THE EFFECT OF ANTIHISTAMINE DRUGS
ON THE EXCRETION
OF
ASCORBIC ACID BY RATS

by

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INTRODUCTION

The role of ascorbic acid as a detoxicating agent in vivo has been the subject of much discussion in the literature during the past ten years. Evidence has accumulated steadily indicating its importanse in detoxication processes, and as yet there is no clear picture of the chemical basis for its action. The body is known to conjugate various toxic substances with a number of detoxifying agents such as glucuronic acid, sulfates, glycine, cysteine, ornithine, and methyl groups, and these conjugates have been isolated from the urine. However, no evidence for the formation of a conjugate of ascorbic acid and a toxic agent has been found.

The work embodied in this thesis was designed to determine the relationship of the excretion of ascorbic acid by the rat to the ingestion of several of the antihistamine drugs, and, is directly related to the work reported by Longenecker[1] and his associates in 1939 and 1940.

These workers investigated the influence of variations in food intake upon vitamin C excretion by rats. In preliminary experiments they found that the excretion rate was fairly high when the animals were maintained on a diet of Purina Dog Chow or on one containing rolled oats. When the animals were fasted for three or four days, the excretion dropped from approximately 2.0 mg. to 0.2 mg. per day. The excretion rate returned to normal when the original diets were resumed but remained low if the animals were fed bran or evaporated milk. The higher excretion on a diet of Dog Chow or oats was found to be due to the presence of volatile lipid constituents in the ether-soluble, unsaponifiable fraction. An inanition period of three days, followed by a diet of evaporated milk,

furnished a good basis for assaying the excretion-stimulating capacity of substances because it resulted in an excretion rate of approximately 0.2 to 0.3 mg. of ascorbic acid per day.

The common fatty acids, sterols, proteins, and sugars did not cause high vitamin C excretion in animals receiving a milk diet. High excretions were caused by the vacuum-distillable fractions from the unsaponifiable matter of alfalfa leaf oil, oat oil, grass leaf oil, and halibut liver oil.

Longenecker and associates isolated a lipid-type substance from alfalfa meal which when added to a milk diet caused an increased rate of ascorbic acid excretion by rats. This work led to further experiments by the same group of investigators which consisted of feeding a number of known, pure organic compounds to rats on a milk diet, and determination of ascorbic acid excretion.

Pure compounds of the terpene and sesquiterpene types resembled the materials found in the lipids of natural products having a stimulatory action. Compounds such as d- and l-carvone, dl-piperitone, α and β-ionone, camphor, thujone, pulegone, and isophorone were administered. A 10- to 100-fold increase in the daily output of ascorbic acid occurred in each case. The terpene alcohols, menthol, isoborneol, cineole, and neridol were found to cause an increased rate of excretion, but of smaller magnitude than produced by the above unsaturated ketone terpenes.

The consideration of the possibility that the animals could use a 6 carbon chain from the stimulating compounds for the specific purpose of ascorbic acid synthesis led to the administration of several C_5 compounds. Cyclopentanone, diethylcarbinol, and dimethylcarbinol showed consistent activity though the response was not large.

Various aliphatic compounds were tested, and diisobutylketone, dipropyl ketone, and dimethylacetylcarbinol were found to be relatively effective.

No conjugation of ascorbic acid with effective compounds could be demonstrated in the urine.

The moderate activity of a few compounds with chains of less than six carbon atoms, and the great variations in molecular structure of the effective compounds, led the authors to believe it improbable that the active substances serve as direct precursors of ascorbic acid. They considered the increased ascorbic acid to arise through stimulation of synthesis from normal metabolites by the active agents.

Longenecker, Fricke and King in 1940 reported further experiments with substances which they felt might accelerate vitamin C synthesis and excretion in rats. They found several new series of compounds which were as active as the terpene-like substances reported in the earlier papers, and individual compounds in the new series were much more active than any used before. Many of these substances are extensively used in clinical and experimental work. Although they differ greatly in chemical structure, they have the common characteristic of functioning as nerve depressants.

A number of barbituric acid derivatives were found to be active in stimulating increased ascorbic excretion. The most active were sodium phenobarbital and calcium ipral.

A similar effect was obtained with a group of chemically unrelated hypnotics. 20 mg. doses of paraldehyde and chloretone raised the average daily excretion of ascorbic acid from 0.2 to 0.3 mg. to 11 and 18 mg. respectively.

Pyrazolone derivatives, aminopyrine and antipyrine, were the most effective of the antipyretics in causing rats to excrete increased amounts of ascorbic acid.

Slight activity was demonstrated with phenols, salicylates, sulfanilamide, and sulfapyridine. A number of alkaloids caused practically no increase in excretion. In this group of compounds no evidence of correlation between nerve depressant action and stimulation of ascorbic acid excretion was noted.

The rat urines from animals receiving narcotine and sodium phenobarbital were biologically assayed for vitamin C activity in guinea pigs. The results were in good agreement with the chemical data.

A report upon the influence of dietary protein, methionine, and cystine on accelerated vitamin C excretion in the rat was published in 1947 by Roberts and Spiegl.[2] A study was made of the effect of changing the dietary protein level on the urinary excretion of vitamin C by rats fed sodium phenobarbital or chloretone. When the casein content of the diet was raised from 5 to 18 per cent the stimulating effect of phenobarbital upon ascorbic acid excretion was increased. Somewhat lower excretion resulted when the casein was further increased to 55 per cent. This effect was also noticed when the dietary level of arachin was increased from 5 to 14 per cent.

Experiments were conducted in which each of the ten essential amino acids individually and cystine were added to the 5 per cent casein diet. A marked accelerating effect upon vitamin C excretion was observed with only cystine and methionine. The excretion of vitamin C caused by chloretone or phenobarbital was increased to about the maximum level when cystine or methionine was added to the 5 per cent casein diet. Methionine

had no effect on vitamin C excretion unless a stimulating agent was also administered. When methionine was added to a milk diet or to the 14 per cent arachin diet supporting a high level of vitamin C excretion, the output of vitamin C was depressed. The authors suggest that cystine and methionine probably do not act as direct precursors of vitamin C, but that the accelerating influence on vitamin C excretion observed in these studies is related to the generally beneficial effects which take place when these amino acids are added to a low protein diet.

