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D H Cowan, … , R C Graham Jr, D Baunach

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Research Article

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The Platelet Defect in Leukemia

PLATELET ULTRASTRUCTURE, ADENINE NUCLEOTIDE METABOLISM, AND THE RELEASE REACTION

DALE H. COWAN, RICHARD C. GRAHAM, JR., and DOROTHY BAUNACH

From the Department of Medicine, Case Western Reserve University School of Medicine at Cleveland Metropolitan General Hospital and University Hospitals of Cleveland, Cleveland, Ohio 44109

ABSTRACT The ultrastructure and adenine nucleotide metabolism of platelets from patients with acute leukemia were studied to elucidate possible mechanisms for the platelet dysfunction observed in this clinical setting. Nonstimulated (resting) platelets from leukemic patients varied greatly in size; exhibited marked variation in the number of alpha granules present per cell; had poorly delineated circumferential bands of microtubules; and often grossly dilated open channel systems or cytoplasmic vacuolization. The intracellular concentrations of ATP and ADP were significantly below normal, and the specific radioactivity of ATP and ADP of nonstimulated platelets in leukemia was equivalent to or exceeded that seen in stimulated normal platelets. Addition of ADP or collagen to platelets from leukemic patients was followed by retarded and incomplete shape change, delayed and incomplete centripetal migration of subcellular organelles, impaired degranulation, and the formation of loose aggregates composed of relatively few platelets. Stimulation of "leukemic" platelets with collagen led to the release of significantly subnormal amounts of ATP and ADP and no significant change in the specific radioactivity of the intracellular nucleotides. In contrast to the results in normal platelets, the conversion of ATP to inosine monophosphate and hypoxanthine in platelets in leukemia failed to increase significantly with collagen stimulation. The results indicate that abnormalities exist in the storage pool of adenine nucleotides and the release mechanism of platelets in acute leukemia. These defects appear to contribute to an impairment in the release reaction in these platelets. Many of the ultrastructural and metabolic defects seen in acute leukemia occur in platelets in preleukemia.

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INTRODUCTION

Patients with acute leukemia may exhibit abnormal bleeding time, tourniquet test, clot retraction, platelet aggregation, platelet factor 3 availability, and nucleotide release (1–8). A major event leading to platelet aggregation and the primary arrest of bleeding is the release of ADP and other cellular constituents from platelets after stimulation with a variety of substances (9). This event, termed the "release reaction" by Grette (10), is part of a secretory-contractile process and is accompanied by biochemical changes which provide for increased availability of metabolically active ATP.

Since the release of endogenous ADP plays a central role in the physiology of platelet plug production, studies were undertaken to define in detail the biochemistry and morphology of the platelet release reaction in acute leukemia. Platelet ultrastructure and adenine nucleotide metabolism were studied before and after stimulation of platelets with collagen. Abnormalities were found in the storage pool of adenine nucleotides, the contractile response to stimulation, and the conversion of ATP to hypoxanthine. These abnormalities appear to underlie the observed reduction in the release of ADP and ATP from stimulated platelets from patients with leukemia.

METHODS

Studies were done using platelets obtained from seven normal subjects and nine patients with acute leukemia (includ-

TABLE I

Clinical Profiles of Patients with Leukemia

Patient	Diagnosis	Hgb	Hct	Leukocytes	Platelets	Blasts	Remarks
		g/100 ml	%		*no./μl*	%	
D. J.	CML-BC	10.4	31.5	24,100	54,000	20	Ph 1 (+)-aneuploidy
J. C.	CML-BC	9.0	29.3	25,400	61,000	40	Ph 1 (+)-aneuploidy
R. T.	CML-BC	10.5	29.8	7,500	333,000	60	Ph 1 (−)
H. J.	AML	9.0	28.6	7,800	92,000	18	—
H. M.	AML	8.8	27.6	6,400	76,000	15	—
E. G.	AMoL	7.7	23.9	17,100	605,000	5	Muramidase-100 μg/ml
E. A.	AMML	11.4	34.3	3,100	70,000	0	Muramidase-100 μg/ml
L. F.	HCL	11.0	32.5	7,600	154,000	0	—
P. M.	HCL	7.8	23.8	10,200	62,000	0	—
Normal		14–16	42–50	4,400–9,500	274,000±48,000	0	

* CML-BC, chronic myelocytic leukemia—blast crisis; AML, acute myelocytic leukemia; AMoL, acute monocytic leukemia; AMML, acute myelomonocytic leukemia; HCL, hairy cell leukemia (leukemic reticuloendotheliosis).

ing three patients with chronic myelocytic leukemia in blast crisis). The patients, grouped according to the cellular type of leukemia, and the pertinent hematologic data are listed in Table I. Patients with clinical evidence of bleeding or infection were excluded. The patients had not ingested drugs presently known to affect platelet function for 7 or more days before the study. All patients were studied before initiation of chemotherapy. All studies were done in accordance with the current HEW guidelines for research in human subjects and were approved by the local hospital and medical school committees on investigations in humans.

The metabolism of adenine nucleotides was studied using a modification of the method described by Holmsen and Weiss (11). Platelets were collected from 1 or 2 U of blood by thrombopheresis using Fenwal packs PA 20 and PA 220 (Travenol Laboratories, Morton Grove, Ill.) from which the acid-citrate-dextrose anticoagulant was removed and to which heat-sterilized EDTA 0.054 in 0.056 M sodium chloride was added. In subjects with normal hematocrits, 56 ml of anticoagulant was added to 444 ml of blood to give a final volume in the pack of 500 ml. In anemic subjects, the amount of anticoagulant used was adjusted in relation to the hematocrit. Blood packs were centrifuged at 900 *g* for 4 min at room temperature. The resulting supernate was spun at 1,500 *g* for 4 min then for 2½ min at 4°C to remove red cells, at 110 *g* for 10 min at 4°C to remove leukocytes, and finally at 1,800 *g* for 15 min at 4°C to sediment the platelets. A portion of the supernatant platelet-poor plasma was then removed and the platelets were gently resuspended in the remaining plasma. The resuspended platelet-rich plasma (PRP) [1] constituted the platelet suspension used in the studies of adenine nucleotide metabolism. The platelet concentration in PRP prepared from normal and leukemic subjects ranged from 0.63 to 1.22×10^9/ml (av 0.94×10^9/ml) and from 0.22 to 1.30×10^9/ml (av 0.74×10^9/ml), respectively. Pilot studies with normal platelets showed that the amounts of ADP and ATP released in response to collagen, determined on a per platelet basis, were similar over the range of platelet counts used in this study. Contamination of the final PRP by red cells and leukocytes was always less than 1/2,000 platelets and 1/10,000 platelets, respectively.

