

## STUDIES OF ASCORBIC ACID AND RHEUMATIC FEVER

### II. TEST OF PROPHYLACTIC AND THERAPEUTIC ACTION OF ASCORBIC ACID<sup>1</sup>

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In the previous communication (Sendroy and Schultz (1936)) evidence was presented indicating that patients with active rheumatic fever utilize ascorbic acid in practically the same manner as do controls without active manifestations of this disease. Apparent abnormalities in excretion of this vitamin were attributable to gastrointestinal abnormalities or to other conditions arising from the diseased state rather than to an essential deficiency on the part of the tissues to handle this substance. As these patients were all suffering from rather severe active rheumatic infection at the time of testing, it was thought that the possible relationship between rheumatic fever and ascorbic acid deficiency might be studied from two other points of view: First, the possible prophylactic effect of administering known quantities of the substance to previously rheumatic subjects, and second, the possible curative effect of large doses given to patients with the active disease.

#### *The effect of administering ascorbic acid to ambulatory subjects previously with rheumatic histories*

Fifty-six patients between the ages of 4 and 19 years, each of whom had experienced one or more attacks of rheumatic fever in previous years, when they were treated in the wards of this hospital, were divided into two groups, each containing 28 members. As nearly as possible these groups represented similar conditions with respect to age, sex, and relative adequacy of their habitual diet in ascorbic acid content, insofar as this last item could be determined by detailed inquiry into the dietary customs of each individual. During the period of observation the members of Group A took 100 mgm. of ascorbic acid daily by

mouth, and those of Group B the same amount of lactose. The ascorbic acid, in the form of Redoxon, and the placebo were administered in capsules. The dose of the vitamin was considered sufficient for protection against scurvy, in view of the estimated minimal daily requirement of 19 to 27 mgm. for adults (Göthlin (1934)) notwithstanding the fact that the requirement for children is believed to be twice that for adults (Falk, Gedda and Göthlin (1932-33)). Each patient, or his parents, kept a continuous record of the medication taken; and in many instances the patients were visited in their homes to insure that members of Group A received the prescribed doses.

Each patient was examined at monthly intervals, and at each visit, in addition to the usual procedures of clinical examination, the degree of capillary fragility was determined as a means of studying the possible incidence of subclinical scurvy. A suction method was used: negative pressures of 0 to 320 mm. of mercury were exerted over different circular areas 1 cm. in diameter for periods of one minute. The cutaneous areas selected for testing were situated on the back within a triangular zone bounded superiorly by a transverse line at the level of the spine of the seventh cervical vertebra, and laterally by the vertebral angles of the scapulae. In this region there is available one of the largest areas of skin of uniform reactivity with respect to this test (Wiemer (1931)). An air reservoir of 4000 cc. was included in the suction system in order to minimize the pressure effects of small leaks<sup>2</sup> (Figure 1). The areas to which suction was applied were examined after one minute with a hand lens of 10 $\times$  magnification at constant focus, and with a constant artificial source of light (Figure 1). The effects of negative pressures

<sup>1</sup> Presented in preliminary form, at the meeting of the American Society for Clinical Investigation, Atlantic City, N. J., May 6, 1935.

<sup>2</sup> A refined instrument of this type has recently been described by Cutter and Johnson (1935).

