AD-A054 345 STRITCH SCHOOL OF MEDICINE MAYWOOD ILL DEPT OF MICRO--ETC F/G 6/15
NEW APPROACHES TO CHEMOTHERAPY OF VIRAL DISEASES.(U)
APR 78 P GORDON N00014-77-C-0507
UNCLASSIFIED NL

| OF |
ADA
054345

END
DATE
FILMED
6-78
DDC

FOR FURTHER TRAN

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: Report No. 1
2. GOVT ACCESSION NO.
3. RECIPIENT'S CATALOG NUMBER

4. TITLE (and Subtitle): New Approaches to Chemotherapy of Viral Diseases.
5. TYPE OF REPORT & PERIOD COVERED: Final; 1 July 77 to 31 December 77
6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s): Paul Gordon Ph. D.
8. CONTRACT OR GRANT NUMBER(s): N00014-77-C-0507

9. PERFORMING ORGANIZATION NAME AND ADDRESS: Department of Microbiology, Loyola University Stritch School of Medicine, Maywood, IL 60153
10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: NR 204-063

11. CONTROLLING OFFICE NAME AND ADDRESS: Office of Naval Research, Arlington, VA.
12. REPORT DATE: 19 April 78
13. NUMBER OF PAGES: 13

14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office): Office of Naval Research, Chicago Branch Office, 536 S. Clark, Chicago IL 60605
15. SECURITY CLASS. (of this report): Unclassified
15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

DISTRIBUTION OF THIS REPORT IS UNLIMITED

Final rept. 1 Jul - 31 Dec 77,

DDC RECEIVED MAY 31 1978 D

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Antiviral drug
Stress
Herpes virus
Macrophage
Guanosine-3',5'-monophosphate

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Two aspects of previously established but insufficiently examined actions of SM-1213 were investigated during the contract period (six months): (a) the interaction of SM-1213 with immunomodulatory stressors; and (b) the effects of SM-1213 on macrophage function. Psychosocial stress induced by housing female mice in pairs and "infection" stress generated with a subclinical herpes simplex type 1 viral infection were examined for their effects on immediate and delayed type hypersensitivity responses to RBC

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE S/N 0102-014-6601

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

407 516

AD A054345

AD No. ____
DDC FILE COPY

20. Abstract (continued)

antigens in the presence and absence of drug treatment (80 mg/kg/day SM-1213) administered ad libitum beginning on Day 6 following immunization. Both classes of stress produced immunosuppression which was restored by treatment with drug; herpes infection lowered antibody levels that were restored by treatment. As expected, high and low psychosocial stress inputs generated animals with significantly different adrenal weights, while SM-1213 treatment tended to prevent the shift in weight produced by change in environment. In mouse macrophages infected by herpes simplex type 2 virus, studies employing fluorescent antibody techniques showed drug to significantly reduce the number of infected macrophages. Other studies showed that SM-1213 enhanced the chemiluminescence in non-elicited mouse macrophages and increased the peroxidase-catalase ratio in oil-elicited guinea pig macrophage phagosomes. A generalized stimulation of protein synthesis and secretion by SM-1213 treated macrophages was also noted.

FINAL REPORT ON ONR CONTRACT N00014-77-C-0507, NR 204-063

PROJECT TITLE: New Approaches to Chemotherapy of Viral Diseases

PRINCIPAL INVESTIGATOR: Paul Gordon, Ph. D.

INSTITUTION: Department of Microbiology, Loyola University Stritch School of Medicine, Maywood, IL 60153

PERIOD OF PERFORMANCE: 1 July 1977 - 31 December 1977

This contract was awarded for the study of a new drug with antiviral activity, called SM-1213.* In our original proposal, SM-1213 was shown to have the capacity to modulate the immune response of mice when the animals were subjected to environmental stress. It was postulated that the immunomodulatory action of SM-1213 might result in the blockade of certain immunosuppressive actions of a viral infection and thus contribute to the drug's antiviral action. In addition, recent work elsewhere has revealed that extremely low concentrations of the drug enhance microbicidal action of macrophages and similar concentrations elevate guanosine-3',5'-monophosphate (cGMP) in these same cells (personal communication, J.W. Hadden, Sloan-Kettering Institute, N. Y.). This report will cover studies in our laboratory which bear on the influence of SM-1213 on adrenal responses to environmental stress and on functional immunosuppression by herpes simplex virus; the drug's effect on virucidal action of mouse macrophages towards this same agent will also be demonstrated. In addition, data showing the drug's action on chemiluminescent activity of macrophages will be presented.

