to 1/1,000 M.L.D. of virus (dilutions of 10^{-7} and 10^{-8}) failed to develop complement-fixing antibodies, although some neutralizing antibodies were detectable. One intraperitoneal inoculation with 100,000 intranasal M.L.D. of virus caused the production of only small amounts of complement-fixing antibodies.

In contrast to results following one inoculation, a second or third inoculation by the intranasal or intraperitoneal routes stimulated the production of complement-fixing antibodies up to titers of 1:64 to 1:128. This marked secondary response occurred in actively immune mice where there was little multiplication of virus or formation of complement-fixing antigen.

In human beings, except the very young, the formation of influenzal complement-fixing antibodies following infection is probably a secondary immune response conditioned by previous exposure to the virus. The results with mice suggest that after the first infection with small amounts of the virus of epidemic influenza, complement-fixing antibodies may not appear in the serum in significant titer.

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Whole Blood and Plasma Ascorbic Acid Concentrations in Patients with Pellagra and Associated Deficiency Diseases.*

VIRGINIA MINNICH, SYDNEY T. WRIGHT, CARL V. MOORE, AND TOM D. SPIES.

From the Department of Internal Medicine, Washington University School of Medicine, and Department of Internal Medicine, University of Cincinnati.

The great majority of patients who attend the Nutrition Clinic of the Hillman Hospital have existed for most of their lives on grossly inadequate diets. Practically all have, or have had, clinical pellagra, beriberi, ariboflavinosis, or other deficiency diseases. Many have clinically recognizable manifestations of several deficiency states. An occasional patient is found to have spongy, bleeding gums which suggest an associated hypovitaminosis C, but advanced cases of scurvy are infrequently seen despite the fact that foods rich in ascorbic acid are available to these people only during the

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late spring and summer.[1] The small quantity of fruits they can afford to preserve is exhausted late in the fall so that for most of the year they have an extremely limited intake of the anti-scorbutic vitamin. In order to determine the degree of their vitamin C depletion, whole blood and plasma ascorbic acid concentrations were measured on 70 of these persons. These observations form the basis of the present report.

Recent experimentation has shown that whole blood or blood cell concentrations of ascorbic acid provide, in all probability, as reliable an index of the adequacy of vitamin C reserves as is available.[2–5] Determination of the amount of ascorbic acid in plasma alone, on the other hand, has been shown to be of very limited value. This fraction is quite labile and changes rapidly with the addition or withdrawal of vitamin C from the diet.[5–8] The ascorbic acid content of the cellular elements of the blood, however, is much more stable.[5, 8] It has been observed that as a state of vitamin C unsaturation develops, ascorbic acid is first depleted from the plasma, then from the red cells, and lastly from the platelet and white cell elements. In the investigation reported by Crandon and Lund[8] in which a normal male subject voluntarily placed himself on a vitamin C free diet, the plasma was free of ascorbic acid within 11 days, but 4 months were required to deplete the white cell-platelet fraction. Conversely, it has been determined that when ascorbic acid is given to a person with scurvy, vitamin C (postabsorptive) first appears in the leucocytes, next in the red cells, and lastly in the plasma.[5] Consequently, a high fasting level of ascorbic acid in plasma provides a good indication that vitamin C stores are adequate while decreased concentrations in the cellular elements of blood serve as an index of vitamin C deficiency or unsaturation. Low levels of plasma ascorbic acid do not necessarily imply a significant depletion of the vitamin.

The methods of Butler and Cushman[5] and of Mindlin and Butler[9]

1 Spies, T. D., Swain, A. P., and Grant, J. M., *Am. J. Med. Sc.*, 1940, **200**, 536.

2 Eekelen, M. von, Emmerie, A., and Wolff, L. K., *Z. Physiol. Vitaminforsch.*, 1937, **6**, 150.

3 Nevweiler, W., *Z. Physiol. Vitaminforsch.*, 1938, **7**, 128.

4 Heinemann, M., *J. Clin. Invest.*, 1937, **17**, 751.

5 Butler, A. M., and Cushman, M., *J. Clin. Invest.*, 1940, **19**, 459.

6 Greenberg, L. D., Rinehart, J. F., and Phatak, N. M., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 135.

7 Mindlin, R. L., *J. Pediat.*, 1940, **16**, 275.

8 Crandon, J. H., and Lund, C. C., *New England J. Med.*, 1940, **222**, 748.

9 Mindlin, R. L., and Butler, A. M., *J. Biol. Chem.*, 1938, **122**, 673.

were used for the determination of whole blood and of plasma ascorbic acid concentrations in 70 patients from the Clinic group and in 20 "normal" adult laboratory workers. No attempt was made to measure separately the apparent ascorbic acid content of leucocytes. For the whole blood determinations, filtrates were used from blood which had been saturated with CO before precipitation with metaphosphoric acid.[5] Determinations, except for a few on the control subjects, were made between June 15 and July 1.

