In Vitro Effects of Priming Agents on Blood Sedimentation Rate and Viscosity

Howard P. Coston and James P. Dearing

Extracorporeal Circulation Technology Program and
The Division of Cardiothoracic Surgery
The Medical University of South Carolina
Charleston, SC

Abstract

Blood from seven male patients undergoing coronary bypass surgery was mixed with four priming agents (hydroxyethyl starch (HES), low molecular weight dextran (LMWD), lactated Ringer's solution (LRS), and 5 percent normal serum albumin (ALB), at concentrations of 1 percent, 10 percent, 20 percent, and 30 percent. Each sample was then assayed for erythrocyte sedimentation rate (ESR), and blood and plasma viscosities.

Results show a positive correlation between increasing HES concentration and ESR, blood viscosity, and plasma viscosity. A positive correlation also exists between increasing LMWD concentration and blood and plasma viscosities. However, a negative correlation was found between LMWD concentration and ESR.

Results indicate a significant red cell aggregating property for HES, and a significant inhibition of red cell aggregation by LMWD.

Introduction

Since the introduction of dextrans as plasma volume expanders in the 1940's, such problems as prolongation of bleeding times [1,2,3] clotting abnormalities [4-9] strong antigenicity [10,11] limited storage life, and aggregation of red cells [12] associated with the use of dextrans have encouraged attempts to develop an even more compatible plasma expander. Amyopectin, a starch, is rapidly degraded in the blood by amylase. However, when hydroxyethylated, it is resistant to the action of amylase[13] and is effective as a plasma expander [14,15,38-41].

Studies of hydroxyethyl starch (HES) have shown it to be stable when stored [16] and non-immunogenic [17,18] though a recent study has reported a very low incidence of anaphalactoid reactions [19]. Prolonged bleeding and increased blood loss has been reported with HES infusion, but is no greater, if not significantly less, than that seen with dextran administration [20]. HES is considered relatively free of problems involving clotting mechanisms, although studies have shown it can coat platelets, leading to decreased platelet adhesiveness [21], alter fibrin structure [22,23], and increase prothrombin and partial thromboplastin times [14]. HES exhibits red cell aggregation effects similar to those seen with dextran 70, a low molecular weight dextran (LMWD), an effect leading to increased erythrocyte sedimentation rates (ESR) [21,27,28], and the basis for its use in leukophoresis [24]. It has also been shown that both red cell aggregation and ESR increase with increasing HES concentrations [27,28]. Conversely, dextran 40, also a LMWD, has been shown to inhibit red cell aggregation [21,29,30]. HES has been used in cryopreservation [25] and as a priming agent for extracorporeal circulation [26,35,37].

In a study by Hussy (unpublished data), it was noted that lactated Ringer's solution (LRS), a crystalloid, and normal serum albumin (ALB), a colloid, produced no significant increase from baseline in ESR at any concentration, while HES caused a significant increase in ESR that increased with increasing HES concentrations, a finding consistent with previously mentioned studies.

Also, a study by Sade [32] on the same three priming solutions noted an interesting effect on blood viscosity changes. LRS and ALB as priming agents both resulted in a drop in blood viscosity of approximately 38 percent from baseline, an effect expected largely as a result of hemodilution. However, HES as a priming agent resulted in only a 19 percent decrease in blood viscosity, a difference in blood viscosity changes of 50 percent less when compared to the effects of LRS and ALB. This

Direct communications to: Howard P. Coston, PSICOR, Inc., 810 E. Grand River, Brighton, MI 48116