THE ANTIHISTAMINE COMPOUNDS

The antihistamine drugs or histamine antagonists are those compounds which have the capacity to diminish or prevent the pharmacological effects of histamine without producing responses diametrically opposed to those which are produced by histamine. For this reason epinephrine and similar drugs are not classed as histamine antagonists. A review of these substances is given by Loew.[3]

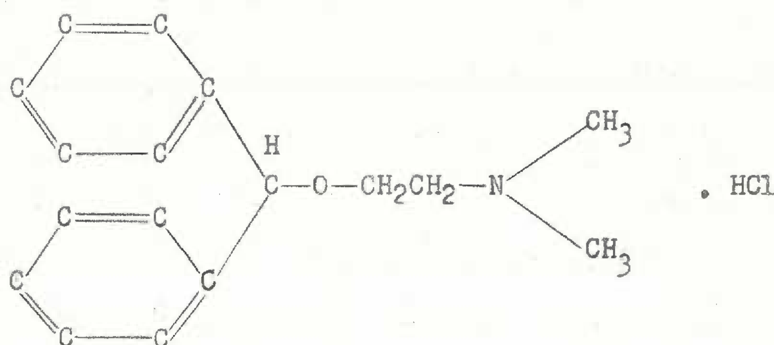
The development of the antihistamine drugs has been of importance because the action of histamine is considered to account for some of the unfortunate symptoms in certain types of disease. These drugs have been particularly useful in the treatment of allergic manifestations.

The first report on certain of the phenolic ethers which are now considered to be antihistaminic compounds was made in 1933 by Fourneau and Bovet.[4] In this report they were considered of interest for their sympatholytic properties and were not mentioned as histamine antagonists. A preliminary report was made in 1937 from Bovet's laboratory at the Pasteur Institute[5] in which the antihistaminic and anti-anaphylactic properties of one of the Fourneau phenolic ethers were described.

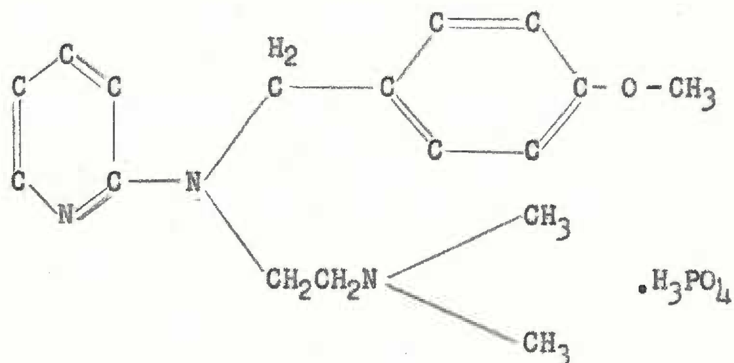
Following this Anne-Marie Staub[6] investigated several of the Fourneau drugs and published extensive data regarding their antihistaminic properties. The toxic side effects of these first compounds prohibited their use therapeutically but did motivate other investigators to attempt to find other compounds which could be used.

Many investigators have worked on the synthesis and testing of antihistamine compounds since the first reports of such drugs were made. Much of the earlier work was done in France and Germany but in the last few years a great deal has been done in this country. At the present time there is a steadily increasing number of drugs in use which belong to several different chemical classes. The experiments reported here were begun shortly after the appearance of Benadryl and Pyribenzamine. The four drugs selected for testing were Benadryl, Neoantergan, Histadyl, and Thephorin, all of which have marked differences in chemical structure.

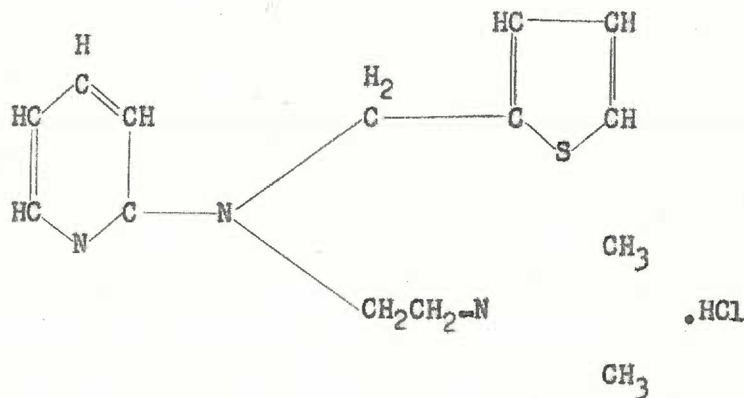
<u>Benadryl</u>: β-dimethylaminoethyl benzhydryl ether hydrochloride.



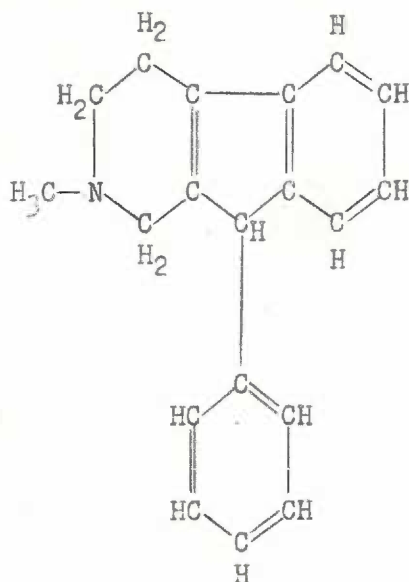
Neoantergan; N-α-pyridyl, N-p-methoxybenzyl-N', N'-dimethylethylene-diamine phosphate.



Histadyl; N-(α-pyridyl)-N', N'-dimethylethylenediamine hydrochloride.



Thephorin; 2-methyl-9 phenyl, tetrahydro-1-pyridinine hydrogen tartrate.



