NUTRITION OF OYSTERS: GLYCOGEN FORMATION AND STORAGE

By Philip H. Mitchell

Contribution from the United States Bureau of Fisheries Biological Station, Woods Hole, Mass., and the Biological Laboratory of Brown University

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INTRODUCTION.

More and more the difficulties of producing well-nourished oysters for market are prone to increase. The continued increment of population along the seaboard causes ever greater pollution of those waters especially suitable for the "fattening" of oysters. Not only does the fresher water of bays and inlets increase the meats of oysters by causing osmosis of water into the tissues, but the actual food for oyster fattening in the true sense tends to be more abundant in such waters. Modern sanitary limitations to oyster culture have therefore made it important to the industry that further information concerning food for oysters and the conditions under which they can be made to yield larger amounts of marketable meat be obtained by investigation. The present paper is one step in that direction.

Chemical analysis of oyster meats would seem to offer the only reliable method of estimating the food value of oysters, since the size and weight of either the entire oyster or the "shucked" meats depend so much on the relative amounts of inorganic matter present as shell and sea salts and on the relative amount of water present that the food value is not truly shown. Estimations of glycogen in the oyster promised an especially fruitful method of studying changes in its nutritive condition, because this substance may constitute the most abundant single constituent when present to the extent of 20 or 25 per cent of the dried meats and because it is subject to very great and comparatively rapid fluctuations in amount. The actual variations in glycogen must be distinguished, of course, from the percentage changes caused by differences in water and salt content of the oyster. It was necessary then to calculate glycogen for the moisture and ash-free constituents of the entire shell contents of the specimens used. Glycogen estimations were made by the well-known Pfluger method. Ash determinations were difficult to make because of the slowness with which some of the organic matter burned and the tendency of some of the inorganic matter to volatilize. The use of porcelain crucibles kept at low red heat during about two hours in a muffle furnace was found to be satisfactory.

[a] The experimental manipulation, aside from chemical analysis, in some of these experiments was conducted by Dr. G. H. Robinson and in others by Dr. W. W. Browne. Three of the chemical analyses were made by Dr. G. H. White.

SEASONAL VARIATIONS IN GLYCOGEN CONTENT OF OYSTERS.

The results indicate a seasonal variation in the glycogen content of oysters. In the specimens observed it was very low during the early summer. This may have been due to spawning or to the fact that the oysters used early in July had been recently transplanted to shallow water, where they would be exposed at very low tides. At any rate the figures show that glycogen may become greatly depleted. It steadily increases, however, during the summer and fall, until just before cold weather it amounts to 20 per cent or more of the dried weight. Lower figures were obtained in the winter. Some analyses to indicate these changes are grouped in Table 1.

TABLE 1.—SEASONAL VARIATION IN GLYCOGEN CONTENT OF OYSTERS.

Source of oysters.	Date.	Oysters used.	Average weight.	Ash in dried meats.	Glycogen in dried meats.	Glycogen in ash-free solids.
			Grams.	*Per cent.*	*Per cent.*	*Per cent.*
Wareham, Mass	July 11	20	88	36.45	2.77	4.37
Do	July 31	14	72	25.30	9.47	12.70
Cotuit, Mass	July 29	12	89	23.60	10.35	13.57
Wareham, Mass	Aug. 6	19	72	30.87	9.69	14.02
Do	Aug. 27	18	55	25.72	11.80	17.38
Narragansett Bay	Nov. 14	15	92	19.05	21.50	26.55
Do	Feb. 3	14	83	22.17	15.52	19.94

These observations were not primarily undertaken for the purpose of studying seasonal variations in the glycogen content of oysters under natural conditions. A large number of determinations on specimens taken directly from the oyster beds are not, therefore, at hand. Those given in Table 1, in fact, are mostly analyses made for comparison with determinations on experimental oysters. Altogether, however, they constitute a sufficiently striking and regular series to be worthy of note and agree with the observations of Milroy.[a] That glycogen would be used up during warm weather might be expected from the author's experiments on the oxygen requirements of oysters,[b] which showed the direct relationship between temperature and oxidation. It is well known that increased oxidation in animals involves the utilization of some of the stored-up glycogen.[c] Further observations on the glycogen variations during winter months would furnish chemical evidence as to whether oysters hibernate or continue to feed during the cold weather. These data, so far as they go, favor the hibernation idea.