1. Interaction of SM-1213 with Immunomodulatory Stressors

A. Social Stress

In our original proposal we described a mouse model wherein hypersensitivity responses to human red blood cell (HuRBC) antigens were evaluated by measuring paw swelling responses. These hypersensitivity responses, which included anaphylaxis (15 minute), Arthus (4 hour), and delayed-type hypersensitivity (24/48 hour) responses, are readily influenced by mild stress, such as housing density variation ranging from 2-12 animals per cage. The immunosuppressive influence of housing female mice at 2 per cage relative to responses in higher densities is illustrated in Table I.

It is established that male mice differ from female mice in their response to selected housing conditions. Male mice in groups continuously develop and maintain social ranking systems through active fighting, and it has been observed that isolated males have significantly higher circulating antibody titers than do grouped mice of this sex (for a typical reference see Vessey, S.H., Proc. Soc. Exper. Biol. Med. 115:252, 1964).

---

* SM-1213 is the code name for the compound 1,2-O-isopropylidene-3-O-3'-(N',N'-dimethylamino-n-propyl)-D-glucofuranose under development by Strategic Medical Research Corp., Greenwich, CT. U. S. Patents #3,939,145; #3,939,146; #3,965,262; #4,016,261; #4,017,608; and #4,056,322.

Presumably, catecholaminic, adrenocortical and other secretions related to stress exert their effects here.

Female mice, in contrast, exhibit a different pattern of response to housing conditions, which the investigator must know about and control. Thus, female mice have been reported to exhibit higher antibody titer when housed in groups than when housed in isolation (Glen, W.G., and Becker, R.E., J. Physiol. Zoology 42:411, 1969).

We have extended our studies to evaluation of adrenal and thymic weights in Ha/ICR female mice housed at either 2 or 12 per cage, immunized with HuRBC and treated with 80 mg/kg of SM-1213 per day in the drinking water beginning Day 6 and continued until sacrifice. Following two paw challenges with HuRBC, the animals were sacrificed by etherization and cardiac puncture on Day 33 (Day 5 following the last challenge) and fresh organ weights were determined. The adrenal and thymic indices (organ weights adjusted for body weight) are shown in Table II.

A reduction of adrenal weight by 35% is seen in control animals simply by housing the animals at a density of 12 per cage rather than 2 per cage. Drug treatment with what we now classify as a high dose (80 mg/kg/day) of SM-1213 reduces adrenal size in the "stressed"* animals (2 per cage) by 20% while elevating the adrenal size in the "unstressed"* animals (12 per cage) by 21%. This modulation of adrenal weight by the drug is very similar to the modulation of immune responsiveness by SM-1213 seen under these conditions of housing density variation, reported in the original proposal.

The only change in thymic weight occurred in the "unstressed" mice, where it was elevated in SM-1213 treated animals by 18%.

These studies are preliminary in nature and their interpretation is complex. However, since we now have evidence that SM-1213 stimulates cGMP formation and have learned from the literature that cGMP appears to act as a second messenger for ACTH action on the adrenal cortex (Perchellet, J.-P., Shanker, G., and Sharma, R.K., Science 199:311, 1978), we can hypothesize that SM-1213 could act by altering ACTH activity and thus corticosterone release from the adrenal. The increased thymic weight in drug-treated, "unstressed" mice may indicate reduced chronic corticosterone

---

* Our use of the terms "stressed" and "unstressed" here refer only to the the parameter of housing density. All of these animals were subjected to considerable handling, anesthesia, and general environmental stress during the course of the immunization experiment, but all animals were handled equally during these procedures.

release in animals not subjected to the additional insult of social (and perhaps thermal) stress* resulting from housing in pairs.

Another aspect of stress which should be considered with respect to the drug effect in this system is that influenced by analgesia. Recently it was shown that direct application of cGMP to the brain generated an analgesic effect similar to that of morphine without the depressant effects of morphine (Cohn, M.L., Cohn, M., and Taylor, F.H., Science 199:319, 1978). It is not yet known whether a drug such as SM-1213 can generate cGMP in the appropriate CNS centers and thus have an analgesic action. However, if it has this action, it would probably influence handling stress as perceived by the mice.

