12. Toolan, H. W., *Bull. N. Y. Acad. Med.*, 1961, v37, 305.

13. Melnick, J. L., Crowther, D., Barrera-Oro, J., *Science*, 1961, v134, 557.

14. Barrera-Oro, J. G., Melnick, J. L., *Tex. Rept. Biol. and Med.*, 1961, v19, 529.

15. Powell, H. M., Culbertson, C. G., McQuire, J. M., Hoehm, M. M., Baker, L. A., *Antibiot. and Chemother.*, 1952, v2, 432.

16. Powell, H. M., Culbertson, C. G., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 161.

17. Cochran, K. W., Brown, G. C., Francis, T., Jr., *ibid.*, 1954, v85, 104.

18. Goulet, N. R., Doctoral Dissertation, Univ. of Michigan, 1958.

19. Cochran, K. W., Headington, J. T., *Fed. Proc.*, 1960, v19, 406.

20. Powell, H. M., Walcher, M. D., Mast, C., *Antibiot. and Chemother.*, 1962, v12, 337.

21. ———, PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 55.

22. Johnson, I. S., Baker, L. A., *Antibiot. and Chemother.*, 1958, v8, 113.

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Bleeding Volume Indices of Hydroxyethyl Starch, Dextran, Blood, and Glucose.* (28945)

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Intravenous administration of selected variations of hydroxyethyl starch in dogs has indicated that their blood stream persistence and tolerance reactions are of the same order as dextran(1). A high grade of tissue compatibility might be expected particularly in the instance of amylopectin which is structurally more closely related to glycogen than any other glucose polymer(2). In experiments with starch as a plasma substitute in World War II, there was no evident means of preventing rapid enzymic degradation of this colloid polymer. Currently, a controlled degree of enzyme resistance is obtained on an industrial scale by introduction of hydroxyethyl groups through interaction with ethylene oxide and alkali. The present series of studies has led to the selection of a "waxy" maize starch consisting largely of the highly branched amylopectin with minimal content of the linear, amylose fraction(3), acid-modified to a stage of low viscosity (specified industrially in this case as 70 or 90 fluidity) and, after gelatinization, derivatized to 0.7 degree of substitution (hydroxyethyl groups per anhydroglucose unit). These and a number of related products were obtained through the cooperation of Drs. T. J. Schoch and E. F. Paschall of the Corn Products Co., Argo, Ill. Biologic variables which have been tested with these products in over 400 experiments have included hemodynamic, respiratory and renal function changes, bleeding times, acid base balance, sedimentation rates, plasma protein values, histamine release and tissue storage(1,4,5). One of the most critical characteristics in terms of possible usefulness is the bleeding volume index. The present study was designed to compare the "bleeding volume indices" of hydroxyethyl starch and dextran as well as that of blood and glucose. This procedure first described by Gordon *et al*(6) represents a simple but significant means of analyzing the relative efficacy of volemic agents in hemorrhagic hypotension.

Methods. Thirty-nine mongrel dogs of both sexes weighing 9.0 to 14.4 kg were anesthetized with 15 mg/kg of sodium pentobarbital and 225 mg/kg of sodium barbital intravenously, a combination producing stable anesthesia for at least 4 hours. Polyethylene catheters were inserted into both femoral arteries and veins and advanced to the thoracic level. Aortic pressures were measured with Statham transducers through one arterial catheter and the contralateral arterial

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catheter was used for bleeding and microhematocrit sampling. The femoral venous catheters were used for infusion of the test solutions.

The dogs were bled into heparinized syringes at the uniform rate of 4.0 ml/kg/min to a mean arterial pressure of 30 mm Hg or slightly less; the mean bleeding volume was 42.0 ± S.D. 6.43 ml/kg. Immediately after the end of bleeding, one of 6 test solutions was infused intravenously by a Sigmamotor pump at rates of 2.2 to 61.5 ml/kg/min. The starch and dextran solutions were infused at about equally matched rates the average of which was 15.9 (7.8 to 61.5) ml/kg/min. Glucose and whole blood were infused at the average rate of 4.0 (2.2 to 11.5) ml/kg/min. The volume of infusion was equal to the blood loss during the initial hemorrhage. Three hours after the end of test solution infusion, a second hemorrhage procedure was performed in the same manner as the first, the bleeding volume was carefully measured, then the blood lost during this second hemorrhage was infused intravenously. The ratio of volume of blood loss during the second hemorrhage to initial blood loss was expressed as a percentage and recorded as the bleeding volume index.