EXPERIMENTAL WORK

GENERAL PLAN OF EXPERIMENTS. The experiments reported in this thesis are similar to those reported by Longenecker and associates.

The animals used were adult female rats of the Sprague-Dawley strain, weighing from 200 to 300 grams. They were kept in individual cages as shown in figure 1. Each rat was housed in a cage made from a glass jug of one gallon capacity. The bottoms were cut from the jugs which were then inverted and fitted with removable floors of galvanized screen wire. The collecting vessels consisted of 125 cc. Erlenmeyer flasks with 3 inch funnels which contained fine galvanized wire screens to separate feces from urine.

The cages were kept in racks of six units. They were covered by perforated wooden lids through which tubes of water bottles were inserted. The feeding vessels were made from 100 cc. beakers which were suspended by wire frames close to the floor of the cages.

Loss of ascorbic acid in the urine samples was prevented by the use of the hydroxyquinoline reagent of Sendroy. 0.05 cc. of 8-hydroxyquinoline solution (1.45 gm. of 8-hydroxyquinoline in 100 cc. of 95% ethyl alcohol) and 0.75 cc. of 5 N sulfuric acid were placed in each flask at the beginning of the collection period.

The rats were placed in their cages and fasted for two days, after which they were maintained on a diet of Pet evaporated milk for the duration of the experiments. After several days on the milk diet, 24 hour urine specimens were collected and analyzed for ascorbic acid until a control period of consistent excretion levels was obtained.

The drug to be tested was then administered by dissolving in the

daily milk ration. Analysis of 24 hour urine samples was continued until maximum rise in ascorbic acid excretion was obtained, or, if no rise occurred the drug was discontinued after ten days and the animals were allowed a rest period of at least five days before another drug was administered. This procedure was varied in one case when it was desired to determine if benadryl would produce the usual rise in excretion after another drug had failed to show activity. The rats in that case were shifted immediately from histadyl to benadryl.

Autopsies were performed at the end of the experiments on all but the first six rats and the last six which are still in use and which will be autopsied when the experiments in progress are finished. At autopsy the blood was collected, hemoglobin was determined, and red and white blood cell counts were done. The animals were dissected, examined for gross pathology, and sections of stomach, pancreas, liver, kidneys, and suprarenals were made.

DETERMINATION OF ASCORBIC ACID. Ascorbic acid was determined by the method of Highet and West[6] based upon the use of a standardized solution of 2,6,-dichlorophenol indophenol in xylene. A modification in the separation of the xylene-dye solution from the water phase was made.

Reagents.

1. 0.03 N HCl.

2. 2,6-dichlorophenol indophenol in xylene. Two 25 cc. portions of water are used to extract 0.1 gm. of 2,6-dichlorophenol indophenol. The solution is filtered, diluted to 200 cc., cooled and acidified with 0.03 N HCl until red. The dye is extracted from the water solution with

FIGURE I

METABOLISM CAGE
FOR RATS

a. water bottle

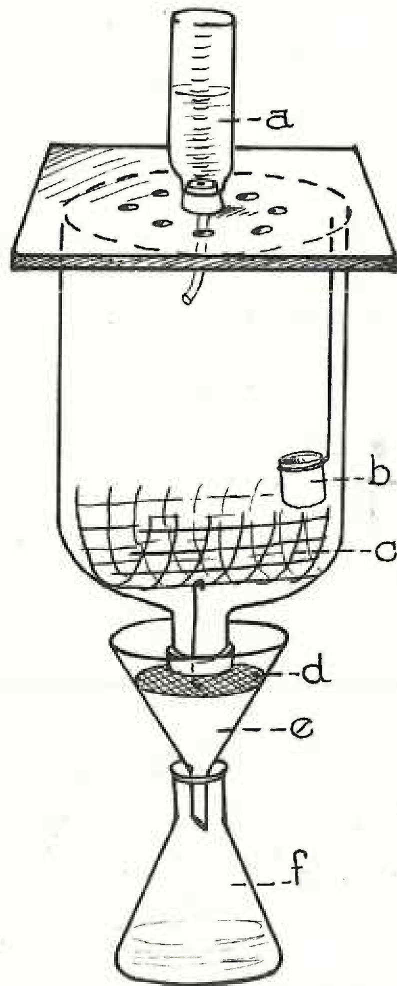
b. feeding vessel

c. removable wire floor

d. screen for separating feces from urine

e. funnel

f. 125 cc. Erlenmeyer flask


a
b
c
d
e
f

200 cc. of xylene. Several 200 cc. portions of 0.03 N HCl are used to wash the xylene-dye solution which is then dried with anhydrous sodium sulfate and filtered. This concentrated xylene-dye solution is used as a stock solution from which the reagent for analysis is prepared by diluting with xylene to give a photoelectric colorimenter reading of 150.

The used xylene-dye solution is recovered for further use by drying with anhydrous sodium sulfate and adding concentrated stock solution to bring the reading up to 150.

3. Anhydrous sodium sulfate.

Procedure for determining ascorbic acid in rat urine. The 24 hour urine samples were collected in the flasks beneath the metabolism cages as described in the preceding section. These were transferred to 100 cc. volumetric flasks and made to volume with water. An aliquot (usually 0.5, 1.0 or 2.0) estimated to contain from 0.01 to 0.06 mg. of ascorbic acid was measured into a 50 cc. glass stoppered graduated cylinder containing 10 cc. of xylene-dye solution reading 150 on the colorimenter. The volume was brought to 25 cc. with 0.03 N HCl. The stoppers were fastened in place and the graduates shaken in horizontal position for three minutes in a mechanical shaker with a speed of 370 R.P.M. In preliminary experiments oxidation of the ascorbic acid was found to be complete in this time.

