9695

The Direct Determination of Ethyl Alcohol in Saliva without Distillation.*

THEODORE E. FRIEDEMANN. (With the technical assistance of Mr. Theodore Brook.)

From the Department of Medicine, University of Chicago.

Saliva is the most dilute of all the secretions, having a solid content of only about 0.5%.[1] It distills with less foaming, and the volatile oxidizable material, determined after a single distillation, is considerably smaller than that of blood or urine.[2] It contains practically no sugar and from 40 to 75% of the nitrogenous non-protein constituents usually found in the blood. Because of its relative simplicity and low organic matter content, most of the oxidizable organic matter of saliva can be removed by precipitation with $CuSO_4$-$HgSO_4$-$Fe_2(SO_4)_3$ followed by $Ca(OH)_2$. Ethyl alcohol is not precipitated by this reagent and can be determined by direct oxidation of the clear supernatant solution.

No special apparatus, such as a still, is required. The precautions as to cleanliness of glassware, noted by Friedemann and Klaas[2] should be carefully observed.

Reagents. 1. Cu-Hg-Fe reagent. 200 cc. of water are added to 40 gm. of $CuSO_4$-$5H_2O$, 40 gm. of $HgSO_4$ (bisulfate) and 15 gm. ferric sulfate. 25 cc. of concentrated H_2SO_4 are added and heat is applied until solution is complete. 800 cc. of water are then added. The solution is kept in a glass-stoppered bottle protected from dust.

2. $Ca(OH_2)$ suspension. Ordinary unslaked lime may contain much organic matter. C. P. CaO (Merck) is therefore recommended. 100 gm. are slaked with a minimum of distilled water. 1000 cc. of water are then added. The creamy suspension is kept in a glass-stoppered bottle protected from dust.

3. Distilled Water. The precautions concerning the purity and method of storage of water noted by Friedemann and Klaas should be carefully observed.

Precipitation of Proteins, Amino-compounds, sugars, etc. 1.0

* A preliminary report of this paper was made in March, 1936, before the American Society of Biological Chemists (*Proc. Am. Soc. Biol. Chem.*, March, 1936, 37).

1 Bodansky, M., *Introd. to Physiol. Chem.*, Wiley and Sons, New York, 2nd Ed., 1930, 147.

2 Friedemann, T. E., and Klaas, R., *J. Biol. Chem.*, 1936, **115**, 47.

cc. of saliva is pipetted into a 100 cc. glass-stoppered volumetric flask. Since saliva is viscous, the pipette should be allowed to drain slowly. 10 cc. of Cu-Hg-Fe reagent and about 50 cc. of water are added. An excess of $Ca(OH)_2$ is added from a pipette, and the contents are mixed by rotation. The volume is then adjusted to the mark; the contents are thoroughly mixed. The flask is allowed to stand several minutes before withdrawing a sample of the clear supernatant solution for analysis.

The ability of $Cu(OH)_2$ to precipitate sugars,[3] as well as the precipitation of proteins and amino-compounds by mercury,[4, 5] has long been known. $Cu(OH)_2$ also precipitates other substances. By combining the 2 reagents,[6] a large part of the organic matter can be precipitated in one operation. $Fe(OH)_3$ precipitates proteins,[7] adsorbs many organic acids, and removes some of the sugar; its presence assures a more rapid settling of the precipitate and a clearer supernatant solution. $HgSO_4$, with H_2SO_4, is preferred to $HgCl_2$ because it yields a more granular precipitate.

Filtration is not recommended. It requires more glassware, it increases the number of manipulations and it introduces a variable amount of soluble oxidizable material from the filter paper.[8] The oxidizable material appears in the first 10 to 25 cc. of filtrate; its effect on the results can be minimized by discarding this portion of the filtrate.

Oxidation. 10 cc. of the clear supernatant solution is withdrawn by means of a pipette and transferred to a carefully cleaned 150 cc. extraction flask. The latter is covered with a 100 cc. beaker. To this are added 15 cc. of water, 10 cc. of 5 *N* NaOH, and, with rotation, exactly 25 cc. of 0.01 *N* $KMnO_4$. The contents are again mixed by rotation. The oxidation and final titration are carried out as directed by Friedemann and Klaas.

If speed is necessary and if only approximate results are desired, the alcohol content may be determined colorimetrically. Depending upon the alcohol content, the color changes are from violet, purple, greenish-purple, green, to brown. The colors may be compared with the colors obtained by oxidation of the supernatant solutions from pure alcohol standards.

