present tests because sera were fully absorbed with A₁ erythrocytes, or human group O mucosa was used for immunofluorescence.

The inconstancy of upper gastrointestinal staining, particularly that of stomach, is deserving of comment. It may be related to the relative solubility of the antigens sought and the secretory state of the mucoid cells of the bowel used for immunohistological preparations. In the human stomach the gastrointestinal antigen has been shown to be inconstant and of patchy distribution in experiments with the heterologous rabbit antigastrointestinal serum (Nairn and de Boer, 1966). Human stomach mucosa was the only part of the gastrointestinal tract stained by one ulcerative colitis serum (Case 5), and it can only be assumed in this case that we are dealing with some unknown antigen specific to stomach, or at least one which is relatively abundant there. Our finding that the antibodies in ulcerative colitis sera which stained human colon and small intestine did not stain normal gastric mucosa is in accord with the observations of Harrison (1965a) and Wright and Truelove (1966).

It is clear that the presence of the intestinal autoantibodies in patients' sera does not explain the selective anatomical localization of ulcerative colitis to the colon, and there is nothing from the results of our tissue-culture studies or those of Broberger and Perlmann (1963) to suggest that the serum antibodies are directly related to the pathogenesis of the disease. While these observations do not support the view that the antibodies are themselves pathogenic, work at present in progress on the reaction in tissue culture between lymphoid cells from ulcerative colitis patients and colon mucosa indicates that cellular immunological activity, as already suggested by Perlmann and Broberger (1963), may play a major part in this disease. It is reasonable to assume that the circulating autoantibodies are a reflection of such an underlying cellular response.

Summary

Sera from 54 patients with ulcerative colitis included 10 giving immunofluorescent staining of human colon mucosa, 13 staining rat colon, and 17 with haemagglutination titres of 16 or more against rat faeces antigen. In contrast with the immuno-

fluorescence reactions against human colon and rat colon, haemagglutinating antibodies to rat colon antigens were no greater in incidence than in 194 control sera. The positive sera reacted with other parts of the gastrointestinal tract, and no evidence of colon specificity was obtained. Moreover, they were not specifically cytotoxic to human colon mucosa in tissue culture. It is suggested that the autoantibodies which react with human intestinal mucosa are not themselves the cause of mucosal damage, but may reflect underlying cellular activity by autoimmune lymphoid cells.

The incidence of antibodies against parietal cells, nuclei, thyroid, and adrenal was low.

We wish to thank Mr. E. S. R. Hughes for sera and surgical specimens; Dr. Peter Perlmann for the rat faeces antigen; Dr. S. Boonpucknavig for amoebiasis sera; Miss L. Canobio, Miss M. Carter, Mrs. M. Cerini, Miss A. Hühn, and Mr. A. Maxwell for technical assistance. The work was supported by grants from the National Health and Medical Research Council, the Anti-Cancer Council of Victoria, and the Australian Research Grants Committee.

REFERENCES

- Asherson, G. L., and Broberger, O. (1961). *Brit. med. J.*, **1**, 1429.
 Broberger, O., and Perlmann, P. (1959). *J. exp. Med.*, **110**, 657.
 ——— (1962). *Ibid.*, **115**, 13.
 ——— (1963). *Ibid.*, **117**, 705.
 Hammarström, S., Lagercrantz, R., Perlmann, P., and Gustafsson, B. H. (1965). *Ibid.*, **122**, 1075.
 Harrison, W. J. (1965a). *Lancet*, **1**, 1346.
 ——— (1965b). *Ibid.*, **1**, 1350.
 Klavins, J. V. (1962). *J. Amer. med. Ass.*, **180**, 759.
 Koffler, D., Minkowitz, S., Rothman, W., and Garlock, J. (1962). *Amer. J. Path.*, **41**, 733.
 Kunin, C. M. (1962). *Arch. intern. Med.*, **110**, 676.
 Lagercrantz, R., Hammarström, S., Perlmann, P., and Gustafsson, B. H. (1966). *Clin. exp. Immunol.*, **1**, 263.
 McGiven, A. R., Datta, S. P., and Nairn, R. C. (1967). *Nature (Lond.)*. In press.
 Nairn, R. C. (1964). *Fluorescent Protein Tracing*, 2nd ed. Edinburgh and London.
 ——— and de Boer, W. G. R. M. (1966). *Nature (Lond.)*, **210**, 960.
 ——— Fothergill, J. E., McEntegart, M. G., and Porteous, I. B. (1962). *Brit. med. J.*, **1**, 1788.
 Perlmann, P., and Broberger, O. (1963). *J. exp. Med.*, **117**, 717.
 ——— Hammarström, S., Lagercrantz, R., and Gustafsson, B. H. (1965). *Ann. N.Y. Acad. Sci.*, **124**, 377.
 Wright, R., and Truelove, S. C. (1966). *Gut*, **7**, 32.
 Zweibaum, A., Oudea, P., Halpern, B., and Veyre, C. (1966). *Nature (Lond.)*, **209**, 159.