When the graduates were removed from the shaker the xylene-dye and water were emulsified. The stability of the emulsion varied with different specimens of urine and in many cases the xylene-dye layer could not be separated even with centrifugation. At this stage the original method was modified by the use of anhydrous sodium sulfate to break the emulsion. In specimens where the globules were large and

partial separation took place it was possible to separate the layers by sifting anhydrous sodium sulfate on top of the mixture in the graduated cylinder without the use of centrifugation. When the mixture was more emulsified it was transferred to a 50 cc. centrifuge tube, anhydrous sodium sulfate was sifted on top of the foamy liquid, the tubes stoppered and centrifuged for 5 minutes at 1900 R.P.M. The xylene-dye layer was poured off and read in the photoelectric colorimeter which had been set at zero for xylene. The colorimeter readings were compared with those on a graph plotted against known ascorbic acid concentrations. The total excretion of ascorbic acid for twenty-four hours was then calculated.

RESULTS

EXPERIMENTS ON RATS TO DETERMINE THE EFFECT OF BENADRYL UPON THE EXCRETION OF ASCORBIC ACID.

A. Six rats (Series I. #1 to #6) which had been placed in individual metabolism cages, fasted two days, and maintained for a control period on evaporated milk, were given 10 mg.* of benadryl per day for 3 days. Only small rises in ascorbic acid excretion were observed. On the fourth day the dose of benadryl was increased to 20 mg. per day and on the sixth day to 30 mg. Each increase in the dosage of benadryl led to an increased output of ascorbic acid.

*Figures indicate approximate amounts, which varied somewhat with variations in food intake.

TABLE I

THE EFFECT OF BENADRYL UPON THE URINARY EXCRETION OF ASCORBIC ACID BY RATS

Values represent mg. per 24 hours

Date	Rat Numbers 1	2	3	4	5	6
Control period						
8/22/47	2.8	2.0	1.2	1.4	1.8	2.6
8/28/47	3.7	1.6	1.2	1.3	1.6	2.0
9/3/47	4.6	3.2	2.6	1.6	2.9	2.7
9/4/47	3.3	2.6	3.2	1.7	2.1	1.4
9/5/47	2.7	2.8	2.7	1.7	2.0	1.5
10 mg. benadryl per day						
9/6/47	3.0	3.4	2.7	2.9	3.7	3.1
9/7/47	3.9	4.7	4.6	3.5	4.2	3.9
9/8/47	3.6	6.2	3.4	2.6	3.6	3.9
20 mg. benadryl per day						
9/9/47	4.6	6.4	3.9	2.4	4.2	4.1
9/10/47	4.2	6.2	4.0	3.0	4.9	4.4
30 mg. benadryl per day						
9/11/47	5.4	4.8	5.1	3.0	5.2	4.0
9/12/47	5.1	5.2	4.0	3.4	4.0	3.5

B. Six rats (Series II. #7 to #12), after the control period, were given 6 mg. of benadryl each for 10 days, 12 mg. per day for 7 days, and then 24 mg. per day for 14 days. Determinations were made for 4 days after discontinuing the benadryl at which time the ascorbic acid excretion had dropped to the original level. The rats were then autopsied.

TABLE II

THE EFFECT OF BENADRYL UPON THE URINARY EXCRETION
OF ASCORBIC ACID BY RATS

Values represent mg. per 24 hours

Date	Rat Numbers					
	7	8	9	10	11	12
Control period						
10/10/47	2.0	1.6	3.1	1.4	0.2	0.6
10/14/47	2.1	1.1	1.9	1.5	0.3	2.6
10/15/47	2.7	1.5	3.1	2.9	0.8	1.7
10/16/47	1.8	1.2	2.0	2.1	0.3	2.1
10/17/47	2.5	2.1	3.1	2.7	0.9	3.3
10/20/47	2.9	1.6	3.2	2.2	1.0	1.4
10/21/47	1.6	0.5	1.8	1.1	0.4	1.1
10/22/47	3.0	1.2	2.7	1.7	0.7	1.3
10/23/47	1.5	0.7	2.0	1.1	0.3	2.7
10/24/47	2.9	1.4	3.1	2.3	0.6	1.6
10/27/47	1.2	0.4	3.0	1.1	0.4	1.8
10/28/47	2.0	0.7	3.1	1.3	0.3	0.6
11/17/47	2.6	0.7	2.1	1.3	0.3	0.6
11/18/47	1.5	0.7	1.4	1.3	0.3	0.8
11/19/47	2.4	1.2	2.1	2.0	0.5	—
6 mg. benadryl per day						
11/20/47	2.0	1.2	2.1	1.7	0.4	1.0
11/21/47	1.3	1.4	1.5	1.2	0.4	0.8
11/24/47	2.2	1.2	2.9	—	0.5	1.6
11/25/47	1.7	1.2	2.8	2.3	0.7	1.7
11/26/47	2.1	1.3	2.8	2.0	0.7	1.5

TABLE II (cont.)

THE EFFECT OF BENADRYL UPON THE URINARY EXCRETION

OF ASCORBIC ACID BY RATS

Values represent mg. per 24 hours

Date	Rat Numbers					
	7	8	9	10	11	12
11/28/47	0.9	0.7	1.7	1.2	0.2	1.0
12/1/47	1.7	1.1	2.9	1.8	0.6	1.6
12/2/47	1.7	0.6	1.7	1.4	0.4	0.8
12 mg. benadryl per day						
12/3/47	2.4	1.2	2.9	2.2	0.8	1.6
12/4/47	1.8	1.2	2.1	2.1	0.7	1.4
12/5/47	1.1	0.9	2.1	1.2	0.4	0.8
12/8/47	3.5	1.4	3.7	1.8	1.0	3.0
24 mg. benadryl per day						
12/9/47	3.2	1.2	2.2	0.9	0.7	3.3
12/10/47	4.0	1.6	3.3	4.3	1.6	4.6
12/11/47	2.9	1.1	3.2	2.4	1.6	3.6
12/12/47	3.2	2.0	4.6	1.8	2.6	5.4
12/15/47	3.2	1.4	5.2	2.8	—	5.2
12/16/47	2.6	0.6	3.8	2.6	3.0	5.8
12/17/47	2.6	0.7	3.8	3.4	3.0	6.4
12/18/47	3.6	—	6.5	3.8	4.8	11.0
12/19/47	4.2	0.4	6.2	6.3	5.1	10.6
12/22/47	4.2	died	6.8	9.4	3.5	10.4
Benadryl discontinued						
12/23/47	2.8	—	3.8	4.0	2.2	4.4

TABLE II (cont.)

