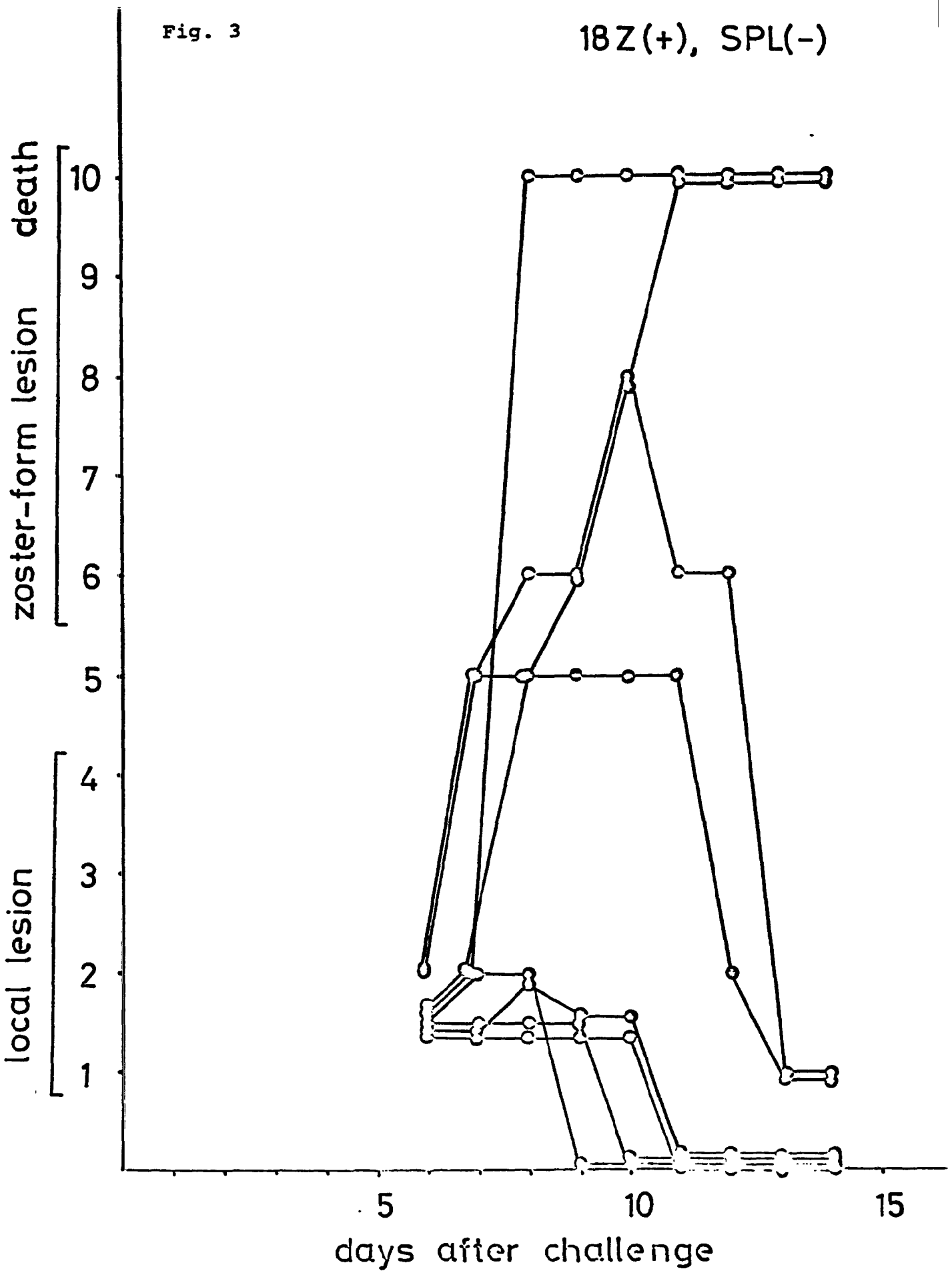

18Z(+), SPL(-)
death
zoster-form lesion
local lesion
10
9
8
7
6
5
4
3
2
1
5
10
15
days after challenge

