rhagica and certain hereditary haemorrhagic states coagulation may be less rapid than normal when venom alone is used, but is not usually different from normal when venom and lecithin are added; the need for lecithin varies at different periods and may run parallel to the tendency to bleed. In prothrombin deficiency coagulation remains impaired despite the addition of venom plus lecithin. If venom is used instead of brain extract to measure prothrombin in the Quick test it must be fortified by the addition of lecithin; in other words, *venom (thrombo-kinase) + lecithin = tissue extract (thromboplastin)*.

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POLYCYTHAEMIA AND VITAMIN C

BY

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Lurgan

For several years past it has been considered that vitamin C plays some part in the regulation of the number of red blood cells in the body. Dunlop and Scarborough (1935) showed that a rise in the red blood count, a decrease in the number of reticulocytes, and a rise in the haemoglobin percentage resulted when vitamin C was given to a patient suffering from scurvy, although the iron content of the diet (10 mg. daily) was not increased. On the other hand, Barron and Barron (1936) observed that vitamin C reduced the red blood count in animals suffering from artificially induced polycythaemia. Waltner and Waltner (1929) noted that this disease could be caused in animals by administering cobalt, which Mascherpa (1930) was able to demonstrate caused an increase in bone marrow activity in dogs. When they simultaneously injected cobalt sulphate and vitamin C intravenously (60 mg. daily) into rabbits Barron and Barron failed to observe any signs of polycythaemia, although this appeared a week after withdrawing the vitamin C.

When vitamin C was injected after the production of polycythaemia a decrease in the red cell count and the haemoglobin percentage resulted, although the effect was temporary. It was found that the percentage of haemoglobin ran parallel with the red blood count. This experimental polycythaemia is probably caused by the cobalt inhibiting the respiratory function of immature red blood cells, which are rapidly replaced in the bone marrow. Vitamin C functions as a regulator of the level of red cells in the circulating blood.

Barron and Barron (1936) produced polycythaemia in rabbits by the daily injection of 0.01 gramme of cobalt sulphate. Reticulocytes and erythroblasts appeared in the circulating blood and there was an increase in respiration of the red blood cells, although the latter was not observed in cases of human polycythaemia vera. The addition of cobalt sulphate to red cell suspensions from the blood of rabbits suffering from cobalt polycythaemia was followed by marked inhibition of respiration, in contrast with the absence of such an effect in similar suspensions prepared from the blood of normal rabbits. This inhibition of respiration was confined to immature red cells, being greater in the bone marrow cells than in the red cells of the kidney and spleen.

According to Barron one of the functions of vitamin C is to maintain the haemoglobin concentration in the blood

at a normal level. This view is based on the following facts: (1) The fall in the percentage of haemoglobin in the early stages of scurvy occurs before clinical symptoms appear, and the haemoglobin returns to normal after the administration of vitamin C, before these symptoms disappear. (2) Cobalt polycythaemia in rabbits is prevented by giving vitamin C. It is also known that vitamin C reduces ferrihaemin to ferrohaemin, which when combined with globin yields haemoglobin. There is some evidence that the utilization of iron is defective when the reserves of vitamin C in the body are depleted (McCollum *et al.*, 1939). Neither large doses of iron nor the substance potent in pernicious anaemia are effective in the scorbutic state.

Kandel and LeRoy (1938) tried the effect of vitamin C on two patients suffering from polycythaemia vera, but reported that it had no influence whatsoever on the red blood count and the haemoglobin percentage.

Encouraged by Barron and Barron's results a study has been made of the vitamin C metabolism in two cases of polycythaemia. I decided to investigate the excretion of the vitamin, the amount necessary to saturate the patient, the result of giving large doses, and finally the effect of raising the threshold of vitamin C excretion by administering sodium bicarbonate.