Whole Blood Ascorbic Acid. The data is summarized in Table I. Patients were classified according to their primary clinical manifestations. Whole blood ascorbic acid concentrations in the control subjects varied from 0.95 mg % to 1.46 mg %. This range of normal values agrees well with those recorded in the literature.[2-5] The average for the clinic group, however, was much lower (0.63 mg % as compared with 1.20 mg %). Eight (11%) of the patients had values less than 0.3 mg % and 51 (73%) had values less than 0.8 mg %. This distribution analysis is expressed in Fig. 1.

Plasma Ascorbic Acid. Plasma ascorbic acid concentrations were, as one would expect, invariably lower than those for whole

TABLE I.
Ascorbic Acid Concentration of Whole Blood and of Plasma in Patients with Deficiency Disease.

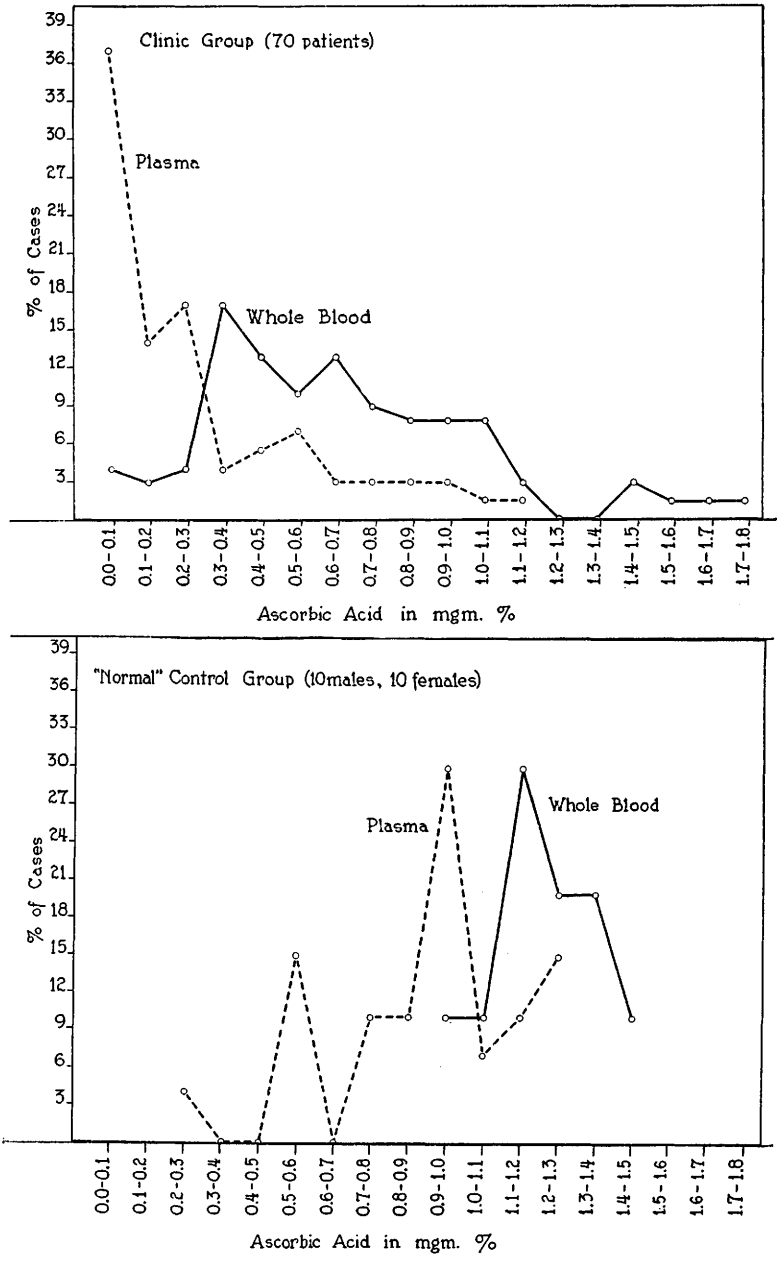
		Whole blood ascorbic acid			Plasma ascorbic acid		
Diagnosis*	No. of cases	Avg mg%	Min. mg%	Max. mg%	Avg mg%	Min. mg%	Max. mg%
Thiamin deficiency	4	0.61	0.46	0.76	0.21	0.0	0.35
Riboflavin deficiency	13	0.74	0.33	1.49	0.33	0.0	0.78
Nicotinic acid deficiency	14	0.47	0.17	0.96	0.15	0.0	0.59
Subclinical nutritional deficiency states†	23	0.79	0.28	1.77	0.32	0.0	1.12
Purpura (nutritional?)	5	0.60	0.07	1.00	0.42	0.0	0.94
Anemias							
Macrocytic	7	0.22	0.0	0.74	0.09	0.0	0.42
Hypochromic, microcytic	4	0.72	0.26	1.52	0.40	0.0	1.02
Summary of all patients studied	70	0.63	0.0	1.77	0.27	0.0	1.12
Normal adult subjects							
Men	10	1.14	0.95	1.46	0.74	0.22	1.10
Women	10	1.27	1.12	1.46	1.03	0.78	1.25

*Many of these patients had clinical manifestations of more than one deficiency disease. To simplify the tabulation, they were classified under the most prominent of their several deficiencies.

