Plasma proteins in vitamin C deficiency

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It has been recorded that in scorbutic guinea-pigs the albumin:globulin ratio is decreased (Böger & Schroeder, 1934; Ciatti & Auerbach, 1936; Garta, 1937) and that the fibrinogen concentration is increased (Marx & Bayerle, 1943). The present study was made in order to obtain additional information on the concentration of plasma proteins in scurvy, by means of electrophoresis on paper. Previous workers, mentioned above, have compared acutely scorbutic with normal guinea-pigs. The work described here includes, in addition, studies on pair-fed controls, and on animals with chronic scurvy (long-standing hypovitaminosis). A claim that the electrophoretic mobility on paper of the serum proteins is decreased in scurvy (Chalopin, 1955) has also been investigated.

EXPERIMENTAL

Animals and diet. Male guinea-pigs of about 250 g body-weight were obtained from Allington Farm, Ministry of Supply, Porton, Wilts, and fed initially on the semi-synthetic diet no. 13 described by Reid & Briggs (1953). Vitamins A, D, E and K were not mixed in the diet but were provided by two drops of halibut-liver oil, two drops of α-tocopheryl acetate (5% (w/v) in arachis oil) and two drops of 2-methyl-1:4-naphthoquinone (0·25% (w/v) in arachis oil) given orally to each animal once weekly. The diet contained 2 g ascorbic acid/kg, giving an intake of ascorbic acid of about 50–70 mg/day.

Acute scurvy. On reaching 300 g body-weight, the guinea-pigs were divided into three groups. A 'scorbutic' group received the above diet with the ascorbic acid omitted; a 'pair-fed control' group received the same quantity of food and water as corresponding animals in the scorbutic group but had ascorbic acid in the diet as before; a 'normal control' group was allowed *ad lib.* the diet containing ascorbic acid. In the scorbutic group, guinea-pigs grew normally for the first 10–12 days and then lost weight. Since death was known to occur at about 190 g body-weight (22–28 days), animals were killed at 230 g (2 days before expected death).

Chronic scurvy. Another series of guinea-pigs was placed on the diet without ascorbic acid for 10 days, divided into three groups and then dosed daily for about 100 days with either 0·4, 1·6 or 6·4 mg ascorbic acid (in 0·5 ml. 1% (w/v) citric acid). Guinea-pigs receiving 1·6 or 6·4 mg ascorbic acid per day grew normally; those receiving 0·4 mg/day did not grow but just maintained their body-weight.

Collection of plasma. Blood was collected carefully from the jugular vein after the animal had been killed by a blow on the head, a few crystals of sodium oxalate being

used as anticoagulant. The blood was centrifuged at room temperature for 30 min and the separated plasma removed. Electrophoresis of the plasma on paper was carried out on the same day.

Total protein. It was determined by the Kjeldahl method as described by King (1951).

Electrophoretic procedure. Electrophoresis on paper was carried out in an apparatus described by Durrum (1951) and supplied by Shandon and Co. Ltd, London. The chamber, which could take six strips 5 cm wide, contained buffer of pH 8·6 and I 0·06 (10·3 g sodium diethyl barbiturate, 1·84 g diethyl barbituric acid in 1 l. solution).

Strips of Whatman no. 1 paper (5 cm wide and 34 cm long) were saturated with 1% (w/v) sodium citrate as described by Hirsch & Cattaneo (1956) before being immersed in the buffer solution described above. Plasma (*c.* 0·015 ml./strip) was applied from a micropipette. The electrophoresis was then conducted with a potential gradient of 150 V and a current of *c.* 1 mA per strip for 16 h. The strips were dried at 100° for 15 min and stained with Naphthalene Black 10 B (obtained from Gurr Ltd, London) by the method of Grassmann & Hannig (1952). They were made translucent with liquid paraffin for evaluation with a photoelectric scanner as described by Grassmann & Hannig (1952) and supplied by Evans Electroselenium Ltd, Harlow, Essex. The Gaussian curves obtained were then traced on transparent paper, cut out and weighed in order to determine the relative areas of each component. It was assumed that the area under each curve was directly proportional to the concentration of each component. From the percentage composition and a knowledge of the protein concentration, it was possible to calculate the concentration of each component.

