BROWN UNIVERSITY

PROVIDENCE, RHODE ISLAND

CONTRIBUTIONS

FROM THE

BIOLOGICAL LABORATORY

(formerly Anatomical Laboratory)

VIII

ISSUED APRIL, 1916

PROVIDENCE, R. I.

U. S. A.

PREFACE

The papers which are collected in this eighth volume of Contributions have been written by officers or students in the Department of Biology of Brown University, and have recently appeared in various scientific journals. In the table of contents and on the title page of each paper will be found the place and time of publication. At the end of the volume is a complete list of the papers published in the preceding volumes of this series.

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BY PHILIP H. MITCHELL AND GEORGE SMITH.

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THE PHYSIOLOGICAL EFFECTS OF ALKALOIDS OF ZYGADENUS INTERMEDIUS.

By PHILIP H. MITCHELL and GEORGE SMITH.

[*From the Biological Laboratory of Brown University.*]

ZYGADENUS intermedius is one of the several varieties of the plants formerly called *Zygadenus venosus* and popularly known as death camas. They have also been designated by a number of other names, among which are wild onion, mystery grass, lobelia, and poison sego lily. Their economic significance, as pointed out by Chestnut and Wilcox,[1] is considerable because of their prevalence on the sheep and cattle ranges of many western states and their highly toxic effect on the animals, especially sheep, which eat them. Starting up in the spring earlier than the grass but resembling it in form and texture, these plants are so attractive to sheep that they are an enemy which the rancher must reckon with. During the season of 1900 in Montana alone 3,030 sheep were reported to be poisoned by various varieties of *Zygadenus*.

Chestnut and Wilcox [1] have carried out some experiments on the injection of watery and alcoholic extracts of *Zygadenus venosus* as well as feeding experiments. They considered that some, at least, of the plants which they used may have been *Zygadenus intermedius*. The sheep and rabbits used in their experiments showed symptoms of poisoning, such as dizzy movements, slow and labored or convulsive respiration, and finally a deep coma which, if sufficient poison had been administered, terminated fatally. The effects were apparently the same after injection of extracts or feeding of plants, and were identical with those observed in animals poisoned on the open ranges. These authors showed that all parts of the plant contain toxic substances, so that the belief formerly held that animals must eat the bulb or root to be poisoned is erroneous. They found that of

[1] Chestnut and Wilcox: U. S. Department of Agriculture Bulletin No. 26, 1901.

the substances which they tried as antidotes potassium permanganate was the most effective. For sheep and cattle they advised giving it in the form of a drench consisting of aluminium sulphate as well as potassium permanganate dissolved in water.

No further work on the effects of this poison has, so far as the authors know, been reported. Little is known of the chemical nature of the active principle. Slade [2] concluded from certain color tests that the plants contained the alkaloids sabadine, sabadinine, and veratralbine. Heyl and Raiford [3] have made alkaloidal preparations from leaves, flowers, bulbs, and roots of the *intermedius* variety and have shown that alkaloids are especially abundant in the flowers.

Some alkaloidal material prepared by them was sent to the authors of the present paper for the purpose of determining its toxicity. The results of injections into guinea-pigs were sufficiently complex and interesting to warrant a more detailed analysis of the pharmacological action of the preparation. This report is concerned with experiments carried out with that preparation only. It consisted of alkaloids, presumably a mixture, prepared from various parts of *Zygadenus intermedius* according to the methods reported by Heyl and Raiford [3] and held in 17.5 per cent aqueous solution in the form of sulphates.

Experiments.

I. **The fatal dose for guinea-pigs.** — To obtain some idea of the relative toxicity of the preparation, intraperitoneal administration to guinea-pigs was employed. Injections of the alkaloidal solution diluted with sterilized physiological saline were made aseptically.

In Table I the results are shown arranged in the order of the dose per 100 gm. of guinea-pig. The fatal dose is seen to be between 4.6 and 5.1 mgm. per 100 gm. of animal. It is probably nearer the lower than the upper of these limits, because the animal which received 4.6 mgm. per 100 gm. recovered only with great difficulty after a long period, eighteen hours, and seemed at least twice during that time to be in its death struggle. This injection, therefore, was probably very

[2] Slade: American journal of pharmacology, 1905, lxxvii, p. 262.

[3] Heyl and Raiford: Journal of the American Chemical Society, 1911, xxxiii, p. 206.

near the fatal dose. In the case of the injection of 5.1 mgm. per 100 gm., on the other hand, death ensued in so short a time, twenty-five minutes, that this dose was perhaps a little more than the fatal one. These results indicate therefore that we have a substance of marked toxicity.

TABLE I.

INTRAPERITONEAL INJECTIONS INTO GUINEA-PIGS.

Weight of guinea-pig.	Volume of solution.	Amount of sulphate.	Dose per 100 gm. of animal.	Result.	Time between injection and death.	Time between injection and recovery.
gm.	c.c.	gm.			min.	hours
485	2.0	0.05	0.0111	Death	19	..
364	1.6	0.04	0.0104	Death	23	..
315	4.0	0.028	0.0090	Death	21	..
322	3.5	0.0245	0.0076	Death	31	..
332	2.9	0.02	0.0060	Death	26	..
290	2.1	0.015	0.0051	Death	25	..
305	2.0	0.014	0.0046	Recovery	..	18
300	1.0	0.0035	0.0011	Recovery	..	3

It seemed important also to test the effect of administration *per os*. The undiluted alkaloidal solution as furnished us was put in small gelatin capsules and fed to guinea-pigs by forcing the capsules into the back of the mouth through a glass tube. The results are arranged in Table II, and apparently indicate that a comparatively large quantity (0.2 gm.) is required for the fatal effect.

The actual fatal dose absorbed from the digestive organs is not, however, as large as these figures would seem to show. A considerable quantity of the material fed was vomited up before it was absorbed, because the substance, as will be explained below, is a very powerful emetic. Another factor tending to diminish the toxicity when fed is the power of the organism to effect an oxidative destruction of the alkaloid before a fatal amount has been absorbed. This effect is illustrated by the rather quick recovery of guinea-pigs from the effects of injection of small doses. For example, in the last experi-

ment recorded in Table I, 3.5 mgm. produced very profound symptoms of poisoning, but these had entirely passed away within three hours. In another experiment, not recorded above, the subcutaneous injection of 3.7 mgm. produced undoubted effects which had all disappeared in an hour and a half. It does not seem necessary then to

TABLE II.

PER OS ADMINISTRATION TO GUINEA-PIGS.

Weight of guinea-pig.	No. of capsules used.	Amount of sulphate.	Dose per 100 gm. of animal.	Result.	Time between feeding and death.	Time between feeding and apparent recovery.
gm.		gm.				min.
334	1	0.0175	0.0053	Recovery	..	30
352	4 [1]	0.140	0.040	Recovery [2]	..	About 12 hours
278	6	0.21	0.0755	Death [2]	About 12 hours	...

[1] Last two capsules given one hour after the first two.
[2] Occurred during the night and was not exactly known.

conclude from these experiments that gastro-intestinal digestion destroys the alkaloid or diminishes its toxicity, but it seems reasonable to conclude that the tendency to vomiting, the slowness of absorption, and the rate of destruction of the alkaloid account for the relatively large quantity required to kill when fed.

II. **The effects on guinea-pigs.** — After subcutaneous or intraperitoneal injection or after feeding, the alkaloid produces the same symptoms of poisoning. There are marked insalivation and frequently repeated vomiting, which begin very soon after the substance is administered and persist nearly as long as any symptoms can be observed. During the first few minutes of the onset of the effects the animal jumps and runs about the cage excitedly in the intervals between vomiting. It soon begins, however, to lose control of its muscles. The hind legs are usually affected first, but finally all the limbs become useless and the guinea-pig lies on its side completely prostrated. The respiration, at first rapid, has by this time become slower than normal and very labored. It is frequently interrupted by short

periods of very convulsive breathing. Although quite unable to make any co-ordinate movements, the animal is exceedingly irritable and responsive to reflex stimuli. The lightest touch will start a struggle, and as the symptom complex advances there comes a stage when the struggles become spasmodic and later typical tetanic spasms occur. The guinea-pig now behaves quite like an animal poisoned with strychnine, and at the slightest irritation, such as a jar of the cage or a breath of wind, exhibits profound tetanus. All of its muscles are rigid and the body stiffened out in an extended position sometimes for ten seconds at a time. The heart rate as detected by allowing the guinea-pig to lie in the observer's hand so that the cardiac impulse may be felt is found to be much slower than normal, and this condition persists until recovery is quite far advanced or death occurs. Defecation takes place at frequent intervals throughout the development of effects. Micturition is also generally observed. If the fatal dose has been given by injection, death occurs after an interval of about twenty to thirty minutes. No relationship between the dosage administered intraperitoneally and the time required to kill is to be seen in our results. Two typical protocols follow:

Experiment No. 1. *Jan.* 30, 1911. — Female guinea-pig; weight, 845 gm. Dose: 0.05 gm.; volume, 2 c.c.

3.25 P. M. Intraperitoneal injection.

3.27 P. M. Vomiting; claws mouth.

3.29 P. M. Spasmodic twitching; retching; jumping; gagging; squealing.

3.30 P. M. Defecation; vomiting; lies on side. Cannot right itself.

3.32 P. M. Respiration very irregular.

3.33 P. M. Short, recurring tetanic spasms.

3.35 P. M. Respiration shallow and infrequent; spasms stop. Trembling.

3.36 P. M. Perfectly limp.

3.38 P. M. Infrequent, spasmodic respiration.

3.40 P. M. Muscular spasms.

3.41 P. M. Respiration, 17 per minute.

3.44 P. M. Lid reflex absent.

3.45 P. M. Tetanus.

3.46 P. M. Dead. Heart in complete diastole. Peristalsis of the intestines very marked.

Experiment No. 2. Jan. 30, 1911. — Male guinea-pig; weight, 352 gm. Dose: 0.0037 gm.; volume, 1.5 c.c.

4.06 P. M. Subcutaneous injection.

4.09 P. M. Insalivated.

4.11 P. M. Vomits a little.

4.13 P. M. Vomits repeatedly.

4.39 P. M. Labored respiration interrupted by rapid respiration. Spasm.

4.40 P. M. Control of limbs is lost. Labored and slow breathing.

4.41 P. M. Respiration, 49–50 per minute.

4.42 P. M. Slight spasm.

4.44 P. M. Respiration very irregular; heart fast. Spasm.

4.46 P. M. Respiration, 24 per minute; sometimes nine seconds elapse between breaths. Tetanic spasms; heart rate, 81 per minute.

4.49 P. M. Respiration, 23 per minute; still tries to right itself although unable to control limbs.

4.52 P. M. Spasms.

4.53 P. M. Tetanus in back muscles when touched; very bad spasms.

4.58 P. M. Heart rate, 42 per minute.

5.00 P. M. Respiration, 29 per minute; lid reflex present.

5.02 P. M. Very acute spasms; tetanus continues for three minutes without stopping.

5.11 P. M. Acute spasms; lid reflex present.

5.14 P. M. Spasms; great respiratory distress.

5.17 P. M. Hyperpnœa alternating with apnœa.

5.21 P. M. Spasms; tetanus; defecation.

5.29 P. M. Heart rate, 48 per minute.

5.32 P. M. Spasms; defecation; much urine passed.

5.33 P. M. Heart very slow.

5.35 P. M. Spasms; eyes closed. Reflex present.

5.39 P. M. Respiration, 32 per minute.

5.42 P. M. Spasms; respiration, 46 per minute.

5.54 P. M. Respiration, 23 per minute.

5.55 P. M. Acute spasms; defecation.

5.58 P. M Spasms; tries to gain feet.

6.01 P. M. Copious flow of urine.

6.12 P. M. Acute spasms; spasms now follow slightest stimulus.

6.30 P. M. Regained control of fore limbs. Spasms no longer follow stimuli.

6.57 P. M. Labored and spasmodic respiration.

6.59 P. M. Respiration easier.
7.01 P. M. Heart beat, 100 per minute.
7.10 P. M. Vomits again. Appears nearly recovered.
Jan. 31, 1911. — 9.00 A. M. Entirely recovered.

