

ON THE VALUE OF BRÜCKE'S METHOD FOR THE
REMOVAL OF INTERFERING SUBSTANCES FROM
URINE IN TESTING FOR GLUCOSE.

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In determining the presence of glucose in diabetic urine by any of the copper tests it is found that, under certain circumstances, some of the normal constituents of urine, such as creatinine and uric acid, interfere with the reaction of these tests. In the ordinary course of a case of diabetes, when the urine contains an abundance of glucose, usually no difficulty is experienced in obtaining the reactions; but after the patient has been restricted to a diet of nitrogenous food alone, great difficulty is often found in making the tests, because of a diminution in the amount of glucose and an increase in the amount of the interfering substances, creatinine, uric acid, etc. It may therefore be necessary to remove these substances before testing for the glucose. The method for their removal suggested by Brücke, and in use many years, consisted in adding neutral plumbic acetate, $Pb(C_2H_3O_2)_2$, to the urine, and immediately afterward adding plumbic tribasic acetate, $Pb_3O_2(C_2H_3O_2)_2$. The precipitate was then separated by filtration and washed with distilled water. The filtrate and washings having been collected in one vessel, ammonium hydroxide was added, which threw down the glucose in combination with the lead in the form of lead saccharate, $(PbO)_3(C_6H_{12}O_6)_2$. This white precipitate was collected on a filter paper, washed, and then suspended in distilled water, and hydrogen sulphide, which decomposed the compound, passed through it. The filtered solution contained the glucose;¹ and it was used for the tests, after the hydrogen sulphide had been driven off by evaporation.² The original paper by Brücke could not be obtained, as it was not to be found in the journal referred to by Neubauer and Vogel. However, Beale gives the same method as the preceding. According

¹ The neutral plumbic acetate removes the sulphates, phosphates, and carbonates. The plumbic tribasic acetate precipitates the coloring matter, and at least a portion of the uric acid and creatinine. Probably a small amount of uric acid is contained in the precipitate of lead saccharate, and the remainder of the uric acid and creatinine, together with the urea, are contained in the filtrate after removal of the lead saccharate.

² Neubauer and Vogel. 8th Ed., 1881, 165.

to Bence Jones, "the seventh of a grain of glucose may be detected, when diluted with upwards of six ounces of water, and two-thirds of the total quantity of glucose present in a solution can be separated by this process."¹

The method of Brücke, as given by Salkowski and Leube,² is as follows: Neutral plumbic acetate is first added to the urine, which is then filtered. Plumbic tribasic acetate and, afterward, ammonium hydroxide are added to the filtrate. After collecting the precipitate on a filter it is washed and suspended in water, hydrogen sulphide passed through it, and the evaporated solution employed for making the tests for glucose.

Although certain statements have been made by different writers as to the amount of glucose recovered after the use of this process, yet, so far as could be learned, no accurate experiments had been made to verify these statements. For this reason the experiments here described were undertaken for the estimation of this amount, and the determination thereby, if possible, of the practical value of the process. For it is readily seen that if a large percentage of the glucose is lost in the procedure, when but a relatively small amount had been present, that which remains may not be sufficient to give the proper reactions. Hence it might be thought that no glucose is present, when it has really been present in the original urine. In making the experiments, fresh normal urine, free from glucose, was used, and to certain quantities of this were added definite amounts of pure crystallised glucose. The first method attempted was the following: Three portions of urine containing definite amounts of glucose were employed: 100 cc. of urine containing 1.0 gram of glucose; 100 cc. of urine containing 0.5 gram of glucose; 100 cc. of urine containing 0.1 gram of glucose. Each of these quantities of urine, after the glucose had been thoroughly dissolved, was then divided into two parts of 50 cc. each, and the two samples worked at the same time. To each of these portions of urine were added 35 cc. of a 10 per cent. solution of neutral plumbic acetate. It was then stirred and filtered, and the precipitate washed with water in six portions of 5 cc. each. The filtrate and wash-water were collected together and stood aside. (For convenience this will be designated filtrate *A*.) The precipitate was suspended in about 50 cc. of water, warmed, and hydrogen sulphide passed through it until all the lead was pre-

¹ Beale, "Kidney Diseases, Urinary Deposits and Calculous Disorders." 3d Ed., 249.

