STUDIES ON THROMBOCYTOSIS. I. HYPERKALEMIA DUE TO RELEASE OF POTASSIUM FROM PLATELETS DURING COAGULATION [1]

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Remarkable elevation of the serum potassium concentration without associated manifestations of hyperkalemia was observed in a patient with an unexplained increase in the blood platelets (1). Studies of the phenomenon in this patient indicated that the excess of potassium was derived from the platelets during the coagulation of the blood. Hyperkalemia was encountered in certain other patients with thrombocytosis. In contrast, no striking elevation of the serum potassium concentration was observed when normal platelets were concentrated *in vitro*.

METHODS AND MATERIALS

Preparation of native blood and plasma specimens. Native (without anticoagulant) blood and plasma specimens were processed by previously described methods with reliance on the use of low temperatures and silicone-treated [2] equipment (2, 3). Platelet-free plasma was prepared from the blood specimen only after prior separation of platelet-rich plasma.

Preparation of acid-citrate-dextrose plasma. Acid-citrate-dextrose [3] (ACD) solution was added to whole blood prior to centrifugation in the proportion of one part of the anticoagulant to four parts of blood.

Isolation of platelets. Blood (300 to 500 ml.) was collected from an antecubital vein into silicone-treated tubes packed in an icebath. Saftidonor [4] sets were used for the collection of the blood in order to minimize trauma and exposure to surfaces such as occurs when blood is collected by multiple syringe technique. The blood was processed with and without the use of ACD anticoagulant. Native (without anticoagulant) and ACD platelet-rich plasmas were prepared by slow speed centrifugation (1,500 to 2,000 rpm) in a refrigerated Servall angle centrifuge. A viscid plug of platelets was isolated from platelet-rich plasma by centrifuging at higher speeds (12,000 rpm). The supernatant plasma was thoroughly removed by draining and wiping the inside of the tube carefully. The platelet plug was placed in a deep-freeze. A small red button (red cells) was noted on the bottom of the platelet plug. In order to obtain a plug free of red and white cells, the red button with a small portion of the adjacent whitish material was removed while the plug was in a frozen state. The material removed was estimated to be less than 2 per cent of the volume of the plug.

The platelet plug isolated from a measured volume of platelet-rich plasma was weighed and its volume determined by centrifugation in a graduated centrifuge tube. Platelet water was determined by allowing the wet plugs to reach constant weight by drying in an oven set at 80° C.

Blood counts. Platelet counts were performed in duplicate by the phase microscopy method of Brecher and Cronkite (4). Counts on undiluted platelet-free plasma and serum were performed as previously described (3). Red and white blood cell counts on platelet-rich and platelet-free plasma and on serum were performed in the routine manner, using appropriate pipet dilutions.

Potassium concentrations in serum, plasma and platelets. Native whole blood or plasma was placed in silicone-treated tubes for one hour at 37° C. The clots were then removed. The red blood cells in the serum from clotted whole blood were removed by high speed centrifugation in silicone-treated equipment at low temperature.

Proteins were removed from serum and plasma specimens by precipitation with 5 per cent trichloracetic acid.

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[2] Syringes and the Lusteroid centrifuge tubes were coated with General Electric Dri-film SC-87 and lubricated with General Electric Silicone Oil SF-96 (200). Other containers and apparatus were coated with Dri-film only.

[3] Acid-citrate-dextrose (ACD) solution consisted of sodium citrate, 1.32 Gm.; citric acid, 0.48 Gm.; and dextrose, 1.47 Gm. in distilled water to a total of 100 ml.

[4] Saftidonor sets were kindly provided by Dr. E. B. McQuarrie, Biochemical Research Division, Cutter Laboratories, Berkeley, Calif. The intravenous needles in these sets had been pretreated with silicone and were of No. 15 or No. 17 gauge. Only the intravenous needle and the attached, proximal 12 inches of plastic tubing were used. The distal segment of tubing and phlanged blood bottle needle were removed prior to venipuncture.

Potassium determinations were carried out in duplicate filtrates by flame photometry with a Beckman model B instrument. The variations between duplicates were as follows: 82 samples, < 0.1 mEq. per liter; 43 samples, 0.1; 19 samples, 0.2; 4 samples, 0.3; and 6 samples, 0.4. The thrombin and ACD solutions and distilled water used in these studies contained no measurable potassium.