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Table 1

MEAN VALUES AND STANDARD DEVIATIONS FOR ESR, BLOOD VISCOSITY, AND PLASMA VISCOSITY

	ESR[a]				BLOOD VISCOSITY[b]				PLASMA VISCOSITY[b]			
	1%	10%	20%	30%	1%	10%	20%	30%	1%	10%	20%	30%
HES	2.00 ±1.00	24.71 ±5.38	64.43 ±4.12	74.29 ±2.14	2.02 ±.20	2.52 ±.26	2.96 ±.19	3.46 ±.27	1.17 ±.04	1.36 ±.02	1.59 ±.11	1.95 ±.04
ALB	1.43 ±.84	1.50 ±.76	1.36 ±.69	1.43 ±.79	2.00 ±.14	2.02 ±.14	2.03 ±.12	2.13 ±.12	1.16 ±.03	1.18 ±.01	1.27 ±.14	1.26 ±.01
LMWD	1.50 ±.76	1.14 ±.69	0.40 ±.34	0.00 ±.00	2.05 ±.17	2.65 ±.18	3.50 ±.19	4.74 ±.28	1.19 ±.04	1.63 ±.03	2.31 ±.08	3.18 ±.13
LRS	1.29 ±.70	1.43 ±.84	1.43 ±.73	1.57 ±.79	1.94 ±.13	1.98 ±.14	1.99 ±.18	2.03 ±.22	1.14 ±.04	1.16 ±.03	1.15 ±.05	1.14 ±.03

a. mm/30 min. b. centipoise

study was then initiated to further investigate the ESR and viscosity changes seen with various priming agents at the full range of concentrations reported in the literature.

Materials and Methods

Sixty-four milliliters of blood from each of seven patients undergoing coronary bypass surgery who exhibited normal ESR values [33] and pre-bypass hematocrits between 40-45 percent were drawn prior to systemic heparinization. The blood was mixed with each of four priming agents (HES[a], LMWD[b], LRS[c], and 5% ALB[d]) at concentrations of 1 percent, 10 percent, 20 percent, and 30 percent. The samples were assayed for ESR, blood viscosity, and plasma viscosity. Samples were prepared in EDTA test tubes[e] (two test tubes per concentration for each agent) such that each tube contained:

1. 2 ml. of blood
2. test agent as required to obtain desired concentration
3. balance 0.9 percent saline to achieve a total volume of 4 ml.

One test tube at each concentration for each agent was centrifuged for five minutes and the plasma layer removed and assayed for plasma viscosity. Three ml. of blood were removed from the second test tube at each concentration for each agent and assayed for blood viscosity. The remaining 1 ml. of blood was used to determine the ESR. Viscosity measurements were done by means of an Ostwald viscometer [f], and the Wintrobe method[34], with one modification, was used to determine the ESR values. The Wintrobe method was modified for this study by reading the ESR values at 30 minutes rather than after 1 hour as called for in the normal protocol. This was necessary because by 1 hour both the 20 percent and 30 percent HES samples had completely settled out, making any differentiation between the two concentrations impossible.

a. Hespan, American Critical Care, McGraw Park, IL 60085
b. Rheomacrodex, Pharmacia Laboratories, Inc., New York, NY 10108
c. Travenol Laboratories, Inc., Deerfield, IL 60015
d. American National Red Cross Blood Services, Washington, D.C. 20006
e. Becton-Dickinson, Rutherford, NJ 07070
f. VWR Scientific, Inc., Rincon, CA 94119

Table 2

STATISTICAL SIGNIFICANCE BETWEEN PRIMING AGENTS

	ESR				BLOOD VISCOSITY				PLASMA VISCOSITY			
	1%	10%	20%	30%	1%	10%	20%	30%	1%	10%	20%	30%
HES vs ALB	S*	S	S	S	NS	S	S	S	NS	S	S	S
HES vs LMWD	NS	S	S	S	NS	NS	S	S	NS	S	S	S
HES vs LRS	S	S	S	S	NS	S	S	S	S*	S	S	S
ALB vs LMWD	NS	NS	NS	NS	NS	S	S	S	S*	S	S	S
ALB vs LRS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	S*	NS
LMWD vs LRS	NS	NS	NS	NS	NS	S	S	S	S	S	S	S

NS: No statistical difference S: $p<.01$ S*: $p<.05$

Randomized complete block analysis of variance and the Bonferroni t-test procedure was used to test for significant differences between agents.[43] Regression and correlation analyses were used to test for relationships between agent concentrations and ESR/viscosity data.

Results

Table 1 lists the mean values and standard deviations for ESR, blood viscosity, and plasma viscosity for each agent at the various concentrations. While these data reveal trends, the significant differences between the priming agents are shown in Table 2. There was no difference in ESR between ALB, LMWD, or LRS at any concentration, while the ESR for HES was significantly higher than each of the other agents at every concentration, with the exception of LMWD at 1 percent. Regression and correlation analyses of these data reemphasize the trends, with HES showing a strong positive correlation between increasing HES concentration and ESR (Figure 1). It was also found that ALB and LRS demonstrated no significant correlation between ESR and concentration. However, though ESR values for LMWD do not significantly differ from those of ALB and LRS, LMWD does exhibit a strong negative correlation between ESR and concentration.