Fig. 4

18 Z(+), SPL(+)

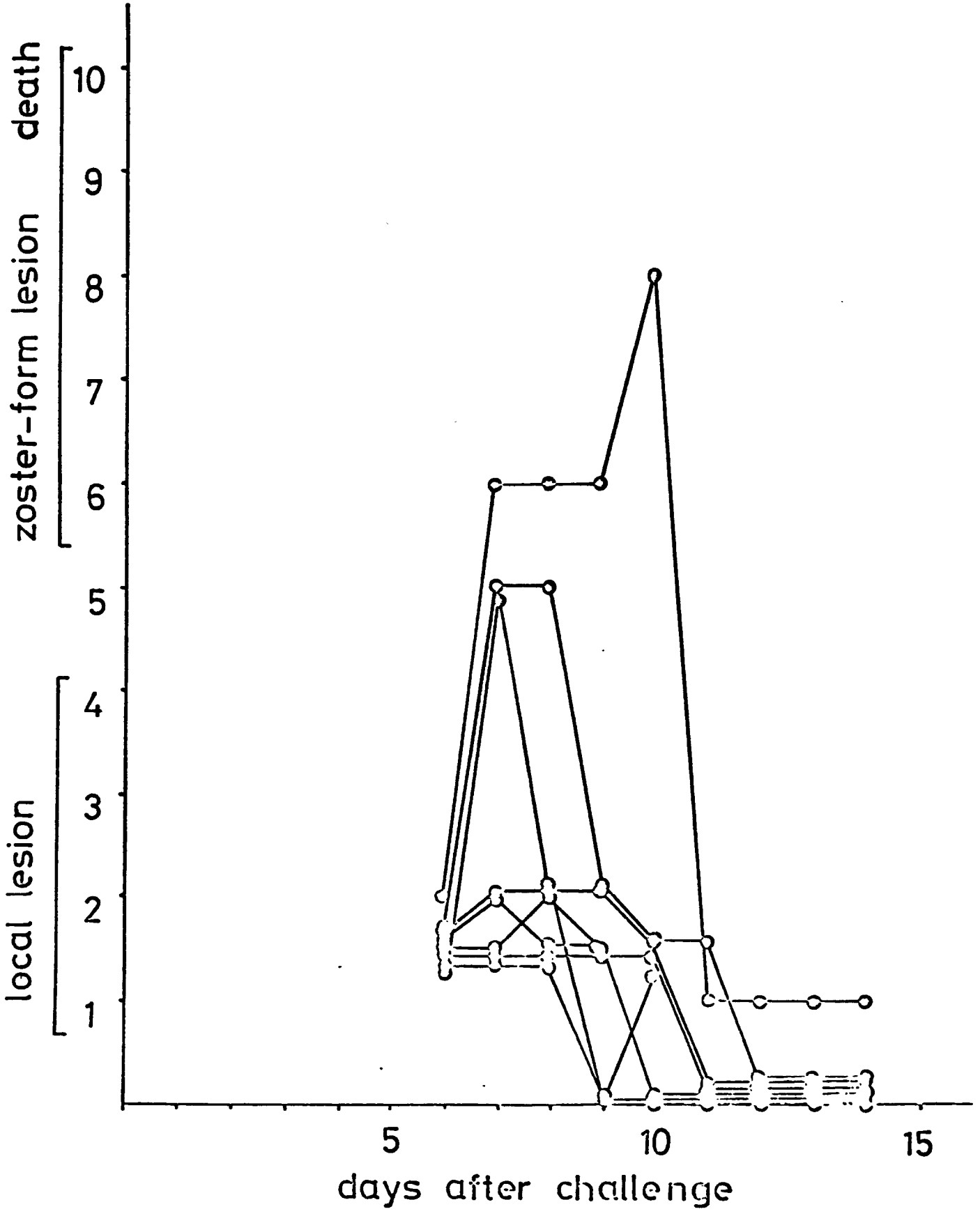
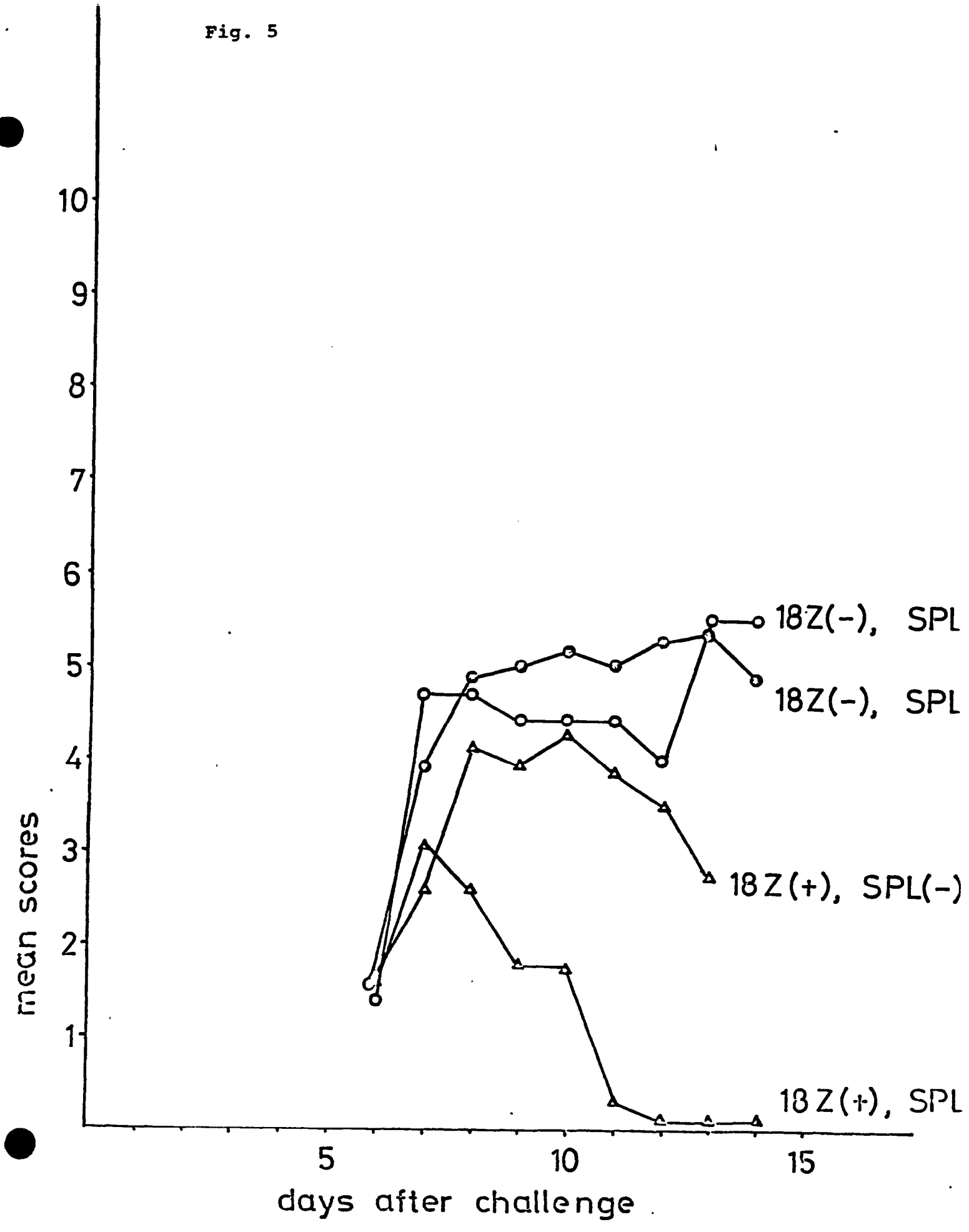

