aminohippurate. Hyperuricemia may initiate a cycle in which the nephrotoxic effects of excess urates in the blood gradually produce detectable impairment of renal function, thereby rendering the afflicted kidney more susceptible to further damage from hyperuricemia and less able to excrete the excess uric acid. Under certain circumstances, it is possible that uricosuric therapy, which increases the urate content in the distal tubules, might be harmful.

Summary. Renal function in dogs, as assessed by the clearances of creatinine and para-aminohippurate, was impaired by hyperuricemia induced by intravenous injections of uric acid. The degree of functional impairment was related to dosage and to frequency of injection and was possibly aggravated by the presence of renal disease. It was more severe in mongrel than in Dalmatian dogs. Small doses, which singly produced no detectable functional impairment, caused renal damage after repeated daily injections, indicating a cumulative effect. The findings suggest that hyperuricemia may produce significant nephropathy by urate blockage of the distal convoluted and collecting tubules. Such hyperuricemic nephropathy, reversible up to a certain point, may become chronic or fatal if permitted to continue unchecked.

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Changes in Platelet Volume Produced by Temperature, Metabolic Inhibitors, and Aggregating Agents.* (30516)

BRIAN S. BULL AND MARJORIE B. ZUCKER

Department of Clinical Pathology, Clinical Center, National Institutes of Health, Bethesda, Md., and American National Red Cross Research Laboratory and Department of Pathology, New York University Medical Center, New York City

Platelets circulate in the blood stream in the form of thin discs(1,2,3); they retain this shape *in vitro* for several hours when citrated blood is kept at 37°C. Aggregation produced by chilling(4), thrombin, or adenosine diphosphate (ADP)(5,6), is accompanied by a change in shape of platelets from discs to spheres with irregular surfaces and a variety of protruding processes(1,3,7,8). The relationship between platelet shape and aggregation is further suggested by the observation

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that prior incubation of platelet-rich plasma with adenosine monophosphate (AMP) prevents both clumping(6) and the change in platelet shape(8) caused by ADP. On the other hand, the association between aggregation and shape change is not invariable. The usual change in platelet shape from disc to sphere takes place even when ADP-induced aggregation is prevented by EDTA(8) or by guanidino compounds such as arcaine(9), or when aggregation is congenitally absent in platelets of patients with thrombasthenia(7). Conversely, platelets retain their disc shape (10) when clumped by epinephrine(11).

The foregoing observations on changes in platelet shape were made by inspection, a subjective technique that makes quantitation impossible. For detailed studies on platelet metabolism and membrane physiology an objective measurement is essential. Changes in platelet volume accompanying the disc-sphere transformation were investigated accordingly. For this purpose, a Coulter Counter and size distribution plotter were employed(12).

Methods. Fresh human blood was collected in acid-citrate-dextrose solution (1 part ACD formula A to 7 parts blood) and centrifuged slowly for a few minutes to prepare platelet-rich plasma (PRP). This was used in all experiments unless otherwise noted. A similar technique was used for specimens in which 1/75th volume 10% dipotassium dihydrogen ethylenediamine tetraacetate (EDTA) was used as anticoagulant. Final EDTA concentration in plasma was approximately 5.9 mM. In a few experiments, blood was drawn into a non-wettable container without any anticoagulant and with minimal exposure to air. After allowing 15-20 minutes for red cell sedimentation, platelet-rich plasma was withdrawn and diluted immediately in albumin-Eagle's solution.

Three types of suspension media were employed: non-lipemic ACD plasma passed several times through a 0.45 μ pore Millipore filter; Eagle's solution diluted 9:1 with distilled water(12) (here called E.S.); and E.S. containing 0.5% human serum albumin (Alb-E.S.). Alb-E.S. was used primarily in experiments designed to determine the effect of temperature on platelet size.

PRP was diluted approximately 1:3000 in the suspension medium, and a particle size distribution curve was plotted using the Coulter Counter Industrial Model B and plotter (12). The instrument was calibrated using a plasma or E.S. suspension of polyvinyltoluene latex particles of 4.51 or 22.26 μ^3 (Dow Chemical Corp.). Compounds to be studied were added in a volume of 0.5 ml or less to a beaker containing about 10 ml of the dilute platelet suspension. In addition, the compounds were often added to a small volume of undiluted PRP in a test tube to give the same final concentration. Aliquots from this tube were observed by phase microscopy to determine changes in platelet shape or were shaken with ADP to determine whether platelet clumping occurred. Compounds were obtained from the following sources: sodium salts of AMP and ADP, Sigma Chemical Co.; epinephrine and thrombin topical, Parke-Davis Co.; arcaine, California Biochemical Co. Highly purified human thrombin was kindly given us by Dr. Kent Miller, New York State Department of Health, Albany.