RESULTS

In guinea-pig plasma, five distinct components could be distinguished corresponding to albumin, α-, β- and γ-globulin and fibrinogen fractions. α-Globulin occasionally showed a small separation into α_1- and α_2-components but the boundaries were usually indistinct so that only the total α-globulin has been calculated.

Table 1. *Percentage of individual plasma proteins in acutely scorbutic guinea-pigs*

Group	No. of animals	Body-weight (g)	Albumin	Globulin α-	Globulin β-	Globulin γ-	Fibrinogen	Albumin: globulin† ratio
(1) Scorbutic	7	223	33·0***	38·2**	7·4	4·1	18·0**	0·49***
(2) Pair-fed controls	7	309	46·7	30·7*	10·6	4·2	7·7	0·89
(3) Normal controls	6	441	44·8	33·2	7·7	3·1	11·1	0·81

Significance of rise or fall in value for groups (1) and (2) compared with group (3): * $0{\cdot}05>P>0{\cdot}01$, ** $0{\cdot}01>P>0{\cdot}001$, *** $P<0{\cdot}001$.

† Globulin includes fibrinogen.

Acute scurvy. The percentage composition of plasma from scorbutic guinea-pigs compared with that from pair-fed and normal controls is shown in Table 1. For normal animals, the values obtained for albumin and α- and β-globulin after subtraction of the fibrinogen were substantially in agreement with the data obtained by Moore (1945) using boundary electrophoresis of serum. For γ-globulin, the mean value shown

Table 2. *Mean values with their standard errors (g/100 g) for the concentration of plasma proteins in acutely scorbutic guinea-pigs compared with pair-fed and normal controls*

Group	No. of animals	Body-weight (g)	Albumin	Globulin			Fibrinogen	Plasma-protein concentration
				α-	β-	γ-		
(1) Scorbutic	7	223	1·31 ± 0·06***	1·52 ± 0·08*	0·30 ± 0·04	0·17 ± 0·02	0·71 ± 0·06**	3·97 ± 0·13
(2) Pair-fed controls	7	309	1·96 ± 0·06*	1·29 ± 0·04	0·45 ± 0·08	0·18 ± 0·04	0·32 ± 0·05*	4·21 ± 0·09
(3) Normal controls	6	441	1·83 ± 0·01	1·35 ± 0·01	0·31 ± 0·03	0·13 ± 0·02	0·46 ± 0·07	4·06 ± 0·07

Significance of rise or fall in value for groups (1) and (2) compared with group (3): * $0{\cdot}05 > P > 0{\cdot}01$, ** $0{\cdot}01 > P > 0{\cdot}001$, *** $P < 0{\cdot}001$.

Table 5. *Mean values with their standard errors (g/100 g) for the concentration of plasma proteins in chronically scorbutic guinea-pigs and controls*

Ascorbic acid in diet (mg/day)	No. of animals	Body-weight (g)	Albumin	Globulin			Fibrinogen	Plasma-protein concentration
				α-	β-	γ-		
(1) 6·4 (surplus)	5	642	1·91 ± 0·03	1·30 ± 0·31	0·34 ± 0·04	0·14 ± 0·02	0·46 ± 0·05	4·21 ± 0·07
(2) 1·6 (just adequate)	5	640	1·98 ± 0·10	1·23 ± 0·30	0·34 ± 0·10	0·15 ± 0·03	0·52 ± 0·05	4·20 ± 0·19
(3) 0·4 (chronic deficiency)	5	320	1·68 ± 0·08*	1·27 ± 0·06	0·33 ± 0·05	0·20 ± 0·01*	0·80 ± 0·08**	4·23 ± 0·11

