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George E. Brown, Grace M. Roth

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Research Article

THE REDUCTION OF HYPERCALCEMIA IN CASES OF POLYCYTHEMIA VERA BY PHENYLHYDRAZINE[1]

By GEORGE E. BROWN and GRACE M. ROTH

(From the Division of Medicine, Mayo Clinic, Rochester, Minn.)

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The range of variation in serum calcium is relatively narrow in health and in most diseases. The accepted normal level is from 9 to 11 mgm. for each 100 cc. of serum or plasma. Lowered values for serum calcium have been found in tetany (parathyroid), rickets, nephritis, osteomalacia and sprue; elevated values are rare. High values have been found by Wells in terminal acidosis and by Coates and Raiment in gout, averaging 19 mgm. for each 100 cc. of serum; these data, however, have been questioned by Cameron. Horowitz has reported values as high as 16.8 mgm. for each 100 cc. of serum in cases of acute gout, and values as high as 16.2 mgm. in five of fourteen cases of arthritis deformans. Hench found that the serum calcium was normal in four cases of gout and in twenty-five cases of infectious arthritis.

The calcium content of the whole blood in healthy subjects has been found by Kramer and Tisdall (16) to vary from 5.3 to 6.8 mgm. for each 100 cc. of blood. Calcium is absent, or present in small amounts, in the corpuscles of the blood.

The calcium content of the whole blood and of the serum in cases of polycythemia vera has not been carefully studied, probably because of the relative rarity of the disease. Rabinowitch (18), employing McCallum's gravimetric method (quantitative determination of calcium as calcium oxide) found lowered serum calcium in two cases of polycythemia vera.

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METHOD OF STUDY

Interest in this study was prompted by the clinical observation of what seemed to be an abnormal degree of calcification in the peripheral arteries in older persons with polycythemia vera, as compared to that occurring in normal persons of like age.

Fourteen typical cases of polycythemia vera were studied from this viewpoint. The spleen was enlarged in all. The number of erythrocytes, both absolute and relative, was greatly increased. The total blood volume, according to the dye method[2] (11), was greatly increased, owing to the absolute increase in the erythrocytes. The patients were hospitalized; three were given the low ionic diet of Keith, Smith and Whelan (11), the others were given the general hospital diet. Determinations were made in each case of the calcium content of the whole blood and serum before and following treatment with phenylhydrazine. The sodium, potassium, magnesium, phosphate, and the sodium chloride contents of the whole blood and serum were made in five cases before and after treatment with phenylhydrazine.

The hemoglobin was determined by the method of Osgood and Haskins. For serum sodium the method of Kramer and Tisdall (14), as modified by Whelan, was used. For calcium and potassium (13), the methods of Tisdall and Kramer were used. Magnesium was determined by the method of Bogert and Plass, which is a combination of Kramer and Tisdall's (15) magnesium method and of Briggs' phosphorus method; sodium sulphite was added to bring out the blue color, as directed by Briggs. In the whole blood a modification[3] of the method of Kramer and Tisdall (16) was used for the direct quantitative determination of sodium, potassium, calcium and

[2] The hermatocrit determinations were made by the dry oxalate method and a correction of 3 per cent in the cell volume was made for shrinkage.

[3] Ten cubic centimeters of blood, accurately measured in a pipette, was laked with about 25 cc. of water in a 100 cc. volumetric flask. From 10 to 15 cc. of 20 per cent trichloracetic acid was added to complete the precipitation of the proteins. After the contents had been made up to volume with water, and filtered, 50 cc. of the filtrate was evaporated to dryness. The method of Kerr was employed in the removal of the trichloracetic acid. After the aliquot had been evaporated to dryness again, the residue was dissolved in 0.2 N hydrochloric acid, transferred to a 10 cc. volumetric flask and made up to volume.