² "Die Lehre vom Harn." 1882, 224.

precipitated, and then filtered. The precipitate was then washed with water in five portions of 5 cc. each, and the filtrate and wash-water evaporated to dryness on a water-bath. The dry residue was extracted with three or more portions of 5 or 8 cc. each of alcohol (to get rid of uric acid). The solution was filtered through a small filter, and the residue washed with about 15 cc. of alcohol. The alcoholic extract with the washings was evaporated to dryness on a water-bath.¹ The dry residue was dissolved in water, the volume of the solution carefully ascertained, and the amount of glucose present determined by means of Fehling's solution or the saccharimeter.

To filtrate *A*, obtained after the addition of neutral plumbic acetate to the urine, were added 15 cc. of plumbic tribasic acetate.² After being stirred and filtered, the precipitate was washed with water in six portions of 5 cc. each. (The filtrate and wash-water will be designated *B*.) The precipitate was suspended in about 50 cc. of water, warmed, and hydrogen sulphide passed through it until all the lead was precipitated. It was then filtered, and the precipitate washed with water in five portions of 5 cc. each. The filtrate and wash-water, collected together, were evaporated to dryness on a water-bath, the residue extracted with methyl alcohol, filtered, washed with more alcohol, and evaporated to dryness on a water-bath. The dry residue remaining was extracted with water, the volume of the solution ascertained, and the amount of glucose present determined as in the previous instance.

To filtrate *B* with wash-water, obtained after the precipitation by plumbic tribasic acetate, was added ammonium hydroxide until the solution had a slight odor of ammonia. The precipitate thus formed was collected on a filter-paper, washed with water in five portions of 5 cc. each, and the filtrate with the wash-water preserved. (It will be designated filtrate *C*.) The filter-paper was pierced, and the precipitate washed into a beaker and diluted with water to a volume of about 100 cc., the mixture warmed, and hydrogen sulphide passed through until all the lead was thrown down. The precipitate was collected on a filter and washed with

¹ Methyl alcohol was here used, glucose being more soluble in it than in the ordinary ethyl alcohol. All the alcohol used was first tested carefully to be sure that it contained no reducing substance, by evaporating a portion to dryness on a water-bath, dissolving the residue in water, and testing it by means of Fehling's solution.

² Prepared as follows: 192 grams of acetate of lead were dissolved in 640 cc. of distilled water, and 64 grams of yellow oxide of lead added. This was allowed to stand (with frequent shaking), until only a small white precipitate remained. The precipitate was then removed by filtration, and the solution was ready for use, its specific gravity being between 1.23 and 1.24

five portions of water of 5 cc. each; the filtrate and wash-water collected together, evaporated to dryness, extracted with methyl alcohol, filtered and washed, evaporated to dryness, extracted with water, and the amount of glucose determined as before. (The amount of glucose found in this instance should be the amount present at the end of the process, while amounts found in each of the other instances would be the losses occasioned by the process.) Filtrate C, with the wash-water obtained after the addition of ammonium hydroxide, was evaporated to dryness, and after being treated as in the other instances, the glucose present was estimated by Fehling's solution or by the saccharimeter.

Certain difficulties, however, were found in this process which rendered it impracticable. It was found, after evaporating the various solutions to dryness, even though done on a water-bath and with greatest care at a low temperature, that a dark brown, or at times almost black, residue was obtained; which partly dissolved in the alcohol, and rendered the final water solution so dark that it could not be used in the saccharimeter, even in some instances preventing the passage of any light at all through the observation tube. The color of the solution also prevented the use of Fehling's solution, for when it was added to the latter reagent a green solution was obtained, and the exact point of the completion of the reaction could not be determined. On account of the very dark color of the solution it was not possible to dissipate the color sufficiently by dilution without carrying it to such a degree that the small amount of glucose present could not be detected. It was thought that the dark color was probably produced by concentration (during evaporation) of the coloring matters of the urine. It was also thought that the acid found to be present in the solution might produce some reaction on certain of the urinary constituents present and thus cause a dark coloration. Therefore, after the excess of hydrogen sulphide had been driven off by evaporation on a water-bath, a saturated solution of sodium bicarbonate was added, until the solution was rendered neutral, and the evaporation then continued to dryness. It was found, however, that this neutralisation of the acid did not prevent the formation of the dark residue, and hence this method was abandoned.

