bocytes. 2. There was no significant difference in these enzyme activities of thrombocytes from normal or thrombocytopenic calves calculated in terms of activity per 10^{10} thrombocytes. 3. There was no significant quantity of deoxypentosenucleic or pentosenucleic acid in bovine thrombocyte concentrates from either normal or thrombocytopenic calves. 4. Thrombocytes released by hypoplastic bonemarrow could not be differentiated from normal thrombocytes by the tests used.

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Relationship of Blood Heparin Levels to Serum Lipoproteins and Cholesterol Levels in Fasting Subjects.* (23936)

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In recent years much of the investigative effort on atherosclerosis has centered about

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studies of lipid metabolism. Since Hahn demonstrated that parenterally-administered heparin had an *in vivo* clearing action upon lipemic blood(1), numerous investigations have been made concerning the nature of this clearing action. Anderson showed that turbidity of lipemic plasma could be cleared *in vitro* by mixing lipemic plasma obtained prior to heparinization with that obtained 5 minutes after heparinization(2). Graham *et al.* demonstrated that heparin administration was followed by decrease in concentration of low-density lipoproteins of high S_f rate together with increase in concentration in low density lipoproteins of low S_f rates(3). The mechanism of the heparin-induced clearing and lipoprotein transformations was shown by Shore *et al.* to be associated with activation of a lipolytic enzyme acting on triglycerides(4). All these observations have been abundantly confirmed. Ability of parenterally-administered heparin to produce such striking effects upon lipids has naturally raised the question of possible relationship of spontaneously-occurring blood heparin to blood lipid and lipoprotein levels. Methods of blood heparin measurement have been reported by Jaques, Mitford and Richer, Jaques, Morehouse and Steward, Monkhouse and Jaques, Bassiouni and Gibson(5,6,7,8,9). Direct investigation of relationships between blood lipids and blood heparin levels have recently been made by Oshima and his associates in Japan. Oshima and associates and Murakami and associates studied the relationship of electrophoretically-determined β/α-lipoprotein ratios with blood heparin levels and concluded that a negative correlation exists between these variables(10,11). Further, Oshima *et al.* have reported a negative correlation between blood heparin level and ultracentrifugally determined $S_f°$ 0-12 and $S_f°$ 12-20 lipoprotein levels, respectively. No significant relationship was found by these workers between blood heparin levels and those of $S_f°$ 20-100 lipoproteins(10).§

It is of interest to know whether or not similar relationships of blood heparin level to blood lipids exist in various "races" of man because of variations in diet and mode of life. Further, confirmation of the general findings described is of value. The present study was designed to determine extent of relationship between blood heparin and blood lipid levels in a sample of males in the United States.

Materials and methods.‖ *Subjects.* 105 male schizophrenic patients, between 40 and 50 years of age, from Stockton State Hospital served as subjects. Blood samples for both heparin and lipid determinations were obtained from fasting subjects. The choice of schizophrenic patients for such a study may be questioned. However, with respect to blood lipid levels, there exists excellent evidence to indicate that no significant difference in blood lipid levels exists between such subjects and those of the population-at-large(12). *Heparin determination.* A modification of the Bassiouni method for determination of heparin (and heparin-like substances) of blood was employed. The modifications introduced provided excellent reproducibility in the heparin assay. *Preparation of samples.* Deliver 10 ml of blood directly into tube containing 5 ml of 3.8% sodium citrate solution and mix. Three ml of this mixture is used for the determination. The blood-citrate mixture is centrifuged for 10 minutes at 1500 rpm in a clinical centrifuge (International Type II) and then the supernatant (plasma-citrate mixture) is pipetted off into a glass-stoppered 25 ml graduated test tube. To the residue, containing blood cellular elements, is added 2 ml of physiological saline solution, tube contents mixed well, and centrifuged as above. The supernatant is pipetted off and combined with bulk of the plasma. This combined plasma sample is made up to 6 ml with physiological saline. The final 6 ml represents the plasma content of 2 ml of blood. *Extraction of Heparin.*¶ To this 6 ml is added 1 ml of 2 N sodium hydroxide, tube is stoppered, and contents mixed well. Then tube is heated in 50°C water bath 40 minutes. Next, with

§ These studies were performed on fasting subjects.

‖ Since heparin and lipid determinations were performed separately and without knowledge of the corresponding findings, this study is essentially a "blind study."

¶ Designation "heparin" includes true heparin and heparin-like substances of blood following heparin through this chemical procedure.