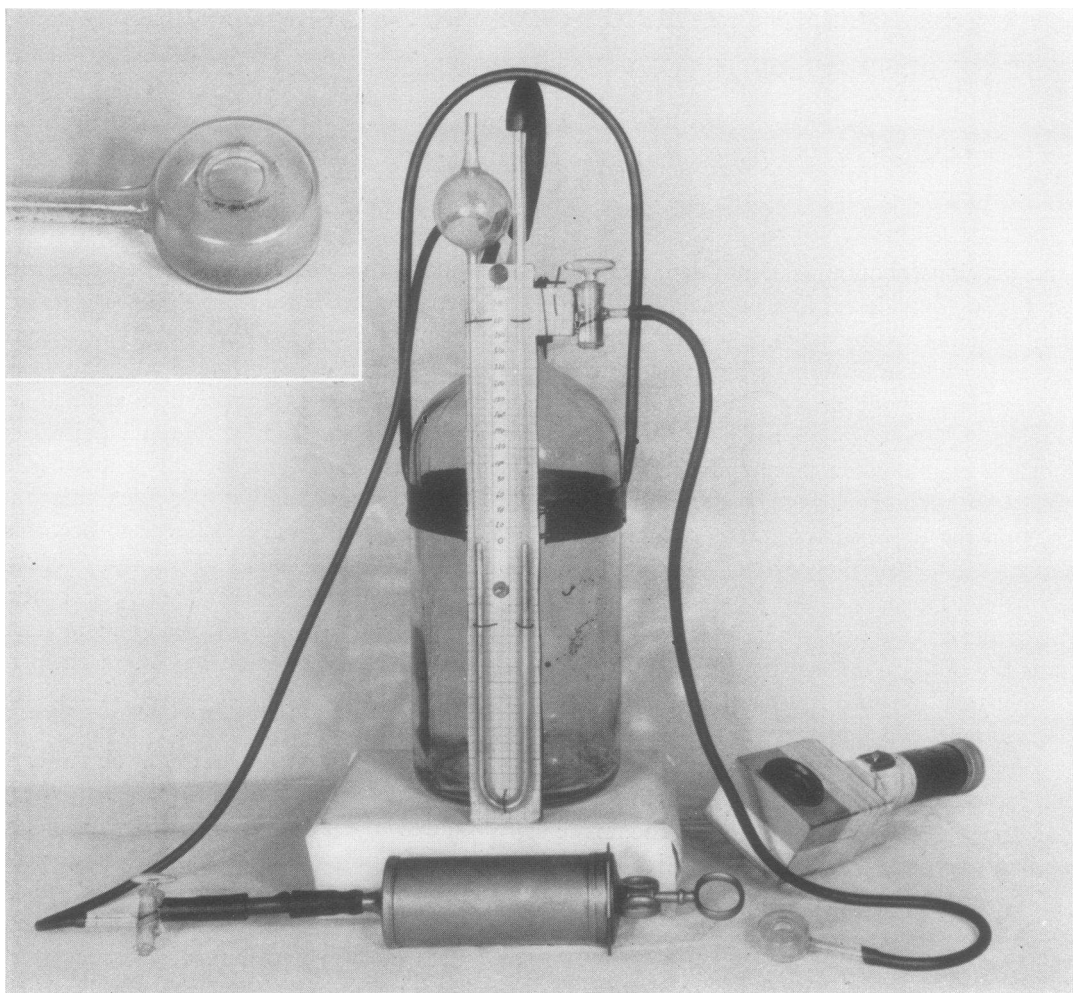


FIG. 1. APPARATUS USED IN THE DETERMINATION OF CAPILLARY PERMEABILITY.

Note detail of suction cup in insert: Diameter of suction area—1 cm. Width of flange resting upon cutaneous surface—1 cm.

at intervals of 10 mm. Hg were investigated; and the reading recorded in each instance was the lowest negative pressure at which discrete capillary hemorrhages appeared.

#### *Incidence of abnormal capillary fragility*

Great individual variations in the amount of negative pressure required to produce capillary hemorrhage were evident. The fact that many racial groups were represented among these patients may, in part, account for this, for it was noted that readings were often high in subjects with heavily pigmented skins. For this reason no absolute standard could be used, and in each subject changes relative to the preliminary deter-

mination in January were taken into account. Readings in patients with intercurrent febrile disease other than rheumatic fever were discarded. As shown in Figure 2, the degree of negative pressure required to produce capillary hemorrhage in the control Group B decreased on the average, especially from January to April, indicating that there was a relative increase in capillary permeability during the late winter and early spring. In contrast there often was noted in the other group, a slight decrease in capillary fragility which usually appeared following the taking of ascorbic acid; and this decrease was maintained during the remainder of the period of observation (see Figure 3). These findings indicate that