There was no difference in blood viscosity between any of the agents at 1 percent, and no difference between ALB and LRS at any concentration. LMWD viscosity was significantly higher than HES at 20 percent and 30 percent. Regression and correlation analyses of blood viscosity data (Figure 2) reveal that neither ALB nor LRS exhibits significant correlation between blood viscosity and concentration, while both HES and LMWD show strong positive correlations between the two parameters, with LMWD viscosity exceeding that of HES at a concentration of approximately 4 percent.

Plasma viscosity was not significantly different between ALB and LRS at any concentration except 20 percent. HES and LMWD had significantly higher viscosities than ALB or LRS at all concentrations, with the exception of HES at 10 percent, 20 percent, and 30 percent. With regression and correlation analysis (Figure 3), LRS exhibited no significant correlation between viscosity and concentration, while HES, ALB, and LMWD each revealed significant positive correlations.

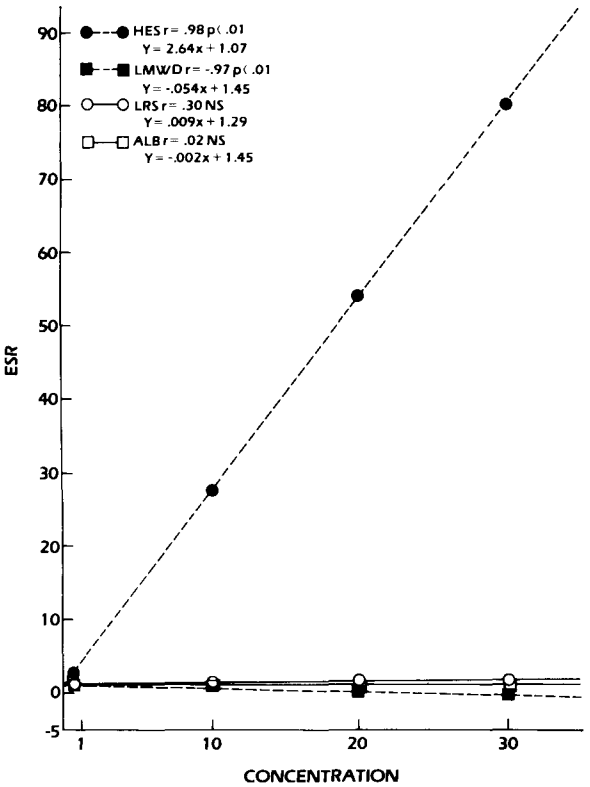

Figure 1: Linear regression lines for ESR versus concentration. The ESR unit is millimeters per thirty minutes (mm/30 min.), and the concentration unit is percentage (%). NS = not statistically significant.

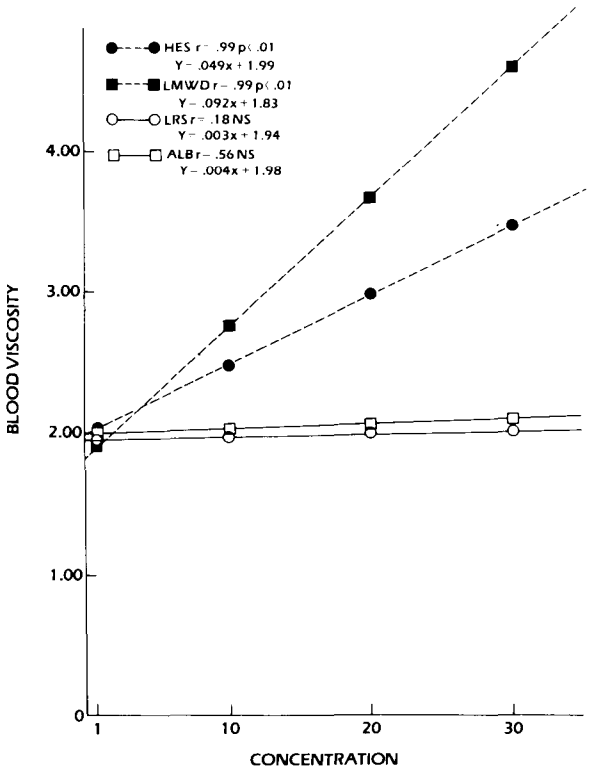

Figure 2: Linear regression lines for blood viscosity versus concentration. The viscosity unit is centipoise (cp), and the concentration unit is percentage (%). NS = not statistically significant.

