

Serum cholesterol in vitamin C deficiency in man

BY B. BRONTE-STEWART*, B. ROBERTS AND V. M. WELLS

*Department of Medicine, University of Cape Town and
Groote Schuur Hospital*

(Received 12 February 1962—Revised 17 August 1962)

Although Cape Town was founded in 1652 to combat the ravages of scurvy in ships at sea, 300 years later scurvy was still rife there. Some 10–15 years ago there was a continuous migration into this urban area of rural Bantu males, whose aim was to make sufficient money as unskilled labourers, in as short a time as possible, so that they might return to their homelands with the requisite marriage fee. This purpose was achieved by restricting variety in food intake, and scurvy resulted. Indeed this situation prompted a study of the anaemia in scurvy, in one report on which the dietary circumstances were fully explained (Bronte-Stewart, 1953). Now scurvy is rapidly becoming a rare disease. Between 1948 and 1953 there were twelve to fifteen admissions annually to this hospital, but over the last 4 years there have been only three admissions.

This rapid decrease in incidence has prompted the publication at this stage of the following findings on serum cholesterol before, during and after recovery from scurvy.

EXPERIMENTAL

Clinical material. Of fourteen adult Bantu males with scurvy, nine (subjects nos. 6–14) were studied in the Metabolism Unit of Groote Schuur Hospital. The clinical features were follicular hyperkeratosis, gingival hyperplasia, ecchymoses, intramuscular haematomas, mild pyrexia and varying degrees of anaemia. For example, the haemoglobin levels on admission of subjects 7 and 9 were 6.0 g/100 ml; of subjects 8, 12 and 13 between 8 and 10 g; of subjects 10 and 14 between 10 and 12 g; and of subjects 6 and 11 above 12 g. As before (Bronte-Stewart, 1953), other forms of avitaminosis were not found, but two further subjects had to be excluded as one was found to have pulmonary tuberculosis, and another had tapeworm infestation. The ages of the subjects ranged from 30 to 60 years.

Method of study. Initial observations were made while the patients ate a diet similar to that on which the disease had developed, except that the maize meal and white bread were supplemented by 50 g casein daily, together with adequate minerals and vitamins, and no unprescribed ascorbic acid was given. The dietary calories were varied between 2300 and 2500 kcal daily to ensure that no marked weight gain or loss occurred throughout the period of study. The daily fat intake was in the region of 3 g and the daily cholesterol intake was 20 mg. The initial period of observation, before treatment with ascorbic acid, was kept to a minimum as scurvy is an unpleasant

* Present Address: M.R.C. Atheroma Research Unit, Western Infirmary, Glasgow, Scotland.

disease. Ascorbic acid (Roche Products (Pty) Ltd), in divided doses amounting to 200 mg daily, was administered either intramuscularly or orally.

For subject 6, 100 g of olive oil were added to the basal diet, and after 3 days ascorbic acid was given. This patient was studied concurrently with a healthy volunteer and the findings have already been reported (Bronte-Stewart, Antonis, Eales & Brock, 1956). Half of the eight remaining subjects received ascorbic acid orally from the outset and the other half received it intramuscularly (see Table 1). For three subjects (7, 8 and 11) the basal low-fat diet was continued throughout the experimental period. For the others, a daily fat supplement was added to the basal diet and, after a further 6 days for subjects 9, 10, 12 and 13, and 10 days for subject 14, the ascorbic acid was administered. The fat supplement for subject 9 was 100 g butter daily and for the others was egg yolk (equivalent to 55 g of fat daily). Before the experiments were terminated the fat supplements were withdrawn.

Table 1. *The plan of the experiment with the eight patients with scurvy studied intensively*

Subject no.	Route of ascorbic acid administration	Dietary fat supplement
7	Oral	None
8	Oral	None
12	Oral	Egg yolk
14	Oral	Egg yolk
11	Intramuscular	None
9	Intramuscular	Butter
10	Intramuscular*	Egg yolk
13	Intramuscular*	Egg yolk

* After 6 days the ascorbic acid was given orally.

