

I should like to take this opportunity to express my sincere thanks to Dr. C. W. Chapman and to Dr. Thomas C. Grubb for their invaluable advice, their suggestions and criticisms, and for their cooperation during the period of this work. I am also very grateful to the Graduate School of the University of Maryland for the privilege of working under its supervision and for the facilities granted me in carrying out this project.

Leonard Karel



THE RELATIONSHIP OF VITAMIN C  
TO SULFANILAMIDE ACTION

By

Leonard Karel

Thesis submitted to the Faculty of the Graduate  
School of the University of Maryland in  
partial fulfillment of the require-  
ments for the degree of Doctor  
of Philosophy, in Pharmacology

1941

UMI Number: DP70027

All rights reserved

INFORMATION TO ALL USERS

The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if material had to be removed, a note will indicate the deletion.



UMI DP70027

Published by ProQuest LLC (2015). Copyright in the Dissertation held by the Author.

Microform Edition © ProQuest LLC.

All rights reserved. This work is protected against unauthorized copying under Title 17, United States Code



ProQuest LLC.  
789 East Eisenhower Parkway  
P.O. Box 1346  
Ann Arbor, MI 48106 - 1346

## TABLE OF CONTENTS

INTRODUCTION TO THESIS.....	i
PART I THE SIGNIFICANCE OF THE VITAMIN C LEVEL OF THE BLOOD PLASMA IN GUINEA PIGS	
Introduction.....	1
Experimental Methods.....	6
Experimental Results.....	12
Discussion.....	12
Summary.....	18
References.....	20
PART II THE EFFECT OF VITAMIN C ON THE DE- TERMINATION OF SULFANILAMIDE	
Introduction.....	22
Experimental Methods and Results..	23
Discussion.....	28
Pathology.....	29
Conclusions.....	36
References.....	38
PART III THE EFFECT OF ASCORBIC ACID ON THE ACUTE TOXICITY OF SULFANILAMIDE FOR GUINEA PIGS	
Introduction.....	40
Experimental Methods.....	41
Experimental Results.....	43

	Discussion.....	47
	Summary.....	48
	References.....	50
PART IV	THE TREATMENT OF A GROUP C STREPTOCOCCUS INFECTION IN GUINEA PIGS WITH VITAMIN C AND SULFANILAMIDE	
	Introduction.....	51
	Experimental Methods.....	56
	Experimental Results.....	62
	Discussion.....	67
	Summary.....	69
	References.....	71
	BIBLIOGRAPHY.....	73
	APPENDIX.....	79
	.....	

## LIST OF TABLES

### PART I

- I Mean Values of Blood Plasma Vitamin C Levels in Normal Guinea Pigs.....3
- II Random Determinations of Vitamin C in the Blood Plasma of Normal Guinea Pigs..7
- III Plasma Vitamin C Levels in Guinea Pigs at Stated Times After Intraperitoneal Injections of 2.5, 5.0, and 10.0 Mgs. of Ascorbic Acid per Animal.....8
- IV Plasma Vitamin C Levels in Guinea Pigs at Stated Times After Intraperitoneal Injections of 10.0 Mgs. of Ascorbic Acid per Animal.....10
- V Significant Difference of Means for Values Given in Table II.....15
- VI Effect of 20 Hours Starvation on the Blood Plasma Level of Vitamin C in Guinea Pigs.....16

### PART II

- I Percentage of Sulfanilamide Recovered in the Presence of Varying Amounts of Vitamin C.....25
- II The Effect of the Vitamin C:Sulfanilamide Ratio on the Recovery of Sulfanilamide.26

PART III

- I Sulfanilamide Mortality of Fasting and Non-Fasting Guinea Pigs in Different States of Vitamin C Nutrition.....44
- II LD<sub>50</sub>'s of Fasting and Non-Fasting Guinea Pigs in Different States of Vitamin C Nutrition.....45
- III Significant Difference of Means Between LD<sub>50</sub>'s of Sulfanilamide for Fasting and Non-Fasting Guinea Pigs in Different States of Vitamin C Nutrition.....46

PART IV

- I Survival Times of Infected Controls and Treated Guinea Pigs.....63
- II Significant Difference of Means for Every Pair of Groups of Inoculated Animals.....64

APPENDIX

- I Formulas.....80
- II Plasma Vitamin C Levels in Guinea Pigs at Stated Times After Feeding Greens...81
- III Vitamin C and Free Sulfanilamide Content of Blood Plasma of Guinea Pigs After Six Doses of Sulfanilamide.....82
- IV Vitamin C and Free and Total Sulfanilamide



	Content of Blood Plasma of Guinea Pigs After Six Doses of Sulfanilamide.....	83
IV-A	Vitamin C, Free and Total Sulfanilamide Content of Blood Plasma of Guinea Pigs After Six Doses of Sulfanilamide.....	84
V	Vitamin C and Free Sulfanilamide Content of the Blood Plasma of Guinea Pigs Under Different Conditions of Ascorbic Acid Treatment.....	85
VI	Mortality of Guinea Pigs Fed Sulfanila- mide Under Varying Conditions.....	86
VII	Protocols for the Determination of the LD <sub>50</sub> 's for Non-Fasting Normal and High Vitamin C Guinea Pigs.....	87
VIII	Protocols for the Determination of the LD <sub>50</sub> 's for Vitamin C Depleted, Normal, and High Vitamin C Guinea Pigs.....	88
IX	Determination of LD <sub>50</sub> 's and Their Range Ninety-Five Times Out of One Hundred...	89
X	Slopes and Their Errors for Fasting and Non-Fasting Guinea Pigs.....	90
XI	Survival Times of Infected Controls and Treated Guinea Pigs.....	91

LIST OF FIGURES

PART I

- I Rate of Disappearance of Blood Plasma Vitamin C in Guinea Pigs Following an Intraperitoneal Injection of 10.0 Mgs. of Ascorbic Acid.....11

PART II

- I Sulfanilamide Recoverable in the Presence of Ascorbic Acid.....27

PART IV

- I Comparison of the Survival Times of Guinea Pigs Infected With Group "C" Streptococci.....66

## INTRODUCTION

A review of the literature on vitamin C (ascorbic acid) reveals that there is no clear cut opinion concerning its various functions in the economy of the animal. All investigators in the vitamin C field agree that vitamin C is directly indicated in the treatment of scurvy. Concerning its role in resistance to spontaneously acquired and induced infections, however, there is widespread disagreement. Similar dissension prevails relative to the use of the vitamin as a detoxifying agent. Much of the indecision concerning the functional capacity of ascorbic acid is the result of the use of too few animals to give statistical significance to the results. Throughout this work, therefore, a sufficient number of animals was used.

Since the advent of the sulfonamide drugs in the chemotherapy of bacterial infections, several studies have been made on the relationship of ascorbic acid to sulfanilamide or its derivatives as a synergic agent, as a detoxifier, and as an adjuvant in its own right as a substance which increases the bodily resistance... (7) (9) (10) (26) (32) (33) (34) (42) (44) (56). In most of these studies, the species of animals used were not fully depletive of vitamin C, and they were, therefore, not so suitable for studies involving the

effect of ascorbic acid as would be animals which can be fully depleted of vitamin C. At the present time, there are known only three mammals which depend on exogenous sources for their nutritive requirements in vitamin C; these are man, the lower primates, and the guinea pig.

With the preceding facts in mind, it was felt that, with guinea pigs as experimental animals, an investigation of the relationship of vitamin C to sulfanilamide action would be interesting.

To ascertain the effects of low and of high vitamin C blood levels in guinea pigs on the sulfanilamide action, it was first necessary to know what the normal vitamin C blood levels were and to what extent these levels could be raised or lowered by the addition or withdrawal of ascorbic acid or of sources of the vitamin. It was, furthermore, considered essential to know to what extent the normal metabolism of the animal was affected by its vitamin C content, inasmuch as any therapeutic agent will have its activity influenced by the general condition of the recipient of the drug at the time that the drug is administered. Consequently, the first study undertaken is concerned with the blood level of vitamin C in the normal, sulfanilamide-untreated guinea pig as an indication of the state of the vitamin C

nutrition of the animal.

Once the vitamin C blood levels of the normal animals had been determined, it became necessary to determine the mutual effects of the sulfanilamide and the ascorbic acid on each other both in the test tube and in the animal organism, in reference to the quantitative determination of these two substances. In vitro and in vivo tests were, therefore, made to learn the effects of different amounts of ascorbic acid on the determination of free and of conjugated sulfanilamide, and the effects of sulfanilamide on ascorbic acid determinations. In conjunction with this phase of the work, there were made, simultaneously, brief pathological studies of the guinea pigs undergoing treatment with sulfanilamide.

Before proceeding to chemotherapeutic studies, it was essential to determine the effect of ascorbic acid on the LD<sub>50</sub>\* of sulfanilamide for guinea pigs. Inasmuch as there were no accurate studies reported in the literature on the acute toxic dose of the drug for guinea pigs, a determination of this dose was undertaken concurrently with the determinations of the effect of various blood levels of ascorbic acid

---

\*The LD<sub>50</sub> is that dose which produces a mortality of 50 per cent under definite experimental conditions.

on the acute toxicity of sulfanilamide.

With the three previous studies as guides, there was conducted a chemotherapeutic investigation of the effect of sulfanilamide alone, of ascorbic acid alone, of ascorbic acid plus sulfanilamide, of vitamin C depletion, and of vitamin C depletion plus sulfanilamide dosage on group C streptococci infections in guinea pigs.

For purposes of clarity, the entire study has been divided into four distinct sections as follows:

- I. THE SIGNIFICANCE OF THE VITAMIN C LEVEL OF THE BLOOD PLASMA IN GUINEA PIGS;
- II. THE EFFECT OF ASCORBIC ACID ON THE DETERMINATION OF SULFANILAMIDE;
- III. THE EFFECT OF VITAMIN C ON THE ACUTE TOXICITY OF SULFANILAMIDE FOR GUINEA PIGS; and
- IV. THE TREATMENT OF A GROUP C STREPTOCOCCUS INFECTION IN GUINEA PIGS WITH VITAMIN C AND SULFANILAMIDE.

THE SIGNIFICANCE OF THE VITAMIN C LEVEL  
OF THE BLOOD PLASMA IN GUINEA PIGS

Introduction

In the published studies (12-14) (48) (51-2) (59-60) (63) (66) on the vitamin C content of the plasma (serum, or whole blood) of the guinea pig determined by chemical methods, only those of Rohmer et al. (51) and of Zilva (66) mention the importance of regulating not only the vitamin intake in determining the "normal" vitamin C content, but also the time interval between administration of the vitamin and the withdrawal of the blood sample from the animal.

Rohmer et al. (51) found only indeterminable traces of the vitamin in the serum of guinea pigs depleted for only four days. After five days, no color at all was given by the serum and the reagent. The curve for vitamin C in guinea pigs given by these workers is in very close accord with that of Figure I. (These authors do not state whether the same animals were used for the vitamin C determinations at the different hours; neither do they give the number of animals used in the experiment.) The highest value, 20 mg. per cent, was reached at approximately one-half hour after sub-cutaneous injection of 200 mg. per kg.

of body weight. At the twenty-fifth hour, the value was about 1.7 mg. per cent.

Zilva (66) took a series of guinea pigs previously kept on a mixed diet with cabbage ad lib. and injected them with 50.0 mg. of l-ascorbic acid. Animals were killed one-half hour, one hour, two hours, two and one-half hours, and four hours after the injection, and the ascorbic acid was determined in the plasma. The values obtained were 32.5, 17.5, 11.0, 7.5, and 3.5 mg. per cent, respectively. He stated that although the vitamin C content of the plasma of saturated guinea pigs is very low, this can, nevertheless, be raised at will by injecting large doses of ascorbic acid.

Table I (on p. 3 ) shows clearly the disagreement existing concerning normal blood vitamin C values. Yet, with only the exceptions already mentioned, none of these authors cites data which would indicate that the time factor was considered in determining normal blood vitamin C values. (See Table I, on p. 3 ).

In the literature pertaining to vitamin C determinations in humans, there are several articles commenting on the inadequacy of making a single, random determination of ascorbic acid in the blood, and emphasizing the importance of accurately controlling



TABLE I

MEAN VALUES OF BLOOD PLASMA VITAMIN C LEVELS IN  
NORMAL GUINEA PIGS

Number of Animals Used		Mean Values Mg. Per Cent Vitamin C	Amount of Daily Vita- min C in Mgs.	References
6	aa	0.00	0.0	(14)Ecker <u>et al.</u>
40		0.02	0.0	(13)Ecker & Pillemer
220		0.09	0.5	(13)ibid.
218		0.13	1.0	(13)ibid.
9	xx	0.14	0.5	(60)Todhunter <u>et al.</u>
-		0.18	2.0	(51)Rohmer <u>et al.</u>
12	yy	0.22	2.0	(59)Todhunter & Brewer
10	**	0.238	-	(52)Sacerdote
9		0.327	-	(52)ibid.
47		0.33	2.0	(13)Ecker & Pillemer
-		0.35	5.0	(51)Rohmer <u>et al.</u>
6		0.40	-	(48)Raabe
29		0.43	2.0	(12)Dobbelstein
-		0.50	*	(66)Zilva
10	zz	0.54	8.0	(59)Todhunter & Brewer
117		0.57	5.0	(13)Ecker & Pillemer
5		0.65	-	(48)Raabe
5		0.68	-	(48)ibid.
6		0.77	-	(63)Wortis <u>et al.</u>
5		0.80	-	(48)Raabe
6	a	0.81	0.0	(14)Ecker <u>et al.</u>
5		0.86	-	(48)Raabe
143		1.03	10.0	(13)Ecker & Pillemer
6		1.06	-	(14)Ecker <u>et al.</u>
85		1.08	20.0	(13)Ecker & Pillemer

- Not Stated

\* Fresh Cabbage ad lib.

\*\* Standard Deviation: 0.011

xx Standard Deviation: 0.05

yy Standard Deviation: 0.03

zz Standard Deviation: 0.11

a Scurvy Diet - 14 days

aa Scurvy Diet - 30 days

both the quantity of the vitamin in the diet and the time elapsing between successive tests for the vitamin in consecutively drawn blood specimens.

Working with humans, Greenberg et al. (23) suggested that "more accurate index of the degree of deficiency existing at the time in any given case can be had by serial determinations following administration of known vitamin C supplements".

Taylor, Chase and Faulkner (58), Wright, Lilienfeld, and MacLenathen (64), and Barron, Brumm, and Dick (3) have graphs showing the rapidity of the rise and fall of vitamin C in the blood. Wright, Lilienfeld and MacLenathen (64) found that after the intravenous injection of 1 gm. of cevitamic acid, the vitamin C content of the blood reached a height which showed some relationship to the previous dietary intake. They stated that the cevitamic acid values of a control blood sample may be misleading regarding the actual state of vitamin C nutrition, since they are subject to sudden fluctuations after temporary dietary changes. For humans, these workers recommend a five hour test for vitamin C after an intravenous test dose of 1 gm. Goldsmith and Ellinger (22), Kellie and Zilva (28), Herlitz (24), Kajdi, Light and Kajdi (25), Kastlin et al. (27), agree with Wright, Lilienfeld and MacLenathen (64) con-

cerning - as Kellie and Zilva (28) phrase it "..... the variation of the ascorbic acid content of the blood with the magnitude of the dose and the time elapsing after taking the dose". Zilva (66) also stated that blood levels determined at random do not indicate the degree of "saturation" of a subject. Of the various reports of investigations, however, only those of Herlitz (24) and of Dagulf (8) present, in addition to dietary and time control, the standard deviation for each experimental group.

It is the purpose of this study to show that vitamin C determinations on the blood plasma of guinea pigs are of value only when the time elapsing between the intake of the vitamin and the withdrawal of the blood sample is taken into consideration; that the initial value is not a reliable index of the state of vitamin C nutrition of the animal; and that, unless the sampling error is known, a correct interpretation of the experimental data cannot be made.

During the course of experiments in which it was necessary first to obtain normal blood plasma vitamin C values in guinea pigs, it was noticed that not only was there a large individual variation in the vitamin C content of the plasma of guinea pigs, but that there was also a considerable variation in the plasma ascorbic acid content in the same animals depending on how soon

after the feeding of greens a determination of the vitamin value was made. The initial series of results which led to the conclusion that the vitamin C content of the blood of guinea pigs not only varies with the amount of vitamin C consumed but also varies with the length of time following the last intake of the vitamin is presented in Table II. (See Table II on p. 7 ).

Subsequent investigations were made with careful timing of the interval elapsing between the administration of the vitamin and the withdrawal of the blood sample from the animal. The latter data are summarized in Tables III and IV, on pp. 8 and 10.

#### Experimental

Guinea pigs of both sexes, weighing approximately 250-450 gms., were fed daily, except Sunday, a diet consisting of ad libidum quantities of hay; rolled oats (100 gms. per guinea pig) mixed with 0.25 gm. sodium chloride, 0.5 gm. of dried brewer's yeast (Fleischmann), one cc. of U. S. P. XI cod liver oil; greens, consisting chiefly of cabbage, celery and carrots; and fresh water. All of the animals were kept at least four days on this diet before being used.