Leucocyte Ascorbic Acid Levels in Patients with Malabsorption or Previous Gastric Surgery

J. M. WILLIAMSON,§ M.B., CH.B., M.R.C.P.ED.; M.R.C.P.GLASG.

A. GOLDBERG,† M.D., D.SC., F.R.C.P.ED., F.R.C.P.GLASG., M.R.C.P.; F. M. L. MOORE,‡ F.I.M.L.T.

Brit. med. J., 1967, **2**, 23–25

In states of malabsorption the clinical syndromes associated with deficiency of the water-soluble vitamins cyanocobalamin and folic acid are well recognized, but clinical scurvy is rare (Badenoch, 1960).

Patients with malabsorption and small-bowel resections have a delayed excretion of ingested ascorbic acid (Stewart and Booth, 1964). These authors have also observed that patients with idiopathic steatorrhoea could be "saturated" with ascorbic acid only by giving them a prolonged course of intravenous injections.

Several patients recently admitted to the Western Infirmary, Glasgow, with idiopathic steatorrhoea or postgastrectomy steatorrhoea were found to have purpura and biochemical evidence of vitamin-C deficiency. It was therefore decided to measure the "leucocyte ascorbic acid" level in patients with malabsorption or with a previous history of gastric surgery, since this level is known to be a more reliable index of ascorbic acid deficiency than the ascorbic acid saturation test or the plasma ascorbic acid level (Crandon *et al.*, 1940; Bartley *et al.*, 1953).

Patients

There were 13 patients with intestinal malabsorption and 13 who had had gastric surgery. The clinical data are summarized in the Table.

* Senior Registrar.

† Reader in Medicine.

‡ Senior Laboratory Technician.

§ University Department of Medicine, and Department of Biochemistry, Western Infirmary, Glasgow W.1.

§ Present address: Southern General Hospital, Glasgow S.W.1.

Summary of Clinical Data

	Diagnosis	No.	Age (years) Mean $\pm$ S.D.	Sex	Leucocyte Ascorbic Acid ($\mu\text{g.}/10^8/\text{W.B.C.}$)	Diet (mg./Vit. C/day) Mean $\pm$ S.D.
Group A (13) Patients with intestinal malabsorption	Idiopathic steatorrhoea	2	50 $\pm$ 12.6	6M 7F	10.7 $\pm$ 5.2	49.1 $\pm$ 30.1
	Unclassified steatorrhoea	5				
	Pancreatic steatorrhoea	1				
	Reticulum-cell sarcoma	1				
	Multiple diverticulæ	1				
	Miscellaneous	3				
Group B (13) Patients after gastric surgery	Gastroenterostomy + vagotomy ..	6	59 $\pm$ 7.5	6M 7F	12.7 $\pm$ 6.8	34.3 $\pm$ 14.9
	Polya partial gastrectomy	4				
	Billroth I gastrectomy	1				
	Total gastrectomy	1				
	Pyloroplasty alone	1				
Groups A and B		26	54.7 $\pm$ 11.36	12M 14F	11.7 $\pm$ 5.9	41.1 $\pm$ 23.8
Group C (26) Control subjects	Laboratory workers	3	54.9 $\pm$ 9.9	12M 14F	20.8 $\pm$ 10.5	60.0 $\pm$ 18.2
	Medical inpatients	2				
	Orthopaedic outpatients (traumatic) ..	16				
	Gen. medical clinic	5				

Idiopathic steatorrhoea was diagnosed when the following criteria were fulfilled: (1) steatorrhoea, (2) a small-bowel biopsy which demonstrated villous atrophy, (3) a characteristic barium-meal follow-through, and (4) abnormal D-xylose excretion. In one case there was a low serum folate level. This estimation was not performed in the second case, since the patient was receiving folic acid.