TABLE 1

Calcium determinations on the same blood with different methods

Case	Serum			Blood		
	Direct precipitation	Ashed sample	Reprecipitation	Ashed sample	Treated with trichloracetic acid	Reprecipitation
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	8.4	8.4	8.4		3.5	3.5
2	9.8	9.8	9.8	3.2	3.7	
3	12.7		12.5	4.7	4.9	4.7
4	12.9		12.5	3.8	4.4	
5	10.2		10.6		6.1	5.7

TABLE 2

Calcium content of blood and serum in cases of polycythemia vera before treatment

Case	Sex	Age	Hemoglobin	Erythrocytes	Cells by the hematocrit	Blood volume		Plasma volume		Calcium			
										Whole blood		S rum	
						Total	Per kilo	Total	Per kilo	Concentration	Total	Concentration	Total
			grams per cent	*millions*	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	*mgm. per 100 cc.*	*mgm.*	*mgm. per 100 cc.*	*mgm.*
1	M	64	23.0	5.76	60	8,580	136	3,430	54	5.2	445	12.6	432
2	M	55	26.0	7.00	75	14,700	180	3,670	46	3.4	504	14.3	524
3	M	50	25.0	8.18	76	14,250	205	3,420	49	4.0	574	17.3	591
4	M	62	21.4	5.70	65	9,900	173	3,460	60			15.2	526
5	M	56	23.4	7.24	70	11,110	173	3,330	52			15.8	526
6	M	25	24.5	7.01	60	11,100	203	3,330	61	5.8	679	18.1	602
7	M	39	27.3	7.60	76	17,250	223	4,140	53	3.5	610	12.9	534
8	F	66	27.2	7.23	70	9,725	191	2,925	58	5.0	488	12.9	387
9	M	58	21.3	8.32	65	13,550	191	4,740	66	4.9	673	11.9	565
10	F	63	13.6	5.80	45	6,560	135	3,600	74			12.6	453
11	M	56	17.2	5.66	52	8,160	145	3,915	52			15.0	459
12	M	59	18.2	6.62	65	9,700	162	3,400	58			11.1	377
13	F	57	20.1	6.69	57	9,640	120	4,180	52			17.9	748
14	M	59	18.2	6.84	61	8,020	140	3,150	54			12.6	397
Average values.....			21.8	6.88	64	10,874	169	3,620	56	5.0	567	14.3	508

magnesium in small amounts of blood. Smith's method was used for chloride determinations in serum and whole blood. The serum protein was determined by the refractometric method.

TABLE 3

Polycythemia vera—studies on the calcium content of the blood and serum before and during treatment with phenylhydrazine

Case	Age	Sex	Date	Hemoglobin	Erythrocytes	Cells by the hematocrit	Blood volume		Plasma volume		Calcium			
											Whole blood		Serum	
							Total	Per kilo	Total	Per kilo	Concentration	Total	Concentration	Total
				grams per cent	*millions*	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	*mgm. per 100 cc.*	*mgm.*	*mgm. per 100 cc.*	*mgm.*
1	64	M	November 14, 1925	23.0	5.76	60	8,580	136	3,430	54	5.2	445	12.6	432
			December 7, 1925	12.8	2.99	35	4,730	75	3,070	48	7.4	350	13.1	402
2	55	M	December 5, 1926	26.0	7.00	75	14,700	180	3,670	46	3.4	504	14.3	524
			January 4, 1926	12.2	2.82	24	5,650	75	4,290	57	7.5	424	10.1	433
3	50	M	January 11, 1926	25.0	8.18	76	14,250	205	3,420	49	4.0	574	17.3	591
			February 4, 1926	15.2	4.51	38	6,450	104	4,000	64	10.0	645	12.6	504
4	62	M	January 28, 1925	21.4	5.70	65	9,900	173	3,460	60			15.2	526
			February 12, 1925	12.2	4.62	40	6,510	116	3,910	70			9.3	364
5	56	M	April 28, 1925	23.4	7.24	70	11,110	173	3,330	52			15.8	526
			May 6, 1925	20.8	4.97	60	8,760	136	3,500	55			11.9	416
			May 15, 1925	12.5	3.28	37	5,055	81	3,185	51			10.6	336
6	25	M	January 2, 1928	24.5	7.01	60	11,100	203	3,330	61	5.8	679	18.1	602
			January 21, 1928	18.1	5.68	53	7,660	150	3,600	76	5.7	440	11.5	415
7	39	M	January 18, 1928	27.3	7.60	76	17,250	223	4,140	53	3.5	610	12.9	534
			February 13, 1928	24.0	7.56	68	13,750	183	4,400	59	3.8	519	9.8	431
8	66	F	October 4, 1927	27.2	7.23	70	9,725	191	2,925	58	5.0	488	12.9	387
			November 10, 1927	15.3	4.24	47	5,565	114	2,950	60	6.1	340	11.4	336