The method next adopted was to make a number of solutions by dissolving a definite quantity of glucose in a small amount of urine, and then adding more urine until a volume of 100 cc. was obtained. A number of such solutions were made of different

strengths, so that 100 cc. would contain 0.05 gram, 0.1 gram, 0.5 gram, 1.0 gram, or 2.0 grams of glucose. Then the strength of each solution was 0.05 per cent., 0.1 per cent., 0.5 per cent., 1.0 per cent., or 2.0 per cent. Each solution was then carefully divided into two parts of 50 cc. each by means of an accurate pipette. In this way two solutions of exactly equal strength were obtained, which could be readily carried through the process at the same time. (In the following description each solution mentioned is to be understood as having had a volume of 50 cc.) To each portion of urine were added 20 cc. of a 10 per cent. solution of neutral plumbic acetate. The mixture was thoroughly stirred and afterwards filtered, and the precipitate washed with 25 cc. of distilled water in portions of 5 cc. each. The filtrate and washings having been collected together, were poured into a graduated cylinder, the volume of the whole ascertained, and the amount of glucose present determined by taking the average of at least ten observations with the saccharimeter. The saccharimeter tube was carefully washed out, the washings saved and placed with the remainder of the solution, to which were then added 15 cc. of plumbic tribasic acetate, the mixture carefully stirred and filtered, and the precipitate washed with 25 cc. of distilled water in portions of 5 cc. each. The filtrate and washings having been collected together, the volume of the whole solution was ascertained, and the amount of glucose present estimated by the saccharimeter as before. To the whole of this solution, which was carefully saved after the estimation, were added 20 cc. of ammonium hydroxide,¹ and the mixture carefully stirred and filtered. The precipitate was washed with 25 cc. of distilled water in portions of 5 cc. each, and the filtrate and wash-water collected, the volume of the whole ascertained, and the amount of glucose present determined by the saccharimeter.

The precipitate caused by the addition of ammonium hydroxide was suspended in distilled water, and hydrogen sulphide passed through it until all the lead was precipitated, then filtered, and the precipitate washed with 25 cc. of distilled water in portions of 5 cc. each. The filtrate and wash-water were collected together, the volume of the whole solution ascertained, and by the saccharimeter the amount of glucose present was determined.

In three cases a saturated solution of potassium carbonate instead of hydrogen sulphide was used to decompose the lead saccharate.

¹ The solution of ammonium hydroxide employed contained 10 per cent. of NH_3 .

In several cases, both neutral plumbic acetate and plumbic tribasic acetate were added before filtration, and the proportion of glucose present then determined, after which the process was carried on as before described.

In a few instances one or more of the determinations were made by Fehling's solution instead of by the saccharimeter.

In some of the solutions the quantity of glucose was not estimated until after the completion of the whole process. Frequently, however, an estimation was also made of the glucose lost in the filtrate after precipitation by ammonium hydroxide.

By these different methods almost all possible conditions of working the original process were obtained, and it was possible to ascertain not only the whole amount of loss caused by the process, but also the steps which involved a loss, and the amount of each.

The saccharimeter used in these experiments was one of the latest Laurent shadow instruments of the largest size.

The 200 mm. tube was frequently used, but when testing a weak solution the 400 mm. tube was used. In every case at least ten observations were made, and the average of these was adopted as showing the amount of glucose present. The glucose was perfectly pure, crystallised and anhydrous, polarising 99.85° to the right as shown by previous experiment.