Relevant to this concept is a study we have performed to measure the emotionality of SM-1213 treated Ha/ICR female mice. Animals housed in groups of 8 were treated with 80 mg/kg/day of SM-1213 ad libitum in acidified drinking water for 14 days. Controls received only acidified drinking water. The animals were then subjected to a single 4-minute trial of open field running supervised by a behaviorist, Dr. M. Preache, of the Illinois Institute of Technology Research Institute. Their exploratory behavior was quantified by a photo-cell system. Treated and control animals demonstrated essentially identical behavior in this system (controls: 272 light beam interruptions/4 minutes; SM-1213 treated: 277 light beam interruptions/4 minutes) and thus the drug does not appear to have a depressant effect at the high dose of 80 mg/kg/day for 2 weeks.

Further analysis of the effect of SM-1213 on the pituitary-adrenal-thymic axis will examine the effect of ACTH directly in drug-treated mice and in adrenal cell cultures. The possible analgesic action of SM-1213 will also be examined.

B. Infection Stress

Noting the interaction of SM-1213 with stress states of mice, we postulated that a possible means by which the drug could exert its anti-viral action was by blocking the immunosuppressive action of the viral infection per se. Such a blockade might render the animal more competent to combat the infection itself, and, potentially, secondary infections by opportunistic pathogens in the environment. In the context of Section IA above, it is pertinent to mention that an acute viral infection by a non-cytopathic virus results in a massive perturbation of the adrenal cortex

---

* We have assumed, but net yet quantified, increased social stress in paired mice due to the increased vocal activity, fighting and fearfulness we have observed in these animals compared to that of animals housed 8-12 per cage. However, mild thermal stress must be considered when animals are housed at low densities. Under these conditions they are unable to huddle for warmth and mice actively use behavioral thermoregulation to compensate for their small body size. The room temperature is maintained at approximately 75°F where these experiments are conducted, and huddling during low activity periods is commonly seen in "high" density housing groups.

as indicated by greatly elevated serum corticosterone levels (Riley, V., and Spackman, D., Fogarty International Center Proceed. No. 28, DHEW Publ. No. (NIH)77-893:319, 1974).

For the study of infection stress we utilized the HuRBC hypersensitivity system employed above in A/J mice infected with a low, subclinical dose of herpes simplex type 1, strain L (HSV-L). (A/J mice were chosen as they are highly susceptible to HSV infections.) The protocol for this experiment is outlined below:

Day 0 - Immunize with 10^9 human type O red cells by the subcutaneous route.
Day 5 - Infect with herpes simplex virus by the intraperitoneal route.
Day 6 - Initiate drug in the drinking water.
Day 10 - Challenge with 10^8 red cells inoculated subcutaneously in one hind paw. Observe for immediate and delayed hypersensitivity.
Day 13 - Bleed for serum and freeze tissue samples for virus titrations.

The results are presented in Table III. The immunosuppressive effect of the virus is evidenced by the reduced delayed-type hypersensitivity response and reduced hemagglutinin antibody response seen in the viral infected, placebo-treated animals. Restoration of these two parameters is achieved by SM-1213 treatment. (The drug effect noted in uninfected controls is commonly seen when baseline paw swelling is at the levels shown for placebo-treated, uninfected mice.)

In the course of confirming these studies we observed that the A/J mice were ill upon receipt from the supplier. Gross pathology in these animals suggested a severe infection with encephalomyocarditis virus. Since this strain is not available from other suppliers, we are currently developing the model in the Balb/c strain which is more resistant to HSV. These studies are in progress.

2. Effects of SM-1213 on Macrophage Function

A. Potentiation of Virucidal Action

In view of the well-recognized central role for the macrophage in defense against herpes simplex viruses in mice, and the information regarding the effect of SM-1213 on microbicidal action of macrophages received from J.W. Hadden of Sloan-Kettering, we examined the influence of the drug on the growth of a highly pathogenic strain of HSV-2 virus in non-elicited mouse peritoneal adherent cells.