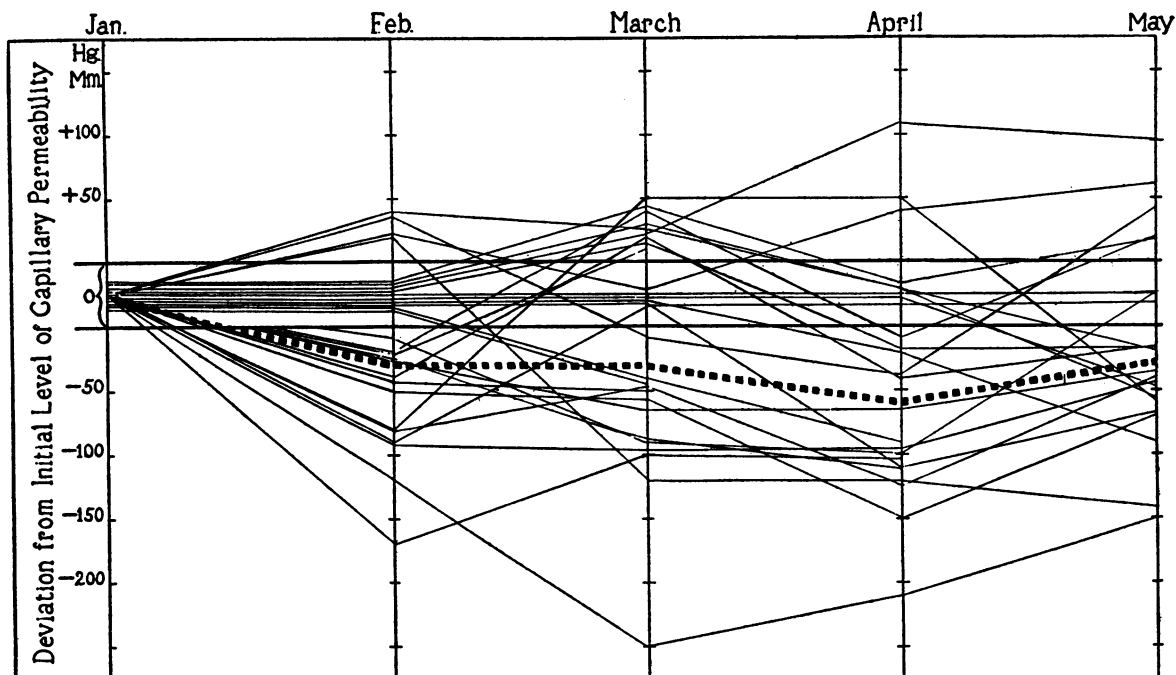


FIG. 2. RELATIVE CHANGE IN CAPILLARY FRAGILITY IN PATIENTS NOT RECEIVING ADDITIONAL ASCORBIC ACID (GROUP B)

Heavy dotted line indicates average for the group.