The platelet plugs were analyzed for sodium and potassium in the following manner: Protein was precipitated with 5 per cent trichloracetic acid or digested with hot nitric acid. The trichloracetic acid precipitates were allowed to stand for 48 hours at 5° C. The suspensions were centrifuged and the supernatant analyzed for sodium and potassium by employing the Beckman DU spectrophotometer with flame attachment.

Red blood cell potassium determinations. Heparinized blood was obtained in a ratio of 1 mg. dried heparin [5] to 5.0 ml. of blood. Red blood cell potassium concentration was calculated from the whole blood and platelet-free plasma determinations, and from the average of triplicate determinations of the volume of packed red blood cells. No correction for "trapped" plasma was employed.

Case report

M. H. (Johns Hopkins Hospital No. 268267), a colored male, was first seen in 1945 at the age of 70 years with symptoms of congestive heart failure and angina pectoris. A diagnosis of hypertensive and arteriosclerotic cardiovascular disease was made. The congestive heart failure responded to therapy which included the occasional use of mercurial diuretics.

The patient's status was reevaluated in March, 1952, because of increasing congestive heart failure. Physical examination revealed grade I vascular changes of the ocular fundi. There were a few fine rales at both lung bases. The heart was enlarged to the left, and there was a soft apical systolic murmur. The pulse rate was 82 and the blood pressure was 164/80 mm. Hg. The liver was palpable 2 cm. below the right costal margin. The spleen was not palpable, and there was no lymphadenopathy. There was bilateral pitting ankle edema. Neurological examination was unremarkable.

From March, 1952, to August, 1952, the following laboratory observations were recorded. The serological tests for syphilis were negative. The volume of packed red blood cells varied between 44 and 50 per cent, and the volume of packed white blood cells and platelets between 4 and 5 per cent. The white blood cell count ranged between 9,000 and 13,000 per cu. mm. Differential white blood cell counts were repeatedly normal except for a persistent basophilia of 2 to 3 per cent. *The platelet count varied between 1,200,000 and 2,000,000 per cu. mm.* Urinalyses were normal except for occasional mild proteinuria and the finding of a rare hyaline cast. Phenosulfonthalein excretion totaled 58 per cent in two hours. *The potassium concentration in serum obtained from clotted whole blood varied between 7.0 and 8.0 mEq. per liter* when tested on five occasions and in two different laboratories. Electrocardiogram revealed no evidence of hyperkalemia. The serum sodium concentration varied between 133 and 138 mEq. per liter. The following blood chemical determinations were repeatedly normal: NPN, serum chloride, CO_2 combining power, bilirubin, cephalin flocculation, thymol turbidity, serum inorganic phosphorus, total serum proteins, albumin-globulin ratio, cholesterol, and serum alkaline phosphatase activity. Bromsulfothalein excretion test revealed 1 mg. per cent retention in 30 minutes.

Both bone marrow aspiration smears and surgical biopsy specimens revealed a marked increase in the proportion of megakaryocytes but no other abnormalities. There was no evidence of myelofibrosis or myelosclerosis.

The period from August, 1952, to the patient's death in January, 1956, was characterized by the following manifestations: increasing difficulty in control of the angina pectoris and congestive heart failure, and progressive enlargement of the liver (to the right iliac crest) and spleen (to 14 cm. below the left costal margin). There was no lymphadenopathy.

A leukocytosis developed and the platelet count and serum potassium concentration gradually fell. A typical blood examination (obtained in March, 1955) revealed the following: volume of packed red blood cells, 51.3 per cent; volume of packed white blood cells and platelets, 1.0 per cent; white blood cells, 18,350 per cu. mm.; platelets, 438,000 per cu. mm.; and serum potassium concentration, 4.8 mEq. per liter. The differential white blood cell count revealed: 1 per cent blasts, 3.5 per cent undifferentiated myelocytes, 2.0 per cent differentiated myelocytes, 6.5 per cent juvenile neutrophils, 62 per cent polymorphonuclear neutrophils, 3 per cent eosinophils, 14 per cent basophils, 6.5 per cent lymphocytes, and 1.5 per cent monocytes. Five nucleated red blood cells were noted per 200 white blood cells. At no time was anemia present.

The patient died in January, 1956. Permission for autopsy was not obtained. Although a tissue diagnosis was not possible, it was thought that the patient did represent an instance of agnogenic myeloid metaplasia.