Platelets were labeled with [8-^{14}C]adenine ([8-^{14}C]adenine CFA 348, 62 mCi/mmol, Amersham/Searle Corp., Arlington Heights, Ill.) by incubating 19 parts of the platelet suspension with 1 part of [8-^{14}C]adenine for 2 h at 37°C. The [^{14}C]adenine was diluted to provide a final concentration in the suspension of 8.1 nmol/ml (= 0.5 μCi/ml).

Platelets were "stimulated" by adding soluble collagen [2] to the platelet suspension at a final concentration of 0.07 mg/ml and the mixture was incubated at 37°C. Tris-saline-EDTA (Tris, 25 mM; NaCl, 140 mM; EDTA, 3.14 mM), pH 7.4, was substituted for collagen in control tubes. All samples were shaken manually throughout the incubation with collagen or buffer. No aggregation was observed in the mixtures. Before and 5 min after addition of collagen or buffer, 1-ml aliquots of the platelet suspension were transferred to iced tubes containing 0.1 ml of 0.077 M EDTA, pH 7.4. The suspensions were centrifuged at 18,400 *g* for 10 min at 4°C. 2 0.4-ml aliquots of the supernates were obtained. One was added to tubes containing 0.4 ml EDTA-ethanol (1 part 100 mM EDTA: 9 parts 96% ethanol) and the other to 0.4 ml perchloric acid, 0.625 M. The sedimented platelets were suspended in 1.35 ml cold Tris-saline-EDTA after which an equal volume of EDTA-ethanol was added. After standing 10 min all tubes were centrifuged at 18,000 *g* for 10 min at 4°C to remove the precipitated proteins. The supernates from samples prepared with EDTA-ethanol were removed and frozen at −85°C until assayed. The supernates from samples treated with perchloric acid were neutralized with K_2CO_3. The resulting precipitates were removed by centrifugation and the supernates were stored at −85°C. Pilot studies showed that the adenine nucleotides and their metabolites were stable during storage at −85°C for up to 6 mo. Determinations of platelet ADP and ATP and of plasma ADP were done using

[1] *Abbreviations used in this paper:* G6P, glucose-6-phosphate; G6PD, glucose-6-phosphate dehydrogenase; HX, hypoxanthine; IMP, inosine monophosphate; LDH, lactate dehydrogenase; PEP, phosphoenolpyruvate; PK, pyruvate kinase; PRP, platelet-rich plasma.

[2] Soluble collagen was kindly provided by Dr. H. Bensusan of the Department of Biochemistry, Case Western Reserve University School of Medicine. It is provided as acid-soluble collagen, 1.3 mg/ml, in 0.1 M acetic acid.

TABLE II

ATP and ADP in Platelets

	Concentration					
	ATP platelets		ADP platelets		ATP/ADP platelets	
	Resting	Stimulated	Resting	Stimulated	Resting	Stimulated
	nmol/10⁹ platelets					
Control (7)*	50.9	34.1	34.2	14.7	1.4	2.1
±1 SD	5.5	5.3	5.2	3.6	0.2	0.2
Leukemic (9)	42.5	38.9	13.7	10.5	3.5	4.3
±1 SD	5.0	6.5	4.5	4.5	0.8	1.0
P‡	<0.01	NS	<0.001	<0.05	<0.001	<0.01

* The numbers in parentheses denote the number of subjects studied.
‡ The P values denote the significance between the results in each column for normal and leukemic subjects.

samples prepared with EDTA-ethanol. Plasma ATP was determined using samples precipitated with perchloric acid.

The adenine metabolites in the EDTA-ethanol extracts were separated on a Shandon high-voltage paper electrophoresis apparatus (Shandon Southern Instruments Inc., Sewickley, Pa.) using the method described by Holmsen and Weiss (11) and the radioactivity of the compounds was counted in a Packard Tri-Carb liquid scintillation counter, model 2425 (Packard Instrument Co., Inc., Downers Grove, Ill.), using Aquasol (New England Nuclear, Boston, Mass.) as scintillation fluid. The counting efficiency of all samples was 90%. Total radioactivity was determined as cpm/10^9 platelets and specific radioactivity was expressed as counts per minute per nanomole. Adenine uptake was measured as the total amount of adenine converted to ATP, ADP, AMP, inosine monophosphate (IMP), and hypoxanthine (HX), the latter including both intra- and extracellular HX (12). The percentage of radioactive adenine converted to HX via the metabolic adenine nucleotide pool was calculated from the formula:

$$\% \ {}^*HX = \frac{\text{cpm } {}^*HX_{\text{platelet + plasma}}/10^9 \text{ platelets}}{\text{cpm } {}^*\text{adenine}/10^9 \text{ platelet}} \times 100.$$

ATP was measured by determining fluorometrically the amount of NADPH produced when the hexokinase-catalyzed conversion, glucose → glucose-6-phosphate (G6P), with ATP as the phosphate donor, is coupled to the conversion G6P → 6-phosphogluconic acid in which $NADP^+$ → NADPH (13). Final concentrations of the reactants were: glucose 4×10^{-3} M, glucose-6-phosphate dehydrogenase (G6PD) 4×10^{-4} mg/ml (140 U/mg), $NADP^+$ 6.1×10^{-5} M, and hexokinase 4×10^{-3} mg/ml (190 U/mg). The reactants were suspended in a buffer containing triethanolamine-HCl, 50 mM; $MgCl_2$, 10 mM; EDTA, 5 mM. The buffer was adjusted to pH 7.4 with KOH before the addition of the reactants. A standard curve was constructed with each assay using ATP in concentrations ranging from 2 to 5×10^{-7} M. The change in NADPH was measured using a Turner fluorometer (model 111, G. K. Turner Associates, Palo Alto, Calif.) with excitation wavelength at 365 nm and fluorescence wavelength of 465 nm. ADP was measured in an analogous manner by determining the amount of reduced NAD (NADH) converted to NAD^+ by the coupled pyruvate kinase (PK)-lactic dehydrogenase (LDH) reaction in which ADP is the phosphate acceptor (13). Final concentrations of the reactants were: phosphoenolpyruvate (PEP), 2.6×10^{-4} M; LDH, 1.6×10^{-3} mg/ml (550 U/mg); and PK, 4×10^{-3} mg/ml (200 U/mg). The reactants were suspended in a buffer containing KH_2PO_4 6.6×10^{-2} M and $MgCl_2$ 5 mM, and adjusted to pH 7.0 with KOH. A standard curve was constructed with each assay using ADP at concentrations ranging from 2 to 5×10^{-7} M.