III. **The effects on frogs.** — The effects of administration to frogs were not very striking. It was thought that because of the tetanic spasms in guinea-pigs this poison might affect the spinal cord somewhat as strychnine does. Experiments with frogs did not fulfil that expectation. Two injections into the dorsal lymph sac of frogs with the brain pithed in which 0.175 gm. of material was administered in one case and twice that amount in the other caused some slight irritability, but the animal soon became entirely limp and unresponsive to stimuli. To another frog about 0.1 gm. was injected without pithing any part of the nervous system. Some twitchings and spasmodic movements occurred, but in a few minutes the animal seemed paralyzed, gave no further response to stimuli, and soon died. In all three frogs there was a noticeable reddening of the skin, and the blood vessels in the web of the foot seen under the microscope appeared to be dilated.

IV. **The effects on dogs.** — To more accurately trace the action of the alkaloidal preparation, intravenous injection into dogs was employed. The animals, seven in all, were anæsthetized in each case with ether followed by A-C-E- mixture. The alkaloidal material diluted with physiological salt solution was injected into the right femoral vein, while a tracing of blood pressure in the left carotid artery was recorded by the aid of a mercury manometer. A record of respiratory movements made by using a simple cord and pulley device to connect a recording lever with a skin suture in the thoracic or abdominal wall of the dog was simultaneously obtained.

The first injection invariably caused a fall in the blood pressure within a few seconds. Its amount was roughly proportional to the amount of material injected. 0.0035 gm. of the alkaloidal sulphate given in 1 c.c. of a fifty-fold dilution of the original preparation to a dog of 13 kilos caused a fall of blood pressure equal to 41.6 mm. of mercury, while the injection of 0.0105 gm. in 5 c.c. of the same dilution in a dog of 20 kilos produced a fall of 82 mm. The proportionality, however, was not always maintained. The quickness of the recovering rise also depended on the amount injected, and if more than 10 mgm. of the alkaloidal sulphate was given the recovery was

very slow. In no case did the blood pressure ever return to quite as high a level as that recorded before the first injection. The cause of this depressor effect is not a simple one. The most potent factor in the initial drop is the marked slowing of the heart rate. That this is due for the most part to an effect on the cardio-inhibitory centre appears from the fact that after section of both vagi an injection of the quantity usually employed did not cause the usual striking depression of the heart rate but only a very slight decrease. A second factor involved in the fall of blood pressure is vaso-dilation. Even when the pulse had quite recovered to its initial rate as seen in several tracings, the blood pressure was found to be still low, and only recovering so slowly that after some half-hour's observation it did not reach its original level. Also, the arterial pressure fell too much when injections were given after double vagotomy to be accounted for apparently by the slight slowing of the heart.

The effect on respiration is, in general, to slow it by a prolongation of the expiratory phase. This effect is slightly modified after the vagi have been severed.

A tracing to show the effect of injection without previous vagotomy is given (Fig. 1). The tracing seems to show that the alkaloid acts on the cardio-inhibitory and respiratory centres, or at least produces effects which involve them.

The results so far discussed are those obtained by an initial injection of small quantities of the alkaloid. Subsequent injections or the administration of larger quantities produced more complex effects. Second, third, and fourth injections of quantities comparable to that ordinarily used for our first, produced a successively smaller effect in decreasing the already lowered blood pressure. After a considerable quantity, for example, 0.0665 gm. had been given, a compensatory hastening of the heart rate produced a slight rise in blood pressure, and this effect was obtained after section of the vagi with comparatively small quantities of the alkaloid. Fig. 2 shows the effect of injection of 0.0105 gm. into a dog of 8.2 kilos after a previous injection, about twenty minutes earlier, of the same quantity. The rise in blood pressure amounts to 36 mm. of mercury, and the change in the heart rate is from 117 per minute before the injection to 157 per minute shortly after. In this case the vagus nerves were severed at the beginning of the experiment and the first injection gave a slight fall in pressure.

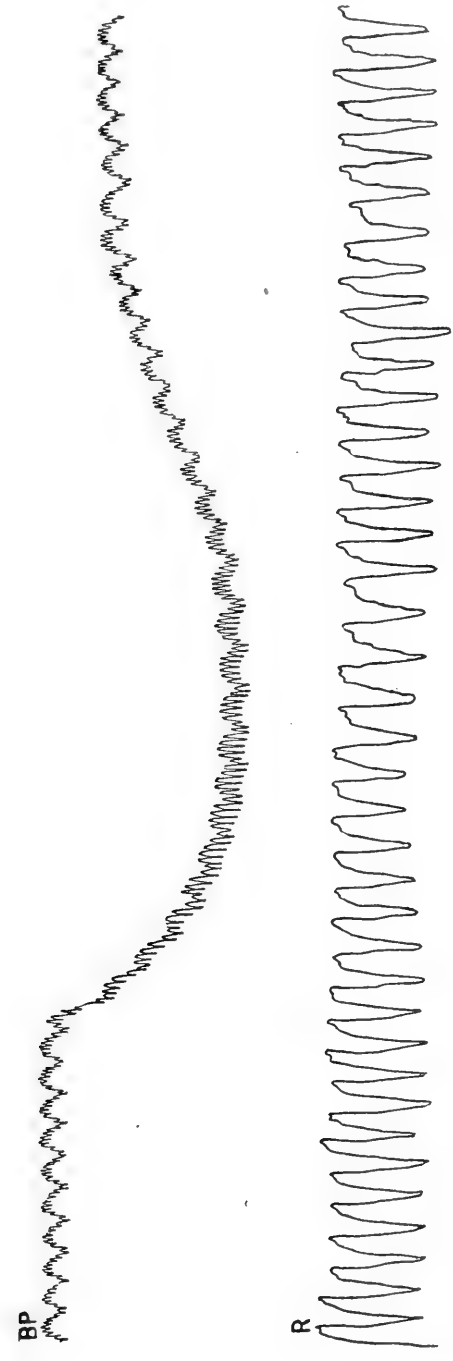

FIGURE 1.—Effect of first injection of small quantity with vagi intact. Upper tracing shows carotid pressure, lower one respiration. Time = 0.6 sec. At *A* 0.0035 gm. of alkaloid in 1 c.c. of saline was injected. Dog weighed 22 kilos.

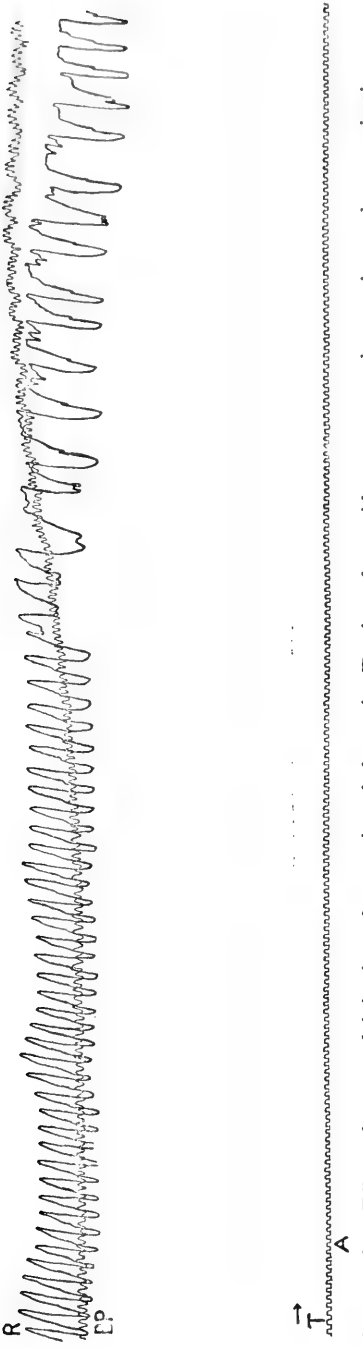

FIGURE 2. — Effect of a second injection, after section of the vagi. Tracing of carotid pressure partly superimposed on respiration curve because the pressure was lowered from the effects of the first injection. Time = 0.5 sec. At *A* 0.0175 gm. of alkaloid in 5 c.c. of saline was injected.

When sufficiently large quantities of the alkaloid had been administered to a dog, its heart showed an effect distinctly different from that produced by the previous injection of small quantities. Instead of the decrease of the heart rate with increase in the force of the beat seen in the initial injections or the increase in rate with slight decrease in force observed after further injections, there appeared in the more advanced stages of poisoning a marked fluttering of the heart. A tracing of such an effect is shown in Fig. 3. Sometimes the heart action as shown by the blood pressure curve would produce a series of very irregular beats interrupted at quite regular intervals by groups of three or four regular but very quick, shallow beats.

The effects of larger quantities of the poison on respiration were also noteworthy. Instead of the mere slowing of respiration, there appeared a considerable irregularity characterized by movements more or less convulsive. This effect in a mild degree can be seen in Fig. 2.

One of the most interesting effects of this substance was that on intestinal peristalsis. Even the smallest dose administered, 0.0035 gm. given

intravenously to a dog of 20 kilos, caused in a few minutes unmistakable intestinal rumblings. In all the dogs used defecation occurred several times, even though stools had previously been passed at the time of etherizing. In the course of an hour, after three or more injections of small quantities, fluid or semi-fluid feces were

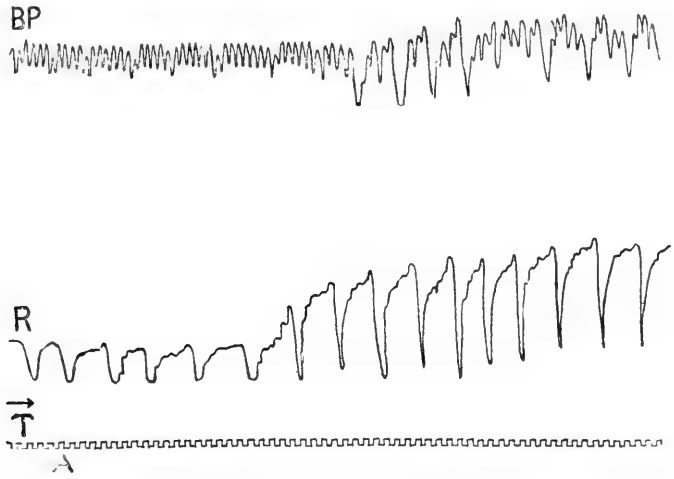

FIGURE 3. — The effect on the heart of cumulative action of the alkaloid. Upper tracing shows carotid pressure, lower one respiration. Time = 0.6 sec. At *A* 0.0175 gm. of the alkaloid in 5 c.c. of saline was injected. The vagi were intact. By means of three previous injections, 0.0315 gm. of the substance had been given.

observed in each case. Micturition also took place in many of the dogs during the experiment. This involuntary evacuation of intestines and bladder in anæsthetized dogs was plainly the result of the action of the alkaloid, because effects followed quickly after injection and in the case of defecation were proportional to the amount given. Such results confirm our observations with guinea-pigs. The influence of the substance on the vomiting mechanism of dogs was not tested, as in guinea-pigs, because no injections were made without previous anæsthesia.

Several of the dogs while still anæsthetized were killed by intravenous injection of a fatal dose. In each case the heart beat became very irregular and soon stopped. Invariably the heart failed before respiration. At the moment respiration ceased, however, the dog passed into a death struggle exhibiting tetanic spasms of the entire

body. The convulsion was always brief, and though in two cases followed by a few spasmodic respiratory movements was never, so far as we observed, succeeded by any revival of the heart beat. The heart was found in the opened thorax in complete diastole, as was seen also in post mortem examination of the guinea-pigs.

No attempt to determine the fatal dose for dogs was made. The smallest quantity which gave a fatal result was 0.105 gm. in 3 c.c. of saline injected intravenously into a dog of 10 kilos without previous injections.

Our experiments have led to the following conclusions:

1. The alkaloidal preparation from *Zygademus intermedius* slows the heart rate by action apparently on the cardio-inhibitory centre.
2. It slows respiration by an effect involving the respiratory centre.
3. It causes vaso-dilation.
4. In quantities approaching the fatal dose it hastens the heart rate and produces both irregularity of the heart beat and convulsive respiration.
5. The fatal dose given intravenously to dogs stops the heart before respiration ceases.
6. The fatal dose for guinea-pigs is between 4.6 and 5.1 mgm. per 100 gm. of animal.
7. It has a very powerful action, whether injected or fed, both as a purgative and an emetic.

The authors wish to acknowledge with thanks the assistance of Mr. H. A. Swaffield, who aided in some of the experiments.