death
zoster-form lesion
local lesion
10
9
8
7
6
5
4
3
2
1
5
10
15
days after challenge

Fig. 5


10
9
8
7
6
5
4
3
2
1
mean scores
5
10
15
days after challenge
18Z(-), SPL
18Z(-), SPL
18Z(+), SPL(-)
18Z(+), SPL

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 $1x10^8$ viable organisms of Staphylococcus aureus (18Z) once a week for 4 or 8 weeks for the pre-treatment. For the mesurement 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, $1x10^6$ 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 assesed on day 10.

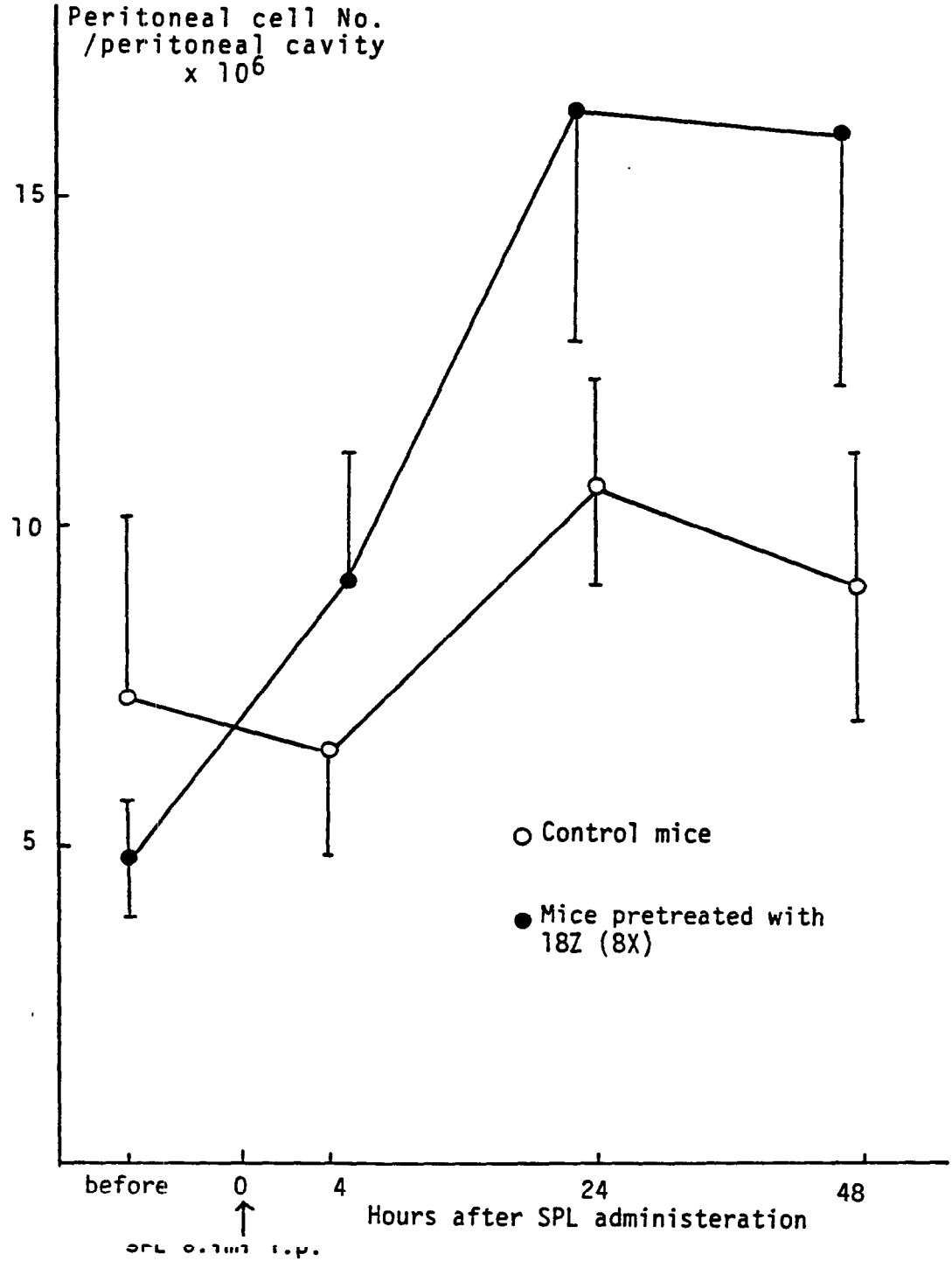
Mice were pretreated with 18Z for 8 weeks and 0.1 ml of SPL was injected intraperitoneally 48 hr after the last inoculation of 18Z. Peritoneal cells were counted before the inoculation of SPL and 4,24 and 48 hr after the inoculation. When SPL was injected into normal controls,

