Ascites-induced LeVeen Shunt Coagulopathy

MARGARET V. RAGNI, M.D., JESSICA H. LEWIS, M.D., JOEL A. SPERO, M.D.

Ten of 11 patients undergoing peritoneovenous (LeVeen) shunt placement for intractable ascites had disseminated intravascular coagulation (DIC) following the shunt procedure. Intraoperative ascitic fluid specimens revealed fibrin split products (FSP) in high titer (1:100–1:1600) in all patients. Endotoxin was found in 6 of 11 ascitic fluid samples but in no plasma samples. Activated clotting factors, clot inhibitors, excess protein, and fibrinolytic activity were not found in ascitic fluid. Clotting factor levels were much lower than in plasma. Bleeding occurred after operation in two patients; this appeared to be related to the severity of liver dysfunction as demonstrated by elevations of bilirubin, serum glutamic oxalocetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and preoperative DIC. It is concluded that the LeVeen shunt coagulopathy is DIC, and may be related to exposure of the systemic circulation to FSP-rich ascitic fluid that may activate the coagulation mechanism. Bleeding complications do not appear to be related to the severity of the post shunt coagulopathy, but rather to the severity of liver dysfunction and presence of preoperative DIC (probably caused by the liver disease).

THE DEVELOPMENT of the peritoneovenous (LeVeen) shunt in 1974,[1] which redirects ascitic fluid from the peritoneal cavity to the superior vena cava via a one-way valve, has provided relief of intractable ascites for many patients, enhancing respiration, mobility, renal function, muscle mass, and overall well-being.[1,2] However, perhaps the most severe and least understood of postoperative complications is the occurrence of a coagulopathy. Because most patients undergoing the shunt procedures have severe liver disease, there are commonly preoperative synthetic defects as well as dysfibrinogenemia and disseminated intravascular coagulation (DIC). After operation, DIC has been described in 20% to 100% of patients.[3–17] The cause presumably is the constant infusion of ascitic fluid. Triggering of the coagulation mechanism has been ascribed variously to thromboplastin,[5,17,18] activated clotting factors,[11,19] endotoxin,[9,20] and/or a plasminogen activator.[14,21] In addition, the rate and volume of the ascitic fluid infusion[6,7] also may determine the incidence of the coagulopathy.

Supported in part by Grant HE 02254 from the National Institutes of Health.

Reprint requests: Margaret V. Ragni, M.D., Central Blood Bank of Pittsburgh, 812 Fifth Avenue, Pittsburgh, PA 15219.

Submitted for publication: December 13, 1982.

From the Department of Medicine, University of Pittsburgh and the Central Blood Bank of Pittsburgh, Pittsburgh, Pennsylvania

Other contributing factors include the severity of underlying liver disease,[3,12] a low plasma antithrombin III level[6,8,19] that is typical of cirrhotics who bleed,[22] and a decreased capacity of the reticuloendothelial system to clear activated clotting factors.[6]

This study was undertaken to ascertain the frequency of DIC, to establish the relationship of DIC to postoperative hemorrhagic tendency, and to investigate the role of ascitic fluid in the development of this coagulopathy.

Methods

Eleven male patients with intractable ascites undergoing LeVeen peritoneovenous shunt insertion between December 1980 and April 1982 at the Presbyterian University Hospital and the Oakland Veterans Administration Hospital were referred for study. The underlying disease process in ten of the patients was cirrhosis, specifically alcoholic in five patients and, in one patient each, associated with regional enteritis, HBsAg(+) hepatitis, intravenous drug abuse, α-1-antitrypsin deficiency, and unknown etiology. The eleventh patient had malignant ascites secondary to colonic adenocarcinoma with hepatic metastases.

After signed informed consent was granted, one preoperative and two postoperative blood samples were drawn. The latter two samples were obtained at 24 or 48 hours after operation in all but one (whose sample was taken at 72 hours) and again 24, 48, or 72 hours later in all but one (whose sample was taken 144 hours later).

