cc). A significant amount of bile pigment was retained by the precipitate which was dissolved in water and tested clinically (Case 540,130). About 60% of the peptide was precipitated by the bile and its potency appeared to be slightly less than that of the original material.

Sodium taurocholate was dissolved in 10 parts of water and brought to pH 3.5 with dilute hydrochloric acid. The peptide (10 g) was dissolved in 20 parts of 3% acetic acid and the taurocholic acid added as long as precipitation occurred. The amount of taurocholic acid necessary was about one-third the weight of peptide. The finely divided precipitate was gently warmed to coagulate it and then after cooling, filtered off and washed with water. To regenerate the peptide, the precipitate was dissolved in a minimum of 60% alcohol, pyridine added (3 cc) to combine with the taurocholic acid, and then a large excess of acetone added. The recovered peptide only represented about 20% of the starting material. The precipitation of the peptide with taurocholic acid is apparently a reversible reaction between the 2 colloids. The very complete precipitation of proteins by taurocholic acid in contrast to its failure to precipitate the simpler peptones was observed by Maly and Emich[4] more than 50 years ago. On testing the material precipitated by taurocholic acid and that left in the filtrate it was found that both chemically and clinically there was no significant difference. (Cases 539,788, 543,019, 546,989, 481,608, 550,302.)

Summary. It has been shown that bile, taurocholic acid, and nucleic acid precipitate liver material active in pernicious anemia from aqueous solution.

10330 P

Changes in Human Blood Preserved for Transfusion.

ELMER L. DEGOWIN, JOHN E. HARRIS AND E. D. PLASS.

From the Departments of Internal Medicine and Obstetrics and Gynecology, College of Medicine, State University of Iowa, Iowa City, Iowa.

The purpose of the present studies was to determine the rate and mode of disintegration of human blood in various preservatives suitable for transfusion.

[4] Maly, R., and Emich, F., *Monatschrift f. Chemie*, 1883, **4**, 89.

TABLE I.
Changes in Human Blood Stored at 2-5°C. (Values are for plasma after cells have been centrifugated out at end of period of storage.)

		Days Storage	Hemoglobin, g%	Potassium, g%	Glucose, g%	pH	Specific Conductance, mho
Exp. 1 (aerobic)		0	<.001	.011	2.36	7.74	.00977
blood	10 parts	5	.005	.037	2.32	7.75	.01044
5.4% glucose	13 "	10	.011	.054	2.33	7.37	.01025
3.2% sodium citrate	2 "	15	.024	.063	2.29	7.39	.01054
		20	.031	.075	2.29	7.55	.01040
		25	.088	.077	2.23	—	.01058
		30	.071	.078	2.18	7.35	.01017
		35	.087	.079	—	7.28	—
Exp. 2 (aerobic)		0	.008	.016	3.39	7.70	.00583
blood	10 parts	5	.015	.035	3.34	7.59	.00582
5.4% glucose	15 "	10	.019	.050	3.15	7.71	.00623
heparin 3 mg/ 5 cc blood		15	.034	.060	2.97	7.36	.00627
		20	.058	.063	3.11	7.39	.00628
		25	.052	.065	3.14	7.47	.00641
		30	.138	.081	3.06	7.39	.00650
Exp. 3 (anaerobic)		0	<.001	.013	2.70	7.08	.00708
blood	10 parts	5	.003	.055	2.66	7.44	.00770
5.4% glucose	13 "	10	.005	.064	2.33	7.46	.00781
3.2% sodium citrate	2 "	15	.006	.066	2.53	7.28	.00774
		20	.007	.065	—	7.69	.00839
		25	.010	.096	—	7.78	—
		33	.016	.077	—	7.18	.00793
			*	*			
Exp. 4 (aerobic)		0	<.001	.012	.62	7.81	.01364
blood	21 parts	5	.051	.107	.61	8.08	.01413
5.4% glucose	2 "	10	.247	.137	.56	7.84	.01382
3.2% sodium citrate	2 "	15	1.013	.154	.51	7.73	.01300
		20	1.690	.162	.49	7.62	.01241
		25	2.370	.161	.47	7.46	.01187
		30	2.380	.167	.49	7.29	.01303
Exp. 5 (aerobic)		0	<.001	.012	.03	7.84	.01804
blood	10 parts	5	.012	.069	.01	7.32	.01830
0.95% NaCl	13 "	10	.112	.087	.00	7.69	.01854
3.2% sodium citrate	2 "	15	.381	.097	.00	7.58	.01778
			†	†			
Exp. 6 (aerobic)		0	<.001	.016	.07	7.93	.01478
blood	23 parts	5	.026	.153	.02	8.03	.01609
3.2% sodium citrate	2 "	10	.214	.179	.01	7.88	.01557
		15	.524	.193	.00	7.64	.01477

*To compare with values in Experiment 1 divide figures in these columns by 2.1.
†To compare with values in Experiment 1 divide figures in these columns by 2.3.

Blood was drawn from healthy adults in amounts of 300 to 600 cc in 1500 cc Erlenmeyer flasks containing the preservatives to be

studied. The blood mixtures were then apportioned aseptically, 50 cc into each 250 cc Erlenmeyer flask, plugged with sterile cotton and stored at 2-5°C. Evaporation was nil. Bacterial contamination was excluded. At 5-day intervals one flask of the series was withdrawn from storage, the cells and plasma thoroughly mixed by careful shaking, and the entire sample centrifugated. The plasma was then pipetted off for analysis.

The preservatives used were: Anhydrous dextrose, U.S.P. (Merck), and anhydrous dextrose (Eastman Kodak Co.). Sodium citrate ($Na_3C_6H_5O_7 + 2H_2O$) (Merck's Reagent Grade). Heparin (Connaught Laboratories).

Plasma hemoglobin was determined by the method of Wu,[1] plasma potassium by the technic of Truszkowski and Zwemer,[2] plasma glucose by the procedure of Shaffer, Hartman, and Somogyi,[3] pH of plasma by the Coleman glass electrode, and specific conductance of plasma by the standard methods.[4]

The Rous-Turner preservative[5] was modified by changing the proportions of blood, glucose, and citrate from 30:50:20 to 40:52:8, thus reducing the concentration of sodium citrate from 0.76% to 0.256%.

Table I presents data obtained from representative experiments. In no blood mixture was there total absence of hemolysis after 5 days' storage. The rates of hemolysis were definitely slower when large amounts of glucose were present. The K ions migrated into the plasma at the same rate in all preservatives; this process was independent of the amount of Hb in the plasma. The migration of K ions was not dependent upon the concentration of Na in the plasma (see No. 5) nor upon the presence of citrate ion (see No. 2). The pH changed from 8.00 to 7.00. Glycolysis proceeded at the temperature of storage for approximately 15 days.

Experiment 3 demonstrates the remarkable inhibition of hemolysis under anaerobic conditions (compare with No. 1), even though the K migration proceeds at the same rate as in blood exposed to air. We have employed the mixture in No. 1 for 35 transfusions without untoward reaction.

---

1 Wu, H., *J. Biochem.*, 1922, **2**, 189.

2 Truszkowski, R., and Zwemer, R. L., *Biochem. J.*, 1937, **31**, 229.

3 Somogyi, M., *J. Biol. Chem.*, 1926, **70**, 599.

4 Hawk and Bergeim, *Practical Physiological Chemistry*, 11th Edition, P. Blakiston's Son & Co., Philadelphia, pp. 42-45.

5 Rous, Peyton, and Turner, J. R., *J. Exp. Med.*, 1916, **23**, 219.