Hawley *et al.* (1936) showed that the amount of vitamin C retained by the body depends upon factors other than the level of the vitamin already in the body. For example, when vitamin C is administered with ammonium chloride to persons already saturated with the vitamin almost 100% of a daily test dose is excreted. If, however, the same amount of vitamin C is given with sodium bicarbonate only 50% is excreted in the urine. Similar experiments with guinea-pigs showed that the administration of large doses of sodium bicarbonate permitted the storage of greater amounts of vitamin C in the adrenals and liver than when the vitamin was given alone or with ammonium chloride.

Dr. J. G. Keohane of Limavady, who had a case of polycythaemia under his care, carried out a similar investigation at my request.

Case Records before starting the Investigation

Case 1.—A fisherman aged 53. This patient was a strong well-built man whose only illness had been pneumonia ten years ago. About two years ago he began to feel a heaviness in the head, and suffered from headaches, lack of concentration, and vertigo. He had pains in his legs and was easily tired. His face became a deep purple colour, particularly so in the lips and cheeks. His eyeballs were bloodshot and the conjunctivae congested. He was admitted to the Royal Victoria Hospital on April 12, 1939, under Prof. W. W. D. Thompson. He then complained of pains in the head and chest and heaviness in the head. His complexion had become more purple. On examination his lips and the conjunctival surfaces of the eyelids were a deep crimson. The conjunctivae were injected and the skin of his body was redder than normal. His apex beat was in the sixth intercostal space, 4 inches from the midline. The blood pressure was 148/95. There were some crepitations at the base of the right lung. The temperature was normal.

The patient had an unhealthy mouth and his abdomen was distended. Palpation revealed a sausage-shaped tumour 4 by 1½ inches in the right iliac fossa. Neither liver nor spleen was palpable. The results of a blood examination were as follows: red cells, 6,290,000 per c.mm.; Hb (Haldane), 106%; colour index, 0.9; white cells, 10,000 per c.mm.; Wassermann negative. Nothing abnormal was found in the cerebrospinal fluid or urine. Cystoscopy and a barium enema also revealed nothing abnormal.

A diagnosis of polycythaemia was made. The patient was advised laparotomy, but refused. On April 19 venesection was performed and 500 c.cm. of blood withdrawn. Another

450 c.cm. of blood was withdrawn on May 10. The patient showed some improvement during his stay in hospital. The headaches and dizziness had gone, and the blood pressure and red cell count were down slightly (138/94 and 5,680,000). The percentage of haemoglobin remained unaltered (106%), and the colour index was 0.93. There was no change in the abdominal tumour.

Case 2.—Dr. Keohane's case. A woman aged 60, who had been ill for seven to eight months, was seen by Prof. W. W. D. Thompson on November 25, 1939. A blood examination made at that time gave: red cells, 6,420,000 per c.mm.; Hb (Haldane), 115%; colour index, 0.9; white cells, 5,700 per c.mm. The red blood cells were of normal size and shape, and the van den Bergh test was negative. The spleen was enlarged and the patient showed signs of arteriosclerosis. There was no evidence of leukaemia. No treatment for the polycythaemia was given before the investigation by Dr. Keohane.

Method of Investigation

The daily excretion of vitamin C in the urine was determined. Then 300 mg. of vitamin C (6 redoxon tablets) was given daily until saturation was accomplished. This dose was then raised to 400 mg. a day and continued later in conjunction with a daily dose of 31 of sodium bicarbonate. In Case 1 the red blood count rose again some time after the investigation had concluded, and so it was repeated; but this time the sodium bicarbonate was administered alone without any previous tests, and after the effect had been noted vitamin C was then given. Both subjects received a good well-balanced diet with plenty of milk, eggs, fruit, vegetables, and, in one case, fish.

During the course of the investigation the following tests were carried out.