Coagulation profiles were performed on both blood and ascitic fluid samples as described previously.[23–25] These included prothrombin time (PT), activated partial thromboplastin time (APTT), recalcification time (clot lysis, MCA lysis), thrombin time (TT), reptilase time (RT), assays of coagulation factors I, II, V, VII, VIII:C, VIIIR:Ag, VIIIR:vW, IX, X, XI, XII, and Fletcher factor, ethanol gel, protamine gel, Wellcotest®, staphylo-

0003-4932/83/0600/0091 $01.05

TABLE 1. *LeVeen Shunt Bleeding and Coagulopathy*

Patient	Age/Race	Diagnosis	DIC Status		Bleeding		Early Postop. Death*
			Preop.	Postop.	Preop.	Postop.	
(1) KW	60 B	Alcoholic cirrhosis†	0	0	+	0	0
(2) HM	68 W	Alcoholic cirrhosis	0	+	0	0	0
(3) CK	69 W	Alcoholic cirrhosis	0	+	0	0	0
(4) MD	67 W	Alcoholic cirrhosis	0	+	0	0	0
(5) SA	61 W	Alcoholic cirrhosis	0	+	0	0	0
(6) HR	56 W	Macronodular cirrhosis, unknown etiology	0	+	0	0	0
(7) JB	66 W	Macronodular cirrhosis, 2° regional enteritis	0	+	+	0	0
(8) WH	53 W	Cirrhosis, 2° to HBsAg(+) hepatitis	0	+	0	0	0
(9) WD	62 W	Colonic adenocarcinoma with hepatic metastases, malignant ascites	0	+	0	0	0
(10) MU	46 B	Micromacronodular cirrhosis, 2° to IV drug abuse†	+	+	0	+	+
(11) SH	27 W	Cirrhosis, 2° to α1-antitrypsin deficiency†	+	+	+	+	+

* Within the first 24 hours after operation.

† Biopsy proven.

coccal clumping titer, euglobulin clot lysis, and the sia test. Bleeding times were done on all patients, and platelet count and platelet aggregation tests (ADP, arachidonic acid, collagen, ristocetin, thrombin) were done on platelet-rich plasma. Antithrombin III (AT-III) was performed immunoelectrophoretically according to the method of Laurell[26] and kinetically by the Ortho® assay system. Plasminogen and antiplasmin were measured photometrically by the method of Friberger,[27] and the presence of endotoxin was determined by the limulus assay of Rojas-Corona.[28] Total protein in ascitic fluid was assayed by the Biuret method.[29]

Liver function tests, specifically albumin, bilirubin, serum glutamic oxalocetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase, gamma glutamyl transpeptidase (GGTP), and lactate dehydrogenase (LDH), were performed at the two hospital laboratories. Results for the latter three tests were expressed as a fraction of the upper limit of normal (×UL) for comparison purposes.

Results

Ten of the 11 patients demonstrated laboratory evidence of DIC after LeVeen shunt surgery (Tables 1 and 2). DIC was said to be present after operation if patients demonstrated two of the following four criteria: fibrin split products (FSP), fibrin monomer, greater than 15% decrease in platelet count, and greater than 15% decrease in fibrinogen level (Table 2).*

Mild preoperative bleeding was noted in three patients (Table I): melena (KW), esophageal variceal bleeding

* A 15% decrease falls outside the range of error of the methods for measuring platelet count and fibrinogen level.

TABLE 2. *Diagnostic Data for DIC*

Patient	Fibrin Split Products (staph clumping titer)		Fibrin Monomer (ethanol gel)*		Platelet ($\times 10^3/mm^3$)		Fibrinogen (mg/dl)		No. of DIC Criteria
	Preop.	Postop.	Preop.	Postop.	Preop.	Postop.	Preop.	Postop.	
(1) KW	0	0	0	0	160	143	295	270	0/4
(2) HM	0	0	0	3+	99	60	230	110	3/4
(3) CK	0	1:32	0	1+	189	117	165	85	4/4
(4) MD	0	0	2+	0	250	140	625	295	2/4
(5) SA	0	1:128	0	0	289	213	165	50	3/4
(6) HR	0	1:4	0	0	144	66	165	130	3/4
(7) JB	0	1:64	0	4+	293	181	255	230	3/4
(8) WH	1:16	1:64	0	1+	322	175	325	270	4/4
(9) WD	1:4	1:128	0	3+	136	51	380	145	4/4
(10) MU	1:8	1:128	1+	4+	90	86	150	60	3/4
(11) SH	1:8	1:32	0	0	163	49	120	140	2/4

* Abnormal = 1 to 4+.