Blood samples were obtained by lightly etherizing the animals and withdrawing 0.5 to 1.0 cc. of

TABLE II

RANDOM DETERMINATIONS OF VITAMIN C IN THE  
BLOOD PLASMA OF NORMAL GUINEA PIGS

	Time of De- termination in hours after feed- ing greens (ad lib.)	Mean in Mg. per- cent	Lowest Value	Highest Value	Standard Deviation	Per Cent Standard Deviation	Number of Animals
Group I	3-6	0.38	0.17	0.76	0.15	39.5	30
Group II	15-17	0.16	0.06	0.24	0.06	37.5	6
Group III	2-3	0.53	0.20	0.82	0.26	49.1	6
Group IV	4-6	0.72	0.06	1.67	0.51	70.8	12
Group V	19-20	0.14	0.02	0.35	0.08	57.1	20
Group VI	42-44 (No greens on Sunday)	0.16	0.00	0.44	0.11	68.8	32
							(106) Total

Weighted Mean For All Animals: 0.30 mg. per cent  
Standard Deviation: 0.20  
Standard Error: 0.02

(See Appendix, Table II--- for Complete Data)

TABLE III

PLASMA VITAMIN C LEVELS IN GUINEA PIGS AT STATED TIMES  
AFTER INTRAPERITONEAL INJECTIONS OF 2.5, 5.0,  
AND 10.0 MGS. OF ASCORBIC ACID PER ANIMAL

Gm.Wt. Sex	Date Experiment Began	Time of Admin. of Ascorbic Acid in Hours	Amt. Vitamin C Injected i.p. per animal	Time Elapsing Between Last Inj. and Detn. of Vit. C	Mg. Per Cent Vitamin C	Detn. of Vitamin C 7 Hours after Last Injection	
Group I	327-F	6/7/40	0,24,72,96	2.5 mg.	24 hours	.09	-
	412-M	"	" " " "	2.5	24 "	.00	-
	305-F	"	" " " "	2.5	24 "	.00	-
	310-M	"	" " " "	2.5	24 "	.00	-
	281-F	"	" " " "	2.5	24 "	.00	-
	293-F	"	" " " "	2.5	24 "	.44	-
	322-F	"	" " " "	2.5	24 "	.08	-
	330-F	"	" " " "	2.5	24 "	.00	-
	364-F	"	" " " "	2.5	24 "	.00	-
					Mean -	.07	
					s.d. -	.15	
Group II	273-F	6/12/40	0,24,48	5.0 mg.	17 hours	.22	-
	382-M	"	" " " "	5.0	17 "	.06	-
	310-F	"	" " " "	5.0	17 "	.14	-
	305-M	"	" " " "	5.0	17 "	.03	-
	275-F	"	" " " "	5.0	17 "	.05	-
	332-F	"	" " " "	5.0	17 "	.13	-
	300-F	"	" " " "	5.0	17 "	.00	-
	326-F	"	" " " "	5.0	17 "	.00	-
	322-F	"	" " " "	5.0	17 "	.07	-
	322-F	"	" " " "	5.0	17 "	.02	-
	330-F	"	" " " "	5.0	17 "	.04	-
	312-M	"	" " " "	5.0	17 "	.00	-
	304-F	"	" " " "	5.0	17 "	.01	-
	340-F	"	" " " "	5.0	17 "	.08	-
					Mean -	.06	
					s.d. -	.06	
Group III	335-F	6/19/40	0 (one inj. only)	10.0 mg.	4 hours	.30	-
	332-M	"	" " " "	10.0	4 "	.28	-
	348-F	"	" " " "	10.0	4 "	.69	-
	362-M	"	" " " "	10.0	4 "	1.34	-
	351-F	"	" " " "	10.0	4 "	.83	-
	373-F	"	" " " "	10.0	4 "	.10	-
	363-F	"	" " " "	10.0	4 "	.06	-
					Mean -	.51	
					s.d. -	.46	
*All animals were fed regular diet plus greens ad lib. On day of vitamin C determination, no greens were fed until after the determination.							
Group IV	323-F	6/19/40	no inj.	----	2 hours	.24	.05
	370-F	"	(Greens fed at 9:45 A.M. vitamin C detn. made at 11:45 A.M. at which time the greens were wholly removed from the cages.)		2 "	.24	.05
	295-F	"			2 "	.42	.10
	335-F	"			2 "	.51	.11
	309-M	"			2 "	.30	.12
	313-F	"			2 "	.20	.04
					Mean -	.32	Mean -.08
					s.d. -	.12	s.d. -.04

blood by cardiac puncture. Inasmuch as only 0.5 cc. to 1.0 cc. of blood was withdrawn at any one puncture, most of the animals remained alive. The survivors were used again after they had been allowed to rest for three or more days.

The vitamin C determinations were carried out according to the directions given by Farmer and Abt (15) (16) for their micro method. A burette made by sealing a stopcock to a 0.2 cc. pipette graduated in thousandths of a cc. was used. A fine capillary tube was connected by gum-rubber tubing to the end of the pipette at which the stopcock had been sealed. This burette was calibrated. Titrations were made with the burette held in a vertical position. Transfers of liquid of 0.2 cc. or less were made with a 0.2 cc. pipette graduated in thousandths of a cc. The same apparatus was used throughout the experiment.

In the titration of 10 unknowns for the purpose of determining the accuracy of this method, the observed values differed from the correct values by not more than 5.4 per cent  $\pm$  3.9 per cent. Determinations were made in triplicate on each sample of plasma tested for its vitamin C content.

Ascorbic acid injections were made intraperitoneally and regardless of body weight, since the ani-

TABLE IV

PLASMA VITAMIN C LEVELS IN GUINEA PIGS AT  
STATED TIMES AFTER INTRAPERITONEAL  
INJECTIONS OF 10.0 MG. OF  
ASCORBIC ACID PER ANIMAL

Date 1940	Sex- Weight in Gms.	Amount Vita- min C I.P. in Mg. Per Pig	Mg. Per Cent Vitamin C in Plasma Hours After Injection		
			1	3	7
6/26	M-257	10.0	0.44	0.25	0.32
	M-342	"	1.04	0.32	0.37
	M-300	"	0.39	0.26	0.09
	M-274	"	2.11	0.66	0.19
	M-320	"	0.74	0.77	0.27
	M-270	"	0.55	0.40	0.26
	M-300	"	0.35	0.36	0.20
	M-257	"	3.66	0.45	0.12
6/27	M-295	"	0.91	0.95	0.13
	M-257	"	0.84	0.74	1.01
	M-280	"	2.11	1.30	0.85
	M-265	"	0.36	0.26	0.17
	F-260	"	0.46	*	*
	M-312	"	0.29	0.33	*
	M-290	"	1.38	0.26	0.52
	F-234	"	2.80	2.08	0.50
	F-256	"	0.08	0.13	0.36
	F-276	"	3.57	0.12	0.07
M-260	"	0.29	0.28	0.10	
Mean**			1.18	0.55	0.33
No. of Animals			19	18	17
Standard Deviation			1.10	0.60	0.30
Standard Error			0.30	0.10	0.10
			$\frac{1}{2}$	1 $\frac{1}{2}$	4
6/21	F-275	10.0	3.04	0.80	0.43
	F-352	"	2.60	1.44	0.74
	F-344	"	4.94	4.82	1.92
	M-335	"	3.60	1.68	0.61
	M-295	"	4.00	0.30	0.10
	F-320	"	4.24	0.72	0.18
	F-347	"	2.70	1.50	0.16
	F-344	"	3.18	2.14	1.61
6/28	F-357	"	1.74	0.52	0.64
	M-320	"	1.31	2.47	0.44
	F-312	"	0.76	0.21	0.18
	M-270	"	1.32	0.33	0.33
	F-287	"	3.34	1.60	1.63
	F-267	"	2.05	0.55	0.39
	F-257	"	0.66	0.21	0.34
	F-268	"	1.78	1.66	0.56
	F-276	"	0.42	0.21	0.21
	F-207	"	1.62	0.58	0.22
Mean**			2.41	1.21	0.59
No. of Animals			18	18	18
Standard Deviation			0.90	0.90	0.60
Standard Error			0.20	0.20	0.10

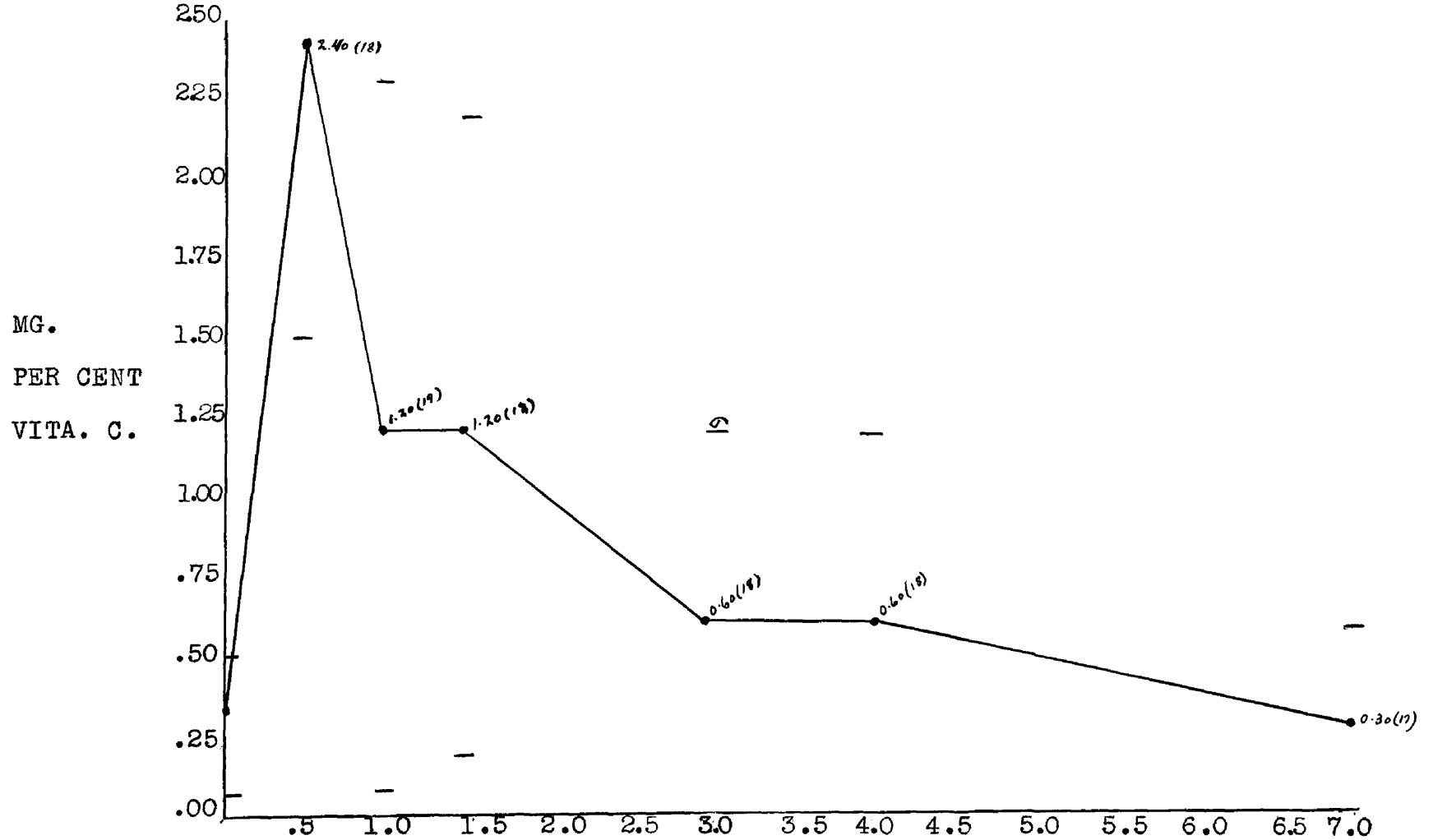
\* Died

\*\* There is a significant difference of means between the values for 1/2 and 1 hour, 1 hour and 3 hours, and 3 hours and 7 hours.



FIGURE I

RATE OF DISAPPEARANCE OF BLOOD PLASMA VITAMIN C IN GUINEA PIGS  
FOLLOWING AN INTRAPERITONEAL INJECTION OF 10.0 MGS. OF ASCORBIC ACID



mals used were all within the same weight range. In the 0.5, 1.5 and 4 hour group and in the 1, 3 and 7 hour group (Table IV, on p.10), the same animal was bled at the time intervals given. In repeating these two experiments, two new groups of guinea pigs were used.

### Experimental Results

The results given in Table II (on p.7 ) represent determinations made without exact time controls and at varying times of day. Table III (on p.8 ) presents data which show the rapid loss of vitamin C in normal animals which were given, in addition to their ad lib. diet of greens, varying intraperitoneal injections of ascorbic acid. The information in Table IV (on p.10) shows the rapidity with which the vitamin disappears from the blood stream. Figure I represents the results accumulated in Table IV.

### Discussion

Although all of the facts gathered in Table II (on p. 7) were obtained on normal animals, the results, if the averages of the separate groups are taken, are not representative of the so-called normal vitamin C content of the plasma of guinea pigs. However, inasmuch as the nutritional state of the animals was controlled and was

known up to the time of cardiac puncture, none of the results represents the plasma value of an actually deficient animal. But, if one determines the standard deviation of the weighted mean value found for all of the non-fasting animals - 106 - on the same vitamin C diet previously mentioned, in 95 per cent of the cases, the mg. per cent of vitamin C found in the blood of a normal animal will be within the range  $0.30 \pm 0.40$  mg. per cent or 0.00 to 0.70 mg. per cent. This means that statistically, under similar experimental conditions, the results will be within this range 19 out of 20 times (17). Therefore, a single determination made without an exact time control relative to the last intake of a given amount of the vitamin by the guinea pig is of no importance in evaluating the nutritional state of the animal.

In Table II (on p. 7), it will be noted that the per cent standard deviation is not constant for the various groups. (As the vitamin content of the plasma approaches zero, the range over which a value can vary is limited more and more as it nears the zero point. Consequently, groups in which there are animals with high values will show greater variation than groups in which the animals have vitamin blood levels that are low. Per cent standard deviation gives a truer picture).

The importance of the preceding considerations is

seen from the data which follow. (Table V, on p.15 ). Groups II, V and VI, in which at least 15 hours had elapsed between the feeding of greens and the determination of vitamin C show statistically no significant difference from each other. Yet the difference between these values and those in Groups III and IV in which the time interval is only 2 to 6 hours is significant. On the other hand, Groups I, III and IV do not differ statistically from one another. (The mean value for the entire group of 106 animals shows a significant difference from all results except the determinations evaluated 2 - 3 hours after feeding greens - Group III - and has been included in order to emphasize the importance of stating time elements in the experiment).

The difference is also significant between the mean of the fasting values (Table VI, on p.16 ) of vitamin C and the non-fasting values determined before feeding of a source of vitamin C (Table II, on p.7 ).

Although in the experiment represented by Table III (on p.8 ) the animals were given the regular diet plus greens ad lib., except on the day of vitamin C determination, when no greens were given, and although the injections of the vitamin C intraperitoneally represent-

TABLE V

SIGNIFICANT DIFFERENCE OF MEANS FOR VALUES\*

GIVEN IN TABLE II

	Group I 0.38 Mg. Per Cent	Group II 0.16 Mg. Per Cent	Group III 0.53 Mg. Per Cent	Group IV 0.72 Mg. Per Cent	Group V 0.14 Mg. Per Cent	Group VI 0.16 Mg. Per Cent
Group I 0.38 Mg. Per Cent	----	2.34	<u>1.15</u>	2.00	2.72	2.48
Group II 0.16 Mg. Per Cent	2.34	----	3.48	3.78	<u>0.63</u>	<u>0.00</u>
Group III 0.53 Mg. Per Cent	<u>1.15</u>	3.48	----	<u>1.06</u>	3.60	3.36
Group IV 0.72 Mg. Per Cent	2.00	3.78	<u>1.06</u>	----	3.94	3.80
Group V 0.14 Mg. Per Cent	2.72	<u>0.63</u>	3.60	3.94	----	<u>0.76</u>
Group VI 0.16 Mg. Per Cent	2.48	<u>0.00</u>	3.36	3.80	<u>0.76</u>	----

\*Any figure larger than 1.96 is considered to indicate a significant difference, since this figure gives a probability of only 1 in 20 that the difference would arise by chance.

TABLE VI

EFFECT OF 20 HOURS STARVATION ON THE BLOOD  
 PLASMA LEVEL OF VITAMIN C IN GUINEA PIGS

Date	Gm. Wt. Sex	Mg. Per Cent Vitamin C in Normal Un- treated Guinea Pigs	Mg. Per Cent Vitamin C after 20 Hours Starvation	Gm. Wt. After 20 Hours Starvation
1.	5/7/40 549-F	0.28	0.09	487
2.	445-F	0.71	0.16	385
3.	442-F	0.45	0.10	410
4.	478-F	0.72	0.12	432
5.	473-F	1.67	0.15	432
6.	321-F	0.85	0.02	274
7.	320-F	0.68	0.06	292
8.	283-F	0.42	0.14	255
9.	355-F	0.90	0.18	306
10.	405-F	0.06	0.16	352
11.	228-F	0.32	0.02	205
12.	471-F	1.63	0.26	412

(A) Mean-Starved Pigs-.12 mg. per cent  
 Standard Deviation-.07 mg. per cent

13.	5/29/40 457-M	0.21	0.03	444
14.	342-F	0.12	0.00	326
15.	352-M	0.23	0.00	350
16.	337-M	0.02	0.00	327
17.	307-F	0.11	0.10	284
18.	329-F	0.11	0.00	306
19.	317-F	0.35	0.04	292
20.	300-F	0.15	0.00	280
21.	268-F	0.23	0.00	255
22.	312-F	0.07	0.00	282
23.	310-F	0.13	0.01	280
24.	316-F	0.10	0.00	290
25.	327-F	0.10	0.00	302
26.	256-F	0.02	0.00	245
27.	208-F	0.19	0.00	281
28.	267-F	0.11	0.00	249
29.	319-F	0.23	0.00	280
30.	309-F	0.11	0.00	288
31.	290-F	0.15	0.08	266
32.	305-F	0.08	0.00	290

(B) Mean-Starved Pigs-.01 mg.per ct. Standard Deviation-.05 ( For all  
 Standard Deviation-.03 mg.per ct. Standard Error -.01) 32 pigs  
 Weighted Mean -.05 (

ed an additional amount of the vitamin in excess of that normally consumed daily, the results for the 2.5 mg. and the 5.0 mg. group, even considering the mean values, are indicative of what has been termed a scorbutic or a partial scorbutic state, if the time element is disregarded. Actually, however, the guinea pigs used were known to be normal. This was shown also by their healthy appearance, their excellent appetites, and their regular increase in weight prior to the vitamin determination.