1. Red blood counts every week in both cases.
2. Vitamin C content of the blood in one case only. The following method was employed. 1 c.cm. of blood serum was mixed with 1 c.cm. of trichloroacetic acid and 1 c.cm. of distilled water to precipitate the proteins; the mixture was filtered, and 1.5 c.cm. pipetted off for titration. The vitamin C was titrated with a solution of dichlorophenolindophenol (Roche) prepared from tablets, each corresponding to 1 mg. of vitamin C. The solution was prepared by dissolving one tablet in 100 c.cm. of distilled water and then filtering it. 1 c.cm. of dichlorophenolindophenol solution is therefore $\equiv$ 0.01 mg. vitamin C. The minimum blood vitamin C is taken to be 12 mg. per litre (Wolff, Banning, and Van Eekelen, 1936).
3. Haemoglobin. By the Dare method: expressed in grammes per 100 c.cm. of blood.
4. Blood pressure.
5. Daily volume of urine.
6. Daily excretion of vitamin C in the urine. Harris's modification of Tillman's method was used. Twenty-four-hour specimens of urine were collected, each specimen being acidified with 10% of its volume of glacial acetic acid just after passing. A solution of dichlorophenolindophenol was then run into a given volume of urine until a light red coloration, persisting at least half a minute, was produced. A daily excretion of less than 13 mg. implies a deficiency of vitamin C (Harris *et al.*, 1936).
7. In Case 1 in the "repeat" a red blood cell fragility test was carried out each week.
8. In the "repeat" test on Case 1 a weekly blood uric acid test was performed.

Results

Case 1.—The red blood counts, vitamin C content of the blood, Hb percentage, blood pressure, volume of urine, and daily excretion of vitamin C are given in Tables I and II. 300 mg. of vitamin C was administered daily from November 8 to 15, 1939. From November 16 onwards the dose was increased to 400 mg. daily. On November 30 31 of sodium bicarbonate was given in addition. This was reduced to 31 daily from December 1.

TABLE I

Date	Red Cells per c.mm.	Vitamin C in Blood mg. per litre	Hb gm. per 100 c.cm.	Colour Index	Blood Pressure
4/11/39					160/110
8/11/39	7,900,000		17.6	0.8	
15/11/39	7,775,000				
23/11/39	8,250,000	16	16.2	0.71	
29/11/39	7,587,500	30	14.8	0.68	
6/12/39	6,934,250		13.8	0.71	
10/12/39		36			
13/12/39	6,500,000	30	13.8	0.77	130/90
20/12/39	6,325,000		14.2	0.8	
12/2/40	7,387,500		12.2	1.6 (?)	130/88

Urine Investigations

TABLE II

Date	Vol. Urine excreted in 24 Hours	Vitamin C excreted in Urine in 24 Hours
4/11/39	1,345 c.cm.	27.24 mg.
5/11/39	1,340 "	29.59 "
6/11/39	1,050 "	25.0 "
7/11/39	1,200 "	36.0 "
8/11/39	900 "	36.0 "
300 mg. vitamin C given daily		
9/11/39	1,100 c.cm.	30.8 mg.
10/11/39	800 "	29.72 "
11/11/39	1,050 "	29.4 "
12/11/39	950 "	55.1 "
13/11/39	1,240 "	99.2 "
14/11/39	950 "	114.0 "
15/11/39	1,050 "	175.3 "
400 mg. vitamin C given daily		
19/11/39	1,100 c.cm.	243.0 mg.
23/11/39	1,100 "	275.0 "
27/11/39	1,150 "	280.4 "
29/11/39	1,100 "	286.3 "
400 mg. vitamin C + 31 sodium bicarbonate given daily		
3/12/39	2,200 c.cm.	199.5 mg.
6/12/39	2,600 "	222.5 "
10/12/39	2,400 "	144.0 "
13/12/39	3,000 "	165.4 "
20/12/39	2,650 "	265.8 "
		(took two aspirins for headache)
26/12/39	2,450 "	162.6 mg.

There was a striking improvement in general health, which the patient dated from the time of taking the bicarbonate and the occurrence of polyuria. There was also a marked change in the viscosity of the blood, as seen by the naked eye, when samples were being taken for investigations.

Vitamin C and sodium bicarbonate were discontinued on December 26. On February 12, 1940, the red cells had increased to 7,387,000. It was decided to repeat the experiment. This time the sodium bicarbonate was first given on February 28, and on March 5 300 mg. of vitamin C was prescribed. Table III gives daily quantities of urine excreted. Results of the blood examination are given in Tables IV and V.