TABLE 3. *Coagulation Studies in LeVeen Shunt Patients ($\bar{x} \pm$ S.D.)**

Test	Normal	Preop	Postop. No. 1	Postop. No. 2
Fibrinogen (mg/dl)	261 ± 60	261 ± 145	210 ± 145	185 ± 80 (8)
F II (U/ml)	0.97 ± 0.19	0.67 ± 0.30	0.62 ± 0.24	0.64 ± 0.22 (8)
F V (U/ml)	0.96 ± 0.19	0.89 ± 0.52	0.61 ± 0.26	0.69 ± 0.22 (8)
F VII (U/ml)	0.99 ± 0.21	0.67 ± 0.30	0.47 ± 0.22	0.61 ± 0.26 (8)
F X (U/ml)	1.02 ± 0.24	0.84 ± 0.20	0.66 ± 0.10	0.78 ± 0.17 (8)
F VIII:C (U/ml)	1.10 ± 0.29	2.93 ± 1.02	1.86 ± 0.81	1.94 ± 0.71 (8)
F VIIIR:Ag (U/ml)	1.00 ± 0.38	6.18 ± 3.95	4.31 ± 2.36	3.84 ± 1.54 (8)
F VIIIR:vW (U/ml)	0.98 ± 0.28	3.46 ± 2.17 (10)	2.87 ± 2.08	2.69 ± 1.30 (8)
F IX (U/ml)	0.99 ± 0.15	1.06 ± 0.49	1.00 ± 0.42	0.96 ± 0.40 (8)
F XI (U/ml)	0.95 ± 0.16	0.83 ± 0.51	0.86 ± 0.40	0.84 ± 0.50 (8)
F XII (U/ml)	0.96 ± 0.26	1.15 ± 0.73	1.24 ± 0.66	1.02 ± 0.64 (8)
FF (U/ml)	1.07 ± 0.28	0.51 ± 0.37	0.57 ± 0.37	0.45 ± 0.22 (8)
APTT (sec)	30.0 ± 2.7	34.8 ± 9.0	37.0 ± 0.6	33.8 ± 6.4 (8)
PT (sec)	10.7 ± 0.4	14.4 ± 5.4	15.3 ± 2.7	14.0 ± 3.3 (8)
AT-III (kinetic) (U/ml)	0.90 ± 0.09	0.73 ± 0.30 (10)	0.70 ± 0.28 (10)	0.66 ± 0.26 (8)
AT-III (immuno) (U/ml)	0.94 ± 0.11	0.70 ± 0.40	0.67 ± 0.35	0.62 ± 0.24 (8)
Antiplasmin (U/ml)	0.90 ± 0.09 (12)	0.34 ± 0.20 (9)	0.34 ± 0.22 (10)	0.45 ± 0.22 (7)
Plasminogen (U/ml)	0.91 ± 0.09 (12)	0.69 ± 0.22 (9)	0.90 ± 0.26 (10)	0.77 ± 0.26 (7)
Thrombin time (sec)	14.8 ± 18.0†	22.8 ± 10.6	30.6 ± 23.8	21.2 ± 6.9 (8)
Reptilase time (sec)	13.0 ± 15.8†	22.8 ± 10.0 (9)	27.7 ± 16.7 (10)	21.7 ± 5.0 (6)
Platelet ($\times 10^3/mm^3$)	150 ± 450†	194 ± 81	126 ± 65	138 ± 62 (8)

* Mean ± standard deviation. Unless specified by a number in parentheses, the means were based on 11 patients or 104 normals.

† Normal range.

(JB), and guaiac-positive stools and nasogastric drainage (SH). Only one of these three patients (SH), however, had preoperative DIC. Postoperative bleeding occurred in the only two patients with preoperative DIC. Their DIC appeared to worsen with surgery and was associated, in one patient, with low-grade fibrinolysis. Both patients died within the first 24 hours after operation.

Coagulation studies revealed, in addition to the DIC, a postoperative decrease in F V, F VIII:C, F VIIIR:Ag, and F VIIIR:vW (Table 3). F VIIIR:Ag was 2 times greater than F VIII:C and F VIIIR:vW in plasma (as well as ascitic fluid samples) both before and after operation. The levels of plasminogen and antiplasmin were not elevated, and euglobulin lysis times were normal in all but patient SH, whose preoperative lysis time of 1½ hours actually improved to 3½ hours after operation.