To measure ADP in the supernatant fractions, ADP was converted to ATP by the PK reaction and the ATP was measured using the coupled hexokinase-G6PD reactions.

For transmission electron microscopy, citrated platelets were fixed in 0.1%, then 3.0% glutaraldehyde, postfixed in 1% osmic acid, dehydrated in ethanol, and cleared in propylene oxide using the methods of White (14). The specimens were embedded in Epon-Araldite. Sections were cut using a diamond knife, stained with lead citrate and uranyl acetate, and examined in an AEI EM-6B electron microscope (AEI Scientific Apparatus Inc., Elmsford, N. Y.). For ultrastructural studies of stimulated platelets, citrated PRP containing 150,000–300,000 platelets/μl was incubated with ADP or collagen at final concentrations of 3.0×10^{-6} M or 35 μg/ml, respectively, at 37°C in an aggregometer (Payton Associates, Buffalo, N. Y.) with the stirrer rotating at 1,100 rpm. Aliquots of PRP were removed for fixation 4 min after addition of ADP and 6 min after addition of collagen.

Platelets were counted by phase microscopy (15). Protein determinations were done by the method of Lowry, Rosebrough, Farr, and Randall (16). The significance of the results was determined using the Student's t test (17).

LDH, rabbit muscle-EC 1.1.1.27, PK rabbit muscle, ammonium sulfate solution—EC 2.7.1.40, PEP, ammonium salt, and G6PD, yeast—EC 1.1.1.49 were purchased from Boehringer Mannheim Corp., New York. NADH and $NADP^+$ were obtained from P-L Biochemicals, Inc., Milwaukee, Wis. Hexokinase, yeast—EC 2.7.1.1, ADP-disodium salt, and ATP-disodium salt were obtained from Sigma Chemical Co., St. Louis, Mo. EDTA, disodium J. T. Baker Chemical Co., Phillipsburg, N. J.

RESULTS

Intracellular concentrations of ATP and ADP

The concentrations of ATP and ADP in resting (nonstimulated) platelets and platelets stimulated by collagen are shown in Table II. The concentrations of ATP and ADP in resting platelets in leukemic patients were significantly less than those observed in normal platelets. The concentration of ADP in stimulated platelets in leukemia was also significantly subnormal ($P < 0.05$) but that of ATP was normal. The ratio ATP/ADP was significantly greater than normal in both resting and stimulated platelets from leukemic patients ($P < 0.001$, $P < 0.01$, respectively).

Amount of ATP and ADP lost from platelets and released to plasma

Fig. 1 depicts the amounts of ATP and ADP lost from platelets stimulated by collagen and released to plasma. Significantly less of both nucleotides were lost from platelets of leukemic patients than from those of normal subjects ($P < 0.001$ for each). Similarly, less ATP and ADP were released to the plasma in leukemia ($P < 0.001$ for each). The ratio of ATP/ADP released from platelets in leukemia (0.5±0.5 SD) was not significantly different from normal (0.5±0.1 SD).

Uptake of [^{14}C]adenine by platelets and the specific radioactivity of ATP and ADP

The uptake of [^{14}C]adenine and the specific radioactivity of adenine nucleotides in resting platelets and platelets stimulated by collagen are listed in Table III. The amount of [^{14}C]adenine taken up by platelets from patients with leukemia was similar to that taken up by normal subjects. The specific radioactivity of ATP in normal platelets was significantly greater after stimulation with collagen than before ($P < 0.01$). The specific radioactivity of ATP in resting platelets from leukemic patients was significantly greater than that found in both resting and stimulated normal platelets ($P < 0.001$, $P < 0.001$, respectively). No significant change in the specific radioactivity of ATP occurred in platelets from leukemic subjects with collagen stimulation.

The specific radioactivity of ADP in resting platelets from patients with leukemia was significantly greater than that found in resting normal platelets ($P < 0.05$) and equivalent to that found in stimulated normal platelets. Stimulation with collagen was associated with a significant increase in specific radioactivity of ADP in normal platelets ($P < 0.001$) but not in platelets from patients with leukemia. The specific radioactivity of both ATP and ADP released to plasma in patients with leukemia was similar to that measured in normal subjects.

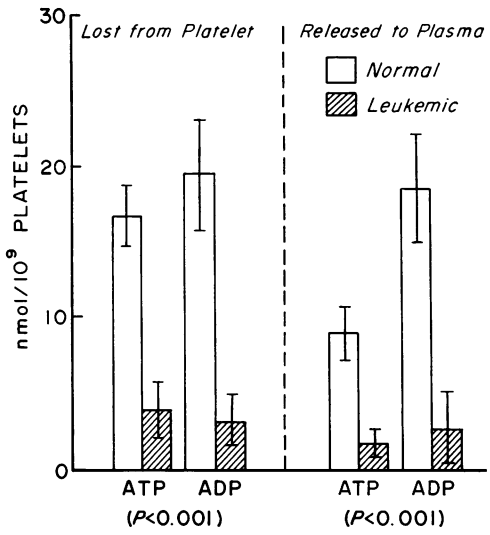

FIGURE 1 Release of ATP and ADP from platelets after collagen stimulation. The vertical lines within each bar denote ±1 SD. The P values denote the significance of the difference between the values for normal and leukemic subjects in each pair of bars.

Conversion of ATP to IMP and HX

Greater than 99% of the [^{14}C]adenine was taken up by the platelets before stimulation by collagen. With collagen stimulation of normal platelets, the radioactivity of intracellular ATP and ADP decreased significantly ($P < 0.05$, $P < 0.01$, respectively) (Table IV). By contrast, significant decreases in the radioactivity of intracellular ATP and ADP were not observed with collagen stimulation of platelets from patients with leukemia. The decreases in the radioactivity of the adenine nucleotides in platelets from both normal and leukemic subjects occurring with stimulation were accompanied by concomitant increases in the radioactivity of inosine and HX (Table IV).

The proportion of labeled adenine converted to HX by resting platelets from both normal subjects and patients with leukemia was similar (Table V). The net increase in the conversion of labeled adenine to HX with collagen stimulation of normal platelets was significant ($P < 0.01$) whereas that observed in platelets from leukemic patients was not significant.