To five other subjects without scurvy the same amount of ascorbic acid was administered while they were on the basal low-fat diet and while they were receiving a mixed high-fat diet of meat, butter, milk and vegetables. Subject 14 after full recovery was placed on the basal diet, and the ascorbic acid supplement was withdrawn. The following 60 days were subdivided for this subject into six 10-day periods, to compare the effects of isoascorbic and ascorbic acids. During the first three periods 60 mg isoascorbic acid were administered orally daily. Neither remedy was given during the fourth period. In the fifth period, ascorbic acid (60 mg orally daily) was given, and it was replaced again by isoascorbic acid in the sixth period. During the first period the patient consumed the basal diet but, from the outset of the second to the end of the sixth period, the egg-yolk supplement was added.

The serum cholesterol level was determined in venous blood drawn every other day. The method was that of Abell, Levy, Brodie & Kendall (1952) as modified by Anderson & Keys (1956). The faecal fat excretion was determined daily by the method of van de Kamer, ten Bokkel Huinink & Weyers (1949). The fat content of the diet was measured gravimetrically after diethyl ether extraction, and the sterol content by the method of Abell *et al.* (1952).

RESULTS

After the intramuscular or oral administration of ascorbic acid to the subjects with scurvy, there was in each instance a prompt clinical response as judged by a fall in temperature, rise in haemoglobin level and subsequent resolution of haematomas, ecchymoses and gingival hyperplasia.

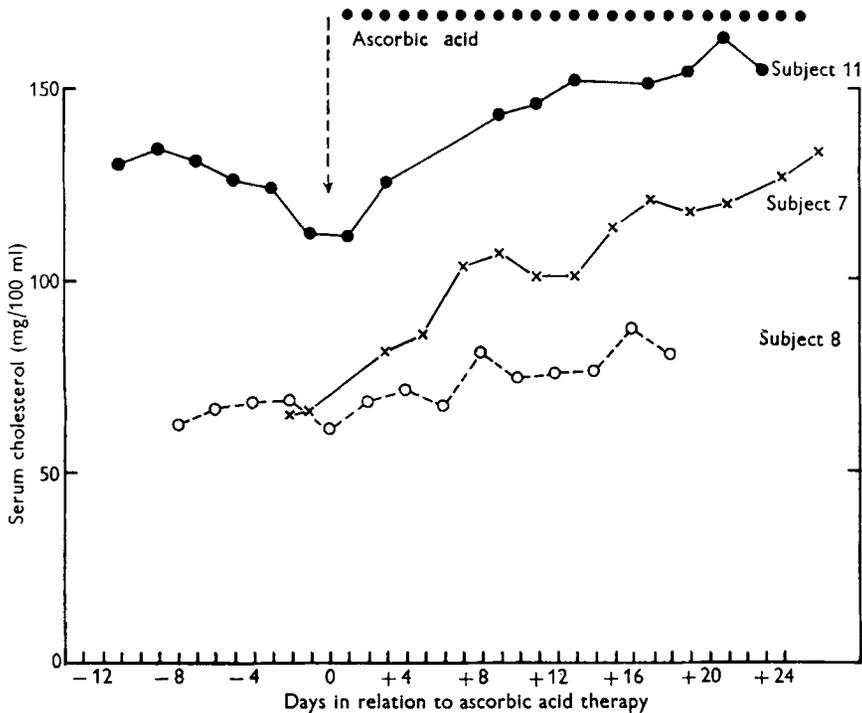


Fig. 1. Serum cholesterol changes in patients with scurvy on a low-fat diet, before and after ascorbic acid administration. To subjects 7 and 11 the ascorbic acid was given orally and to subject 8 it was given intramuscularly.

Serum cholesterol levels before treatment with ascorbic acid. The initial serum cholesterol levels of thirteen out of fourteen patients admitted with scurvy ranged from 58 mg to 116 mg/100 ml. The remaining patient had a level of 189 mg/100 ml. The mean level for these fourteen subjects was 99.0 and the standard deviation 30.6 mg/100 ml, which was significantly lower ($P < 0.001$) than the mean level (140.4 ± 36.5 mg/100 ml) of a group of sixty-six apparently healthy men within the same age range, of the same race and of the same low socio-economic class. There was no correlation between the haemoglobin levels and the serum cholesterol levels in these fourteen patients.

