of ovomucoid, mucoid of the thyroid gland and of a mucoid tumor is now in progress.

I was prompted to make the present communication by the fact that about ten days ago an article appeared in Hoppe-Seyler's *Zeitschrift*. The author analyzed an ovarial mucoid and found a substance similar to chondroitinsulphuric acid. His findings on the latter mucoid coincide with mine on several mucins and justify the conclusions I was ready to draw from my work, that mucins are not simple compounds of proteids and carbohydrates, but are proteid derivatives of an ethereal sulphuric acid.

I consider it premature at this moment to state positively the nature of this combination.

BIBLIOGRAPHY.

Chittenden and Gies : *Journal of Experimental Medicine*, Vol. 1.
Folin : *Ztschr. phys. Chem.*, Bd. 23.
Hammarsten : Pfluger's *Arch.*, Bd. 36.
Landwehr: *Ztschr. physiol. Chem.*, Bd. 5, 6, 8, 9, Pflüger's *Arch.*, Bd. 39.
Loebisch : *Ibid.*, Bd. 10.
Hanzer: *Ibid.*, Bd. 28.
Schmeideberg : *Arch. exp. Pathol. and Pharm.*, Bd. 28.

[CONTRIBUTION FROM THE CHEMICAL DIVISION, U. S. DEPARTMENT OF AGRICULTURE, NO. 36. Sent by H. W. Wiley.]

THE DETERMINATION OF GLYCOGEN AND RELATIVE QUANTITIES OF GLYCOGEN IN DIFFERENT PARTS OF THE FLESH OF A HORSE.

BY J. K. HAYWOOD.

Received January 16, 1900.

A GREAT many methods have been proposed for the estimation of glycogen,[1] but on trying them I found that nearly all were open to some serious objection, which rendered their employment either entirely out of the question or the results obtained very doubtful. I have, therefore, worked out a method which, although not new in principle, is new in many of the details which go to making up a fairly accurate and speedy procedure. But first I will speak of some of my preliminary experiments to show future workers in this field, at least, what *not to do*.

[1] Wiley's "Principles and Practice of Agricultural Analysis", 3, 554.

Having seen in a paper by Von Wittich[1] that glycogen might be determined by digesting the sample of meat on the steam-bath for two or three hours, with water containing a few drops of sulphuric acid, filtering, neutralizing, filtering again, determining the reducing sugar in the filtrate by Fehling solution, and from that figure calculating the glycogen, I thought I would try this, as it seemed the easiest way. I added, however, more than a few drops of sulphuric acid,—in fact, made a 3 per cent. solution; for it is well known that glycogen is more resistant to the action of acids than starch, and a few drops of sulphuric acid would not convert starch in this time. I also continued the digestion for six hours. I encountered a difficulty at once; *i. e.*, the mixture would not filter after digestion, except very slowly,—so slowly that a practical use of the method was impossible. I therefore neutralized immediately after digestion, made to a volume, and filtered off an aliquot portion, which still took a long enough time, but was much shorter than filtering the whole. Using a dried, ether-extracted liver, I obtained results of 1.27 and 1.29 per cent. glycogen, which looked encouraging from the closeness of the duplicates. The Fehling solution filtrate, however, looked dirty, and the copper suboxide precipitated was not red, but a dirty green, such as no chemist likes to see.

I therefore carried through a sample of the liver, using the above method, except that at the end the solution was clarified with lead subacetate, the excess of lead removed by sodium sulphate, and the dextrose determined in the filtrate. In this way a clear Fehling filtrate was obtained, but the results did not at all compare either with each other or with those (1.29 and 1.27) mentioned above. In one case I obtained a result of 0.87 per cent. glycogen, and in a duplicate 0.00 per cent. glycogen.

Thinking from the work of Külz and Bornträger[2] that hydrochloric acid might be a better agent to convert the glycogen to dextrose, I next boiled some of the liver for three hours with 200 cc. of water and 20 cc. of hydrochloric acid (sp. gr. 1.125), neutralized with sodium carbonate, and filtered just as in the determination of starch. The result on a non-clarified portion was 4.72 per cent. glycogen, and on a portion clarified by lead

1 *Central-Blatt. f. d. med. Wissenschaft*, **13**, 292 (1875).

2 *Archiv. f. d. ges. Physiologie*, **24**, 28.

subacetate, and the excess of lead removed by sodium sulphate, the result was 1.25 per cent. glycogen. I again determined the glycogen as above, except that the solution at the end was clarified with neutral lead acetate, and results of only 0.03 and 0.04 per cent. glycogen were obtained.