TABLE III

Date	Volume of Urine excreted in 24 Hours	Date	Volume of Urine excreted in 24 Hours
23/2/40	1,120 c.cm.	25/2/40	1,060 c.cm.
24/2/40	950 "	26/2/40	1,355 "
(Average 1,121 c.cm.)			
31 of sodium bicarbonate daily			
28/2/40	1,510 c.cm.	2/3/40	2,470 c.cm.
29/2/40	1,810 "		(2 bottles stout to-day)
1/3/40	1,850 "	3/3/40	1,770 c.cm.
		4/3/40	1,800 "
(Average 1,748 c.cm., excluding 2/3/40)			
300 mg. of vitamin C daily in addition to sodium bicarbonate			
5/3/40	2,330 c.cm.	16/3/40	2,600 c.cm.
6/3/40	1,800 "	17/3/40	1,830 "
7/3/40	1,980 "	18/3/40	1,570 "
8/3/40	1,860 "	19/3/40	2,260 "
9/3/40	2,070 "	20/3/40	1,660 "
10/3/40	1,700 "	21/3/40	1,740 "
11/3/40	1,600 "	22/3/40	2,100 "
12/3/40	1,800 "	23/3/40	1,700 "
13/3/40	1,710 "	24/3/40	1,650 "
14/3/40	1,850 "	25/3/40	2,180 "
15/3/40	2,100 "		
(Average 1,913 c.cm.)			

TABLE IV

Date	Blood Uric Acid	Date	Red Cells
26/2/40	4.33 mg. per 100 c.cm.	21/2/40	7,987,500
11/3/40	5.30 " " " "	4/3/40	7,750,000
17/3/40	4.80 " " " "	10/3/40	7,337,000
26/3/40	6.11 " " " "	18/3/40	6,079,000
		26/3/40	5,488,000

TABLE V

Date	Blood Fragility	Date	Haemoglobin
26/2/40	Partial 0.40% saline	21/2/40	13.4 gm. per 100 c.cm.
	Complete 0.35 " "		
4/3/40	Partial 0.45 " "	4/3/40	15.6 " " "
	Complete 0.40 " "		
11/3/40	Partial 0.40 " "	10/3/40	15.0 " " "
	Complete 0.35 " "		
18/3/40	Complete haemolysis 0.45 " "	18/3/40	15.2 " " "

This subject felt better after taking the sodium bicarbonate, and the addition of vitamin C caused a marked improvement in general health; but even after the blood count had fallen the facial appearance did not return to normal, although the colour was not so deep.

Case 2.—Treatment was begun on January 8, 1940. An excretion test on urine voided after a dose of 300 mg. of vitamin C (6 redoxon tablets) showed a definite deficiency. From January 10 to 24 the patient was given 400 mg. vitamin C daily. From January 17 she also received 2 drachms of sodium bicarbonate thrice daily. The following investigations were made (Tables VI and VII).

TABLE VI

Date	Red Cells	Date	Blood Pressure
5/1/40	5,250,000	25/11/39	170/115
12/1/40	5,150,000	15/1/40	170/110
21/1/40	5,000,000	29/1/40	155/110
31/1/40	4,500,000	2/2/40	145/110

Urine Investigations

TABLE VII

Date	Vol. Urine Excreted in 24 Hours	Excretion of Vitamin C in 24 Hours	Date	Vol. Urine Excreted in 24 Hours	Excretion of Vitamin C in 24 Hours
7/1/40	1,136 c.cm.		19/1/40	1,988 c.cm.	Saturated
8/1/40	568 "		20/1/40	1,704 "	
9/1/40	1,136 "		21/1/40	1,704 "	
10/1/40	1,136 "		22/1/40	1,420 "	
11/1/40	852 "	24.5 mg.	23/1/40	1,704 "	
12/1/40	1,136 "	26.0 "	24/1/40	1,136 "	
13/1/40	1,136 "	28.0 "	25/1/40	1,136 "	
14/1/40	1,136 "	34.0 "	26/1/40	1,136 "	
15/1/40	852 "	31.0 "	27/1/40	568 "	
16/1/40	852 "	35.0 "	28/1/40	1,136 "	
17/1/40	1,704 "	35.0 "	29/1/40	1,136 "	
18/1/40	1,988 "	40.0 "	30/1/40	1,420 "	
			31/1/40	852 "	