Significance of rise or fall in value for groups (2) and (3) compared with group (1): * $0{\cdot}05 > P > 0{\cdot}01$, ** $0{\cdot}01 > P > 0{\cdot}01$, *** $P < 0{\cdot}001$.

here is only half that given by Moore (1945) but the standard error of his value was large. From Table 1, it can be seen that in the scorbutic animals as compared with controls the percentage of albumin decreased, whereas the percentage of α-globulin and fibrinogen increased; the albumin:globulin ratio also decreased. These changes were not shown in the pair-fed controls, which differed from normal only in having a lower percentage of α-globulin. Since the plasma-protein concentrations of the three groups were the same, the actual concentrations of each of the components showed similar trends (Table 2). Thus, in the scorbutic group, the albumin concentration was decreased by 25%, the fibrinogen increased by 50%, and the α-globulin increased by 10%. The only difference between the pair-fed controls and the normal group was a slight increase in albumin concentration in the former. The mobilities of the plasma-protein components are shown in Table 3. Owing to electro-osmosis, fibrinogen and γ-globulin had negative mobilities. No differences were observed between the three groups. In addition, a few samples of plasma from scorbutic animals were placed on

Table 3. *Mean values with their standard errors for the electrophoretic mobilities on paper of plasma proteins in acutely scorbutic guinea-pigs compared with pair-fed and normal controls*

(The values quoted are the distances (mm) travelled in 16 h at a gradient of 150 V and 1 mA in strips 34 cm long and 5 cm wide)

Group	No. of animals	Body-weight (g)	Albumin	Globulin α-	Globulin β-	Globulin γ-	Fibrinogen
Scorbutic	7	223	61·4±3·7	29·9±3·2	12·1±2·0	−15·0±1·0	−2·6±0·3
Pair-fed controls	7	309	62·9±4·1	30·9±2·4	11·3±1·1	−16·6±1·5	−2·8±0·5
Normal controls	6	441	61·6±2·0	29·0±1·2	10·8±1·4	−14·8±1·7	−3·0±0·5

Table 4. *Mean percentage of individual proteins in the plasma of chronically scorbutic guinea-pigs and controls*

Ascorbic acid in diet (mg/day)	No. of animals	Body-weight (g)	Albumin	Globulin α-	Globulin β-	Globulin γ-	Fibrinogen	Albumin:globulin† ratio
(1) 6·4 (surplus)	5	642	45·4	31·3	8·3	3·4	11·4	0·83
(2) 1·6 (just adequate)	5	640	47·0	29·4	7·7	3·5	12·3	0·89
(3) 0·4 (chronic deficiency)	5	320	39·5***	29·8	7·6	4·8*	19·0*	0·66***

Significance of rise or fall in value for groups (2) and (3) compared with group (1): * $0{\cdot}05 > P > 0{\cdot}01$, ** $0{\cdot}01 > P > 0{\cdot}001$, *** $P < 0{\cdot}001$.

† Globulin includes fibrinogen.

the same sheet of paper as samples from normal and pair-fed controls. The mobilities were always identical. These results do not confirm the work of Chalopin (1955) who claimed that the serum-protein components from scorbutic guinea-pigs had a slower mobility than normal.

Chronic scurvy. The percentage of each plasma protein in chronic scurvy is shown in Table 4. In the scorbutic group, increases occurred in the percentages of fibrinogen and γ-globulin and decreases in the albumin and in the albumin:globulin ratio. Plasma from animals receiving 1·6 mg ascorbic acid per day resembled that from those receiving 6·4 mg/day. As shown in Table 5, in the scorbutic group, the albumin

concentration was decreased by 15%, a somewhat smaller reduction than was found in acute scurvy. There was no increase in α-globulin concentration but the increase in fibrinogen (70%) was greater than in acute scurvy. Another difference was an increase of 40% in the γ-globulin concentration.