RESULTS

A. Patient studies

Results of studies shown in Table I demonstrate that the remarkable findings of thrombocytosis and hyperkalemia noted in Patient M. H. were related. Serum obtained from clotted whole blood or platelet-rich plasma had a markedly elevated potassium concentration, *i.e.*, 7.2 to 9.6 mEq. per liter. In contrast, serum obtained from clotted *platelet-free* plasma had a normal potassium concentration, *i.e.*, 4.2 to 5.4 mEq. per liter. When plasmas with intermediate concentrations of plate-

[5] Heparin sodium, 1,000 U.S.P. units (10 mg.) per ml. was kindly supplied by the Upjohn Company, Kalamazoo, Mich.

TABLE I

The relationship between platelet levels and serum potassium concentrations

Patient and diagnosis	Platelet-rich plasma		Whole blood		Platelet-free plasma	
	Platelets ($\times 10^3$) *per cu. mm.*	Serum potassium *mEq./L.*	Platelets ($\times 10^3$) *per cu. mm.*	Serum potassium *mEq./L.*	Platelets ($\times 10^3$) *per cu. mm.*	Serum potassium *mEq./L.*
A. Patients with thrombocytosis						
M. H. Myeloid metaplasia (?)	3,120	9.6	1,210	7.2	<1	5.2
	3,470	8.8		7.8	<1	5.0
	3,220	8.0			<1	5.4
	1,610*	7.0				
	3,250	8.6			<1	4.2
	406*	4.8				
	102*	4.3				
	4,350	9.6				
R. W. Thrombocytosis, cause undetermined	2,200	7.4	1,820	6.5	1.0	4.7
R. C. Myeloid metaplasia, postsplenectomy	2,440	5.8	1,740	5.5	2.8	4.5
	2,900	6.8			<1	4.8
	2,160	6.2			<1	4.1
E. T. Myeloid metaplasia (?)	5,530	5.6	3,880	5.3	<1	4.3
M. M. Myeloid metaplasia, postsplenectomy	2,730	5.2	2,000	5.3	2.8	4.2
H. L. Chronic myeloid leukemia	1,630	5.8	978	5.4	1.7	5.1
W. B. Thrombocytosis, cause undetermined	1,210	5.6	966	5.9	<1	5.0
E. H. Chronic myeloid leukemia	1,530	4.8	1,090	5.0	<1	4.7
M. W. Myeloid metaplasia (?)	2,850	5.4	1,210	5.5	<1	5.2
B. Postsplenectomy thrombocytosis						
B. W. Idiopathic thrombocytopenic purpura	1,200	5.5	490	4.9	<1	4.8
H. M. Acquired hemolytic anemia	1,050	5.5	640	5.4	<1	5.3
N. V. Idiopathic thrombocytopenic purpura	970	5.7	541	5.3	4.0	4.8
C. Thrombocytopenic patients						
T. K. Idiopathic thrombocytopenic purpura			3.6	4.4	<1	4.3
B. W. Idiopathic thrombocytopenic purpura			4.5	4.5	<1	3.8
N. V. Idiopathic thrombocytopenic purpura			89	4.8	<1	5.2
D. Normal subjects						
S. M.	462	4.6			<1	3.9
R. H.	172	4.4	146	4.3	<1	4.5
E. N.	349	5.0	201	5.0	<1	5.3
J. G.	381	4.5	180	4.5	<1	4.6
L. C.	506	4.0	150	3.5	<1	3.6

* To obtain these levels the platelet-rich plasma was diluted with an appropriate amount of platelet-free plasma.

lets were obtained by diluting platelet-rich with platelet-free plasma prior to clotting, the resulting serum potassium concentrations were likewise intermediate in value, *i.e.*, 4.3 to 7.0 mEq. per liter. These results demonstrated that the apparent elevation of serum potassium concentration actually represented a *spurious hyperkalemia,*[6] and that the circulating *plasma* potassium concentration was normal.