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 administeration of SPL (0.1 ml) in normal and 18Z-pretreated mice. (mean of 5 ± S.D.)



ivity of SPL against Sarcoma 180 in ddY mice.

arcoma 180 mplantation ell No./body)	SPL (ml/body)	Volume of ascitic fluid	Total packed cell volume	Cell growth (T/C)	Activity
i.p.	i.p.	ml	ml	%	
	1.00	0	-	-	+++
$1x10^6$	0.30	0.55	0.12	16.9	++
	0.10	1.08	0.35	49.3	+
	0	1.98	0.71	100	-

ivity of SPL against Sarcoma 180 in ddY mice.

arcoma 180 mplantation ell No./body) i.p.	SPL (ml/body) i.p.	Volume of ascitic fluid ml	Total packed cell volume ml	Cell growth (T/C) %	Activity
$1x10^6$	1.00	0	-	-	+++
	0.30	0.75	0.27	33.8	++
	0.10	1.92	0.64	80.0	±
	0	2.30	0.80	100	-

MASON RESEARCH INSTITUTE

Worcester, Massachusetts 01608

CONCEPT OF INDUCTION AND ELICITATION

by Arthur E. Bogden, Ph.D.
Director of Immunobiology

The hypothesis that recognition and destruction of transformed cells as they arise, is also effected by macrophages, is based upon experiments by Alexander and Evans (1) in which macrophages were shown to become cytotoxic to a range of sarcomata and lymphoma cells following exposure *in vitro* and *in vivo* to endotoxin and dsRNA at very low concentrations. Interestingly, lymphocytes are not involved in this reaction and macrophages from T-cell deprived mice, that are unable to mount specific immune reactions, can be rendered cytotoxic by such treatments.

Hibbs in 1973 (2) showed that the cytotoxic activity of endotoxin-treated macrophages was selective for transformed cells and left normal cells intact. Such tumoricidal macrophages were referred to by Alexander and Evans as being "activated" (1).

As in infection (3), so also in cancer (4) macrophages can affect immunologically specific cytotoxic reactions by acting in cooperation with lymphocytes that have been triggered by specific antigen. After recognizing tumor-specific antigens lymphocytes arm *in vitro* and *in vivo* macrophages that then acquire the capacity to kill the specific target cell. These specifically cytotoxic macrophages, as opposed to those generally tumoricidal, are only produced in animals with functioning T cells (5). Most importantly, such specific macrophages can be rendered generally tumoricidal by contact with a specific antigen (6) and by lymphokines released when antigens meet immune lymphoid cells (7,8). Consequently, as was shown by Hibbs, Lambert

Presented at the G.U. Cancer Immunology Workshop, Iowa, August 1976

and Remington (9), animals with persistent infection are frequently more resistant to cancer and their macrophages are tumoricidal.

The dangers involved and the lesions resulting from repeated injection of living microorganisms, such as BCG, into cancerous subjects having not only impaired immunological response capabilities but also undergoing an immunosuppressive therapy, is well documented. There is an inherent risk which may not always be acceptable in the use of living organisms, albeit non-pathogeneic in non-cancerous subjects, to stimulate cell mediated immunological responses under these conditions.

The concept of "Induction and Elicitation" propounded by Stuart Mudd, circumvents the dangers of infecting the immunologically impaired individual without abrogating the effectiveness of the antigenic stimulus provided by an initial infection with living organisms. Mudd's concept utilizes a naturally established hypersensitivity to Staphlococcus aureus as the primary induction step for the more effective use of bacterial fractions, such as staphylococcal phage lysate, in the subsequent elicitation of a non-specific, cell-mediated immune response.

The multifactorial pathogenesis of staphylococcal infection and the bodily responses thereto have been reviewed by Mudd (10). Staphylococcus aureus is a gram-positive, pyogenic, opportunist invader, and colonization, carrier states and inapparent infections, as well as manifest infections with S. aureus, occur throughout life. In fact, the close association of human subjects and staphylococci may be considered as beginning with infeciton of the umbilical stump and colonization on the skin of the newborn, and continuing thereafter

in the form of nasal and nasopharyngeal carriage, as well as various inapparent and apparent infections. It is not surprising, therefore, that delayed-type hypersensitivity to staphylococcal antigens is found to be as common in human populations (>90%) as were positive tuberculin reactions in urban populations two generations ago (11).

Therefore, the ubiquitous delayed-type hypersensitivity to S. aureus evident in man (11), affords the primary induction step in immunologically competent, non-cancerous subjects, for the subsequent and more effective use of bacterial fractions as eliciting agents in immunotherapy.

By elicitation is meant the evocation of lymphokines and the activation of macrophages in response to antigens homologous with the pathogenic agent. In this context, elicitation is produced by a staphylococcal phage lysate (SPL), a potent immunizing agent commercially available and FDA approved for clinical and experimental use[1]. Elicitation by staphylococcal antigens is being practiced effectively in human subjects having diseases other than malignancies (12,13,14, 15).