Ultrastructure of resting and stimulated platelets

The ultrastructure of resting platelets from normal subjects and from patients with acute leukemia is shown in Fig. 2. Normal platelets (Fig. 2A) are disk shaped and have a number of readily defined intracellular structures including a circumferential band of microtubules, an open channel system, glycogen, alpha granules, dense bodies, and mitochondria. In contrast to the uniform appearance of normal platelets, platelets from patients

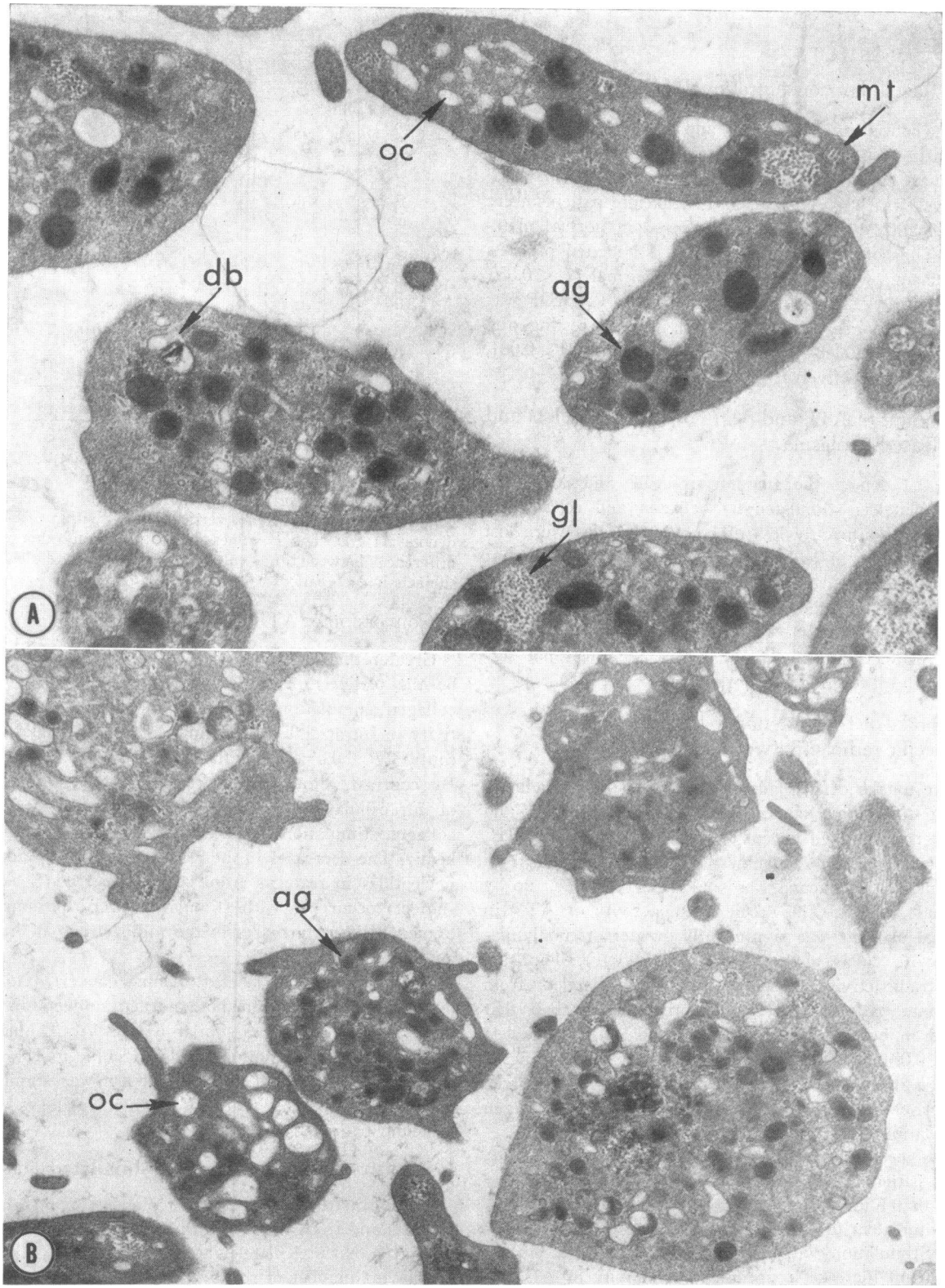

FIGURE 2 (A) Normal platelets. Cytoplasmic structures visible include alpha granules (ag), a dense body (db), profiles of the open channel system (oc), glycogen particles (gl) and the circumferential band of microtubules (mt). × 23,000. (B) Platelets from patient with acute myelomonocytic leukemia. Individual platelets are quite variable in size and shape. Some platelets contain an unusually large number of alpha granules (ag), many of which are abnormally small. One platelet contains an unusually dilated open channel system (oc). × 17,000.

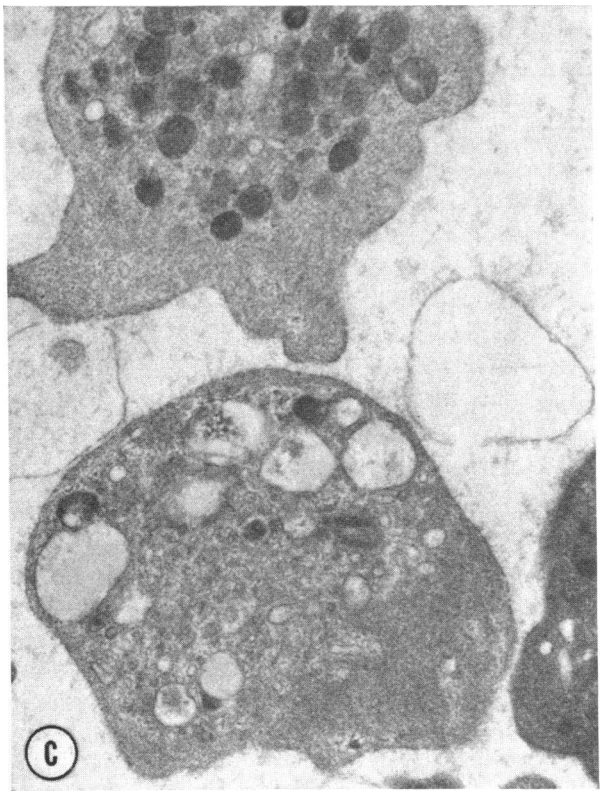

FIGURE 2 (C) Platelets from patient with acute myelomonocytic leukemia. The cytoplasm of one platelet, which contains a variety of disorganized membranous structures, is virtually devoid of alpha granules. × 20,000.

with acute leukemia (Fig. 2B) varied greatly in size and internal structure. Alpha granules varied greatly in size; many were unusually small, although very large granules, sometimes of unusual shape, also were encountered. The number of alpha granules per platelet was highly variable, with some cells having an unusually large number of small granules while others appeared virtually devoid of granules (Fig. 2C). The number of dense bodies in platelets from leukemic patients appeared to be similar to that in normal platelets, although actual quantification was not attempted. Some platelets in leukemia had an unusually extensive, often dilated, open channel system. The circumferential band of microtubules was poorly defined in some leukemic platelets, while in others it was readily discernible.

