ords taken from both the central and the peripheral end. Those from the central end still presented regular oscillations of a frequency equalling the pitch of the recorded voice sound. The range of frequencies observed so far lies between 380 and 1800 oscillations per second. We, therefore, assume that the observed potential changes are travelling from the central organ to the larynx.

We do not feel justified in considering these regular action-potentials as originating in the higher centers of coordination. We studied the superior laryngeal nerve as the possible afferent branch for a proprioceptive reflex mechanism containing the inferior laryngeal nerve as efferent branch. Records taken from the superior laryngeal nerve do not show the observed regular oscillations but irregular potential changes of small amplitude and low frequency, which indicates that this nerve is largely sensory.

Transsection of the superior laryngeal nerve leads to a definite change in the action current picture recorded for the inferior laryngeal nerve. It seems that the regular oscillations described above disappear after this procedure on the side of the transsected nerve. Although this does not prove that the superior laryngeal nerve serves as the afferent branch in the reflex arc, it favors the explanation offered for the regular oscillations observed in the inferior laryngeal nerve. Preisendorfer[1] has described action current records obtained from the calf musculature while the subject is pressing his toes against a vibrating object. The usual irregular grouping of large and small oscillations was replaced by regular oscillations corresponding in frequency to the vibration of the object. He considers his picture as a series of proprioceptive reflexes. We believe that a similar mechanism is responsible for the regular oscillation observed in the laryngeal nerve. Further research will be necessary to study the suggested proprioceptive control of the action of the vocal chords.

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The Micro Determination of Blood Sugar.

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A technically simple and rapid procedure for the determination of the blood sugar which gives results which are little, if at all,

[1] Preisendorfer, *Z. f. Biologie*, 1919, lxx, 505.

affected by the non-sugar reducing substances of the corpuscles and plasma is presented. The problem of non-sugar reducing substances has been approached in 2 ways—by using copper solutions which are effective on the glucose of the blood only (Folin, S. R. Benedict) and by obtaining protein-free filtrates from which the non-sugar reducing substances are removed along with the protein (Somogyi). The second procedure is obviously the more practical, and is attained in our methods by increasing the proportion of tungstate and sulfuric acid in relation to the volume of blood precipitated.

Dr. J. S. Boyd (1923), working in this laboratory, adapted the Folin-Wu method for finger-tip blood specimens to meet the increase in the number of blood-sugar determinations necessitated by insulin control of diabetics. We are now doing over 4200 blood sugar determinations yearly in the routine diabetic service alone. The procedure may be used for venous specimens as well. About 2.5 times the amounts of sodium tungstate and sulfuric acid over that in the original Folin-Wu procedure in proportion to the volume of blood taken were used as the precipitant. As completely glycolized specimens of blood give no color with Boyd's method, Shrader[1] added increasing amounts of glucose to blood after glycolysis and determined these. Calculated values for the glucose found over a range of 70 to 140 mgm. percent corresponded closely to the amounts of sugar added, with progressively increasing variation above and below these figures.

Shrader prepared a table for the glucose in mgm. percent corresponding to colorimetric readings (in mm.) over the ordinary clinical range of blood sugar values from a graph constructed from his data. The method was practical and gave results somewhat lower than could be obtained with other procedures. From some figures obtained in studying the distribution of glucose between the plasma and corpuscles in diabetic patients receiving insulin and glucose, 10 analyses gave an average corpuscle-plasma glucose ratio of 0.74, the normal ratio according to Somogyi[2] being 0.77.

However, the color developed tends to fade on standing. Substitution of S. R. Benedict's arseno-tungstate reagent with added formaldehyde[3] gives a color which is permanent and more easily matched. Both blank reagent mixtures and glycolized blood give

[1] Gibson, R. B., Mitchell, K. Z., and Larimer, R. N., *J. Iowa State Med. Soc.*, 1925, xv, 225.

[2] Somogyi, M., *J. Biol. Chem.*, 1928, lxxviii, 117; *Arch. Int. Med.*, 1928, lxiii, 931.

[3] Benedict, S. R., *J. Biol. Chem.*, 1925, lxiv, 207.

a trace of blue color, and results for added sugar (100 mgm., to glycolized blood) are about 10 mgm. too high.

A table of glucose values corresponding to the colorimetric readings when the arseno-tungstate sugar reagent was employed was prepared by Madge L. Baltimore from determinations of known amounts of glucose added to completely glycolized blood. Her analyses were in duplicate and results obtained with 3 specimens of glycolized blood were averaged.

TABLE I.

Blood sugar values from colorimetric readings (std. at 10 mm.). Micro-adaptation for finger tip blood of the combined Folin-Wu and Benedict procedures; from sugar added to glycolized blood (M.L. Baltimore).