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SEASONAL VARIATION IN THE BACTERIAL CONTENT OF OYSTERS.

BY FREDERIC P. GORHAM.

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SEASONAL VARIATION IN THE BACTERIAL CONTENT OF OYSTERS†

FREDERIC P. GORHAM,

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In the course of the investigation of the sanitary condition of the oyster layings in Narragansett Bay and its tributaries by the Rhode Island Shellfish Commission,* it became apparent that analyses of oysters from a certain bed made in the summer did not agree with analyses of oysters from the same bed made in the winter. With the advent of cold weather there seemed to be so great an improvement in the sanitary quality of the oysters that oysters taken from beds in close proximity to the outfalls of large sewers showed in the colder months entire absence of any evidence of contamination, if judged solely by the results of analyses.

The question of the cause of this improvement during the winter at once arose. Did it mean that the bacterial content of the salt water varied with the season to such an extent as to cause this difference, or did it mean that the bacterial content of the oysters themselves varied at different seasons?

An investigation was immediately begun to answer these questions, if possible. The work of the summer of 1910 showed that all of the oysters on beds in the Providence and Warren Rivers and in the upper parts of Narragansett Bay were so badly polluted by sewage that they would be condemned as unfit for food even when judged by the most lenient standards. The analyses of oysters from these beds showed the presence of the colon bacillus in the shell water of every oyster in amounts as small as one one-hundredth of a cubic centimeter and even less. Analyses of the water, both chemical and bacteriological, showed it to be heavily charged with sewage.

In December, 1910, it appeared that oysters from these polluted areas gave on analysis very different results. Indeed it was impossible to find a single laying in any of these localities which would not pass inspection if judged by the analysis alone. The condition of the water however seemed to be unchanged.

In December, 1910, two of these Providence River beds, numbers 8 and 44 on the 1910 map of the Rhode Island Shellfish Commission were selected for the purpose of studying the results of analyses made during the succeeding five months, or until the waters warmed up and assumed their normal summer condition. Similar studies were made of two other beds in the Warren River, numbers 204 and 205, for the purpose of camparison with conditions in the Providence River because the City of Providence began during this period the routine disinfection of its sewage by means of bleaching powder. But as the sewage from the City of Providence was not the only source of pollution in the Providence River, later examinations showed that this disinfection of Providence sewage had very little effect on the total colon content of the waters of the river. Bac-

† Read before the Laboratory Section of the American Public Health Association, Havana, Cuba, December, 1911.

* See Preliminary Report upon the Sanitary Condition of Rhode Island Oyster Beds, by F. P. Gorham, in the Annual Report of the Rhode Island Commissioners of Shell Fisheries made at the January Session of the Legislature, 1911.

terial analyses of the water over these beds were made at the same time that the oysters were examined, and records were made of the temperature and density of the water, conditions of the tides, winds, etc.

Bed number 8 is about one-third of a mile from the main sewer outlet of the City of Providence which discharges an average of 21,000,000 gallons of disinfected sewage per day into a river already heavily polluted. Bed 204, in the Warren River, is nearly opposite the town of Warren which discharges its untreated sewage into the river by several small sewers. Bed number 44, in the Providence River, and bed number 205, in the Warren River, are some distance below any direct sewage contamination, but the results of the analyses made in the summer showed these to be so badly polluted that they would be condemned under the standards adopted by the State of Rhode Island and the United States Pure Food Board.

All analyses were made according to the Standard Methods adopted by the Laboratory Section of the American Public Health Association. The average total count gives the total number of bacteria present in the shell water of the five oysters examined in each lot. The presence of B. coli in the five oysters examined was tested by the bile tube and subsequent isolation and identification of the organism.

SEASONAL VARIATION IN THE BACTERIAL CONTENT OF OYSTERS.

Date.	Average Total Bacterial Count of Shell Water of Five Oysters.	Proportion of Five Oysters showing B. coli in 1cc.	0.1cc.	0.01cc.	Score.	B. coli present in water in	Temperature of water, C.
	Bed No. 8. Providence River.						
Dec. 20, 1910.....	1000	3	1	0	4	0.01cc.	—1°
Jan. 14, 1911.....	750	5	3	1	41		
Jan. 25	80	4	3	0	23	0.01cc.	1°
Jan. 27	23	5	3	0	32		
Feb. 10	130	2	2	0	4	1cc.	0.1°
Feb. 28	140	0	0	0	0	0.0001cc.	1°
Mar. 11	200	5	4	0	41	0.01cc.	1.75°
April 14	275	5	2	0	23	0.01cc.	8.5°
April 28	700	5	5	4	410	0.0001cc.	12.5°
May 12	1700	5	5	5	500	0.0001cc.	15°
	Bed No. 44. Providence River.						
Jan. 7, 1911.....	425	5	5	1	140		0.25°
Feb. 10	250	4	0	0	4		0°
Feb. 28	240	5	1	0	14		0.5°
March 11	100	5	2	0	23		2°
April 14	210	2	0	0	2		8.5°
April 28	1000	5	5	4	410		11.75°
May 12	1100	5	5	4	410		14.75°
	Bed No. 204. Warren River.						
Jan. 25, 1911.....	600	5	4	1	50		0°
Feb. 10	140	0	0	0	0	1cc.	0°
Feb. 28	400	0	0	0	0	0.01cc.	0.75°
March 4	750	3†	3†	0†	†		0.75°
March 11	60	1	0	0	1	0.01cc.	3°
March 14	3400	0	0	0	0	0.01cc.	8.75°
April 28	1050	5	5	4	410	0.01cc.	13°
	Bed No. 205. Warren River.						
Dec. 22, 1910.....	250	3	0	0	3		—1°
Feb. 10, 1911.....	325	0	0	0	0	1cc.	0°
Feb. 28	450	4	2	0	14	0.01cc.	1°
March 4	600	2	2	1	5		0.75°
March 11	85	2	1	0	3	0.01cc.	2°
April 14	325	1	1	0	2	0.01cc.	8.25°
April 28	4000	5	5	5	500	0.01cc.	11.5°

† Only three oysters used.

Five oysters were examined in each case and the accompanying table shows the number in which B. coli was found in one, one-tenth and one one-hundredth cubic centimeter amounts of the shell water. The score of the several lots of oysters is the numerical method of indicating the results of the colon test as adopted by the Committee on Standard Methods. A perfect score, that is no colon bacilli found, would be zero, while if every oyster of the five showed colon bacilli present in one one-hundredth of a cubic centimeter of the shell water the score would be 500. The standard adopted by the United States Pure Food Board is that all oysters showing a score of 32 or above be condemned. The temperature of the water ranged from —1 degree C. to 15 degrees C., from the low temperatures of the winter to the higher temperatures of the spring.

The conclusions to be drawn from these observations seem to be somewhat as follows: The number of B. coli in the water of these rivers during the winter apparently does not decrease and this organism is present practically all the time with such variations as we might expect with tidal conditions. More extended series of observations might show that the temperature had some affect on the colon content but this is not apparent from these investigations.

The most interesting and important results are seen in the B. coli content of the shell water of the oyster. During the cold weather the number present varies somewhat as might be expected in the examination of different lots of the same oysters, but is never very high, and in most cases these oysters would pass the standard required by the government and state authorities. But just as soon as the water begins to warm up the number gradually increases, until with the warming of the water to 12 or 15 degrees the number reaches the maximum, and practically every oyster shows the presence of B. coli in one one-hundredth of a cubic centimeter. This is the condition which holds throughout the summer.

We must conclude then that the reduction of the number of B. coli in the oyster in cold weather is not due to any decrease in the number present in the seawater but is due to some peculiar condition in the oyster itself.

The only reasonable explanation of such a condition to my mind is that with the advance of the cold weather, the oyster assumes a condition of rest or hibernation, during which time the ciliary movement ceases, the current of water over the gills stops, and the process of feeding is suspended. No organisms are taken in from the water outside, and those inside are gradually eliminated so that the total number of organisms is reduced very considerably and the oyster becomes practically free from B. coli.

These observations enable us to give a reasonable scientific explanation for the common belief that oysters are not fit to eat in the summer time, but improve greatly in the winter. Perhaps it would be well, as the result of these observations, to limit the oyster season even more than at present, particularly in the case of oysters from polluted areas, by excluding the months of September and October, March and April, from the open season.

It is a fact well known to all oyster growers that there are two periods in the year when oysters are fat and two when they are lean. Oysters all fatten up in the late spring preparatory to the discharge of their sexual products in July and August. After these products are discharged there is a period of leanness

which continues until the advent of the colder weather, about November and December, when the oysters fatten up again and are supposed to be in the very best condition for marketing. It is at this time that they apparently store up a reserve of material to carry them through the period of hibernation during the rest of the winter.

Of course, these conditions would apply only to oysters in our cold northern waters. There is some evidence to show however that a similar period of hibernation occurs in oysters growing farther south, but further observations are necessary to confirm this.

From these facts we must conclude that in warm weather the results of bacterial analysis tally very well with the actual conditions as determined by the sanitary survey and therefore analyses may be used to determine whether or not certain oysters may be sold for human consumption. But during the cold weather oysters judged by analyses alone would be pronounced good although they came from within a short distance of a larger sewer outfall.

We probably do not want to eat oysters which come from the immediate neighborhood of sewer outlets, even though they appear on analysis to be free from colon bacilli. Therefore, in order to exclude these oysters from the market in the winter time, the only reasonable method would be to set definite limits from sewer outlets within which it shall be unlawful to take oysters or other shellfish for use as food.

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THE SANITARY REGULATION OF THE OYSTER INDUSTRY.

BY FREDERIC P. GORHAM.

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THE SANITARY REGULATION OF THE OYSTER INDUSTRY.

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There are two distinct questions involved in any attempt to regulate the sanitary quality of shellfish. First, the question of protection of the public against shellfish which are actually dangerous, that is, those which may contain disease germs and therefore may be the cause of the spread of disease; and second, the question of the protection of the public against shellfish which are merely dirty, which, in the language of the United States Pure Food ruling, consist "in whole or in part of a filthy, decomposed or putrid animal or vegetable substance," which is interpreted as meaning shellfish taken from water which contains sewage material even though this material has been so diluted or disinfected that it carries no disease-producing organisms.

I believe that we should separate these two ideas in our minds very clearly. For I am sure that we are all of the same mind in regard to the first question, but in regard to the second, we may differ in our interpretation of what is meant by "dirty." And when it comes to the enactment of laws which shall properly regulate these matters we should certainly consider the distinction between *dangerous* and *dirty*. For what is dangerous should certainly be prohibited, but where between the different degrees of dirtiness shall we draw the line showing which should be used as food and which should not?

In regard to the first of these questions, the protection of the public health, we have some accurate scientific data on which to base our opinions. Let me briefly review them.

Many shellfish, oysters in particular, are often eaten raw. These shellfish are sometimes taken from sewage-polluted waters and contain therefore the organisms which are present in sewage. These organisms may in certain cases be pathogenic. As long ago as 1880 it was suspected that shellfish might occasionally be the cause of epidemics of typhoid fever. Observations have been continued since then and now we have on record several well-authenticated cases where typhoid fever epidemics have been traced to the eating of raw polluted shellfish.

But we must consider all the facts in these cases and be perfectly fair in our conclusions. We must not condemn altogether the eating of raw shellfish, thus denying ourselves the enjoyment of a most delectable delicacy, and at the same time doing damage to a very large industry, until all the evidence is in. For as far as our knowledge goes at present no epidemics thus far studied have been due to eating shellfish *grown* in polluted waters, but in every case to eating shellfish removed from their growing beds and *"floated," "fattened"* or *"freshened"* in polluted waters. The reason for this is apparent at once. Beds on which oysters are grown are rarely situated directly at the mouths of sewers. While

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the presence of a little sewage in the water may hasten the growth of oysters, any considerable amount of it exhausts the oxygen, deposits a smothering mud upon the bottom, and makes it impossible for oysters to live. The typhoid organism is not an hardy one. It probably lives but a short time under the unfavorable conditions found in sea-water. Those that do survive are so diluted in the enormous amount of water ebbing and flowing with the tides, that few if any of them reach the oyster beds. And if a few should reach the oysters I have an idea that they would be digested and destroyed by the feeding apparatus of the oyster himself long before such oysters reach the market.