Stimulation of normal platelets with ADP or collagen was accompanied by a change in the shape of the platelets from disks to spheres, an inward migration of the granules, degranulation, and formation of aggregates in which the outer membranes of adjacent cells could not be distinguished (Fig. 3A). Stimulation of platelets from leukemic patients was associated with a less uniform shape change, incomplete centripetal migration of granules, incomplete degranulation, and the formation of small, loose aggregates (Fig. 3B). Additionally, some platelets showed persistence of the dilated open channel systems (Fig. 3C). Since the concentrations of platelets in PRP from normal and leukemic subjects were similar the differences in the size of the platelet aggregates could not be related to differences in the number of platelets present.

Comparative studies in preleukemia

Adenine nucleotide metabolism. Adenine nucleotide metabolism and platelet ultrastructure also were studied in two patients with preleukemia diagnosed using the criteria discussed by Linman and Saarni (18). The concentrations and specific radioactivities of ATP and ADP in resting and collagen-stimulated platelets and the amounts and specific activities of these nucleotides released to plasma after stimulation are listed in Table VI. The concentrations of ATP and ADP in resting platelets from both patients with preleukemia and the amounts of these nucleotides released to plasma after collagen stimulation were significantly subnormal (> 2 SD below normal).

The uptake of [^{14}C]adenine by platelets from the two patients with preleukemia was normal. The specific radioactivity of ATP in resting platelets from one patient with preleukemia (L. F.) was subnormal and in platelets from the other patient (R. B.) was greater than normal. The specific radioactivity of ADP in resting platelets from the patients with preleukemia exceeded by more than 2 SDs that measured in normal resting platelets. Additionally, the specific activity of ADP in the resting platelets from one of the patients (R. B.) exceeded by more than 2 SDs the levels measured in stimulated normal platelets. The specific radioactivity of ADP increased 2.5-fold after collagen stimulation of platelets from this patient. A similar increase in the specific radioactivity of ATP was not observed. There was no consistent abnormality in the specific activities of the nucleotides released to plasma.

The conversion of [^{14}C]adenine to HX by nonstimulated platelets in the patients with preleukemia (49.7, 52.8%) significantly exceeded (> 2 SD) normal (31.5±7.9 SD). The net increase in the conversion of labeled adenine to HX that occurred with collagen stimulation was normal in platelets from both patients with preleukemia.

Platelet ultrastructure. Many platelets from the patients with preleukemia showed structural abnormalities similar to those described in platelets in acute leukemia, while others appeared to have normal structure (Fig. 4A). When stimulated, these platelets formed somewhat loose aggregates in which degranulation of a portion of the platelets occurred. Other platelets, which were abnormal in structure, not only failed to degranulate but

TABLE III

Uptake of [^{14}C]Adenine and Specific Radioactivity of ATP and ADP in Platelets

	Uptake of [^{14}C]adenine	Specific radioactivity					
		ATP			ADP		
		Platelets		Released to plasma	Platelets		Released to plasma
		Resting	Stimulated		Resting	Stimulated	
	nmol/10⁹ platelets/2 h	*cpm/nmol*			*cpm/nmol*		
Control (7)*	3.5	4,303	4,923	503	1,015	1,710	13
±1 SD	0.9	168	173	269	122	183	12
Leukemic (9)	4.0	6,861	7,311	312	2,261	2,918	3
±1 SD	1.1	588	695	162	1,318	1,108	2
P‡	NS	<0.001	<0.001	NS	<0.05	<0.05	NS

* The numbers in parentheses denote the number of subjects studied.
‡ The P values denote the significance between the results in each column for normal and leukemic subjects.

showed little evidence of centripetal migration of granules (Fig. 4B).

DISCUSSION

The present studies of nucleotide metabolism were done using platelets anticoagulated with EDTA. EDTA was used in order that PRP from thrombocytopenic patients could be centrifuged and the platelets resuspended in smaller volumes of plasma to provide suspensions with adequate numbers of platelets for study. Although EDTA diminishes the release of radioactive serotonin by collagen (19), the amounts of ATP and ADP released from normal platelets by collagen in the present study were similar to those released by collagen from normal platelets anticoagulated with sodium citrate (12, 20).

The results of the present studies suggest that in platelets from patients with acute leukemia, several biochemical and ultrastructural abnormalities accompany the marked reduction in the amounts of ATP and ADP released after stimulation with collagen. The concentrations of ATP and ADP in platelets in leukemia were significantly reduced and the specific radioactivities of these nucleotides in resting platelets in leukemia were equivalent to or greater than the values servation, when made in platelets from patients with a observed in stimulated normal platelets. The latter obfamilial disorder of the release reaction, was interpreted by Holmsen and Weiss (12) as indicating a selective depletion of storage (i.e. nonmetabolic) nucleotides. Thus, a storage pool defect similar to that described by

TABLE IV

Radioactivity of Adenine Nucleotides and Metabolites before and after Collagen Stimulation

	Platelet					Plasma	Total radioactivity
	ATP	ADP	AMP	IMP	HX	HX	
	% of total radioactivity						*cpm/10⁹ platelets*
Control (7)*							
Resting	58.6	8.1	0.8	1.0	1.4	30.1	441,072
(±1 SE)	6.7	0.4	0.1	0.1	0.2	3.1	46,983
Stimulated	43.0	5.5	1.0	2.9	5.0	42.6	430,070
(±1 SE)	6.6	0.8	0.1	0.4	0.8	5.8	62,752
Leukemic (9)							
Resting	60.4	6.4	1.0	1.0	1.9	29.4	513,279
(±1 SE)	10.5	1.3	0.1	0.2	0.5	5.6	93,902
Stimulated	50.2	6.0	1.3	2.2	4.6	35.7	509,022
(±1 SE)	6.5	0.7	0.1	0.5	1.2	8.5	88,826

* Number of subjects studied.
‡ Adenine radioactivity was found only in the plasma and was always <1% of the total radioactivity. Total radioactivity here refers to the Σ-radioactivity (ATP+ADP+AMP+IMP+HX).