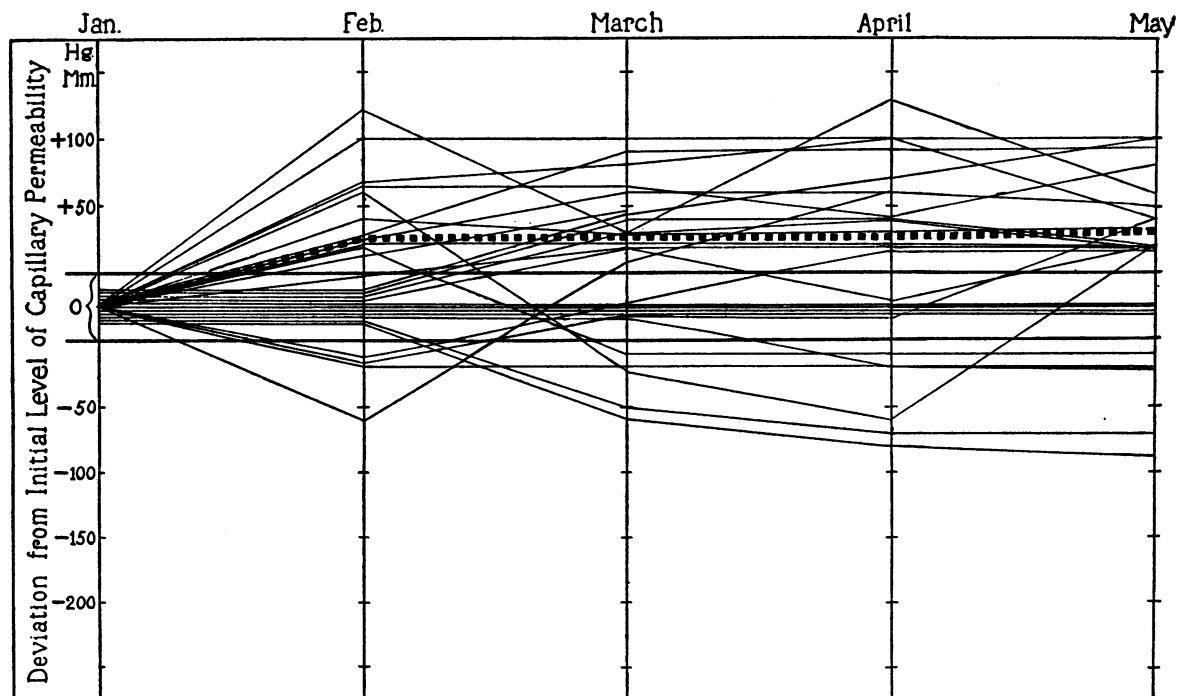


FIG. 3. RELATIVE CHANGE IN CAPILLARY FRAGILITY IN PATIENTS RECEIVING 100 MG. ASCORBIC ACID DAILY (GROUP A)

Heavy dotted line indicates average for the group.

TABLE I  
*Manifestations of rheumatic fever in ambulatory patients*

Case number	Group "A" Treated with ascorbic acid															Group "B" Received lactose									
	1	2	3	4	5	6	8	12	14	17	21	22	23	25	1	5	6	7	8	12	17	20	22	28	
Joint and muscular pains . . . . .	+			+		+			+									+							
Chorea . . . . .	+																			+					
Precordial pain . . . . .		+		+	+	+			+		+	+					+							+	
Epistaxis . . . . .				+	+	+	+		+									+	+					+	+
Gastro-intestinal symptoms . . . . .			+	+	+	+			+		+							+	+				+	+	
Change in cardiac murmurs . . . . .	+			+	+	+			+									+					+	+	
Gallop rhythm . . . . .				+		+			+				+	+	+			+					+	+	
Erythema multiforme . . . . .						(+)																		+	
Palpable spleen . . . . .			+	(+)	+					+	+			+											
Pleurisy . . . . .									+		+						+								
Leukocytosis . . . . .				+		+			+								+			+	+				
Rapid erythrocyte sedimentation rate . . . . .	+			+	+	+	+	+	+	+	+	+	+			+	+	+	+	+	+	+	+	+	+
Fever . . . . .	+	+	+	+	+	+	+	+	+	+	+	+	+			+	+	+	+	+	+	+	+	+	+
Failure to gain weight . . . . .	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+
Tachycardia . . . . .	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Summary																									
Acute rheumatic fever . . . . .				+		+			+																
Active rheumatic fever . . . . .	+	+			+			+			+	+	+			+		+		+	+		+	+	
Probably active rheumatic fever			+				+				+			+	+	+	+	+	+			+		+	

instances of ascorbic acid deficiency were probably present in both groups during January; and furthermore, that, judged by this test, members of Group A were protected against the development of subclinical scurvy, while this condition appeared in several members of Group B. Alterations in degree of capillary permeability could not be correlated with the occurrence of rheumatic activity in these patients. None of them showed any lesions, such as gingivitis, characteristic of scurvy.

#### *Incidence of active rheumatic disease*

Among the 56 subjects investigated, 14 of Group A and 10 of Group B showed evidence of active infection, probably attributable to the presence of rheumatic fever, at some time during the period of observation. The evidence of disease activity is detailed in Table II.

In 4 of each group the symptoms and signs were so slight or so few in number that the designation of "probably rheumatic fever" must be given them. On the other hand, 7 patients in Group A and 6 in Group B had definite evidence of low grade rheumatic activity; while 3 additional members of Group A developed severe acute rheumatic fever during the time they were receiving fairly large amounts of ascorbic acid. The comparative incidence of rheumatic activity among the treated and untreated patients during the various months is shown in Table II. In the

TABLE II  
*Evidence of rheumatic fever in treated and untreated groups*

Number of		January	February	March	April	May
Patients showing some degree of rheumatic activity	Treated with ascorbic acid	9	12	9	6	7
	Given lactose	4	7	7	5	1
Patients with acute rheumatic fever	Treated with ascorbic acid	0	1	1	3	3
	Given lactose	0	0	0	0	0

group receiving ascorbic acid there were twice as many active cases when medication was begun in January as there were in the group receiving the placebo. This greater tendency to develop recurrences doubtless accounts for the higher incidence of rheumatic activity in this group. Nevertheless, if latent scurvy were an important element in calling out relapses it would be probable that measures which are effective in eliminating scurvy would also prevent the rheumatic relapses, but this was not the case. With the exception of a greater relative incidence of recurrences in Group A no differences were apparent in the behavior of the two groups.