We have evidence which bears on this point in the City of Providence. The "floating" of oysters is not practiced in Rhode Island, and all oysters come directly from the growing beds. Until last winter there was nothing to prevent the use of oysters from beds in the upper part of Narragansett Bay and from the Providence River, which were badly polluted with sewage. Yet in 1910 Dr. Chapin wrote in regard to Providence: "raw oysters are very popular; they are consumed in restaurants in large numbers, and form a course in a large proportion of banquets and dinners. Many oysters are grown in the upper part of the bay in water grossly polluted with sewage and in the water and in the oysters colon bacilli are found. Yet Providence has a typhoid death-rate less than half that of the average American city. Oysters are not eaten to any extent in August when typhoid fever begins to increase and they are largely consumed in winter and spring when there is little of the disease. Very few oysters are eaten by laboring people, but at present laboring people furnish fully their share of typhoid fever."

Taking these facts altogether, then, it seems to me that there is no evidence whatever that oysters taken directly from their growing beds, even though these beds may be polluted by sewage to a certain extent, have ever caused disease.

But we cannot say the same of "floated" oysters. It is to them that the finger of suspicion points. For here we have very different conditions. Here we find instances where oysters have been placed directly at the mouths of typhoid-laden sewers or in typhoid-laden creeks for hours just previous to sale. No better scheme for the direct infection of such shellfish could be found unless the germs were inserted directly between the shells.

As to whether or not the practice of floating in itself is a proper one is a subject which has been hotly discussed for some time. But in regard to floating in polluted water there can certainly be no difference of opinion.

The arguments usually advanced in favor of floating are that it removes the grit and mud and slime from the interior of the oysters and improves their flavor and keeping qualities. Those advocating the practice usually fail to add also that it bloats and swells the meats by the absorption of a large amount of water which can then be sold at the price of oysters. It has been said that the profit from this increase in bulk is often sufficient to pay all expenses of opening the oysters. From this standpoint then floating constitutes fraud and adulteration under the Pure Food and Drugs Act. Those who are unbiased in their opinion all agree that the only real advantage gained by floating is the cleansing from mud and slime, and this can be accomplished just as well by holding the oysters in clean salt water for a while. The fact that it is practically impossible

to find any stream of fresh water which is free from all pollution should deter any conscientious oyster grower from carrying on this practice. That floating is indeed dangerous and accomplishes nothing in the improvement of the stock is recognized by the Pure Food Board because they are at present considering the withdrawal of their sanction of the practice in any form and prohibiting it altogether.

I think you will all agree with me then in answering the first question, when I say that as far as the public health is concerned, for the present at least, all that is necessary is to prohibit this practice of floating oysters. Until further evidence is produced oysters taken directly from the beds on which they are cultivated may be considered safe in practically all cases.

Investigations have not been carried far enough as yet to say whether the same applies to clams, quahogs, and mussels or to the natural set of oysters which are occasionally found in small quantities in heavily and directly polluted waters.

This brings us to a consideration of the second question. How much sewage pollution, not in itself dangerous, but merely dirty, shall we permit in our oysters or other shellfish before we prohibit their sale? This opens up the whole matter of standards of purity. Think of the years of discussion on this subject in our studies of water supplies. Shall colon bacilli in 1 cubic centimeter, 10 cubic centimeters, or 100 cubic centiemters condemn water? It was only when the sanitary survey came to our rescue that we were able to answer this question satisfactorily. We are at present trying to work out standards in our milk analyses. Shall it be 500,000 bacteria per cubic centimeter, 100,000 per cubic centimeter or 10,000 per cubic centimeter? The conditions which enter into the shellfish question are just as complicated as in water and in milk. Before satisfactory laws can be passed regulating the industry much more light must be thrown on the subject than we have at present.

At first the Pure Food and Drug Board adopted the practice of condemning a lot of oysters if three out of five examined showed colon bacilli in one-tenth of a cubic centimeter of the shell liquor. We in Rhode Island followed their lead, and under the law passed at the January session, 1910, we passed or condemned oyster beds in Narragansett Bay by this standard. This gave us results which coincided with the conditions shown by the sanitary survey.

The examination of beds was made in the summer so that the certificates could be issued at the opening of the oyster season in the fall. But on examining some of these beds again later in the winter it was found that if this same standard was used every bed in Narragansett Bay and Providence River would easily pass inspection. Indeed beds in immediate proximity to sewer outlets showed the absence of colon bacilli in all five oysters examined even in cubic centimeter amounts of the shell liquor, a far better result than that given by oysters from far down the Bay, beyond all sources of immediate pollution during the summer.

Obviously the same standard could not be used in winter as in summer, and the question immediately arose ought we to prohibit the use of oysters when they come from near a sewer outlet when analyses show them to be as free from the colon bacillus as many of our best drinking waters? Simply because they

are in polluted water should they be condemned though analysis shows them to be better than what we consider the best oysters in the summer? Under such conditions our sanitary survey fails us. We have no standard by which to judge the purity of such shellfish. We are at a loss as how to proceed. Must we have a separate standard for summer and winter?

Of course in a measure we have this now, for no very considerable quantity of oysters is marketed at present in the warmer weather. The old idea as to the oysters being fit to eat only in the R months seems in a way to be borne out by this observation of seasonal variation in the bacterial content.

Almost the same question has arisen in regard to the use of oysters grown in waters into which disinfected sewage is discharged. Disinfected sewage is not dangerous but is it dirty? Ought such oysters to be used for human food?

I believe that the only satisfactory way to handle this matter is to adopt a certain distance from discharging sewers within which it shall be unlawful to take shellfish for food.

These are some of the problems which are troubling us at present. But while we are working at them a very considerable amount of good has already been accomplished in the improvement of the industry along sanitary lines.

In the first place we have seen placed on the statute books of the State of Rhode Island laws which regulate the industry and protect it. Pollution of the waters of the State in which shellfish are grown is prohibited under severe penalty. The State Shellfish Commission must make examinations of and issue sanitary certificates for any bed on which oysters are grown for consumption as food before such oysters can be taken from the beds and sold.

The Commissioners are required to inspect all opening and packing houses and the methods in use therein and must issue certificates as to their sanitary condition before shellfish can be sold therefrom. They are also given the power to make any regulations concerning the construction and operation of such oyster houses as they may see fit in order to place them in proper sanitary condition.

As a result of such legislation we have been able to make a series of analyses of oysters from all the beds in Narragansett Bay and its tributaries at different seasons; also chemical and bacteriological analyses of the water at the surface, at the bottom, and of the mud at the bottom of a considerable portion of the Bay; a study of the tides, currents, temperatures, and densities of these waters; and a complete sanitary survey showing every sewer outlet, every source of pollution entering the waters of the Bay.

In addition to this, the oystermen have been awakened to the seriousness of the matter. Because of the activities of the United States authorities and those of the State, they have been forced to examine into their methods of conducting business and to make every effort to improve their methods along sanitary lines. They have co-operated nobly in the matter and have helped to bring about a complete revolution in their business, which has in many cases involved changes from methods which were as old as the industry itself.

But best of all has been the effect this campaign for clean shellfish has had upon the entire removal of some of the sources of pollution from the tidewaters of the State. Ultimately, of course, this is the goal which we seek, the return of our bays and streams to their earlier condition of purity. It may be a long

time indeed before this can be accomplished, but we are glad to record that a beginning has been made.

The Commissioners of Shellfish summoned before them the parties interested in sewage disposal in the several cities and towns which were using Narragansett Bay as a common sewer. At this hearing they were required to tell what efforts they were making to comply with the law which prohibited the pollution of the tidewaters. They were informed that they must take steps immediately to remedy the conditions. As a result already some of them have moved in the matter. The City of Providence is already conducting an investigation of its methods of sewage disposal and has begun the disinfection of its effluent. Other towns are doing something. Much remains to be done. Rhode Island even has a bone to pick with Massachusetts, for there seems to be some interstate traffic in sewage which we hope in time to see remedied.

In conclusion, then, were I asked along what lines at present can we best effect improvement of the shellfish industry I would say:

1. Prohibition of the practice of floating oysters.

2. Prohibition of the use for food of shellfish taken from waters directly polluted with sewage, that is, within a certain distance of discharging sewers.

3. Careful sanitary regulation and supervision of the methods of handling and packing shellfish.

4. Efforts to secure the removal of sewage pollution from the tidewaters where shellfish are grown, or at least the disinfection of all sewage effluents which enter tidewater.

Discussion.

Prof. WHIPPLE. The subject of oyster sanitation is a most interesting one and it may interest you to know that a committee has been studying the subject, in the American Public Health Association, for the past year or two. A preliminary report was made at Havana a few weeks ago and this will be published in due time. (This Journal, January, 1912.—Ed.)

What Prof. Gorham has said in regard to the winter conditions of oysters seems to be true not only of oysters in the Providence River but also of oysters found in this country farther south. During the winter season the bacteriological analyses of all oysters seem to be better than during the summer season. It looks as if the oyster was a more intelligent being than we have given him credit for, because, knowing that we appreciate his society only in the winter, he apparently takes particular care to protect himself against sewage and other filth during this season in order that he may not do us damage or injure his own reputation. I have sometimes wondered why, if that is the case, it is necessary to go to the expense of disinfecting sewage in the way that Prof. Phelps has outlined. If the oyster protects himself why is it necessary to spend money to further protect him? In reply, there is this to be said,—that the oyster is no Pharisee. Neither during the winter, nor at any time, does he attempt to keep clean the outside of his shell and there is a real danger at all times that polluted mud that has not been thoroughly washed off may get into the half shell in which the oyster is served. Many oysters have a small cavity near the hinge which is difficult to clean and which is often filled with mud when served. If this mud gets into the bowl, as it may easily do, there is a danger that it may also get into one's mouth.

Then of course there is the further question of general cleanliness. No one desires to eat food that is unclean. Our committee is trying hard to devise some practical standard which can be applied to bring this about. Prof. Gorham has marked out some of the ways in which this can be done. One other method has been suggested and the committee is working along this idea at the present time and will doubtless report upon it later, viz., a method of "scoring" the oyster beds and the oyster houses, very much as milk farms are now scored. By this method some appropriate authority would give a good score to the oysterman who raises oysters on clean grounds and who harvests and markets his oysters in a clean way, and a low score to the man who is careless in handling his goods and grows his oysters very near a sewer.

I think the whole question of the sanitation of the oyster is one that is rapidly clearing itself up. To some it may seem to be growing more and more complicated, but now that the oystermen are co-operating with the sanitarians we are learning many facts hitherto unknown, and I think that in a short time we shall be able to establish certain standards that will satisfy the sanitarians and that can be lived up to by the oyster growers.

Mr. Williams. When I accepted the invitation of my local board of health to attend this meeting I knew that I would be greatly rewarded by listening to the papers that were to be read here, and I was especially interested, of course, in the paper that Professor Phelps was to read. I have had a number of very interesting meetings with Professor Phelps and Professor Sedgwick with regard to the disinfection of sewage.

New Bedford, as you may know, has been doing its best to become a large city and in doing so it has had to meet a few of the ills that come with municipal growth and development, one of which is the very serious pollution of the river upon which it is located. This body of water which we call the Acushnet river, is really only a tidal arm of Buzzard's Bay. But we have succeeded in polluting it to such an extent that a few years ago the State Board of Health prohibited the further taking of shell fish from its waters. These shell fish, of course, did not include oysters. They did include the famous little neck clams, which, I understand, we have distributed to quite a considerable extent over various parts of the country.

In order to relieve ourselves from a very serious situation and to save a business which furnishes employment to a great many of our citizens the city undertook, two years ago, to provide for a further disposal of its sewage. We have now started upon the construction of an intercepting sewer which will collect all the sewage of the city and deposit it at a point in Buzzard's Bay something over half a mile from the most extreme point of land in New Bedford. Furthermore the sewage will be delivered in water about 30 feet deep at low tide where it will also come in contact with the great tidal movement of the water of Buzzard's Bay.

When we announced our intention some of our neighboring shore towns became seriously alarmed as to what the effect might be upon their shores, and so, to relieve them of any cause for alarm, we consulted with Professor Sedgwick and Professor Phelps and thereby learned of their great interest in the disinfection of sewage. Disinfection of sewage, of course, has been generally

known about in one way or another for a good many years, but up to this time none of us knew of any practicable way of disinfecting raw sewage.