Unstimulated peritoneal adherent cells from Balb/c mice were cultured in a rich medium consisting of 50% HI-W05/BA2000 supplemented with 50% fetal calf serum and 50 μg Gentamycin/ml for 24-48 hours in Lab-Tek chamber slides. The adherent cell monolayers, consisting of approximately 80%

macrophages, 15% mast cells, and 5% non-macrophage mononuclear cells, were infected (or sham-infected) with 1.2 x 10^6 PFU HSV-2 (Chang strain) for 1 hour. The viral inoculum was removed and the cells were fed with the same medium supplemented with conditioned medium and various concentrations of SM-1213. Following appropriate incubation in the drug-supplemented medium, the slides were washed and fixed with acetone, reacted with guinea pig serum containing antibodies to HSV, and overlaid with fluorescine isothiocyanate-labeled rabbit anti-guinea pig IgG. Without knowledge of the treatment, fluorescent cells were enumerated in 5-6 fields of view, counting about 500 total cells. The data from these studies is presented in Table IV.

Note that, while the drug effect at a concentration of 0.1 μg/ml is somewhat erratic, a definitive and highly statistically significant ($p < 0.001$) reduction of viral-infected adherent cells is seen when the lower dose of 0.01 μg/ml SM-1213 is applied. Because this reduced viral intracellular antigen is seen at 6 hours, a very early stage in the infection, it would appear that otherwise susceptible cells are made resistant to viral replication although the drug is not added until after viral adsorption and probably penetration have occurred. This suggests that the virus is destroyed before it initiates replication, perhaps by enzymatic action of the cells. Studies of SM-1213 action of macrophage metabolism are reported in the final section.

B. Macrophage Metabolism

Certain antimicrobial actions of phagocytes have been attributed to the action of oxidizing systems that locate in phagosomes during and after phagocytosis, and which generate singlet oxygen, superoxide and hydrogen peroxide that can oxidize and irreversibly injure microbial surfaces. The existence of superoxides and related highly reactive oxidative free radicals can be determined by their chemiluminescence. On the other hand, enzyme control over antimicrobial oxidative processes is exerted in part by catalase and peroxidase activities. Of these enzymes, catalase, by converting hydrogen peroxide directly into oxygen and water, may act to limit the oxidation by peroxide of substrates such as bacterial or viral components; while peroxidase acts to enhance the rate of peroxide oxidation of substrate by generating an activated halogen intermediate or by facilitating the oxidative capacity of hydrogen peroxide on substrates directly.

We were interested, therefore, in determining whether SM-1213 might enhance the capacity of macrophages to generate antimicrobial oxidative metabolites. Towards this end, studies were carried out on peritoneal macrophages from unstimulated Balb/c female mice in which the chemiluminescent metabolites induced by phagocytosis of heat-killed Candida albicans were estimated by the chemiluminescence of harvested macrophages under specified incubation conditions. (Chemiluminescence quantifies the generation of biologically active free radicals derived from O_2, such as superoxide, singlet oxygen, etc.) Chemiluminescence was determined by scintillation counting in the coincident mode at room temperature following incubation of 2 x 10^7 cells in duplicate in HI-WO_5/BA_{2000} medium under

CO_2 for the cited times at 37°C. The results of a typical study are displayed in Table V.

We were also interested in determining whether SM-1213 might alter enzyme activity in macrophage phagosomes. Towards this end, a series of studies was carried out in which guinea pig peritoneal macrophages were harvested after paraffin oil induction, and cultured for 24 hours in the presence of different concentrations of SM-1213. Medium was separated from cells and cells processed by Dounce homogenization and discontinuous gradient ultracentrifugation for phagosomes according to the method of Stossel (Stossel, T.P., Mason, R.J., Hartwig, J., and Vaughan, M., J. Clin. Invest. 51:615, 1972). Cells per flask varied between 4.2 and 6 million; all data were corrected to reflect a 5 million cell count. The enzyme assays carried out on appropriate fractions included: catalase according to the method of Beers and Sizer (Beers, R.F., Jr., and Sizer, I.W., J. Biol. Chem. 195:133, 1952); peroxidase employing 4-aminoantepyrine as a hydrogen donor as described by Decker (Worthington Enzyme Manual, ed. L.A. Decker, Worthington Biochemical Corp., Freehold, N.J., 1977, pp. 66-70); medium lactic acid dehydrogenase, a measure of nonspecific cell leakage, by the method of Wacker (Wacker, W.E.C., and Dorfman, L.E., JAMA 181:972, 1962) employing a Sigma Chemical Co. reagent kit. The protein content of all fractions was determined by the method of Lowry (Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., J. Biol. Chem. 193:265, 1951).

Of this ongoing work, three experiments are reported in Table VI.