Effect of ascorbic acid without dietary supplements of fat. There was a gradual but definite rise in serum cholesterol levels after treatment with ascorbic acid (see Fig. 1), but with the two subjects who received ascorbic acid orally (nos. 7 and 11) the rise was more rapid than with subject 8 who received it intramuscularly. Amongst the

remaining eleven patients there were ten whose mean serum cholesterol level at the outset could be compared with that at the termination of the studies, the nature of the diet on each occasion being similar. Before treatment the mean total serum cholesterol level was 93 mg/100 ml and after at least 3 weeks of ascorbic acid administration this level was 119 mg/100 ml.

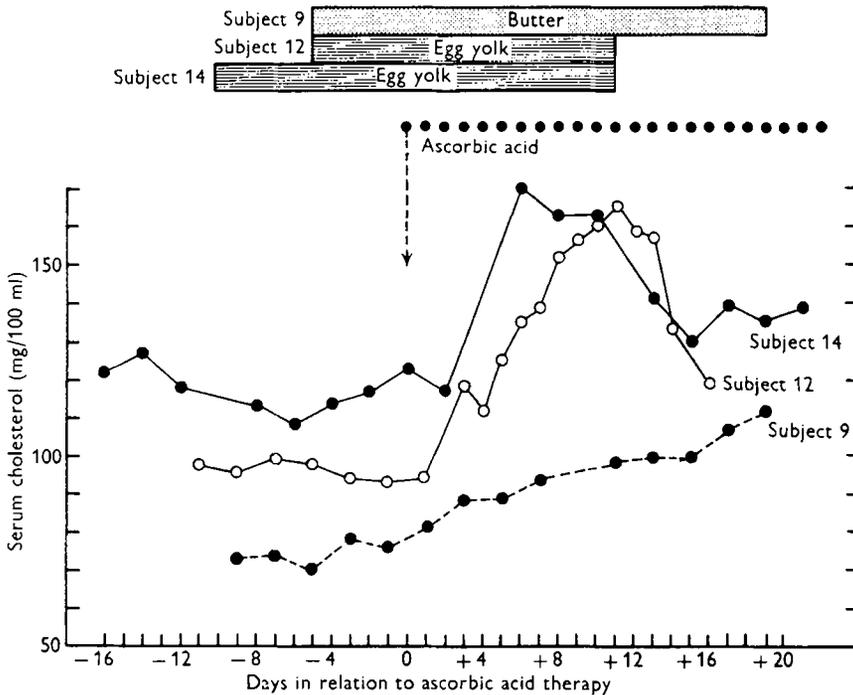


Fig. 2. Serum cholesterol changes in patients with scurvy on a high-fat diet, before and after ascorbic acid administration. To subjects 12 and 14 the ascorbic acid was given orally, and to subject 9 it was given intramuscularly.

Effect of ascorbic acid with dietary supplements of fat. No rise in serum cholesterol level occurred when either egg yolk or butter was added to the basal diet before treatment with ascorbic acid. The response to the treatment appeared to depend on the route of administration of ascorbic acid. In subjects 12 and 14 who received ascorbic acid by mouth an immediate rise in serum cholesterol level of about 60 mg/100 ml occurred. The level fell on the withdrawal of egg yolk from the diet. In subject 9 who received ascorbic acid intramuscularly throughout the experimental period the rate of rise of serum cholesterol level was very gradual (see Fig. 2). In subjects 10 and 13 only a minor rise in level occurred during the period when ascorbic acid was given intramuscularly. When the route of administration was changed and ascorbic acid given orally, however, an immediate and marked rise in serum cholesterol level occurred, in subject 13 while the egg-yolk supplement was continued and in subject 10 when the egg-yolk supplement was re-introduced after its withdrawal (see Figs. 3 and 4).