Ascitic fluid samples, obtained just prior to shunt insertion, revealed FSP in high titer (1:100 to 1:1600) by staphylococcal clumping assay in all patients (Table 4). There was no correlation between the presence or titer of FSP in ascitic fluid (Table 5) and that of the systemic

TABLE 4. *Ascitic Fluid Findings*

Patient	Fibrin Split Products* (staph clumping titer)	Presence of Endotoxin	Recalcification Time† (seconds)	Total Protein (mg/dl)
(1) KW	1:400	0	170	3.2
(2) HM	1:200	0	170	2.0
(3) CK	1:100	+	170	0.4
(4) MD	1:1600	+	195	3.5
(5) SA	1:100	0	200	3.5
(6) HR	1:100	+	180	2.6
(7) JB	1:400	0	176	1.2
(8) WH	1:100	+	165	0.9
(9) WD	1:400	+	170	0.6
(10) MU	1:100	+	176	1.0
(11) SH	1:100	0	175	0.5

* Normal titer in fast serum <1:4.

† 0.1 ml normal plasma + 0.1 ml ascitic fluid or saline + 0.1 ml 0.02 M $CaCl_2$ (saline = 120–130 sec).

TABLE 5. *Coagulation Studies in Ascitic Fluid of LeVeen Shunt Patients ($\bar{x} \pm$ S.D.)**

Test	Ascitic Fluid
Fibrinogen (mg/dl)	0 ± 0†
F II (U/ml)	0.15 ± 0.10
F V (U/ml)	0.05 ± 0.00
F VII (U/ml)	0.10 ± 0.00
F X (U/ml)	0.04 ± 0.00
F VIII:C (U/ml)	0.15 ± 0.22
F VIIIR:Ag (U/ml)	0.37 ± 0.35
F VIIIR:vW (U/ml)	0.16 ± 0.10
F IX (U/ml)	0.13 ± 0.14
F XI (U/ml)	0.46 ± 0.36
F XII (U/ml)	0.49 ± 0.24
FF (U/ml)	0.18 ± 0.10 (10)
APTT (sec)	>150
PT (sec)	>150
Thrombin time (sec)	>120
Reptilase time (sec)	>120 (10)
AT-III (kinetic) (U/ml)	0.20 ± 0.10 (10)
AT-III (immunologic) (U/ml)	0.20 ± 0.14
Antiplasmin (U/ml)	0.05 ± 0.00 (10)
Plasminogen (U/ml)	0.29 ± 0.24 (10)

* Mean ± standard deviation. Unless specified by a number in parentheses, the mean was based on 11 patients. Normal values are found in Table 3.

† Two of 11 patients had minimal fibrinogen present when assayed for evidence of clot formation with thrombin.

TABLE 6. *Liver Function Tests in LeVeen Shunt Patients ($\bar{x} \pm$ S.D.)**

Liver Test	Postoperative Bleeder (Nos. 10, 11)	Postoperative Nonbleeder (Nos. 1–9)
Albumin (mg/dl)	2.3 ± 0.5	3.2 ± 0.7
Total protein (mg/dl)	5.5 ± 1.3	6.8 ± 0.8 (8)
SGOT (×UL)	7.0 ± 0.9	1.6 ± 0.8 (8)
SGPT (×UL)	3.7 ± 0.5	0.9 ± 0.4 (8)
Alk. phos. (×UL)	0.9 ± 0.8	2.1 ± 1.9
Total bili. (mg/dl)	7.2 ± 0.8	1.6 ± 1.6
Indirect bili. (mg/dl)	4.4 ± 2.3	0.8 ± 1.1
GGTP (×UL)	1.9 ± 1.0	3.7 ± 1.4 (4)
LDH (×UL)	1.8 ± 0.5	2.6 ± 2.7 (6)

* Mean ± standard deviation. Unless specified by a number in parentheses, the mean was based on two patients in the "Bleeder" column and on nine in the "Nonbleeder" column.

circulation (postoperative plasma samples) (Table 2). Endotoxin was found in six of the 11 ascitic fluid specimens but was present in none of the seven preoperative and six postoperative plasma samples available for testing. No activated clotting factors, determined by the recalcification time, nor clot inhibitors, evaluated in an APTT test system using a 1:1 mix with normal plasma, were detected in the ascitic fluid. Levels of clotting factors in ascitic fluid were much lower than those of plasma (Table 5), although F VIIIR:Ag, F XI, and F XII were just below the normal (plasma) range. Total protein levels were not elevated markedly.