THE FORMATION OF GLYCOGEN FROM DEXTROSE.

The formation of glycogen from dextrose was tested out by putting oysters into large, shallow glass dishes containing sea water of known specific gravity and known amounts (usually 0.25 per cent) of either pure dextrose or crude glucose. It was found that larger amounts of sugar were toxic. Experiments employing 1 per cent and even some with 0.5 per cent of dextrose had to be discarded because of high mortality among the oysters. The toxicity of an unphysiological abundance of dextrose is in accord with common observations on higher animals. Oxygen was constantly furnished

[a] Milroy: Seasonal variation in the quantity of glycogen present in samples of oysters. Department of Agriculture and Technical Instruction for Ireland. Fisheries Branch. Scientific Investigation, 1907. No. IV.

[b] Mitchell: Oxygen requirements of shellfish. Bulletin United States Bureau of Fisheries, vol. XXXII, 1912, p. 209.

[c] Lusk: Elements of the science of nutrition. 1909.

throughout the experiment by bubbling air from an aspirator bottle through glass tubes reaching to the bottom of the dish. At the end of a period, varying from two to five days, the entire shell contents of the oyster were dried down and analyzed. Analyses, for comparison and control, were made on oysters taken from the same source as those used for feeding with sugar, but analyzed at the beginning of the experiment; and also analyses of oysters from the same lot kept meanwhile in aerated sea water containing no sugar. In one experiment a further control consisted in the analyses of oysters having the same origin as the others, but kept during the time of the experiment in a wire cage suspended in the water near the Government docks at Woods Hole Harbor. Here with garbage and animal and plant life in very great abundance, feeding conditions were about as rich as could be obtained. The results of the experiments are shown in Table 2.

TABLE 2.—FORMATION OF GLYCOGEN FROM DEXTROSE.

Experiment No.[a]	Oysters used.	Average weight of oyster.	Treatment of oysters.	Ash in dried meats.	Glycogen in dried meats.	Glycogen in ash-free solids.
			GROUP I.			
		Grams.		*Per cent.*	*Per cent.*	*Per cent.*
1	20	88	Analyzed at beginning of experiment to be compared with treated oysters of Nos. 2, 3, and 4	36.45	2.77	4.37
2	17	80	In sea water, sp. gr. 1.023, containing ¼ per cent of dextrose during 48 hours	33.70	4.11	6.20
3	15	59	In sea water, sp. gr. 1.015, containing ¼ per cent of dextrose during 48 hours	29.45	4.38	6.21
4	15	66	In running sea water in aquarium for 15 days, then treated exactly like No. 3	23.68	8.55	11.21
			GROUP II.			
5	14	72	Analyzed as soon as brought from beds, to be compared with Nos. 6, 7, 8, and 9	25.30	9.47	12.70
6	20	73	In sea water, sp. gr. 1.015, containing ¼ per cent dextrose during 48 hours	21.65	11.30	14.43
7	20	70	In sea water, sp. gr. 1.015, with no dextrose, treated like No. 6 during 60 hours	24.35	9.43	12.47
8	19	71	Taken from running sea water in aquarium at time No. 6 ended. Control of Nos. 6 and 7	30.08	8.63	12.36
9	19	72	Kept in wire cage in Woods Hole Harbor during 50 hours before analysis	30.87	9.69	14.02
			GROUP III.			
10	20	58	Analyzed as soon as brought from beds, to be compared with Nos. 11, 12, and 13	25.57	9.28	12.47
11	20	62	In sea water, sp. gr. 1.015, containing ¼ per cent crude glucose during 48 hours	14.86	12.08	14.19
12	19	55	In running sea water in aquarium 48 hours, then treated with glucose like No. 11, 72 hours	19.00	12.75	15.74
13	20	55	In sea water, sp. gr. 1.015, without sugar during 76 hours. Had been in aquarium 48 hours. Control for No. 12	23.12	10.05	13.07
			GROUP IV.			
14	15	92	Analyzed as soon as brought from beds, to be compared with Nos. 15 and 16	19.05	21.50	26.55
15	15	92	In sea water, sp. gr. 1.022, containing ¼ per cent dextrose during 65 hours	18.33	22.77	27.90
16	15	92	In sea water, sp. gr. 1.022, without dextrose during 67 hours	19.99	21.26	26.33

[a] All oysters used in experiments of the several groups came from the same beds at the same time.

