TABLE II. Recovery of Cholesterol Added to Tissues.

Tissue	Rat No.	Cholesterol added (μg)	Cholesterol recovered (μg)
Liver	1	250	242
	2	250	238
	1	1500	1494
	2	1500	1418
Serum	1	250	229
	2	250	255
	1	1500	1419
	2	1500	1530

described. After very gentle mixing of the digest, an aliquot was removed for analysis; another was taken from the reheated digest after vigorous stirring which produced the greenish color; and a third aliquot was taken for analysis after 14 days storage in the freezer. The cholesterol concentrations were 263, 264 and 262 mg/100 g of liver, respectively. Recovery of added cholesterol from serum and liver was also studied. Results presented in Table II indicate that good recoveries are obtained.

Summary. A simple and sensitive method for determination of serum and tissue total cholesterol is described.

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Glycolytic Intermediates of Human Platelets: Their Separation and Identification.* (22993)

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Studies of human platelets have indicated the presence of an active carbohydrate metabolism(1,2) with a respiratory quotient of 0.96. Direct investigation of this metabolic system has demonstrated the presence of carbohydrate intermediates, following which partial separation and identification of the esters was obtained. Addition of radioactive phosphorous to the platelets prior to separation led to knowledge of the esters of highest specific activity, and thus to their place in the cycle. Important differences were found between fresh normal platelets, normal stored platelets, and platelets obtained from patients with hematologic disease.

Materials and methods. (a) Preparation of Platelets. Blood was obtained using silicone coated surfaces with one-tenth volume of 1% EDTA as the anticoagulant. Platelet rich plasma was carefully separated by differential centrifugation at 4°C. The volume of platelet rich plasma was standardized at 100 ml, the platelet count adjusted to 275,000/mm^3 and the pH maintained at 7.4 with .001M phosphate buffer. One millicurie of radioactive phosphorous was added and the platelet rich plasma incubated for 2 hours at 23°C. Platelets were separated by centrifugation at 1500 PM for 15′ and then washed twice with 100 ml of normal saline at 4°C. (b) Extraction of glycolytic intermediates from the washed platelet mass was obtained through modification of a method previously used in the study of erythrocytes (Fig. 1 and 2)(3,4). The platelets were ground after adding 25 ml of 4°C, 7.5% trichloracetic acid, the extract separated by centrifugation at 4°C, and the residue extracted with 25 ml of 4°C, 5% trichloracetic acid. The com-

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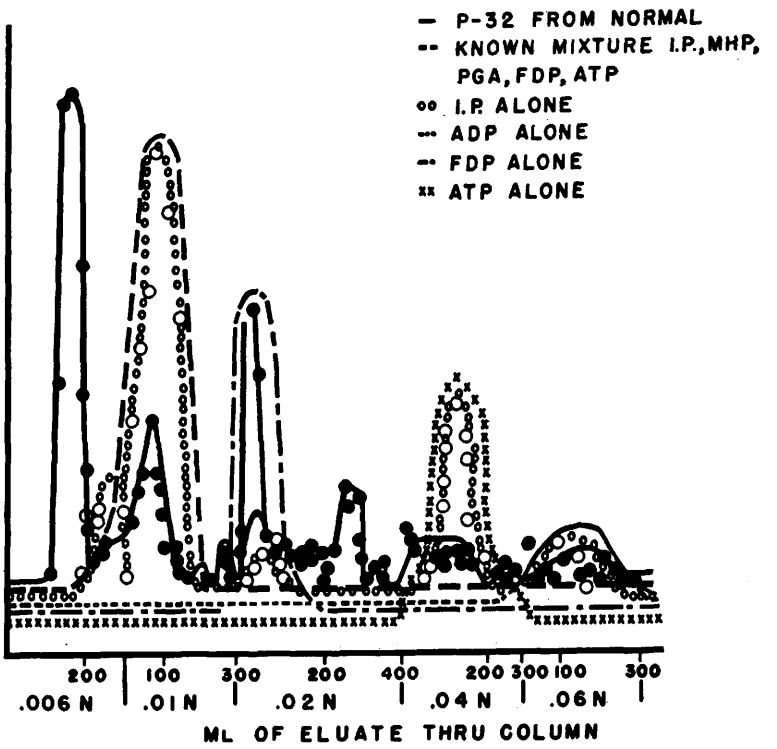