#### *The effect of diet*

Even after detailed inquiry it was impossible to grade accurately all of the habitual diets according to their relative ascorbic acid content. A

TABLE III

*Relationship between adequacy of ascorbic acid in diet and occurrence of rheumatic relapses*

Approximate ascorbic acid content of habitual diet	Group "A" in January*		Group "B" during observation		Totals		
	Number of cases	Number with active rheumatism	Number of cases	Number with active rheumatism	Number of cases	Number with active rheumatism	Per cent active rheumatism
Definitely deficient.....	3	2	4	1	7	3	42.8
Intermediate.....	13	4	11	4	24	8	33.3
Adequate.....	12	3	13	5	25	8	31.2
	28	9	28	10	56	19	

\* Before administration of ascorbic acid.

few were identified as definitely deficient in this respect, but almost half of them seemed adequate. The remainder were graded as intermediate. The incidence of active rheumatic disease in these three categories, as indicated in Table III was slightly higher in the individuals taking diets deficient in ascorbic acid. The significance of this correlation is clouded by the fact that these diets were without exception deficient in other respects. Among the patients falling into this "deficient" classification, furthermore, were those living under the most unsatisfactory general hygienic environment.

*The therapeutic effect of ascorbic acid in rheumatic fever.* The therapeutic effect of ascorbic acid medication was tested in 20 hospital patients with rheumatic fever. Three of them had received daily doses of 100 mgm. of ascorbic acid for 1 to 3 months before they were admitted to the wards because of acute illness. All were given ascorbic acid in conjunction with antipyretics when indicated, beginning on the third hospital day and continuing for varying periods of time. Seventeen patients received 250 mgm. of ascorbic acid daily by mouth or intravenously for from 1 to 5 months (average  $2\frac{1}{2}$  months), and 7 (including 4 previously given synthetic ascorbic acid without demonstrable benefit) were fed 200 cc. of orange juice daily for two months. In 11 patients the oral doses of ascorbic acid were replaced by intravenous injections of an equivalent quantity for periods of ten days each during different phases of the disease. Meanwhile, the diets contained liberal quantities of other accessory foodstuffs, or were supplemented by potent yeast and cod liver oil preparations. None of

these measures exerted a demonstrable beneficial influence upon the clinical picture of rheumatic fever. During the course of these treatments there were several instances in which each of the following manifestations of the disease appeared in characteristic fashion: arthritis, carditis, erythema marginatum, subcutaneous fibroid nodules, and prolonged low grade fever. In several patients all other treatment was withheld except a high intake of ascorbic acid combined with adequate caloric diets, and the rheumatic condition progressed, so that eventually the usual antirheumatic therapy had to be instituted in order to relieve the unpleasant symptoms.

#### DISCUSSION

The importance of the disease rheumatic fever warrants a thorough investigation of any valid suggestion concerning possible etiological factors. That made by Rinehart (1933) and his coworkers seemed of sufficient import to excite considerable interest, especially in view of the newer knowledge concerning the structure and action of ascorbic acid. We have, therefore, attempted to apply all possible techniques to the investigation of the relationship between ascorbic acid deficiency and rheumatic fever. Our data concerning the synergic effect of ascorbic acid deficiency and streptococcal infection in guinea pigs, in general agree with Rinehart's, but we differ in our interpretation concerning the resemblance of the lesions so induced with those of human rheumatic fever, and feel that it is too remote to be more than suggestive (Schultz (1936)).