In Experiment 1, macrophages were harvested from guinea pigs 72 hours after the intraperitoneal injection of 20 cc of Fisher paraffin oil. Harvested cells were washed 3 times with phosphate buffered saline and were cultured in Eagles MEM with 20% fetal calf serum and SM-1213 at 0.1 and 1.0 μg/ml.

In Experiment 2, macrophages were harvested 96 hours after intraperitoneal paraffin oil injection. Harvested cells were washed as in Experiment 1, but culture medium was International Scientific Industries defined cell growth medium HI-WO_5/BA_{2000}, which is serum free. Here, drug concentrations were 0.001, 0.01, 0.1 and 1.0 μg/ml.

In Experiment 3, three guinea pigs (identified as A, B, and C) were harvested for macrophages 96 hours following paraffin oil injection; one guinea pig (D) was harvested at 72 hours; one guinea pig (E) was harvested for unelicited macrophages. Culture of macrophages was carried out as in Experiment 2. For guinea pigs A and B, the 1.0 μg/ml dosage level of SM-1213 was dropped and the effects of 0.001M imidazole were assessed because of the capacity of this drug input to elevate cGMP, a possible second messenger route by which function-enhancing drug influences might be communicated. For guinea pig E, only the 1.0 μg/ml dosage level of previous studies was tested along with a dose level 50 times greater. Medium was examined for peroxidase activity and lactic acid dehydrogenase activity.

The data displayed in Table VI concerning drug effects on macrophage biochemistry reveal that drug treatment increases the peroxidase-catalase ratio in phagosomes, and that drug induces a specific secretion of peroxidase in the absence of lactic acid dehydrogenase leakage from macrophages. Drug is also found to increase net protein synthesis in macrophages and to increase the amount of protein secreted by macrophages into the medium.

In presenting all of the above data, we have included data generated from research supported by other funds so as to give ONR reviewers a more complete picture of the action of SM-1213.

PUBLICATIONS OR REPORTS DERIVED FROM ONR CONTRACT N00014-77-C-0507

Majde, J.A., and Gordon, P. (1977) Maintenance of immune responsiveness during a sub-clinical herpesvirus infection in mice by SM-1213, a drug with antiviral activity. Abstract 127, 17th interscience Conference on Antimicrobial Agents and Chemotherapy, New York, N. Y.

TABLE I

These data illustrate the dependence of immune response intensity of mice on housing conditions.

DAY 14 CONTROL PAW RESPONSES

	12 mice/cage (n = 24)	8 mice/cage (n = 24)	2 mice/cage (n = 16)
15-minute responses	56.75 ± 10.24	18.83 ± 4.18	5.00 ± 1.94
4-hour responses	57.83 ± 11.38	21.75 ± 7.07	2.00 ± 0.54
24/48-hour responses	94.33 ± 20.63	53.71 ± 8.93	38.38 ± 8.81

Summary of statistical analysis:

$56.75 > 18.83$, $57.83 > 21.75$, $P < 0.01$
$18.83 > 5.00$, $21.75 > 2.00$, $P < 0.02$

TABLE II

The effect of SM-1213 therapy (80 mg/kg/day for 27 days) on adrenal and thymic weight indices of Ha/ICR female mice housed at different densities (n = 12)

	Adrenal Index	Thymic Index
2 mice/cage - Placebo	0.34 ± 0.05	1.81 ± 0.06
- SM-1213	0.27 ± 0.02	1.86 ± 0.11
12 mice/cage - Placebo	0.22 ± 0.01	1.83 ± 0.08
- SM-1213	0.28 ± 0.03	2.24 ± 0.11

Summary of statistical analysis:

$0.34 > 0.22$, $1.86 < 2.24$, $P < 0.05$
$1.83 < 2.24$, $P < 0.01$

TABLE III

RED CELL IMMUNE RESPONSES OF
A/J MALE MICE INFECTED WITH 300 PFU HSV-L I.P.