I next added 1 per cent. of starch to a sample of meat which gave no glycogen by the above method, and 1 per cent. of glycogen to another portion of the same meat. The samples thus prepared were treated just as in the determination of starch, and at the end clarified with neutral lead acetate, and the excess of lead removed. Proceeding in this way I could only recover 0.08 per cent. starch and 0.00 per cent. glycogen.

With results thus varying in the case of a sample of liver from 0.00 per cent. to 4.72 per cent., and results of 0.00 per cent. and 0.08 per cent., where 1 per cent. of glycogen and 1 per cent. of starch respectively had been added, it appeared that the method was absolutely worthless ; so it was abandoned.

It may seem strange, at first sight, that no better results than the above could be obtained following after Külz & Bornträger[1] who had worked out a method based on the inversion of glycogen to dextrose and the determination of the latter by Fehling solution ; but this apparent contradiction will disappear when I call attention to the fact that Külz only worked on *pure* glycogen, and in no case mixed his glycogen with meat and recovered it.

I did not make an attempt to estimate glycogen by means of the polariscope, as also given by Külz,[2] because Külz had worked with rather large quantities, so that if only 0.00 to 1.5 per cent. of glycogen were present the determination would be very uncertain, since if a workable quantity of the meat were used (say 50 to 60 grams), the reading on the sugar scale would be only a few tenths of a degree.

Estimating glycogen by the depth of its color with iodine, according to Goldstein,[3] was not employed, as it has been shown to be worthless by many subsequent investigators.

A qualitative test for glycogen by the test of Bräutigam and

[1] *Archiv. f. d. ges. Physiologie*, **24**, 28.

[2] *Ibid.*, **24**, 87 and **24**, 90.

[3] *Verhandlungen der physik-med. Gesell. in Würzburg*, **7**, 1.

Edelmann,[1] by means of iodine, was not employed, because subsequent investigators have been able to obtain the same test with other meats besides horse meat, notably on beef.

I therefore decided to try the method of Brücke,[2] which appeared to promise better results than any of the other gravimetric methods. Brücke digests the meat on the steam-bath with water, filters, precipitates the proteid matter in hydrochloric acid solution with the double iodide of potassium and mercury, again filters, precipitates the glycogen in the filtrate with alcohol. filters on a weighed filter, washes first with 60 per cent. alcohol, then with 95 per cent. alcohol, then with ether, dries at 115° C., and weighs. This method has been examined by many later investigators. Külz[3] found that an extraction of the meat with water was not sufficient, but that it was better to employ a weak solution of potassium hydroxide. Kratschmer[4] found that the hydrochloric acid of the Brücke reagent acted on the freshly precipitated glycogen ; also that it was best to first dry the glycogen at 80°–100° C., and then at 115°. I took all of these points into consideration when working out the method.

But first of all a serious difficulty was met with. It was found impossible to filter the meat digested with potassium hydroxide in a reasonable length of time, and nearly impossible to get off any fair-sized aliquot portion. Instead of filtering after the digestion with a weak solution of potassium hydroxide, the mixture was made immediately acid with hydrochloric acid, in the presence of the unattacked residue, Brücke's reagent added to precipitate proteid matter, the whole made to a volume, and an aliquot portion filtered off. In this way a very easily filterable mass was obtained. After an aliquot portion, say 250 cc. out of 500 cc., had been thus obtained, it was neutralized with a solution of potassium hydroxide (using a drop of phenolphthalein as indicator, and noting the amount of hydroxide employed) to get rid of the hydrochloric acid, which would, according to Kratschmer, act on the glycogen. When exact neutralization had been obtained, small flakes of some non-glycogen material would usually separate out. These were filtered

1 *Ztschr. anal. Chem.*, **33**, 98.

2 *Central-Blatt f. d. med. Wissenschaft*, 1871, 388.

3 *Ztschr. für Biologie*, **22**, 161.

4 *Archiv. f. d. ges. Physiologie*, **24**, 134.

off, a few drops of hydrochloric acid added to the filtrate, and twice the volume of 92 to 95 per cent. alcohol added. The precipitated glycogen was, at the end of a few hours, filtered off through a weighed filter, washed first with 60 per cent. alcohol, then with 95 per cent. alcohol, then with ether, dried at 80°-100° C., then at 115° C. and weighed again, the gain representing glycogen. In this way a result of 0.51 per cent. was obtained on a dry roast beef, and where 0.87 per cent. and 1.61 per cent. glycogen were added, 0.82 per cent. and 1.54 per cent. respectively were recovered. In each of the above determinations, however, something seemed to come down besides the glycogen, and on treating the filter with hot water this would mostly remain as a residue, and the glycogen be dissolved. It appeared from this, then, that a much better method than the one above was to weigh the filter and contents after drying, then dissolve out glycogen with water, dry and weigh again, and thus determine glycogen by the difference in weight. To test this I carried through a sample of roast beef, and on the water-free, fat-free sample by first method obtained a result of 0.3 per cent. glycogen ; by second, 0.17 per cent. glycogen ; on a liver I obtained 1.53, 1.56 per cent. by the first, and by second, 1.04, 1.10 per cent.; on a chicken I obtained 0.35 per cent. by the first, and by the second 0.26 per cent. It would therefore appear that all of the proteid matter was not precipitated by the double iodide of potassium and mercury, but that some which was nearly entirely insoluble in water came down with the glycogen and remained on the filter when the glycogen was dissolved.