In view of the lack of any suitable subject among the usual laboratory animals for testing this hypothesis further, it seemed to us that the final evidence would have to be obtained from a study of human subjects in whom the disease existed or was likely to occur. We have, therefore, attempted to determine whether the mode of utilization of ascorbic acid was different in rheumatic patients from non-rheumatics, and also whether this substance had any prophylactic or therapeutic influence in this disease. As already noted, the answer to the first of these questions was in the negative insofar as utilization can be determined in terms of intake and output of definite quantities of ascorbic acid (Sendroy and Schultz (1936)). The methods available gave us no evidence con-

cerning the intermediate utilization of this substance, and we can simply state that apparent disturbances in rheumatic subjects follow the same pattern that exists among non-rheumatics. While ascorbic acid subnutrition seemed to occur more frequently among the rheumatics than among the controls, this was by no means a characteristic finding, and when such a state was detected it was attributable to the patient's economic condition rather than to any particular diseased state. When the subjects, both rheumatic and non-rheumatic, received adequate preliminary doses they showed a high degree of saturation with respect to this substance. Although others (Schroeder (1935); Gabbe (1934)) have suggested that in certain diseased states anomalies in ascorbic acid metabolism may occur, definite proof of this has not been presented. In this connection the difficulty of measuring the excretion of the substance in the urine of patients with jaundice should be mentioned; and also the fact that gastro-intestinal disturbances apparently interfere with its absorption from the stomach or bowel. Febrile states or other conditions accompanied by gastro-intestinal upsets may, therefore, apparently affect the excretion of this substance, but when such conditions exist the intravenous administration of ascorbic acid is followed by a normal pattern of excretion.

It was thought that contributory evidence concerning the relationship between subclinical scurvy and rheumatic fever might be obtained by measuring the capillary permeability of the subjects under investigation. Divers states may be accompanied by a decrease in this permeability (Dall-dorf (1933); Cutter and Marquardt (1930); Stephan (1921)); and scurvy has long been recognized as one of them. Particularly through the work of Göthlin and his collaborators the significance of increased capillary fragility as an early manifestation of mild scurvy has been emphasized. The results of applying this test to children in this country have led to different conclusions concerning the prevalence of subclinical scurvy among them (Dalldorf (1933); Stocking (1933)). Various authors agree that coincident with rheumatic carditis—among other febrile states—capillary permeability is increased (Dall-dorf (1933); Cutter and Marquardt (1930); Stephan (1921)). Frontali (1927) and Simon

(1929–30), on the other hand, found an unaltered permeability in patients with simple polyarthritis uncomplicated with carditis or cardiac decompensation. On the basis of this test our findings indicate the existence of subclinical scurvy in some, but not all, of the rheumatic children studied. It is probable that this increase in permeability was an expression of dietary deficiency rather than of a rheumatic state, because no characteristic change in capillary permeability accompanied the onset or disappearance of signs of rheumatic activity in either group.

The taking of adequate quantities of ascorbic acid over periods of several months did not prevent the recurrence of rheumatic activity among subjects who would otherwise have been expected to develop the disease, even though the results of capillary permeability tests indicated that individuals so treated were removed from a state of subclinical scurvy. This appears to us to be most significant evidence that ascorbic acid deficiency is not an important factor in the etiology of this disease. The failure of large doses adequately taken into the circulation, either enterally or parenterally, to alleviate the symptoms of rheumatic fever, or to prevent the appearance of new symptoms, is further proof in this respect.

It has been recently reported (Euler and Malmberg (1934); Euler, Söder and Malmberg (1935)) that fruit juices contain an "anti-infective vitamin" "J." In addition to pure ascorbic acid, a number of our patients were given large amounts of orange juice and other fruit juices without any appreciable benefit.

Evidence based on the study of the dietary habits of patients in respect to ascorbic acid is, naturally, difficult to evaluate, for a group of persons who have a low intake of this substance probably are in economic conditions where other vitamins and also the total caloric value of their food is subnormal. Faulkner (1935), in a preliminary report of a study of 27 cases of rheumatic fever treated with ascorbic acid, has found large doses of this substance to be without effect on the course of the disease. Warner, Winterton and Clark (1935) have recently investigated in detail the dietary habits of a large group of rheumatic children compared with non-rheumatic controls, and found no significant difference in the two groups in respect to ascorbic acid. We are in agreement

with their conclusions that deficiency of ascorbic acid intake is not a characteristic phenomenon in rheumatic fever, and therefore feel that subclinical scurvy is not a necessary factor in the causation of this disease.