Liver dysfunction (Table 6) was more severe in the two patients with postoperative bleeding and subsequent death than in the nine patients with no postoperative bleeding.

Discussion

Although the existence of a postoperative coagulopathy in patients undergoing LeVeen peritoneovenous shunt insertion has been described previously,[3-17] the etiology has remained obscure, and the relationship to bleeding has been unclear.

DIC occurred in ten of the 11 patients after LeVeen shunt surgery in this study; eight of the nine patients with normal baseline preoperative coagulation profiles developed DIC within 72 hours after operation, and the two patients with preoperative DIC showed worsening of their consumptive coagulopathy.

Intraoperative ascitic fluid samples revealed high-titer (1:100 to 1:1600) FSP. These ascitic fluid FSP were markedly higher than the plasma (systemic circulation) FSP, and there was no correlation between the ascitic fluid FSP and plasma FSP levels. This lack of correlation might suggest that systemic FSP are not acquired passively from the ascitic fluid, and that the practice of replacing discarded ascitic fluid with saline during operation, prior to opening the shunt, may not prevent the postoperative coagulopathy. While this remains unproven, a patient not in this study, who recently underwent saline replacement at surgery, developed postoperative DIC.

The origin of FSP in the ascitic fluid of LeVeen shunt patients is not known; the very low or absent levels of fibrinogen in ascitic fluid suggests that the FSP may represent rapid lysis of fibrinogen in the fluid. Alternatively, FSP could have been absorbed from a necrotic or bleeding site; however, measurable circulating FSP were found in only one of the three patients with documented preoperative bleeding.

In the absence of clot inhibitors or accelerators, fibrinolysis, or excess protein content in any ascitic fluid sample, it would appear that the post LeVeen shunt coagulopathy, DIC, results from infusion of FSP-rich ascitic fluid into the systemic circulation. While endotoxin, present in six of 11 ascitic fluid samples, may play a role, as suggested by Harmon et al.,[9] it certainly is not important in all of the patients. Lack of endotoxin in the plasma of any tested patient argues against its importance in the development of DIC.

There may be no relationship between the presence of postoperative DIC and bleeding. Only two patients developed postoperative bleeding, and both had more severe liver disease than the nine nonbleeders, as determined by bilirubin, SGOT, and SGPT. They also were the only two with preoperative DIC and, thus, therapeutic intervention, as previously proposed with heparin,[6,9,16] e-aminocaproic acid (EACA),[10] and anti-thrombin III[8] may be unnecessary or even dangerous.

The occurrence of preoperative DIC in the only two patients with postoperative bleeding and early postoperative deaths would support the need for cancellation of shunt surgery until preoperative DIC resolves, as suggested previously,[18] and/or until liver function improves, as recommended by others.[12]

Acknowledgments

The authors are indebted to Dr. John Stremple, Dr. Charles Cobb, and Dr. James Riley for patient referral. Special thanks is given to Ms. Beverly Schreiner for preparation of the manuscript.

References

1. LeVeen HH, Christoudias G, Ip M, et al. Peritoneovenous shunting for ascites. Ann Surg 1974; 180:580–591.
2. LeVeen HH, Wapnick S, Grosberg S, Kinney MJ. Further experience with peritoneovenous shunt for ascites. Ann Surg 1976; 184:574–581.
3. Fullen WD. Hepatorenal syndrome: reversal by peritoneovenous shunt. Surg 1977; 82:337–341.
4. Ansley JD, Bethel RA, Bowen PA, Warren WD. Effect of peritoneovenous shunting with the LeVeen valve on ascites, renal function, and coagulation in six patients with intractable ascites. Surgery 1978; 83:181–187.
5. Lerner RG, Nelson JC, Corines P, del Guercio LRM. Intravas-