Results. Calculation and normal variations in platelet volume. A typical graph of the distribution of platelets by size is shown in Fig. 1. Due to the low noise characteristic of the Industrial Model B Coulter Counter it was possible to obtain good graphical separation between the peaks produced by platelets and those caused by background noise, even when plasma was used as the diluent. The resultant platelet size distribution curves were markedly asymmetrical, presumably because they represented the distribution of the volumes of particles whose diameters were normally distributed. Approximate normalization of these curves could be achieved by plotting peak height against the logarithm of window numbers, using semilog paper. The mode of the normalized plot was used to determine absolute size or relative volume change. Absolute size in cubic microns was determined by comparison with the mode observed when control suspensions of polyvinyltoluene particles of known volume were measured. However, since the present investigation was concerned mainly with relative changes in platelet volume under specific con-

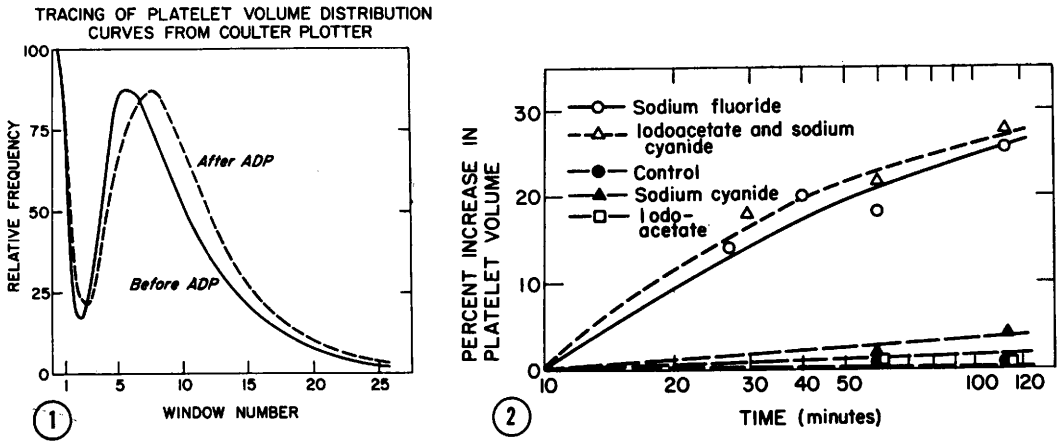

FIG. 1. Typical graph demonstrating effect of ADP on volume distribution of platelets suspended in ACD plasma. The shift represents an increase of about 25% in platelet volume; this occurs within 3 min and is slowly reversed. Machine settings were: amplitude ¼, aperture current 0.707, fine gain 70, and aperture matching switch 64L.

FIG. 2. Typical experiment demonstrating gradual increase in volume of platelets incubated in ACD plasma containing either sodium fluoride or a combination of sodium iodoacetate and cyanide. Volume of platelets incubated in plasma alone or plasma containing either iodoacetate or cyanide remained essentially constant during the 2-hr period of study.

ditions, usually the mode for each experimental curve was simply compared with that of the appropriate control curve and the result expressed as per cent change. A maximum variation of 5% occurred in consecutive plots due to machine variability. Consequently, duplicate determinations were made and the results averaged.

Platelets obtained by slow centrifugation of ACD blood from 6 normal donors averaged 5.8 μ^3 (range 5.2-6.9) when measured at 37°C. The size was the same whether E.S. or plasma was used as the suspension medium, but platelets obtained by sedimentation were 5-10% larger than those recovered by centrifugation. The effect on platelet volume of changes in anticoagulant and temperature will be reported below. Except for a slight (5%) decrease during the first half-hour, platelet volume did not change for approximately four hours provided the temperature of the suspension was maintained at 37°C. Thereafter, the platelets began to swell and the PRP had to be discarded.

Effect of various agents on platelet volume. Temperature. Early attempts to study the effect of temperature change on platelet volume were complicated by the observation that the apparent size of polyvinyltoluene latex particles increased with decreasing temperature in plasma, but decreased with decreasing temperature in E.S. These artifacts are unexplained, but presumably result from changes of viscosity and/or ionic strength of the suspension medium. Since the apparent size of polyvinyltoluene latex particles did not change in Alb-E.S. from 5° to 40°C, Alb-E.S. was used in experiments relating platelet size to temperature.

Platelets from PRP anticoagulated with ACD and incubated at room temperature had a volume of 7.1 μ^3. When half of this preparation was incubated at 37°C for 10 minutes, the average platelet size was 6.5 μ^3; after 30 minutes, it was 6.3 μ^3. When the other half was incubated at 5-10°C, the platelets increased steadily in size so that after 10 minutes they measured 7.6 μ^3 and after 30 minutes, 7.8 μ^3. Because of this marked effect of temperature on platelet volume, both the PRP and the suspensions used for measurement of volume were kept at 37°C. In addition, platelets in each suspension were sized both before and after addition of the compound to be tested so that each suspension served as its own control.