You probably understand that our situation was such that we could not very well introduce sand filters, because we didn't have the sand areas suitable for the purpose, and contact filters were also a very expensive method of treatment for a city situated as we are. And so we have decided that the proposition of Professors Sedgwick and Phelps was worthy of our consideration. We have gone into the subject to a considerable extent; and we have decided that we will provide at a point near the outlet of our intercepting sewer for disinfecting the crude sewage by the addition of chloride of lime, or ordinary bleaching powder if later it is definitely decided to adopt this method of treatment. While our plant is not yet built we are preparing plans with that object in view.

Gentlemen, that, in a few words, places New Bedford's position before you. I thank you very much.

Prof. PHELPS. There have been, Mr. Chairman, two things said here about the oyster that I want to say do not apply to the clam. First, Professor Gorham says the oyster loves clean waters and will not grow near sewers. That certainly does not apply to the soft shell clam. Then Professor Whipple tells us the oyster shuts up and fasts in winter. That, according to my observation, the soft shell clam does not do. And so, however we may remove suspicion from the oyster, I think this clam situation is going to still be with us. It is certainly true that the clam is inherently a filthy beast. He seems to prefer polluted waters and grows fatter and faster in them. The oyster may be wise and we may possibly trust to his discretion, but the clam, proverbially happy, needs the guardianship of the State to keep him from falling into evil ways.

Mr. STEPHEN DEM. GAGE. I have been much interested in Professor Gorham's paper and am particularly pleased that he has drawn the distinction so sharply between polluted or dangerous oysters and oysters that are merely dirty. The shellfish situation in Massachusetts is quite different from that in Rhode Island and in the States further south. In these States the oyster is the only mollusk of any economic importance. The hard and soft clams apparently are unable to grow south of New Jersey and the few beds of these clams which existed at one time along the shores of Long Island Sound and in Narragansett Bay have been practically depleted. Even the clams for the famous Rhode Island clambakes are now brought in from Massachusetts sources. On the other hand, north of Massachusetts, the oyster industry is so small as to be negligible, but the gathering of hard and soft clams and of scallops is an important industry. Massachusetts lies intermediate between these zones and along different portions of her coast all of these various types of shellfish are gathered in greater or less quantities. In 1909, the Massachusetts Fish and Game Commission published a special report on the Mollusk Fisheries in the State and some statistics from that report showing the relative amount and value of these different shellfish gathered annually may be of interest. The number of men employed in the oyster industry was 159, in the scallop industry 645, in quahaug or hard clam industry 745, and in soft clam industry was 1,360. The catch of oysters is about 115,000 bushels per year valued at $148,000, of scallops 86,000 bushels valued at $164,000 of hard clams 144,000 bushels valued at $195,000,

and of soft clams about 154,000 bushels valued at about $150,000. It will be observed that the value of both the hard and soft clam industries is greater than that of the oyster industry and that the number of bushels of hard and soft clams produced is each much greater than the number of bushels of oysters produced. Furthermore, there is a still greater difference in the food value of these various shellfish owing to differences in thickness of shell and differences in actual food value of their bodies. Computed on this basis the oyster industry produces only about 8 per cent., while the hard clam industry produces about 17 per cent. and the soft clam industry produces over 37 per cent. of the food value of the entire shellfish industry.

For more than ten years the State Board of Health has been studying the question of the pollution of these various kinds of shellfish, and in certain instances, areas which are dangerously polluted have been closed. The question of the pollution of clam flats is the most serious problem, however, as not only is the soft clam industry more important from an economic viewpoint, but the flats from which soft clams are found are widely distributed along the shores of the State and as has already been stated, the soft clam will grow and thrive in shallow water in close proximity to public sewers, while the other mollusks mentioned are natural inhabitants of deeper waters where pollution is reduced to a greater or less extent by dilution.

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SUPERIOR SANITARY QUALITY OF RHODE ISLAND OYSTERS.

BY FREDERIC P. GORHAM.

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SUPERIOR SANITARY QUALITY OF RHODE ISLAND OYSTERS

By FREDERIC P. GORHAM, BROWN UNIVERSITY
Sanitary Expert, Rhode Island Shellfish Commission

It is a very serious matter when any suspicion is cast on the fair name of the Rhode Island oyster. It is no wonder then that all lovers of this most delicious shellfish were up in arms at once when Dr. Wiley and his associates on the Board of Food and Drug Inspection descended upon these Plantations and informed us that certain of our Rhode Island oysters must not be shipped out of the State because they were polluted with sewage.

We have known for some time that a constantly increasing amount of sewage was being poured into Narragansett Bay, but we had failed to realize that this sewage might injuriously affect the shellfish. Indeed, as long ago as 1904 in a paper in this Journal by Dr. C. A. Fuller* the conditions existing at that time in reference to the pollution of the oyster beds was pointed out, and the suggestion made that something should be done to prohibit the sale of polluted shellfish and to prevent the further increase of the pollution. We did not realize that the time had come for action until the Washington authorities brought the matter most forcibly to our attention by prohibiting the sale of certain Rhode Island oysters in interstate trade and haling some of the oyster growers into court.

By a ruling of the United States Board of Food and Drug Inspection oysters which are grown in sewage-polluted waters, or which have been fattened by the process of "floating" or "drinking" in sewage-polluted waters, are prohibited from interstate trade because they contain an added "poisonous or other added deleterious ingredient which may render such article injurious to health" or because they consist "in whole or in part of a filthy, decomposed or putrid animal or vegetable substance."

Whether such oysters would be the cause of disease depends entirely on whether typhoid germs are present in the sewage. In

*Providence Medical Journal, 5, 1904, 39

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every community of any size there is a possibility that typhoid germs may be present in the sewage. It is true these germs die out rather rapidly in the sewage, and particularly when it is discharged into salt water. There is no evidence whatever to show that oysters taken directly from beds on which they are grown have ever been so polluted as to be the cause of disease; but there is very good evidence to show that oysters which have been removed from these beds for purposes of "floating," and placed at the mouths of sewers or in waters where the pollution is immediate and direct, have caused serious epidemics of typhoid. As the practice of "floating" is not carried on in Rhode Island, there is no danger from this source. And there is no evidence to show that there has ever been any disease caused by eating oysters from Rhode Island waters. Indeed, Dr. Chapin in his recent book on the "Sources and Modes of Infection" says, "raw oysters are very popular; they are consumed in restaurants in large numbers, and form a course in a large proportion of banquets and dinners. Many oysters are grown in the upper part of the bay in water grossly polluted with sewage, and in the water and in the oyster colon bacilli are found. Yet Providence has a typhoid death rate less than half that of the average American city. Oysters are not eaten to any extent in August, when typhoid fever begins to increase, and they are largely consumed in winter and spring, when there is little of the disease. Very few oysters are eaten by laboring people, but at present laboring people furnish fully their share of typhoid fever."

But whether dangerous or not, oysters grown in sewage-polluted waters are dirty and we do not want them on the market, so any reasonable steps taken by the federal authorities to limit the sale of such oysters will be welcomed by all advocates of pure and clean food.

When the United States authorities made the first move toward condemning certain shipments of oysters and ordered certain dealers to appear in court, the oyster growers of the state were immediately aroused. They at once appealed to the Rhode Island Shellfish Commission for assistance. This body is charged by the state with the care of its shellfish industries. Last year the Shellfish Commission paid into the treasury of the state $133,341 as rentals from the 20,846 acres under cultivation. The capital invested in the business was stated by the last census to be $1,031,738, with a total value of all products, shell and shucked oysters and seed, of $2,930,750. In the neighborhood of 25,000 gallons of oysters were opened in the state on every day of the oyster season. This places the oyster industry as the third or fourth on the list of large industries of the state.

It is no wonder then that the appeal of the oyster growers to the Shellfish Commission met with ready response. The importance of the industry warranted thorough investigation of any criticism of the product, and also warranted every effort to correct whatever injurious conditions might be found.

The Shellfish Commission set to work immediately. They secured an appropriation from the legislature for the work, and, acting under the existing laws, which were sufficient to cover the matter, they began a thorough investigation of the whole situation.

In order to secure accurate information as to the conditions in Narragansett Bay with reference to sewage pollution at the present time, a comprehensive plan for a complete sanitary survey was outlined and work begun. The engineer of the commission undertook to locate and map every discharge pipe of whatever sort emptying into tidewater. Twelve plans were prepared, on which the sewer outlets were indicated. These covered practically the whole shore line of the state, so that the sources of pollution are known at least. In addition to this the sewer commissioners of the largest towns concerned in polluting the bay were summoned before the Shellfish Commission at a public hearing, and under oath testified to the conditions in their several towns, the number of sewers, the ownership thereof, the number of houses connected with the several sewers, the presence or absence of sewage treatment plants and the efficiency of those plants. These hearings called the attention of the authorities and the public to the conditions, and because of this publicity it was hoped to secure the active co-operation of the towns concerned in remedying the unsatisfactory conditions.

Under the direction of the writer a careful study of the distribution of this pollution in the waters of the bay, with special reference to the oyster beds, was undertaken. A series of "stations" covering the entire upper part of the bay were located by the engineer, and at these stations observations were made as to the amount of pollution and as to other conditions which might affect shellfish. Chemical analyses of the water at the surface and at the bottom were made at these stations under different conditions of tide; bacteriological analyses of the surface and deep water were made also under different tidal conditions. Samples of the mud and shellfish at the bottom were also analyzed bacteriologically, with special attention to the presence or absence of sewage organisms. Besides the above analyses, data in regard to wind, weather, tide, salinity and temperature were secured.

Very early in the work it became apparent that very many factors enter into the question as to whether the water and shellfish at a given locality are polluted or not. Besides wind and tide, the temperature of the water appears to have a very considerable effect, so that it became necessary to extend observations throughout the year, so as to include all seasons. Obviously it would be unfair to the oyster grower to condemn a given locality from observations in the summer time, if that same locality in the winter time, when the oysters are marketed, is entirely free from contamination. Such conditions seem to hold in certain parts of the bay.

A new law was passed which required that sanitary certificates be issued by the Shellfish Commission for every oyster bed in the state, and it prohibited the sale of oysters from beds which did not hold a certificate showing that the given bed has passed inspection. For the purpose of issuing these certificates samples of oysters were taken from each of the 296 leased oyster beds of the state and subjected to bacteriological analysis. The regular methods used by the Federal authorities and recommended by the American Public Health Association Committee on Standard Methods of Shellfish Analysis were used. The standard adopted was that used by the Federal Laboratory also, and required that the colon bacillus be absent from amounts as large as one-tenth of a cubic centimeter in three out of five oysters.

In our study of these oysters from the different beds it soon became apparent that we could not draw a hard and fast line between the beds which passed and those which did not pass. We found an intermediate zone of beds which could not be definitely put into either of the above classes. In these intermediate beds we found conditions variable, different samples, taken on different days, might vary, the oysters might be bad and yet the water analysis might be good. Obviously such beds were on the border line between the polluted area and the unpolluted area, and different conditions of wind and tide determined the different conditions at any one time. For such beds as these we determined to issue conditional certificates, which stated that "the bed or beds covered by this certificate are in a questionable area and are still under investigation. This certificate is therefore temporary and a final certificate will be issued later." This meant that the use of oysters from such a bed must be determined by the conditions at the time, and if the oysterman wished to make sure that a lot of oysters from such a bed was in proper condition to be marketed he must have an examination of the oysters made at the time

they were taken. With an improvement of the conditions of sewage pollution in the bay, such beds as these ought to be the first to show changes for the better.

As a result of our examination of the oyster beds of the state, we made three classes. First, there were those which were so badly contaminated that they were refused all certificates; second, those which were questionable, and third, those which were free from pollution. Certificates were refused for 3,986 acres; conditional certificates were given for 3,188.2, leaving 12,864.8 acres which were given full certificates as to their sanitary quality.

While the Shellfish Commission did not attempt to supervise the taking of oysters from these beds, indeed exercised no police duty at all in the matter, yet we believe that to a very considerable extent the oystermen co-operated in the matter and refrained from marketing oysters from prohibited areas. Indeed we know that many of the larger dealers went to a very considerable expense to remove their oysters from polluted beds to clean water before marketing them, and also we know that in some cases oysters were marketed from non-certified beds; but on the whole the oystermen co-operated, and we believe that the oyster shipments from Rhode Island were, on the average, of better sanitary quality during the past year than ever before. At any rate the oyster growers had the information at hand for knowing the exact condition of their oysters, and it was at their own risk, and it was endangering their business reputation, if they shipped oysters from polluted areas.