Three dogs received solutions of 70-fluidity hydroxyethyl corn starch (70F-CS), 7 received 90-fluidity hydroxyethyl corn starch (90F-CS), and 6 received 90-fluidity hydroxyethyl waxy maize starch (90F-WS). "Fluidity" here is the reciprocal of viscosity with the fluidity of water being 100. Eleven dogs received dextran (Gentran, Baxter) 6 g/100 ml in normal saline, 5 received dextrose, U.S.P., 6 g/100 ml in water, and 7 received fresh autologous whole blood containing 2 mg/100 ml of heparin sodium, U.S.P. The starch solutions were prepared as 6 g/100 ml in Tyrode's balanced saline solution modified by the replacement of glucose and magnesium by equal osmolar amounts of sodium chloride. The starches were gelatinized in a boiling water bath with intermittent magnetic stirring and then were autoclaved for 20 minutes at 15 psi. Some of these resulting solutions contained sediment after a few days and all solutions used in the present study were filtered through 2 thicknesses of Whatman No. 5 filter paper immediately before use. Solutions of hydroxyethyl waxy starch reautoclaved after filtration did not develop turbidity or other gross signs of deterioration during the 2½ year period of observation.

Results. Bleeding volume indices were not significantly different in dogs receiving whole blood or the colloidal solutions, but glucose solutions produced significantly lower values. These relationships among the group were calculated according to the method of Duncan(7) at the significance level $P<.05$. Using the same procedure, there was a significant difference in the instance of glucose when calculating mean arterial pressures after the second infusion and in the instance of both blood and glucose in the calculation of arterial hematocrit values at the 3 hour interval.

MEAN BLEEDING VOLUME INDICES

	Mean	S.E.±		Mean	S.E.±
Glucose	49.9%	2.3	90F-WS	85.3%	4.2
70F-CS	82.4%	1.2	90F-CS	90.3%	8.1
Blood	84.9%	7.2	Dextran	100.9%	2.2

Mean femoral arterial pressures following infusion of starch solutions, dextran, and whole blood quickly returned to the range of control levels. With glucose, mean arterial pressures were significantly lower during the first hour after infusion and progressively approached control levels in the second and third hours. After the second hemorrhage, the arterial pressure responses to reinfused blood were significantly lower in those animals which had received glucose than in those which had received the other solutions.

MEAN ARTERIAL PRESSURES 30 MIN AFTER SECOND INFUSION

% of initial prehemorrhage mean pressures

Glucose	70.3%	90F-WS	91.0%
70F-CS	90.1%	Blood	94.2%
90F-CS	90.3%	Dextran	96.7%

After the initial infusion, pulse pressures were lowest in dogs that received glucose solution and fresh autologous whole blood. The highest pulse pressures occurred with the most viscous product, 70-fluidity corn starch.

FEMORAL ARTERIAL PULSE PRESSURES 90 MIN AFTER INITIAL INFUSION
% of prehemorrhage pulse pressures

Glucose	88%	Dextran	139%
Blood	116%	90F-CS	156%
90F-WS	130%	70F-CS	242%

Pulse pressures following the second hemorrhage-infusion procedure were similar to those during the interval between the bleedings.

Heart rates were reduced to 70 to 88% of prehemorrhage values immediately following infusion, but increased to 101 to 146% of prehemorrhage values 3 hours after the initial infusion. There were no significant differences in mean heart rates among the 6 groups.

Prehemorrhage arterial hematocrit values averaged 37.4 ± S.D. 5.63 and no significant differences were observed among the 6 control groups. After infusion of the test solutions there were significant differences in arterial hematocrits. During the 3-hour interval between bleedings, arterial hematocrits increased in each of the 6 groups. The increments were minimal in the case of the colloidal solutions and whole blood, but in dogs that received glucose solution, arterial hematocrits 3 hours after infusion of 42 ml/kg of glucose were equal to the prehemorrhage hematocrits.

ARTERIAL HEMATOCRIT VALUES 3 HR AFTER INITIAL INFUSION
% of prehemorrhage values

90F-WS	48.4%	Dextran	56.6%
90F-CS	49.3%	Blood	102.8%
70F-CS	52.0%	Glucose	103.5%

Discussion. The bleeding volume indices obtained in our study following infusion of hydroxyethyl starch solutions are comparable to those reported by others after infusions, in dogs, of albumin, plasma, dextran, and gelatin derivatives. In the studies of Morrison *et al*(8) and Lawson and Rehm(9), dogs were bled initially to 60 mm Hg mean arterial pressure, but during the second hemorrhage period, they were bled to death. An average value for the residual blood volume of dogs bled to 60 mm Hg was used to calculate the blood loss that would have occurred if the dogs had been bled to death initially. The studies by Parkins *et al*(10-15) were conducted by two procedures: dogs were bled to 20 mm Hg and immediately infused with test solution or dogs were held at 30 mm Hg mean arterial pressure for 60 minutes before infusion. The hypotensive period resulted in decreased values of the bleeding volume index, but the relationships between the bleeding volume indices after infusion of blood, colloidal solutions, or saline solutions were not altered by the hypotensive interval. The relatively high bleeding volume indices reported by Govier and Colovos(16) may be attributed to rapid bleeding, to respiratory arrest, and rebleeding after an interval of only 30 minutes. In general, the bleeding volume indices reported by others were lowest with saline and intermediate in the case of polyvinylpyrrolidone.