THE EFFECT OF BENADRYL UPON THE URINARY EXCRETION OF ASCORBIC ACID BY RATS

Values represent mg. per 24 hours

Date	Rat Numbers					
	7	8	9	10	11	12
12/24/47	1.9	—	1.3	2.7	0.6	4.9
12/26/47	1.7	—	3.4	1.0	0.7	2.3
12/27/47	0.9	—	2.1	1.2	0.3	1.0

C. Six rats (Series III. #13 to #18), after being subjected to the usual control period and a period of 12 days on histadyl, which caused no rise in ascorbic acid excretion, were shifted, without interval to 18 mg. of benadryl per day for 7 days. The excretion of ascorbic acid rose steadily during this period and dropped to a level slightly above the original normal level 4 days after discontinuing benadryl.

TABLE III

THE EFFECT OF BENADRYL, HISTADYL, AND NEOANTERGAN UPON THE URINARY EXCRETION OF ASCORBIC ACID BY RATS

Values represent mg. per 24 hours

Date	Rat Numbers					
	13	14	15	16	17	18
Control period 1/14/48	1.0	0.6	0.9	0.9	0.3	0.5
1/15/48	1.5	1.4	2.1	2.3	0.7	0.8

TABLE III (cont.)

THE EFFECT OF BENADRYL, HISTADYL, AND NEOANTERGAN

UPON THE URINARY EXCRETION OF ASCORBIC ACID BY RATS

Values represent mg. per 24 hours

Date	Rat Numbers					
	13	14	15	16	17	18
1/16/48	1.4	1.2	1.7	2.3	0.6	0.8
1/19/48	1.6	1.4	1.3	1.7	0.5	0.9
1/20/48	1.4	1.9	1.5	2.0	0.7	1.1
1/21/48	1.6	1.4	1.3	1.7	0.5	--
18 mg. histadyl per day						
1/22/48	1.6	2.2	2.0	2.3	0.8	1.1
1/23/48	1.1	1.5	1.4	2.3	0.8	1.0
1/26/48	0.6	0.5	0.9	1.3	0.3	0.6
1/27/48	0.8	0.6	1.4	1.7	0.4	1.0
1/28/48	0.9	0.9	1.4	1.5	0.5	0.9
1/29/48	1.3	1.4	1.9	1.4	0.9	1.4
1/30/48	1.4	1.5	1.9	2.1	1.2	1.5
2/2/48	1.2	1.3	1.5	1.8	0.4	0.7
18 mg. benadryl per day						
2/3/48	1.5	2.2	1.6	2.0	0.6	0.8
2/4/48	2.0	3.2	2.5	2.3	1.0	1.8
2/5/48	2.7	2.6	2.9	3.0	1.2	2.4
2/6/48	3.3	3.3	3.2	2.7	1.7	2.6
2/9/48	8.8	8.4	7.2	7.2	5.8	9.6
Benadryl discontinued						
2/10/48	4.2	5.0	4.4	4.2	2.8	4.4
2/11/48	2.8	3.7	2.8	3.1	1.9	1.9

TABLE III (cont.)

THE EFFECT OF BENADRYL, HISTADYL, AND NEOANTERGAN UPON THE URINARY EXCRETION OF ASCORBIC ACID BY RATS

Values represent mg. per 24 hours

Date	Rat Numbers					
	13	14	15	16	17	18
2/12/48	1.7	1.9	3.4	3.9	1.6	2.1
2/18/48	1.2	1.4	2.5	2.1	1.2	1.4
2/19/48	2.5	3.0	2.5	3.2	1.1	1.3
2/20/48	1.9	2.4	1.7	3.0	0.9	1.0
3/1/48	2.2	2.9	2.5	2.8	0.8	1.7
3/3/48	2.6	3.0	2.3	1.3	1.2	1.1
3/4/48	1.3	1.3	0.9	3.8	0.4	0.4
3/5/48	1.8	2.4	1.8	3.0	1.2	1.3
3/8/48	2.6	3.5	2.4	2.0	1.2	1.3
3/9/48	1.5	1.2	1.3	—	0.3	0.6
20 mg. neoantergan per day						
3/10/48	2.0	2.7	2.0	2.6	0.6	1.1
3/11/48	2.2	2.7	2.0	2.0	0.6	0.5
3/12/48	2.7	3.3	2.4	2.8	1.4	1.4
3/15/48	2.5	2.5	2.7	1.7	1.7	1.0
3/16/48	2.7	3.0	2.9	1.5	2.5	1.5
3/18/48	3.3	3.0	2.5	1.0	1.5	1.5
3/19/48	3.1	3.0	2.2	0.7	1.7	1.4
3/22/48	3.4	3.7	3.2	0.7	1.2	1.6
18 mg. benadryl per day						
3/23/48	3.8	3.3	2.9	died	1.6	1.8

TABLE III (cont.)

THE EFFECT OF BENADRYL, HISTADYL, AND NEOANTERGAN

UPON THE URINARY EXCRETION OF ASCORBIC ACID BY RATS

Values represent mg. per 24 hours

Date	Rat Numbers					
	13	14	15	16	17	18
3/24/48	4.4	4.1	3.0	—	2.0	1.9
3/25/48	5.4	6.0	4.4	—	4.2	4.0
3/26/48	5.4	6.2	5.0	—	6.4	4.6
3/30/48	7.0	4.2	3.8	—	4.4	2.6
Benadryl discontinued						
3/31/48	5.2	5.8	2.8	—	3.3	2.0
4/6/48	4.8	3.9	1.9	—	1.3	0.6
4/7/48	4.5	4.2	1.4	—	1.5	0.7
4/13/48	3.8	3.0	0.9	—	1.2	died
4/20/48	2.7	2.7	1.3	—	0.9	—

D. Five of the six rats (Series III. #13 to #18, #16 died of a respiratory infection just prior to the begining of this part of the experiments) used in part C were again placed on benadryl after a ten day period on neoantergan, which showed no activity in raising ascorbic acid excretion. They were given from 17 to 19 mg. of benadryl per day for seven days and showed an increase in ascorbic acid excretion comparable with previous similar periods. After discontinuing the drug the excretion rate slowly returned to a high normal level. Table III.