Discussion

By Stokes' Law it can be shown that the sedimentation rate of any suspension is primarily a measure of the size of the sedimenting particles [31]. Although changes in red cell shape and size, plasma viscosity, and cell and plasma density may have minor effects, red cell aggregation and rouleaux formation play the major role in increasing particle size and increasing ESR [31]. Therefore, ESR effectively becomes a measure of the tendency of red cells to stick together [31]. The results of this study indicate a significant red cell aggregating property of HES, and a significant ability by LMWD to inhibit red cell aggregation. These results are in agreement with the findings of other authors [21,27-30]. While an increased ESR is generally believed to indicate a change in the surface properties of the red cell membrane, usually through some inflammatory process, the significance of an HES mediated increase in ESR during bypass is not known. It is not known whether this red cell aggregation occurs while blood is flowing through the bypass system or is initiated upon blood stasis such as occurs within the extracorporeal circuit upon termina-

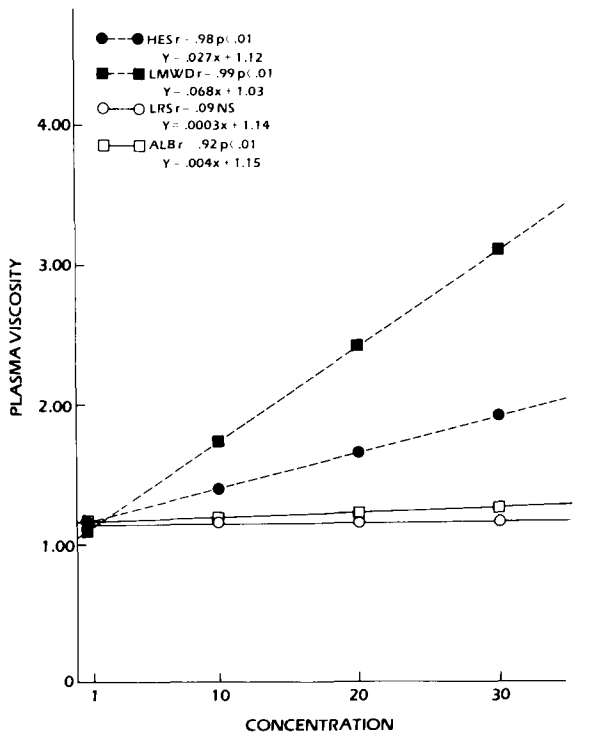

Figure 3: Linear regression lines for plasma viscosity versus concentration. The plasma viscosity unit is centipoise (cp), and the concentration unit is percentage (%). NS = not statistically significant.

tion of bypass. One concern may be whether the red cell aggregating properties of HES play any role in the decreased platelet counts post-bypass reported in several recent articles studying HES used as a priming agent for cardiopulmonary bypass [32,42]. Another possible concern of HES users during bypass may be its use in conjunction with micro-capillary, hollow-fiber membrane oxygenators. Is there any possibility that red cell aggregates and sedimentation could affect the operating parameters of such oxygenators, particularly in the case where bypass may need to be re-established following a period of low or even no blood flow?

Increased viscosity is important in that it can indirectly result in increased myocardial work. Viscosity has a linear relationship to vascular resistance, which is directly related to vascular pressure, and vascular pressure is directly related to myocardial work. Sade and associates [32], in evaluating HES, ALB, and LRS as priming fluids, measured a number of hemodynamic variables, and found no statistically or clinically significant differences between HES, ALB, and LRS. However, several interesting trends did occur. Both the left atrial and pulmonary artery pressures were higher immediately post-bypass in the HES population, while the cardiac index remained lower through the first hour following transfer to the Surgical Intensive Care Unit (SICU). Furthermore, the systemic vascular resistance index remained higher in the HES population through the eighth hour after transfer to SICU, and through postoperative day one for the pulmonary vascular resistance index.

