

ART. VII.—*Contributions from the Sheffield Laboratory of Yale College. No. XXXV.—On Glycogen and Glycocol in the Muscular Tissue of Pecten irradians*; by R. H. CHITTENDEN, Assistant in Physiological Chemistry.

THE genus *Pecten* is world-wide in its distribution. The species *irradians* is entirely American, being found most abundantly on the eastern shores of the United States. It is closely allied to the European and English species *opercularis* and *maximus*. The large central muscle which closes the valves of this mollusk is highly valued as an article of food, although its peculiar sweet taste is objectionable to some.

With this central muscle the following experiments were made:

Glycogen.—By extracting the edible portion of the scallop with cold water, a milky opaque fluid with slight acid reaction is obtained, and an insoluble residue consisting principally of syntonin or fibrin mixed with inorganic matters. The strong opacity of the aqueous solution is not due to an emulsion of fatty matters. On boiling the solution with or without the addition of acetic acid, a large amount of albumin is precipitated, leaving the fluid still opalescent. On treating the fluid, after the removal of albumin, with a small amount of ninety-five per cent alcohol, a light flocculent precipitate is obtained which dissolves by agitation, leaving the fluid unaltered in appearance; but if three or four volumes of the alcohol are added, a copious, permanent precipitate settles, leaving the supernatant fluid perfectly clear. This precipitate is of snowy whiteness, except when previous to precipitation the fluid has been boiled considerably, in which case both filtrate and precipitate assume a yellow or brownish color, from which the latter can be freed by solution in cold water and reprecipitation by alcohol. The precipitate, if allowed to dry in contact with air, after having been washed with alcohol merely, soon becomes translucent on the edges and finally is transformed completely into a gummy mass, which is sticky when moistened; but if after precipitation it is washed with ether thoroughly, it loses this property of becoming gummy, which seems to be due to the presence of water and of albuminous matters in small quantity. This gum-like mass when hard is brittle and yields on trituration a white hygroscopic powder showing under the microscope no distinct structure. A portion of the precipitate so prepared, dried in the air, yielded by analysis:

	1.	2.	Calculated. $C_6H_{10}O_5 + H^2O$ or $C_6H_{12}O_6$
C	39.52	39.56	40.00
H	6.62	6.55	6.60
O	53.86	53.89	53.34

In this state it is not quite pure, giving with Millon's reagent a strong reaction for albumin and containing some inorganic matter, one specimen 1.57 per cent, another 1.38 per cent, consisting in all cases, so far as were examined, of calcium phosphate. From this analysis it is seen that the substance has the formula of the sugars, or that of the starch group plus a molecule of water. The substance is tasteless, gummy when moistened and gives an opaque fluid with water, seemingly a true solution, which passes unchanged through filter paper and animal charcoal, and shows no particles under the microscope with a half inch objective. When this aqueous solution is boiled, thin films separate, forming a scum on the top of the fluid, which goes into solution again as the liquid becomes cool.

The substance is insoluble in alcohol and ether, has no reducing action with cupric sulphate and sodium hydroxide, but when boiled with a few drops of dilute hydrochloric acid gives a clear fluid which has strong reducing action. This same reaction takes place also with nitric and sulphuric acids, but not so readily as with the former. A portion of the substance was treated with a small quantity of saliva at the ordinary temperature, and at 40° C., and in both cases the ptyalin acted immediately upon it and sugar was formed. Treated with a solution of iodine in potassium iodide, a brownish red or maroon color was obtained. These and other reactions pointed to glycogen. It was yet to be ascertained whether the sugar formed by the action of acids and ferments was glucose, also to examine the action of boiling dilute nitric acid upon it, and to determine whether the different formulæ of glycogen could be obtained by drying it at different temperatures. A portion of the substance was then boiled with hydrochloric acid until alcohol produced no precipitate in a sample tested, the excess of acid removed by oxide of silver and the sugar obtained by evaporation. The product had all the properties of glucose, was intensely sweet, reduced alkaline solutions of copper and silver and yielded Pettenkofer's reaction. Analyzed, it gave the following result:

	1.	2.	Calculated. $C_6H_{12}O_6 + H_2O$
C	36.15	36.17	36.36
H	6.82	6.88	7.07
O	57.03	56.59	56.57