Treatment	15 Minute Reaction	4 Hour Reaction	24 Hour Reaction	HA Antibody (log_2)
Control + placebo	47	34	67	5.5
Control + SM-1213*	23	21	67	5.0
HSV-L + placebo	41	39	38	3.5
HSV-L + SM-1213	41	25	58	6.0

* 80 mg/kg/day one day post-infection

TABLE IV

INCIDENCE OF HSV-2 INFECTED ADHERENT CELLS

	No Treatment	SM-1213, 0.1 μg/ml	SM-1213, 0.01 μg/ml
6 Hours Post-Infection	20.4%	15.5% (-24.0%)	1.5% (-92.6%)
12 Hours Post-Infection	22.9%	20.9% (-8.7%)	5.3% (-76.6%)
	15.1%	5.6% (-62.9%)	3.4% (-77.4%)
Average ± SE	19.4 ± 2.3	14.0 ± 3.6	3.4 ± 0.9

TABLE V

	Counts per 2 Minutes - Average of Duplicates				
	Before Candida Addition	Post Candida Addition			
		1 Hour	2 Hours	3 Hours	4 Hours
Control	6	6	6	12	0
SM-1213, 0.2 μg/ml	15	23	11	3	0

TABLE VI

	Phagosomal Catalase (units)*	Phagosomal Peroxidase (milliunits)*	P/C Ratio	Ratio Group Average	Drug Effect	Catalase Flux in Medium (units)*	Catalase Group Average	Peroxidase Flux in Medium (milliunits)*	Peroxidase Group Average
EXPERIMENT 1									
Control-1	2.52	3.09	1.23	1.55		+13.32	+12.31	- 2.64	-4.96
Control-2	1.94	3.59	1.86			+11.29		- 7.27	
SM-1213 (μg/ml)									
0.1 - 1	0.58	3.79	6.55	4.58	+195%	+11.84	+12.17	+ 7.93	+5.62
0.1 - 2	1.08	2.81	2.60			+12.49		+ 3.30	
1.0 - 1	1.18	2.73	2.31	2.09	+ 35%	+12.77	+12.91	- 6.61	+9.08
1.0 - 2	1.58	2.94	1.86			+13.04		+24.78	
EXPERIMENT 2									
Control pool	0.042	0.035	0.83						
SM-1213 (μg/ml)									
0.001 pool	0.022	0.123	5.49		+561%				
0.01 pool	0.033	0.039	1.16		+ 40%				
0.1 pool	0.025	0.063	2.50		+201%				
1.0 pool	0.034	0.058	1.69		+104%				

* Data given per 5×10^6 cells.

EXPERIMENT 3

TABLE VI
(continued)

	% Change in Enzyme Activity Secreted into Medium, relative to control		% Change in Medium Protein of Cell Origin, relative to control	Significance
	Peroxidase	Lactic Acid Dehydrogenase		
Guinea Pig A				
SM-1213				
0.001 µg/ml	+125	0	+ 22.2	
0.01 µg/ml	+ 25	+12.5	- 33.3	
0.1 µg/ml	+125	- 6.3	+ 48.1	
Imidazole, 1 mM	+ 75	-12.5	+200.0	
Guinea Pig B				
SM-1213				
0.001 µg/ml	+280	- 8.6	+287.5	
0.01 µg/ml	+ 44	+23.9	0	
0.1 µg/ml	+280	+17.2	+602.9	
Imidazole, 1 mM	+ 63	0	+370.2	
Guinea Pig E				
SM-1213				
1.0 µg/ml	--	--	+ 77.0	
50.0 µg/ml	--	--	+276.4	
Average ± SE	+127.1 ± 33.4	+3.3 ± 4.4	+184.2 ± 60.5	127.1>3.3, P< 0.01 184.2>3.3, P< 0.02
SM-1213 only	+146.5 ± 41.6	+6.5 ± 5.0	+159.0 ± 71.5	146.5>6.5, P<0.01 159.0>6.5, P<0.1
SM-1213, excluding 0.01 µg/ml	+202.5 ± 38.8	+0.6 ± 5.1	+217.5 ± 82.4	202.5>0.6, P<0.001 217.5>0.6, N.S.
All drugs, excluding 0.01 µg/ml SM-1213	+158.0 ± 36.6	-1.7 ± 3.9	+234.4 ± 64.4	158.0>-1.7, P<0.01 234.4>-1.7, P<0.01

EXPERIMENT 3

TABLE VI
(continued)

SUMMARY FOR SM-1213 OF % CHANGE IN INTRACELLULAR PROTEIN
GROUPING DATA FROM GUINEA PIGS A-E

SM-1213			
0.001 µg/ml	-2.0, +1.5, +10.7	=	+ 3.4 ± 3.1
0.01 µg/ml	+5.9, +17.1, +12.3	=	+11.8 ± 2.7
0.1 µg/ml	-0.4, -23.3, +17.6, +8.1	=	+ 0.5 ± 7.6
1.0 µg/ml	+22.8, +25.2, +4.9	=	+17.6 ± 5.2
50.0 µg/ml	+17.1	=	+17.1