Thus, the second stage in eliciting cell mediated resistance requires bringing about contact of the homologous antigen with reticuloendothelial cells which are committed to reaction with the antigens of the infectious agent in question. As with all immunoadjuvants, dosage, timing and route of administration are important. Under appropriate circumstances contact between committed reticuloendothelial

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[1] Staphage Lysate, Delmont Laboratories, Swarthmore, Pa.

cells and homologous antigen brings about an activation of the effector cells, the monocytes, and macrophages. In their activated state the "angry macrophages", as termed by Mudd, are non-specific in their augmented capacity to destroy intracellular agents (15).

The purpose of the study which follows was (a) to determine the response of normal rats to repeated injections of living Staphylococcus aureus, in terms of health and well being; (b) to test the effect of such treatment on tumor metastases with surgical excision of the primary tumor mass as adjunct therapy; and (c) to determine whether the elicitation step with staphylococcal phage lysate would provide any enhancement of immunotherapeutic effects.

This was a preliminary probe to determine toxicity and to get a feel of rat response to the induction and elicitation agents and no attempt was made to optimize any conditions nor to ascertain whether a hypersensitivity had, in fact, been induced against Staph. aureus.

For induction, Staphylococcus aureus, strain 18Z organisms, were injected, s.c., left side, at a concentration of 10^8 viable units per ml, at weekly intervals, for a period of 6 weeks prior to tumor implantation.

Specific elicitation was induced by injecting staphylococcal phage lysate i.p.

The results are summarized in the next slide.

SLIDE No. 1

Several observations can be made. For one, early surgery has a better prognosis in terms of "cure" than if surgery is delayed. Induction alone did not enhance the surgical "cure" rate. Induction followed by elicitation as an adjuvant therapy to surgery, effectively increased the number of "cures" over that of surgery alone.

Of particular interest, was the observation that there was regrowth of tumors at the implant site in several animals of each surgically treated group. However, only in animals receiving some form of immunotherapy was there evidence of regression of some of these tumors.

Although only a probe, the experimental evidence suggests that the concept of "induction and elicitation" using delayed hypersensitivity to the ubiquitous Staph. aureus, and the harmless staphylococcal phage lysate for eliciting the "angry macrophages", may be effectively applied as an immunotherapeutic modality in cancer.

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INDUCTION AND ELICITATION AS AN IMMUNOTHERAPEUTIC MODALITY IN METASTATIC MAMMARY CANCER

Group No.	Treatment	No. Alive / No. Treated	Percent "Cures"	Dying From Metastases Percent Increased Life Span
I	Tumor bearing, untreated Control	0/12	0	-
II	Surgery day 18	2/12	17	133
III	Surgery day 25	0/11	0	123
IV	Induction + Surgery day 18	2/12	17	150
V	Induction + Surgery day 18, plus elicitation days 12, 15, 17, 19 and 23	4/12	33	128
VI	Induction + Surgery day 18, plus elicitation every 4 days beginning day 12 to survival	3/11	27	180

Percent increased life span = test/control

215 KINGSWOOD 3-3368 215 KINGSWOOD 3-2747

DELMONT LABORATORIES, INC.

BIOLOGICAL SPECIALTIES

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

(Ref. to Esber Protocol
dated Jan. 24, 1978)

Tues., 1-31-78

Re: Phone communication from Arthur G. Bogden, Ph.D., Director of Immunobiology, and Henry Esber, Ph.D., Mason Research Institute, Worcester, Mass. to S. J. DeCourcy, Jr., D.Sc., Director and Responsible Head, Delmont Laboratories, Inc., Swarthmore, Pa. received 10:15 AM, Tues 1-31-78.

Subject: Progress report of near-completed assay for efficacy of SPL being carried out at the Mason Research Institute. (The test is the same as that used for testing the efficacy of MER as an immunoadjuvant for the National Cancer Institute). SPL was tested as to its protective efficacy against challenge with Klebsiella and Staphylococcus in mice.

The study will be completed by the end of this present week, at which time the data will be assembled, and a report will be forthcoming.

As of the time of the phone communication, results are already showing positive protection against challenge with both Klebsiella and Staphylococcus in mice induced with S. aureus and elicited with SPL. It was noted, however, that protection against challenge with S. aureus (homologous organism) was a bit more pronounced than against Klebsiella, which is to be expected.

Based on this study and previous experience with SPL*, Drs. Bogden and Esber classify SPL as an effective immune adjuvant (enhanced macrophage activity).

The test itself takes 10 days.

The overall test, including the time necessary to induce the animals to a state of staphylococcal hypersensitivity prior to administration of SPL and challenge, takes approximately 2 months.

Respectfully submitted,

Samuel J. DeCourcy, Jr., D.Sc.

(* see attached paper by Bogden)

EG&G MASON RESEARCH INSTITUTE

57 Union Street Worcester, Massachusetts 01608 (617) 791-0931

AN EQUAL OPPORTUNITY EMPLOYER

24 January 1978

Dr. Samuel J. DeCoursey, Jr.
Director of the Laboratory
Delmont Laboratories, Inc.
Biological Specialties
P.O. Box AA
Swarthmore, Pennsylvania 19081

JAN 26 1978

Dear Dr. DeCoursey:

Enclosed you will find a copy of the experimental protocol of a study entitled, "Host Resistance Enhancing Activity by Staphage Lysate in Mice". This protocol is designed in a similar fashion to the protocol used by the NCI for testing the immunostimulating activity of MER-BCG.

I hope to have the results by the middle of February. I will send you a complete report at that time.

If I can be of help, do not hesitate to contact me.

Sincerely yours,

Henry J. Esber, Ph.D.