Mm.	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
2						400	385	372	362	349
3	337	325	316	306	297	289	281	273	266	259
4	253	247	241	236	231	226	221	217	212	208
5	204	200	196	192	189	186	182	179	176	173
6	170	167	165	162	159	157	155	152	150	147
7	145	142	140	138	135	133	131	129	126	124
8	122	120	118	116	114	112	110	109	107	105
9	104	102	100	99	98	96	95	94	92	91
10	90	89	87	86	85	84	82	81	80	79
11	78	77	75	74	73	72	71	69	68	67
12	66	65	64	63	62	61	60	59.5	59	58
13	57	56	55	54	53	52.5	52	51	50	49.5
14	49	48	47	46.5	46	45	44	43.5	43	42
15	42	41	40	39.5	39	38.5	38	37	36.5	36
16	35.5	35	34.5	34	33	32.5	32.5	32	31	31
17	30	29.5	29	29	28.5	28	27	27	26.5	26
18	25.5	25	24.5	24	24	23.5	23	23	22.5	22
19	22	21.5	21	20.5	20	20	19.5	19	19	18.5
20	18	18	17.5	17	17	17	16.5	16	16	15.5

Blood specimens from our psychopathic service probably more closely approximate normal venous blood values. For evaluation of the blood sugar method, 107 specimens of blood from these patients for the 2nd half of the year 1929 have shown an average of 91 mgm. percent with extremes of 66 to 122 mgm. A second routine series beginning January 1, 1930, of 25 specimens shows an average of 85 mgm. percent with extremes of 72 to 104 mgm. percent. The distribution of glucose in the venous blood during a sugar tolerance test on a mild diabetic gave a corpuscle-plasma ratio of 0.76 as an average for 6 blood specimens taken. The corpuscle glucose content was calculated from analyses of whole blood, plasma, and the hematokrit value.

Draw 0.2 cc. of blood from a finger tip puncture (we use a pointed interchangeable surgical knife blade) into a serological or special pipette, graduated to the tip, as in drawing blood for the

Sahli hemoglobin determination. Discharge the blood into a centrifuge tube containing 4.3 cc. of 1.25% sodium tungstate solution and 0.5 cc. of 2% (volume) sulfuric acid. Centrifuge after standing 15 minutes; there will be sufficient supernatant fluid for duplicate determinations. Take 2 cc. of the supernatant fluid in a Folin-Wu sugar tube graduated at 10 and 25 cc. and 2 cc. of 0.01% glucose standard solution in a second tube; add 2 cc. of the alkaline copper-tartrate solution to each tube, and heat for exactly 6 minutes in the boiling water bath. Cool, and then add 2 cc. of sugar reagent to each tube and mix by inclining the tubes and tapping the bulbs of the tubes sharply against the palm of the hand until gas ceases to form. Dilute the blood sugar tube to the 10 cc. mark and the standard tube to 25 cc., mix, and read in the colorimeter with the standard at 10 mm. Take the result from tabulated values for the ordinary range of readings. Blood specimens sent to the laboratory may be determined by the above procedure; 0.05 gm. of sodium fluoride per 5 cc. of blood is preferred as a preservative and anticoagulant.

For bloods with blood sugar values over 300 mgm., the blood tube may be diluted to the 25 cc. mark and the calculated result multiplied by 2.5. For hypoglycemic bloods a double strength alkaline copper solution is used; for 2 cc. of the supernatant fluid, add 1 cc. of the double strength copper solution and 1 cc. of a 0.004% glucose solution and subtract 50 mgm. from the blood sugar figure obtained.

Solutions. 1. Sodium tungstate, 1.25% solution.

2. 2% sulfuric acid by volume (approximately 2/3 normal).

3. Alkaline copper-tartrate solution: dissolve 16 gm. of anhydrous sodium carbonate in 160 cc. of water in a 400 cc. beaker, add 3 gm. of tartaric acid, and when dissolved add 1.8 gm. of crystalline copper sulfate (grind in a mortar after weighing); mix and make up to 400 cc. (filter if necessary). A double strength copper solution, twice the above ingredients made up to 400 cc., should be kept on hand.

4. Standard sugar solution (stock): 1 gm. of glucose to 100 cc.; add NaF as a preservative and keep in the ice-box. Dilute 1 cc. of the stock solution to 100 cc. to give a 0.01% solution. Dilute 1 cc. to 250 cc. for a 0.004% solution.

5. Arseno-phosphotungstic acid reagent: 100 gm. pure sodium tungstate in a liter flask with 600 cc. water and dissolve. Add 50 gm. of pure arsenic pentoxide, 25 cc. of 85% phosphoric acid, 20 cc. of conc. hydrochloric acid. Boil 20 minutes. Cool and dilute to 1000 cc. Add 5 cc. of 40% formaldehyde solution for each 100 cc. of the reagent.