Of the five patients grouped under "unclassified steatorrhoea," all had steatorrhoea, and a barium-meal follow-through examination showed characteristics of steatorrhoea. Three had abnormal D-xylose tests and three a low serum folate level. The result of the Crosby capsule biopsy was normal in two cases; it was not performed in three cases.

Pancreatic steatorrhoea was diagnosed on the basis of excess faecal fat excretion, pancreatic calcification, and a diabetic glucose-tolerance curve. Reticulum-cell sarcoma was diagnosed on the histological appearance of the Crosby capsule biopsy.

The patient with small-bowel diverticulosis had a characteristic radiological picture, a macrocytic anaemia, low serum vitamin-B₁₂ level, an abnormal Schilling test not corrected by intrinsic factor, but normal faecal fat excretion and D-xylose test.

The "miscellaneous" group comprised three patients who had been regarded as suffering from adult coeliac disease, and all had responded clinically to a gluten-free diet. Small-bowel biopsies were not available. Two had biochemical evidence of steatorrhoea, one an abnormal follow-through appearance, and one a low serum folate level. One patient showed biochemical evidence of osteomalacia.

Patients with Previous Gastric Surgery.—The diagnoses of these 13 patients are shown in the Table. Of this group the radiological appearances of the small bowel were abnormal in three, seven had steatorrhoea, and in six a D-xylose test was abnormal.

Control Group.—Twenty-six subjects without alimentary disease were used as controls, and were matched for age and sex with the malabsorption and surgical groups. Three were laboratory workers, two were inpatients with cardiac and respiratory disorders, five attended a general medical clinic with cardiac or respiratory disease, and 16 were orthopaedic outpatients.

Methods

Faecal Fat Estimation.—The amount of fat in two three-day collections of faeces of the patients on a ward diet was estimated by the method of Harrison (1947), the normal value being taken as less than 5 g. of fat excreted per day.

D-Xylose Excretion.—The amount of D-xylose in a five-hour collection of urine after an oral dose of 25 g. in 500 ml. of water was estimated by the method of Roe and Rice (1948). The

excretion of less than 4 g. was taken as evidence of malabsorption (Varley, 1962). In two cases it was not possible to carry out the five-hour test, and a two-hour serum xylose of less than 25 mg./100 ml. was accepted as abnormal (Varley, 1962).

Serum folate was estimated by the method of Spray (1964).

Leucocyte Ascorbic Acid.—The method of Denson and Bowers (1961) was used.

Diet Histories.—Estimations of the ascorbic acid content of the diet were based on the tables of McCance and Widdowson (1960).

Results

The mean leucocyte ascorbic acid level in the patients with malabsorption or previous gastric surgery was 11.7 $\mu\text{g.}/10^8$ W.B.C. (S.D. $\pm$ 5.9), while that in the controls was 20.8 $\mu\text{g.}/10^8$ W.B.C. (S.D. $\pm$ 10.53). This difference is statistically significant ($P < 0.01$).

When the results in patients with malabsorption or previous gastric surgery were analysed separately a statistically significant difference was observed between the mean of each group and that of the control group. In the malabsorption group the mean leucocyte ascorbic acid level was 10.7 $\mu\text{g.}/10^8$ W.B.C. (S.D. $\pm$ 5.2), and in the gastric surgery group it was 12.7 $\mu\text{g.}/10^8$ W.B.C. (S.D. $\pm$ 6.8), P being < 0.01 and < 0.02 respectively.

The mean leucocyte ascorbic acid level in all patients with steatorrhoea, whether in the malabsorption group or surgical group, was 10.0 $\mu\text{g.}/10^8$ W.B.C., which is not significantly different from the two groups as a whole.