In fact, we believe that these certificates had a very considerable educational as well as commercial value. The public became immediately interested in knowing whether the oysters which they were buying were from certified beds, and the oystermen were educated to the fact that unless their oysters were from certified beds the public didn't want them and the Federal Pure Food Inspectors did. So on the whole the certificate matter was a good thing all around, even though strict police supervision was not had to enforce the exact provisions of the law. We believe that in the future certified oysters will be the only oysters that can secure a market either in our own state or in interstate trade.

The new law required also that every opening house be inspected by the commissioners and that oysters must not be opened for market in a house which had not received a certificate showing that it was in proper sanitary condition. This regulation of the opening houses

was, perhaps, a more difficult matter than the certification of the beds. It required not only inspection of the houses, but education of the owners. They must be told what to do to improve the insanitary conditions. One inspection was not sufficient, the houses must be visited again and again to see that the requirements had been carried out. But the commissioners went immediately about the work.

A book of instructions was drawn up by the sanitary expert of the commission, placards were prepared showing the rules which must be complied with, and the official inspector began his visits. He visited every house in the state, talked with the owners, informed them of the conditions which were wrong, told them how to improve them, gave them the literature describing the requirements, and posted the placards on the walls of the opening houses. He promised to return shortly to see what improvements had been effected.

Of course, everything could not be accomplished in one year. A very satisfactory beginning was made, however. Many of the owners spent thousands of dollars in putting their houses into better shape. In the majority of cases an attempt was made to comply with every request of the authorities, and on their side the authorities tried to make every request a reasonable one. Of course, there was some opposition, and several visits were required to some houses to get the owner started on the improvements. Much remains to be done, but with the co-operation of federal and state authorities to make only reasonable requirements, and the oystermen to try to carry out these requirements at the earliest possible moment, the opening houses of Rhode Island should soon be above all suspicion.

But the efforts of the state authorities did not stop with the attempt to regulate the sanitary condition of the industry. They went still further, and made an attempt to prohibit the further pollution of the waters of the state, and so far as possible to abate the pollution already present. After the facts of the case had been secured by the sanitary survey and by the public hearings, the authorities of the several towns were warned by letters from the attorney general of the state that present conditions were contrary to law, and that they must take steps to remove their sewage from tide water at the earliest possible moment or prosecution would follow. Indeed an earnest attempt on the part of the Shellfish Commissioners, the law department of the state, and the oyster-growers was made to bring about an improvement in conditions.

Here again work went on slowly, but we can see some gain.

The city of Providence, the greatest offender in the matter, was the first to co-operate. Not only did they begin at once a very general overhauling of their sewage system, but they installed a disinfecting plant at the Field's Point precipitation works. Recent experiments have shown that ordinary bleaching powder, if used in proper amounts, will so effectually disinfect a sewage effluent as to render it free from all objectionable micro-organisms and therefore of such a quality that it may be discharged immediately into a stream or into salt water. Experiments carried on in co-operation between the city of Providence and the State Shellfish Commission at the Field's Point plant showed that it was possible without great expense to so treat the Providence sewage that there could be no objection to it on the ground that the effluent contained injurious bacteria.

The Providence authorities also began plans for the installation of additional sewers, which at the earliest possible moment would remove all the sewage from tide water and carry it to the disposal plant, where it could be properly treated. We could have asked no heartier co-operation than that we secured from the city of Providence. Some of the other towns also began to inquire into the problem, and appeared to be somewhat interested in the matter. Others have not yet moved at all. Indeed some of them have even tried to block further activities of the Shellfish Commission. Of course, the installation of sewage disposal plants in towns is slow work, particularly during the period when a study of the situation is going on, but we have every hope that the ball which has been set rolling will gather momentum as it goes, that the examples already set will be followed by others. There is no reason why Narragansett Bay should be turned into an open sewer because the several towns are unwilling to expend the small sum necessary for the proper disposal of their wastes. If we could but secure united action by all the parties concerned we would soon find the waters of our beautiful bay returning to their original pure condition, to the advantage of the shellfish, the summer boating, the summer residents, and the general good health of the state.

Many new facts regarding the oyster and its relation to sewage pollution have been brought out by these investigations. Many problems have arisen in the course of the work. The relation of the oysters themselves to the colon bacilli, whether these organisims multiply in the oyster or whether they tend to die out, their distribution in the oyster and the length of time required for oysters to purify

themselves when removed to clean water; all of these problems have begun to receive attention. And then the matter of standards by which to judge the purity of oysters, both in the shell and shucked, has received much attention. At first we were inclined to believe that the standards used by the government authorities were too high. When we began work during the summer months, there were many localities far removed from all sources of pollution where oysters did not come up to the standard. But when we followed up these localities during the winter, when oysters were being marketed, we found that the oysters from these same localities easily measured up to the standard required. In fact, from our year's work, we are inclined to believe that the government standard is a good one if used during the summer season, but localities must not be condemned for winter use by examinations made during the warmer weather. I believe that oysters which cannot pass the government standard in the colder months are certainly growing too near to sources of pollution to be used for food. In fact, we found in the winter many oyster beds situated very near to sewer outlets which easily passed this standard, and in the end it may be necessary to abandon the methods of judging oysters by the results of analysis, and adopt a regulation prohibiting the taking of shellfish from within a certain distance of a sewer outlet.

In conclusion then, we believe that in Rhode Island, in the short space of one year, a great deal has been accomplished in the campaign for clean oysters, simply by active co-operation on the part of all concerned. Government and state officials, oyster-growers and those concerned in sewage disposal must all work together to bring about, in the shortest possible time, in the tidal waters of our entire coast, a condition most favorable to the further development of the oyster industry.

I believe that it can be truthfully said that never before has such a superior quality of oysters been marketed as those which came from Rhode Island waters this year. No other state has so successfully and energetically attacked the problem of clean oysters, and nowhere has so much been accomplished as here.

If those efforts are continued, and if, as soon as possible, the present sources of pollution are removed, we shall see a remarkable extension of the oyster industry in the state, and the Rhode Island oyster will come to occupy a position unequalled by any oysters in the world.

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SOME BIOCHEMICAL PROBLEMS IN BACTERIOLOGY.

BY FREDERIC P. GORHAM.

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SOME BIOCHEMICAL PROBLEMS IN BACTERIOLOGY[1]

THE Society of American Bacteriologists stands, primarily, for pure as distinguished from applied bacteriology. In these days when the applications of the science are becoming so immensely important, and therefore so enticing to the investigator, there is danger that our thoughts turn not often enough to the broader aspect of the science, upon which, as a foundation, all of its applications must ultimately rest. We as a society must make it our special duty to see that these foundations are laid broad and firm, upon the very bed rock of truth itself.

We have as a society been interested for some time in the preparation of standard and uniform methods of describing bacterial species. This is of fundamental importance, leading as it does to uniformity of method and completeness and comparability of results. When we couple with this the use of the standard methods of the laboratory section of the American Public Health Association we have gone a long way toward the standardization of our work, and have begun the foundation upon which can be built the science of pure bacteriology.

But we must ever beware that we be-

[1] President's address before the Society of American Bacteriologists at the Washington meeting, December 28, 1911.

come not slaves to standardization and uniformity. It is well enough to proceed by standard methods, but we must not be tied by them. We must ever be ready to abandon the old and adopt the new, when the new marks the way of progress.

At this time I wish to bring to your attention some of the lines along which we are making little progress, I believe, because of the false security which we are taking in our standard methods.

First, suppose we consider our ordinary culture media. We have drawn up ridiculously exact procedures for mixing and boiling and filtering and titrating our broth, agar and gelatin, and when we are through we believe we have a standard, uniform product. But indeed it is not so. Not only are the results obtained in different laboratories unlike, but two lots of the same medium made at different times in the same laboratory are extremely unlike, when measured by the delicate physiological properties of organisms which respond to the slightest of chemical differences. Far better, it is true, are the results secured at the present time by the use of standard methods, than before their introduction, and I do not for a moment want to decry our standard methods, but I simply want to warn against the false security which their use may give. For however careful we may be in the process, the final result can never be uniform as long as the ingredients used are themselves variable. No medium can be standardized, that is, can be exactly duplicated at another time or place, if it contains such variable materials as beef extract, either

freshly made or commercial, peptone, gelatin, agar, blood serum, bile, etc. I am inclined to think that in order to get uniform results, particularly in our study of the delicate physiological properties upon which we depend so largely for the differentiation of bacterial species, the time has come for us to abandon altogether the use of all complex and variable animal and vegetable products, and in their places to substitute materials of definite, known, chemical composition. From my work of the past few years I am led to believe that for every organism it is possible to prepare a synthetic medium containing chemically pure salts, upon which these organisms will grow and grow well. Such a medium as this we are able to duplicate exactly anywhere and at any time.

In the past we have been inclined to think that the physiological properties of many organisms were too variable to be of much use in species determination. I am coming more and more to think that it is not so much the properties of the bacteria which are variable as the environment in which we have attempted to study them. We talk about rejuvenation of organisms to restore them to normal conditions. Our attempts at rejuvenation are attempts to make normal organisms adapt themselves in short order to an abnormal environment. Could we but supply the proper environment we should find the organisms responding in an entirely normal and uniform manner. It is the environment, our culture media, that need rejuvenating and not the organisms. This rejuvenation will come about, I believe, through the

adoption of synthetic media of absolutely known chemical composition. Then the physiological properties of organisms will come into their proper place in species differentiation, for then we can substitute the exact qualitative and quantitative tests of the chemist for the inexact determination of the present-day bacteriologist.

There is scarcely a physiological property of the bacteria that is to-day accurately measurable. Variations in the media are so great that measurements at present amount to nothing. Chemistry advanced to its present position as a science only when it became quantitative. Lord Kelvin said: that "When you can measure what you are speaking about, and express it in numbers, you know something about it; but when you can not measure it, when you can not express it in numbers, your knowledge is of a meager and unsatisfactory kind; it may be the beginning of knowledge, but you have scarcely in your thoughts advanced to the stage of science." Bacteriology must then become quantitative before the real foundations of the science are laid, before it can take its proper place among the sciences. And it can not become quantitative until we can measure its reactions in media of known chemical composition, by methods as exact and definite as any known to the chemist. We are always dealing, it is true, with living protoplasm, but if we place it under definitely determined environment, recent experiments lead us to believe that living substance always responds in a perfectly

definite way both qualitatively and quantitatively.

The very complexity of the society's card for the description of bacterial species is to me its own condemnation. It is our admission of ignorance. Are not the real diagnostic characters of a species lost in a maze of unessential characters? Aside from the morphological data, which I believe is all-essential, the cultural and biochemical data could, I am sure, be simplified to a very considerable extent as far as species differentiation is concerned. The forms of cultures and colonies are but functions of the morphology and methods of subdivision of the organisms themselves, as has been so well shown by the recent work of Graham-Smith.[2] We might then eliminate from the card many of the complex descriptions of cultures and colonies on agar and gelatin, retaining perhaps the agar streak and the gelatin stab, and substitute therefore information in regard to their determining factors. And then among the biochemical data if we could eliminate all but a few deep-seated physiological characters which are accurately measurable, and which can be easily determined by means of accurate chemical tests on synthetic media of known composition, we would have a simple, accurate, all-sufficient description of a bacterial species.

One of the weakest parts of bacteriology to-day is its taxonomy. Our methods of classification of bacteria are practically

[2] Graham-Smith, G. S., "The Division and Post-fission Movements of Bacilli when Grown on Solid Media," *Parasitology*, 3, 1910, 17.

the same to-day that they were in the earliest days of the science. Migula, it is true, systematized the scheme of classification to a certain extent, Chester contributed to its more accurate terminology, and the Winslows gave it a new impetus by the introduction of the methods of biometry. But when we think of the thousands of described species among which but two or three genera are recognized, it must be apparent at once that some of our generic names are seriously overworked. In other biological sciences the classification into species, genera, families, orders, classes, etc., is not only of great convenience, but it also expresses for us something of the relationship of the different groups, something of their probable ancestry and line of evolution. I see no reason why the bacteria should not be classified in the same way. The bacteria are not exceptions to the general biological laws. Variation, selection, heredity, are the factors of evolution here as elsewhere. It is true among the unicellular forms we are free from many of the complications which enter into our discussions of the origin of species among multicellular animals and plants. Sexual reproduction is absent, there is no differentiation into germplasm and somatoplasm to prevent the acquisition of new characters, the environment presses very closely upon these unicellular forms and they respond more directly to it. Generation follows generation with startling rapidity, elimination of the unfit proceeds rapidly, the struggle for existence is more severe because of the enormous numbers concerned. In the bacteria we ought

almost to be able to see the actual process of evolution since we can place under observation untold numbers of generations as compared with the comparatively few generations of multicellular forms which we are able to observe. Eons of multicellular time are literally compressed into a few unicellular days.