These results show clearly that glycogen may be formed in the oyster from dextrose, while experiment No. 9 shows that even richest feeding in excellent normal conditions with abundant tide flow did not cause as much glycogen formation as that obtained by treatment during the same time with a 0.25 per cent dextrose solution and under somewhat adverse conditions at that. The oysters were crowded into a dish in water that was not changed throughout the time of the experiment. Indeed, experiments Nos. 7 and 8, compared with No. 5, show how adverse conditions in the laboratory may tend to lower the

glycogen content. The great variations in ash percentages are due not only to the differences in density of water, but also to variations in the amount of sea water included in the shell contents used for analysis. The metabolic changes in glycogen, therefore, are more fairly represented by the percentages in the ash-free solids than by those in the total solids of the oyster. It apparently makes no difference whether the oysters at the start contain much or little glycogen. The amount formed in any case is about the same for two or three days' treatment with dextrose, i. e., 1.5 to 2.5 grams of glycogen to every 100 grams of organic matter. The numbers of oysters used in these experiments would seem to exclude the possibility of accounting for variations by mere individual differences, especially since the dried shell contents of all oysters used in any one experiment were very thoroughly ground and mixed before analysis.

The storage of glycogen can not continue indefinitely under the adverse conditions used in these experiments. In one case oysters kept in aerated but unchanged sea water, containing 0.25 per cent of dextrose, during five days, yielded glycogen to the amount of 11.12 per cent of the ash-free solids, but the corresponding figure for some of the same oysters analyzed at the beginning of the experiment was 13.57 per cent.

Further experiments to test this point were made as shown in Table 3.

TABLE 3.—EFFECT OF VARYING DURATIONS OF DEXTROSE FEEDING ON GLYCOGEN FORMATION.

Experiment No.	Duration of treatment.	Treatment of oysters (15 used for each analysis).	Ash in dried meats.	Glycogen in dried meats.	Glycogen in ash-free solids.
	Days.		*Per cent.*	*Per cent.*	*Per cent.*
25	1	In aerated sea water containing ¼ per cent dextrose during 20 hours	32.17	6.07	8.94
26	2	Same as No. 25 but continued during 44 hours	31.12	5.16	7.49
27	3	Same as Nos. 25 and 26 but continued during 68 hours	30.35	4.34	6.23
28	.....	From same lot as Nos. 25, 26, and 27, analyzed as a control	34.40	4.84	7.39
29	.....	Analyzed at beginning of experiments Nos. 30 to 34 as a control	25.56	3.74	5.01
30	1	In aerated sea water containing ¼ per cent dextrose, 1 day	33.46	5.06	7.60
31	2	Same as No. 30, 2 days, water (¼ per cent dextrose) renewed second day	31.22	6.11	8.87
32	3	Same as Nos. 30 and 31, 3 days, water (¼ per cent dextrose) renewed daily	29.85	4.68	6.68
33	4	Same as No. 32, 4 days	30.60	5.03	7.25
34	5	Same as No. 33, 5 days	29.50	4.89	6.97
35	.....	From same lot as Nos. 29 to 34 analyzed at end of No. 34 as control	34.70	3.96	6.06

These experiments show that the maximum glycogen formation under these circumstances occurs in about two days. Although in experiments Nos. 30 to 34 the water containing dextrose was daily renewed and constantly aerated, yet glycogen did not show a progressive daily increase. The control oysters (experiment No. 29) were analyzed immediately after they were brought from the beds at Wareham, Mass. There the specific gravity of the water is lower than at Woods Hole. This explains why the second set of control oysters (experiment No. 35), analyzed after remaining five days in Woods Hole water, gave a higher ash yield than the first lot (experiment No. 29), though taken from the same beds at the same time. What effect this may have had on glycogen formation is discussed later. That the oysters of experiments No. 31 to 34 did not have as high an ash content as those of No. 35 may indicate a failure to remain open and feed continuously under the experimental conditions. With smaller concentration (0.1 per cent) of dextrose, glycogen formation may continue for three days. (See experiments Nos. 37 and 38, Table 4.)