FIG. 1 and 2. Studies on carbohydrate intermediates of human platelets.

bined extracts were neutralized with concentrated ammonium hydroxide, 5 ml of saturated barium chloride was added, and ethonal solution added to final concentration of 80%. Care was taken to minimize hydrolysis by rapid neutralization and low temperatures while the esters were in solution. A minimum time of 2 hours at 4°C was allowed for precipitation of barium salts of the esters. The salts were separated by centrifugation and reconverted to esters by a batch process using hydrogen cycle Dowex-50 which was separated by filtration. The solution of esters was neutralized with ammonium hydroxide and an excess of 1 ml was added. The final volume was adjusted to 10 ml with distilled water. 1.5 ml was retained for P_{32} assay and

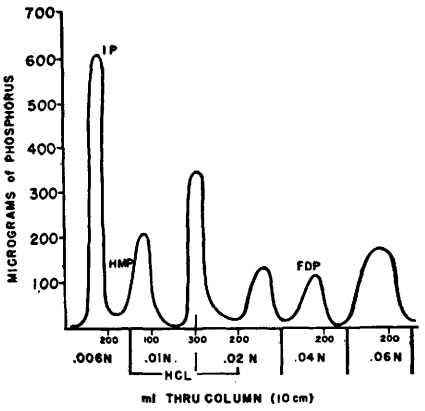

FIG. 3. Phosphate partition of normal human platelets.

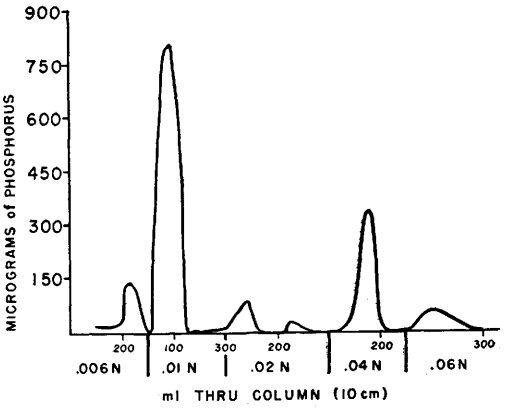

FIG. 4. Phosphate partition of known compounds.

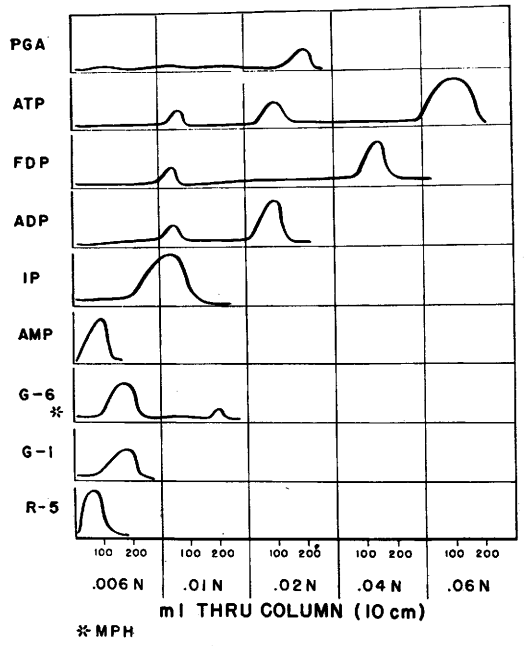

FIG. 5. Composite of data on known compounds by HCl method.

phosphorous analyses, and 8.5 ml was absorbed on a column 1.11 cm^2 x 10 cm of chloride cycle Dowex-1 anion exchange resin. Chloride was removed by repeated washing with distilled water and the esters were eluted serially with increasing concentrations of hydrochloric acid. P_{32} was assayed by direct G-M count using a 1.5 mg/cm^2 end window tube in conjunction with atomic scaler; phosphorous was determined by the method of Fiske-Subbarow(4). P_{32} activity was corrected for decay to day zero in each experiment and specific activities (S.A.) then calculated.