Because of this lack of the regulating influence of reproduction and the greater influence of the environment and the rapidity of reproduction, we might expect to find among unicellular organisms a series of intergrading forms without divisions into groups that resemble the species of higher forms. But this is not so. Among the protozoa differentiation has followed definite lines, and classes, orders, families, genera and species are well marked. The species of protozoa are, for the most part, based on morphological differences, it is true, but among the bacteria a morphological basis of classification fails us. The only morphological differences are the three main divisions as to shape, the round, the rod and the spiral, with slight modifications as to size, arrangement of flagella, formation of spores, chemical composition, etc. But in spite of this lack of morphological basis for classification we find as distinct groups as among the protozoa. As the Winslows say:[3]

> Typhoid germs descend from typhoid germs, tubercle bacilli from tubercle bacilli. The same yellow coccus falls on gelatin plates exposed to the air all over world. The same spore-forming aerobes occur in every soil, the same colon bacilli

[3] Winslow and Winslow, "Systematic Relationships of the Coccaceæ," p. 1, John Wiley and Sons, 1908.

crowd the intestines of animals and man in every clime. These fundamental types can not be transformed into each other.

And yet to a considerable extent these fundamental types are based not on morphology, but upon physiological differences.

It is among the bacteria that for the first time among living forms we find physiological differences made a basis for classification. Are physiological properties valid criteria for the separation of species? We are accustomed to think of morphological characters as fairly stable, rather difficult to modify, but of physiological characters as easily modified and directly dependent upon the environment. But have we any reason to assume that physiological characters are not deep seated also, are not stable, fully as much as morphological characters? Are we not in reality dealing with characters of the same sort as morphological characters except that we can not see their ultimate basis in structure? I am not sure but what physiological characters are even of greater importance than those of form and external appearance, especially in such simple and undifferentiated forms as the bacteria, since they testify to deep modifications in the chemistry and vital properties of the protoplasm itself. It is upon such chemical properties as these that these simple organisms depend for their very existence, and not upon a modification of external appearance.

But before we can proceed far in the use of physiological differences for species determination we must be able accurately

to determine these delicate characters. Hitherto this has been impossible because of the variable and uncertain culture media in which we have attempted to study them. With the adoption of media of definite chemical composition for making our determinations and measurements, the physiological characters of the bacteria will assume the importance of the morphological characters of higher organisms. Then and then only shall we able to arrive at a natural classification of bacterial species which shall express for us their true relationship.

With the adoption of such accurate chemical tests of the physiological characters of the bacteria much of the present apparent variation will pass away and we shall find the physiological characters of the different groups as stable as any of their morphological characters. And what little variation remains—and we shall always find some variation as long as we deal with living organisms—we can handle easily by the method of biometry. For, as you know, the methods of statistical variation can apply only in characters which are measurable.

This matter of better methods of species identification and a new taxonomy will be one of the first outcomes of the adoption of simple chemical culture media.

Another important result will be the increased ease with which certain biochemical problems in bacteriology can be attacked. Think for a moment of our endeavors to find a suitable method of isolating the colon bacillus. Litmus-lactose-agar, endo, esculin, neutral red, mala-

chite green, phenol, bile and bile salts and their various combinations are but expressions of our total ignorance of the chemistry involved. The problem should be attacked in an entirely different way. First, we should determine the simplest synthetic medium upon which the colon bacillus will grow rapidly and well. Then by adding to it the chemical body which is the inhibiting agent in the phenol or in the bile we ought to have the ideal medium for colon isolation. A beginning along this line has been made by Dolt[4] in his ammonium lactate, glycerin and malic acid media. But further work must be done until we have a simple synthetic substitute for the complex, variable and inconvenient media upon which we depend so much at present.

And then take the differentiation of the colon and typhoid groups by cultural characters. There must be certain chemical differences inherent in these organisms which could be easily determined by the use of synthetic media adapted to each organism.

Another desideratum is a simple synthetic medium to be used as a substitute for blood serum when used for the diagnosis of diphtheria. We have to depend at present upon the uncertain and unsatisfactory supply of blood from the abattoirs. How much better would it be to find a chemical substitute which would be just as certain for diagnosis and much easier to

[4] Dolt, M. L., "Simple Synthetic Media for the Growth of *B. coli* and for its Isolation from Water," *Journal of Infectious Diseases,* V., 1908, 616.

prepare? I believe that such a substitute will soon be forthcoming.

We have been gradually accumulating a knowledge of a considerable number of important chemical products which are produced by the activities of bacteria either by synthesis or decomposition. Many of these substances are of great importance. Many are produced only as far as we know at the present time by bacteria. How little at present we know of the chemistry of these products! How much can be learned by a study of the formation of these substances in culture media of known composition! The chemistry of ptomaine and toxin formation, of pigment formation, of enzyme production, may be worked out in this way. The chemist laughs at our present methods of testing the production of gas, the reduction of nitrates, the production of indol, the fixation of nitrogen. And yet when these processes are tested in synthetic media how simple are the chemical tests involved and how accurate may be the results!

Slowly and laboriously the physiological chemist is now trying to work out the chemistry of protoplasm, of the proteins, such as the albumens, the peptones and proteoses. His principal line of attack is by a study of their decomposition products. The brilliant work of Fischer opened up an entirely new field of research when he undertook the study of the synthetic production of the polypeptides from amino acids by an amide link. Still more light might be thrown upon this important problem by the study of the growth of

bacteria in simple chemical solutions. For in the synthetic culture medium we would be able to study step by step the synthesis of protein under conditions accurately controlled and completely known. For when bacteria are growing on simple chemical media and are building up untold millions of bacterial bodies from the simple salts present, we can almost see protoplasm in the making.

And finally, aside from the important chemical information which may in this way be obtained, I believe that some most interesting biological information lies along this path. Who would dare to deny that some day it might be possible by some such method as this to discover the secret of the very origin of life itself!

These then are some of the lines of work which appear to me to mark progress in the science of pure bacteriology. Brilliant as may be the results of the study of the applications of bacteriology, fully as interesting, and hardly less important, will be the results that come from the application of exact chemical methods to our at present inexact and rather uncertain bacteriological procedures.

F. P. Gorham

Brown University

A CONTRIBUTION TO OUR KNOWLEDGE OF THE GAS METABOLISM OF BACTERIA.

First Paper—The Gaseous Products of Fermentations of Dextrose, by B. coli, by B. typhosus and by Bact. Welchii.

Second Paper—The Absorption of Oxygen by Growing Cultures of B. coli, and of Bact. Welchii.

BY FREDERICK G. KEYES AND LOUIS J. GILLESPIE.

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A CONTRIBUTION TO OUR KNOWLEDGE OF THE GAS METABOLISM OF BACTERIA.

FIRST PAPER.

THE GASEOUS PRODUCTS OF FERMENTATIONS OF DEXTROSE BY B. COLI, BY B. TYPHOSUS AND BY BACT. WELCHII.

BY FREDERICK G. KEYES AND LOUIS J. GILLESPIE.

(*From the Biological Laboratory of Brown University.*)

(Received for publication, October 9, 1912.)

Earlier investigations.

B. coli. The literature on the gases produced by *B. coli* on various culture media has already been reviewed by one of us.[1] It was found that very little work had been attempted on this subject by investigators familiar with the properties of gases.

Harden[2] found that, when *B. coli* was grown anaërobically on a non-albuminous dextrose medium based on asparagine, the asparagine was reduced to ammonium succinate with a consequent lessened evolution of hydrogen. He therefore made use of media made of beef broth and of Witte's peptone and concluded, from a study of the solid, liquid and gaseous products, that on these media dextrose was decomposed with the formation (if not evolution) of equal volumes of hydrogen and of carbon dioxide. His gas analyses showed for the most part more hydrogen than carbon dioxide.

One of us[3] determined for several strains of *B. coli* the quantities and the composition of gas obtained in various incubation periods

[1] Keyes: *Journ. of Med. Res.*, xxi (N. S. xvi), p. 69, 1909. In this communication the composition of the culture medium was unfortunately given incorrectly. The medium always contained, in addition to the constituents noted, 1 per cent of Merck's "Highest Purity" dextrose.

[2] Harden: *Trans. Jenner. Inst.*, 1899, ii, p. 126; *Journ. Chem. Soc.* (Transactions), lxxix, p. 610, 1901.

[3] Keyes: *Journ. of Med. Res.*, xxi (N. S. xvi), p. 69, 1909.

by an exact method from vacuum fermentations of dextrose. A non-albuminous asparagine medium was used for the most part, but a dextrose meat bouillon was also tested; and it was found that a larger percentage of hydrogen was indeed evolved from the albuminous medium but that even on this medium carbon dioxide was produced in considerably greater volume than hydrogen.[4]

B. typhosus. Hesse[5] studied the gas metabolism of this organism by growing it in the presence of air and drawing off samples of gas from day to day for analysis. He stated that carbon dioxide is evolved and that oxygen is absorbed but he did not calculate his analytical results. We calculate from his protocols that in one instance as much as 18 cc. of carbon dioxide was evolved aërobically from 33 cc. of "glycerin agar-agar."

Harden[6] detected no production of gas from anaërobic fermentations of dextrose by *B. typhosus* and Pakes and Jollyman[7] detected no formation of gas from sodium formate—the supposed intermediate product between dextrose and carbon dioxide and hydrogen.

Bact. welchii. Dunham[8] analyzed the gas evolved by this organism from 1 per cent dextrose bouillon in Smith fermentation tubes. The ratio CO_2:H_2 was 0.43, but his results are not comparable with ours because he disregarded the loss of carbon dioxide due to solution in the medium and to diffusion from the open end of the fermentation tube.

Technique.

Organisms. Cultures were kept in a vigorous state of growth by means of frequent transfers and isolations by the plate method. The medium used for *B. typhosus* and *Bact. welchii* was the ordinary meat extract peptone agar; in the case of *B. coli* it consisted

[4] We shall give below an analysis from a fermentation on a Witte's peptone medium, which gave practically the same figures as the meat bouillon. We have always found more CO_2 than H_2, and attribute Harden's contrary result, as also his negative result with *B. typhosus*, to insufficient removal of dissolved carbon dioxide from the culture fluid.

[5] Hesse: *Zeitschr. f. Hyg.*, xv, p. 17, 1893.

[6] Harden: *Journ. Chem. Soc.* (Transactions), lxxix, p. 610, 1901.

[7] Pakes and Jollyman: *Journ. Chem. Soc.* (Transactions), lxxix, p. 459, 1901.

[8] Dunham: *Johns Hopkins Hospital Bull.*, viii, p. 68, 1897.

of 1 per cent dextrose, 0.2 per cent disodium phosphate and 1.5 per cent agar, with either 1 per cent asparagine for experiments on asparagine media or 1 per cent ammonium lactate for experiments on lactate media, and was made neutral to litmus with sodium hydrate. Test cultivations of *B. coli* or of *B. typhosus* were started from a loopful of culture taken from the surface of a twenty-four-hour growth on slant agar. *Bact. welchii* fermentations were started from stab cultures. Subcultures from the test cultivations were never used for further tests. The organisms answered the usual identification tests.

Culture fluids. The culture fluids were sterilized in the absence of air in fermentation bulbs (to be described) in streaming steam by the intermittent method. The reaction after boiling was slightly acid to litmus, except in certain experiments (noted in the protocols) in which the reaction was made just neutral to phenolphthalein by the addition of normal sodium hydrate. When alkali was added, the amount of carbon dioxide thus introduced was calculated from a determination of the actual volume of carbon dioxide liberated from a sample of the sodium hydrate solution upon acidification *in vacuo* with sulphuric acid. Since the medium was subsequently exposed only momentarily to the atmosphere, the correction applied (a small one) was exact.