From January 24 the patient was kept on a maintenance dose of 50 mg. of vitamin C daily. There was very little change in the spleen; if anything it was slightly smaller. There was a great improvement in the patient's general condition.

A control test was carried out on a healthy person to observe the effect of sodium bicarbonate on the threshold of vitamin C excretion in the urine (Table VIII).

TABLE VIII

Dose of Vitamin C	Day	Vol. Urine in c.cm. per 24 Hours	Vitamin C Excreted in mg.
	1st day	1,350	10.8
	2nd "	1,400	8.4
	3rd "	1,300	10.4
300 mg.	4th "	1,300	5.2
300 mg.	5th "	1,650	201.3
300 mg.	6th "	1,480	195.36
300 mg.	7th "	1,400	223.7
300 mg.	8th "	1,250	225.0
300 mg. + $\frac{1}{2}$ sod. bicarb.	9th "	1,850	104.4
300 mg. + $\frac{1}{2}$ sod. bicarb.	10th "	1,700	100.8

Conclusions

It would appear that vitamin C has some influence in regulating the number of red blood cells in the circulation, and that in polycythaemia there is a defect in the metabolism of vitamin C. Both the subjects had a definite polycythaemia and excreted considerable amounts of vitamin C. They were deficient in the latter, however, since they did not excrete 40% of the test dose for several days (Harris *et al.*, 1936). Neither was affected by large doses of the vitamin, but when the threshold of excretion was raised by the administration of sodium bicarbonate there was a fall in the red cell count and a marked polyuria. This was especially noticeable in Case 1, and was not altogether due to the sodium bicarbonate, since in the repeat experiment a higher average excretion followed on the administration of vitamin C than was found when sodium bicarbonate was given alone. There was a definite improvement in the general condition of the subjects. In both cases a fall in blood pressure coincided with a fall in the red cell count. In Case 1 the haemoglobin percentage fell in the first experiment, but was unaffected in the repeat experiment. Neither the blood fragility nor the blood uric acid showed much variation with the fall in the red cell count. Case 1 relapsed when the vitamin C and sodium bicarbonate were withdrawn, but improved when these were given again. Further consideration of these facts would suggest that vitamin C and sodium bicarbonate might be used in the treatment of polycythaemia. The material available would not permit of more definite claims being made.

Summary

The literature on vitamin C as a regulator of the number of red blood cells in the circulating blood is reviewed.

The results are given of an investigation of the relation of vitamin C to polycythaemia in two cases.

Vitamin C alone had little effect on the condition, but in conjunction with sodium bicarbonate caused an improvement in the general health and a marked fall in the red cell count. The latter rose in one case after the experiment had concluded, but fell again when vitamin C and sodium bicarbonate were readministered.

A marked diuresis occurred after giving vitamin C and sodium bicarbonate.

It is suggested that this method merits more extensive trial in the treatment of polycythaemia.

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Dr. Paul Michael (*J. Amer. med. Ass.*, 1940, **115**, p. 286) investigated the blood sugar of thirty male golfers of ages ranging from 30 to 45 years and possessing handicaps of from 10 to 27. It was found that after lunch and under ordinary conditions the blood sugar of the players dropped to hypoglycaemic levels between the ninth and fifteenth holes in a foursome and between the eleventh and fifteenth holes in a twosome. These periods corresponded to the time of lessened efficiency of the players. It was found that "more ketogenic types of lunch" and the eating of sugar or candy at the seventh or ninth hole diminished symptoms and improved the scores.