By the action of boiling dilute nitric acid, oxalic acid was formed and separated. A different sample of the original substance, dried over sulphuric acid until a constant weight was obtained, yielded:

	1.	2.
C	43.81	43.90
H	6.43	6.46
O	49.76	49.64

A sample dried at 100° C. gave by analysis:

	1.	2. Analysis of starch dried at 100° C. by Mulder
C	43.86	43.89
H	6.41	6.38
O	49.73	49.73

A sample dried at 140° C.:

	1.	2. Analysis of starch dried at 140° by Mulder
C	44.32	44.40
H	6.38	6.41
O	49.30	49.19

On treating an aqueous solution of the substance at the ordinary temperature with an excess of a saturated solution of

barium hydroxide, a heavy white precipitate was obtained, soluble in water, insoluble in baryta water and alcohol. This precipitate was dissolved in water, the baryta removed by a little dilute sulphuric acid in the cold and then reprecipitated by an excess of alcohol.

Prepared thus, it seemed to have lost the property of becoming gummy so readily as before, and on examination was found to be completely free from albuminous matters, giving no reaction even with Millon's reagent and also contained only 0.61 per cent of ash. The substance dried at 100° C. gave by analysis the following result, agreeing closely with that of the preceding preparation dried at the same temperature :

	1.	2.
C	43.93	43.91
H	6.45	6.40
O	49.62	49.69

Another sample, prepared in the same way and dried between 110°–120° C., gave :

	1.	2.
C	43.56	43.63
H	6.71	6.71
O	49.73	49.66

Casting a backward glance, we see that the analysis of the air-dried substance corresponds with the formula $C_6H_{12}O_6$, that of the substance dried at 140° C. with $C_6H_{10}O_5$, which requires 44.44 C. 6.11 H. These results agree with glycogen, which in different states of hydration has been found to have the formulæ $C_6H_{10}O_5$, $C_6H_{12}O_6$ and $C_6H_{14}O_7$. But the results obtained by the analyses of the substance dried at 100° C. and 110°–120° C. do not agree closely with any of the above formulæ. The same is true of members of the starch group to which glycogen is closely related, and lately Dr. Nägeli* has published a paper in which he points out that the elementary composition of starch, dextrin and "amylo-dextrin" dried at temperatures not exceeding 116°, agrees better with the formula $C_{36}H_{62}O_{31}$, which requires 43.63 C. 6.3 H, than with $C_6H_{10}O_5$, and that after exposure to a temperature of 140 C., when the composition corresponds to $C_6H_{10}O_5$, we probably do not deal with undecomposed starch. The analysis of amylo-dextrin by Nägeli (loc. cit. p. 35), dried in a stream of hydrogen, at a temperature of 112° to 116° C, gave the following result :

	1.	2.
C	43.58	43.85
H	6.86	6.58

* Beiträge zur näheren Kenntniss der Stärke Gruppe. Dr. Walter Nägeli. 1874.

A sample of dextrin dried at the same temperature gave Nägeli as a mean of two analyses, C. 43.52. H. 6.78, in both cases agreeing closely with my analyses of glycogen dried at 110°–120° C. Thus this substance, which is without doubt glycogen, coincides in this respect with its neighbors, dextrin, amylo-dextrin, etc.

A sample of glycogen, prepared by the preceding methods and dissolved in water, on treatment with basic lead acetate, with the application of a gentle heat, yielded a heavy gelatinous precipitate, which, when filtered off by the aid of a pump and washed with water, was found to contain lead. Dried at 100° C, it gave by analysis the following result:

	1.	2.
C	21.62	21.78
H	2.91	2.96
Pb	48.39	48.34
O	27.08	26.92

Since this result was obtained, I find that M. Bizio * has already discovered glycogen in some invertebrates. Among the Mollusks, he found it in considerable quantity in the oyster.