In the experiment (see Table III, on p.8 ) in which 10.0 mg. was given to the animals, the ascorbic acid value subsequently found gave a mean of only 0.51, but even here, two of the seven values were unusually low and, on the basis of a random, single determination, indicate depletion. However, when the time element and the sampling error are considered, it can be seen that the values obtained, standing alone, are of no significance.

The fourth experiment (see Table III, on p.8 ) of the series, where greens were fed at 9:45 and were removed at 11:45 - the time of the first determination - gives data for the second vitamin C determination which, had they been obtained with the history of the previous background lacking, would have led to the erroneous assumption of partial depletion.

If one considers individual values apart from the time element, the fifth experiment (Table IV, on p. 10 and Figure I, on p. 11) would furnish misleading data at the seventh hour as well as at the fourth hour. Yet, considering the experimental data pertaining to vitamin C requirements of the guinea pig (11) (13) (15) (16) (20) (21) (65) (66) no one would seriously assume on the basis of all the facts presented that any of the animals here used was depleted of vitamin C or that the animal passed from "super-saturation" at the end of one hour to a state of partial depletion at the end of the seventh hour, or even the twenty-fourth hour. It is clear, therefore, that in addition to time, the extent of the sampling error must be stated also, and that in random determinations under circumstances where the standard deviation is great, it is almost impossible to designate the state of vitamin C nutrition on the basis of the determination of the blood plasma level of the vitamin.

#### Summary

1. A single, random determination of the ascorbic acid content of the plasma of guinea pigs is not indicative of the state of vitamin C saturation of the tissues of the animal.



2. To determine the state of saturation or of depletion of guinea pigs, one must take into consideration the vitamin C intake of the animal and the time elapsing between the intake of the vitamin and the withdrawal of blood from the animal.
3. Because of individual variations (which may be caused, for example, by different rates of metabolic activity or difference in rate of excretion of the vitamin) one should present, for all experiments, along with the other evidence referred to, the deviation from the mean value found for each experiment. In this way the degree of accuracy of the experiment is indicated.

## REFERENCES

- ( 3) Barron, E.S.G., Brumm, H.J. and Dick, G.F.  
J.Lab. and Clin. Med. 23:1226, 1938.
- ( 8) Dagulf, H. Klin.Wochschr. 18:669, 1939.
- (11) Dann, B. and Cowgill, G.R. J. Nutrition. 9:4,  
1935.
- (12) Dobbelstein, O. Klin. Wochenschrift. 18:610, 1939.
- (13) Ecker, E.E. and Pillemer, L. Proc. Soc. Exper.  
Biol. and Med. 44:262, 1940.
- (14) Ecker, E.E., et al. J. Immunol. 34:39, 1938.
- (15) Farmer, C.J. and Abt, A.F. Proc. Soc. Exper.  
Biol. and Med. 32:1625, 1934-5.
- (16) Farmer, C.J. and Abt, A.F. Proc. Soc. Exper.  
Biol. and Med. 34:146, 1936.
- (20) Giroud, A., Leblond, C.P., Ratsimamanga, R.  
Yale J. Biol. and Med. 9:573, 1937.
- (21) Giroud, A., Santos-Ruiz, A., Leblond, P. and  
Ratsimamanga, A.R. Bull soc. chim. biol. 18:750,  
1936.
- (22) Goldsmith, G.A. and Ellinger, G.F. Arch.Int.  
Med. 63:531, 1939.
- (23) Greenberg, L.D., Rinehart, J.F., and Phatak, N.M.  
Proc. Soc. Exper. Biol. and Med. 35:135, 1936-37.
- (24) Herlitz, C.W. Acta Paediatrica. 23:43, 1939.
- (25) Kajdi, L., Light, J., and Kajdi, C. J. Pediatrics.  
15:197, 1939.
- (27) Kastlin, King, et al. Am. J. Clin. Path. 10:882,  
1940.
- (28) Kellie, A.E. and Zilva, S.S. Biochem. J. 33:153,  
1939.
- (43) Mitolo, M. Atti Accad. Lincei, Classe Sci. 28:  
31, 1938.
- (48) Raabe, S. Biochem. Ztschr. 299:141, 1938.

- (51) Rohmer, P., Bezssonoff, N., Stoerr, E., and Perier, J. *Compt. rend. soc. biol.*, 1935. 118:1090.
- (52) Sacerdote, P. *Ann. Physiol. Physicochimie Biol.* 14:271, 1938.
- (58) Taylor, F.H.L., Chase, D., and Faulkner, J.M. *Biochem. J.* 30:1119, 1936.
- (59) Todhunter, E.N., and Brewer, W. *Am. J. Physiol.* 130:310, 1940.
- (60) Todhunter, E.N., Robbins, R.C., Ivey, G., and Brewer, W. *J. Nutrition.* 19:113, 1940.
- (62) Wachholder, K., Baucke, J., and Podesta, H.H. *Arch. ges. Physiol. (Pflüger's)*. 241:495, 1939.
- (63) Wortis, H., Wortis, S.B., and Marsh, F.I. *Arch. Neurol. and Psychiatry.* 39:1055, 1938.
- (64) Wright, I.S., Lilienfeld, A. and MacLenathen, E. *Arch.Int.Med.* 60:264, 1937.
- (65) Zilva, S.S. *Biochem. J.* 30:1419, 1936.
- (66) Zilva, S.S. *Brit. J. Exper. Path.* 18:449, 1937.
- (17) Fisher, R.A. *Statistical Methods for Research Workers* (Oliver and Boyd) 1934.

THE EFFECT OF VITAMIN C ON THE DETERMINATION  
OF SULFANILAMIDE

Introduction

Because the therapeutic effectiveness of sulfanilamide is dependent on the attainment of a definite minimum concentration of the free drug in the blood and because too much of the free drug in the blood leads to toxic reactions, it has become customary to make routine determinations of the free sulfanilamide present in the blood of patients undergoing treatment (42) (35). Although it is known that certain substances interfere with the determination of the drug by the method of Marshall(et al.) (37), there have appeared no reports in the literature pertaining to the effect of vitamin C on the sulfanilamide determinations, despite the fact that there have recently appeared reports dealing with the combined ascorbic acid-sulfanilamide (or its derivatives) therapy (9) (56) (7) (33) (34) (32). Obviously, if there is any appreciable interference by the vitamin present in the blood with the determination of free sulfanilamide, serious consequences may follow for the patient.

That large amounts of vitamin C may appear in the blood as a result of massive dose administration of ascorbic acid is shown by the works of several authors (51)

(66) (58) (57) (64) (3). In fact, blood levels as high as 25 mg. per cent have been reported in humans.

It was felt, because of the possibility of interference between ascorbic acid and sulfanilamide, that it would be desirable to determine the effects of these two substances on each other in chemical tests.

### Experimental

The methods used for the determination of sulfanilamide were those of Marshall et al. (37) (39) (6). Vitamin C determinations were made by the methods of Farmer and Abt (15) (16).

For the in vivo experiments, blood was obtained by cardiac puncture from guinea pigs which were lightly etherized. The animals used were of both sexes and weighed approximately between 275-425 gms. The sulfanilamide was given by mouth by means of a rubber catheter attached to a suitable syringe. The drug was given as a 15 per cent water suspension in the amount of 323 mgs. of sulfanilamide per kg. of guinea pig; and doses were administered at 0, 3, 7, 11, 15 and 19 hours (2). Ascorbic acid was administered intraperitoneally concurrently with the per os sulfanilamide dosage. Blood samples were obtained immediately after the 19th hour dose was given, and the samples were prepared at once for the determination of

vitamin C and sulfanilamide.

The in vitro experiments were made with a constant amount-10 mg. per cent-of sulfanilamide throughout, except as noted in the tabulation of results. Also, the dye (N-1-naphthyl ethylene diamine dihydrochloride) was added, except as noted in Table I, after the final sulfanilamide-ascorbic acid solution (containing trichloroacetic acid in the same proportion as outlined in the method of Bratton and Marshall (6)) had first been diluted 1:10, giving thereby a 1:20 dilution of the original 20.0 mg. per cent sulfanilamide stock solution. The preparation of the sulfanilamide-ascorbic acid solutions were made by adding to 5.0 cc. of the 20.0 mg. per cent stock solution of sulfanilamide used as the standard the requisite amount of the vitamin contained in 5.0 cc. of a solution of trichloroacetic acid made sufficiently concentrated with respect to the trichloroacetic acid so that in the final sulfanilamide-ascorbic acid solution, the percentage of the trichloroacetic acid would be the same as that in the blood filtrates treated according to the method of Bratton and Marshall (6).

Several ascorbic acid-sulfanilamide-trichloroacetic acid solutions prepared as previously described were refluxed on a water bath for one hour, at the end of which time the solutions were cooled and readjusted to the pre-refluxing volume by the addition of distilled water. The

PERCENTAGE OF SULFANILAMIDE RECOVERED IN  
THE PRESENCE OF VARYING AMOUNTS  
OF VITAMIN C

TABLE I

Mg. Per Cent Sulf.	Mg. Per Cent Reduced Vitamin C	Ratio C : S*	Per Cent of Sulf. Recovered	Mg. Per Cent Oxid. Vitamin C	Per Cent Sulf. Recovered	Mg. Per Cent Reduced Vitamin C Refluxed with Sulf.	Per Cent of Sulf. Recovered	Mg. Per Cent Oxid. Vitamin C Refluxed with Sulf.	Per Cent of Sulf. Recovered	2.0 cc. of Dye Added Before 1:20 Dilution	
										Mg. Per Cent Reduced min C Refluxed with Sulf.	Per Cent of Sulf. Recov.
10.0	0.125	1:80	95.2								
10.0	.25	1:40	97.4								
10.0	.50	1:20	96.3			.50	92.9				
10.0	.75	1:13	95.0								
10.0	1.00	1:10	97.7			1.00	96.0				
10.0	1.25	1:8	97.7								
10.0	1.50	1:7	96.5								
10.0	1.75	1:6	98.0								
10.0	2.00	1:5	98.0			2.00	90.0				
10.0	2.25	1:4	97.5								
10.0	2.50	1:4	99.4								
10.0	2.75	1:4	97.4								
10.0	3.00	1:3	99.8			3.00	83.1				
10.0	5.00	1:2	93.3			5.00	77.8				
10.0	10.00	1:1	91.6	10.00	93.8	10.00	76.4			10.00	59.3
10.0	15.00	3:2	92.0								
10.0	20.00	2:1	89.8	20.00	93.4	20.00	68.3	20.00	75.6	20.00	37.7
10.0	25.00	2.5:1	85.2								
10.0	30.00	3:1	84.2	30.00	88.9	30.00	62.2	30.00	66.3	30.00	22.0
10.0	40.00	4:1	77.3	40.00	73.2	40.00	57.6	40.00	56.7	40.00	15.8
10.0	50.00	5:1	71.5	50.00	63.1	50.00	56.6	50.00	50.7	50.00	9.7
10.0	100.00	10:1	56.1	100.00	65.0						
10.0	150.00	15:1	49.6								
10.0	200.00	20:1	24.5								
10.0	250.00	25:1	Trace								
10.0	300.00	30:1	00.0								

\*Ratio of Vitamin C to Sulfanilamide

THE EFFECT OF THE VITAMIN C:SULFANILAMIDE  
RATIO ON THE RECOVERY OF SULFANILAMIDE

TABLE II

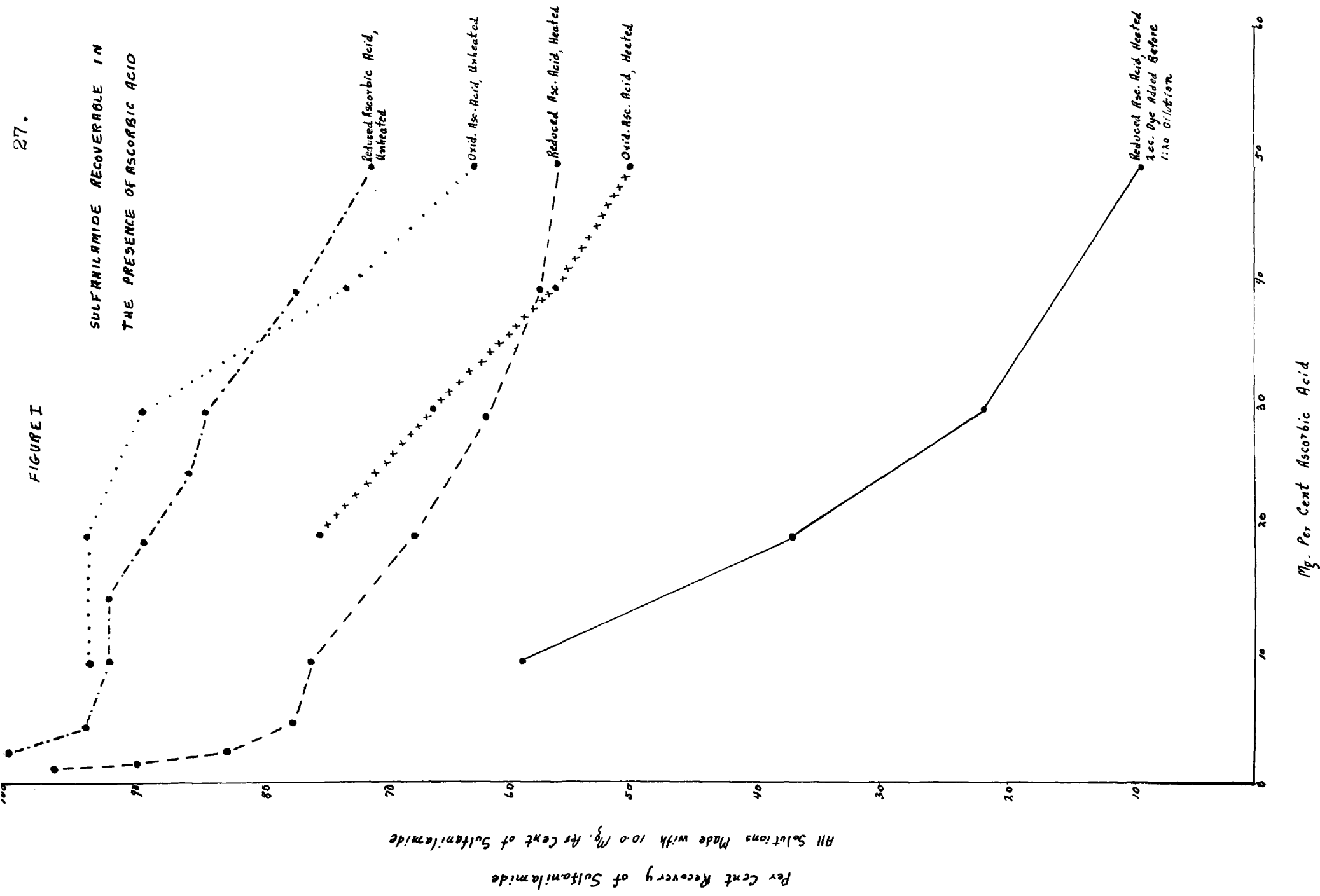
Mg. Per Cent Sulfanilamide	Mg. Per Cent Reduced Vita- min C	Ratio C : S*	Per Cent of Sulfanilamide Recovered
1.0	1.00	1:1	90.4
1.0	2.00	2:1	86.3
1.0	3.00	3:1	84.0
2.0	2.00	1:1	96.3
2.0	3.00	3:2	95.3
2.0	4.00	2:1	85.6
50.00	50.00	1:1	93.7
50.00	150.00	3:1	76.9

\*Ratio of Vitamin C to Sulfanilamide



FIGURE I

SULFANILAMIDE RECOVERABLE IN  
THE PRESENCE OF ASCORBIC ACID



effect of this manner of heating was determined for mixtures of sulfanilamide and reduced ascorbic acid and mixtures of sulfanilamide and oxidized ascorbic acid.

Ascorbic acid solutions were oxidized by heating a distilled water solution of the vitamin under reflux on a water bath for one hour. The vitamin oxidized in this manner was compared with the reduced form of the vitamin in regard to their respective effects on the determination of sulfanilamide.

Sulfanilamide solutions alone when refluxed on a water bath for one hour and tested for the purpose of recovering the sulfanilamide known to be present gave complete recovery within the limits of error previously found as the result of determinations made on a series of 10.0 mg. per cent, separately prepared, unrefluxed, sulfanilamide solutions compared with a standard.

All determinations of sulfanilamide were made in a Klett colorimeter with glass cups. All experiments were repeated at least once, and at least four readings were averaged for each sample read on the colorimeter.

#### Discussion

The determinations of free sulfanilamide in the

in vivo experiments, which were based on those of Armstrong and Thompson(2), confirm the results of these workers both in respect to the highly erratic percentages of the drug found in the blood and in respect to the amounts of sulfanilamide present as a result of the recommended dosage (see Table III, Appendix, and Tables IV, IV-A, and V, on pp. 82, 83, 84, 85). However, it was found that dissolving and reprecipitating the drug before administering it may result in traumatic injury to the stomach because of the presence of sharp-pointed crystals. This type of injury did not occur when the finely powdered sulfanilamide (Merck) was used. Adherence to the schedule of treatment of the guinea pigs recommended by these authors - with only the slight modifications mentioned in a preceding section, resulted in a number of deaths (approximately 10 per cent) within 48 hours. Autopsy, and subsequent sectioning of the organs of several of the animals, revealed the following pathology, which was not, however, present in its entirety in every case examined:

Stomach - Chemical, coagulation necrosis. Hemorrhage; erosion of wall (destroyed epithelium); sub-mucosa thickened, apparently by gas; blood vessels in sub-mucosa eroded, with hemorrhage into mucosa; acute inflammatory phenomena in larger vessels of

sub-mucosa; muscular layer thin because of stretching.

Small Intestine - Mucosa eroded; inflammatory cells; sub-mucosa dilated because of gas; blood vessels intensely engorged; chronic inflammatory cells in the mucosa.

Large Intestine - Essentially same as the small intestine - intense infiltration with inflammatory cells; blood vessels intensely dilated; erosion; involuntary muscle stretched; etc.