Similar studies were carried out on eight other patients with thrombocytosis (Table I, Group A). In two (R. W. and R. C.) of the eight patients, the potassium concentration in serum obtained from whole blood or platelet-rich plasma was more than 6.0 mEq. per liter. In these two patients, the difference in potassium concentration in serum obtained from platelet-rich plasma and from platelet-free plasma ranged from 1.3 to 2.7 mEq. per liter. In the other six patients this difference was less than 1.3 mEq. per liter. Studies (not shown in Table I) on another patient, W. S., with agnogenic myeloid metaplasia, were of particular interest. Examination of the peripheral blood sug-

[6] The term *spurious hyperkalemia,* while possibly open to criticism from a purist point of view, was used to avoid repetition of such lengthy phrases as "hyperkalemia due to release of potassium from large numbers of platelets during coagulation." The term does not imply error in potassium determinations. Arbitrarily the term was used only when serum from clotted platelet-rich plasma had a potassium concentration more than 1.3 mEq. per liter above that of serum from platelet-free plasma.

gested megakaryocytic leukemia. An estimated platelet count was 642,000 per cu. mm. Innumerable giant abnormal platelets were noted on the smear, and there were 200 megakaryocytes and megakaryocytic fragments per 100 white blood cells. The white blood cell count was 9,900 per cu. mm. and the volume of packed white blood cells and platelets was 5 per cent. The potassium concentration in serum obtained from clotted whole blood was 10.0 mEq. per liter, but there were no associated manifestations of hyperkalemia. Following five weeks of therapy with Myleran,® the platelet count fell to 126,000 per cu. mm. There were 66 megakaryocytes and megakaryocytic fragments per 100 white blood cells. The white blood cell count was 1,800 per cu. mm. and the volume of packed white blood cells and platelets was 0.7 per cent. At this time the potassium concentration in serum obtained from clotted whole blood was 5.4 mEq. per liter. There were, therefore, four patients (M. H., R. C., R. W., and W. S.) with thrombocytosis and an associated *spurious hyperkalemia.*

Three patients with mild postsplenectomy thrombocytosis (Table I, Group B), three with thrombocytopenia (Group C), and five normal subjects (Group D) were similarly studied. In all instances the differences in the potassium concentration in serum obtained from whole blood, platelet-rich and platelet-free plasma were less than 1.0 mEq. per liter.

Results of two determinations of red blood cell potassium in one of the patients (R. C.) with *spurious hyperkalemia* were 102.8 and 105 mEq. per liter. Red blood cell potassium concentration in five normal subjects ranged between 89 and 104.8 mEq. per liter. These values agree well with those reported by Knowles, Alverson, and Rubenstein (5). Thus, the red blood cell potassium content was not strikingly increased in this patient with *spurious hyperkalemia.*

B. Special studies

Factors influencing the release of potassium from platelets

The effect of incubation of *clotted* plasma prepared from a patient (M. H.) with *spurious hyperkalemia* was studied as follows: Platelet-rich and platelet-free ACD plasma samples were clotted by the addition of thrombin solution. The clotted platelet-free plasma and a portion of the clotted platelet-rich plasma were incubated at 37° C. for one *hour* after addition of the thrombin. Thereafter, the clots were removed and the potassium concentrations were determined on the sera. The clots were removed from the other portion of platelet-rich plasma one *minute* after the addition of thrombin, and the potassium concentration was determined on the serum. Results are shown in Table II. The increase in potassium concentration in sera from platelet-rich plasma occurred within one *minute* after coagulation. These data indicate that a large amount of potassium was released rapidly from platelets into serum after coagulation had occurred. Additional incubation of clotted platelet-rich plasma at 37° C. was associated with release of still more potassium into the serum.

The effect of platelet disruption by freezing was

TABLE II

The effect of coagulation, clot retraction, and freezing on the serum potassium concentration

			Serum obtained from clotted platelet-rich plasma			Serum obtained from platelet-free plasma (platelets = <1,000 per cu. mm.)
	Platelet count of platelet-rich plasma ($\times 10^3$)	Whole platelet-rich plasma	Plasma frozen and thawed five times before coagulation with thrombin*	Clot removed 60 min. after coagulation with thrombin (clot had retracted)	Clot removed one min. after coagulation with thrombin (no clot retraction)	Clot removed 60 min. after coagulation with thrombin (no clot retraction)
	per cu. mm.	*mEq./L.*	*mEq./L.*	*mEq./L.*	*mEq./L.*	*mEq./L.*
Patient M. H.	3,250		9.6	8.6	7.0	4.2
	3,226			8.0	7.2	5.4
Patient R. C.	2,730	7.4	7.3	5.8		4.5

* The results were essentially the same whether the platelets were removed by centrifugation prior to clotting by thrombin or not.