The optimum concentration of dextrose for glycogen formation was found to be about 0.25 per cent. It was found that 1 per cent of dextrose was toxic. Oysters exposed to it showed inability to close their shells normally after 24 hours' exposure and rapidly died off in about two or three days. If removed from the sugar-containing water soon after signs of distress appeared, the oysters recovered their normal behavior in the course of a few days' immersion in running sea water. Some experiments contrasting the glycogen formation in 0.25 per cent dextrose with that in 0.1 per cent dextrose are given in Table 4.

TABLE 4.—THE EFFECT OF DIFFERENT CONCENTRATIONS OF DEXTROSE ON GLYCOGEN FORMATION.

Experiment No.	Concentration of dextrose.	Treatment of oysters (15 used for each analysis).	Ash in dried meats.	Glycogen in dried meats.	Glycogen in ash-free solids.
	Per cent.		*Per cent.*	*Per cent.*	*Per cent.*
36	0.1	Control analysis to be compared with Nos. 37 and 38	35.50	5.48	8.49
37	.1	In sea water, sp. gr. 1.015, with 0.1 per cent dextrose, 36 hours, aerated	30.72	6.34	9.15
38	.1	Same as No. 37, but continued for 72 hours	28.65	7.44	10.45
39	.....	Control analysis to be compared with Nos. 43 and 44	31.92	7.79	11.50
43	.1	In sea water, sp. gr. 1.022, with 0.1 per cent dextrose, 24 hours, aerated	32.95	9.90	14.76
44	.25	Same as No. 43, but with 0.25 per cent dextrose	31.50	12.70	18.53
45	.....	Same as Nos. 43 and 44, but without dextrose	32.90	8.98	13.38

By comparison of experiments Nos. 37 and 38 with 6, 11, and 12 (shown in Table 2), evidence of more rapid formation of glycogen in 0.25 per cent dextrose than in 0.1 per cent is seen in addition to the data shown in Table 4.

The effect of different specific gravities of the sea water containing dextrose on glycogen formation was studied. Fresh or nearly fresh water did not permit glycogen formation. The evidence points to the fact that any change in the salt concentration at the time oysters are transferred from ordinary sea water to dextrose-containing water is a deterrent to maximum glycogen formation. Experiments bearing on this point are reported in Table 5.

TABLE 5.—THE EFFECT OF VARYING SPECIFIC GRAVITY OF THE SEA WATER ON GLYCOGEN FORMATION.

Experiment No.	Specific gravity of the water.	Treatment of oysters (15 used for each analysis).	Ash in dried meats.	Glycogen in dried meats.	Glycogen in ash-free solids.
			Per cent.	*Per cent.*	*Per cent.*
39	1.023	Control analysis to be compared with Nos. 40 to 45	31.92	7.79	11.50
40	1.000	In aerated fresh water containing 0.1 per cent dextrose, 24 hours	27.55	8.75	12.08
41	1.010	In aerated water (¼ fresh, ¾ sea water) with 0.1 per cent dextrose, 24 hours	22.00	10.79	13.84
42	1.016	In aerated water (¼ fresh, ¾ sea water) with 0.1 per cent dextrose, 24 hours	27.75	10.57	14.64
43	1.023	In aerated sea water with 0.1 per cent dextrose, 24 hours	32.95	9.90	14.76
44	1.023	In aerated sea water with 0.25 per cent dextrose, 24 hours	31.50	12.70	18.53
45	1.023	Same as Nos. 43 and 44, but without dextrose, control	32.90	8.98	13.38
46	1.023	Control analysis to be compared with Nos. 47 to 49	34.20	4.20	6.39
47	1.000	In aerated fresh water with 0.25 per cent dextrose, 24 hours	31.62	3.82	5.60
48	1.000	Same as No. 47, but continued 72 hours, water changed daily	24.84	4.02	5.36
49	1.023	Control analysis, same lot as Nos. 46 to 48, in sea water until end of experiment No. 48	37.60	3.51	5.64
50	1.023	Control analysis to be compared with Nos. 51 and 52	35.75	4.54	7.08
51	1.006	In aerated water (¼ sea water, ¾ fresh) with 0.1 per cent dextrose, 36 hours	26.35	5.36	7.28
52	1.006	Same as No. 51, but continued 72 hours	19.90	5.41	6.75
53	1.023	Control analysis to be compared with Nos. 54 and 55	37.48	3.69	5.90
54	1.010	In aerated water (½ fresh, ½ sea water) with 0.1 per cent dextrose 72 hours	23.55	4.45	5.82
55	1.023	Control analysis, same lot as Nos. 53 to 54, in sea water until end of experiment No. 54	36.73	4.12	6.50