DISCUSSION

The decrease in albumin and increase in α-globulin which I have found in acute scurvy is non-specific, since similar changes occur in a large number of unrelated diseases such as nephritis, diabetes mellitus, rheumatic fever, acute and chronic infections (Sunderman & Sunderman, 1957) and many others (Gutman, 1948). Although many factors may influence the concentration of these proteins, the above-mentioned changes appear to be associated with considerable inflammation or tissue destruction irrespective of the cause (Shedlovsky & Scudder, 1942; Gutman, 1948). My results accord with this view, since, as is well known, scurvy in guinea-pigs is characterized by a rapid weight loss and tissue haemorrhages.

A further change was a rise in fibrinogen, which has been previously reported in acute scurvy (Marx & Bayerle, 1943). It may be connected with a raised metabolism of certain coagulation factors caused by the haemorrhagic condition. Thus, the platelet count is also increased (Hess, 1920; Barkhan & Howard, 1958).

Pair-fed controls did not show similar abnormalities. In contrast, these animals had a slightly increased albumin and a decreased fibrinogen concentration but were otherwise normal. A lowered food intake was therefore not responsible for the abnormal distribution of plasma proteins in acute scurvy. My results are in agreement with those of other authors (Henschel, Mickelsen, Taylor & Keys, 1947; Keys, Taylor, Mickelsen & Henschel, 1946) who found that a large group of volunteers maintained on a semi-starvation diet for several months showed no pronounced hypoalbuminaemia.

In chronic scurvy, the composition of the plasma proteins was similar to but not identical with that in acute scurvy. Thus, there was a decrease in albumin, somewhat smaller than was found in acute scurvy, but the α-globulin was not significantly higher than normal. Fibrinogen was, however, increased to a greater extent than in acute scurvy. Another difference was a small rise in γ-globulin which may have been caused by increases in antibodies associated with a chronic infection. Only scorbutic animals showed changes in their plasma proteins. Thus guinea-pigs receiving 1·6 mg ascorbic acid/day, which is just adequate, showed no differences from those receiving 6·4 mg/day.

Previous workers have found in scurvy a decreased albumin:globulin ratio (Böger & Schroeder, 1934; Garta, 1937) but no change in total protein concentration (Garta, 1937). My results are in agreement with these findings.

Chalopin (1955) has claimed that the electrophoretic mobility of scorbutic guinea-pig serum is lower than normal, and suggested that the structure of the serum proteins is altered in scurvy. Using plasma, I have been unable to confirm this observation and have found the mobilities to be normal in scurvy. Since Chalopin examined only two specimens of pooled serum, it is possible that some factor other than scurvy may have influenced his results.

SUMMARY

1. Nineteen guinea-pigs of body-weight 300 g were divided into three groups: group 1 (acute deficiency) was placed on a scorbutogenic diet; group 2 was pair-fed with the first group, but had ascorbic acid in the diet (2 g/kg); group 3 received *ad lib.* the diet containing ascorbic acid. After 22–26 days, the animals were killed and the plasma was examined by means of paper electrophoresis.

2. In a second experiment, fifteen guinea-pigs of body-weight 300 g were placed on the scorbutogenic diet for 10 days, divided into three groups and dosed with either 0·4 mg ascorbic acid/day (chronic deficiency) or 1·6 mg/day or 6·4 mg/day. After 100 days, the animals were killed and the plasma was examined as before.

3. In acute scurvy, a decrease in albumin (25%), an increase in fibrinogen (50%) and an increase in α-globulin (10%) were found, although the total protein concentration was unchanged. These changes were not observed in pair-fed animals receiving ascorbic acid.

4. The electrophoretic mobility on paper of plasma proteins from scorbutic guinea-pigs did not differ from normal.

5. In chronic scurvy the albumin was decreased (15%), the fibrinogen increased (70%), the γ-globulin increased (40%) but there was no change in the α-globulin. No differences were observed between animals receiving 1·6 mg ascorbic acid/day and those receiving 6·4 mg ascorbic acid/day.

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