2. The electrical variation has the form of a ventricular premature beat and not that of a ventricular beat due to an auricular impulse (Figs. 1-4).

3. The P-wave occurs after the premature ventricular complex and the spacing of the P-wave remains normal, showing that the auricular rhythm has not been disturbed (Figs. 1, 2-4).

4. The premature contraction when not producing fibrillation is followed by a full compensatory pause which, although possible in case of auricular premature systoles, is a rare occurrence (Fig. 1).

5. A stimulus applied a little too early in ventricular systole to produce an extra contraction never induces premature auricular contraction as it should if it were spreading to the atria.

When ventricular fibrillation follows as in Figs. 2, 3, 4, the undisturbed and regular sequence of the P waves and of small pressure elevations of atrial origin shows that the stimulus acts directly on the ventricle.

The conclusion is reached that a single short strong localized shock applied during the latter portion of systole is directly responsible for the premature contraction or ventricular fibrillation.

11165 P

A New Reagent for Quantitative Estimation of Estrone.

CLARA M. SZEGO AND LEO T. SAMUELS.

From the Department of Physiological Chemistry, University of Minnesota, Minneapolis.

Most present colorimetric methods of assay[1-4] for the estrogenic hormones have two serious defects: a lack of specificity and the production of interfering colors with inactive impurities. Bachman,[5] however, has recently reported a modification of the Kober reaction which appears to be specific for estriol.

This report deals with an investigation of guaiacolsulfonic acid:

1 *a.* Kober, S., *Biochem. Z.*, 1931, **239**, 209—modified by: *b.* Cohen, S. L., and Marrian, G. F., *Biochem. J.*, 1934, **28**, 1603; *c.* Cartland, G. F., Meyer, R. K., Miller, L. E., and Rutz, M. H., *J. B. C.*, 1935, **109**, 213; *d.* Venning, E. H., Evelyn, K. A., Harkness, E. V., and Browne, J. S. L., *J. B. C.*, 1937, **120**, 225; *e.* Bachman, C., *J. B. C.*, 1939, **131**, 455.

2 Zimmerman, W., *Z. physiol. Chem.*, 1935, **233**, 257; 1936, **245**, 47.

3 Smulovitz, M. J., and Wylie, H. B., *J. Lab. and Clin. Med.*, 1935, **21**, 210.

4 Kober, S., *Biochem. J.*, 1938, **32**, 357.

5 Bachman, C., *J. B. C.*, 1939, **131**, 463.

$(CH_3.O)$ (HO) $(C_6H_3.SO_3H)$, substituted for phenolsulfonic acid as the color reagent in the Kober[1] reaction. The reagent was prepared by dissolving the potassium salt, thiocol, in concentrated sulfuric acid.

Conditions for the reaction with crystalline estrone* in both 1.0 cc and 7.5 cc final volumes have been studied.

Guaiacolsulfonic acid gave a pink color with estrone which was similar in intensity to that given by phenolsulfonic acid. The resultant color of the reaction in the 1 cc final volume was more stable than that given by the Cartland[1] phenolsulfonic acid procedure. The color in the 7.5 cc final volume was at least as stable as that given by the Venning[1] phenolsulfonic acid procedure modified to that volume.

Beer's law applied to the reaction with the new reagent over at least 25-fold variation in hormone concentration.

A 10% solution of thiocol and concentrated sulfuric acid was found to be most satisfactory for the 1 cc procedure. This procedure involves the same series of steps as the Kober[1] reaction except that the optimum times for each step were as follows:

1. Six minutes initial heating in the boiling water bath.
2. Cooling in ice water 5 minutes.
3. After dilution, heating in a boiling water bath for 7 minutes.
4. Cooling again in ice water for 5 minutes before the final dilution.

Concentrations of guaiacolsulfonic acid were compared for the reaction in the 7.5 cc volume and it was found that 20% thiocol was more satisfactory than the 10% reagent used in the 1 cc technic. A one-minute initial heating time, and a 2-minute reheating time proved best for this amount. Except for reagents and time conditions as given, volumes of reagents for the 7.5 cc procedure were one-half those used by the Venning group,[1] and the technic of color development was the same.

The error in the determination was less than a maximum of $\pm 5\%$ with 5μg, and $\pm 2\%$ with 15μg quantities of estrone. As little as 1 μg in a final volume of 7.5 cc could be detected.

The guaiacolsulfonic acid reagent is less viscous than the phenolsulfonic acid reagent, making pipetting easier. The reproducibility of the new reagent is better than that of the latter because thiocol is easily weighed and is not hygroscopic.

It reacts with estrone and at slower rate with estriol, but not with estradiol, androsterone, sodium pregnandiol glycuronidate or chol-

* Estrone and estriol for this study were kindly supplied by Parke, Davis and Company. Estradiol and androsterone were furnished by the Schering Corporation. Equilen and equilenin were given us by Prof. Oskar Wintersteiner of Columbia University.

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.

11166

Observations on Human Blood Stored at 4° Centigrade.

WILLIAM F. LIPP AND ROGER S. HUBBARD.
From the Buffalo General Hospital, Buffalo, N. Y.

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.