EDTA. Platelets from blood collected in EDTA were consistently larger than those from ACD blood. The swelling was proportional to the time the platelets had been ex-

posed to EDTA and approached a maximum of 30% at 60 minutes. It was partially reversed when the suspensions were made in ACD plasma and incubated at 37°C for 30 minutes. Incubation in Alb-E.S. had no effect.

Although platelets from blood collected in EDTA consistently showed a pronounced increase in size, addition of similar quantities of EDTA (final concentration of 3.2-10.8 mM) to suspensions of platelets from blood collected in ACD had no consistent effect. No change in volume occurred when EDTA was added to E.S. suspensions of such platelets from two donors. Platelets from a third donor increased 29% in volume in E.S., 28% in Alb-E.S. and 9% in ACD plasma when EDTA was added. Platelets from 2 other donors suspended in ACD plasma showed maximum increases of 7 and 14% when EDTA was added.

Inhibitors of metabolism. Fig. 2 illustrates the effect of inhibitors added in a final concentration of 3 mM to PRP diluted in ACD plasma. Sodium cyanide or iodoacetate alone had little effect on platelet volume in 2 hours, although considerable swelling was observed in 3 hours. However, when cyanide was added simultaneously with iodoacetate or after 35-40 minutes of pretreatment with iodoacetate, marked swelling was observed within one hour. Fluoride caused lysis in one experiment, and swelling in two experiments.

ADP, inhibitors of ADP clumping, thrombin and epinephrine. After obtaining several control curves, ADP was added to PRP diluted in E.S. in a final concentration of 0.5-2 μM/l. Swelling was apparent within 30 seconds, and platelet volume increased within 3 minutes to an average of approximately 26% of the initial value (range of 14 tests, 20-28%). The volume remained elevated for over an hour. Similar concentrations added to a suspension of erythrocytes had no effect. ADP caused a similar swelling when platelets were diluted in plasma (Fig. 1), but the volume returned to the control value in an hour. Comparable reversibility was noted when ADP was added to PRP and the mixture kept at 37°C for an hour before sizing the platelets.

PRP which had been pre-incubated for 10 minutes with 1 mM/l AMP(6) was used to study the inhibitory effect of AMP; ADP failed to produce aggregation in this PRP. For platelet volume measurements, pre-incubated PRP was diluted in either plasma or E.S. which also contained 1 mM/l AMP. Neither AMP alone nor the subsequent addition of ADP caused any increase in platelet volume.

Arcaine was added only to the final plasma or E.S. suspension of platelets since pre-incubation does not affect its inhibitory action. A final concentration of 1 mM/l was used which completely inhibited aggregation of platelets in undiluted PRP. Arcaine alone did not alter platelet size, but the expected increase in volume after the addition of ADP was only half that expected.

The effect of EDTA on the ADP response was variable. In two experiments using E.S. in which EDTA alone did not alter platelet volume, subsequent addition of ADP produced not only swelling but also a progressive increase in small particles, indicating that platelets were undergoing dissolution. In a third experiment in E.S., 2 experiments in Alb-E.S. and 3 experiments in plasma, EDTA alone caused some swelling and ADP caused further swelling without evidence of platelet rupture.

Thrombin was tested only in a final concentration of 10 units per ml; this was added to platelets suspended in E.S. In 6 experiments with bovine thrombin and 2 with human thrombin, platelet volume increased an average of 19% (range 16 to 23%). Epinephrine, in a final concentration of 5 μM/l, caused no change in volume of platelets suspended in either plasma or E.S.

Discussion. The data indicate that the volume of normal human platelets is approximately 5.8 μ^3 at 37°C. Because several variables were not rigorously controlled, this figure cannot be delineated more precisely. An average value of 7.3 μ^3 was obtained for platelets in a saline-glucose-metaphosphate mixture by Olef(13) who reviewed the earlier literature, and a value of 16.3 μ^3 was reported by Rebuck *et al*(14) using a high concentration of EDTA as anticoagulant. Our results indicate that future estimates will

have to be made in a medium and at a temperature which does not alter platelet volume. Other factors of importance are the anticoagulant employed and the time elapsed after venipuncture. McDonald, Odell and Gosslee(15) used the Coulter Counter to compare washed rat platelets of different ages, did not report results in cubic microns.

The change of platelet shape from smooth disc to spiny sphere was accompanied by an increase in volume of at least 15% under all circumstances tested in this study. It seems likely that active metabolic processes are necessary to maintain disc shape since platelets swell slowly at low temperature and with some enzyme inhibitors. Gorstein and Carroll(16) found that the metabolic inhibitors sodium cyanide or iodoacetate caused little inhibition of potassium flux but that a combination of the two was strongly inhibitory. Our data indicate that no change in volume results from an hour's exposure to either iodoacetate or cyanide alone but that a combination of both results in marked platelet swelling. A similar combination of the 2 inhibitors is necessary to cause platelet sphering (Jerushalmy and Zucker, unpublished results).

