esterol. Phenolsulfonic acid on the other hand, reacts with estrone, estriol, and estradiol.

As mentioned above, the reaction with estrone is complete in one minute while estriol requires longer initial heating to give a significant color. The rate of reaction of guaiacolsulfonic acid with estriol indicates a preliminary dehydration to estrone in the hot concentrated sulfuric acid during the initial phase of the reaction, the estrone so formed subsequently taking part in the dye complex. It was noted above that there is no significant reaction with estradiol, a compound which has the same structure as estriol except that the hydroxy group on the 16-carbon is missing. The two adjacent hydroxy groups on the 16, 17 carbons of estriol have been shown to be converted to a 17-ketonic group under dehydration conditions.[6]

The reaction of the guaiacolsulfonic acid reagent with equilin and equilenin in the 7.5 cc technic is faintly positive when a 6-minute initial heating time is used. On the other hand, as with estriol, the color is negligible at a 1-minute initial heating time.

Under the conditions standard for the 7.5 cc technic (one minute initial heating and 2 minutes reheating), therefore, the guaiacolsulfonic acid reaction appears to be specific for estrone. This greater degree of differentiation may be of importance in the possible application of the method to mixtures of the estrogenic hormones. Its usefulness may become more apparent as advances in purification methods make possible reliable studies upon urine and tissue extracts.

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Observations on Human Blood Stored at 4° Centigrade.

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A study was made of citrated blood preserved for transfusion purposes. Two 500 cc quantities of blood were collected from apparently healthy donors under aseptic conditions. In each instance, 50 cc of a 2.5% solution of sodium citrate were used to prevent coagulation, bringing each specimen to a total of 550 cc of the blood-citrate mixture, approximately a 9% dilution of the blood.[1] The blood was stored in sealed flasks at a temperature of 4°C.[2] On the days the tests

6 Butenandt, A., and Hildebrandt, F., *Z. physiol. Chem.*, 1931, **199**, 243.

1 Lewisohn, R., *Med. Rec.*, 1915, **87**, 141.

2 Fantus, B., *J. Am. Med. Assn.*, 1937, **109**, 128.

were made, the flasks were removed from the refrigerator, agitated for 3-4 minutes, the required amount of blood withdrawn, and the flasks sealed and returned to the refrigerator.

Over a period of 29-36 days determinations were made of: the plasma proteins using modified micro-Kjeldahl methods; red blood cell fragility; the sedimentation rate of the red blood cells using the Wintrobe tube; the hematocrit value; the total erythrocyte count; the hemoglobin (Newcomer); and the total leucocyte count. The results of these studies are given in Tables I and II. Cultures of the blood at the end of the second and fourth weeks were sterile. Although only 2 specimens of blood were studied, the findings paralleled each other.

There was an appreciable increase in the fragility of the erythrocytes apparent at the fifth day, becoming marked at the end of the third week. This confirms the results recently reported by Belk, Henry, and Rosenstein.[3] The actual number of red cells destroyed

TABLE I.
Observations on Stored Citrated Blood.
Specimen 1.

Day	1	3	5	8	15	22	29	36
Albumin %	3.7	3.9	3.9	3.9	3.6	4.1	5.9	5.2
Globulin %	1.5	1.4	1.2	1.3	2.0	1.8	2.0	3.6
Fibrinogen %	0.195		0.17	0.16	0.16	0.25	0.34	0.39
Fragility (% NaCl)	.46-.30	.46-.32	.50-.32	.50-.34	.54-.36	.85-.38	.85-.38	
Sedimentation rate (mm/hr)	4	1	1	1	0	0	0	
Hematocrit %	40	41	41	41	42	41	38	
Red blood count (millions)	4.2	4.0	4.0	4.0	4.4	4.3	4.0	
Hemoglobin %	76	75	75	79	75	74	78	
White blood count (thousands)	8.9	7.4	8.0	7.2	6.4	4.0	3.3	

TABLE II.
Observations on Stored Citrated Blood.
Specimen 2.

Day	1	3	5	8	15	22	29	36
Albumin %	4.2	3.5	3.7	3.4	3.6	4.4	6.0	6.1
Globulin %	2.1	2.1	2.1	2.0	1.8	1.6	2.6	3.3
Fibrinogen %	0.26		0.28	0.29	0.25	0.36	0.43	0.55
Fragility (% NaCl)	.46-.30	.46-.32	.50-.32	.50-.34	.54-.36	.85-.38	.85-.38	
Sedimentation rate (mm/hr)	8	3	2	3	0	0	0	
Hematocrit %	42	42	42	42	45	44	41	
Red blood count (millions)	4.2	4.0	4.1	4.2	4.3	4.2	4.1	
Hemoglobin %	74	78	78	80	80	78	76	
White blood count (thousands)	9.8	8.5	8.1	7.7	5.8	3.2	2.5	

must have been small, for the total red cell count and hematocrit determinations did not show significant changes. The appearance of hemoglobin in the plasma, which is probably associated with this increased fragility, was first grossly apparent on the fifth day.[4] The total leucocyte count showed a decrease at the end of the second week which became more marked by the third and fourth weeks.[3, 4]

Changes in the concentration of proteins other than hemoglobin in the plasma have not, as far as the authors can determine, been reported previously. There was a significant increase in all of the protein fractions. The actual change in concentration was approximately the same for both albumin and globulin, but the percentage increase in globulin was greater. The change in globulin could not be explained by the hemoglobin content of the plasma, for there was only a trace of visible color at the end of the experiment.

It is interesting to note that not only did the proteins classified as "albumins" and "globulins" increase during the storage of the blood, but that portion described as "fibrinogen" also showed an increase. The nature of this compound cannot be decided definitely, but the increase of the insoluble nitrogen content of the clot formed on the addition of calcium chloride to give a total concentration of less than 0.1% is unquestioned.

It is worth noting that in spite of the changes in the albumin-globulin ratio noted in the first specimen and the increase in apparent fibrinogen observed in both experiments, the sedimentation rate of the erythrocytes did not increase during the period of observation. Instead, there was a significant decrease in the rate from that observed when the specimens were first obtained. The rate became unmeasurable by the method used after the blood had been stored for 2 weeks. The authors were unable to offer any explanation for this unexpected finding, which has apparently not been described.

In addition to the changes in the chemical properties of stored "bank" blood which have previously been reported, an increase in the precipitable nitrogen in the supernatant plasma can be demonstrated. This change is shown by the 3 protein fractions, albumin, globulin, and fibrinogen, after the blood has been preserved for one month. The change is too great to be attributed to the amount of hemoglobin which is present in the plasma. A significant decrease in the rate of sedimentation of the erythrocytes also developed during the period of observation.

[3] Belk, W. P., Henry, N. W., and Rosenstein, F., *Am. J. Med. Sci.*, 1939, **198**, 631.

[4] Kolmer, J. A., *Am. J. Med. Sci.*, 1939, **197**, 442.