While these results were not statistically significant, and while it is recognized that a number of other factors interrelate with viscosity in a complex way to affect arterial resistance and pressure, the question remains as to what degree the increased viscosity associated with HES and LMWD may affect the myocardial workload. Should the increased viscosity associated with these agents play even a small role in increasing myocardial work, the use of these agents may be contraindicated for the critically ill patient, particularly the patient with severely compromised ventricular function. It is felt that these are issues worthy of further investigation.

References

1. Weil, P.G., Webster, D.R.: Studies on the bleeding tendency following dextran infusion. *Surg. Forum.* 6:88, 1965.
2. Langdell, R.D., et al.: Dextran and prolonged bleeding time. *JAMA* 166:346, 1958.
3. Adelson, E.: Bleeding time prolongation after dextran infusion. *Bibl. Haemat.* 7:275, 1958.
4. Bergentz, S.E., Eiken, O., Nilsson, I.M.: The effect of dextran of various molecular weights on the coagulation in dogs. *Thromb. Death. Haemorrh.* 6:151, 1961.
5. Carbone, J.V., Furth, F.W., Scott, E., Jr., Crosley, W.H.: A hemostatic defect associated with dextran infusion. *Proc. Soc. Exp. Biol. & Med.* 85:101, 1954.
6. Seegers, W.H., Levine, W.G., Johnson, S.A.: Inhibition of prothrombin activation with dextran. *J. Appl. Physiol.* 7:617, 1955.
7. Jacobaeus, U.: The effect of dextran on the coagulation of blood. *Acta Med. Scand.* 151:505, 1955.
8. Jacobaeus, U.: Studies on the effect of dextran on the coagulation of blood. *Acta Med. Scand.* 322:1, 1957 (Suppl.)
9. Nilsson, I.M., Eiken, O.: Further studies on the effect of dextran of various molecular weight on the coagulation mechanism. *Thromb. Diath. Haemorrh.* 11:38, 1964.
10. Thompson, W.L.: Plasma Substitutes. *J.S. Carolina Med. Assoc.* 56:456-472, 1960
11. Gronwall, A.: Dextran and its use in colloidal infusion solutions. New York: Academic Press, Inc., 1957, pp. 93-97.
12. Knox, R.J., Nordt, F.J., Seaman, G.V.F., Brooks, D.E.: Rheology of erythrocyte suspensions: dextran-mediated aggregation of deformable and nondeformable erythrocytes. *Biorrheology.* 14:75-84, 1977.
13. Ziese, W.: Action of amylases on hydroxyethyl starch. Zeitscher. *Physiol. Chem.* 229:235, 1935.
14. Lee, W.H., Jr., Cooper, N., Weidner, M.G., Jr., Murner, E.S.: Clinical evaluation of a new plasma expander, hydroxyethyl starch. *J. Trauma.* 8:381-393, 1968.
15. Solanke, T.F.: Clinical trial of 6% hydroxyethyl starch (a new plasma expander). *Brit. Med. J.* 3:783-785, 1968.
16. Lee-Benner, L., Walton, R.P.: Comparative stability of hydroxyethyl starch and dextran solutions in storage. *Third Conference on Artificial Colloid Agents.* Washington, D.C.: National Academy of Sciences and National Research Council, 1965, pp. 54-55.
17. Brickman, R.D., Murray, G.F., Thompson, W.L., Ballinger, W.F., II: The antigenicity of hydroxyethyl starch in humans. Studies in seven normal volunteers. *JAMA* 198:1277-1279, 1966.
18. Maurer, P.H., Berardinelli, B.: Immunologic studies with hydroxyethyl starch (HES). A proposed plasma expander. *Transfusion.* 8:265-268, 1968.
19. Ring, J., Seifert, J., Messmer, K., Brendel, W.: Anaphylactoid reactions due to hydroxyethyl starch infusion. *Eur. Surg. Res.* 8:389-399, 1976.
20. Karlson, K.E., Garzon, A.A., Shaftan, G.W., Chu, C.J.: Increased blood loss associated with administration of certain plasma expanders: dextran 70, dextran 40, and hydroxyethyl starch. *Surgery.* 62:670-678, 1967.
21. Lewis, J.H., Szeto, I.L.F., Bayer, W.L., Takaori, M., Safar, P.: Severe hemodilution with hydroxyethyl starch and dextrans. Effects on plasma proteins, coagulation factors, and platelet adhesiveness. *Arch. Surg.* 93:941-950, 1966.
22. Gollub, S., Schaefer, C.: Structural alteration in canine fibrin produced by colloid plasma expanders. *Surg. Gynecol. Obstet.* 127:783-793, 1968.
23. Wallenbeck, I.A.M., Tangen, O.: On the lysis of fibrin formed in the presence of dextran and other macromolecules. *Thromb. Res.* 6:75-86, 1975.
24. Mishler, J.M.: Hydroxyethyl starch as an experimental adjunct to leukocyte separation by centrifugal means: review of safety and efficacy. *Transfusion.* 13:449-460, 1975.
25. Baar, S.: Albumin and hydroxyethyl starch in the cryopreservation of red cells—an in-vitro study. *Transfusion.* 13:73-83, 1973.
26. Lee, W.H., Jr., Rubin, J.W., Huggins, M.P.: Clinical evaluations of priming solutions for pump oxygenators perfusions. *Ann. Thorac. Surg.* 19:529-536, 1975.
27. Corry, W.D., Jackson, L.J., Seaman, G.V.F.: The effect of hydroxyethyl starch on the rheological properties of human erythrocyte suspensions. *Biorheology.* 18:517-529, 1981.