Amongst the total series of fourteen patients there were four who received ascorbic acid intramuscularly and four who received it orally at the commencement of treat-

ment. Two of each were given the egg-yolk supplement before treatment, and for the remaining two in each group the basal diet, low in fat, was continued throughout. The mean increase in serum cholesterol level during the 1st week after the commencement of treatment in the intramuscularly treated group was 12.3 mg/100 ml and, in the orally treated group, it was 32.4 mg/100 ml; the difference was highly significant ($P < 0.001$).

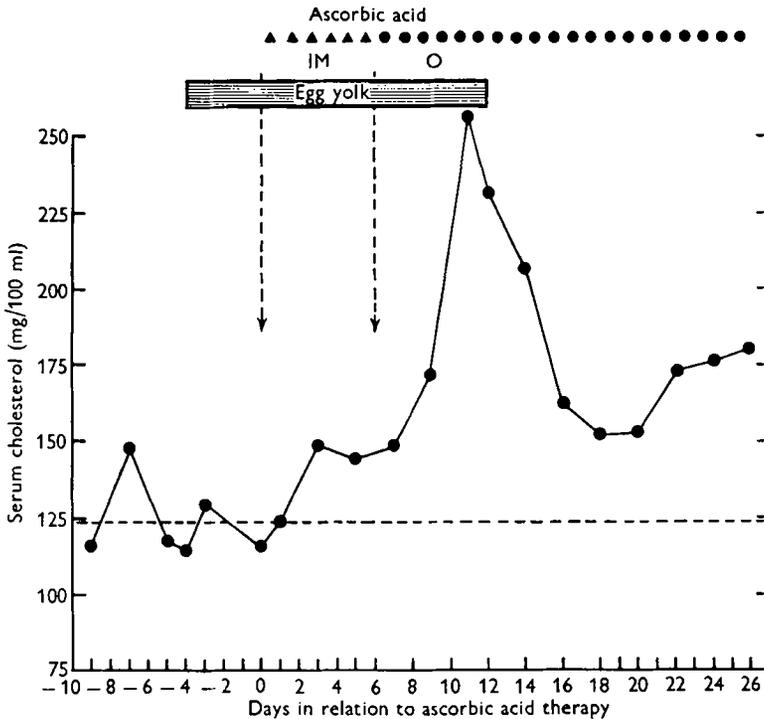


Fig. 3. Effect of the route of administration of ascorbic acid on the serum cholesterol level of a patient with scurvy (no. 13) while egg yolk was added to the diet. IM, intramuscularly; O, orally.

Faecal fat excretion before and after treatment with ascorbic acid. With four (nos. 9, 10, 11 and 14) of the five subjects whose daily faecal fat excretion was estimated, the studies were sufficiently prolonged to make it possible to compare the faecal fat excretion on the low-fat basal diet and during the period of fat supplementation, in each instance before and after treatment. In all these subjects there was a significant fall in excretion after treatment. On the basal diet the mean daily faecal fat excretion before treatment was 1.4 g and it fell to 0.6 g in the second basal period when full clinical remission had occurred. The values when the fat supplements were given were 2.7 g daily before, and 1.5 g daily after, treatment. Soon after the administration of ascorbic acid a transitory increase in faecal fat excretion occurred in some subjects. This trend showed no correlation with the changes in serum cholesterol level and in no instance did the faecal fat excretion exceed 5% of the amount of fat in the diet during the periods when the fat supplements were given.

Effect of ascorbic acid and isoascorbic acid in the absence of scurvy. No significant effects on the serum cholesterol levels or faecal fat excretion were seen on administering ascorbic acid to individuals on high-fat or low-fat diets who had no clinical features of scurvy. Likewise isoascorbic acid (60 mg daily) with an ascorbic acid-free diet, with and without egg-yolk supplements, failed to have any effect on the serum cholesterol levels.