In two solutions containing 0.05 per cent. of glucose, the average loss caused by the process was found to be 41.00 per cent., the tests being made by Fehling's solution.¹

A solution containing 0.1 per cent. of glucose showed a loss of 52.60 per cent. by the neutral plumbic acetate and plumbic tribasic acetate, when both were added before the first filtration, and a loss of 66.80 per cent. by the whole process, leaving 14.20 per cent. as the loss caused by the precipitations by ammonium hydroxide and by hydrogen sulphide. However, as only a trace of glucose was found in the filtrate and washings obtained after the precipitation by ammonium hydroxide, this loss must have been caused almost entirely by the precipitation by hydrogen sulphide. In one solution containing the same amount of glucose, only 2.60 per cent. was lost as determined by Fehling's solution in employing the method, and only a trace of glucose was found in the filtrate and washings obtained after precipitation by ammonium hydroxide. In four solutions of the above strength, no glucose was found in the

¹ In the estimations following, the saccharimeter was invariably used, unless Fehling's solution is specially mentioned as having been employed.

filtrate and washings obtained after precipitation by ammonium hydroxide. The average loss in the six solutions employed, containing this amount (0.1 per cent.) of glucose, was 59.16 per cent.

In two solutions, containing 0.5 per cent. glucose, it was found that the average loss in the precipitation by neutral plumbic acetate was 28.22 per cent., while in the precipitation by plumbic tribasic acetate it was 2.76 per cent., making the loss in the two precipitates 30.98 per cent. In the same solutions the average loss by the whole process, as determined by Fehling's solution, was 50.26 per cent., leaving a loss of 19.28 per cent. to be accounted for by the precipitation by ammonium hydroxide and by hydrogen sulphide. But the average loss found in the filtrates obtained after precipitation by ammonium hydroxide was 6.85 per cent., so that the loss caused in the precipitation by hydrogen sulphide was 12.70 per cent. In two solutions, containing the same amount of glucose, both neutral plumbic acetate and plumbic tribasic acetate were added before the first filtration, and the average loss was then found to be 37.82 per cent., and the loss by the whole process was 54.46 per cent., leaving 16.64 per cent. as the loss occasioned by ammonium hydroxide and hydrogen sulphide; but no glucose was found in the filtrates obtained after precipitation by ammonium hydroxide, so that this percentage must represent the loss in the precipitation by hydrogen sulphide. In two solutions, also of this strength, the total loss was found to be 40.20 per cent., both neutral plumbic acetate and plumbic tribasic acetate having been added before filtration. From the six solutions of this strength (0.5 per cent.), the average loss occasioned by the process was 47.33 per cent., while in four of them the loss caused by neutral plumbic acetate and by plumbic tribasic acetate was 34.40 per cent., and the loss to be accounted for by the precipitation by ammonium hydroxide and hydrogen sulphide was 17.96 per cent. But the average loss caused by ammonium hydroxide was 3.29 per cent., leaving 14.67 per cent. as the average loss occasioned by hydrogen sulphide.

In two solutions, containing 1.0 per cent. of glucose, the loss occasioned by neutral plumbic acetate was 8.09 per cent., and that occasioned by plumbic tribasic acetate was 6.85 per cent., making the total loss up to this part of the process 14.94 per cent. After the addition of ammonium hydroxide to these solutions the lead was removed from one of them by a saturated solution of potassium carbonate, and the total loss of glucose was found to be 76.10 per cent., a loss of 62.90 per cent. having been caused by ammo-

nium hydroxide and by the solution of potassium carbonate. No glucose being found in the filtrate from the precipitation by ammonium hydroxide, this average must represent the loss taking place in the precipitation of the lead by potassium carbonate. From the other solution the lead was removed by hydrogen sulphide, and the total loss of glucose was 49.98 per cent., the loss caused by the precipitation by ammonium hydroxide and hydrogen sulphide being 33.30 per cent. But as 8.34 per cent. was found in the filtrate after precipitation by ammonium hydroxide, the remainder (24.98 per cent.) must have been caused by hydrogen sulphide. In a third solution a loss of 30.02 per cent. was caused by the precipitations by ammonium hydroxide and by hydrogen sulphide, and a loss of 40.34 per cent. by the whole process, a loss of 2.02 per cent. by the precipitation by ammonium hydroxide, and this taken from 10.32 per cent., which was the loss occasioned by both ammonium hydroxide and hydrogen sulphide, leaves 8.30 per cent. as the loss caused by the latter. In two solutions, also containing 1.0 per cent. of glucose, the average loss occasioned by the whole process was 64.33 per cent., and the average loss in the five solutions, from which the lead was removed by hydrogen sulphide, was 50.87 per cent.