All determinations were carried out in duplicate on fasting blood, and in several cases the direct method was checked by the trichloracetic acid technic of Kramer and Tisdall (16). Control deter-

minations in normal subjects by the same methods showed values within the accepted normal range; control determinations of calcium by direct precipitation in the serum checked against the "ashed method" showed close approximation (table 1). To rule out possible adsorption of calcium by protein, the sample after the first titration with potassium permanganate was treated with excess potassium permanganate to oxidize all organic matter completely. The calcium in this solution was then reprecipitated with ammonium oxalate after adjustment of the hydrogen-ion concentration. The results obtained in the two precipitations were identical.

In the determinations of calcium in the whole blood, it was necessary to use the trichloracetic acid method since in the ash method the large number of erythrocytes increased the iron content to such a degree that it interfered with the permanganate titration.

RESULTS

Calcium content of serum. The calcium content of the serum previous to treatment or early in treatment is shown in table 2. The average value for the group was 14.3 mgm. for each 100 cc. of serum. The range was from 11.1 to 18.1 mgm. In twelve of the fourteen cases values were in excess of 12 mgm. In six cases the values were 15 mgm. or more.

Calcium content of the whole blood. The calcium content of the whole blood averaged 5.0 mgm. for each 100 cc.; the range was from 3.4 to 5.8 mgm. (normal values). The total circulating calcium in the serum and whole blood was calculated in seven cases (table 2). The difference in the actual amount of calcium in the serum and in the whole blood represents the calcium contained in the corpuscles. In three cases calcium was not found; in four cases, the total calcium content of the corpuscles ranged from 77 to 108 mgm. or for each 100 cc. of corpuscles from 0.67 to 1.7 mgm., amounts within the probable error of the method.

Changes in the calcium levels following treatment with phenylhydrazine (table 3). The percentage calcium content of the serum decreased in seven of eight cases following destruction of erythrocytes by phenylhydrazine, but the values after treatment did not fall below the accepted normal level. In all but one case the calcium values

TABLE 4

Polycythemia vera—Studies on the mineral substances of the blood before and during treatment with phenylhydrazine