With glycogen from these sources he prepared a lead compound by means of tribasic acetate of lead, and says its "analysis has given me the formula $C_{12}H_{18}PbO_{10}$," which requires:

C	27.22
H	3.40
Pb	39.13
O	30.24

It will be seen at once that my result does not agree with this formula. I therefore made some further lead precipitates from the same and other preparations of glycogen, and in these simply determined the lead as follows:

Pb.	1st Prep.	2d Prep.	3d Prep.	4th Prep.
No. 1.	48.39	53.63	51.45	50.27
No. 2.	48.34	53.58	51.45	50.28

Some glycogen was also prepared from the liver of an ox by the usual method and dried at 100°. It yielded by analysis:

	1.	2.	Calculated. $C_{12}H_{22}O_{11}$
C	41.87	41.90	42.11
H	6.35	6.38	6.43
O	51.78	51.72	51.46

A lead preparation made from this gave:

	1.	2.
Pb	61.99	61.94

* Comptes Rendus, lxx, 175. Zeitschrift für Chemie, 1867, 745.

These results indicate that the composition of the precipitate is not constant.

The amount of glycogen occurring in this muscular portion of the scollop is quite large; at one time, from three quarts 160 grams were obtained; at another, two quarts yielded 70 grams.

Glycocol.—On evaporating the alcoholic filtrate from the precipitated glycogen until quite concentrated and adding neutral lead acetate, a heavy white precipitate is produced, which is a combination of inorganic matters with the lead. The excess of lead is then removed from the filtrate by hydrogen sulphide, and after concentration the liquid is decolorized by animal charcoal. On further evaporation the fluid deposits white prismatic crystals. The crystals have a sweet taste, but upon ignition with soda lime, ammonia is evolved, evincing the presence of nitrogen, which at once separates it from the saccharine group. The crystals first obtained were not quite pure, but after treatment with animal charcoal and recrystallization gave by analysis a result corresponding to the composition of glycocol. Two more distinct preparations were made and gave by analysis:

	1st Prep.		2d Prep.	3d Prep.	Calculated. $C_2H_3O_2NH_2$.
	1.	2.			
C	31.98	31.99	32.09	31.97	32.00
H	6.88	6.84	6.79	6.81	6.66
N	18.57	18.58	18.49	18.45	18.66
O	42.57	42.59	42.63	42.67	42.66

The impurities which seemed to be the most difficult to remove were coloring and inorganic matters. The crystals were soluble in water and weak alcohol, insoluble in ether and absolute alcohol. An aqueous solution, when treated with sulphate of copper and sodium hydroxide, assumed an azure blue color without separation of cuprous oxide on heating. The substance melted at about $180^{\circ}C$, then decomposed. With nitric acid fine crystals corresponding to nitrate of glycocol were obtained. These analyses and reactions identify the substance as glycocol, which I believe has never before been found in nature.*

A preparation was now made in which the alcoholic filtrate from the precipitated glycogen was evaporated without the addition of any reagents, and here the same crystals were obtained mixed with a considerable quantity of inorganic matters and some dextrose.

The amount of glycocol occurring in the tissue is small, although where two or three quarts of material are used a fine crop of crystals may be obtained.

* *Lehrbuch der Physiologischen Chemie*, Gorup-Besanez, page 236. 1876.

The quantitative analysis of the edible or muscular portion of the scollop, as obtained at the market, is as follows :

1ST ANALYSIS.

	1.	2.
Water,-----	79·60	79·66
Solids,-----	20·40	20·34
Ash,-----	1·26	1·26
Nitrogenous matters ($=N \times 6·4$),	15·68	15·68
Ether extract,-----	·33	·28
Non-nitrog. by difference,-----	3·13	3·12

2D ANALYSIS.

	1.	2.
Water,-----	80·25	80·25
Solids, ---	19·75	19·75
Ash, -----	1·24	1·22
Nitrogenous matters,-----	15·04	15·04
Fatty "-----	·32	·24
Non-nitrog. "-----	3·15	3·25

Total amount of nitrogen in the substance dried at 100° C. :

	1.	2.
N	11·35	11·37

The percentage of glycogen was determined in two separate quantities.

	1.		2.	
	1.	2.	1.	2.
Glycogen,---	2·43	2·40	1·98	2·19

The percentage of glycocoll was determined, but owing to the inaccuracy of the method, can be considered only as an approximation to the truth.

	1.	2.	3.	4.
Glycocoll,-----	·46	·68	·71	·39

The ash of the muscle consisted of the bases, soda, potash, magnesia and lime ; acids, chlorine, sulphuric and phosphoric.

In conclusion, I wish to express my obligations to Prof. S. W. Johnson for advice freely given.