The loss caused by neutral plumbic acetate in two solutions, containing 2.0 per cent. of glucose, was 8.30 per cent., and that caused by plumbic tribasic acetate was 8.28 per cent., the sum of these losses being 16.58 per cent., while the whole loss was 75.60 per cent., the lead having been precipitated by a saturated solution of potassium carbonate, leaving a loss of 59.02 per cent., which was caused by ammonium hydroxide and by potassium carbonate. By testing the filtrate obtained after precipitation by ammonium hydroxide, a loss was found in one case of 57.82 per cent., and in the other of 20.89 per cent., making an average for the two of 39.35 per cent., which would leave a loss of 19.67 per cent., which must have taken place in the precipitation by potassium carbonate. In two solutions, also containing 2.0 per cent. of glucose, the average total loss was 52.30 per cent., the lead having been precipitated by hydrogen sulphide.

By referring to Table I, it is seen that in one of these solutions (*V*) hardly any loss took place at this part of the process that was not accounted for. If the loss caused by both neutral plumbic acetate and by plumbic tribasic acetate (17.44 per cent.) be subtracted from the total (75.60 per cent.) loss, 58.16 per cent. is left

to be accounted for by ammonium hydroxide and by potassium carbonate. But in the filtrate obtained after precipitation by ammonium hydroxide, 57.82 per cent. of the total amount of glucose was found, leaving only 0.34 per cent. which has not been accounted for. In another solution (*W*) it is observed that although a loss of 59.89 per cent. must have been caused by ammonium hydroxide and by potassium carbonate, yet only 20.89 per cent. was found in the filtrate after precipitation by ammonium hydroxide, and hence there still remains to be accounted for 39.00 per cent. It was thought that some glucose might have been lost in the precipitate caused by the addition of a solution of potassium carbonate, and hence in each case this precipitate was diffused in water, and hydrogen sulphide passed through it until all the lead was precipitated. The precipitate of lead sulphide was collected by filtration, and washed with 25 cc. of water in five portions of 5 cc. each. The filtrate and washings were tested, but no glucose was found. However, before passing hydrogen sulphide through the precipitate of lead carbonate diffused in water, the mixture was warmed on a water-bath, and it was then noticed that a dark coloration appeared. No doubt this was caused by the action of an excess of alkali on the glucose present, causing the formation of melassic acid, etc.

By examination of the tables it will be seen that the process does not give satisfactory results if the lead is precipitated by a saturated solution of potassium carbonate, for in the three solutions in which it was tried the average loss was 75.77 per cent. The average loss discovered in the filtrates obtained after precipitation by ammonium hydroxide was 26.23 per cent., leaving 49.54 per cent. as the percentage probably lost in the precipitation caused by the solution of potassium carbonate.

It is evident, then, that less than 25.00 per cent. of the glucose, originally present in the urine, can be expected to be found after employing the process in this way.

It is also evident that in all of the processes employed there is a steady loss taking place throughout. By referring to Table III it is observed that after precipitation by neutral plumbic acetate, in two solutions, containing each 0.25 gram of glucose, only 71.78 per cent. was recovered; and in two solutions containing 0.5 gram of glucose only 91.91 per cent., and in two solutions containing 1.0 gram of glucose, 91.70 per cent. was recovered, giving an average of 85.13 per cent. recovered in the six solutions tested.