Results.† In an initial experiment using normal human platelets (Fig. 3) consecutive 10 ml samples were collected and an aliquot from each assayed for P_{32}. If P_{32} was detected an aliquot was analyzed for phosphorous. Close correlation occurred as would be expected. On the basis of this experiment, a solution containing a mixture of known esters was absorbed and eluted by the same technic (Fig. 4). Subsequently, individual esters of the known mixture were absorbed and the sites of eluation were identified and were consistent with results of Bartlett and Savage (3). A composite of these data was compiled (Fig. 5).

† The following abbreviations have been used: R-5 (ribose 5-phosphate), MHP (mixed hexose phosphates), I.P. (inorganic phosphorous), A.D.P. (adenosine diphosphate), A.T.P. (adenosine triphosphate), A.M.P. (adenosine monophosphate), P.G.A. (phosphoglyceric acid), and F.D.P. (fructose diphosphate).

TABLE I. Partition Data from Normal Platelets.*

Fraction	1		2		3		4		5	
	μg P/10^{10}	S.A.	μg P/10^{10}	S.A.	μg P/10^{10}	S.A.	μg P/10^{10}	S.A.	μg P/10^{10}	S.A.
.006†(0-100)‡ AMP or R-5					5.45	580	8.65	1805	11.6	1865
.006 (100-200) MHP	19.8	670	17.2	400	4.7	27.2			5.2	254
.006 (200) + .01 (0-100) IP	31.2	247	17.2	283	8.45	51.6	6.4	73.3	7.2	341
.02 (0-100) ADP	11.0	345	11.0	296	3.52	175	7.05	651	6.4	190
.02 (150-200) PGA					5.45	21.6	4.3	570	4.8	51.4
.04 (100-200) FDP	3.3	135	7.7	76.3	4.3	13.1	3.84	24.7	4.0	111
.06 (0-150) ATP	4.6	156	6.1	119	5.13	9.0	5.45	3.3	23.7	50.4
Total platelets ext./10^{10}	125		89		54.6		54.6		61.1	
% platelets recovered	65		—		72.5		72		100	

Fraction abbreviations—see (†) of text.
* Values in $\mu g/10^{10}$ platelets. † Molarity of HCl. ‡ Vol of sample in ml.