In 24 of the 26 patients investigated the diet history was thought to be reliable, and in these cases the mean daily intake of vitamin C was 41.1 mg./day (S.D. $\pm$ 23.8), range 12–91 mg./day. None of the patients had a dietary intake of vitamin C below 10 mg./day, the minimal protective level against scurvy (Bartley *et al.*, 1953). A diet history was available in 12 of the 26 controls, the mean intake of vitamin C being 60.0 mg./day (S.D. $\pm$ 18.2).

The platelet count, measured at the same time as the leucocyte ascorbic acid level, was within the normal range in all patients.

Hess's test for capillary fragility was positive in five patients—two in the malabsorption group and three in the surgical group. In addition, all three patients in the surgical group had purpura of the arms and/or legs; the thrombotest was normal in all five patients.

Discussion

The results of the leucocyte ascorbic acid tests, the most reliable measure of the tissue content of the vitamin, show a significantly lower level in patients with malabsorption or after gastric surgery than in the control group. Badenoch (1960) has

noted that leucocyte ascorbic acid levels might be low in patients with steatorrhoea, but did not give figures or details of the dietary intake of vitamin C. The diet histories of the patients show a mean intake of ascorbic acid of the order of 40 mg. daily, which is above the level recommended by the B.M.A. (1950). It is noteworthy that the mean leucocyte ascorbic acid level in the control group (mean age 54.9 years) is $20.8 \mu\text{g./}10^8$ W.B.C. Bowers and Kubik (1965) observed that the leucocyte ascorbic acid levels in elderly patients living at home (mean age 76.2 years) were lower than in a group of patients of working age. Andrews and Brook (1966) have confirmed that low leucocyte ascorbic acid levels are encountered in the elderly, but they suggest that this is possibly due to poor dietary intake of ascorbic acid, and point out that the low leucocyte ascorbic acid levels of old people can, by giving oral ascorbic acid, be raised quickly to levels comparable to those found in younger people. The available diet histories in the present control group indicate a satisfactory intake of ascorbic acid.

The most likely cause of the low levels of leucocyte ascorbic acid is malabsorption of this water-soluble vitamin, but there may be an increased demand for ascorbic acid. Phenolic acids are present in excess in the urine of patients with steatorrhoea, and Boscott and Cooke (1954) suggested that there is an abnormal demand for ascorbic acid for their detoxification. In addition, one specific action of ascorbic acid is its ability to protect an enzyme, *p*-hydroxyphenyl pyruvic acid oxidase, from its substrate, a phenolic acid (Goodman and Gilman, 1965). These mechanisms may partly account for the low leucocyte ascorbic acid levels in the patients of the present series.

Only five of the 26 patients investigated showed, in fact, clinical evidence of vitamin-C deficiency. The significance of subclinical ascorbic acid deficiency is not yet certain, though there is a significant correlation between low levels of leucocyte ascorbic acid and sublingual petechiae (Andrews and Brook, 1966), and also between such levels and a high incidence of wound dehiscence in surgical patients (Crandon *et al.*, 1961).

It is known that vitamin-C deficiency occurs more commonly in the elderly (Bowers and Kubik, 1965) and in those subsisting on poor diets, whether by reason of food faddism or apathy or poverty. It would seem from the present series that patients who have intestinal malabsorption or who have had gastric surgery should be added to those groups in whom ascorbic-acid deficiency may be more readily suspected. In states of malabsorption or after gastric surgery the leucocyte ascorbic acid should therefore be routinely measured, and, where low, oral vitamin-C supplements should be given. If such a test is not

possible it would seem reasonable to give routine oral vitamin C to all patients, especially elderly ones.

Summary

Twenty-six patients who either had intestinal malabsorption or had undergone gastric surgery had significantly lower "leucocyte ascorbic acid" levels than 26 control subjects. The mean leucocyte ascorbic acid level in the controls was $20.8 \mu\text{g./}10^8$ W.B.C. and in the patients $11.7 \mu\text{g./}10^8$ W.B.C. ($P < 0.01$).

Five of the 26 patients had clinical evidence of increased capillary fragility and three had purpura.

The estimated dietary intake of vitamin C in all these patients was within the accepted normal limits.