After precipitation by plumbic tribasic acetate in one solution, containing 0.05 gram of glucose, 47.40 per cent. was recovered. In four solutions, each containing 0.25 gram, 65.60 per cent. was recovered. In three solutions, each containing 0.5 gram, 80.04 per cent. was recovered, and in two solutions, each containing 1.0 gram, 83.42 per cent. was recovered. In these ten solutions, then, the average amount recovered was 71.67 per cent.

At the end of the process in two solutions, each containing 0.025 gram of glucose, 59.00 per cent. was recovered. In six solutions, each of which contained 0.05 gram of glucose, 40.84 per cent. was recovered. In six solutions, each of which contained 0.25 gram, 51.69 per cent. was recovered. In five solutions, each of which contained 0.5 gram of glucose, 49.13 per cent., and in two solutions, each containing 1.0 gram of glucose, 47.70 per cent. was recovered.

When hydrogen sulphide is used to precipitate the lead, it is certain that no one step in the process can be said to be specially faulty. The loss occasioned by the precipitation by neutral plumbic acetate (as seen by Table V) is greater than that caused by plumbic tribasic acetate, but neither of these losses is equal to that which takes place at later steps in the process.

Moreover, the losses are not uniform, but very variable in different solutions. And in the experiments this variation was found to exist, even though the solutions were subjected to exactly the same conditions, and even when the corresponding precipitates, in each case, were washed the same number of times and with equal volumes of water. Hence, from any amount of glucose found after employing the process, it could not be determined how much had originally been present in the urine.

The process certainly removes the interfering substances from the urine; but even for qualitative work, if no reaction for glucose could be obtained after making use of the process, it would be by no means certain that no glucose had been originally present in the urine.

In completing this paper the writer desires to express his great indebtedness to Dr. John Marshall, Demonstrator of Chemistry in the Medical Department of the University of Pennsylvania, for his valuable suggestions in carrying on the work, and to whom any success attained in the experiments is largely due.

TABLE I.—Showing Loss after Each Step of the Process and Per Cent. of that Loss.

	Cc. of Solution Employed.	Amount of Glucose Present.	Loss after Plumbic Acetate.	Per Cent. of Previous Loss.	Loss Caused by Tribasic Acetate.	Per Cent. of Loss.	Sum of Previous Losses.	Per Cent. of Losses.	Total Loss.	Per Cent. of Total Loss.	Amount Finally Present.	Amount Finally Present when K_2CO_3 was used.	Loss in NH_4OH Filtrate.	Per Cent. of Loss.	Loss caused by NH_4HO , H_2S or K_2CO_3 (Estimated).	Per Cent. of Loss.
A	50	0.025	0.0097	38.80	0.0153 ¹
B	50	0.025	0.0108	43.20	0.0142 ¹
C	50	0.05	0.0305	61.00	0.0195	0
D	50	0.05	0.0419	83.80	0.0081	0
E	50	0.05	0.0013	2.60	0.0487 ¹	Trace.
F	50	0.05	0.0263	52.60	0.0334	66.80	0.0166	Trace.	0.0071	14.20
G	50	0.05	0.0354	70.80	0.0146
H	50	0.05	0.0350	70.00	0.0150	0
I	50	0.25	0.0808	32.32	0.0039	1.56	0.0847	33.88	0.1203	50.52	0.1237 ¹	0.0141	5.64	0.0416	16.64
J	50	0.25	0.0603	24.12	0.0099	3.96	0.0702	28.08	0.1250	50.00	0.1250 ¹	0.0188	7.52	0.0548	21.92
K	50	0.25	0.0944	37.76	0.1110	44.40	0.1390	0	0.0160	6.64
L	50	0.25	0.0947	37.88	0.1613	64.52	0.0887	0	0.0666	26.64
M	50	0.25	0.1126	45.04	0.1374
N	50	0.25	0.0884	35.36	0.1616
O	50	0.5	0.0351	7.02	0.0309	6.18	0.0660	13.20	0.3805	76.10	0.1195	0	0.3145	62.90
P	50	0.5	0.0458	9.16	0.0376	7.52	0.0834	16.68	0.2499	49.98	0.2501	0.0417	8.34	0.1605	33.32
R	50	0.5	0.1501	30.02	0.2017	40.34	0.2983	0.0101	2.02	0.0516	10.32
S	50	0.5	0.1718	34.30	0.3282
T	50	0.5	0.2856	57.12	0.2144
U	50	0.5	0.3627	72.54	0.1373
V	50	1.0	0.1157	11.57	0.0587	5.87	0.1744	17.44	0.7560	75.00	0.2440	0.5782	57.82	0.5816	58.16
W	50	1.0	0.0504	5.04	0.1068	10.68	0.1572	15.72	0.7561	75.61	0.2439	0.2089	20.89	0.5989	59.89
X	50	1.0	0.4919	49.19	0.5081
Y	50	1.0	0.5560	55.60	0.4440