Platelets from blood collected in EDTA undergo marked swelling; this is in accord with observations on shape change(3). The swelling is reversed when PRP from EDTA blood is diluted with large amounts of ACD plasma and is minimal when EDTA is added to PRP from ACD blood. Aster and Jandl (17) observed a similar "protective effect" of citrate on platelet shape which was more marked at pH 6.5 than 7.4, but pointed out that citrate did not counteract the harmful effect of EDTA on platelet viability. They commented that reduction of the concentration of divalent cation cannot be the sole cause of the swelling induced by EDTA.

ADP causes very rapid swelling. This observation suggests that ADP has a metabolic action(18,19) rather than participating in bridge formation(20). Our results also support previous studies(8,9,19) which indicate that inhibitors of ADP-induced clumping may have different modes of action. Thus, although AMP, arcaine, and EDTA all inhibit platelet aggregation in the concentrations used here, only AMP prevents swelling.

Summary. The volume of normal human platelets, measured with a Coulter Counter, was approximately 5.8 μ^3 when the platelets were disc-shaped. Platelet volume increased gradually between 15 and 30% when platelets from ACD blood were maintained at less than 10°C or incubated with fluoride, or iodoacetate plus cyanide. Platelets from blood anticoagulated with EDTA swelled progressively; this was partially reversed in ACD plasma. Adenosine diphosphate or thrombin in concentrations causing aggregation in platelet-rich plasma induced rapid platelet swelling. ADP-induced swelling was prevented by adenosine monophosphate, but not by arcaine or EDTA, although all three compounds prevented ADP-induced clumping. Epinephrine in a concentration capable of causing aggregation did not produce swelling. All of the conditions which caused platelet swelling were associated with change in shape of platelets from smooth discs to spiny spheres. The technique described for measuring rapid changes in platelet volume should prove useful for studying membrane physiology.

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Voluntary Sodium Chloride Intake of Two Strains of Rats with Opposite Genetic Susceptibility to Experimental Hypertension.*(30517)

GEORGE WOLF, LEWIS K. DAHL AND NEAL E. MILLER

Department of Anatomy, School of Medicine, Yale University; Medical Department, Brookhaven National Laboratory; and Department of Psychology, Yale University

Chronic excess salt (NaCl) ingestion will induce permanent hypertension in rats(1) and has been implicated as an etiological factor in human essential hypertension(2,3). However, it is not known to what extent innate differences in salt appetite, if such differences indeed exist, are related to the development of experimental or human hypertension. The present study is concerned with possible relationships between salt appetite and genetic susceptibility to hypertension in rats. A number of investigators have reported relationships between various forms of experimental hypertension and salt appetite. In rats, desoxycorticosterone (DOC) hypertension is accompanied by enhanced voluntary intake of sodium salts(4,5) and there is suggestive evidence that adrenal regeneration hypertension may be associated with enhanced sodium appetite in this species(6). On the other hand, rats with renal(7) or with metacorticoid(5) hypertension manifest relative aversion for sodium salts. Fregly (8-10) has studied thoroughly the NaCl appetite of rats with renal hypertension and has concluded that the aversion of renal hypertensive rats is not specific for NaCl but includes non-sodium salts as well.

Recently, 2 strains of rats have been evolved by selective inbreeding, one of which rapidly develops severe hypertension with various experimental techniques which are almost ineffective in the other strain. While the 2 strains were selected initially on the basis of their respective capacities to develop hypertension from NaCl ingestion(11), it was observed subsequently that this difference in potential was demonstrable by means of other techniques as well; the strain genetically predisposed to develop NaCl hypertension had a similar predisposition to develop hypertension after DOC acetate plus NaCl, as well as after unilateral renal artery compression without NaCl(12). The other strain proved much more resistant to developing hypertension using these techniques. In recent unpublished work a similar disparity in the tendency to develop hypertension has been observed with cortisone and with adrenal enucleation in these two strains. The divergent response to salt in these strains suggested that they might manifest additional disparities. In the present study it was found that rats from the strain with a genetic predisposition to hypertension voluntarily ingested significantly *less* NaCl (as saline) than did the ones with a genetic resistance to developing hypertension.

Method. Subjects. Forty-six female rats between 1 and 2 months of age and weighing 100 to 150 g were used. Twenty-three animals were derived from the so-called *Sensitive* strain, *i.e.,* those with a strong genetic predisposition to hypertension, and 23 were derived from the *Resistant* strain, *i.e.,* those with genetic resistance to developing hyper-

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