Lungs - Broncho-pneumonia (In addition to broncho-pneumonia, all of the larger arteries were tightly contracted).

Spleen - Chronic passive congestion with areas of necrosis in the red pulp.

Liver - Parenchymatous degeneration and engorgement on venous side; early chronic passive congestion; very early fatty degeneration (at periphery of lobules); number of K<sup>u</sup>pffer cells unaltered (Therefore, no effect on reticuloendothelial system).

Kidney - Parenchymatous degeneration; no necrosis; vessels dilated, showing considerable amount of blood in organ; around some glomeruli, infiltration of inflammatory cells as a result of chemical

causes.

All of the above pathology occurred when the re-crystallized drug was used, but no recurrence of these conditions in their entirety appeared with the use of the finely powdered compound. However, a large number of the cases autopsied grossly either as a result of death within 48 hours after the beginning of the experiment or as a result of sacrificing the animals at the end of 48 hours showed hemorrhagic areas in the muscular layers of the stomach, almost always in conjunction with hemorrhagic lungs. The most frequently occurring pathological symptom was hemorrhage in one or more lobes of the lungs.

The animals used in these experiments were distributed as follows:

- 27 guinea pigs of both sexes-200--299 gms. in weight, inclusive
- 60 guinea pigs of both sexes-300--399 gms. in weight, inclusive
- 24 guinea pigs of both sexes-400--499 gms. in weight, inclusive
- 2 guinea pigs, 1 male, 1 female-500--599 gms. in weight, inclusive

Although different conditions in regard to the vitamin C level of the blood were produced in the animals, no correlation whatsoever was found between the amount of free or of conjugated sulfanilamide in the blood and the vitamin C content of the blood. (Tables III,

IV, IV-A, and V, in App., on pp. 82, 83, 84, 85.)

However, inasmuch as the vitamin C in the blood was consistently very low at the time that the blood was withdrawn for the purpose of making tests for both sulfanilamide and vitamin C, it was thought that perhaps the sulfanilamide interfered with the vitamin either by affecting its determination by the method of Farmer and Abt (16), or by causing a change in the in vivo metabolism of the vitamin. The in vitro tests with pure sulfanilamide-ascorbic acid showed, however, that there was no interference between the acid and the sulfanilamide when determinations of ascorbic acid were made with 2,6 dichlorophenol-indophenol as the indicator. On the other hand, the low content of the vitamin in the blood can readily be explained by the fact that within three hours after the drug has been administered, the animals are no longer in a condition to partake of food, as they exhibit spastic paralysis, convulsions, rigidity, ataxia, purposeless movements, retching, and drop in body temperature. The animals remain for the duration of the experiment in a state in which they cannot take nourishment voluntarily. It was shown in part I that under conditions of 20 hours starvation the blood level of vitamin C detectable by the micro method here used drops to an extremely low value. Con-

sequently, although the drop in blood ascorbic acid values may be the result of increased excretion of the vitamin because of the effect of the sulfanilamide (53), nevertheless, the low blood vitamin C values can be explained by the lack of food intake by the guinea pigs.

The in vitro experiments were undertaken to check the results of the in vivo work, and to determine by controlled addition of ascorbic acid to given concentrations of sulfanilamide to what extent there was interference, if any, by ascorbic acid in the determination of sulfanilamide, or by sulfanilamide in the determination of ascorbic acid. (Inasmuch as no interference was given by the sulfanilamide in the determinations of known amounts of ascorbic acid, this phase of work will not again be referred to).

Sulfanilamide determinations in the Klett colorimeter were made to an accuracy of 5 per cent. If the limits of accuracy of the determinations are considered, it can be seen (Table I, Figure I, and Table II) that there is no interference with the drug when a solution contains 10.0 mg. per cent of sulfanilamide and up to 3.0 mg. per cent of the reduced form of ascorbic acid. Above 3.0 mg. per cent, reduced ascorbic acid in the presence of 10.0 mg. per cent of sulfanil-

amide interferes with the sulfanilamide determination increasingly as higher quantities of the vitamin are present along with the constant amount of sulfanilamide. Reference at this point to the data presented in Table I, Figure I, and Table II, shows that interference with the color production is proportional not to the quantity of vitamin C present, but to the ratio of ascorbic acid to sulfanilamide. Thus, if the ratios of reduced ascorbic acid to sulfanilamide in respect to the 1.0 mg. per cent, 2.0 mg. per cent, and 10.0 mg. per cent sulfanilamide solutions are compared, there will be found to exist no significantly different results between the corresponding ratios.

When the reduced form of the vitamin is heated with sulfanilamide on a water bath for one hour under reflux, there takes place a reaction between the ascorbic acid and the sulfanilamide. Evidence for this lies in the facts that the percentage of sulfanilamide recoverable is considerably reduced; that complete recovery can be obtained when sulfanilamide is heated alone; that if the ascorbic acid solution is first oxidized by heating on a water bath for one hour under reflux and is then added to the sulfanilamide solution, the recovery of sulfanilamide (which has not been heated with the oxidized vitamin) is ap-



proximately equal to the amount recoverable when mixtures of sulfanilamide and reduced ascorbic acid (unheated) are tested; and that heating the sulfanilamide with previously oxidized ascorbic acid gives results not appreciably different from those obtained when the reduced ascorbic acid is heated with the drug.

These latter facts are particularly of interest in regard to the determination of the total amount of sulfanilamide in solutions in which either the oxidized or the reduced form of vitamin C may be present, inasmuch as the presence of amounts of ascorbic acid less than 3.0 mg. per cent cause definite interference in attempts at recovery of the sulfanilamide. This amount of ascorbic acid, it will be recalled, does not interfere in the determination of the free drug when the latter is present in the amount of 10.0 mg. per cent.

With respect to the effect of oxidized ascorbic acid on the determination of free sulfanilamide or of combined sulfanilamide, it is seen from Table I and Figure I that the oxidized form of the vitamin does not differ significantly from the reduced form. One additional fact deserves mention. If amounts of the dye at least equivalent to that necessary to react fully with the sulfanilamide are used, the results will vary according to the extent of the dilution of

the sulfanilamide-ascorbic acid solution to which the dye is added. For example, if the dye is added before the 1:20 dilution of the mixture is made, the results in recovery will differ from those obtained when the dye is added after the 1:20 dilution is made. (See Table I and Figure I). The results are more accurate, quantitatively, the greater the dilution before the dye is added.

### Conclusions

- 1 - Both the oxidized and the reduced forms of ascorbic acid interfere with the determination of sulfanilamide by the method of Marshall et al. when the ratio of ascorbic acid to sulfanilamide is 1:2 or greater. Complete inhibition of the reaction does not occur, however, until the ratio reaches a value of 25:1 or greater (250-300 mg. per cent of ascorbic acid in the presence of 10.0 mg. per cent of sulfanilamide).
- 2 - Heating the ascorbic acid and the sulfanilamide together on a water bath for one hour under reflux results in a considerable lowering of the amount of sulfanilamide recoverable even when the ratio of ascorbic acid to sulfanilamide

is as low as 1:20. The amount which can be recovered is here less than that which can be recovered as a result of treating the sulfanilamide with ascorbic acid minus the heat.

- 3 - To get comparable results, one must always add the dye used in the coupling to a definite dilution of the ascorbic acid-sulfanilamide mixture.

## REFERENCES

- ( 2) Armstrong, A.R. and Thompson, J.L. J. Lab. and Clin. Med. 24:1203, 1939.
- ( 3) Barron, E.S.G., Brumm, H.J. and Dick, G.F. J. Lab. and Clin. Med. 23:1226, 1938.
- ( 6) Bratton, A.C. and Marshall, E.K., Jr. J. Biol. Chem. 128:537, 1939.
- ( 7) Cooper, F.B. and Gross, P. Proc. Soc. Expl. Biol. Med. 36:774, 1937.
- ( 9) Dainow, I. Ann. d. Mal. Vener. 34:513, 1939.
- (15) Farmer, C.J. and Abt, A.F. Proc. Soc. Exper. Biol. and Med. 32:1625, 1935.
- (16) Farmer, C.J. and Abt, A.F. Proc. Soc. Exper. Biol. and Med. 34:146, 1936.
- (32) Locke, A. J. Immunol. 36:159, 1939.
- (33) Locke, A., Locke, R.B., Bragdon, R.J., and Mellon, R.R. Science 86:228, 1937.
- (34) Locke, A., Main, E.R., and Mellon, R.R. J. Immunol. 36:183, 1939.
- (35) Long and Bliss. Clinical Use of Sulfanilamide and Sulfapyridine, etc. The Macmillan Company, 1939.
- (37) Marshall, E.K., Jr. Proc. Soc. Exper. Biol. and Med. 36:422, 1937.
- (39) Marshall, E.K., Jr. and Cutting, W.C. J.H.H. Bull. 63:328, 1938.
- (42) Mellon, Gross and Cooper. Sulfanilamide Therapy of Bacterial Infections. Charles C. Thomas, 1938.
- (51) Rohmer, P., Bezssonoff, N., Stoerr, E., and Perier, J. Compt. rend. soc. biol. 1935, 118:1090.
- (53) Samuels, L.T., Ritz, N.D., and Poyet, E.B. J.P.E.T. 68:465, 1940
- (56) Steinbach, M.M. and Duca, C.J. Proc. Soc. Exper. Biol. and Med. 44:133, 1940.

- (57) Stephens, D.J. and Hawley, E.E. J. Biochem. 115:653, 1936.
- (58) Taylor, F.H.L., Chase, D. and Faulkner, J.M. Biochem. J. 30:1119, 1936.
- (64) Wright, I.S., Lilienfeld, A., and MacLenathen, E. Arch. Int. Med. 60:264, 1937.
- (66) Zilva, S.S. Brit. J. Exp. Path. 18:449, 1937.

THE EFFECT OF ASCORBIC ACID ON THE ACUTE  
TOXICITY OF SULFANILAMIDE FOR  
GUINEA PIGS

Introduction

It has been suggested that the effect of sulfanilamide might be influenced by the ascorbic acid content of an animal. Dainow (9) reported that in all cases where an intravenous injection of ascorbic acid was given along with sulfanilamide, it was possible to administer relatively large doses of sulfanilamide or its derivatives without provoking manifestations of intolerance which these drugs generally cause when administered alone. Dainow and Zimmet (10) stated that toxic symptoms caused by sulfanilamide derivatives are the result of a vitamin C hypovitaminosis engendered by the drugs and that appropriate administration of ascorbic acid will alleviate the symptoms. Vauthey (61) attributes to ascorbic acid the power of diminishing the toxicity of chemotherapeutic compounds. The literature contains also several reports dealing with the administration of ascorbic acid along with sulfanilamide, or its derivatives, in chemotherapeutic studies (56) (33) (7) (32) (34).

Although the mouse and the rat have been used most

frequently in experimental work in sulfanilamide, these animals are not suitable for vitamin C investigations, inasmuch as they cannot be fully depleted of vitamin C. It was, consequently, necessary to use the guinea pig, which is susceptible to scurvy.

Because a search of the literature pertaining to sulfanilamide failed to reveal an accurate determination of the acute toxic dose of the drug for guinea pigs\* a determination of this dose was undertaken and was compared with the effect of the vitamin C content of guinea pigs on the acute toxic dose of sulfanilamide for these animals.

#### Experimental

In the determination of the acute toxic dose of sulfanilamide, a single dose of the drug as a 20 per cent suspension in water was administered orally to guinea pigs. The dose was calculated in terms of grams per kilogram of body weight of animal. This method of giving the drug was followed throughout all of the experiments. All animals were observed for seven days.

The toxic dose was determined in terms of LD<sub>50</sub> which is the dose that is lethal for 50 per cent of

---

\*In a personal communication, Dr. G.M. Findlay states that in his laboratories (unpublished data), the LD<sub>50</sub> of sulfanilamide in guinea pigs was found to be 2.50 grams per kilogram of body weight.

the animals under the conditions of the experiment. The calculations for its determination were carried out according to the methods of Gaddum (18) and of Bliss (4) (5).

Ascorbic acid (Cebione, Merck) was administered intraperitoneally in water solution. The solution was freshly prepared prior to each series of injections, and the pH was adjusted to approximately 7.4. The total dose varied according to the experiment.

To determine the effect of vitamin C on the acute toxicity of sulfanilamide, animals receiving large doses of vitamin C and animals depleted of vitamin C were compared with normal groups. To keep a group of guinea pigs at a high vitamin C blood level, it was necessary to inject the ascorbic acid solution several times during the experiment. One series of animals was, therefore, injected with ascorbic acid one-half hour prior to the administration of the single dose of sulfanilamide, one hour after the sulfanilamide was given, and again two and one-half hours after the sulfanilamide had been given. A second group of animals receiving large amounts of vitamin C was given a fourth injection of ascorbic acid on the day of the sulfanilamide dosage and three injections daily for two successive days. Each injection consisted of 10.0 mgs. of ascorbic acid.



Inasmuch as there was no significant difference between these two series of guinea pigs treated with ascorbic acid, they were combined and referred to as the "high vitamin C" group.

The depleted guinea pigs were kept for ten days on the diet described below, minus greens. Animals used as normals for comparison were given the same diet as previously referred to.

Guinea pigs used in these experiments belonged to both sexes and weighed approximately 300 grams. All animals were kept at least three days, after their arrival in this laboratory, before being used.

The LD<sub>50</sub> was determined for both fasting and non-fasting animals. Non-fasting animals were fed on a diet of mixed grains, cod liver oil, brewer's yeast (Fleischmann), and greens ad lib. Food was removed from the cages of the fasting animals about fifteen hours before treatment was begun. In the case of depleted animals, the diet was the same except for the removal of the greens.

### Results

In Tables VII to X in the appendix, and in Table I of the text, will be found the protocols for these experiments and the formulas<sup>\*</sup> used in the calculation of the LD<sub>50</sub>'s, the slopes of the curves, and the significant

---

\*See Table I, p. 80, in Appendix

TABLE I

## SULFANILAMIDE MORTALITY OF FASTING AND NON-FASTING GUINEA

## PIGS IN DIFFERENT STATES OF VITAMIN C NUTRITION

<u>Non-Fasting Guinea Pigs</u> (May-July, 1940)			<u>Fasting Guinea Pigs</u> (August-November, 1940)		
<u>Dose-Gms.</u> <u>Per Kg.</u>	<u>Mortality</u>	<u>Per Cent</u> <u>Mortality</u>	<u>Dose-Gms.</u> <u>Per Kg.</u>	<u>Mortality</u>	<u>Per Cent</u> <u>Mortality</u>
<u>Normal Animals</u>			<u>Normal Animals</u>		
1. 3.90	3/4	75	3.87	10/11	91
2. 3.56	1/4	25	3.35	13/14	93
3. 3.45	4/7	57	2.92	12/14	86
4. 3.38	19/23	83	2.58	24/31	77
5. 3.31	3/8	38	2.41	16/20	80
6. 3.21	1/4	25	2.24	4/11	36
7.			1.90	2/11	18
<u>High Vitamin C</u>			<u>High Vitamin C</u>		
1. 3.45	4/5	80	3.87	15/16	94
2. 3.38	15/30	50	3.35	16/19	84
3.			2.92	17/19	89
4.			2.24	4/22	18
5.			1.90	7/22	32
<u>Vitamin C Depleted</u>			<u>Vitamin C Depleted</u>		
1.			2.58	6/7	86
2.			2.24	8/12	67
3.			1.90	4/13	31

TABLE II

LD<sub>50</sub>'s OF SULFANILAMIDE FOR FASTING AND NON-FASTING GUINEA  
PIGS IN DIFFERENT STATES OF VITAMIN C NUTRITION

<u>Groups</u>	<u>b±σ</u>	LD <sub>50</sub> *	Range of LD <sub>50</sub> 95 Times in 100
Non-Fasting Normals	7.90 ± 9.35	3.13	2.78 - 3.51
Non-Fasting High Vitamin C	91.75 ± 75.20	3.29	2.90 - 3.73
Fasting Normals	7.77 ± 1.81	2.16	1.97 - 2.36
Fasting High Vitamin C	7.65 ± 1.40	2.40	2.19 - 2.63
Fasting Vitamin C Depleted	12.11 ± 4.81	2.05	1.78 - 2.35

\*All LD<sub>50</sub>'s calculated with composite "b", which is equal  
to 7.937 ± 1.154

TABLE III

SIGNIFICANT DIFFERENCE OF MEANS\* BETWEEN LD<sub>50</sub>'s OF  
 SULFANILAMIDE FOR FASTING AND NON-FASTING  
 GUINEA PIGS IN DIFFERENT STATES OF  
 VITAMIN C NUTRITION

		<u>Non-Fasting</u>		<u>Fasting</u>		
		Normals	Vitamin C	Normals	Vitamin C	Vitamin C Depleted
Non-Fasting	Normals	----	0.56	4.46	3.28	4.40
	Vitamin C	0.56	----	5.37	3.62	4.67
Fasting	Normals	4.46	5.37	----	1.51	0.61
	Vitamin C	3.28	3.62	1.51	----	1.88
	Vitamin C Depleted	4.40	4.67	0.61	1.88	----

\*Prob. = .01 (t=2.75) for 30 or more animals

difference of means.

### Discussion

Reference to Table II for the non-fasting and the fasting groups indicates that the slopes of the various determinations - which are a measure of the uniformity of the animals used - do not differ significantly from each other. Although the "b" value - slope - for the non-fasting high vitamin C experiment was not accurately determined, the standard deviation of the slope is so large that this "b" does not differ significantly from the other "b's". Consequently, a weighted mean "b" was calculated from the data presented in Table I for all of the experiments, both in the fasting and in the non-fasting groups. This composite "b" is hereafter referred to as " $b_c$ ". The  $LD_{50}$  for each one of the different experiments was calculated by means of Bliss' equation #28(5), with " $b_c$ " used in all instances.

In the non-fasting groups, the  $LD_{50}$ 's were 3.13 and 3.29 grams per kilogram of guinea pig for the normal and the high vitamin C animals, respectively (Table II). Determination of the significant difference of means for these values showed that they do not differ significantly from each other when a probability of 0.01 and a "t" (17) value of 2.75 are used (Table III). This

means that there is only one chance in 100 that the difference in these LD<sub>50</sub> values is not a result of normal sampling error.