---

3 Van Slyke, D. D., and Fitz, R., *J. Biol. Chem.*, 1917, **32**, 455.

4 Patein, G., and Dufau, E., *J. pharm. et chim.*, 1902, **15**, 221.

5 West, E. S., Scharles, F. H., and Peterson, V. L., *J. Biol. Chem.*, 1929, **82**, 137.

6 Freeman, S., and Friedemann, T. E., *J. Biol. Chem.*, 1935, **108**, 471.

7 Somogyi, M., *J. Biol. Chem.*, 1931, **90**, 725.

8 Friedemann, T. E., and Graeser, J. B., *J. Biol. Chem.*, 1933, **100**, 292.

The supernatant solution contains a considerable quantity of calcium salts which, by reaction with NaOH, lower the alkalinity. This effect is not pronounced with the 10 cc. sample recommended. However, a larger sample reduces the alkalinity below the optimum necessary for maximum oxidation in the 20-minute heating period. For larger samples more alkali should be added.

Blanks. Blank determinations with the water and reagents should be run simultaneously.

Sample Calculation. 1 cc. sample diluted to 100 cc.; 10 cc. analyzed. Blank titrations: 24.85, 24.90 cc. 0.01 *N* thiosulfate. Titration of samples: 20.37, 20.36 cc. 0.01 *N* thiosulfate. 24.88-20.37 = 4.51. 1 cc. 0.01 *N* thiosulfate is equivalent to 0.042 mg. ethyl alcohol.

$$\text{Mg. \% alcohol} = \frac{4.51 \times 0.042 \times 100}{0.1} - 11 = 178 \pm 4$$

The reagent does not precipitate all of the oxidizable substances of saliva, but the residual material is small and relatively constant. Saliva was collected from 20 subjects and analyzed by the direct method as given above. The results, expressed as mg. % of ethyl alcohol, were: 11, 11, 7, 14, 8, 17, 2, 0, 9, 10, 14, 16, 13, 16, 10, 10, 5, 7, 16, 13. The arithmetic mean is 10.5, with a standard deviation of ±4.6. The deviation from the mean corresponds to a ±0.1 cc. error in titration.

From 25 to 75 cc. of pure grain alcohol, diluted with water, were given to 9 subjects. Twenty-two samples were collected and analyzed by the distillation procedure of Friedemann and Klaas and by the direct method of the present paper. The results given by the 2 methods were: 142, 152; 60, 67; 66, 78; 98, 106; 89, 98; 81, 97; 98, 113; 57, 71; 50, 61; 66, 83; 63, 76; 78, 88; 64, 79; 99, 106; 103, 108; 91, 103; 69, 83; 91, 117; 94, 106; 106, 118; 95, 104; 80, 88. The alcohol concentrations varied from 50 to 142 mg. %. In every instance a higher result was obtained by the direct method. The differences varied from +5 to +26; the latter represented an unusual variation. The arithmetic mean of all determinations is 11.9, with a standard deviation of ±4.4. By excluding the single high result, the arithmetic mean becomes 11.2 ± 3.3.

Summary. All but a small, but relatively constant, quantity of the oxidizable material of normal saliva may be removed by the addition of 2 reagents: (1) a solution of $CuSO_4$, $HgSO_4$, and ferric sulfate, and (2) a suspension of $Ca(OH)_2$. The residual material

from normal saliva is equivalent to 10.5 ± 4.6 mg. % of ethyl alcohol.

A procedure for the direct determination, without distillation, of ethyl alcohol in saliva is described. The results are 11.2 ± 3.3 mg. % higher than by the method of Friedemann and Klaas.

9696

Inhibitory Effects of Male Sex Hormone on Human Menstruation and Their Evaluation by Vaginal Smears.*

GEORGE N. PAPANICOLAOU, HERBERT S. RIPLEY AND EPHRAIM SHORR.

From the Departments of Anatomy, Psychiatry, and Medicine, Cornell University Medical College, and the New York Hospital.

The suppressive effect of the secretions of the male gonads on the female sexual functions of mammals has been known for a long time. Prior to the isolation of the active principle of the testis, evidence of this effect was obtained from studies of cases of hermaphroditism, gonadal transplants, studies on freemartins, and experiments with parabiotic twins.

With the isolation of the male sex hormone, experimental evidence has been obtained of the inhibitory effect of this hormone on the sex cycle of female rodents.[1] Recently a similar effect on menstruation in primates has been demonstrated.[2]

The absence of comparable studies in the human has prompted this report on the effect of the male sex hormone on the menstruation of a young woman. The results obtained with the treatment of this case are in agreement with the experimental findings in animals. Furthermore, they were complemented by observations of significant morphological changes occurring in the vaginal fluid which were revealed by studies of vaginal smears.

The subject was a young woman of 18 with a history of premenstrual depression, dysmenorrhea, moderate menorrhagia, and occasional premenstrual bleeding. Physical examination was es-

* This work has been aided by grants from the Committee for Research on Sex Problems, of the National Research Council, and from the Josiah Macy, Jr., Foundation.

1 Robson, J. M., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 49.

2 Zuckerman, S., *Lancet*, 1937, **233**, 676; Hartman, C. G., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 87.