Holmsen and Weiss (11, 12) in patients with storage pool disease would appear to be one mechanism for reduced nucleotide release in leukemia.

The impaired centripetal migration of granules in platelets from patients with acute leukemia after stimulation with collagen resembles that observed by Horowitz, Stein, Cohen, and White (21) studying the effect of guanidinosuccinic acid and by Weiss and Ames (22) studying the effect of aspirin on normal platelets. The latter authors also reported "paralysis" of the release reaction in one patient who subsequently developed acute myeloblastic leukemia. Impaired centripetal migration of subcellular organelles is suggestive of a defect in the release mechanism of platelets (22). Failure of reorientation of organelles to occur in response to collagen stimulation may be another defect contributing to the impaired release reaction in platelets in leukemia.

The dilation of the open channel system in some of the platelets from patients with acute leukemia and preleukemia resembles that described by Haguenau, Hollmann, Levy, and Boiron (23) in blast crisis of chronic myelocytic leukemia, by Weiss and Ames (22) in the patient who developed acute myelocytic leukemia, and by Maldonado, Pintado, and Pierre (24) in platelets from patients with chronic myeloproliferative disorders. The open channel system in platelets, the membrane of which appears to be continuous with the outer membrane, transmits stimuli which induce release, and conveys the released organellar constituents to the outside of the cell (25–27). The relationship between the structurally abnormal open channel system in platelets in leukemia and the impairment in the release mechanism is not known. Conceivably, the markedly dilated open channel system may be ineffective in transmission of release-inducing stimuli and thereby impair the release mechanism.

Day and Holmsen (9, 28) suggested that a portion of the ATP in platelets provides the energy for the release reaction and is rapidly degraded to IMP and HX. In the present study the loss of radioactive ATP from platelets from leukemic patients and the net increase in the conversion of ATP to HX after stimulation were significantly less than normal. Although the relationship between ATP degradation and the release reaction is not yet understood, it is possible that the subnormal degradation of metabolic ATP observed in platelets in acute leukemia may contribute to the defective release reaction.

In normal platelets, the decrease in metabolically active ATP that occurs rapidly after release induction appears to precede and to stimulate glycolysis and glucose oxidation, resulting in increased rates of production of CO_2 and lactate over those seen in resting platelets (9). Earlier studies from this laboratory showed that the increases in the production of CO_2 and lactate by stimulated platelets from leukemic patients were subnormal (29). The present data suggest that the reduced augmentation of glycolysis and glucose oxidation in stimulated platelets in leukemia may be due to a reduction from normal in the amount of metabolically active ATP degraded after release induction. The find-

TABLE V

Conversion of [^{14}C]Adenine to Hypoxanthine by Platelets in Acute Leukemia

	Platelets + plasma	
	Resting	Stimulated
	% Conversion	
Control (7)*	31.5	47.6
±1 SE	3.3	6.6
	$P < 0.01$	
Leukemic (9)	31.2	40.3
±1 SE	6.1	9.7
	NS	

* Number of subjects studied.

TABLE VI

Adenine Nucleotides in Platelets in Preleukemia

	Platelet					
	Resting		Stimulated		Released to plasma	
	ATP	ADP	ATP	ADP	ATP	ADP
Amount of nucleotide, *nmol/10^9 platelets*						
Control ±1 SD	50.9±5.5	34.1±5.3	34.2±5.2	14.7±3.6	9.0±2.3	18.6±4.7
RB	25.6	13.8	17.9	8.0	3.4	6.1
LF	33.5	15.1	26.9	9.1	2.1	6.8
Specific radioactivity, *cpm/nmol*						
Control ±1 SD	4,303±168	1,015±122	4,923±173	1,710±183	503±269	13±12
RB	5,507	4,754	5,612	11,371	0	0
LF	2,623	2,442	3,281	2,010	1,375	0