THE EFFECT OF HISTADYL UPON THE EXCRETION OF ASCORBIC ACID.

Six rats (Series III, #13 to #18) were fasted two days and maintained for a control period of 6 days on evaporated milk and were then given 18 mg. of histadyl per day for 12 days. No rise in ascorbic acid excretion was observed. Table III.

THE EFFECT OF NEOANTERGAN UPON THE EXCRETION OF ASCORBIC ACID.

A. Six rats (Series III, #13 to #18) after being subjected to a control period of 6 days, 12 days on 18 mg. of histadyl, 7 days on 18 mg. of benadryl, a 20 day rest period, and a 10 day control period were placed on 20 mg. of neoantergan per day for 10 days. No rise in ascorbic acid excretion was observed. Table III.

B. Six rats (Series IV, #19 to #24) which had been fasted two days and placed on evaporated milk for a c ontrol period of 8 days were given 28 mg. of neoantergan per day for 8 days. No rise in ascorbic acid excretion was observed.

TABLE IV

THE EFFECT OF NEOANTERGAN AND THEPHORIN UPON THE URINARY EXCRETION OF ASCORBIC ACID BY RATS

Values represent mg. per 24 hours

Date	Rat Numbers					
	19	20	21	22	23	24
Control period						
4/27/48	2.4	3.9	2.2	2.0	1.6	2.7
4/28/48	2.9	4.5	3.6	2.8	2.0	2.6
4/29/48	2.9	3.5	2.7	1.1	1.8	2.2

TABLE IV (cont.)

THE EFFECT OF NEOANTERGAN AND THEPHORIN UPON THE URINARY EXCRETION OF ASCORBIC ACID BY RATS

Values represent mg. per 24 hours

Date	Rat Numbers					
	19	20	21	22	23	24
4/30/48	3.1	3.5	2.4	1.8	1.8	2.2
5/3/48	3.5	4.4	3.0	2.0	2.0	3.1
5/4/48	2.4	3.2	2.0	1.1	1.0	1.4
28 mg. neoantergan per day						
5/5/48	3.2	4.1	2.5	1.8	1.0	1.9
5/6/48	3.1	3.8	1.9	1.9	1.6	2.5
5/7/48	2.7	3.2	2.0	1.4	1.5	2.1
5/10/48	2.8	3.4	3.3	2.5	1.3	2.9
5/11/48	2.6	2.7	2.0	1.8	1.2	2.6
5/12/48	3.5	3.9	2.3	2.6	1.7	2.6
Neoantergan discontinued						
5/17/48	2.9	3.2	2.3	1.8	1.8	2.7
Thephorin administered						
5/18/48	1.8	2.7	1.0	1.0	0.5	1.5
5/20/48	1.3	5.4	1.6	1.7	1.5	3.2
5/21/48	1.6	7.4	2.6	1.6	1.4	3.2
5/22/48	1.0	7.2	2.4	2.2	2.2	3.2
5/24/48	2.1	6.6	5.0	3.8	3.4	4.0
5/25/48	2.5	8.8	4.4	3.2	4.2	4.4
5/26/48	4.5	9.2	5.2	2.4	3.8	5.0
5/27/48	5.2	10.0	4.6	3.0	3.2	5.4

TABLE IV (cont.)

THE EFFECT OF NEOANTERGAN AND THEPHORIN UPON THE URINARY EXCRETION OF ASCORBIC ACID BY RATS

Values represent mg. per 24 hours

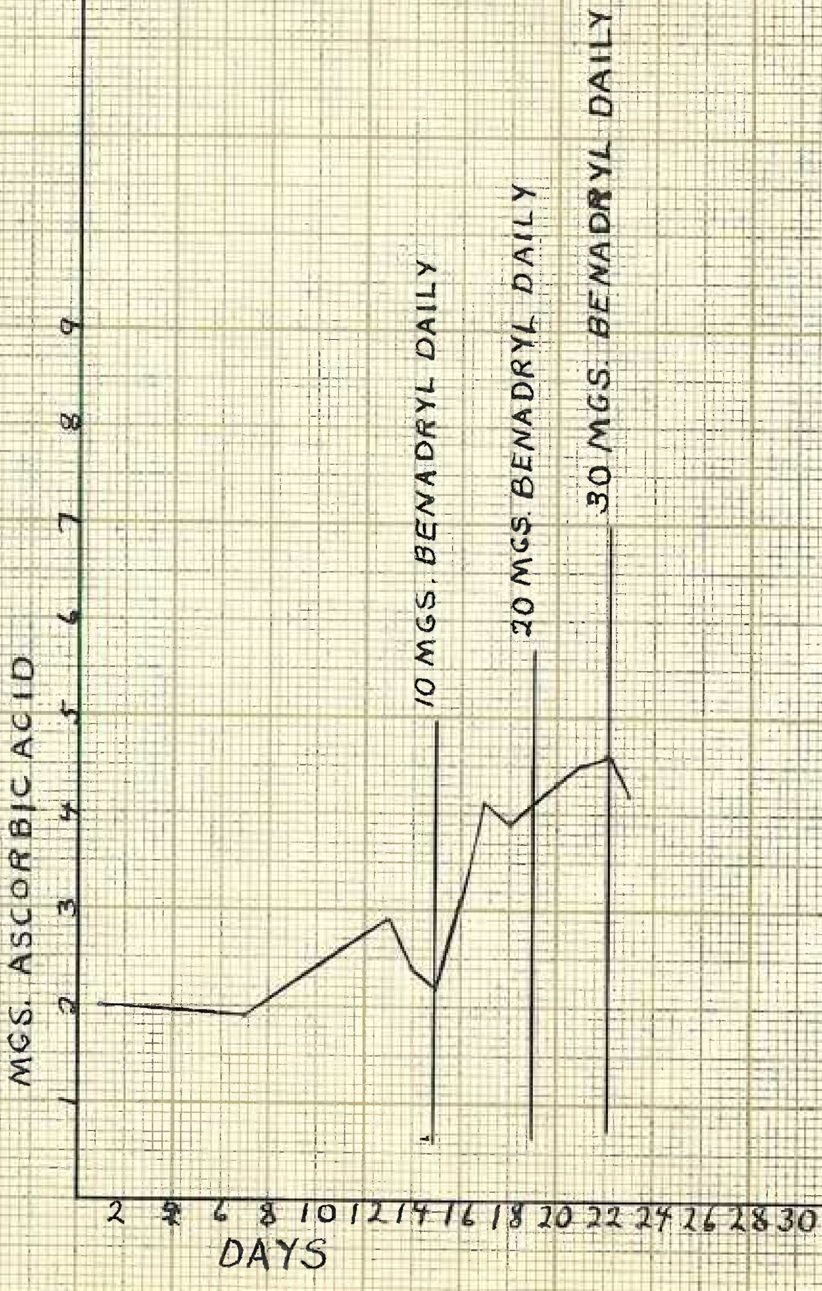
Date	Rat Numbers					
	19	20	21	22	23	24
5/29/48	---	10.2	5.0	4.6	4.2	4.6
6/1/48	6.8	10.0	5.4	5.0	4.8	7.0
Thephorin discontinued						
6/2/48	5.8	10.4	5.8	5.4	5.6	7.6
6/3/48	6.6	9.0	5.4	3.6	5.0	8.4