It is seen from Table 5 that the greatest glycogen formation from dextrose occurred in experiment No. 44. Over 60 per cent increase occurred in 24 hours. Sea water from the harbor where the oysters had been kept previous to the experiment was used, so that during the sugar feeding (0.25 per cent dextrose in the water) the oysters experienced no considerable change in the density of the medium. With lower densities some glycogen formation appeared to occur in experiments Nos. 40, 41, and 42; but the results of experimets Nos. 40 and 41 when compared with the control, experiment No. 45, are not significant increments. This series of experiments (Nos. 39 to 45) was done early in September when glycogen formation in oysters is at its height under natural conditions, so that the oysters kept in a small amount of aerated but unchanged sugar-free sea water were able to show a significant increase in glycogen during 24 hours. Water of specific gravity of 1.015 to 1.016 does not so completely inhibit glycogen formation in oysters that have previously been in water of specific gravity of 1.022 to 1.023. Examination of the results of experiments reported in Table 2 yields additional evidence on this point. An increase in the specific gravity of the sugar-containing medium may also, within certain limits, allow considerable glycogen formation. This is shown by a comparison of the results of experiments Nos. 29, 30, 31, and 35 as reported in Table 3.

An attempt to study the effect of the varying concentrations of specific inorganic salts was made. The immediate object was to find the effect on glycogen storage of adding phosphates to sugar-containing water whose density was low enough to check the glycogen process. The experiments as reported in Table 6 show a tendency of phosphates to interfere with glycogen storage. The experiments, Nos. 61, 62, and 63, in which phosphates were added, all resulted in a lowered glycogen content of the oysters although sugar was plentifully furnished.

TABLE 6.—THE EFFECT OF PHOSPHATES ON GLYCOGEN FORMATION FROM SUGAR IN DILUTED SEA WATER.

Experiment No.	Treatment of oysters (15 used for each analysis).	Ash in dried meats.	Glycogen in dried meats.	Glycogen in ash-free solids.
	In experiments Nos. 61 to 65 a mixture of equal parts of sea and fresh water (sp. gr. 1.010) was used.	*Per cent.*	*Per cent.*	*Per cent.*
60	Control analysis to be compared with Nos. 61 to 65	29.27	10.53	14.90
61	In aerated, diluted sea water, with 0.15 per cent disodium phosphate and 0.25 per cent dextrose, 24 hours	21.37	9.25	11.77
62	In aerated, diluted sea water, with 0.5 per cent disodium phosphate and 0.25 per cent dextrose, 24 hours	24.07	7.32	9.65
63	In aerated, diluted sea water, with 0.5 per cent disodium phosphate, 0.25 per cent dextrose, and 0.25 per cent calcium chloride, 24 hours	19.77	10.76	13.40
64	In aerated, diluted sea water, with 0.25 per cent dextrose, 24 hours	21.21	12.50	15.88
65	In aerated, diluted sea water, without addition of salt or sugar, 24 hours, as a control to Nos. 61, 62, 63, and 64	23.30	10.67	13.91

The possibility of the formation of glycogen from dextrose by oyster meats separated from the shells was tested, because in the present-day practice of the oyster industry the meats, before packed for shipment, are washed freely with tap water during periods varying in different establishments from a few minutes to several hours. If the glycogen increment, then, could be obtained during this process by addition of glucose to the wash water, an obvious economy would be attained. Only one such experiment is reported, as time and opportunity for conducting others have not yet been found. The result of this, shown in Table 7, was entirely negative. This might be expected physiologically. With the circulation destroyed, death coming on in the tissues, and metab-

olism coming to a stop, it is hardly likely that a synthetic process like glycogen formation would occur with sufficient activity to overbalance the hydrolytic process of glycogenolysis known to occur in various dying animal tissues.