studied as follows: Platelet-rich and platelet-free ACD plasmas were rapidly and repeatedly frozen and thawed using an alcohol-dry-ice mixture. Some of the frozen and thawed platelet-rich plasma was centrifuged to remove platelet fragments. All of the plasma specimens were clotted by the addition of thrombin solution. After incubation for one hour at 37° C., the clots were removed and potassium concentrations determined. Results are shown in Table II. Disruption of the platelets by freezing released into the serum an additional increment of potassium (1.0 to 1.5 mEq. per liter) over and above that released by coagulation alone. The potassium concentrations in whole platelet-rich *plasma* and in *serum* obtained from frozen and thawed platelet-rich plasma were essentially identical, suggesting that freezing released virtually all of the potassium from platelets (see Table II, Patient R. C.). An aqueous suspension of platelets prepared from the blood of Patient R. C. was frozen and thawed five times and then centrifuged at high speed. The supernatant was removed and hydrochloric acid was added to the sediment in a volume equal that of the supernatant. This was allowed to stand at 5° C. for four weeks. The potassium concentration of the aqueous supernatant was 2.4 mEq. per liter and that of the hydrochloric acid used for leaching the sediment was less than 0.5 mEq. per liter. These data indicate that disruption of platelets by freezing releases most of the potassium from the platelets.

The effect of incubation of *unclotted* platelet-rich plasma prepared from Patient R. C. was studied as follows: Platelet-rich ACD plasma was incubated at 37° C. and at 2° C. At intervals, aliquots were removed and centrifuged at high speed to remove the platelets. Potassium concentrations were determined in the supernatant *platelet-free* plasma. The whole platelet-rich plasma had a potassium concentration of 6.8 mEq. per liter. Platelet-free plasma specimens, whether freshly prepared or the supernatant from incubated and centrifuged platelet-rich plasma, had a potassium concentration of 5.4 ± 0.1 mEq. per liter. It is apparent that no measurable amount of potassium was released from the platelets during incubation of unclotted plasma for 60 minutes either at 37° C. or at 2° C.

The platelets of Patient R. C. with *spurious hyperkalemia* were subjected to various experimental conditions *in vitro, e.g.,* incubation at 37° C. for several days, exposure to glass surfaces in rotating flasks, and incubation in hypotonic solutions. Survival, as measured by clot retraction (6), was not different from that of normal platelets. This patient's platelets likewise showed normal *in vivo* survival when transfused into a patient with severe thrombocytopenia. Thus there was no evidence of abnormal fragility or shortened potential lifespan.

Platelet potassium and sodium concentrations

Platelet potassium concentration was originally estimated in terms of platelet counts in plasma containing more than several million thrombocytes per cu. mm. These data suggested that the platelets in some cases of thrombocytosis might contain as much as twice the amount of potassium as normal platelets. This was not explained by a larger size of the average platelet since one of the patients with *spurious hyperkalemia* had platelets which appeared smaller than normal. Furthermore, our experience indicated that it was difficult to obtain accurate platelet counts in platelet-rich plasma containing greater than two million per cu. mm. The need for more precise measurement was obvious.

Table III shows the results of the direct determination of potassium in platelet plugs isolated from normal subjects and from patients with chronic myeloid leukemia, myeloid metaplasia and polycythemia vera. The potassium concentration of normal platelets had a mean value of 69.1 mEq. per Kg. of platelet mass (wet weight). A slight but definite increase in platelet potassium was observed in the patients with myeloid metaplasia and polycythemia vera. In chronic myeloid leukemia there was little change from the normal.

The amount of sodium in platelets was also measured. No significant difference was observed in the sodium concentration of platelets isolated from normal subjects and from thrombocythemic patients. The water content of platelets from normal subjects and thrombocythemic patients was 78.8 to 80.9 and 79.2 to 80.0 per cent, respectively. The concentrations of potassium and sodium in terms of platelet water are presented in Table III.