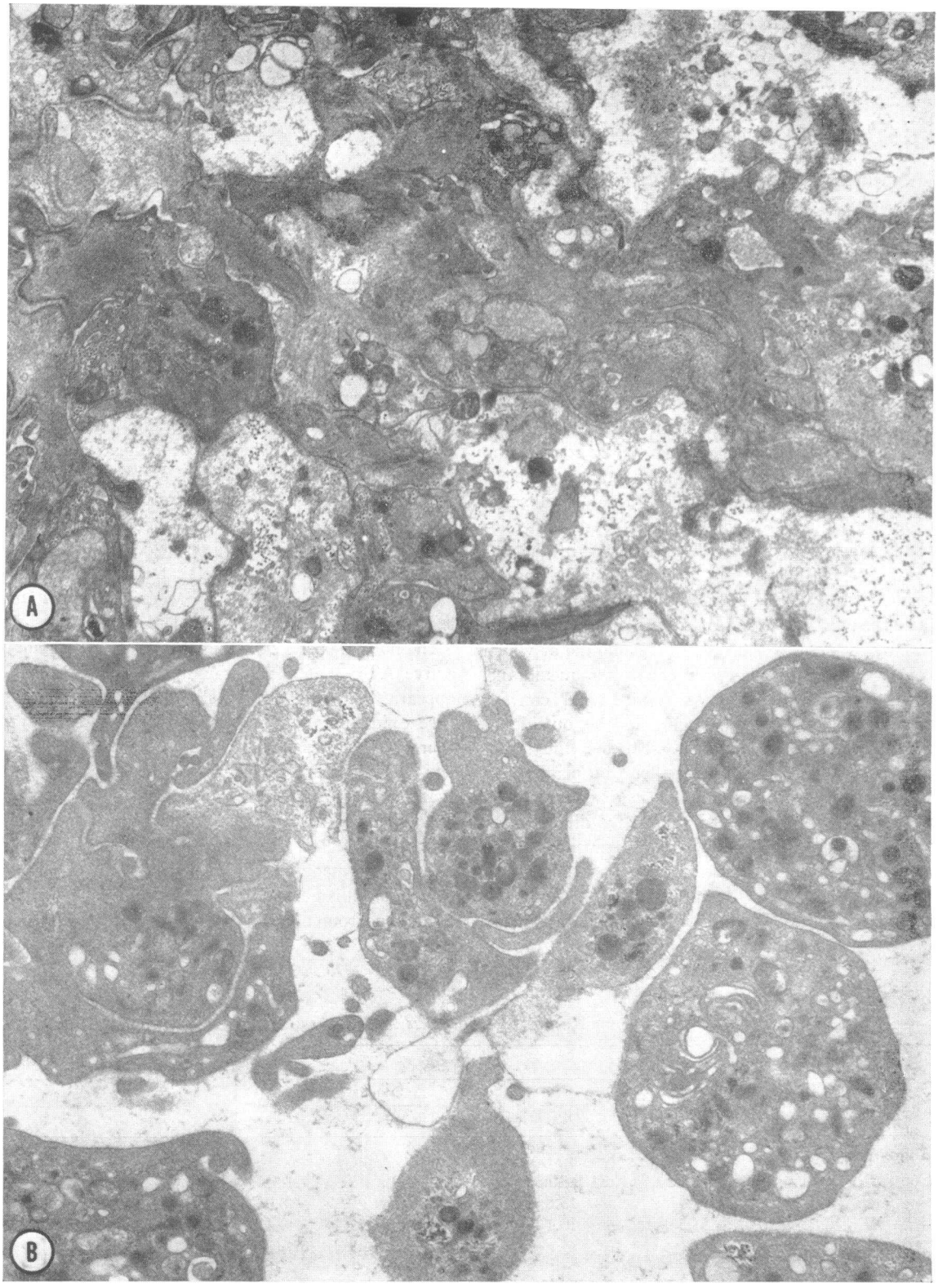

FIGURE 3 (A) Normal platelets following stimulation by ADP, 3×10^{-6} M. The platelets are closely approximated and degranulation is almost complete. Few alpha granules remain. × 17,000. (B) Platelets from patient with acute myelomonocytic leukemia after stimulation with ADP, 3×10^{-6} M. Small platelet aggregates show partial degranulation. Other platelets show no discernible alteration, with no centripetal migration of the numerous small alpha granules. × 17,000.

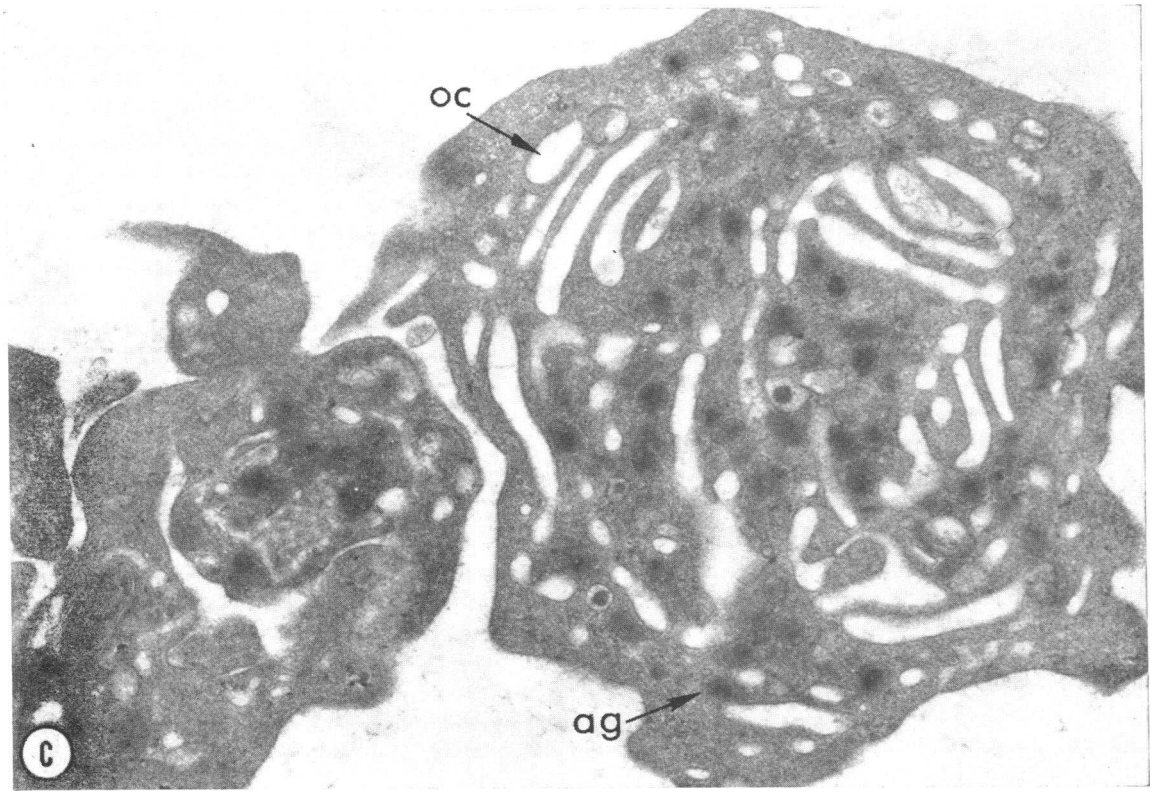

FIGURE 3 (C) Platelets from patient with acute myelomonocytic leukemia after stimulation with ADP, 3×10^{-6} M. Persistence of an unusually extensive open channel system (oc) is apparent in the platelet on the right. Extremely small alpha granules (ag) show no evidence of centripetal migration. × 30,000.

ings lend support to the suggestion that a relationship exists between the amount of ATP metabolized and the stimulus for ATP generation.

The studies of adenine nucleotide metabolism in platelets in preleukemia gave results similar to those observed in acute leukemia and suggest that reduced nucleotide release in preleukemia appears due in part to a decrease in the total cellular nucleotide concentration and to a specific deficit of storage pool ADP. These findings could account for the deficiency in the release reaction and the impairment in platelet function described in patients with this clinical condition (30). In contrast to the findings in acute leukemia, however, the apparent increase in ATP degradation associated with collagen stimulation of platelets in preleukemia was normal. A possible defect in the metabolism of metabolically active adenine nucleotides may nonetheless exist in platelets in preleukemia since the conversion of adenine to HX by resting platelets was significantly greater than normal.

The ultrastructural observations of platelets in preleukemia are consistent with the possibility that in this disorder both normal and abnormal populations of platelets are present in the peripheral blood. Maldonado et al. (24) recently reported similar morphologic abnormalities in platelets from patients with chronic myeloproliferative disorders. The similarities between the structural, functional, and biochemical changes in platelets in preleukemia and those in acute leukemia support the suggestion advanced by Maldonado et al. (24) that the morphologic changes in platelets in the myeloproliferative disorders relate to the hemostatic abnormalities. Although the present data provide some explanation for the defects in platelet function previously reported in acute leukemia (1–8), the clinical relevance of these findings to the bleeding diathesis in leukemia cannot be ascertained since the patients selected for study were not bleeding.