cular coagulation complicating peritoneal-atrial shunts. Blood (Suppl) 1977; 50:274.
6. Lerner RG, Nelson JC, Corines P, del Guercio LRM. Disseminated intravascular coagulation: complication of LeVeen peritoneovenous shunts. JAMA 1978; 240:2064–2066.
7. Matseshe JW, Beart RW, Bartholomew LF, et al. Fatal disseminated intravascular coagulation after peritoneovenous shunt for intractable ascites. Mayo Clin Proc 1978; 52:526–528.
8. Boyer C, Wolf M, Lavergne JM, et al. Prevention of disseminated intravascular coagulation after LeVeen peritoneovenous shunting by infusion of AT-III (No. 218) (Abst). Fifth International Congress on Thrombosis of the Mediterranean League Against Thromboembolic Diseases, Monte Carlo, October 23–25, 1980.
9. Harmon DC, Demirjian Z, Ellman L, Fischer JE. Disseminated intravascular coagulation with the peritoneovenous shunt. Ann Intern Med 1979; 90:774–776.
10. Lewis RT. Severe coagulopathy followng insertion of the LeVeen shunt: a potentially fatal complication. Can J Surg 1979; 22:361–363.
11. Phillips LL, Rodgers JB. Procoagulant activity of ascitic fluid in hepatic cirrhosis: in vivo and in vitro. Surgery 1979; 86:714–721.
12. Schwartz ML, Swaim WR, Vogel SB. Coagulopathy following peritoneovenous shunting. Surgery 1979; 85:671–677.
13. Grieg PD, Langer B, Blendis LM, et al. Complications after peritoneovenous shunting for ascites. Am J Surg 1980; 139:125–131.
14. Henderson JM, Stein SF, Kutner M, et al. Analysis of twenty-three plasma proteins in ascites. Ann Surg 1980; 192:738–742.
15. Raaf JH, Phil D, Stroehlein JR. Palliation of malignant ascites by the LeVeen peritoneovenous shunt. Cancer 1980; 45:1019–1024.
16. Stein SF, Fulenwider JT, Ansley JW, et al. Accelerated fibrinogen and platelet destruction after peritoneovenous shunting. Arch Intern Med 1981; 114:1149–1151.
17. Stein SF, Harker LA. Kinetic and functional studies of platelets, fibrinogen, and plasminogen in patients with hepatic cirrhosis. J Lab Clin Med 1982; 99:217–230.
18. Stanley MM. Treatment of intractable ascites in patients with alcoholic cirrhosis by peritoneovenous shunting (LeVeen). Med Clin North Am 1979; 63:523–536.
19. Giles AR, Sauder D, Seaton TL, et al. Changes in the coagulation status of patients undergoing autotransfusion of concentrated ascitic fluid as treatment of refractory ascites. Blood (Suppl) 1977; 50:267.
20. Puig JG, Anton FM, Gonzales JM, et al. Peritoneovenous shunt and bacterial endotoxin. Mayo Clin Proc 1979; 54:133.
21. Levy VG, Buffet C, Conard J. Troubles de la coagulation au cours des reinjections du liquide d' ascite. Fibrinolyse ou coagulation intra-vasculaire. Nouv Presse Med 1973; 2:446.
22. Ragni MV, Lewis JH, Spero JA, Hasiba U. Bleeding and coagulation abnormalities in alcoholic cirrhotic liver disease. Alcoholism Clin Exp Res 1982; 6:267–274.
23. Lewis JH, Spero JA, Hasiba U. Diagnostic methods: laboratory tests. *In* Bleeding Disorders. Garden City: Medical Examination Publishing Co. Inc, 1978; 22–34.
24. Lewis JH. Coagulation defects. JAMA 1961; 178:1014–1020.
25. Lewis JH. Hemostasis and hemorrhage. Scientific Clinician 1971; 1:1–66.
26. Laurell CB. Electroimmunoassay. Scand J Clin Lab Invest 1976; 29:21–37.
27. Friberger P, Knos M, Gustavsson S, et al. Methods for determination of plasmin, antiplasmin, and plasminogen by means of substrate S-2251. Haemostas 1978; 7:138–145.
28. Rojas-Corona RR, Skarnes R, Tanakuma S, Fini J. The limulus coagulation test for endotoxin. A comparison with other assay methods. Proc Soc Exp Biol Med 1969; 132:599–601.
29. Kingsley GR. The determination of serum total protein, albumin, and globulin by the Biuret reaction. J Biol Chem 1939; 131:197–200.