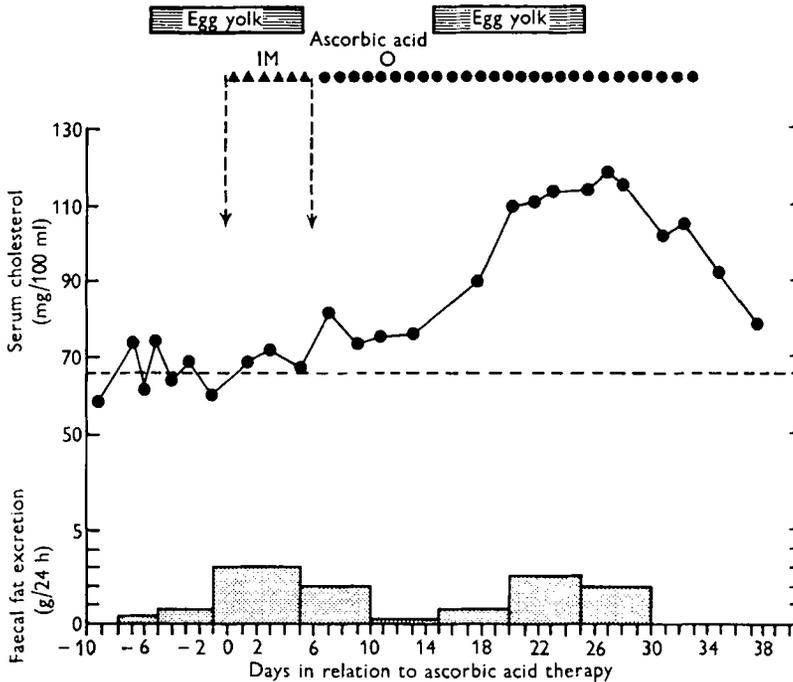


Fig. 4. Comparison of the serum cholesterol changes of patient 10 when egg yolk was added to the basal diet before and after treatment with ascorbic acid given intramuscularly (IM) and after treatment with ascorbic acid given orally (O).

DISCUSSION

It is unlikely that the low levels of serum cholesterol found in our patients on admission resulted from the very low fat content of the type of diet that would predispose to the development of scurvy. Before treatment with ascorbic acid, the giving of fats known to raise serum cholesterol levels in healthy individuals failed to influence these levels in scurvy. Treatment with ascorbic acid on a diet very low in fat was associated with a slow rise in serum cholesterol level.

Perhaps the most remarkable observation was that, in the depleted state, the expected rise in serum cholesterol level did not occur when a dietary fat such as egg yolk or butter was added to the diet. From our experience on non-scorbutic individuals, we expect a rise in serum cholesterol level within 48–72 h after the giving of such fats. This rise continues rapidly to reach a peak on about the 7th or 8th day, and thereafter flattens to a plateau (Bronte-Stewart *et al.* 1956). Subject 14 exemplified this in particular. A year previously we had observations on him when he had no scurvy. On each occasion he consistently responded to egg-yolk supplements with a rise in

serum cholesterol level of approximately 60 mg/100 ml over a 10-day period. When he had scurvy no rise occurred until ascorbic acid had been given.

The route of administration of ascorbic acid appeared to have a striking influence on the rate of response. In a series as small as this, however, the more rapid response after oral administration may be merely fortuitous. On the other hand, the explanation could be that those subjects treated orally from the outset had less severe scurvy but, if clinical assessment can be used as a measure, it was not so. The dose used was 200 mg daily, and studies that others have done on vitamin C saturation, using three and four times this dose daily, showed that it takes about 5 days for the tissues to be replete (Wright & Lilienfeld, 1936; Bartley, Krebs & O'Brien, 1953).

It is for these reasons, together with the fact that there was a slight but significant fall in faecal fat excretion after ascorbic acid repletion, that our attention has been focused on the gut as the site of action of vitamin C in this aspect of cholesterol metabolism.

Our studies are inadequate to assess the mode of action, but the importance of ascorbic acid in oxidation-reduction reactions (Harris, 1953) might suggest a part in digestive enzyme efficiency. There is some evidence available to support this view. For instance, in the previous study Bronte-Stewart (1953) observed a high frequency of achlorhydria in patients with scurvy. In some patients, acid had returned after 3 weeks of treatment with ascorbic acid. In the guinea-pig, a decrease in activity of several enzyme systems occurred as scurvy developed, amongst them being intestinal phosphatases (Harrer & King, 1941).