From all of the above work I have settled upon the following method for determining glycogen. From 50 to 60 grams of meat, after having been run through a sausage grinder, is treated in an evaporating dish with 300 cc. of a 1 per cent. potassium hydroxide solution, and heated on the steam-bath for about six hours, water being added from time to time, so that the volume never becomes less than 150 cc. Finally the water is removed by evaporation until about 150 cc. remain. This is made slightly acid with hydrochloric acid (1–5), and hydrochloric acid and double iodide of potassium and mercury[1] added alternately until all pro-

[1] The double iodide of potassium and mercury is prepared by first precipitating a solution of mercuric chloride with potassium iodide, washing the precipitated mercuric iodide till free of chlorides, then saturating a 10 per cent. potassium iodide solution with the mercuric iodide at the boiling temperature.

teid matter is precipitated. The hydrochloric acid is added about 2 cc. at a time, and the double iodide of potassium and mercury about 10 cc. at a time. Usually about 20-25 cc. acid (1–5) and 70–100 cc. of the iodide solution are required. When the proteid matter separates, and leaves a clear liquid layer above, a small amount of this is carefully poured off and tested for complete precipitation. If the precipitation is not complete the liquid is returned, and the proteid precipitant added until the clear liquid above the proteid matter gives no precipitate with hydrochloric acid and the double iodide solution. Sometimes, not often, the proteid matter will not separate. In this case follow Külz's method of nearly neutralizing with potassium hydroxide and adding again hydrochloric acid, and the precipitate will usually flocculate. The proteid matter being now precipitated as completely as possible, the whole is transferred to a 500 cc. flask, made to the mark with water, well shaken, and an aliquot portion (say 250 cc.) filtered through a fluted filter. A drop or two of phenol phthalein is now added, and the solution titrated to exact neutrality with a concentrated solution of potassium hydroxide, noting the amount used. If a slight amount of flakey-looking matter separates at this point, the liquid is again passed through a fluted filter, and such a volume taken as will correspond to 2/5 of the original material, of course taking into consideration the number of cubic centimeters of potassium hydroxide used to neutralize the hydrochloric acid. Three or four drops of concentrated hydrochloric acid are now added, and twice the volume of from 93 to 95 per cent. alcohol. After standing two or three hours the precipitated glycogen is filtered off through a paper filter, washed with 60 per cent. alcohol, then with 95 per cent. alcohol, then with ether; dried at 80°–100° C., then at 115° C., and weighed in a weighing tube. The filter is then extracted thoroughly with boiling water, dried again at 115° C., and again weighed in a weighing tube, the difference in weight representing glycogen.

Proceeding in this way, I obtained on roast beef 0.17 per cent. glycogen, calculated to the dry, fat-free basis; where 0.87 per cent. glycogen was added, 0.78 per cent. was recovered; and where 1.72 per cent. glycogen was added, 1.57 per cent. was

recovered. Close duplicates were also obtained by this method, as shown from the following work :

On a liver the results were 1.04 and 1.10 per cent. calculated to dry, fat-free basis.

On a chicken the results were 0.26 and 0.29 per cent. calculated to dry, fat-free basis.

On an ox tongue the results were 0.27 and 0.30 per cent. calculated to dry, fat-free basis.

On another ox tongue the results were 0.65 and 0.62 per cent. calculated to dry, fat-free basis.

Following will be found the analysis of three parts of three different horses, the glycogen of which was determined by the above method. First I will mention, however, that in addition to the direct determination of glycogen I also estimated the reducing sugars in a water extract of each sample of meat, as suggested by Niebel[1] to find the amount of glycogen that had been converted to dextrose. This was done by boiling the meat three separate times with 500 cc. portions of water, pouring off the water each time, evaporating to a small volume, clarifying with neutral lead acetate, removing excess of lead with sodium carbonate, making to a volume, filtering off an aliquot portion, and determining the dextrose in the filtrate by Allihn's method.