#### CONCLUSIONS

1. Two comparable groups of rheumatic children, one of them receiving daily doses of ascorbic acid, were observed at intervals during late winter and early spring. As indicated by tests of capillary permeability, the development of subclinical scurvy was prevented in the treated group, but the incidence of active rheumatic fever was not favorably affected by this medication.

2. The clinical manifestations of acute rheumatic fever were not demonstrably affected by the oral or intravenous administration of ascorbic acid over periods of several months. Large doses of orange juice were also ineffective.

3. These data are additional evidence that ascorbic acid deficiency is not a necessary factor in the etiology of rheumatic fever.

#### BIBLIOGRAPHY

- Cutter, I. S., and Johnson, C. A., Studies on capillary fragility: A device for the study of capillary hemorrhage. *J. A. M. A.*, 1935, **105**, 505.
- Cutter, I. S., and Marquardt, G. H., Studies in capillary fragility. *Proc. Soc. Exper. Biol. and Med.*, 1930, **28**, 113.
- Dalldorf, Gilbert, A sensitive test for subclinical scurvy in man. *Am. J. Dis. Child.*, 1933, **46**, 794.
- Euler, H. v., and Malmberg, M., Ein Anti-Pneumonie-Faktor in C-Vitamin-haltigen Früchten. *Naturwiss.*, 1934, **22**, 205.
- Euler, H. v., Söder, H., and Malmberg, M., Die Wirkung des Nahrungsfaktors J auf die Entwicklung der Pneumonie von Meerschweinchen. *Ztschr. f. Hyg. u. Infektionskr.*, 1935, **116**, 672.
- Falk, G., Gedda, K. O., and Göthlin, G. F., An investigation into the strength of the skin capillaries and indirectly into the vitamin C standard of school-children in the District of Norrbotten, North of the Arctic circle. *Upsala läkaref. förh.*, 1932, **38**, 1.
- Faulkner, J. M., The effect of administration of vitamin C on the reticulocytes in certain infectious diseases. *New Eng. J. Med.*, 1935, **213**, 19.
- Frontali, Gino, I capillari nel bambino. Studi sull'aspetto microscopico, sulla resistenza, sulla permeabilità e sulla pressione capillare nel vivente. *Arch. di pat. e clin. med.*, 1927, **6**, 1.
- Gabbe, E., Bestimmung von Vitamin C im Blutserum. *Klin. Wchnschr.*, 1934, **13**, 1389.
- Göthlin, G., Human daily requirements of dietary ascorbic acid. *Nature*, 1934, **134**, 569.
- Rinehart, J. F., and Mettier, S. R., The joints in experimental scurvy and in scurvy with superimposed infection with a consideration of the possible relation of scurvy to rheumatic fever. *Am. J. Path. (Proc.)*, 1933, **9**, 952.
- Schroeder, H., Die Ausscheidung der Ascorbinsäure im Gesunden und Kranken Organismus. *Klin. Wchnschr.*, 1935, **14**, 484.
- Schultz, M. P., Cardiovascular and arthritic lesions in guinea pigs with chronic scurvy and streptococcal infections. *Arch. Path.*, 1936, **21**, 472.
- Sendroy, J., Jr., and Schultz, M. P., Studies of ascorbic acid and rheumatic fever. I. Quantitative index of ascorbic acid utilization in human beings and its application to the study of rheumatic fever. *J. Clin. Invest.*, 1936, **15**, 369.
- Simon, Irma, Über die Resistenz der Hautcapillaren bei den rheumatischen Herzerkrankungen. *Ztschr. f. Kinderh.*, 1929-30, **48**, 437.
- Stephan, R., Ueber das Endothel-Symptom. *Berl. Klin. Wchnschr.*, 1921, **58**, 317.
- Stocking, R. E., Application of the capillary resistance test as a measure of vitamin C nutrition. *Arch. Pediat.*, 1933, **50**, 823.
- Warner, E. C., Winterton, F. G., and Clark, M. L., A dietetic study of cases of juvenile rheumatic disease. *Quart. J. Med.*, 1935, **4**, 227.
- Wiemer, P., Das Endothelsymptom. *Ztschr. f. d. ges. exper. Med.*, 1931, **78**, 229.