Case	Age	Sex	Date	Hemoglobin	Erythrocytes	Cells by the hematocrit	Blood volume		Plasma volume		Sodium		Potassium		Magnesium		Phosphate		Calcium		Sodium chloride	
							Total	Per kilo	Total	Per kilo	Serum	Blood	Serum	Blood	Serum	Blood	Serum	Blood	Serum	Blood	Serum	Blood
				gms. per cent	*millions*	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
1	64	M.	November 14, 1925	23.0	5.76	60	8,580	136	3,430	54	325	160	20.1	270	2.2	2.4	2.7	2.1	12.6	5.2	565	305
			December 7, 1925	12.8	2.99	35	4,730	75	2,070	48	346	224	19.7	157	2.9	2.3	2.7	4.5	13.1	7.4		525
2	55	M.	December 5, 1926	26.0	7.00	75	14,700	180	3,670	46	425	194	35.0	270	3.7	6.7	2.7	2.6	14.3	3.4		
			January 4, 1926	12.2	2.82	24	5,650	75	4,290	57	340	114	19.4	135	3.2	5.0	2.5	2.8	10.1	7.5	660	580
3	50	M.	January 11, 1926	25.0	8.18	76	14,250	205	3,420	49	518	158	50.4	280	3.1	2.7	2.1	3.2	17.3	4.0	657	420
			February 4, 1926	15.2	4.51	38	6,450	104	4,000	64			22.7	89.2	1.6	6.3	3.3	2.8	12.6	5.3	585	520
4	62	M.	January 28, 1925	21.4	5.70	65	9,900	173	3,460	60	420		28.0		5.0		3.9		15.2			
			February 12, 1925	12.2	4.62	40	6,510	116	3,910	70	337		21.0		2.8		3.2		9.3			
5	56	M.	April 28, 1925	23.4	7.24	70	11,110	173	3,330	52	394				4.6		2.6		15.8		610	
			May 6, 1925	20.8	4.97	60	8,760	136	3,500	55	401		22.0		1.8		2.4		11.9		570	
			May 15, 1925	12.5	3.28	37	5,055	81	3,185	51	358				2.5		2.9		10.6		580	

remained slightly higher than the upper limit of normal. The greatest decrease occurred in Case 6, from 18.1 to 11.5 mgm. for each 100 cc. of blood, or a decrease of 187 mgm. in the total serum calcium. The total circulating calcium in the serum decreased in every instance but not as much in proportion as the percentage values.

The percentage calcium content in the whole blood increased in four of six cases following treatment with phenylhydrazine; there was a slight decrease in the total circulating calcium of the whole blood in all but one case. There were no significant changes in the total calcium content of the corpuscles following treatment.

A close correlation could not be demonstrated in the degree of change in the content of the serum calcium and that occurring in the blood volume.

Other inorganic constituents (table 4). Sodium, potassium, magnesium, phosphate and chloride values of the serum and whole blood were determined in five cases, before and after treatment. Abnormally high values were obtained for the percentage content of serum potassium in cases 2 and 3, which returned to normal after treatment.[4] In case 4 there was a moderate increase in the serum potassium; in case 5 a normal value for the serum potassium was found but the determination was made after considerable destruction of cells had occurred. The potassium in the whole blood was studied in three cases, all of which showed increased concentration; following treatment normal or subnormal values were found. The concentration of sodium, magnesium, phosphate and sodium chloride in the whole blood and serum were normal.

DISCUSSION

The basis of the hypercalcemia in polycythemia vera is not known. In the light of our recent knowledge of the rôle of the parathyroids in the regulation of the calcium in the blood, one would be tempted to theorize on functional overactivity of these glands in polycythemia vera. There is no evidence that hyperfunction ever occurs; the premise, therefore, is probably false. A second conception would be

[4] Hemolysis in the serum was not ruled out by spectroscopic examination. That hemolysis produced the elevated values for potassium is unlikely, since lower values were found after treatment.

the assumption that the increased calcium is related to an increase in he serum protein with variation in the colloid-calcium combination. In two cases studied, slight increases in the serum proteins were found. Studies have not been carried out in our cases to determine whether the diffusible or the nondiffusible fractions, or both, are increased. Methods for the study of this question are being developed.

The most logical explanation for the hypercalcemia in this disease is that it is related in some manner to the great increase in the absolute number of erythrocytes, since a definite decrease in the calcium levels in the serum follow the reduction in the number of erythrocytes. Apparently a relative increase in the ratio of corpuscles to plasma is not accompanied by an increase in the calcium, since normal values were obtained in a case of relative polycythemia vera due to dehydration, in which there were 60 per cent of cells by the hematocrit. The absence of an increase in the serum calcium in relative polycythemia vera is in accord with the work of Van Slyke. He showed that change does not occur in the inorganic ratios with variations in the relative amount of cells or plasma, since the concentration of the inorganic substances remains the same.