No. of sample.	Part of horse.	Water. Per cent.	Fat. Per cent.	Glycogen. Per cent.	Glycogen corresponding to dextrose. Per cent.	Glycogen in each of the preceding columns calculated to fat-free, water-free basis. Per cent.	
1	chuck	70.57	9.01	0.30	0.00	1.47	0.00
2	chuck	74.30	4.63	0.48	0.00	2.28	0.00
3	chuck	77.22	5.84	0.86	0.00	5.08	0.00
4	rib	66.12	12.51	0.61	0.00	2.85	0.00
5	rib	72.87	4.54	0.54	0.00	2.39	0.00
6	rib	76.31	1.24	0.79	0.00	3.52	0.00
7	flank	57.93	25.01	0.42	0.00	2.46	0.00
8	flank	71.79	7.66	0.33	0.00	1.61	0.00
9	flank	76.39	1.16	0.53	0.00	2.36	0.00

In four of the above samples a determination was made of the amount of nitrogen in the glycogen dissolved from the filter, to

[1] *Ztschr. f. Fleisch. u. Milch. Hygiene*, 1, 185, 210, and 5, 86, 130.

see how much, if any. proteid matter had not been removed, but still contaminated the glycogen. In four more of the above samples a determination was made of the amount of ash in the glycogen to see if a correction was necessary. The results are given below.

No. of sample.	Amount of original beef used.	Amount of beef used in actual determination, being 2/5 of original.	Amount of glycogen found.	Amount of proteid matter 6.25 × N.	Amount of ash.
	Grams.	Grams.	Grams.	Grams.	Grams.
1	60	24	0.0717	0.0009	....
2	60	24	0.1148	....	0.0048
3	60	24	0.2070	....	0.0065
4	60	24	0.1467	0.0018	....
5	60	24	0.1304	0.0017	....
6	60	24	0.1898	0.0024	....
7	60	24	0.0999	....	0.0030
9	60	24	0.1282	....	0.0025

It will thus be seen that only a small percentage of the glycogen consists of proteid matter and ash, and when these two are added together, and calculated back as per cent. of the original substance, the figure for glycogen will only be altered by about 0.02 per cent.

In several other determinations of glycogen, the glycogen which was dissolved from the filter was evaporated to a small volume and tested with Brücke reagent and with bromine, to see if any proteid matter was precipitated. In no case did any come down.

From this work it will be seen that the glycogen obtained is quite pure, only a small portion consisting of ash and proteid matter. The small plus error caused by these substances, in all probability, about balances the negative error caused by the action of hydrochloric acid on the glycogen during the precipitation of proteid matter.

While the above method is, I think, much more accurate than the original Brücke method, it does not possess the accuracy of a chlorine or sulphuric acid determination, but this is not necessary, since the end of the work is not to know the amount of glycogen to 0.01 per cent., but to distinguish horse meat from other meats by its high content of glycogen.

Appended will be found analyses of several parts of a single horse. These are not given to show anything about the accuracy of the method, but to increase the data relating to the amount of glycogen in different cuts of horse meat. The horse was one which had been killed in an accident, three days before the analysis was made.

No. of sample.	Part of horse.	Water.	Fat.	Glycogen.	Glycogen corresponding to dextrose.	Glycogen in each of the preceding columns, calculated to fat-free, water-free basis.	
		Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	
1	Second cut of round	74.36	3.27	0.49	0.00	2.19	0.00
2	First cut of round	73.77	3.23	0.27	0.05	1.17	0.22
3	Shoulder clod	73.54	5.27	0.58	0.05	2.73	0.23
4	Cross ribs	73.86	6.30	0.32	0.04	1.62	0.20
5	Chuck	68.00	15.39	0.34	0.00	2.05	0.00
6	Plate	52.16	33.66	0.41	0.00	2.89	0.00
7	Brisket	66.70	12.16	0.46	0.006	2.17	0.03

I would like to express my thanks to Dr. W. D. Bigelow, of this Division, for putting at my disposal a very complete bibliography of glycogen. Since his work will shortly appear in print, I will not attempt to go into the literature of the subject.

[CONTRIBUTIONS FROM THE HAVEMEYER LABORATORIES OF COLUMBIA UNIVERSITY, NO. 15.]

THE ELECTROLYTIC DEPOSITION OF BRASS.

BY J. LIVINGSTON R. MORGAN.

Received January 19, 1900.

BRASS plating by electrolysis was discovered in 1841, and the original process, with a few modifications, is still in use at the present time. The formulas for the preparation of the bath are entirely empirical, the subject, so far as I know, having never been treated from the theoretical standpoint. The purpose of this paper is to consider the reaction by the light of our present knowledge of electrochemistry, and to present the theory of the process which results.

When a metal with a high electrolytic solution pressure (Zn) is placed in an acid solution and connected by a wire to a plate of platinum, immersed in the same liquid, hydrogen gas is