In the non-fasting groups, the LD<sub>50</sub>'s for the normals, the high vitamin C, and the vitamin C depleted guinea pigs were, respectively, 2.16, 2.40, and 2.05 grams per kilogram of body weight of animal (Table II). Here, too, there was no significant difference between any two of these values (Table III).

Reference to Table III shows, however, that there is a highly significant difference between any value of the non-fasting groups and any value of the fasting groups. It is apparent, therefore, that fasting animals have a lower LD<sub>50</sub> for sulfanilamide than do non-fasting animals and that, consequently, sulfanilamide is more toxic to fasting than to non-fasting guinea pigs. It is further obvious that the presence or absence of vitamin C in the blood stream does not, at least under the conditions of these experiments, affect the toxicity of sulfanilamide. (It is interesting to note that Marshall and Litchfield (38), working with mice, have also found that the presence or absence of food in the gastro-intestinal tract influences the toxicity of sulfanilamide).

#### Summary

1. From these experiments, it can be concluded

that vitamin C has no influence on the acute toxicity of sulfanilamide for guinea pigs.

2. The LD<sub>50</sub> for fasting guinea pigs is significantly different from that for the non-fasting animals.
3. A total of 363 guinea pigs was used in these studies.

## REFERENCES

- ( 4) Bliss, C.I. Ann. Applied Biol. 22:134, 1935.
- ( 5) Bliss, C.I. Ann. Applied Biol. 22:307, 1935.
- ( 7) Cooper, F.B. and Gross, P. Proc. Soc. Exper. Biol. and Med. 36:774, 1937.
- ( 9) Dainow, I. Ann. malad. vener. 34:513, 1939.
- (10) Dainow, I. and Zimmet, D. Compt. rend. soc. biol. 130:846, 1939.
- (17) Fisher, R.A. Statistical Methods for Research Workers (Oliver and Boyd) 1934.
- (18) Gaddum, J.H. Medical Research Council, Special Report Series, 1933. #183
- (32) Locke, A. J. Immunol. 36:159, 1939.
- (33) Locke, A., Locke, R.B., Bragdon, R.J. and Mellon, R.R. Science. 86:228, 1937.
- (34) Locke, A., Main, E.R. and Mellon, R.R. J. Immunol. 36:183, 1939.
- (38) Marshall, E.K., Jr. and Litchfield, J.T., Jr. J.P.E.T. 67:454, 1939.
- (56) Steinbach, M.M. and Duca, C.J. Proc. Soc. Exper. Biol. and Med. 44:133, 1940.
- (61) Vauthey, M. Ann. malad. vener. 32:98, 1937: also Prat. Med. Franc. 18:107, 1937.



THE TREATMENT OF A GROUP C STREPTOCOCCUS INFECTION  
IN GUINEA PIGS WITH VITAMIN C AND SULFANILAMIDE

**Introduction**

The role of vitamin C in the infected animal has been studied by many investigators, and excellent reviews of the literature on this subject have been published, (50) (46) (36) (19) (1) (47) (29) (41). Despite the numerous investigations on this problem, opinion is still divided as to whether vitamin C does or does not give any degree of protection to the infected animal. In reviewing the subject of vitamins and resistance to infection, Robertson (50) stated that relatively few investigators had brought forward evidence to the effect that a deficiency of vitamin C does not lead to a lower resistance to infection. She also stated that numerous reports demonstrating the effect of vitamin rich diets in clinical tuberculosis have been published, but that it is impossible to decide what role vitamin C plays in such treatment.

Perla and Marmorston (46) in their review concluded that latent scurvy in animals and man is associated with marked increase in their susceptibility to drugs and bacterial toxins...the scorbutic state is associated with increased susceptibility to spontaneous

infection. They asserted that what part is due solely to vitamin C deficiency, what part to under-nutrition, and what part to concomitant deficiencies of other dietary factors is not well established. The authors stated that indications are that complete deficiency may lower natural resistance to subsequently induced, acute infection. It has not been unequivocally established what effect chronic insufficiency of vitamin C in the diet has on natural resistance. They ended their review by saying that the prophylactic or therapeutic value of an excess of vitamin C needs further confirmation through experimentation.

Mandillon (36) asserted that sub-acute deficiency of vitamin C may cause syndromes which clinically have no relationship with frank deficiency. He stated, further, that vitamin C is not a universal panacea but that it can be used to aid in restoring health in many instances.

On the other hand, A. Giroud (19) said that the activity of the organism; that is, exercise, fever, hormone action (thyroxin), increases destruction of the vitamin. "There is a manifest relationship", he stated, "between ascorbic acid content and phenomena of recuperation.....As one can see, ascorbic acid and certain of its derivatives constitute a therapeutic

element of the first order, whether one utilizes it as a pharmacological agent or as a vitamin."

According to Abt and Farmer (1) and King (29), vitamin C seems important in increasing immunity, resistance, and wound healing.

Peters and Davenport (47) asserted that it is quite clear to an impartial observer that no case has yet been made out for ascorbic acid as an anti-toxic agent per-se; that is, apart from its influence as an antiscorbutic agent. They continued by stating that there is no evidence from animal experimentation that saturation, as such, matters. They added that as a qualification to this Perla reported one unpublished experiment indicating that extra vitamin C protected guinea pigs against trypanosome infection.

In his recent review of the subject, McCullough (41) made the following statements: "...it was felt that guinea pigs on a vitamin C deficient diet for longer than one week prior to inoculation were useless as test animals, since the acute scurvy itself would limit the length of survival and mask any effects of the infectious agent.

"As pointed out in the historical review, the role of vitamin C in resistance to infection has been obscured by the technic of investigation. Most previous

workers have used virulent organisms for experimental purposes. The experimental animal would then die from a summation of the injuries - the infectious process and the vitamin deficiency. As stated earlier, the author does not feel that such a technic gives results that can be satisfactorily evaluated. A scorbutic guinea pig is a very sensitive animal. Almost any injury in addition to the existing injury of the vitamin C deficiency will result fatally for the animal.

"Avirulent human strains that did not produce infection in guinea pigs on a vitamin C adequate diet, produced minor abscesses in the extremely scorbutic guinea pig. Animals with a subacute scurvy did not develop infection. The slightly more virulent animal strains, which produced transient abscesses in the control animals, gave a more severe degree of infection in the vitamin C deficient guinea pigs. The lesions persisted and the guinea pigs died sooner than did uninfected vitamin C deficient animals. A severe scorbutus was necessary before a drop in resistance to *Bacterium necrophorum* became evident."

If it is assumed that an adequate vitamin C level is an important defensive mechanism in the infected host, the observation by Dainow and Zimmet (10) that

the ingestion of sulfanilamide causes a diminution in the vitamin C content of the testicles, liver and brain of all the animals treated is highly significant. Furthermore, Dainow (9) reported that the purpuras, gingival hemorrhages, gastro-intestinal disturbances, cutaneous eruptions, hematurias, epistaxis and anemia all resulting from sulfanilamide therapy, are each characteristic signs of scurvy, and that the concomitant administration of ascorbic acid prevents these toxic manifestations. Moreover, Mouriquand et al. (44) reported a scurvy-like syndrome resulting from doses of 0.5 gm. of sulfanilamide per kg. of body weight. On the other hand, Vauthey (61) attributes to ascorbic acid the power of diminishing the toxicity of chemotherapeutic compounds.

Search of the available literature pertaining to the administration of vitamin C with sulfanilamide or its derivatives in experimental animals revealed only the reports of Steinbach and Duca (56) and the associates of Mellon (7) (32) (33) (34). Steinbach and Duca found the administration of sulfapyridine alone or in conjunction with vitamin C to be ineffective in the treatment of guinea pigs infected with human tubercle bacilli. Locke et al. (32) (33) (34) reported that in rabbits infected with the type I pneumococcus

and treated with sulfanilamide, the administration of vitamin C lowered the mortality rate. However, when the rat was used as the experimental animal, Cooper and Gross (7) did not find that the administration of vitamin C in addition to sulfanilamide had any effect on the mortality rate in type II pneumococci infections.

On the basis of the above observations, it was believed that an experimental investigation of the relationship between vitamin C and sulfanilamide in the treatment of an infectious disease should prove of interest. Since Seastone (54) had reported that group C streptococci infections in guinea pigs were amenable to sulfanilamide therapy, it was concluded that this combination of organism and experimental animal would be the most effective to employ, because guinea pigs, in contrast to rats and mice, can be depleted of vitamin C. Admittedly, it is more desirable to use a group A streptococcus as the infecting agent, but guinea pigs are not usually susceptible to the human strains.

### Experimental

Grouping of animals: One hundred and ninety-one guinea pigs of both sexes weighing between 250-400 grams were grouped as follows according to the treatment received:

**Infected Animals:**

- Group I - 30 animals not receiving ascorbic acid or sulfanilamide (infected controls)
- Group II - 30 animals receiving sulfanilamide
- Group III - 30 animals receiving ascorbic acid
- Group IV - 30 animals receiving ascorbic acid and sulfanilamide
- Group V - 20 animals depleted of vitamin C (for 10 days)
- Group VI - 20 animals depleted of vitamin C (for 10 days) and receiving sulfanilamide

**Non-infected Animals:**

- Group VII - 7 animals depleted of vitamin C (for 10 days) and untreated
- Group VIII - 24 normal animals

Diet: The diet of all animals, except those in the depleted groups, was prepared daily and consisted of the following ingredients:

Rolled oats	100.	gms.
Cod liver oil	1.	gm.
Brewer's yeast (Fleischmann)	0.5	gm.
Sodium chloride	0.25	gm.

This diet was supplemented with water and green food ad lib. throughout the experiment.

The animals in the depleted groups (V,VI,VII) were given a similar diet except that for three days preceding inoculation and for seven days thereafter green food was eliminated from the diet. On the eighth day after

inoculation, green food was again given to these animals. In this way, deaths from scurvy in the inoculated animals were avoided as attested by the fact that the animals in the control group (VII) were in good health without loss of weight or appetite at the end of the experiment even though the blood level of vitamin C was low. Normal control animals (group VIII) were kept under the same conditions as the experimental animals, and all survived the experiment, indicating that intercurrent infections probably did not play a part in the experimental results obtained. Depletion of the animals by withdrawal of greens three days before inoculation is ample time to remove ascorbic acid from the blood, inasmuch as previous titrations in these laboratories have shown that only traces of ascorbic acid can be detected in the blood 24 hours after elimination of greens from this guinea pig diet.

Infective agent: All animals in groups I to VI were inoculated subcutaneously with a 19-24 hour broth culture of a group C hemolytic streptococcus\* containing from 4 to 16 million organisms per cc: (12/3/40 - 4,200,000 per cc., 12/12/40 - 16,160,000 per cc., 1/14/41 - 4,000,000 per cc.). One-tenth cc. of the broth culture

---

\*I am indebted to Dr. J. Howard Brown of the Johns Hopkins Medical School for typing this organism.



per 100 grams of body weight of the animals was injected. The streptococcus, which was isolated from the heart's blood of a naturally infected guinea pig in the laboratory colony, fermented sorbitol but not trehalose and was thus classed as an animal group C streptococcus. It was heavily encapsulated and occurred most frequently in either grape-like clusters or with occasional chains containing from three to ten cocci. The strain used to inoculate all experimental animals had been rapidly passed through three guinea pigs to enhance the virulence of the organism. When this organism was injected subcutaneously into guinea pigs, septicemic death in more than fifty per cent of the animals occurred within 72 hours (Table I). Occasionally, the infection assumed a more chronic course with the production of abscesses under the pericardium, in the inguinal and cervical lymph nodes, and at the site of inoculation. The streptococcus was always isolated from these abscesses.

Culture medium: All cultures for inoculation and subcultures for isolation of the organism at autopsy were grown in an infusion broth having the following composition: beef infusion, 1000cc., bacto neopeptone, 20 grams; sodium chloride, 5 grams; and dextrose, 5 grams. The reaction was adjusted to pH 7.6 before

autoclaving.

Administration of Ascorbic Acid and Sulfanilamide: An aqueous solution of ascorbic acid containing 15 mgms. per cc., and an aqueous suspension of sulfanilamide containing 50 mgms. per cc. were prepared each time immediately before feeding these substances to the animals. Guinea pigs receiving both ascorbic acid and sulfanilamide were fed an aqueous mixture containing 15 mgms. of ascorbic acid plus 50 mgms. of sulfanilamide per cc.

One hour after the infective agent had been inoculated, each animal received 1 cc. of the ascorbic acid solution, 1 cc. of the sulfanilamide suspension, or 1 cc. of the ascorbic acid-sulfanilamide mixture according to the groups into which the animals had been divided, as previously described. The animals were fed perorally by means of a small rubber catheter attached to a suitable glass syringe. Approximately three and one-half hours later, the initial dosage was repeated; and this, in turn, was followed by a third dose three and one-half hours after the second. This schedule of feeding was repeated for the two days following infection, but on the fourth, fifth, seventh, and eighth days following infection, only 1 cc. of ascorbic acid, sulfanilamide, or 1 cc. of the mixture was fed per diem.

The dosage of sulfanilamide used here was chosen on the basis of the results of several workers (54) (49) (2) (55). The ascorbic acid doses employed were based on blood titrations made in previous work in these laboratories and were calculated to produce a high vitamin C blood level of approximately 3.5 mgms. per cent at the time the blood of the guinea pigs had its highest sulfanilamide content.

Because of the number of animals involved, it was found necessary to divide the work into three separate experiments. In the first experiment, there were 10 animals in each of the groups I, II, III, and IV; in the second experiment, there were 12 animals in each of the groups I, II, III, and IV; in the third experiment, there were 8 animals in each of the groups I, II, III, and IV; 20 animals in each of the groups V and VI; 7 animals in group VII; and 24 animals in group VIII.

The time interval between the first and last experiments was approximately six weeks, but since the virulence of the organism and the sensitivity of the animals to the drugs during this time did not change as indicated by the control animals included in each experiment, it was considered valid to combine the results of these three series of experiments, as shown in Table I.

After inoculation, the number of deaths during each eight or twelve hour period of the experiment was recorded as indicated in Table I. All dead animals were autopsied, and a loopful of the heart's blood was inoculated into infusion broth which was incubated at 37°C. until growth appeared except as noted below. Streptococci were observed in 111 cultures (94.0 per cent), while two cultures (1.7 per cent) contained gram positive bacilli, and five (4.3 per cent) were sterile.

All animals were observed for a period of two weeks (336 hours) which was arbitrarily considered the end point of the experiment, inasmuch as observations continued for several weeks following this two-week period did not significantly change the results.

### Results

The combined results of the three experiments with the inoculated animals (groups I-VI) are presented in Table I and Figure I.

The results for the two-week observation period have been calculated as per cent mortality and as weighted mean survival time for each group. The per cent mortality does not give an adequate picture of the results, since animals dying at the beginning and

TABLE I  
SURVIVAL TIMES OF INFECTED CONTROLS  
AND TREATED GUINEA PIGS

Observation Period		Group I	Group II	Group III	Group IV	Group V	Group VI
D A Y S	H O U R S	Con- trols	Sulfanil- amide	Ascorbic Acid	Ascorbic Acid and Sulfanil- amide	Deplet- ed Con- trols	Depleted Sulfanil- amide
I	8	0	0	0	0	0	0
	16	0	0	0	0	0	0
	24	0	0	1	0	2	0
	32	1	0	7	1	4	0
II	40	2	0	1	1	0	0
	48	13	1	9	1	3	1
	56	0	2	4	0	0	1
III	64	0	0	0	1	0	0
	72	1	0	0	1	3	1
	80	1	1	0	0	0	1
IV	88	0	0	0	1	0	0
	96	1	2	1	1	2	1
	104	2	0	0	0	0	0
V	112	2	0	0	0	0	0
	120	1	0	1	0	0	0
	128	1	0	2	0	0	0
VI	136	1	0	1	0	5	1
	144	1	0	0	0	0	0
	152	0	0	0	0	0	0
VII	160	0	0	1	1	0	0
	168	2	0	0	1	0	0
	180	0	0	0	2	0	0
VIII	192	0	1	2	1	0	3
	204	0	0	0	0	0	0
IX	216	0	0	0	0	0	1
	228	0	0	0	0	0	0
X	240	0	0	0	0	1	1
	252	0	0	0	0	0	0
XI	264	0	1	0	1	0	0
	276	0	0	0	0	0	1
XII	288	0	1	0	1	0	0
	300	0	0	0	0	0	0
XIII	312	0	0	0	1	0	0
	324	0	1	0	0	0	0
XIV	336	0	2	0	0	0	0
Mortality		29	12	30	15	20	12
Number of Animals Infected		30	30	30	30	20	20
Per Cent Mortality		96.7	40	100	50	100	60
Total Hours Survival		2,616	7,884	2,096	7,224	1,648	4,484
Weighted Mean Sur- vival Time in Hours		87.20 (26.0%)	262.80 (78.2%)	69.87 (20.8%)	240.80 (71.4%)	82.40 (24.5%)	224.20 (66.7%)

(See Appendix, Table XI---for Complete Data)

TABLE II

SIGNIFICANT DIFFERENCE OF MEANS FOR EVERY  
PAIR OF GROUPS OF INOCULATED ANIMALS

	Group I. Con- trols	Group III. Ascor- bic Acid	Group V. Deplet- ed Con- trols	Group II. Sulfanil- amide	Group IV. Ascorbic Acid and Sulfanil- amide	Group VI. Depleted Sulfanil- amide
Group I. Controls	---	1.2	0.3	7.9	6.5	10.0
Group III. Ascorbic Acid	1.2	---	0.8	8.9	7.6	13.1
Group V. Depleted Controls	0.3	0.8	---	8.0	6.5	9.5
Group II. Sulfanil- amide	7.9	8.9	8.0	---	0.8	1.8
Group IV. Ascorbic Acid and Sulfanil- amide	6.5	7.6	6.5	0.8	---	0.8
Group VI. Depleted Sulfanil- amide	10.0	13.1	9.5	1.8	0.8	---

at the end of the experiment are given equal weight. However, a much more accurate picture of the results is obtained by using the weighted mean survival time, inasmuch as by this method animals are then weighted according to their respective survival times. The weighted mean survival time for each group was calculated by multiplying the time interval elapsing between inoculation and death of the animals by the number of animals dying in this interval. The number of animals living at the end of the experiment was multiplied by the total observation time of 336 hours. The sum of the products thus obtained was divided by the total of number of animals used in the group.