HJE/mb

enclosure

MASON RESEARCH INSTITUTE

EXPERIMENTAL PROTOCOL

Group	Number of Animals	Treatment
A	10	Saline controls, 0.2 ml, i.p., 24 and 3 hrs. prior to infection.
B	10	Induction with 0.1 ml of S. aureus 18Z (10^8) s.c., at 28, 21 and 14 days prior to infection.
C	10	Elicitation with 0.1 ml of SPL, i.p., at 24 and 3 hours prior to infection.
D	10	Elicitation with 0.2 ml of SPL, i.p., at 24 and 3 hours prior to infection.
E	10	Elicitation with 0.1 ml of SPL, i.p., at 24 and 3 hours prior to infection and daily days 2-10 after infection.
F	10	Induction with 0.1 ml of S. aureus 18Z (10^8) s.c., at 28, 21 and 14 days prior to elicitation. Elicitation with 0.1 ml of SPL, i.p., at 24 and 3 hours prior to infection.
G	10	Induction with 0.1 ml of S. aureus 18Z (10^8) s.c., at 28, 21 and 14 days prior to elicitation. Elicitation with 0.2 ml of SPL, i.p., at 24 and 3 hours prior to infection.
H	10	Induction with 0.1 ml of S. aureus 18Z (10^8) s.c., at 28, 21 and 14 days prior to elicitation. Elicitation with 0.1 ml of SPL, i.p., at 24 and 3 hours prior to infection and daily days 2-10 after infection.
I	10	Induction with 0.1 ml of S. aureus 18Z (10^8) s.c., at 28, 21 and 14 days prior to elicitation. Elicitation with 0.2 ml of SPL, i.p., at 24 and 3 hours prior to infection and daily days 2-10 after infection.
J	10	Zymosan 3 mg/0.2 ml, i.p., 48 hours prior to infection.

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INJECTION SCHEDULE

Induction (S. aureus 18Z)	12/21/77 12/28/77 1/4/78	*DPI - 28 DPI - 21 DPI - 14
Elicitation (SPL)	1/23/78 1/24/78	DPI - 1 DPI - 0
Infection	1/24/78	DPI - 0
Elicitation (SPL)	1/25/78-2/2/78	DPI + 2 to DPI + 10

Induction: 0.1 ml., s.c., near axillary lymph nodes.

Infection: 0.1 ml. of a 6 hour culture of either pathogenic K. pneumoniae or pathogenic S. aureus.

Endpoints: Survival time over a 10 day period (daily observations).

* DPI = Days prior to infection
(De Courcy)

85. Measurement of nonspecific resistance to Escherichia coli induced in mice by repeated staphylococcal infections. MEHDI SHAYEGANI and LINDA M. PARSONS* Divisi[illegible] of [illegible] Research, New York State Department of [illegible], Albany, N.Y. [illegible].

Nonspecific resistance was induced in mice by a weekly subcutaneous injections of 10^6 viable staphylococcus aureus. One week after the last injection, the hypersensitized mice were elicited by two subcutaneous injections of staphylococcal bacteriophage lysate at 48 and 24 hours before challenge with E. coli. The E. coli strain was isolated in our laboratory from an infant with diarrhea. This strain is virulent in mice having an LD_{50} of 5×10^7 organisms by both the intraperitoneal and intravenous routes. However, it has proved to be negative for invasiveness, and for heat-labile and heat-stable enterotoxins. It also could not be serotyped with antisera commonly used for typing enteropathogenic strains. The resistance of infected mice to E. coli was compared to that of normal mice using a quantitative measure of the number of organisms found in blood, liver, and spleen at various time intervals after intraperitoneal challenge. The counts from all organs in treated and untreated mice were similar at ½ and 2 hours. At 6 hours, the counts from the normal mice had risen to the 10^8 range, while the infected mice had counts that peaked in the 10^7 range. At 24 hours, all normal mice were already dead, while the infected mice survived. The counts on these surviving mice had dropped to the 10^5 range in the liver and spleen and to the 10^1 to 10^3 range in the blood. Intravenous challenge with E. coli was also studied. Although a similar pattern of outcome was observed, there were differences in survival time of the normal mice and in the number of organisms recovered.

RES, Dec, 1976

Official Abstract Form

(Read all instructions before typing)

Development of Delayed-type Hypersensitivity and Non-specific Protection in Mice by Repeated Staphylococcal Infections. M. SHAYEGANI,* L. M. PARSONS and P. S. MAUPIN. NYS Department of Health, Albany, NY.

The development and duration of delayed-type hypersensitivity (DTH) and non-specific protection was investigated in mice repeatedly infected with 10^8 live Staphylococcus aureus. Eight weekly subcutaneous injections resulted in a gradual increase of DTH in the infected mice, measured by footpad swelling. The increase in DTH reactions paralleled a continual increase in the survival of the infected mice when, after elicitation with staphylococcal antigens*, they were challenged intraperitoneally with a lethal dose of Escherichia coli. A quantitative measure of E. coli recovered from blood, spleen and liver after challenge showed the infected and elicited mice had significantly lower numbers of systemic E. coli than did normal controls.

The DTH response and non-specific protection in these infected mice have been recalled by injections of staphylococcal antigens* for periods up to 3 months after the 8th staph injection.

The DTH response to staphylococcal antigens was transferred from mice infected 8 times to normal mice by spleen cell transfer. We are currently studying the transfer of non-specific protection against E. coli challenge using spleen or lymph node cells.