†Patients who had been on inadequate diets and who complained of anorexia, burning of the tongue and "in the stomach," burning of the skin over the face, neck, and arms, paraesthesias of the hands and feet, nervousness, irritability, etc. Nearly all the members of this group had had pellagra in previous years.

Fig. 1

Distribution of Plasma and of Whole Blood Concentrations of Ascorbic Acid



blood. One of the "normal" subjects, a healthy male, had only 0.2 mg % ascorbic acid in his plasma; 3 other members of the control group had between 0.5 and 0.6 mg %; the other 16 had more than 0.8 mg %. For the patient group, there were 26 (37%) with less than 0.1 mg % plasma ascorbic acid, 48 (69%) with less than 0.3 mg %, 55 (79%) with less than 0.5 mg % and 64 (91%) with less than 0.8 mg %. (Fig. 1).

Since the diets of these patients provided them with only very inadequate amounts of vitamin C, the low values of plasma ascorbic acid were to be expected. It is of interest that even though the average concentration in whole blood was only half that of the control series, and even though 55 of the total number of 70 patients had whole blood ascorbic acid levels below the lowest value recorded for a normal subject, that there were only 8 patients lower than 0.3 mg %.† That is, if the amount of ascorbic acid in whole blood really constitutes an index of the adequacy of vitamin C nutrition, as seems to be the case, only 15 of the patients studied had normal vitamin C reserves, but in only 8 were the levels low enough to suggest that the depletion was advanced. The 4 normal subjects with low concentrations of plasma ascorbic acid were males; there was no significant variation in their whole blood ascorbic acid values from the rest of the members of the control group. From Table I it is obvious that there was no recognizable difference in the vitamin C blood levels of the patients with B_1 deficiency, ariboflavinosis, pellagra, etc. The low average for the macrocytic anemia group was caused by the low concentrations found in several patients critically ill with pernicious anemia, each of whom had approximately one million red cells.

Five of the 12 children studied had spongy, bleeding gums suggestive of a scorbutic change. Their whole blood vitamin C concentration, however, averaged no lower than that for the group as a whole (0.58 mg % with variation from 0.44 mg % to 0.98 mg %). Plasma ascorbic acid values for these 5 children ranged from 0.06 mg % to 0.71 mg %, averaged 0.31 mg %.

It is significant that these determinations were made during the last half of June. At that time tomatoes had not yet ripened. Wild blackberries had become edible only during the last few days of June. But fresh vegetables had been available to most patients for a month and many had had strawberries for 2 weeks. It is probable,

† This figure was arbitrarily chosen because it represented a fairly sharp dividing level, in terms of frequency, between values below and those above it.

therefore, that the blood levels of ascorbic acid were even lower before May 1st.

The evidence accumulated in this investigation that patients with clinical deficiencies of the vitamin B complex frequently have an associated partial depletion of vitamin C is in agreement with the results reported by Goldsmith, Ogaard, and Gowe.[10] These workers studied the vitamin C nutrition of 18 pellagrins, and on 14 of these performed either oral or intravenous tolerance tests. Eight of their patients showed evidence of an ascorbic acid deficiency. Whole blood determinations of the vitamin were not done.

Summary and Conclusions. Whole blood and plasma ascorbic acid determinations made on 70 patients in the nutrition clinic of the Hillman Hospital indicate that 55 of these persons had a whole blood concentration of ascorbic acid lower than in normal controls. In 8 of the 70 cases, the concentration was so low as to suggest that depletion may have been advanced. Most of these patients had clinical evidence of other deficiencies, such as pellagra, beriberi and riboflavin deficiency, but no significant correlation could be made between the blood ascorbic acid values and the symptoms of these deficiency states, nor indeed of scurvy itself. These observations seem pertinent in view of the fact that they were made in late June, at a period when leafy vegetables and berries had been available to the patients for some time, and probably indicate that ascorbic acid deficiency is still greater at other periods. These studies give strong support to the concept that natural-occurring deficiency diseases exist as complexities rather than as single entities.

[10] Goldsmith, J. A., Ogaard, A. T., and Gowe, D. F., *Am. J. Med. Sc.*, 1940, **200**, 244.