In normal individuals receiving dextran there is substantial expansion of the blood volume in excess of the infused volume(17). Pirani *et al*(18) noted that the colloidal osmotic pressure of 6% dextran in saline is 50 to 100% greater than that of plasma, and Thorsen(19) found that the colloidal osmotic pressure of plasma-dextran mixtures was greater than the sum of the osmotic pressures measured in pure solutions. Metcalf and Rousselot(20) reported that the plasma volume increment following infusions of dextran in normovolemic patients was directly proportional to the initial plasma protein concentration.

Summary. Solutions of hydroxyethyl starch were approximately equivalent to dextran and autologous whole blood in restoring and maintaining near normal arterial pressures in bled dogs. Bleeding volume indices with hydroxyethyl starch solutions, dextran, or whole blood were not significantly different. With glucose solution, arterial pressures and bleeding volume indices were significantly lower. During the interval between bleedings, arterial hematocrit values were maintained at about 50% of prehemorrhage levels by hydroxyethyl starch and dextran, whereas with glucose solutions hematocrits progressively rose to levels in the range of prehemorrhage values.

1. Thompson, W. L., Britton, J. J., Walton, R. P., *J. Pharmacol. Exp. Therap.,* 1962, v136, 125.

2. Manners, D. J., in *Advances in Carbohydrate Chemistry,* Academic Press, N. Y., & London, 1962, v17, 373.

3. Schoch, T. J., in *Starch and Its Derivatives,* Radley, J. A., ed., John Wiley & Sons, N. Y., 1954, p123.

4. Walton, R. P., Richardson, J. A., Thompson, W. L., *J. Pharmacol. Exp. Therap.,* 1958, v124, 223.

5. Thompson, W. L., Walton, R. P., in *Artificial Colloids for Intravenous Use,* Nat. Acad. Sci., Nat. Research Council, Washington, D.C., 1962, p168.

6. Gordon, H., Hoge, L. J., Lawson, H., *Am. J. Med. Sci.,* 1942, v204, 4.

7. Duncan, D. B., *Biometrics,* 1955, v11, 1.

8. Morrison, A. E., Jr., Lundy, J. S., Essex, H. E., *Circulation,* 1952, v5, 208.

9. Lawson, H., Rehm, W. S., *Am. J. Physiol.,* 1943, v140, 431.

10. Parkins, W. M., Saxe, L. H., Vars, H. M., *Am. J. Med. Sci.,* 1944, v207, 414.

11. Parkins, W. M., Perlmutt, J. H., Vars, H. M., *Am. J. Physiol.,* 1953, v173, 403.

12. Elder, J. D., Parkins, W. M., Vars, H. M., *Bibliotheca. Haematol.,* 1958, v7, 278.

13. Hamilton, A. S., Parkins, W. M., Waltzer, F., *Am. J. Physiol.,* 1947, v150, 641.

14. Parkins, W. M., Perlmutt, J. H., Vars, H. M., *ibid.,* 1952, v170, 351.

15. Vars, H. M., Parkins, W. M., Perlmutt, J. H., *Ann. N. Y. Acad. Sci.,* 1952, v55, 496.

16. Govier, W. M., Colovos, G. C., *ibid.,* 1952, v55, 491.

17. Meyer, L. M., Berlin, N. I., Hyde, G. M., Parsons, R. J., Whittington, B., *Surg. Gynecol. Obstet.,* 1952, v94, 712.

18. Pirani, C. L., Juster, R., Froeb, H. F., Consolazio, C. F., Ingraham, R. C., *J. Appl. Physiol.,* 1955, v8, 193.

19. Thorsen, G., *Surg. Gynecol. Obstet.,* 1959, v109, 43.

20. Metcalf, W., Rousselot, L. M., *Surg. Forum.,* 1954, v5, 514.

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Effects of Blood Taken from Patients with Neoplastic Disease on the Chick Embryo.* (28946)

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Handler *et al*(1) reported that whole blood or blood fractions, obtained from patients with various forms of neoplastic disease, were toxic to chick embryos when inoculated onto the chorioallantoic membrane (CAM) of the chick embryo at the 6th to 9th day of incubation. They state that a comparison of the toxicity in chick embryos of blood from patients with and without neoplastic disease revealed that blood from 186 of 187 patients with neoplastic disease tested was toxic in eggs. Of 35 samples of normal blood, 5 were toxic; 3 of these patients had hairy or pigmented nevi, one had an osteochondroma with fever, and one a laceration, presumably under treatment. The toxic factor was present in whole blood, plasma and plasma suspensions of leukocytes. Handler(1) suggests that a subcellular factor exists in the blood of patients with neoplastic disease that is involved in the high rate of chick mortality, and furthermore, that the utilization of these techniques may be a possible tool for clinical diagnosis. Because of the important implications of this report, their technique has been used in an attempt to confirm the presence of a factor in the blood of patients with neoplastic disease toxic to the 8-day chick embryo.

Materials and methods. Fertile chicken eggs were obtained from a commercial source and incubated at 38°C. The embryos were checked for viability on the 8th day. A window was made over a major blood vessel and the CAM was dropped. Blood was obtained

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