The volume of the packed platelet mass isolated from 40.0 ml. of normal platelet-rich plasma was 0.2 to 0.4 ml. Patients with thrombocythemia had

TABLE III

Quantitative measurement of platelet potassium and sodium

Number of experiments	Patient and diagnosis	Potassium *mEq./Kg.* platelets (wet weight)	Potassium *mEq./liter* of platelet water	Sodium *mEq./Kg.* platelets (wet weight)	Sodium *mEq./liter* of platelet water
9	Seven normal subjects	69.1 (65–71)*	86.4 (81.1–88.8)	27.0 (25–28)	33.7 (31.6–35.0)
4	Chronic myeloid leukemia (Patients B. C. and A. W.)	71.2 (67–72) $p > 0.1$†	89.0 (83.5–90) $p > 0.1$	25.0 (23–26) $p > 0.1$	31.2 (28.7–32.5) $p > 0.1$
8	Myeloid metaplasia (Patient R. C.)	74.8 (72–76) $p < 0.01$	93.4 (90–95.2) $p < 0.01$	24.5 (22–25) $p > 0.1$	30.6 (27.5–31.6) $p > 0.1$
6	Polycythemia vera (Patient C. N.)	76.1 (73–80) $p > 0.001$	96.1 (91.2–100) $p > 0.001$	25.2 (24–27) $p > 0.1$	31.5 (29.9–33.7) $p > 0.1$

* Figures in parentheses represent the range of determined values.
† p values refer to significance of the variation from the values of normal platelets.

platelet volumes of 1.0 to 3.0 ml. per 40 ml. of platelet-rich plasma. A knowledge of this volume factor plus the potassium concentration per liter of platelet mass allows one to calculate the amount of potassium contributed to platelet-rich plasma by platelets. For example, in a patient with chronic myeloid leukemia the potassium concentration of platelet-rich plasma was 8.8 mEq. per liter and that of platelet-free plasma 5.6 mEq. per liter. Forty ml. of this patient's platelet-rich plasma yielded 2.0 ml. of platelets. Hence, the volume of platelet mass per liter of plasma was 50 ml. The concentration of platelet potassium in this patient was 70.1 mEq. per liter of platelet mass (wet weight). Therefore, the amount of potassium contributed by this volume of platelets to a liter of plasma was 3.5 mEq. per liter. This figure agrees quite well with the experimentally determined value, 3.2 mEq. of potassium per liter of plasma (platelet-rich plasma K^+ minus platelet-free plasma K^+).

Influence of red blood cells on serum potassium concentration

The potassium concentration of red blood cells is approximately 25 times greater than that in plasma. Escape of potassium from the red blood cells into plasma or serum *in vitro* may significantly alter potassium levels. In the present studies, however, serum from platelet-rich plasma prepared from the patients with *spurious hyperkalemia* (M. H., R. C., and R. W., Table I) consistently had higher potassium concentrations than did serum from whole blood. During the processing of the specimens, the platelet-free plasma was actually in contact with the red cells much longer than was the platelet-rich plasma. Had the escape of potassium from the red cells been a significant factor, hyperkalemia would also be present in platelet-free plasma. Such was never the case.

Red blood cell counts in platelet-rich plasma ranged from 0 to 5,200 per cu. mm. in 16 of 18 experiments, and in two instances, the counts were 8,800 and 10,000 per cu. mm. Red blood cell counts in 14 different samples of *platelet-free* plasma were: 9 samples, 0 per cu. mm.; 4 samples, less than 20 per cu. mm.; and 1 sample, 3,400 per cu. mm.

Small amounts of normal blood (1 part to 1,000 to 5,000 parts) were added to normal ACD *platelet-free* plasma to simulate the concentrations of red cells present in platelet-rich plasma. Aliquots were frozen and thawed repeatedly with an alcohol-dry-ice mixture in order to hemolyze the red blood cells. Following clotting by thrombin and incubation of the clots at 37° C., potassium was determined on the serum. Results are shown in Table IV. An increase in serum potassium concentration occurred only after hemolysis of approximately 100,000 red blood cells per cu. mm.,

and visible hemolysis was obvious. No increase in serum potassium concentration was noted after freezing and thawing samples containing 12,500 red blood cells per cu. mm. although hemolysis was still clearly visible. Similar results were obtained in experiments performed on adjusted platelet-free plasma from two of the patients with *spurious hyperkalemia* (M. H. and R. C.). In the present studies, all plasma samples contained less than 10,000 red blood cells per cu. mm., and there was no visible hemolysis in any plasma or serum sample. These data clearly indicate that potassium escape from red blood cells did not influence the potassium determinations.