TABLE 7.—TREATMENT OF SHELLED OYSTERS WITH DEXTROSE.

Experiment No.	Oysters used.	Total weight of oysters.	Treatment of oysters.	Ash in dried meats.	Glycogen in dried meats.	Glycogen in ash-free solids.
		Grams.		*Per cent.*	*Per cent.*	*Per cent.*
21	14	125	Opened at packing house of Narragansett Bay Oyster Co. (analyzed at once)	11.22	21.58	24.31
22	14	125	In tap water containing 0.5 per cent of dextrose during 22 hours	8.42	22.22	24.26
23	14	.....	In tap water containing no dextrose during 22 hours	7.90	22.85	24.80

THE FORMATION OF FAT FROM CARBOHYDRATE NUTRIMENT.

The formation of fat from carbohydrate nutriment was also observed in oysters, though experiments on this point were not as numerous as on glycogen formation. Results are shown in Table 8.

TABLE 8.—FORMATION OF FAT FROM DEXTROSE.[a]

Experiment No.	Treatment of oysters.	Fat in dried meats.	Fat in ash-free solids.
		Per cent.	*Per cent.*
[b] 8	In Woods Hole Harbor 50 hours before analysis	2.55	3.25
[b] 6	In sea water containing ¼ per cent dextrose, 48 hours	3.13	3.98
[c] 10	Analyzed as soon as brought from beds	3.41	4.58
[c] 11	In sea water containing ¼ per cent glucose, 48 hours	6.36	7.47

[a] Further data on these analyses are contained in Table 2.
[b] Oysters used in experiments Nos. 6 and 8 were from the same lot.
[c] Oysters used in experiments Nos. 10 and 11 were from the same lot.

LACK OF GLYCOGEN FORMATION FROM DEXTRIN.

The failure of oysters to form glycogen from dextrin was observed in two experiments. This possibility was tested because only crude commercial glucose, which always contains dextrin, could be used economically in practical work with oysters. Kahlbaum's purified dextrin was used. Results are shown in Table 9.

TABLE 9.—LACK OF GLYCOGEN FORMATION FROM DEXTRIN.

Experiment No.	Oysters used.	Average weight of oysters.	Treatment of oysters.	Ash in dried meats.	Glycogen in dried meats.	Glycogen in ash-free solids.
		Grams.		*Per cent.*	*Per cent.*	*Per cent.*
17	14	84.9	In sea water, sp. gr. 1.022, with no dextrin. Treated like No. 18 during 60 hours	21.55	17.22	22.01
18	14	82.8	In sea water, sp. gr. 1.022, containing 0.5 per cent dextrin, during 60 hours	21.00	16.86	21.34
19	14	83.5	Analyzed as soon as brought from beds	22.17	15.52	19.90
20	14	83.4	In sea water, sp. gr. 1.022, containing ¼ per cent dextrin, during 60 hours	23.04	14.80	19.23

NUTRITION BY SEAWEEDS AND PROTOZOA.

The nutrition of oysters by fragments of seaweed was also studied. This idea seems to have been little considered because of the generally prevailing view that algæ and other microscopic life constitute the food of oysters.[a] In these experiments oysters were fed in glass jars of aerated water with sea lettuce (*Ulva lactuca*) chopped into fine fragments and added fresh at intervals of one or two days. It was found that the material had to be in fresh condition, because if it darkened and decomposed, or if the water in the containers was not changed frequently enough, the oysters would die. That this material was ingested was shown by repeated microscopic observations of the stomach contents of oysters so fed. All the sea water used was carefully filtered through several thicknesses of fine filter paper so that, as shown by subsequent microscopic examination, it was freed from algæ. It is true that some algæ clung to the sea lettuce, and Protozoa and bacteria from the same source were also present in the preparation. The multiplication of these organisms provided, therefore, a part of the nutrition for the oysters. Several experiments were started, but only one was successfully continued long enough to be of value. The result is shown in Table 6. It indicates that the seaweed fragments contributed to the nourishment of the oyster.