On the assumption that the site of action was within the gut, the attempt was made to find a possible antagonist that could be administered orally to patients and thereby lower the serum cholesterol levels. As isoascorbic acid is almost ineffective against scurvy, it was considered that it would presumably not be built into the enzyme systems which play a part in the prevention of scurvy (W. Leigh, personal communication, 1961). Other than this, there is no evidence that isoascorbic acid is antagonistic to vitamin C. In the study now described it was administered with meals to a repleted individual on a vitamin C-free diet supplemented by egg yolk. No significant effects were observed. Apparently the difficulty in finding an antivitamin is that the participation of vitamin C in many enzyme reactions is not specific (Harris, 1953).

We are unaware of any other findings that relate vitamin C and cholesterol metabolism in man. In animals an interrelationship between ascorbic acid and cholesterol does appear to exist, certainly in the adrenal gland. Becker, Burch, Salomon, Venkita-subramanian & King (1953) record accelerated conversion of ^{14}C -labelled acetate into cholesterol as scurvy develops in the guinea-pig. In his review Meiklejohn (1953) emphasizes that depletion of cholesterol in the adrenal glands is not seen until the late stages of scurvy.

It is unlikely that this relationship will be noted in man unless there is an advanced stage of vitamin C depletion. In all subjects studied here the scurvy was fairly severe. We are in agreement with Anderson, Grande & Keys (1958), who were unable to show any effect of ascorbic acid administration on the serum cholesterol levels in healthy men.

SUMMARY

1. Before treatment the mean serum cholesterol levels of fourteen adult males with scurvy were significantly lower than the mean for a group of apparently healthy individuals on the same socio-economic plane.
2. Treatment with ascorbic acid alone led to a rise in serum cholesterol level in patients receiving a diet to which no fat supplements had been added.
3. Until ascorbic acid was given, the serum cholesterol level failed to respond to fats known to elevate it in healthy subjects.
4. In those patients given ascorbic acid orally, the rise in serum cholesterol level was more rapid than in those given ascorbic acid intramuscularly.
5. Ascorbic acid and isoascorbic acid given to healthy individuals failed to influence the level of the serum cholesterol.

This work was undertaken as part of the programme of the Clinical Nutrition Research Unit which is under the direction of Professor J. F. Brock, and is supported in the University of Cape Town by the Council for Scientific and Industrial Research of South Africa. It was also supported in part by research grant H-3316 from the National Heart Institute, Public Health Service, USA.

Thanks are due to Dr W. Leigh of Roche Products (Pty) Ltd, Johannesburg, for supplies of and data on isoascorbic acid, and to Mr G. Schoonraad, S.R.N., the charge nurse of the Metabolism Unit.

REFERENCES

- Abell, L. L., Levy, B. B., Brodie, B. B. & Kendall, F. E. (1952). *J. biol. Chem.* **195**, 357.
 Anderson, J. T., Grande, F. & Keys, A. (1958). *Fed. Proc.* **17**, 468.
 Anderson, J. T. & Keys, A. (1956). *Clin. Chem.* **2**, 145.
 Bartley, W., Krebs, H. A. & O'Brien, J. R. P. (1953). *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 280.
 Becker, R. R., Burch, H. B., Salomon, L. L., Venkitasubramanian, T. A. & King, C. G. (1953). *J. Amer. chem. Soc.* **75**, 2020.
 Bronte-Stewart, B. (1953). *Quart. J. Med.* **22**, 309.
 Bronte-Stewart, B., Antonis, A., Eales, L. & Brock, J. F. (1956). *Lancet*, **270**, 521.
 Harrer, C. J. & King, C. G. (1941). *J. biol. Chem.* **138**, 111.
 Harris, L. J. (1953). *Proc. Nutr. Soc.* **12**, 128.
 Meiklejohn, A. P. (1953). *Vitam. & Horm.* **11**, 61.
 van de Kamer, J. H., ten Bokkel Huinink, H. & Weyers, H. A. (1949). *J. biol. Chem.* **177**, 347.
 Wright, I. S. & Lilienfeld, A. (1936). *Arch. intern. Med.* **57**, 241.