¹ These estimations were made by means of Fehling's solution.

TABLE II.—Averages of the Amounts of Glucose Recovered and Lost at Different Stages of the Process.

Number of Solutions represented in Averages.	Corresponding Solutions in Table I.	Amount of Glucose in each Solution.	Amount Present after use of Lead Acetate.	Loss.	Per Cent. after use of Tribasic Acetate.	Averages of Amounts Present.	Loss.	Averages of Loss.	Amount Finally Present.	Averages of Amounts Finally Present.	Final Losses.	Averages of Final Losses.	Number of Solutions represented in Previous Averages.
2	A, B	0.025	0.0147	0.0147	0.0103	0.0103	2
1	F	0.05	0.0237	0.0237	0.0263	0.0263	0.0166	0.0166	0.0334	0.0334	6
5	C, D, E, G, H	0.05	0.0212	0.0204	0.0288	0.0296	6
2	I, J	0.25	0.1794	0.0706	0.1725	0.1639	0.0775	0.0861	0.1243	0.1292	0.1257	0.1208	6
2	K, L	0.25	0.1554	0.0946	0.1138	0.1362	6
2	M, N	0.25	0.1495	0.1005	6
2	O, P	0.5	0.4595	0.0405	0.4253	0.4001	0.0747	0.0999	0.2591	0.2456	0.2099	0.2544	5
1	K	0.5	0.3499	0.1501	0.2983	0.2017	5
3	S, T, U	0.5	0.2266	0.2734	5
2	V, W	1.0	0.9170	0.0830	0.8342	0.8342	0.1658	0.1658	0.2440	0.7560	2
2	X, Y	1.0	0.4760	0.4760	0.5240	0.5240	2

1 0.2501 is the amount recovered in one of the solutions. The other solution was not considered, as in it the lead was precipitated by K_2CO_3 instead of by H_2S .
 2 0.2440 was not used in making up the average, as in these solutions the lead was precipitated by K_2CO_3 .

TABLE III.—Averages of the Percentages Recovered and Lost at Different Stages of the Process.

Number of Solutions represented in the Averages.	Corresponding Solutions in Table I.	Amount of Glucose in each Solution.	Per Cent. Present after use of Lead Acetate.	Per Cent. of Loss.	Averages of Percentages Present.	Per cent. of Losses.	Average of percentage Lost.	Percent-ages Finally Present.	Average Percentage Finally Present.	Per Cent. of Total Losses.	Average Per Cent. of Total Losses.	Number of Solutions represented in Previous Averages.
2	A, B	0.025	59.00	59.00	41.00	41.00	2
1	F	0.05	47.40	47.40	52.60	52.60	33.20	40.84	66.80	59.16	6
5	C, D, E, G, H	0.05	42.30	40.84	57.64	59.16	6
2	I, J	0.25	71.78	28.22	69.02	30.98	34.40	49.74	51.69	50.26	48.31	6
2	K, L	0.25	62.18	37.82	45.54	51.69	54.46	48.31	6
2	M, N	0.25	59.80	51.69	40.20	48.31	6
2	O, P	0.5	91.91	8.09	85.06	14.94	19.96	150.02	49.13	149.98	50.87	5
1	K	0.5	69.98	30.02	59.66	49.13	40.34	50.87	5
3	S, T, U	0.5	24.40	75.60	54.67	5
2	V, W	1.0	91.70	8.30	83.42	16.58	16.58	47.60	47.60	52.40	52.40	2
2	X, Y	1.0	2

1 50.02 is the percentage recovered in one of the solutions. The other solution was not considered, as in it the lead was precipitated by K_2CO_3 instead of by H_2S .
 2 In these solutions the lead was precipitated by K_2CO_3 and 24.40 per cent. was not considered in making up the average.