☐ A

☐ R

☐ P

☐ PS

*Staphage Lysate (Delmont)

Ref. Proc. Nat'l Mtg. A.S.M., Las Vegas, N.M., May 1978 (To be presented)

Authors: Shayegani, M., Parsons, L.M., and Maupin, P.S.
Division of Laboratories and Research, N.Y.
State Department of Health, Albany, N.Y. 12201

III. MEDICAL RATIONALE AND PURPOSE

III. Medical Rationale and Purpose

Staphage Lysate (SPL) are prepared by lysing parent cultures of *Staphylococcus aureus*, Serologic Types I and III, with a polyvalent staphylococcal bacteriophage. After ultrafiltration, the lysates, which are staphylococcal vaccines, contain active bacteriophage and heat labile and heat stable antigenic fractions plus the extracellular and intracellular enzymes of *S. aureus* and culture medium ingredients (sodium chloride, beef heart infusion: Bactor Tryptose). To maintain maximum immunogenic potency, no preservative is added to SPL.

SPL is standardized on the basis of bacterial cell content before lysis. Each milliliter contains the lysate of 120-180 million colony-forming units of *S. aureus*, and 100-1000 million staphylococcus bacteriophage plaque-forming units.

At one time, clinical benefits associated with administration of SPL were attributed to its ability to induce antibacterial antibodies. Recent animal studies and clinical investigations in humans suggest, however, that SPL's effectiveness instead depends on its favorable stimulating effect on the cell-mediated immunity of the host to *Staphylococcus* -- *i.e.*, that SPL acts as an immunopotentiator.

Cellular hypersensitivity to S. aureus is exhibited by most human subjects. Its augmentation may have beneficial results in various Staphylococcal conditions. A variety of clinical studies have reported favorable effects when SPL was employed against a variety of diseases. The work of Salmon and Symonds is particularly significant. (Salmon, G. and Symonds, M., "Staphage Lysate Therapy in Chronic Staphylococcal Infections," J. Med. Soc. N.J., 60: 180-193 (1963).) Patients treated had a variety of chronic staphylococcal infections, all of which were characterized by colonization of the host with staphylococcal pyogenes. A total of 607 patients were studied. Most patients had received courses of tetracycline, penicillin, chloromycetin, and a sulfa derivative and had proved refractory. This prior treatment served, in effect, as a treatment control or a historical control. Other studies include Baker, A.G., "The Treatment of Chronic Bronchial Asthma," Amer. Prac. Dig. Treat. 9: 591-598 (1958); Baker, A.G., Staphylococcus bacteriophage Lysate: Topical and Parenteral Use in Allergenic Patients," Penn. Med. J. 66: 25-28 (1963); and Mudd, S., Taubler, J.H., and Baker, A.G., "Delayed-Type Hypersensitivity to Staphylococcus aureus in Human Subjects," J. Retculoend. Soc., 8: 493-498.

A concomitant, though not necessarily causally related, increased capability of the macrophages to inactivate

Staphylococci has been demonstrated in the laboratory following SPL treatment. (Lenhart, N. and Mudd, S., "Staphylococcidal Capability of Rabbit Peritoneal Macrophages in Relation to Infection and Elicitation: Induction and Elicitation of Activated Macrophages," Infect. Immunity, 5: 763-768 (1972); Mudd, S. "Resistance Against Staphylococcus aureus, JAMA 218: 1671-1673 (1971); Shayegani, M., DeCourcy, S.J., Jr., and Mudd, S., "Cell-Mediated Immunity in Mice Infected with S. aureus and Elicited with Specific Bacterial Antigens," J. Res. 14: 44-51 (1973); Mudd, S. and Shayegani, "Delayed-Type Hypersensitivity to S. aureus and Its Uses," Ann. N.Y. Acad. Sci. 236: 244-251 (1974).) In addition, SPL has been shown to stimulate lymphoproliferative responses to both T and B cell subpopulations present in peripheral and cord blood in normal human subjects. (Dean, J.H., et al., "In Vitro Human Reactivity to Staphylococcal Phage Lysate," J. Immunol. 115: 1060-1064 (1975); Dean, J.H., et al., "Functional Activities of Rosette Separated Human Peripheral Blood Leukocytes," J. Immunol. 115: 1449-1455 (1975).) These findings support the interpretation that SPL in staphylococcal hypersensitive subjects acts as an immunopotentiator of nonspecific cell immunity.

The recently reported clinical work of Tsuda and Minami of Kurume University in Japan is also significant. (Tsuda, S. and Minami, K., "Immunochemotherapy for Infections --

with Particular Reference to Staphage Lysate," item 7 accompanying letter dated December 28, 1977, from Yozo Mitsubara to Charles E. Lincoln, included in section II of this submission.)

Of great potential significance is the clinical study being conducted by John S. Silva, M.D., at the Veterans Administration Hospital in Beloxi, Mississippi, to test the effectiveness of SPL in prevention of wound infections in surgical patients. A protocol for the study is included with this submission.

The data from the studies described above support a conclusion that Staphage Lysate products are effective in the treatment of Staphylococcal diseases. Although the more recent studies have altered our understanding about the products' mode of action, they have not changed the medical community's perception that SPL is effective in the treatment of significant disease entities.

IV. SIGNED STATEMENT OF RESPONSIBLE OFFICIAL

215 KINGSWOOD 3-3365 215 KINGSWOOD 3-2747

DELMONT LABORATORIES, INC.
BIOLOGICAL SPECIALTIES
P. O. BOX AA, SWARTHMORE, PENNSYLVANIA 19081, U.S.A.