28. Metcalf, W., Papadopoulos, A., Tufaro, R., Barth, A.: A clinical physiologic study of hydroxyethyl starch. *Surg. Gynec. Obstet.* 131:255-267, 1970.

29. Gelin, L.E.: Disturbance of the flow properties of blood and its counteraction in surgery. *Acta Chir. Scandinav.* 122:287, 1961.

30. Gelin, L.E., Ingelman, B.: Rheomacrodex - a new dextran solution for rheological treatment of impaired capillary flow. *Acta Chir. Scandinav.* 122:294, 1961.

31. Burton, A.C.: Physiology and biophysics of the circulation. Chicago: Year Book Publishers, Inc., 1972, pp. 15-16.

32. Sade, R., Dearing, J.P.: Priming fluids: a prospective randomized study. In press.

33. Treseler, K.M.: Clinical Laboratory tests - significance and implications for nursing. Englewood Cliffs: Prentice-Hall, Inc., 1982, pp. 148-149.

34. Wintrobe, M.M.: *Clinical Hematology*, 7th edition. Philadelphia: Lea and Febiger, 1974, pp. 125.

35. Sade, R.M., Crawford, F.A., Jr., Dearing, J.P., Stroud, M.: Hydrowyethyl starch in priming fluid for cardiopulmonary bypass. *J. Thorac. Cardiovasc. Surg.* 84:34-38, 1982.

36. Mishler, J.M., Nicora, R.W., Yoshitake, T., Oishi, K., Kawasaki, T., Shimizu, K.: Hemodilution with hydroxyethyl starch during cardiopulmonary bypass. Review of a multi-institutional study. *JECT* 7:140-149, 1975.

37. Palanzo, D.A., Parr, G.V.S., Bull, A.P., Williams, D.R., O'Neill, M.J., Waldhansen, J.A.: Hetastarch as a prime for cardiopulmonary bypass. *Ann. Thorac. Surg.* 34:680-683, 1983.

38. Lee, W.H., Jr., Cooper, N., Weidner, M.G., Jr., Murner, E.S.: Clinical evaluation of a new plasma expander, hydroxyethyl starch. *J. Trauma.* 8:381-393, 1968.

39. Lamke, L.O., Liljedahl, S.O.: Plasma volume changes after infusion of various plasma expanders. *Resuscitation.* 5:93-102, 1974.

40. Shatney, C.H., Deepika, K., Militello, P.R., Majerus, T.C., Dawson, R.B.: Efficacy of hetastarch in the resuscitation of patients with multisystem trauma and shock. *Arch. Surg.* 118:804-809, 1983.

41. Puri, V.K., Paidipaty, B., White, L.: Hydroxyethyl starch for resuscitation of patients with hypovolemia and shock. *Crit. Care. Med.* 9:833-837, 1981.

42. Palanzo, D.A., O'Neill, M.J., Jr., Williams, D.R., Parr, G.V.S.: Hetastarch as a clear prime for cardiopulmonary bypass: an update. *JECT.* In press.

43. Duncan, Robert C., Knapp, Rebecca G. and Miller, M. Clinton III: *Introductory Biostatistics for the Health Sciences.* New York: John Wiley and Sons, 1977. pp. 151-155.