Influence of white blood cells on serum potassium concentration

Six of the nine patients with thrombocytosis had white blood cell counts greater than 10,000 per cu. mm. However, the two patients with the most marked *spurious hyperkalemia* (M. H., Table I; and W. S.) had normal white blood cell counts at the time of study. Furthermore, the only patient (H. L., Table I) with a white blood cell count more than 35,000 per cu. mm. did not have hyperkalemia. The peripheral white blood cell count in Patient R. C. (Tables I and II) ranged between 25,000 and 35,000 per cu. mm. Over two million platelets per cu. mm. were present in three samples of platelet-rich plasma prepared from Patient R. C., and the white blood cell counts on these plasmas were 2,000, 50, and 20 per cu. mm., respectively. Hyperkalemia was present in the serum obtained from these platelet-rich and white blood cell-deficient plasmas (Table II). These results suggest that the white blood cells present during coagulation did not influence the potassium determinations.

Formed elements in isolated platelet plugs

The red and white cell counts on platelet-rich plasma from which platelet plugs were isolated were in all instances less than 222 and less than 329 per cu. mm., respectively. In addition, the button of red cells and surrounding whitish material were cut away from the frozen platelet plug before cation determinations.

Influence of formed elements in serum on potassium determinations

The presence of large numbers of formed elements in *serum* could influence the potassium determinations by virtue of their content of this electrolyte. Serum obtained from clotted platelet-rich and platelet-free plasma was examined by phase microscopy in 14 experiments, including studies on three patients (M. H., R. C., and H. L.) with spontaneous thrombocytosis and one patient (N. V.) with postsplenectomy thrombocytosis. Less than 20 red blood cells per cu. mm. of serum were seen in 13 of 14 samples, and in one there were 2,000 red blood cells per cu. mm. No white blood cells were seen in the serum in 8 of 14 experiments. In the remaining six instances the white blood cell counts were: 2,900, 156, 150, 100, 50, and 6 white blood cells per cu. mm. of serum. No platelets were seen in the serum in 11 of 14 experiments. In three instances the platelet counts were: 4,000, 3,000, and 2 platelets per cu. mm. of serum. Thus, no formed elements were present in the serum prepared from most samples of platelet-rich and platelet-free plasmas. The few

TABLE IV

Effect of red blood cells on serum potassium concentration

Red cells per *cu. mm.*	112,000	12,500	5,800	1,096	3
White cells per *cu. mm.*	203	20	10	2	0
Platelets per *cu. mm.*	3,700	594	286	128	161
I. Untreated plasma					
Serum potassium (*mEq./L.*)	3.6	3.5	3.5	3.5	3.6
Appearance of serum	Not hemolyzed	Not hemolyzed	Not hemolyzed	Not hemolyzed	Not hemolyzed
II. Plasma frozen and thawed					
Serum potassium (*mEq./L.*)	4.4	3.5	3.5	3.5	3.5
Appearance of serum	Cherry red	Slightly red	Slightly pink	Not hemolyzed	Not hemolyzed

formed elements seen in occasional specimens were insufficient to influence the serum potassium determinations. Serum from clotted whole blood contained many formed elements, particularly red blood cells. These, however, were removed from the serum by high speed centrifugation prior to the potassium determinations.

DISCUSSION

The studies presented clearly indicate that some patients with thrombocytosis may have an associated *spurious hyperkalemia* in the absence of symptoms or signs of hyperkalemia. The circulating plasma in these patients contained a normal potassium concentration, as demonstrated by the fact that sera prepared from platelet-free plasmas had normal potassium concentrations. Initially, the etiology of the thrombocytosis in the first patient (M. H.) studied was obscure. Subsequently the patient developed the picture of agnogenic myeloid metaplasia. The thrombocytosis was associated with agnogenic myeloid metaplasia in three of the four patients with *spurious hyperkalemia,* and the fourth patient had thrombocytosis of undetermined etiology. The elevated potassium concentration in serum prepared from clotted blood returned to normal in one patient (W. S.) with myeloid metaplasia when the platelets were suppressed by therapy with Myleran.® A similar observation has been reported following therapy with radioactive phosphorus (7).

In any patient with an unexplained hyperkalemia, platelet concentrations should be measured by platelet count or determination of the volume of packed platelets. If the platelets are elevated, potassium concentration should be measured in serum obtained from carefully prepared platelet-free plasma. Others (7) have reported elevated potassium levels in platelet-free as well as platelet-rich plasma from similar patients, but it is not clear that the methods employed were adequate to prevent escape of potassium from the platelets and red blood cells during the preparation of platelet-free plasma.