February 3, 1978

Mr. John J. Singleton
Bureau of Biologics
HFB 620
6110 Executive Blvd.
Rockville, Maryland 20857

Dear Mr. Singleton:

This submission includes in full (or incorporates by reference as permitted in Par. 314.200 (c) (2)) all studies and information specified in Par. 314.200 (d).

Respectfully submitted,
DELMONT LABORATORIES, INC.

Samuel J. DeCourcy, Jr.
Samuel J. DeCourcy, Jr., D.Sc.
Responsible Head

SJDC/sl

215 KINGSWOOD 3-3365 215 KINGSWOOD 3-2747

DELMONT LABORATORIES, INC.
BIOLOGICAL SPECIALTIES
P. O. BOX AA, SWARTHMORE, PENNSYLVANIA 19081, U.S.A.

February 3, 1978

To Whom It May Concern:

Study of Longevity of Phage activity in Filing Samples of SPL.

by S. J. DeCourcy, Jr., DSc. and D. Gottlieb, B.S.

The attached chart demonstrates quite conclusively that the phage counts in lots assayed as long as 18 months following the expiration date are no lower than the corresponding phage counts obtained from assays of the same lots made at the time of completion of the fill. Thus, these results will answer the question posed in the Federal Register, Nov. 8, 1977, Part II, Page 58284, Column 3, par. 5 ("It is also significant that -----etc.).

Respectfully submitted,

Samuel J. DeCourcy, Jr., D.Sc.

Study of Longevity of Phage in Filing Samples of SPL

(Types I and III Vials)

S. J. DeCourcy, Jr. D.Sc. + Doris Gottlieb, B.S.
Delmont Labs., Inc.

Production Lots Used in Fills (Bottle #)	Fill # number	Designated number	Fill Date	Expiration date of Fill Lot.	Initial ∅ count of Production Lots PFU/ml	∅ Count of Fill Lot. PFU/ml	∅ count of Expired Lots (till) PFU/ml	Elapsed time between Prod Lot and Exp. Date	Elapsed time between Fill Lot and Exp. Date.
I 4120994(4) III 5021099(1)	4120901	1	3-11-75	Mar. 9, '76	I .7 X 10^9 III 1.47 X 10^9	.47 X 10^9	11/30/77 .52 X 10^9	15 mo.	~ 1 yr
I 4022572(1) III 4012169(4)	4012176	2	3-28-74	Apr. 21, '75	I 1.39 X 10^9 III 1.06 X 10^9	.4 X 10^9	11/30/77 .13 X 10^9	15 mo	~ 1 yr
I 4022572(4) III 4032595(2)	4022581	3	5-21-74	May 25, '75	I 1.09 X 10^9 III 1.1 X 10^9	.52 X 10^9	12/1/77 .30 X 10^9	15 mo.	~ 1 yr.
I 4040 392(2) III 4032595 (4)	4032584	4	7-9-74	June 25, '75	I 1.1 X 10^9 III 1.1 X 10^9	.4 X 10^9	12/1/77 .42 X 10^9	15 mo	~ 1 yr. (2 wks)
I 4081286 (1) III 4040177 (2)	4040190	5	9-25-74	July 1, '75	I 1.02 X 10^9 III 1.0 X 10^9	.3 X 10^9	12/1/77 .32 X 10^9	15 Mo	9 mo
I 4181286(3) III 4040177(3)	4040195	6	12-12-74	July 1, '75	I 1.18 X 10^9 III 1.1 X 10^9	.24 X 10^9	12/1/77 .28 X 10^9	15 mo.	7 mo.
I 4120994(1) III 4093089(2)	4093096	7	1-17-75	Dec. 30, '75	I 1.1 X 10^9 III 1.01 X 10^9	.43 X 10^9	11/77 .35 X 10^9	15 mo	~ 1 yr
I 5021700(1) III 5021099(3)	5021004	8	6-8-75	May 10, '76	I 1.03 X 10^9 III 1.3 X 10^9	.69 X 10^9	11/77 .45 X 10^9	15 mo	1 yr
I 5031700(4) III 5021099 (5)	5021006	9	6-26-75	May 10, '76	I 0.9 X 10^9 III 1.1 X 10^9	.51 X 10^9	12/13 .56 X 10^9	15 mo	10½ mo.
I 5071407 (1) III 5072808(2)	5071411	10	9-~~30~~ 13-75	Sept 25, 1976	I .63 X 10^9 III .97 X 10^9	.55 X 10^9	7/13 .54 X 10^9	15 mo	~ 1 yr
I 5072808(3) III 5071407(3)	5071414	11	9-30-75	Oct 14, 1976	I .6 X 10^9 III 1.03 X 10^9	.5 X 10^9	7/14 .34 X 10^9	15 mo	~ 1 yr
I 5072808(5) III 5071407(5)	5071421	12	2-4-76	Oct 14, 1976	I .8 X 10^9 III 1.01 X 10^9	.5 X 10^9	7/14 .30 X 10^9	15 mo.	8 mo.
I 5092912(1) III 5100613(1)	5092916	13	11-5-75	Dec. 29, 1976	I 1.05 X 10^9 III 1.24 X 10^9	.55 X 10^9		15 mo.	14 mo.
I 5092912(2) III 5100613(2)	5092916	13	11-5-75	Dec 29, 1976	I 1.16 X 10^9 III 1.18 X 10^9	"		15 mo.	"