It is suggested that patients with intestinal malabsorption and those who have been subjected to gastric surgery, especially if they are elderly, should have their diet supplemented with vitamin C. If leucocyte ascorbic acid levels can be measured, and are found to be low, the administration of ascorbic acid would seem to be obligatory.

We are indebted to Dr. E. B. Hendry, Biochemistry Department, Western Infirmary, Glasgow, in whose department the "leucocyte ascorbic acid" levels were estimated; to Dr. H. E. Hutchinson, Haematology Department, Western Infirmary, Glasgow, for platelet counts and serum folate estimations; and to Miss C. Leahy, chief dietitian, Western Infirmary, Glasgow, for the diet histories.

REFERENCES

- Andrews, J., and Brook, M. (1966). *Lancet*, 1, 1350.
 Badenoch, J. (1960). *Brit. med. J.*, 2, 879.
 Bartley, W., Krebs, H. A., and O'Brien, J. R. P. (1953). *Spec. Rep. Ser. med. Res. Coun. (Lond.)*, No. 280.
 Boscott, R. J., and Cooke, W. T. (1954). *Quart. J. Med.*, 23, 307.
 Bowers, E. F., and Kubik, M. M. (1965). *Brit. J. clin. Pract.*, 19, 141.
 British Medical Association (1950). *Report of the Committee on Nutrition*. London.
 Crandon, J. H., Lennihan, R., jun., Mikal, S., and Reif, A. E. (1961). *Ann. N.Y. Acad. Sci.*, 92, 246.
 ———, Lund, C. C., and Dill, D. B. (1940). *New Engl. J. Med.*, 223, 353.
 Denson, K. W., and Bowers, E. F. (1961). *Clin. Sci.*, 21, 157.
 Goodman, L. S., and Gilman, A. (1965). *The Pharmacological Basis of Therapeutics*, 3rd ed. London.
 Harrison, G. A. (1947). *Chemical Methods in Clinical Medicine*, 3rd ed., p. 490. London.
 McCance, R. A., and Widdowson, E. M. (1960). *Spec. Rep. Ser. med. Res. Coun. (Lond.)*, No. 297.
 Roe, J. H., and Rice, E. W. (1948). *J. biol. Chem.*, 173, 507.
 Spray, G. H. (1964). *J. clin. Path.*, 17, 660.
 Stewart, J. S., and Booth, C. C. (1964). *Clin. Sci.*, 27, 15.
 Varley, H. (1962). *Practical Clinical Biochemistry*, 3rd ed., p. 356. London.

Plasma Cortisol Levels in Heart Failure

C. K. CONNOLLY,* M.A., M.B., M.R.C.P.; M. R. WILLS,* M.D., M.C.PATH.

Brit. med. J., 1967, 2, 25-27

Cushing's syndrome is generally agreed to be the result of an excessive endogenous secretion of cortisol (Longson and Christy, 1964). Therefore the definitive test in the diagnosis of Cushing's syndrome is the determination of the cortisol secretion rate. The methods available for the determination of that rate are too complex for routine use. The diagnosis of Cushing's syndrome is therefore made, indirectly, by measurement of the concentration of cortisol and its metabolites in blood and urine (Ross, Marshall-Jones, and Friedman, 1966). In normal subjects there is a circadian rhythm of the concentration of cortisol in blood with the minimum value about midnight and maximum value

between 06.00 and 09.00 hours (Mills, 1966). In Cushing's syndrome there is abolition of the cortisol circadian rhythm due to elevation of the midnight level (Doe, Vennes, and Flink, 1960; Ekman, Håkansson, McCarthy, Lehmann, and Sjögren, 1961). Ross *et al.* (1966) reported that this occurred in eight out of nine patients with Cushing's syndrome and stated that "absence of a diurnal rhythm is very helpful in diagnosis."

Congestive cardiac failure may occur in patients who have many of the clinical features of Cushing's syndrome. We have recently found raised plasma cortisol levels at midnight in patients with congestive cardiac failure. This investigation was started after we had found a high midnight plasma cortisol level in a patient with congestive cardiac failure who had some

* From the Departments of Medicine and Chemical Pathology, Royal Free Hospital, London W.C.1.