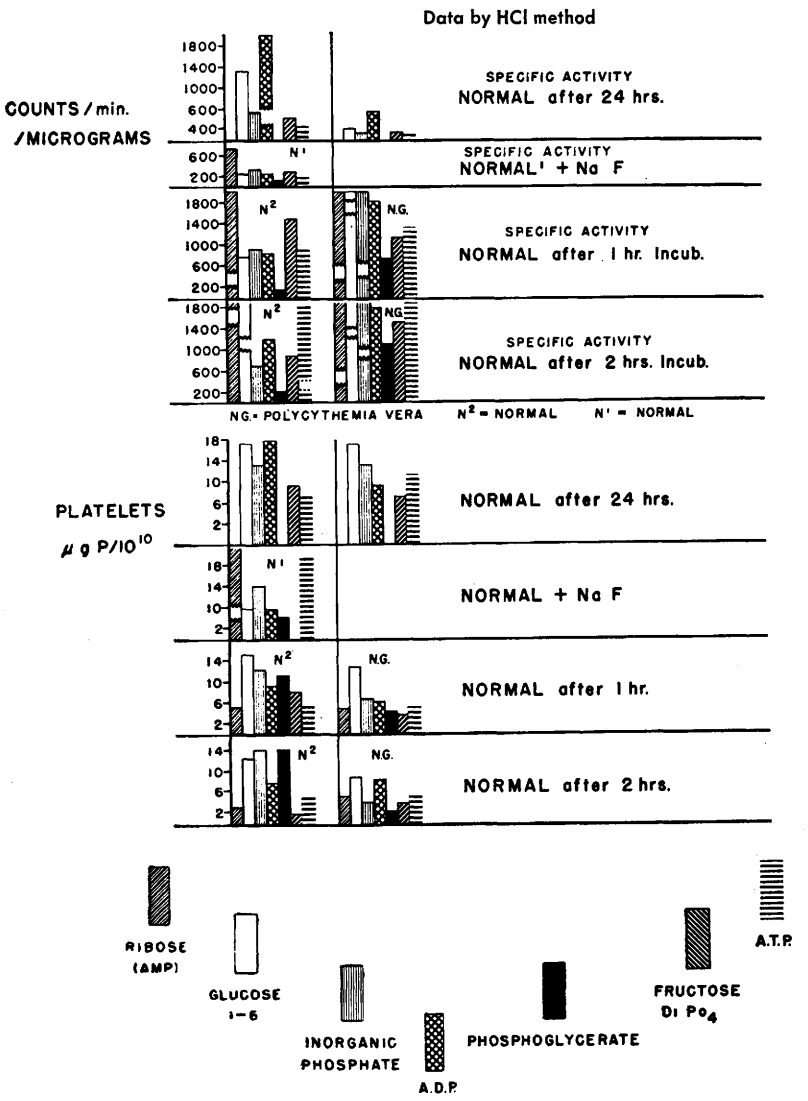

FIG. 6. Glycolytic intermediates of normal human platelets.

Extraction of normal platelets from fresh blood (Table I) yielded 55 μg of phosphorous/10^{10} platelets with a recovery of 70% of the total esters. The relation of specific activities of the identifiable fractions was R-5, MHP, ADP, IP, PGA, FDP and ATP in descending order with some variation. Numerical differences occurred in all experiments but these values were obviously less important than their relative differences and order of specific activity. In all experiments, HMP and ADP were of nearly equal activity.

Study of freshly collected, normal platelets stored for 24 hours at 4°C revealed some differences (Fig. 6). From initial data the total amount of phosphate esters of glycolytic intermediates extracted was decreased with recovery of 75-85% of the esters. The decrease in total amount of esters extracted suggested a leakage of phosphorous from platelets into the medium during storage. Notably, the quantity of ADP differed with a marked increase in the specific activity as compared with minimal difference in MHP indicating a qualitative change. The PGA fraction was also decreased.