Since the life span of platelets in acute leukemia is short (8, 31) it could be proposed that the present findings are not specific for leukemia but are common to other thrombocytolytic states associated with large platelets. In studies to be published elsewhere (32), we observed that platelets from a patient with alcohol-related thrombocytopenia had subnormal amounts of intracellular ADP, a deficit in storage pool ADP, impaired release of ADP, and ultrastructural abnormalities similar to those described in acute leukemia. The con-

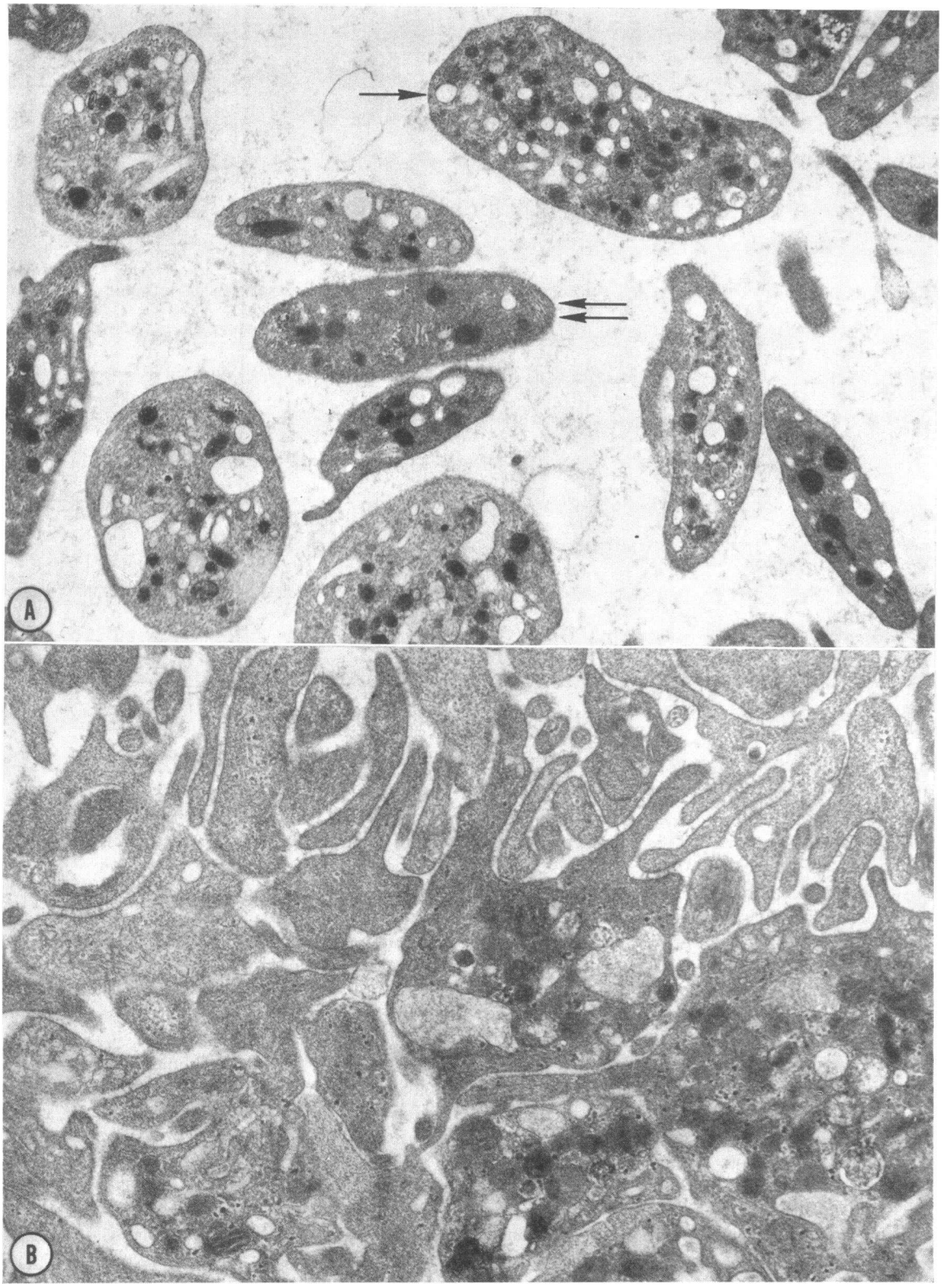

FIGURE 4 (A) Platelets from patient with preleukemia. Some of the platelets, which have numerous small alpha granules and an unusually extensive open channel system (single arrow), resemble platelets from patients with acute leukemia. Other platelets (double arrow) are not detectably abnormal. × 17,000. (B) Platelets from patient with preleukemia after exposure to ADP, 3×10^{-6} M. Platelets are aggregated, but distinct cell boundaries remain visible. No alpha granules are apparent in some of the platelets, while others contain numerous granules with no evidence of centripetal migration. ×29,000.

centration, release, and metabolic degradation of ATP, however, were not impaired. Platelets from two patients with immune thrombocytopenic purpura had normal concentrations of ATP and ADP and an apparent deficit of storage pool ADP, but showed no reduction in the amounts of ATP or ADP released with collagen stimulation or in the apparent conversion of metabolic ATP to HX (*32*). Additionally, although resting platelets in immune thrombocytopenic purpura showed greater variability in size than is seen in normal platelets, the morphologic alterations after stimulation by ADP and collagen were normal. While results are not available for all situations associated with large platelets and short platelet life span, these data lend support to the suggestion that the abnormalities observed in acute leukemia constitute a more extensive derangement of platelet structure, function, and metabolism than that seen in other clinical disorders.

The pathogenesis of the several alterations in platelet structure and function in acute leukemia remains a subject of conjecture. Data from this laboratory suggest that extracellular factors may promote some of the functional changes seen in platelets in acute leukemia (*7*). However, Van Der Weyden, Clancy, Howard, and Firkin (8) found platelet-poor plasma from patients with acute leukemia had no effect on ADP-induced aggregation of normal platelets. Alternatively, both structural and metabolic-functional abnormalities in platelets in acute leukemia may result from defective platelet production and reflect primarily a disturbance in megakaryocytic structure or development. Zittoun, Bernardou, and Samama (4) have suggested that the megakaryocytic line is affected by the leukemic process. A third possibility that could account for all of the biochemical, structural, and functional defects observed here is that the platelets may have undergone the release reaction during circulation. Additional studies are required to assess these possibilities.

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