TABLE IV.—*Amount of Glucose Present in Each Solution after Each Step in the Process.*

	Volume of each Solution.	Amount of Glucose in each.	Amount Present after use of Lead Acetate.	Amount Present after the use of Tribasic Acetate.	Final Amount Present.	Final Amount Present when K_2CO_3 was used.
A	50 cc.	0.025	0.0153 (F)
B	50 cc.	0.025	0.0142 (F)
C	50 cc.	0.05	0.0195
D	50 cc.	0.05	0.0081
E	50 cc.	0.05	0.0487 (F)
F	50 cc.	0.05	0.0237	0.0166
G	50 cc.	0.05	0.0146
H	50 cc.	0.05	0.0150
I	50 cc.	0.25	0.1692	0.1653	0.1237 (F)
J	50 cc.	0.25	0.1897	0.1798	0.1250 (F)
K	50 cc.	0.25	0.1556	0.1390
L	50 cc.	0.25	0.1553	0.0887
M	50 cc.	0.25	0.1374
N	50 cc.	0.25	0.1616
O	50 cc.	0.5	0.4649	0.4340	0.1195
P	50 cc.	0.5	0.4542	0.4166	0.2501
R	50 cc.	0.5	0.3499	0.2983
S	50 cc.	0.5	0.3282
T	50 cc.	0.5	0.2144
U	50 cc.	0.5	0.1373
V	50 cc.	1.0	0.8843	0.8256	0.2440
W	50 cc.	1.0	0.9496	0.8428	0.2439
X	50 cc.	1.0	0.5081
Y	50 cc.	1.0	0.4440

(F) These results were obtained by the use of Fehling's solution.

TABLE V.—*Average Percentages of Losses Caused at Different Stages of the Process.*

No. of Solutions.	Percentage of each.	Amount of Glucose in each.	Average Loss after use of Lead Acetate.	Average Loss caused by use of Tribasic Acetate.	Sum of these Losses.
2	0.5	0.25	28.22	2.76	30.98
2	1.0	0.5	8.09	6.85	14.94
2	2.0	1.0	8.30	8.28	16.58
Average of above losses.....			14.87	5.96	20.83

TABLE VI.—*Average Percentages of Losses at Different Stages of the Process in all of the Solutions.*

No. of Solutions.	Amount of Glucose Present after use of Lead Acetate.	Loss after Lead Acetate.	Per Cent. of Loss by Tribasic Acetate.	Per Cent. of Amount Present after Tribasic Acetate.	Sum of Previous Losses.	Per Cent. of Total Loss.	Per Cent. Finally Present.
6	85.13	14.87	5.96	79.17	20.83
4	60.44	39.56
	Average of above percentages			71.67	28.33	52.36 ¹	47.64
14						50.52	49.48
	Average of above percentages ²					51.71	48.29

TABLE VII.—*Average Amounts of Glucose Recovered after the Process, with Percentages.*

No. of Solutions.	Per Cent. of Glucose in each Solution.	Amount of Glucose in Grams in each Solution.	Amount of Glucose Recovered.	Per Cent. of Glucose Recovered.
2	0.05	0.025	0.0147	59.00
6	0.1	0.05	0.0204	40.84
6	0.5	0.25	0.1292	51.69
5	1.0	0.5	0.2456	49.13
2	2.0	1.0	0.4760	47.60

¹ Only seven of the solutions were considered in making this average, as in the remaining three of the ten solutions the lead was precipitated by K_2CO_3 instead of by H_2S .

² Viz. : in all of the twenty-one solutions employed, in which the lead was precipitated by hydrogen sulphide.