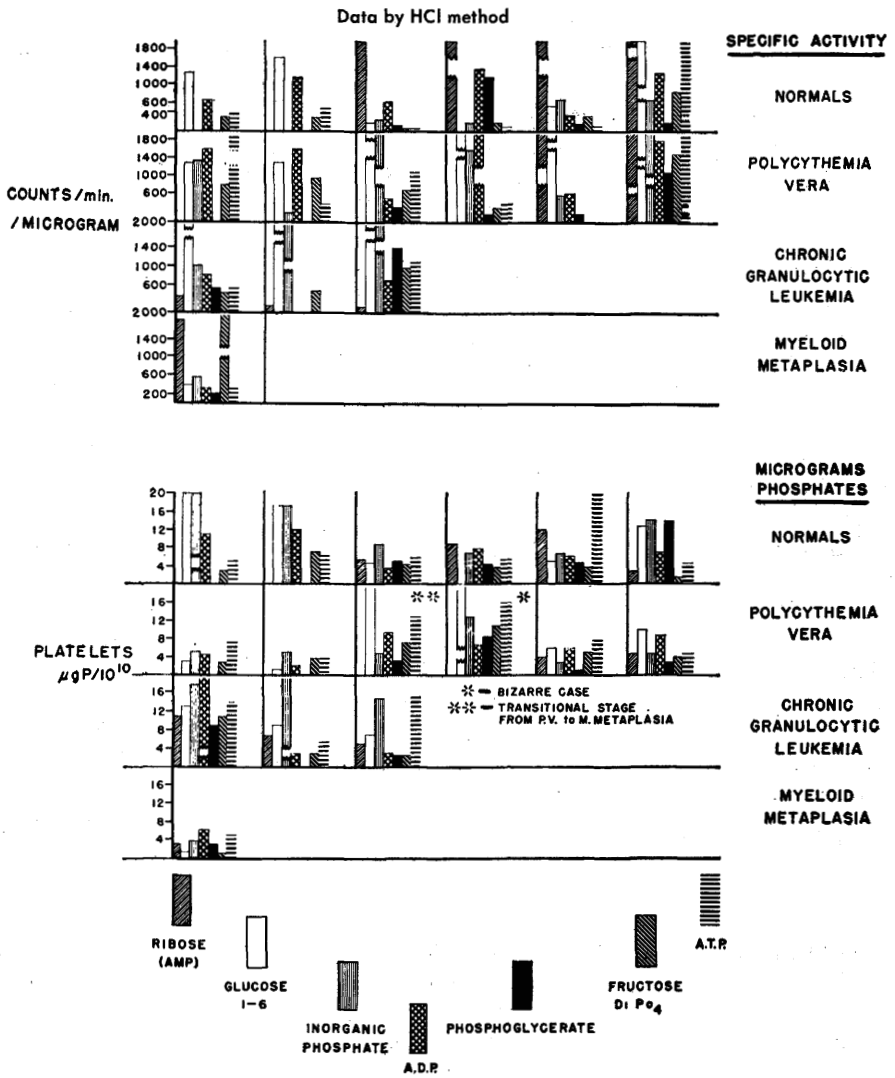

FIG. 7. Comparative study of the glycolytic intermediates of platelets in hematologic diseases.

Similar study of platelets obtained from individuals having polycythemia vera, chronic granulocytic leukemia and myeloid metaplasia (Fig. 7) revealed striking differences in platelets in these different diseases as compared with normals. In polycythemia vera 40 μg of Phosphorous/10^{10} platelets was obtained as compared with 119 mg in the platelets of chronic granulocytic leukemia.

A decrease in quantity of I.P. was obtained from platelets of 6 individuals with polycythemia while a significant increase in those from chronic granulocytic leukemia was observed. A decrease of similar magnitude was detected in the ADP, PGA, FDP, and ATP fractions. The specific activities of the esters of polycythemic platelets indicated a reversal from normal in R-5, and in MHP with an increase in ATP although there was considerable variation in different individuals. In platelets from 5 cases of chronic granulocytic leukemia the specific activity of R-5 was very markedly decreased. Several fractions were separated which have not been identified but had higher specific activities than the identified esters. Study of platelets of 3 cases of

myeloid metaplasia have revealed very bizarre individual variations suggesting marked variations in this syndrome.

Discussion. Direct study of carbohydrate metabolism of human platelets through fractionation of glycolytic intermediates revealed important differences as between fresh normal platelets, stored normal platelets, and the platelets of individuals with certain hematologic disorders (Fig. 7). (Individual variations have occurred and may be explained by variation in differences in hematologic syndromes.)

Comparison of carbohydrate intermediates from fresh normal and stored normal platelets revealed that storage resulted in a decrease in total amount of extractable esters. This suggested a leakage of phosphorous from platelets to plasma. The simultaneous increase in ADP with its marked increase in specific activity as compared with MHP suggested that stored platelets had undergone a qualitative metabolic change. Changes in specific activity of the various fractions indicated a qualitative change in this cycle. The striking qualitative and quantitative variations from normal values in platelets of chronic granulocytic leukemia suggested the possibility of an abnormal carbohydrate metabolism. Of particular interest was the increase of inorganic phosphorous and the presence of several unidentified fractions.

Summary. Presence of active degree of carbohydrate metabolism and identification and separation of some intermediates of this system has been directly demonstrated in human platelets. The use of radioactive phosphorous to determine specific activity of some fractions has yielded important additional data. Preliminary studies have indicated quantitative and qualitative alterations in carbohydrate metabolism during storage of normal platelets and particularly in platelets obtained from certain hematologic disorders, notably chronic granulocytic leukemia.

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Dye-Dilution Curves from Left Heart and Aorta for Localization of Left-to-Right Shunts and Detection of Valvular Insufficiency. (22994)

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The precise localization of left-to-right shunts whether intra- or extracardiac, may sometimes be difficult or even impossible with conventional diagnostic technics. A similar problem is presented in the detection of mitral or aortic valvular insufficiency. Dilution curves recorded from a peripheral artery following injection of indicator into a peripheral vein, the right heart, or pulmonary artery are helpful in establishing the presence of a left-to-right shunt(1). They are of relatively little value, however, in determining its precise location(2).

The technics for catheterization of the left side of heart(3,4,5) and of the central aorta (6) have made possible the injection of an indicator into the left atrium, left ventricle, and various sites in the aorta. Dilution curves obtained from these injection sites have been recorded in patients with various types of left-to-right shunts or valvular insufficiency.

Methods. Left heart catheterization was usually performed by the transbronchial route(5). In several patients the catheter was passed into the left heart retrograde from