Potassium was released from the platelets during or after the process of coagulation. It was, however, not necessary for the clot to undergo retraction in order to bring about release of potassium from the platelets. The potassium concentration was markedly elevated in serum from platelet-rich plasma within one minute after coagulation by thrombin (Table II). Previous studies have suggested that platelets undergo profound changes within seconds after coagulation occurs using similar experimental conditions (6). Clot retraction was associated with a further release of potassium into the serum (Table II). Whether this was due to clot retraction *per se* or simply to the additional period of incubation required for retraction to occur is not known.

Vasoconstrictor (serotonin) activity has been reported to be low in serum obtained from some patients with thrombocytosis (8, 9). The biological assay used is based on the degree of constriction of vascular and other muscle tissue suspended in the serum. The potassium concentration in fluid media is known to influence muscular activity. The *spurious hyperkalemia* present in some cases of thrombocytosis might influence serum vasoconstrictor activity determinations.

The method employed in isolating platelet plugs provides a means of measuring platelet cations under the most physiological conditions possible. The isolation of platelets from blood in the absence of an anticoagulant was necessary, since use of the anticoagulant, ACD, was found to reduce platelet potassium by as much as 5 to 10 per cent. A similar loss of potassium has been demonstrated in red cells collected in ACD solution (10).

When platelet potassium was estimated on the basis of platelet counts, the data suggested that the concentration of this cation in thrombocythemic platelets might be much greater than that in normal platelets. On the other hand, direct determination of potassium in isolated platelets showed only a relatively slight increase above normal in patients with thrombocytosis (Table III). This increase could not account for the abnormally high serum potassium observed in such patients. It is concluded from these studies that the *spurious hyperkalemia* was mainly due to an increased platelet mass per unit of blood or plasma, and only slightly to increased platelet potassium concentration.

The initial misleading estimates based on platelet counts were apparently due to difficulty in counting all of the platelets in thrombocythemic plasma. When such large numbers of platelets are concentrated in platelet-rich plasma during centrifugation, there appears to be great likelihood that these elements will collide and form clumps.

Under such circumstances platelet counts may greatly underestimate the number of platelets present. In patients with thrombocytosis, it appears essential to employ platelet volume or weight studies in order to ascertain platelet mass.

The sodium, potassium and water contents of platelets observed in the present study are interesting. The water content (80 per cent) was found to be similar to that of the white blood cell (11) but different from that of the red blood cell (65 per cent). The concentrations of the cations, sodium and potassium are only slightly different than in the red blood cell. The present study demonstrates that the cation composition of human platelets shows the pattern of intracellular fluid, *i.e.,* a preponderance of potassium and a relatively small amount of sodium. These findings suggest that platelets may be enveloped by a semipermeable membrane, and that they may possess a sodium and potassium pump mechanism. Experiments are in progress to test the latter hypothesis.

SUMMARY

Elevated serum potassium concentrations were noted in 4 of 13 patients with thrombocytosis in the absence of symptoms or signs of hyperkalemia. This represented a *spurious hyperkalemia* due to release of potassium from the platelets during coagulation, since serum prepared from platelet-free plasma had a normal potassium concentration.

Coagulation, incubation of clotted plasma, and disruption of platelets by freezing were associated with release of additional increments of potassium from the platelets. Potassium was released from the platelets immediately after coagulation and prior to retraction of the clots. Potassium was not released from the platelets during incubation of unclotted plasma for a period of one hour.

The mean potassium concentration of normal platelets was found to be 69.1 mEq. per Kg. of platelets (wet weight) or 86.4 mEq. per liter of platelet water. There was no appreciable increase in platelet potassium in chronic myeloid leukemia. Platelet potassium was relatively increased in patients with polycythemia vera and myeloid metaplasia. This increase, however, was insufficient to account for the *spurious hyperkalemia* present in some patients with thrombocytosis. It was concluded that the hyperkalemia was mainly due to an increased platelet mass per unit volume of blood or plasma, and only slightly due to increased platelet potassium concentration.

Platelets were found to have a water content of approximately 80 per cent and to contain small amounts [25 mEq. per Kg. of platelets (wet weight)] of sodium. The method employed provides a means of measuring platelet cations under the most physiological conditions possible.

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