The data presented in Table I have been graphically portrayed in Figure I, so that one may more readily comprehend the difference in survival times of the animals in the various groups.

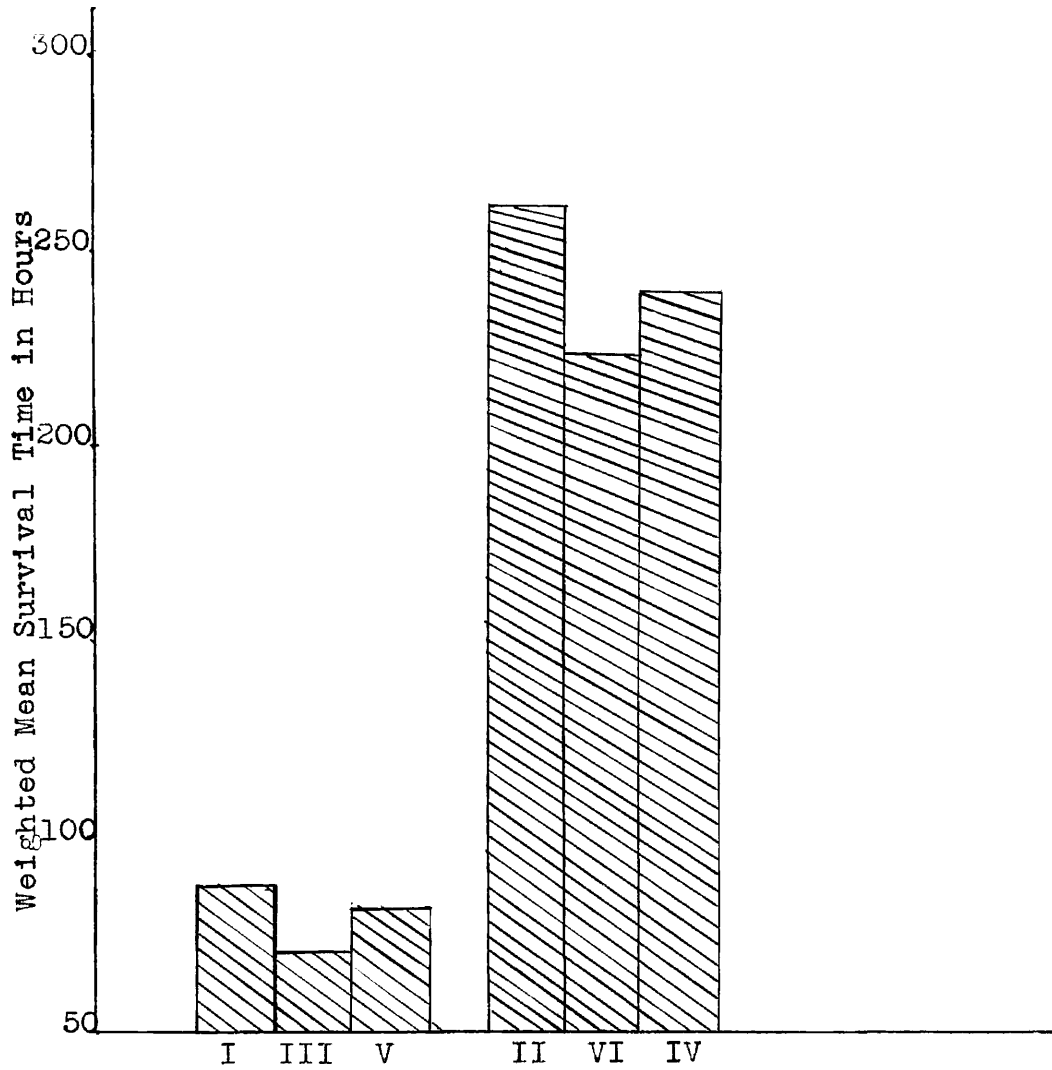
In order to determine whether the differences in the survival times of the various groups were statistically significant, the significant difference of means\* (17) was calculated for every pair of groups of inoculated animals. The results of these calculations are presented in Table II.

---


$$* \frac{m_1 - m_2}{\sqrt{\epsilon_1^2 + \epsilon_2^2}}$$

FIGURE I

COMPARISON OF THE SURVIVAL TIMES OF GUINEA  
PIGS INFECTED WITH GROUP "C" STREPTOCOCCI



Groups According to Treatment Received

Legend

I	Untreated	II	Sulfanilamide Only
III	Ascorbic Acid Only	VI	Vit. C Depl. and Sulf.
V	Vitamin C Depleted	IV	Asc. Acid and Sulf.



This table has been arranged so that one can determine whether or not the difference in weighted mean survival time of any two groups of animals in the experiment is statistically significant. Any figure larger than 2.9 is considered to indicate a significant difference, since with groups of 20 or more animals, this figure gives a probability of only 1 in 100 that the difference would arise by chance (17).

From Table II it will be observed that in these experiments, the only significant difference in survival time occurs between those groups receiving (II, IV, and VI) and those not receiving (I, III, and V) sulfanilamide. The presence or absence of ascorbic acid in animals also receiving sulfanilamide (Groups IV and VI) did not significantly change the survival time in comparison with the normal group (I) receiving sulfanilamide only. Furthermore, there is no significant difference between the control group (I) and the group receiving ascorbic acid only (III), and the depleted control group (V).

#### Discussion

The results of these experiments, establishing the effectiveness of sulfanilamide in the treatment of group C streptococci infections in guinea pigs, confirmed the results of Seastone (54).

Locke et al. (32) (33) (34) reported that in rabbits infected with type I pneumococci, the administration of ascorbic acid in addition to sulfanilamide lowered the mortality rate. While these results do not agree with those reported in this paper, the experiments are not comparable, because these workers used a different organism and test animal.

While the results in the present report showing that ascorbic acid has no effect agree with those of Cooper and Gross (7), again a comparison is not valid, because these workers used a different test organism and animal.

All available articles studied revealed that no work has been previously reported showing the therapeutic effect of sulfanilamide on vitamin C depleted animals.

In regard to the in vitro effect of vitamin C on the bactericidal power of sulfanilamide, it was recently reported that ascorbic acid has no effect on the drug (26).

In view of the controversy regarding the relationship between vitamin C itself and resistance to infection, it was considered of interest to observe whether there was any difference in the survival time of the animals receiving ascorbic acid (III), those depleted of

ascorbic acid (V), and those in the control groups with so-called normal blood levels of ascorbic acid (I). Although the weighted mean survival times of these groups were 69.87, 82.40, and 87.20 hours, respectively, these differences are not statistically significant (Table II); they indicate, rather, that the blood level of vitamin C apparently has no effect on resistance to infection, at least under the condition of these experiments.

Robertson (50) and Perla and Marmorston (46), in considering the effect of vitamin C on infection, point out that a great deal of the work which has been done on this problem loses much of its weight, because the results were not statistically significant. In the experiments reported in this paper, a sufficient number of animals were used to obtain significant results.

#### Summary

- 1 - In 160 guinea pigs infected with a group C streptococcus, the course of the infection and the effectiveness of sulfanilamide were studied in relation to the vitamin C content of the animals. Thirty-one non-infected animals with a normal or low vitamin C content were included as controls.
- 2 - The weighted mean survival time for the various

groups of guinea pigs was determined at the end of 14 days (336 hours) of observation.

- 3 - Sulfanilamide was effective in the treatment of group C streptococci infections in guinea pigs, but neither high ascorbic acid content nor 10 days of vitamin C depletion influenced the effect of sulfanilamide.
- 4 - In the absence of sulfanilamide treatment, the course of the infection as measured by survival time was not influenced by either high ascorbic acid content or by 10 days of vitamin C depletion.

## REFERENCES

- ( 1) Abt, A.F. and Farmer, C.J. J.A.M.A. 111:1555, 1938.
- ( 2) Armstrong, A.R. and Thompson, J.L. J. Lab. and Clin. Med. 24:1203, 1939.
- ( 7) Cooper, F.B., and Gross, P. Proc. Soc. Exper. Biol. and Med. 36:774, 1937.
- ( 9) Dainow, I. Ann. d. Mal. Vener. 34:513, 1939.
- (10) Dainow, I. and Zimmet, D. Compt. Rend. Soc. Biol. 130:846, 1939.
- (17) Fisher, R.A. Stat. Meth. for Research Workers (Oliver and Boyd) 1934.
- (18) Gaddum, J.H. Reports on Biol. Standards. Med. Res. Council S.R.S. No. 183, 1933.
- (19) Giroud, A. Presse Med. 46:1838, 1938.
- (26) Kalmanson, G.M. J. Bact. 40:817, 1940.
- (29) King, C.G. Ann. Rev. Biochem. 8:371, 1939.
- (32) Locke, A. J. Immunol. 36:159, 1939.
- (33) Locke, A., Locke, R.B., Bragdon, R.J. and Mellon, R.R. Science. 86:228, 1937.
- (34) Locke, A., Main, E.R., and Mellon, R.R. J.Immunol. 36:183, 1939.
- (36) Mandillon, G. Rev. de Med. 55:552, 1938.
- (41) McCullough, N.B. J. Infect. Dis. 63:34, 1938.
- (44) Mouriquand, G., Edel, V., Tete, H., and Dauvergne, M. Compt. Rend. Acad. d. Sc. 210:515, 1940.
- (46) Perla, D. and Marmorston, J. Arch. Path. 23: 543, 683, 1937.
- (47) Peters, R.A. and Davenport, H.W. Ann. Rev. Biochem. 7:329, 1938.
- (49) Rich, A.R. and Follis, R.H., Jr. Bull J.H.H. 62:77, 1938.

- (50) Robertson, E.C. *Medicine* 13:123, 1934.
- (54) Seastone, C.V. *J. Immunol.* 33:403, 1937.
- (55) Siebert, W.J. and Loose, F. *J. Lab. and Clin. Med.* 25:1062, 1940.
- (56) Steinbach, M.M. and Duca, C.J. *Proc. Soc. Exper. Biol. and Med.* 44:133, 1940.
- (61) Vauthey, M. *Ann. d. Mal. Vener.* 32:98, 1937.  
Also, *Prat. Med. Franc.* 18:107, 1937.

## BIBLIOGRAPHY

- (1) Abt, A.F., and C.J. Farmer. Pharmacology and Therapeutics of Vitamin C. *J.Am.Med.Assoc.*, 1938. 111:1555.
- (2) Armstrong, A.R., and J.L Thompson. The Optimum Dose of Sulfanilamide for Guinea Pigs. *J.Lab. Clin. Med.*, 1939. 24:1203.
- (3) Barron, E.S.G., H.J. Brumm and G.F. Dick. Ascorbic Acid in the Blood and Urine after the Intravenous Injection of Sodium Ascorbate-A Clinical Test for Determining Vitamin C Deficiency. *J.Lab.Clin.Med.*, 1938. 23:1226.
- (4) Bliss, C.I. The Calculation of the Dosage Mortality Curve. *Ann.Applied Biol.*, 1935. 22:134.
- (5) Bliss, C.I. The Comparison of Dosage Mortality Data. *Ann.Applied Biol.*, 1935. 22:307.
- (6) Bratton, A.C., and E.K. Marshall, Jr. New Coupling Component for Sulfanilamide Determination. *J.Biol.Chem.*, 1939. 128:537.
- (7) Cooper, F.B., and P. Gross. Sulfanilamide, Anti-pneumococcus Serum and Vitamin C Therapy in Type II Pneumococcal Pneumonia of Rats. *Proc.Soc.Expl.Biol.Med.*, 1937. 36:774.
- (8) Dagulf, H., Zur Frage der C-Hypovitaminose. *Klin. Wochschr.*, 1939. 18:669.
- (9) Dainow, I. Prevention des Accidents dus aux Sulfamides per les Injections Intraveineuses d'Acide Ascorbique. *Ann.malad.vener.*, 1939. 34:513.
- (10) Dainow, I., and D. Zimmet. Hypovitaminose C Localisee au Testicule, au Foie, et au Cerveau par les Derives Benzeniques du Souffre. *Compt.rend.soc.biol.*, 1939. 130:846
- (11) Dann, B., and G.R. Cowgill. The Vitamin C Requirement of the Guinea Pig. *J. Nutrition*, 1935. 9:4
- (12) Dobbelstein, O. Experimentelle Untersuchungen Zur Frage des Erhöhten C-Vitaminverbrauches

bei Fieber. *Klin.Wochschr.*, 1939. 18:610.

- (13) Ecker, E.E., and L. Pillemer. Vitamin C Requirement of the Guinea Pig. *Proc.Soc.Expl.Biol. Med.*, 1940. 44:262
- (14) Ecker, E.E., L. Pillemer and D. Wertheimer. Complementing Activity and Ascorbic Acid Content of Guinea Pig Serum Following Ether Anesthesia. *J.Immunol.*, 1938. 34:39.
- (15) Farmer, C.J., and A.F. Abt. Ascorbic Acid Content of Blood. *Proc.Soc.Expl.Biol.Med.*, 1934-35. 32:1625.
- (16) Farmer, C.J., and A.F. Abt. Determination of Reduced Ascorbic Acid in Small Amounts of Blood. *Proc.Soc.Expl.Biol.Med.*, 1936. 34:146.
- (17) Fisher, R.A. *Statistical Methods for Research Workers* (Oliver and Boyd)1934.
- or
- Fisher and Yates. *Statistical Tables for Biological, Agricultural and Medical Research.* London, 1938.
- (18) Gaddum, J.H. *Reports on Biol. Standards. III. Methods of Biological Assay Depending on a Quantal Response.* Medical Research Council, Special Report Series, 1933. #183.
- (19) Giroud, A. L'Acide Ascorbique. *Presse Med.*, 1938. 46:1838.
- (20) Giroud, A., C.P. Leblond and R. Ratsimamanga. The Vitamin C Requirements of the Guinea Pig. *Yale J.Biol. Med.*, 1937. 9:573.
- (21) Giroud, A., Santos-Ruiz, A., P. Leblond, and A.R. Ratsimamanga. Le Taux de l'Acide Ascorbique Chez les Animaux Carences. *Bull.soc. chim. biol.*, 1936. 18:750.
- (22) Goldsmith, G.A., and G.F. Ellinger. Ascorbic Acid in Blood and Urine after Oral Administration of a Test Dose of Vitamin C. *Arch. Internal Med.*, 1939. 63:531.
- (23) Greenberg. L.D., J.F. Rinehart and N.M. Phatak,



---. Studies on Reduced Ascorbic Acid of the Blood Plasma. Proc.Soc.Expl.Biol.Med., 1936-37. 35:135

- (24) Herlitz, C.W. Investigations of the C Vitamine Standard in Healthy Children and in Children Suffering from Gingivitis. Acta Paediatrica, 1939. 23:43
- (25) Kajdi, L., J. Light and C. Kajdi. A Test for the Determination of the Vitamin C Storage. Vitamin C Index J. Pediatrics, 1939. 15:197
- (26) Kalmanson, G.M. The Bactericidal Power of Sulfanilamide Under Anaerobic Conditions. J. Bact., 1940. 40:817.
- (27) Kastlin, G.J., C.G. King, C.R. Schlesinger and J.W. Mitchell. Chemical Methods for the Determination of Clinical Vitamin C (Ascorbic Acid) Deficiency. Am.J.Clin.Path., 1940. 10:882.
- (28) Kellie, A.E. and S.S. Zilva. The Vitamin C Requirements of Man. Biochem.J., 1939. 33:153.
- (29) King, C.G. The Water Soluble Vitamins. Ann.Rev. Biochem., 1939. 8:371.
- (30) Kolmer, J.A. Progress in Chemotherapy of Bacterial and Other Diseases, with Special Reference to the Prontosils, Sulfanilamide and Sulfapyridine. Arch.Int.Med., 1940. 65:671.
- (31) Litchfield, J.T., Jr. H.J. White and E.K. Marshall, Jr. The Experimental Basis for a Method for the Quantitative Evaluation of the Effectiveness of Chemotherapeutic Agents against Streptococcus Infection in Mice. J. Pharmacol., 1939. 67:437.
- (32) Locke, A. Non-Specific Factors in Resistance. I. Capacity to Sustain Effective Circulation. J. Immunol., 1939. 36:159.
- (33) Locke, A., R.B. Locke, R.J. Bragdon and R.R. Mellon. Fitness, Sulfanilamide and Pneumococcus Infection in the Rabbit. Science, 1937. 86:228.

- (34) Locke, A., E.R. Main and R.R. Mellon. Non-Specific Factors in Resistance - III. Capacity for Retarding Bacterial Proliferation. *J. Immunol.*, 1939. 36:183.
- (35) Long, P.H. and E.A. Bliss. The Clinical and Experimental Use of Sulfanilamide, Sulfapyridine and Allied Compounds. The Macmillan Company, 1939.
- (36) Mandillon, G. Therapeutique et Vitamines. Notions Recentes Sur La Vitamine C et son Emploi. *Rev.med.*, 1938. 55:552.
- (37) Marshall, E.K., Jr. Quantitative Determination of Sulfanilamide in Blood and Urine. *Proc. Soc.Expl.Biol.Med.*, 1937. 36:422.
- (38) Marshall, E.K., Jr. Bacterial Chemotherapy: The Pharmacology of Sulfanilamide. *Physiol. Rev.*, 1939. 19:240.
- (39) Marshall, E.K., Jr. and W.C. Cutting. Absorption and Excretion of Sulfanilamide in the Mouse and Rat. *Bull. Johns Hopkins Hospital*, 1938. 63:328.
- (40) Marshall, E.K., Jr. and J.T. Litchfield, Jr. Some Aspects of the Pharmacology of Sulfapyridine. *J. Pharmacol.*, 1939. 67:454.
- (41) McCullough, N.B. Vitamin C and Resistance of the Guinea Pig to Infection with Bacterium *Necrophorum*. *J. Infectious Diseases*, 1938. 63:34.
- (42) Mellon, R.R., P. Gross and F.B. Cooper. Sulfanilamide Therapy of Bacterial Infections. Charles C. Thomas, 1938.
- (43) Mitolo, M. Possible Synthesis of the Antiscorbutic Vitamin in Guinea Pigs. *Atti accad.Lincei, Classe sci., fis., mat., nat.*, 1938. 28:31. (cf. C.A. 2185<sup>8</sup>, 1939)
- (44) Mouriquand, G., V. Edel, H. Tete and M. Dauvergne. Syndrome Scorbutiforme dans l'Intoxication Experimentale par les Sulfamides. *Compt. rend.*, 1940. 210:515.