It could be assumed that the increased concentration of calcium represents an effort to maintain the normal inorganic ratios in the blood. Against this premise is the presence of increased values for serum calcium associated with normal values for serum potassium. There is no explanation for the increase in serum potassium in the cases in which it is observed, since it has been shown that this electrolyte does not pass through the cell membrane.

The decrease in the percentage concentration of calcium following treatment is due to a decrease in the total calcium of the serum and to dilution from the increased amount of plasma. As an example (case 2, table 3), the total serum calcium decreased 17 per cent, the actual plasma volume increased 17 per cent, and the concentration of serum calcium decreased from 17.3 to 12.6 mgm. (30 per cent). Approximately half of the percentage change in calcium was related to the variation in the plasma. Either the concentration of calcium in the tissue fluid was low or an abnormal excretion of calcium occurred during hemolysis. Data regarding the excretion of calcium during the period of blood destruction are lacking.

There are two clinical features in polycythemia vera that may be directly related to the hypercalcemia: the apparent increase in the tendency to calcification in the peripheral vessels, and, besides the high viscosity and slowing of the flow of blood, susceptibility to thrombosis. Routine roentgen-ray examination of the arteries of the extremities in this disease gives the impression that calcification of the vessels is present in a greater degree than that occurring in normal subjects of comparable ages. Hueper's investigation on the metastatic calcification in the organs of the dog following injection of parathyroid hormone is suggestive in this connection. He stated that serum calcium may be maintained at levels varying from 10 to 15 mgm. for a long time without metastatic calcification, but values in excess of this are dangerous. Since the introduction of the parathyroid hormone, its therapeutic use in a large number of cases has been reported; untoward effects of the increase in serum calcium from its use for comparatively short periods have not been reported. The work of Hunter and Aub (9) on this agent in the treatment of lead intoxication has further confirmed the high degree of tolerance exhibited by human subjects to increases in the serum calcium for periods of from three days to three weeks. Changes in the physical properties of the blood were noticeably absent.

Collip (5) defined a syndrome which he ascribed to hypercalcemia occurring in dogs following the injection of a parathyroid hormone. Marked asthenia, vomiting, diarrhea, coma, dehydration, pneumonia and hemorrhages comprise the symptoms of this intoxication; death may follow. Polycythemia vera was not observed. Collip definitely stated that with enormous doses of this hormone, hypercalcemia can be induced in normal rabbits without ill effects. He concluded that hypercalcemia alone is not deleterious in this animal but the urgent symptoms are related to the increase of phosphorus and to terminal acidosis. Apparently the tolerance of the different species of mammals to hypercalcemia varies widely, at least as far as the acute effects are concerned. The long-continued elevation of the serum calcium as obtained in polycythemia vera, perhaps a useful or compensatory adaptation, may in itself induce certain pathologic disturbances, and be productive of harm to the organism.

SUMMARY AND CONCLUSIONS

Fourteen subjects presenting the classic picture of polycythemia vera showed an increase in the serum calcium above the accepted range of normal. The values in this substance ranged from 11.1 to 18.1 mgm. for each 100 cc. of serum. The average value was 14.3 mgm.

Following treatment with phenylhydrazine and destruction of corpuscles to approximately normal or even subnormal values, the percentage concentration of serum calcium decreased to levels slightly above normal.

The basis of the hypercalcemia is not known. It may represent a compensatory effort to maintain the inorganic ratios of the blood. Hypercalcemia in the human subject can be tolerated without grave disturbance to the organism. The susceptibility of patients with polycythemia vera to thrombosis and to high grades of calcification in the peripheral vessels in some cases of polycythemia vera may be late results of hypercalcemia.[5]

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[5] We have encountered three cases of mild polycythemia vera during the last three months with volumes of blood, 128, 131 and 140 cc. for each kilogram, with serum calcium values of 10.6, 10.4 and 11.3 mgm. respectively. These data indicate that there are cases of mild degrees of polycythemia vera in which hypercalcemia is probably not present at this stage.

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