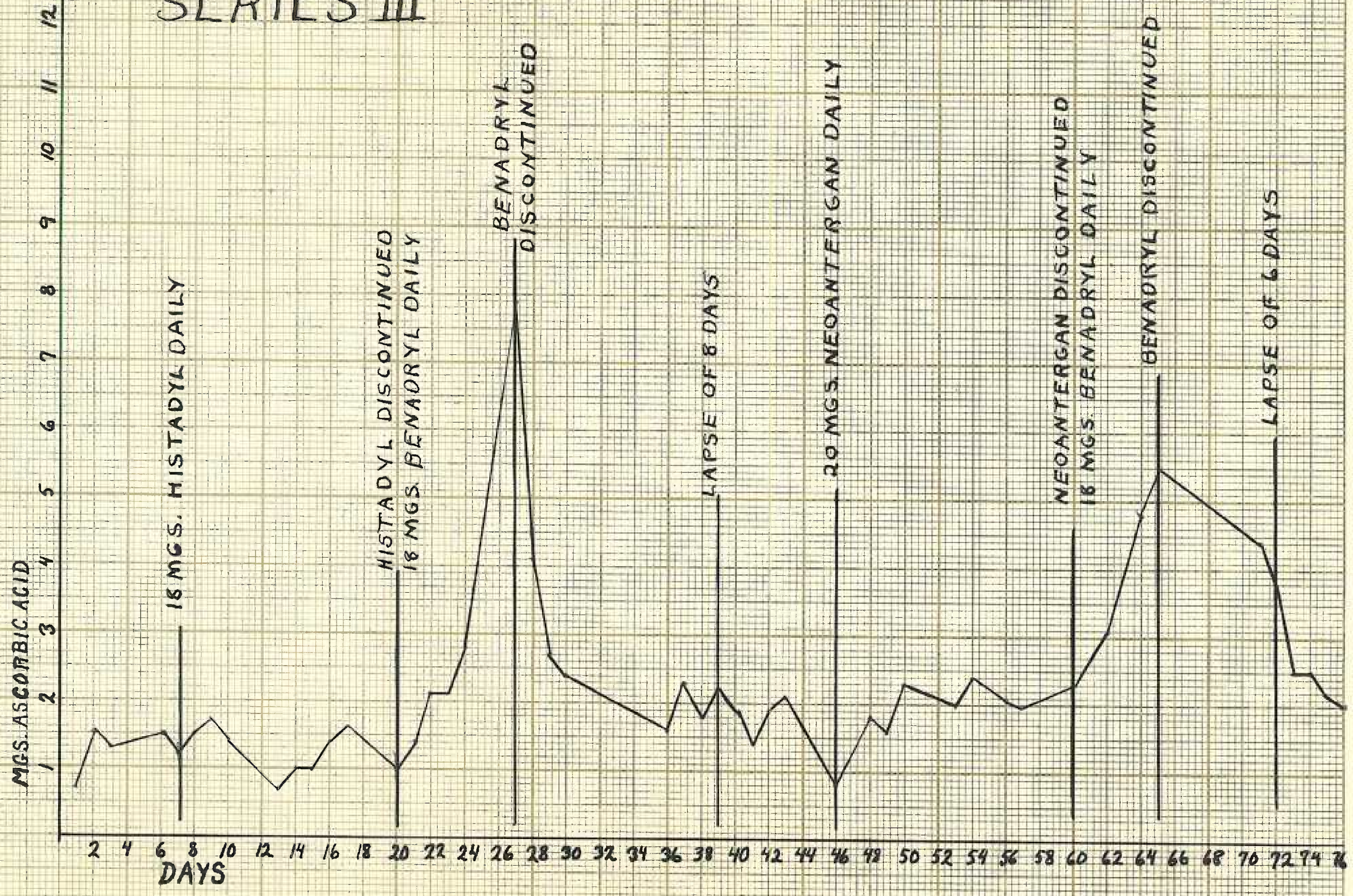
THE EFFECT OF THEPHORIN UPON THE EXCRETION OF ASCORBIC ACID.