Whether accumulations of seaweed on oyster beds may cause the death of oysters is a question of practical interest to oystermen. Gorham[b] observed the death of oysters on beds where seaweeds in which *Ulva* predominated had lodged in masses sufficient to cause putrefaction. In his opinion the seaweed was the cause of death.

The following experiments on this point were undertaken: Wooden boxes about 30 inches square and 3 inches deep were filled with sand and gravel, and 25 oysters were laid on top of the gravel in each box. A basket of galvanized wire netting was made so as to fit over the top of each box with a space of 3 or 4 inches above the oysters. Two such boxes, one with the wire basket loosely filled with seaweeds and the other without them, were then anchored together in each of several locations on the shores of Narragansett Bay in waters suitable for oyster culture. The seaweeds used were mostly *Ulva* with some eelgrass. In one case *Ulva* alone was used. Four such experiments were carried out. In two of them the oysters were taken up for observation after 29 days, in one after 25 days, and in one after 14 days. In every case the oysters under seaweeds were found to be dead and badly decomposed, with the meats running out of the shell. Of the 100 oysters so treated only 7 were found alive and 6 of these had discolored meats showing signs of incipient putrefaction. All the control oysters showed no pathological conditions or signs of decay, and from the new shell formation during the progress of the experiment, showed abundant evidence of flourishing growth. It would seem, then, that although seaweed may serve as food for oysters, accumulations of it in places where the tide does not keep it sufficiently in motion must be guarded against in oyster culture.

The nutrition of oysters by Protozoa was also investigated. Rich cultures of various Protozoa were added daily to a dish of filtered, aerated sea water containing oysters. As algæ were excluded from this experiment, only Protozoa and bacteria, but with Protozoa predominating, served as food for the oysters. They appeared after 17 days,

[a] Brooks, William K.: The Oyster. 225 p., 1905. Baltimore.
[b] Gorham: Annual Report, Rhode Island Shellfish Commission, 1914.

when the experiment was ended, to be in a nutritive condition comparable to the oysters from the same source examined before the experiment. The result of chemical analysis is shown in Table 10.

TABLE 10.—THE NUTRITION OF OYSTERS BY SEAWEED AND PROTOZOA.[a]

Treatment of oysters.	Ash in dried meats.	Glycogen in dried meats.	Glycogen in ash-free solids.
	Per cent.	*Per cent.*	*Per cent.*
Analyzed as soon as brought from beds. (See experiment 1, Table 2)	36.45	2.77	4.37
Fed with *Ulva lactuca* during 14 days	32.56	7.03	10.43
Fed with Protozoa cultures during 17 days	36.70	4.55	7.18

[a] The oysters used in these experiments were from the same lot.

An attempt was made to follow the nutritive condition of the experimental oyster by determinations of nitrogen in the dried tissues. From the results of 20 analyses no definite conclusions could be drawn. In the various artificial feedings, changes in the nitrogen content of the tissues were observed, but they were always small and in most cases merely percentage changes attributable to the altered proportions of salts and glycogen, rather than to a change in the actual amount of protein present.

SUMMARY.

A summary of the results of this work follows:

1. Evidence was obtained of a seasonal variation in the glycogen content of oysters. A depletion occurs in the warm weather but is followed by a storing-up process in the latter part of the summer and in the fall. Glycogen seems to decrease during the coldest weather. This favors the idea of hibernation. The results on seasonal variation agree with the results of Milroy.

2. Glycogen may be formed in the oysters from dextrose. Fat storage may occur simultaneously.

3. The optimum conditions for this process are: (*a*) Duration of dextrose feeding two to three days; (*b*) concentration of dextrose equal to about 0.25 per cent; and (*c*) water density not greatly different from that in which the oysters have previously been.

4. Excess of sodium phosphate in the medium may check glycogen formation from sugar.

5. Formation of glycogen from dextrin was not obtained.

6. Failure of glycogen formation from sugar by oysters taken from the shells was observed.

7. Evidence that Protozoa and fragments of seaweed (*Ulva lactuca*) may serve as food for oysters is given.

8. Decaying seaweeds lodged above oysters that are under otherwise good growing conditions may cause their death.