SM-1213		
All Doses	8.4 ± 3.2	
0.1 µg/ml and less	4.8 ± 3.6	N.S.
1.0 µg/ml and more	17.5 ± 3.9	$P < 0.05$

EXPERIMENT 3

TABLE VI
(continued)

SUMMARY FOR SM-1213 OF % CHANGE IN MEDIUM PROTEIN OF CELL ORIGIN
GROUPING DATA FROM GUINEA PIGS A-E

SM-1213			
0.001 µg/ml	+22.2, +287.5	=	+154.9 ± 94.1
0.01 µg/ml	-33.3, 0	=	- 16.7 ± 11.8
0.1 µg/ml	+48.1, +602.9	=	+325.5 ± 196.7
1.0 µg/ml	+77.0	=	+ 77.0
50.0 µg/ml	+267.4	=	+267.4

SM-1213		
All Doses	+159.0 ± 71.5	
Excluding 0.01 µg/ml	+217.5 ± 82.4	P = 0.05

OFFICE OF NAVAL RESEARCH
MICROBIOLOGY PROGRAM
STANDARD DISTRIBUTION LIST

Number of copies:

(12) Administrator, Defense Documentation Center
Cameron Station
Alexandria, VA 22314

(6) Director, Naval Research Laboratory
Attention: Technical Information Division
Code 2027
Washington, D.C. 20390

(6) ~~Director, Naval Research Laboratory~~
~~Attention: Library Code 2029 (ONRL)~~
~~Washington, D.C. 20390~~
Code 1021P (ONRL Doc)
Office of Naval Research
800 N. Quincy St.
Arlington, VA 22217

(3) Office of Naval Research
Department of the Navy
Code 443
Arlington, Virginia 22217

(2) Director, Research Division (Code 00)
Naval Medical Research and Development Command
National Naval Medical Center
Bethesda, Maryland 20016

(2) Technical Reference Library
Naval Medical Research Institute
National Naval Medical Center
Bethesda, Maryland 20016

(1) Office of Naval Research
Department of the Navy
Code 200
Arlington, Virginia 22217

(1) Office of Naval Research Branch Office
~~666~~495 Summer Street
Boston, Massachusetts 02100

(1) Office of Naval Research Branch Office
536 South Clark Street
Chicago, Illinois 60605

OFFICE OF NAVAL RESEARCH
MICROBIOLOGY PROGRAM
STANDARD DISTRIBUTION LIST (Cont'd)

Number of copies:

(1) Office of Naval Research Branch Office
1030 East Green Street
Pasadena, California 91101

(1) Office of Naval Research
Contract Administrator - Southeastern Area
2110 G. Street, NW
Washington, D.C. 20007

(1) Commanding Officer
U.S. Naval Medical Research Unit #2
Box 14
APO, San Francisco 96263

(1) Commanding Officer
U.S. Naval Medical Research Unit #3
FPO, New York 09527

(1) Commanding Officer
U.S. Naval Medical Research Unit #5
APO, New York 09319

(1) Officer in Charge
Submarine Medical Research Laboratory
U.S. Naval Submarine Base, New London
Groton, Connecticut 06342

(1) Scientific Library
U.S. Naval Medical Field Research Laboratory
Camp Lejeune, North Carolina 28542

(1) Scientific Library
Naval Biosciences Laboratory
Naval Supply Center
Oakland, California 94625

(1) Scientific Library
Naval Aerospace Medical Research Institute
Naval Aerospace Medical Center
Pensacola, Florida 32512

(1) Commanding Officer
U.S. Naval Air Development Center
ATTN: Aerospace Medical Research Department
Johnsville, Warminster, PA 18974

OFFICE OF NAVAL RESEARCH
MICROBIOLOGY PROGRAM
STANDARD DISTRIBUTION LIST (Cont'd)

Number of copies:

(1) Commanding General
U.S. Army Medical Research and
Development Command
Forrestal Building
Washington, D.C. 20314
Attn: MEDDH-SR

(1) Director of Life Sciences
Air Force Office of Scientific Research
Bolling Air Force Base
Washington, D.C. 20032

(1) STIC-22
4301 Suitland Road
Washington, D.C. 20390

(1) Director
Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington, D.C. 20012