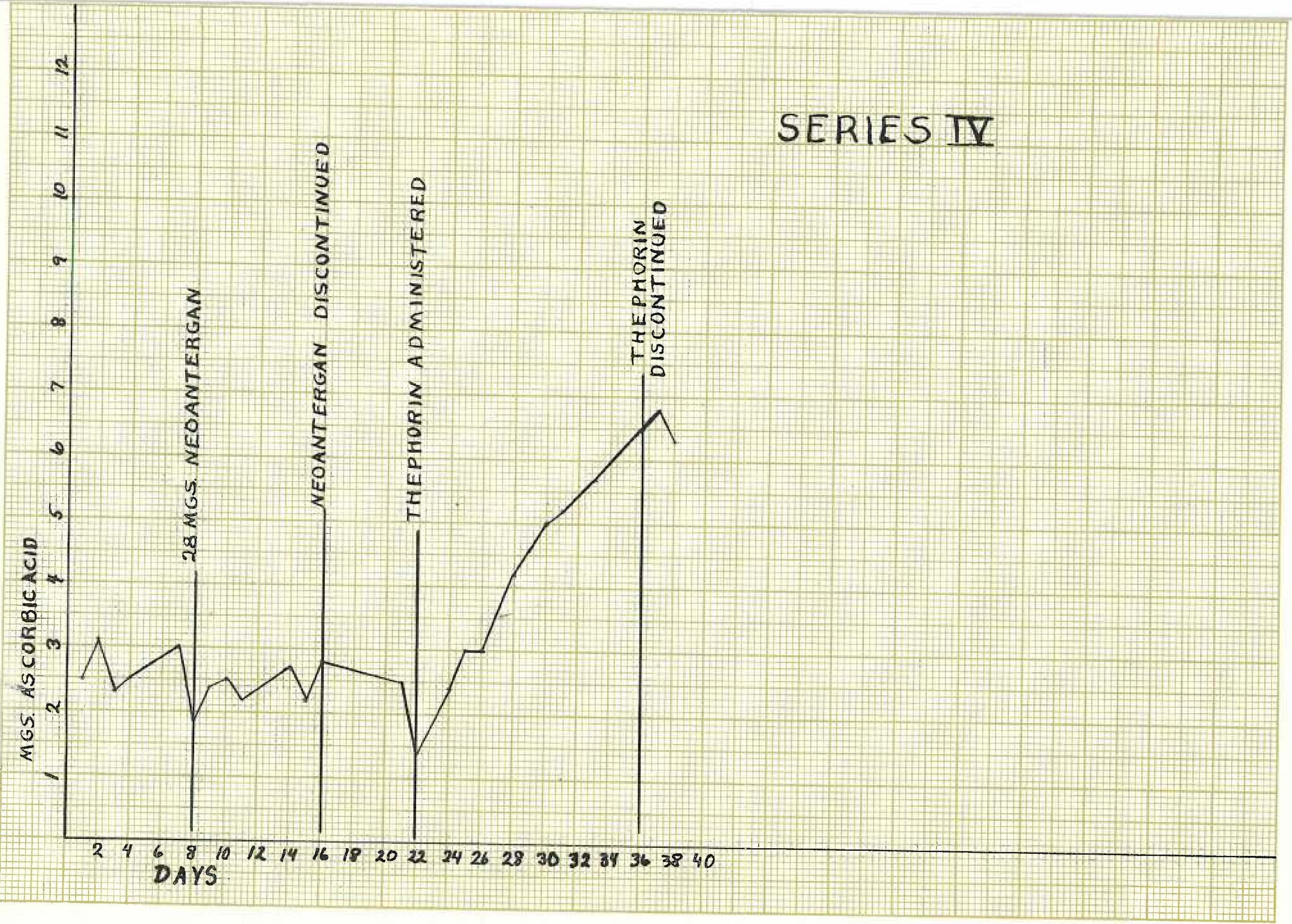
Six rats (Series IV, #19 to #24) which had been subjected to the usual control period, 10 days on neoantergan, and a 5 day rest period were placed on thephorin. At first, consumption of the milk containing the drug by the animals was erratic, none eating the normal amount. The rat taking the largest quantity showed an early rise in ascorbic acid excretion. As the animals became accustomed to the taste of the milk, the intake of milk and drug increased and the ascorbic acid excretion of all animals rose. The results of this experiment are given in Table IV.

The results of this investigation are graphically shown in the curves of figures 2-5.

SERIES I




SERIES III
MGS. ASCORBIC ACID
DAYS
18 MGS. HISTADYL DAILY
HISTADYL DISCONTINUED
18 MGS. BENADRYL DAILY
BENADRYL
DISCONTINUED
LAPSE OF 8 DAYS
20 MGS. NEOANTERGAN DAILY
NEOANTERGAN DISCONTINUED
18 MGS. BENADRYL DAILY
BENADRYL DISCONTINUED
LAPSE OF 6 DAYS



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AUTOPSY FINDINGS

Autopsies showed no pathology in the blood or tissues of rats except multiple lung abscesses in the three which died and in two of those killed at the end of Series III.

The writer is indebted to Dr. Frank B. Queen of the Pathology Department for performing the autopsies.

DISCUSSION AND CONCLUSIONS

As noted in the introduction, ascorbic acid has been shown to be of importance in increasing the resistance of the animal body to toxic agents. In this connection the following specific findings may be cited.

Ascorbic acid has been shown to be beneficial in the treatment of lead poisoning[7] and to protect the body against the toxicity of a number of drugs.[8,9,10,11] It is also involved in the resistance of the body to infection.[12,13,14,15]

The fact that certain drugs cause prolonged excretion of large excesses of ascorbic acid by the rat indicates excess synthesis and not merely increased excretion of the preformed vitamin.[1] It appears valid to assume that this excess synthesis of ascorbic acid represents a defense reaction of the organism for protection against the toxic effects of the administered drug.

Upon this basis we have found that benadryl and thephorin, of the antihistamine drugs tested, cause the production of excess ascorbic acid by the rat, and presumably require it for their metabolism, while this is not the case for the drugs histadyl and neoantergan.

Benadryl for these experiments was donated by Parke, Davis and Company; Neoantergan by Merck and Company; Histadyl by Eli Lilly and Company; and Thephorin by Hoffmann-La Roche, Inc.

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