tube still warm, 5.5 g of ammonium sulfate crystals are added, the mixture shaken vigorously, and returned to 50°C water bath for 20 minutes. Temperature is then rapidly increased to 75°C in boiling water. After vigorous shaking, mixture is filtered through sintered-glass, yielding approximately 8-9 ml of filtrate. The residue is washed with 3 ml of saturated ammonium sulfate solution, filtered, and filtrate combined with original filtrate. Distilled water is added to make up to 23 ml. Pressure is then gradually reduced by pumping until no gas bubbles appear even with vigorous tube tapping. Distilled water is added to make up to 23 ml. *Dyeing, precipitation and colorimetry.* 0.2 ml of 0.1% dimethyl thionine solution is added to 15 ml of filtrate, the combined solution mixed and stored 48 hours at room temperature in the dark. The precipitate formed is centrifuged at 3000 rpm for 40-60 minutes and the supernatant decanted. The precipitate is washed with 2 ml of 90% saturated ammonium sulfate solution and re-centrifuged at 3000 rpm for 30-40 minutes. After decanting supernatant, 4 ml of buffer solution (1 vol. acetone mixed with 2 vol. 0.1 N glycine in 0.4 N sodium hydroxide) is added to dissolve precipitate. After dissolving, tube is spun in clinical centrifuge and the supernatant decanted and read within 30 minutes at 526 mμ in a spectrophotometer (Beckman). *Blank.* To 11.5 ml of saturated ammonium sulfate solution is added 1 ml of 2 N sodium hydroxide and distilled water to make 23 ml. This mixture is heated to 75°C and treated like the plasma samples. *Calibration.* Solutions of 0.01, 0.02, 0.05, 0.10 and 0.15 mg/ml of heparin (calcium heparin, 105 units/mg from Southern California Gland Co.) are each mixed with 1 ml of 2 N sodium hydroxide, 11.5 ml of saturated ammonium sulfate, and distilled water to make up to 23 ml, and then treated like the blanks. Recovery of heparin added to plasma in 6 cases averaged 88%, utilizing 0.05 mg of heparin per sample. *Reproducibility.* Reproducibility, tested on 28 pairs of blind duplicates, showed a technical error of less than 5% (0.08 mg/100 ml for a mean level of 2.58 mg/100 ml). Our method yields experimental values of blood "heparin" larger than anticipated from Bassiouni's original method, probably the result of a higher percentage recovery of "heparin." *Lipid determinations.* High and low density lipoproteins were determined by the method of de Lalla and Gofman(13) and serum cholesterol by that of Colman and MacPhee(14).

TABLE I. Mean Values and Correlations of Variables Studied (105 Cases*).

Variable	Mean and stand. dev., mg/100 ml of serum	Correlation with blood "Heparin" r (Pearson)	Significance test for r
"Heparin"	3.07± 1.12†		
S_f 0- 12	379.3 ± 92.7	−.49	$p < .001$
S_f 12- 20	47.6 ± 24.4	−.34	"
S_f 20–100	80.8 ± 60.5	−.01	N.S.
S_f 100–400	40.2 ± 8.3	+.13	"
Cholesterol	242.6 ± 47.6	−.47	$p < .001$
HDL_1	24.8 ± 8.7	−.01	N.S.
HDL_2	62.4 ± 52.2	.00	"
HDL_3	210.5 ± 39.5	−.13	"

* All variables were studied in 105 cases except the 3 high density lipoproteins, HDL_1, HDL_2 and HDL_3, which were studied in 99 cases.
† mg/100 ml of blood rather than serum.

Results. In Table I are presented means and standard deviations for all variables measured and the Pearson correlation coefficient, r, between each lipid variable and blood "heparin." Significant inverse correlations were demonstrable for the following pairs of variables:

S_f 0-12 *vs* heparin	$r = -.49$ ($p < 0.001$)
S_f 12-20 *vs* heparin	$r = -.34$ "
Serum cholesterol *vs* heparin	$r = -.47$ "

Neither the $S_f°$ 20-100, $S_f°$ 100-400, nor any of the 3 high-density lipoproteins showed any provably significant relationship with blood "heparin" level. The relationship of serum cholesterol with blood "heparin" is undoubtedly a reflection of corresponding relationship of $S_f°$ 0-12 and $S_f°$ 12-20 lipoproteins with "heparin." These lipoproteins represent the major cholesterol-bearers of blood.

While pharmacologic studies with parenteral heparin indicate that lipoproteins of $S_f°$ 20 and higher are those largely affected by heparin-activated lipase, this study shows no quantitative relationship between blood "he-

parin" and such lipoproteins. On the other hand, pharmacologic studies *in vivo* and *in vitro* indicate low-density lipoproteins of $S_f°$ 20 and lower may be a product of action of this lipase and this study shows an appreciable inverse correlation of levels of $S_f°$ 0-20 lipoproteins with blood "heparin." None of the high density lipoproteins (HDL_1, HDL_2 or HDL_3) show quantitative relationship to blood "heparin." While mechanism remains obscure, it does appear that levels of spontaneously occurring heparin-like substances are quantitatively related to levels of certain of the blood lipoproteins.

Discussion. These findings on fasting blood from a sample of U.S. males are consistent with those of Oshima *et al.* on fasting blood from a Japanese population sample. Engelberg, studying non-fasting bloods of a mixed group of subjects, preponderantly atherosclerotics, reported a very low order inverse correlation of $S_f°$ 0-12 lipoproteins with blood heparin levels(15). The population sample studied, the heparin method, and the use of non-fasting subjects all render it difficult to compare his results with those reported here. However, it is of interest that Engelberg's study and this one point to an inverse relationship of $S_f°$ 0-12 lipoproteins with blood heparin levels.

Summary. 1. A highly reproducible modification of the Bassiouni method for determination of spontaneously-occurring blood heparin-like substances has been described and evaluated. 2. A mean level of 3.07 mg/100 ml of blood heparin-like substances was determined for a sample of 105 fasting U.S. schizophrenic males 40 to 50 years old. This sample group showed lipoprotein and cholesterol values very close to those characteristic for other samples of U.S. males previously studied. 3. Highly significant negative correlations were demonstrated for the following pairs of variables, $S_f°$ 0-12 lipoproteins versus blood "heparin," $S_f°$ 12-20 lipoproteins versus blood "heparin," and serum cholesterol versus blood "heparin."

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