- (45) Northey, E.H. Structure and Chemotherapeutic Activities of Sulfanilamide Derivatives. *Chem.Rev.*, 1940. 27:85
- (46) Perla, D. and J. Marmorston. Role of Vitamin C in Resistance. *Arch.Path.*, 1937. 23: 543-575 and 683-712.
- (47) Peters, R.A. and H.W. Davenport. Vitamin C (Ascorbic Acid, Cevitamic Acid). *Ann. Rev. Biochem.*, 1938. 7:325.
- (48) Raabe, S. Studien über Funktionen von Ascorbinsäure und Glutathion beim Lebenden Tier unter Besonderer Berücksichtigung des Esterase-haushaltes. *Biochem.Zeit.*, 1938. 299:141.
- (49) Rich, A.R. and R.H. Follis, Jr. The Inhibitory Effect of Sulfanilamide on the Development of Experimental Tuberculosis in the Guinea Pig. *Bull. Johns Hopkins Hosp.*, 1938. 62:77.
- (50) Robertson, E.C. Vitamins and Resistance to Infection. *Medicine*, 1934. 13:123.
- (51) Rohmer, P., N. Bezssonoff, E. Stoerr and J. Perier. La Teneur en Vitamine C du Sang et des Urines apres Injections Massive. *Compt.rend.soc.biol.*, 1935. 118:1090.
- (52) Sacerdote, P. Variations du Taux de l'Acide Ascorbique du Sang et des Tissues au Cours de l'Anoxemie. *Ann.physiol.physicochim.biol.*, 1938. 14:271.
- (53) Samuels, L.T., N.D. Ritz and E.B. Poyet. The Effect of Drugs on Vitamin C Excretion. *J. Pharmacol.*, 1940. 68:465.
- (54) Seastone, C.V. The Effect of Sulfanilamide (Para-Amino Benzenesulfonamide) on Group "C" Hemolytic Streptococcus Infections. *J. Immunol.*, 1937. 33:403.
- (55) Siebert, W.J. and F. Loose. Observations on a Sulfanilamide Solution. *J.Lab.Clin.Med.*, 1940. 25:1062.
- (56) Steinbach, M.M. and C.J. Duca. N'Dodecanoyl-

- sulfanilamide, and Sulfapyridine Plus Vitamin C, in Experimental Tuberculosis in Guinea Pigs. *Proc.Soc.Expl.Biol.Med.*, 1940. 44:133.
- (57) Stephens, D.J. and E.E. Hawley. The Partition of Reduced Ascorbic Acid in Blood. *J.Biol. Chem.*, 1936. 115:653.
- (58) Taylor, F.H.L., D. Chase and J.M. Faulkner. The Estimation of Reduced Ascorbic Acid in Blood Serum and Plasma. *Biochem.J.*, 1936. 30:1119.
- (59) Todhunter, E.N. and W. Brewer. The Ascorbic Acid, Phosphatase and Calcium Content of the Blood of Guinea Pigs with Varying Degrees of Scurvy. *Am.J.Physiol.*, 1940. 130:310.
- (60) Todhunter, E.N., R.C. Robbins, G. Ivey and W. Brewer. A Comparison of the Utilization By Guinea Pigs of Equivalent Amounts of Ascorbic Acid (Vitamin C) in Lemon Juice and in the Crystalline Form. *J. Nutrition*, 1940. 19:113.
- (61) Vauthey, M. Vitamine C (Acide l-Ascorbique) et Tolerance des Medicaments Chimique. *Ann. malad.vener.*, 1937. 32:98. Also, *Prat. med.franc.*, 1937. 18:107.
- (62) Wachholder, K. J. Baucke and H.H. Podesta. Nachweis Zusätzlicher Entstehung von Vitamin Im Organismus, Insbesondere Bei Mangelhafter Zufuhr Mit Der Nahrung. *Arch.Ges.Physiol. (Pflüger's)*, 1939. 241:495
- (63) Wortis, H., S.B. Wortis and F.I. Marsh. Role of Vitamin C in Metabolism of Nerve Tissue. *Arch.Neurol.Psychiat.*, 1938. 39:1055.
- (64) Wright, I.S., A. Lilienfeld and E. MacLenathen. Determination of Vitamin C Saturation ( - A Five Hour Test after an Intravenous Test Dose) *Arch.Int.Med.*, 1937. 60:264.
- (65) Zilva, S.S. Vitamin C Requirements of the Guinea Pig. *Biochem.J.*, 1936. 30:1419.
- (66) Zilva, S.S. The Alleged Antitoxix Action of Vitamin C in Diphtheria. *Brit. J.Expl.Path.*, 1937, 18:449.

## APPENDIX

TABLE I

## FORMULAS

- $x$  = Logarithm of the dose to the base 10  
 $y$  = Normal deviation equivalent to the percentage mortality  
 $\bar{x}$  = The weighted means of the observed values of  $x$   
 $\bar{y}$  = The weighted means of the observed values of  $y$   
 $n$  = The number of animals receiving the same dose  
 $LD_{50}$  = The dose corresponding to 50 per cent of responses  
 $m$  =  $\text{Log}_{10}(LD_{50})$   
 $b$  = The slope of the line connecting  $y$  and  $x$   
 $B$  = The 'weight factor' corresponding to the observed result  
 $\sigma$  = The theoretical standard deviation  
 $S(-)$  = The sum of all values of any expression included in the bracket

$$\bar{x} = \frac{S(Bnx)}{S(Bn)} \qquad \bar{y} = \frac{S(Bny)}{S(Bn)}$$

$$b = \text{slope} = \frac{S[Bny(x-\bar{x})]}{S[Bn(x-\bar{x})^2]}$$

$$\log \bar{m} = \bar{x} - \frac{\bar{y}}{b} \quad (y=N.E.D.) = \text{WEIGHTED } LD_{50}$$

$$\sigma_b^2 = \frac{1}{S[Bn(x-\bar{x})^2]} = \text{variance}$$

$$\sigma_b = \frac{S\left[\left(\frac{1}{\sigma_b^2}\right)\right]}{S\left[\frac{1}{\sigma_b^2}\right]^2}$$

$$b_c = \frac{S\left[b\left(\frac{1}{\sigma_b^2}\right)\right]}{S\left(\frac{1}{\sigma_b^2}\right)}$$

$LD_{50} \pm 2$  std. deviations (Bliss-equation #28)

$$\log LD_{50} = x = \bar{x} + \frac{B(Y-a)}{b^2 - t^2 V(b)} \pm t \frac{\sqrt{V(b)(Y-a)^2 + V(a)[b^2 - t^2 V(b)]}}{b^2 - t^2 V(b)}$$

$$a = \bar{y}$$

$$Y = 5.000$$

$$t = 1.96 \text{ (Fisher)}$$

$$t^2 = 3.8414$$

$$V(b) = \frac{1}{S[Bn(x-\bar{x})^2]} = \sigma_b^2 = \text{variance}$$

$$V(a) = \frac{1}{SW} = \frac{1}{SBn}$$

Bliss, C.I. (5)

Gaddum, J.H. (18)

TABLE II-

PLASMA VITAMIN C LEVELS IN GUINEA PIGS AT  
STATED TIMES AFTER FEEDING GREENS

Dates	Time of De-termination in Hours after Feeding Greens (ad lib.)	Mg.Per Cent Vita-min C	Dates	Time of De-termination in Hours after Feeding Greens (ad lib.)	Gm.Wt. Sex	Mg.Per Cent Asc-orbic Acid		
3/22/40 to 4/8/40 incl.	3-6	0.69	4/18/40	15-17	300-F	0.18		
		0.21			315-F	0.16		
		0.33			321-F	0.24		
		0.29			316-F	0.18		
		0.31			260-F	0.06		
		0.33			320-F	0.18		
		0.66						
		0.32			Mean -	0.16		
		0.24			s.d. -	0.06		
		0.60						
		0.17						
		0.35			4/27/40	2-3	412-M	0.65
		0.26					300-F	0.53
		0.36					243-F	0.72
		0.53					314-F	0.23
0.17	307-M	0.82						
0.30	372-M	0.20						
0.52								
0.76	Mean -	0.53						
0.37	s.d. -	0.26						
0.39								
0.25	5/7/40	4-6	549-F	0.28				
0.17			445-F	0.71				
0.37			442-F	0.45				
0.46			478-F	0.72				
0.42			473-F	1.67				
0.26			321-F	0.85				
0.42			320-F	0.68				
0.42			228-F	0.32				
0.51			283-F	0.42				
			355-F	0.90				
Mean -			405-F	0.06				
0.38			471-F	1.63				
s.d. -			Mean -	0.72				
0.15			s.d. -	0.51				
Wt. range: 300-450 gms.								
			5/29/40	19-20	475-M	0.21		
					342-F	0.12		
					352-M	0.23		
					337-F	0.02		
					307-F	0.11		
					329-F	0.11		
					317-F	0.35		
					300-F	0.15		
					268-F	0.23		
					312-F	0.07		
					316-F	0.10		
					310-F	0.13		
					327-F	0.10		
					256-F	0.02		
					308-F	0.19		
					267-F	0.11		
					319-F	0.23		
					309-F	0.11		
					290-F	0.15		
					305-F	0.08		
					Mean -	0.14		
					s.d. -	0.08		
			6/3/40	42-44 (no greens) (on Sunday)	301-M	0.29		
					362-M	0.08		
					317-M	0.10		
					270-M	0.44		
					345-M	0.05		
					327-M	0.23		
					318-M	0.17		
					342-M	0.16		
					395-F	0.30		
					312-M	0.17		
					320-F	0.11		
					345-M	0.17		
					348-F	0.12		
					327-M	0.00		
					340-M	0.11		
					432-M	0.10		
					422-M	0.19		
					368-M	0.26		
					290-M	0.05		
					290-F	0.08		
					302-F	0.07		
					354-F	0.23		
					302-M	0.00		
					280-F	0.08		
					307-F	0.09		
					332-F	0.11		
					298-M	0.08		
					340-F	0.35		
					277-M	0.11		
					302-F	0.22		
					302-F	0.35		
					314-F	0.23		
For All Animals	- (106) :				Mean -	0.16		
Weighted Mean	- 0.30 mg.%				s.d. -	0.11		
Standard Deviation	- 0.20							
Standard Error	- 0.02							

TABLE III

VITAMIN C AND FREE SULFANILAMIDE CONTENT  
OF BLOOD PLASMA OF GUINEA PIGS  
AFTER 6 DOSES OF SULFANILAMIDE \*

Date	Gm.Wt. Sex	Mg. Per Cent Vitamin C 20 Hours After 1st Sulf. Treatment	Mg. Per Cent Sulf. 20 Hours After 1st Sulf. Treatment
1. 4/9/40	301-F	.17	29.0
2.	330-M	.42	18.0
3.	413-F	.15	53.0
4.	427-F	.13	19.0
5.	414-M	.33	11.0
6.	420-F	.20	12.0
7.	421-M	.32	13.0
8.	430-F	.27	15.0
9. 4/15/40	266-F	.11	27.0
10.	306-F	.10	31.5
11.	371-M	.08	27.6
12.	389-M	.22	19.7
13.	482-F	.14	20.3
14.	433-F	.04	27.3
15.	542-M	.06	26.3
16.	336-F	.11	0.0
17.	291-F	.06	39.0
18.	272-F	.11	49.7
19.	270-F	.09	32.3
20.	294-F	.07	36.9

\*15 gms of sulfanilamide suspended in 100.00  
cc. of H<sub>2</sub>O - 32.3 mgs. sulfanilamide per  
100.00 gms of guinea pig per dose.



TABLE IV

VITAMIN C AND FREE AND TOTAL SULFANILAMIDE  
 CONTENT OF BLOOD PLASMA OF GUINEA PIGS  
 AFTER 6 DOSES OF SULFANILAMIDE\*\*

Date	Gm.Wt. Sex	Mg. Per Cent Vitamin C in Normal Un- treated Guinea Pigs	Mg. Per Cent Vitamin C After 20 Hours Star- vation	Gm. Wt. After 20 Hours Starvation	*Sulfanilamide Dose To Star- ved Guinea Pigs	Mg. Per Cent Vitamin C 20 Hours after 1st Sulf. Dose	Free Sulfan- ilamide 20 Hours after 1st Sulf. Dose	Total Sulfan- ilamide 20 Hours after 1st Sulf. Dose
1.	5/7/40 549-F	0.28	0.09	487	1.00 cc.	0.02	25.2 mg.%	35.1 mg.%
2.	445-F	0.71	0.16	385	0.83	0.00	59.7	69.0
3.	442-F	0.45	0.10	410	0.88	0.00	40.4	48.2
4.	478-F	0.72	0.12	432	0.92	0.00	27.6	31.7
5.	473-F	1.67	0.15	432	0.92	0.00	35.1	41.2
6.	321-F	0.85	0.02	274	0.59	0.00	42.6	48.2
7.	320-F	0.68	0.06	292	0.63	0.00	19.9	23.8
8.	283-F	0.42	0.14	255	0.55	0.06	25.3	31.5
9.	355-F	0.90	0.18	306	0.66	0.00	39.6	45.5
10.	405-F	0.06	0.16	352	0.76	0.00	59.7	75.5
11.	471-F	1.63	0.26	412	0.89	0.00	23.2	45.5
12.	5/29/40 457-M	0.21	0.03	444	0.96	0.00	22.9	44.3
13.	342-F	0.12	0.00	326	0.70	0.00	16.4	21.7
14.	352-M	0.23	0.00	350	0.75	0.09	23.9	31.5
15.	337-F	0.02	0.00	327	0.70	0.09	20.5	37.0
16.	329-F	0.11	0.00	306	0.63	0.05	14.5	37.4
17.	317-F	0.35	0.04	292	0.66	0.00	17.5	25.1
18.	300-F	0.15	0.00	280	0.60	0.00	15.1	37.7
19.	268-F	0.23	0.00	255	0.55	0.30	9.6	25.3
20.	312-F	0.07	0.00	282	0.60	0.07	14.7	29.2
21.	316-F	0.10	0.00	290	0.62	0.00	15.5	31.7
22.	256-F	0.02	0.00	245	0.53	0.10	17.0	33.1
23.	308-F	0.19	0.00	281	0.60	0.06	17.9	48.8
24.	267-F	0.11	0.00	249	0.54	0.02	19.4	39.6
25.	319-F	0.23	0.00	280	0.60	0.05	12.3	45.5
26.	309-F	0.11	0.00	288	0.62	0.11	38.1	61.5
27.	290-F	0.15	0.08	266	0.57	0.10	22.9	39.2
28.	305-F	0.08	0.00	290	0.62	0.05	24.1	63.5

\*\*Animals were fed ad lib. 1 hour after 1st sulfanilamide dose. Sulfanilamide was given as follows: Hours-0, 3, 7, 11, 15, 19, 23, 27, 31.

(For method of sulfanilamide determination see (6))

\*15 gms. of sulfanilamide suspended in 100.00 cc. of H<sub>2</sub>O - 32.3 mgs. sulfanilamide per 100.00 gms. guinea pig per dose.

TABLE IV-A

VITAMIN C, FREE AND TOTAL SULFANILAMIDE CONTENT  
OF BLOOD PLASMA OF GUINEA PIGS AFTER 6  
DOSES OF SULFANILAMIDE\*

Date	Gm.Wt. Sex	Mg. Per Cent Vitamin C Prior to Sulf. Dosage	Dose Sulf. in cc.	Mg. Per Cent Vitamin C 20 Hours after 1st Sulf. Dose	Free Sulf. 20 Hours after 1st Sulf. Dose	Total Sulf. 20 Hours after 1st Sulf. Dose
1.	6/3/40 301-M	0.29	.65 cc.	0.01	33.0 mg. %	40.8 mg. %
2.	362-M	0.08	.78	0.00	34.8	53.4
3.	317-M	0.10	.67	0.00	44.2	60.6
4.	270-M	0.44	.58	0.00	33.7	51.0
5.	345-M	0.05	.74	0.00	27.3	45.8
6.	327-M	0.23	.70	0.00	27.9	43.4
7.	318-M	0.17	.70	0.00	19.0	26.6
8.	395-F	0.30	.85	0.00	36.9	44.2
9.	312-M	0.17	.67	0.00	20.0	29.6
10.	320-F	0.11	.69	0.01	19.5	31.2
11.	345-M	0.17	.74	0.00	32.8	40.6
12.	348-F	0.12	.75	0.00	17.3	28.8
13.	327-M	0.00	.70	0.00	28.4	50.5
14.	432-M	0.10	.93	0.00	25.8	28.8
15.	422-M	0.19	.91	0.00	38.0	43.5
16.	368-M	0.26	.80	0.00	30.0	39.0
17.	302-F	0.07	.65	0.00	24.7	44.4
18.	354-F	0.23	.80	0.00	36.4	40.0
19.	302-F	0.00	.65	0.00	25.5	26.5
20.	307-F	0.09	.66	0.00	30.0	54.1
21.	332-F	0.11	.71	0.00	59.7	77.9
22.	298-M	0.08	.64	0.00	62.5	80.5
23.	340-F	0.35	.73	0.05	35.1	36.8
24.	302-F	0.22	.65	0.02	42.1	34.5
25.	302-F	0.35	.65	0.07	33.6	40.7
26.	314-F	0.23	.67	0.03	20.4	22.4

\*15 gms. of sulfanilamide suspended in 100.00 cc.  
of H<sub>2</sub>O - 32.3 mgs. sulfanilamide per 100.00 gms.  
guinea pig per dose.

TABLE V

VITAMIN C AND FREE SULFANILAMIDE CONTENT OF THE  
BLOOD PLASMA OF GUINEA PIGS UNDER DIFFERENT  
CONDITIONS OF ASCORBIC ACID TREATMENT

Date	Gm.Wt. Sex	Mg. Per Cent Vitamin C in Ascorbic Acid Depleted Guinea Pigs	Mg. Per Cent Vitamin C 20 Hours after 1st Sulf. Treatment	Mg. Per Cent Sulf. 20 Hours after 1st Sulf. Treatment	
4/19/40	430-M	.01	.13	15.5	#1
	416-M	.04	.00	13.4	2
	361-M	.03	.00	4.7	3
	411-M	.03	.01	14.2	4
	480-M	.04	.06	16.3	5
	453-M	.00	.00	17.4	6
4/27/40	282-F	.10	.04	20.0	7
	288-F	.02	.00	13.2	8
	233-F	.04	.00	19.0	9
	317-F	.02	.00	20.2	10
	327-F	.07	.00	16.4	11
	252-F	.18	.00	14.2	12

Date	Gm.Wt. Sex	Mg. Per Cent Vitamin C in Normal Un- treated Animals *	Mg. Per Cent Vitamin C 20 Hours after 1st Sulf. Treatment	Mg. Per Cent Sulf. 20 Hours after 1st Sulf. Treatment	
4/19/40	315-F	.16	1.79	9.2	#1
	321-F	.24	1.46	13.9	2
	316-F	.18	.91	20.0	3
	260-F	.06	1.71	20.3	4
	320-F	.18	.78	17.8	5

\*10 mg. vitamin C i.p. - 2½ hours after 1st dose  
and every 4 hours thereafter.

Date	Gm.Wt. Sex	Mg. Per Cent Vitamin C in Normal Un- treated Animals *	Mg. Per Cent Vitamin C 20 Hours after 1st Sulf. Treatment	Mg. Per Cent Sulf. 20 Hours after 1st Sulf. Treatment	
4/27/40	412-M	.65	.80	14.3	#6
	300-F	.53	.07	17.2	7
	243-F	.72	1.52	19.4	8
	314-F	.23	.73	11.8	9
	307-M	.82	1.29	15.9	10
	372-M	.20	.57	15.4	11

\*2.5 mg. vitamin C sub. cut. along with the  
sulfanilamide per os.



TABLE VII

PROTOCOLS FOR THE DETERMINATION OF THE LD<sub>50</sub>'s FOR  
NON-FASTING, NORMAL AND HIGH VITAMIN C  
GUINEA PIGS

Dose in Grams Per Kg.	Log Dose	No. of Guinea Pigs	Per Cent Response	N.E.D.	Weight. Coeff.	(3x6)	(7x2)	(7x5)	(9x10)	(7x(10) <sup>2</sup> )			
x		n	y	B	Bn	Bnx	Bny	(x- $\bar{x}$ )	log(x- $\bar{x}$ ) <sup>2</sup>	Bny(x- $\bar{x}$ )	Bn(x- $\bar{x}$ ) <sup>2</sup>		
<u>Non-Fasting Normals</u>													
<u>May-July 1940</u>													
1.	3.45	.5378	7	57	.1764	.627	4.389	2.3605	.7742	.0045	5.3064	.00348	.00009
2.	3.38	.5289	23	83	.9542	.439	10.097	5.3280	9.6345	-.0044	5.2870	-.04239	.00020
3.	3.31	.5198	8	38	-.3055	.616	4.928	2.5616	-1.5055	-.0135	4.2606	.02032	.00090
4.	3.21	.5065	4	25	-.6745	.532	2.128	1.0770	-1.4350	-.0268	4.8562	.03845	.00153
5.	3.56	.5514	4	25	-.6745	.532	2.128	1.1730	-1.4350	.0181	4.5154	-.02597	.00070
6.	3.90	.5911	4	75	.6745	.532	2.128	1.2580	1.4350	.0578	3.5238	.09631	.00801
							25.798	13.7581	7.4682			.09020	.01143
$\bar{x} = .5333$													
$b = 7.898$													
$\bar{y} = .2896$													
$\sigma_b = 9.35$													
<u>Non-Fasting High Vitamin C</u>													
1.	3.45	.5378	5	80	.8416	.503	2.515	1.3536	2.1166	.00767	5.7696	.01623	.00015
2.	3.38	.5289	30	50	.0000	.637	19.110	10.1070	0.0000	-.00123	6.1798	.00000	.00003
							21.625	11.4606	2.1166			.01623	.00018
$\bar{x} = .53013$													
$b = 91.75$													
$\bar{y} = .097892$													
$\sigma_b = 75.2$													

TABLE VIII

PROTOCOLS FOR THE DETERMINATION OF THE LD<sub>50</sub>'s FOR  
VITAMIN C DEPLETED, NORMAL, AND HIGH  
VITAMIN C GUINEA PIGS

Dose in Gms. Kg.	Log Per Dose	No. of Guinea Pigs	Per Cent Response	N.E.D.	Weight Coeff.	(3x6)	(7x2)	(7x5)	(9x10)	(7x(10) <sup>2</sup> )			
x		n	y	B	Bn	Bnx	Bny	(x-x̄)	log(x-x̄) <sup>2</sup>	Bny(x-x̄)	Bn(x-x̄) <sup>2</sup>		
<u>Fasting-Vitamin C Depleted Guinea Pigs</u>													
1.	2.58	.4116	7	86	1.0803	.439	3.073	1.265	3.318	.0803	3.8094	.26650	.01981
2.	2.24	.3502	12	67	.4399	.601	7.212	2.523	3.168	.0189	4.5530	.05987	.00258
3.	1.90	.2788	13	31	-.4959	.581	7.553	2.118	-3.744	-.0525	3.4404	.19660	.02082
							17.838	5.906	2.742			.52297	.04321
						$\bar{x} = \frac{5.906}{17.838} = .3313$							
						$b = \frac{.52297}{.04321} = 12.11$							
						$\bar{y} = \frac{2.742}{17.838} = .1538$							
						$\sigma_b^2 = \frac{1}{.04321} = 23.14$							
						$\sigma_b = 4.810$							
<u>Fasting Normals</u>													
1.	3.87	.5877	11	91	1.3408	.336	3.696	2.172	4.956	.1758	2.4900	.8712	.11420
2.	3.35	.5250	14	93	1.4758	.267	3.740	1.965	5.540	.1131	2.1010	.6266	.04785
3.	2.92	.4654	14	86	1.0803	.439	6.146	2.861	6.641	.0535	3.4568	.3554	.01760
4.	2.58	.4116	31	77	0.7388	.532	16.492	6.788	2.183	-.0003	8.9542	-.0037	.000001
5.	2.41	.3820	20	80	0.8416	.503	10.060	3.841	8.467	-.0299	4.9514	-.2532	.00899
6.	2.24	.3502	11	36	-0.3585	.601	6.611	2.316	-2.370	-.0617	3.5806	.1462	.02517
7.	1.90	.2788	11	18	-0.9154	.471	5.181	1.444	-4.743	-.1331	2.2484	.6314	.09179
							51.926	21.387	30.674			2.3739	.30560
						$\bar{x} = .4119$							
						$b = 7.768$							
						$\bar{y} = .5907$							
						$\sigma_b^2 = 3.2723$							
						$\sigma_b = 1.809$							
<u>Fasting High Vitamin C</u>													
1.	3.87	.5877	16	94	1.5548	.238	3.808	2.238	5.917	.1842	2.5306	1.0900	.1292
2.	3.35	.5250	19	84	.9945	.439	8.343	4.380	8.296	.1215	2.1690	1.0080	.1231
3.	2.92	.4654	19	89	1.2265	.370	7.031	3.273	8.626	.0619	3.5834	.5339	.02695
4.	2.24	.3502	22	18	-.9154	.471	10.360	3.628	-9.484	-.0533	3.4534	.5055	.02943
5.	1.90	.2788	22	32	-.4677	.581	12.780	3.563	-5.978	-.1247	2.1916	.7454	.1987
							42.322	17.082	7.377			3.8828	.50738
						$\bar{x} = .4035$							
						$b = 7.652$							
						$\bar{y} = .1744$							
						$\sigma_b^2 = 1.404$							
						$\sigma_b = 1.971$							

TABLE IX

DETERMINATION OF LD<sub>50</sub>'s AND THEIR RANGE

95 TIMES OUT OF 100\*

$$x = \bar{x} + \frac{b(y-a)}{b-t^2V(b)} = t \frac{\sqrt{V(b)(y-a)^2 + V(a)[b^2 - t^2V(b)]}}{b-t^2V(b)}$$

$$\begin{aligned} a &= \bar{y} \\ y &= 5.000 \\ t &= 1.96 \\ t^2 &= 3.8414 \end{aligned}$$

$$V(b) = \text{variance}(b) = \frac{1}{S[Bn(\bar{x}-\bar{x})^2]} = \sigma_b^2$$

$$V(a) = \frac{1}{SW} = \frac{1}{S(Bn)}$$

---

Fasting Normals  $x = .4119 + \frac{7.937(5.000-5.5907)}{(7.937)^2 - 3.8416(\frac{1}{1.329})} \pm 1.96 \frac{\sqrt{\frac{1}{(1.329)}(5.000-5.5907)^2 + (\frac{1}{51.926})[(7.937)^2 - 3.8416(\frac{1}{1.329})]}}{(7.937)^2 - 3.8416(\frac{1}{1.329})}$

$x = .3339 \pm .03886$

Antilog  $x = LD_{50} = 2.157$

Range of LD<sub>50</sub> 95 times in 100 is 1.973 to 2.359

---

Fasting High Vita-  
min C  $x = .4035 + \frac{7.937(5.000-5.1744)}{(7.939)^2 - 3.8416(\frac{1}{1.329})} \pm 1.96 \frac{\sqrt{\frac{1}{(1.329)}(5.000-5.1744)^2 - (\frac{1}{42.522})[(7.937)^2 - 3.8416(\frac{1}{1.329})]}}{(7.937)^2 - 3.8416(\frac{1}{1.329})}$

$x = .38047 \pm .03919$

LD<sub>50</sub> = 2.4014

Range of LD<sub>50</sub> is 2.1942 to 2.6282

---

Fasting Vitamin C Depleted  $x = .3313 + \frac{7.937(-.1538)}{60.1064} \pm 1.96 \frac{\sqrt{(\frac{1}{60.1064})(-.1538)^2 + (\frac{1}{17.838})}}{60.1064}$  (60.1064)

$x = .3110 \pm .06015$

LD<sub>50</sub> = 2.046

Range of LD<sub>50</sub> is 1.7817 to 2.3504

---

Non-Fasting Normals  $x = .5333 + \frac{7.937(-.2896)}{60.1064} \pm 1.96 \frac{\sqrt{(\frac{1}{60.1064})(-.2896)^2 + (\frac{1}{25.798})(60.1064)}}{60.1064}$

$x = .49506 \pm .05044$

LD<sub>50</sub> = 3.1265

Range of LD<sub>50</sub> is 2.783 to 3.5116

---

Non-Fasting High Vita-  
min C  $x = .53013 + \frac{7.937(-.09789)}{60.1064} \pm 1.96 \frac{\sqrt{(\frac{1}{60.1064})(-.09789)^2 + (\frac{1}{21.625})(60.1064)}}{60.1064}$

$x = .51720 \pm 0.54444$

LD<sub>50</sub> = 3.290

Range of LD<sub>50</sub> is 2.9024 to 3.7294

---

\* Bliss' Equation #28 (5)

TABLE X

SLOPES AND THEIR ERRORS FOR FASTING AND  
NON-FASTING GUINEA PIGS\*

	b (slope)	$\frac{1}{\sigma_b}$	$b\left(\frac{1}{\sigma_b}\right)$
Fasting Normals	7.768	0.30560	2.3749
Fasting Vitamin C Depleted	12.110	0.04321	0.5232
Fasting High Vitamin C	7.652	0.50738	3.8830
Non-Fasting Normals	7.898	0.01142	0.0902
Non-Fasting High Vitamin C	91.75	$\frac{0.00018}{0.86779}$	$\frac{0.0165}{6.8878}$

$$\text{composite } b = \frac{S[b(\frac{1}{\sigma_b})]}{S(\frac{1}{\sigma_b})} = b_c = \frac{6.8878}{0.86779} = 7.937$$

$$\text{variance} = \sigma_b^2 = \frac{1}{S[bn(x-\bar{x})^2]} = \sigma_b^2 = 1.1524$$

$$\sigma_{b_c} = \frac{S(\frac{1}{\sigma_b})}{S(\frac{1}{\sigma_b})} = 1.1524$$

---

\* $\sigma_{b_c}$  = extent of error



TABLE XI

SURVIVAL TIMES OF INFECTED CONTROLS  
AND TREATED GUINEA PIGS

Observation Period	Group I Controls			Group II Sulfanilamide			Group III Ascorbic Acid			Group IV Ascorbic Acid and Sulfanilamide			Group V Depleted Controls		Group VI Depleted Sulfanilamide			
	DAYS	HOURS	TOTAL	DAYS	HOURS	TOTAL	DAYS	HOURS	TOTAL	DAYS	HOURS	TOTAL	DAYS	HOURS	TOTAL	DAYS	HOURS	TOTAL
I	8 16 24																	
II	32 40 48	1 1 2	1 1 4	1 2 13	1	1	3	5 1 4	2 1 2	7 1 9	1 1 1	1 1 1	4 3	4 3	1	1		
III	56 64 72		1 1	1	2 2	2	2	2	2	4	1 1	1 1	3 3	3 3	1 1	1 1		
IV	80 88 96	1	1	1	1	1	2	2	1	1	1 1	1 1	2 2	2 2	1 1	1 1		
V	104 112 120	2 2	1	2 2 1				1		1								
VI	128 136 144	1	1	1 1 1			2		1	2 1			5 5	5 5	1	1		
VII	152 160 168		1 1	2			1			1	1 1							
VIII	180 192				1	1	1	1	1	2	1 1	2 1			3	3		
IX	204 216														1	1		
X	228 240												1	1	1	1		
XI	252 264				1						1	1						
XII	276 288				1		1				1	1			1	1		
XIII	300 312										1	1						
XIV	324 336				1 1	1	1	2										
Mortality				29			12			30			15		20			12
No. Animals Infected				30			30			30			30		20			20
Per Cent Mortality				96.7			40			100			50		100			60
Total Hours Survived				2,616			7,884			2,096			7,224		1,648			4,484
Weighted Mean Survival Time in Hours				87.20			262.80			69.86			240.80		82.40			224.20

TABLE II-

PLASMA VITAMIN C LEVELS IN GUINEA PIGS AT  
STATED TIMES AFTER FEEDING GREENS

Dates	Time of De-termination in Hours after Feeding Greens (ad lib.)	Mg. Per Cent Vita-min C	Dates	Time of De-termination in Hours after Feeding Greens (ad lib.)	Gm. Wt. Sex	Mg. Per Cent Asc-orbic Acid		
3/22/40 to 4/8/40 incl.	3-6	0.69	4/18/40	15-17	300-F	0.18		
		0.21			315-F	0.16		
		0.33			321-F	0.24		
		0.29			316-F	0.18		
		0.31			260-F	0.06		
		0.33			320-F	0.18		
		0.66						
		0.32				Mean - 0.16		
		0.24				s.d. - 0.06		
		0.60						
		0.17						
		0.35			4/27/40	2-3	412-M	0.65
		0.26					300-F	0.53
		0.36					243-F	0.72
		0.53					314-F	0.23
		0.17					307-M	0.82
		0.30					372-M	0.20
		0.52						
		0.76						Mean - 0.53
		0.37						s.d. - 0.26
0.39								
			5/7/40	4-6	549-F	0.28		
					445-F	0.71		
					442-F	0.45		
					478-F	0.72		
					473-F	1.67		
					321-F	0.85		
					320-F	0.68		
					228-F	0.32		
					283-F	0.42		
					355-F	0.90		
					405-F	0.06		
					471-F	1.63		
							Mean - 0.72	
							s.d. - 0.51	
			5/29/40	19-20	475-M	0.21		
					342-F	0.12		
					352-M	0.23		
					337-F	0.02		
					307-F	0.11		
					329-F	0.11		
					317-F	0.35		
					300-F	0.15		
					268-F	0.23		
					312-F	0.07		
					316-F	0.10		
					310-F	0.13		
					327-F	0.10		
					256-F	0.02		
					308-F	0.19		
					267-F	0.11		
					319-F	0.23		
					309-F	0.11		
					290-F	0.15		
					305-F	0.08		
		Mean - 0.14						
		s.d. - 0.08						
			6/3/40	42-44 (no greens) (on Sunday)	301-M	0.29		
					362-M	0.08		
					317-M	0.10		
					270-M	0.44		
					345-M	0.05		
					327-M	0.23		
					318-M	0.17		
					342-M	0.16		
					395-F	0.30		
					312-M	0.17		
					320-F	0.11		
					345-M	0.17		
					348-F	0.12		
					327-M	0.00		
					340-M	0.11		
					432-M	0.10		
					422-M	0.19		
					368-M	0.26		
					290-M	0.05		
					290-F	0.08		
302-F	0.07							
354-F	0.23							
302-M	0.00							
280-F	0.08							
307-F	0.09							
332-F	0.11							
298-M	0.08							
340-F	0.35							
277-M	0.11							
302-F	0.22							
302-F	0.35							
314-F	0.23							
For All Animals	- (106) :							
Weighted Mean	- 0.30 mg. %				Mean - 0.16			
Standard Deviation	- 0.20				s.d. - 0.11			
Standard Error	- 0.02							