Oxygen. Oxygen was prepared by heating potassium permanganate *in vacuo* and was purified by passing over phosphorus pentoxide and over sodium hydrate (not that purified by alcohol). It was measured dry, the pressure being read on a barometer column. Generator, burette and barometer were permanently incorporated in the pump system (fig. 2) by fused glass joints. The generator was exhausted by the mercury pump and then rinsed out twice with small quantities of oxygen before the gas was generated for use in the work.

Control of gaseous environment and collection and analysis of the gases. Fermentations were conducted either *in vacuo*[9] or in the presence of gases admitted in known quantities after a vacuum had first been obtained.

The fermentation bulbs (fig. 1) fitted with stopcocks of a special form described by one of us,[10] were about half or three-quarters filled with cul-

[9] That is, in an atmosphere of water vapor only.

[10] Keyes: *Science* (N.S.), xxviii, p. 17, 1908.

ture medium, and were sterilized. After sterilization, the stopcocks of the bulbs were wiped dry with sterile rolls of filter paper and re-greased with sterile lubricant. The lubricant was the non-volatile non-antiseptic mixture described by one of us.[11] The medium was then inoculated.

The bulbs were then freed from gases. It was found that this could be done to any desired degree of completion by the use of a good Sprengel water pump, which was capable of maintaining the pressure at from 9 to 15 mm. (The use of a mercury pump gave no better results and was moreover unnecessarily troublesome.) This method of exhaustion depends upon rinsing out the gas with water vapor. In extracting dissolved gases from liquids the driving force becomes less as the process becomes more nearly complete and the process comes practically to an end while detectable amounts of gases are still dissolved, unless by vigorous agitation the liquid is kept well mixed and many new boundary surfaces thus continually formed. We found that, without agitation, pumping for an hour after most of the air had been removed was not so efficacious as five or ten minutes' vigorous shaking interrupted by a few exposures to the action of the pump. This experience led us to think that the degree of exhaustion is controlled, in cases where the space above the liquid is kept as free from gases as in these experiments, by the thoroughness of agitation. The bulbs were therefore subjected alternately to the action of the pump, by opening the stopcock of the bulb for an instant, and to a vigorous shaking. This was repeated until a metallic sound was emitted upon striking the bulb hard with the finger ends[12] and was then further continued until this effect (which cannot be produced until the vacuum is rather good) was produced only with great difficulty. As the liquid was shaken, falling drops of liquid clicked metallically against the sides of the bulb. We believe that we were able to treat the bulbs with practical uniformity and that before we finished the process the gas that remained in any bulb was negligible in quantity so far as our analyses were concerned.[13]

If gas was to be admitted, the bulb was now connected to the pump system by a short piece of heavy walled rubber tubing, which was well painted with hot adherent grease. Both ends of the tube were bound tightly on with copper wire. The tubing connecting the bulb with the gas burette was evacuated by the use of the mercury pump, and the gas, having been measured, was pushed over into the bulb. By careful manipulation practically all the gas could be passed over into the bulb without admitting any mercury.

[11] Keyes: *Journ. Amer. Chem. Soc.*, xxxi, p. 1271, 1909.

[12] Special experiments have shown us that this sound is occasioned by the tearing apart of the liquid with the liberation of a bubble or two of gas and the consequent collapse of the newly formed walls of liquid. Thus the range of concentrations of dissolved gases at which this effect is possible, should be, as it in fact is, limited in both directions.

[13] Except possibly in the case of *B. typhosus*, where the total gas evolution was very small.

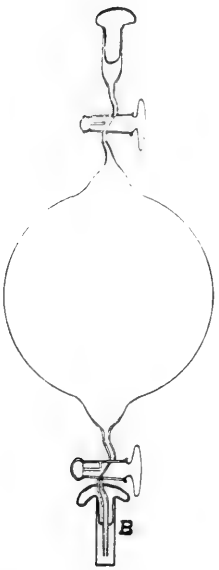

FIG. 1. FERMENTATION BULB FITTED WITH VACUUM STOPCOCKS.

B, bottle, secured by cotton plug, serving to keep sterile the capillary lead. Capacity of bulb, about 300 cc.

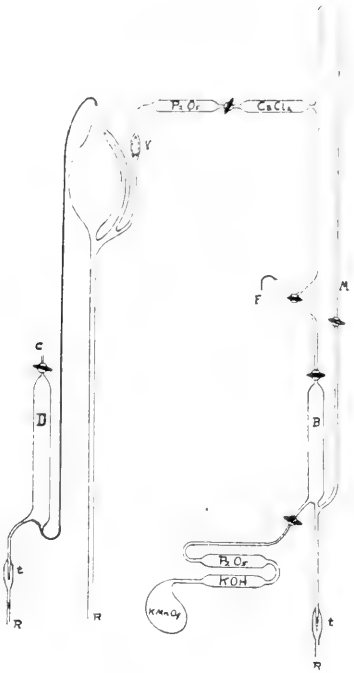

FIG. 2. THE PUMP SYSTEM.

B, oxygen measuring burette; *D*, delivery tube—it connects with gas analysis apparatus at *C*; *M*, manometer column, provided with meter stick; *t*, *t*, air traps; *V*, valve tight to ascending mercury. Fermentation bulb connects at *F*. Mercury reservoirs connect by heavy rubber tubing at *R*, *R*, *R*.

After incubation at about 37°C., the gases in the bulb were recovered by the mercury pump and analyzed. In every instance, the whole obtainable quantity was pumped out and the whole quantity was used for the analysis or else a measured portion after the gases were well mixed. The gas absorptions were conducted according to the method of Hempel. The burettes were filled with mercury. Carbon dioxide was absorbed by strong caustic soda solution (after the total volume had been measured dry); oxygen, by phosphorus or when necessary by alkaline pyrogallol; and hydrogen

was determined by exploding with a quantity of air sufficient to avoid the burning of nitrogen. Other gases were absent. Sometimes the Hempel pipettes were used for the reagents; but when small amounts of gas were to be analyzed, they were generally measured under diminished pressure, and treated with absorbing reagents in tubes inverted over mercury, as in the method given by Travers.[14] Sometimes the diminution of pressure was read on an open manometer; and sometimes the actual pressure was read on a barometer (the space of which was saturated with water vapor) sealed to the burette. The work was done in a cellar where the temperature was sufficiently constant. All gas volumes have been calculated for 0°C., 760 mm. and dryness. The medium was always strongly acid after incubation so that no carbon dioxide was retained chemically.

Experiments with B. Coli.

Experiment 1. *B. coli* was grown *in vacuo* for 48.2 hours on 250 cc. of a medium containing 1.00 per cent each of Witte's peptone and Merck's "Highest Purity" dextrose in distilled water, prepared hot and filtered.

Total gas	137.0 cc.
CO_2	56.1 per cent.
H_2	43.0 per cent.
CO_2 per gram of dextrose	30.7 cc.
Ratio, CO_2:H_2	1.31

The ratio 1.28 was found by one of us[15] for beef infusion broth containing 1 per cent each of dextrose and Witte's peptone.

It seemed better to work with a medium containing no substances of unknown composition. One such, described by Dolt,[16] has the advantage that no amino group is present so that retention of hydrogen by the type of reaction found by Harden for asparagine is excluded. It consisted of 1.00 per cent ammonium lactate, 1.00 per cent dextrose and 0.200 per cent disodium phosphate in distilled water, prepared without heating. In table I are given some experiments upon this medium, arranged according to the length of incubation.

The influence of phosphate, of nitrate and of increased dextrose percentage is shown in table II. The medium contained 1.00 per

[14] Travers: *Study of Gases*, 1901, p. 28.
[15] Keyes: *Journ. of Med. Res.*, xxi (N. S. xvi), p. 69, 1909.
[16] Dolt: *Journ. Inf. Dis.*, v, p. 616, 1908.

TABLE I.

B. coli in vacuo.

EXP. NO.	VOLUME OF MEDIUM	INCUBATION AT 37°	TOTAL GAS	CO_2	H_2	CO_2 PER GRAM DEXTROSE	$\frac{CO_2}{H_2}$
	cc.	*hours*	*cc.*	*per cent*	*per cent*	*cc.*	
2	100	23.3	27.60	51.1		14.10	
3	25	40.0	7.21	49.5	48.1	14.30	1.03
4	100	44.3	28.40	51.3	48.1	14.55	1.06
5	100	58.2	28.60	51.1	47.0	14.60	1.09

cent of ammonium lactate and various quantities of dextrose, sodium phosphate and ammonium nitrate, as noted. In experiments 7, 8 and 9 Merck's "Highest Purity" dextrose was used; in experiment 6, the same recrystallized from alcohol; in experiment 10, a nitrate-free dextrose (Merck's "Pure") recrystallized.

The effect of an increase of phosphate is to increase the total gas formation, probably by delaying acid inhibition, and to increase the formation of carbon dioxide more than hydrogen.

The effect of nitrate is to decrease slightly the carbon dioxide formation and to use up most (or all) of the hydrogen that would otherwise be produced. The latter effect has been noted by Pakes and Jollyman.[17] Merck's "Highest Purity" dextrose was found by the phenolsulphonic acid method to contain 0.04 per cent of sodium nitrate, which could be reduced to 0.01 per cent by recrystallization. Assuming proportionality, the result of experiment 9 would indicate that the amount of nitrate present in 1 gram of the "Highest Purity" dextrose could use up 0.012 cc. of hydrogen, a quantity which is negligible for *B. coli* fermentations but which would be of great significance in *B. typhosus* fermentations. The ammonium lactate and sodium phosphate were found free from nitrates by the same (controlled) test.

In another part of our work, to be communicated in a second paper, we have studied somewhat fully the effect of oxygen upon *B. coli* fermentations of dextrose in an asparagine medium. The following figures show the results for an aërobic fermentation by *B. coli* of a medium composed of 1.00 per cent "Highest Purity"

[17] Pakes and Jollyman: *Journ. Chem. Soc.* (Transactions), lxxix, p. 386, 1901.

TABLE II.

B. coli in vacuo.

EXP. NO.	COMPOSITION OF MEDIUM*			VOLUME OF MEDIUM	INCUBATION AT 37°	TOTAL GAS	CO_2	H_2	CO_2 PER GRAM DEXTROSE	$\frac{CO_2}{H_2}$
	Dextrose	Na_2HPO_4	$(NH_4)NO_3$							
	per cent	*per cent*	*per cent*	*cc.*	*hours*	*cc.*	*per cent*	*per cent*	*cc.*	
6	1.00	0.10		25	42.8	3.72	48.3	41.9	7.20	1.15
7	1.00	0.60		100	48.2	36.4	63.5		23.10	
8	1.00	0.60		100	52.7	68.3	63.5		43.40	
9	1.00	0.20	0.40	100	72.0	14.0	95.8		13.40	
10	4.00	0.20		50	580.	20.4	50.3	47.2	5.12	1.07

*Exclusive of 1.00 per cent ammonium lactate.

TABLE III.

Gas production of B. typhosus in vacuo.*

EXP. NO.	VOLUME OF MEDIUM	INCUBATION AT 37°	TOTAL GAS	CO_2	H_2	RESIDUAL GAS	SUM†	PER GRAM OF DEXTROSE		$\frac{CO_2}{H_2}$
								CO_2	H_2	
	cc.	*hours*	*cc.*	*per cent*	*per cent*	*per cent*	*per cent*	*cc.*	*cc.*	
1	213	48.2	3.26	92.4	2.4	5.1	99.9	1.41	0.038	38.5
2	193	72.2	3.40	92.10	1.96	5.98	100.04	1.63	0.033	47.0
3	250	360.	5.44	97.10	2.13	0.76	99.99	2.12	0.044	45.6
4	150	384.	3.22	93.25	2.03	4.44	99.72	1.99	0.042	46.0
5	150	108.	2.01	92.15	4.90	2.47	99.52	1.24	0.066	18.8

*In experiments 1, 2 and 5 strain "A" was used; in experiments 3 and 4, strain "B."
† The figures in this column represent the sums of the analytical percentages recorded in the three preceding columns.

dextrose, 1.00 per cent ammonium lactate and 0.100 per cent disodium phosphate:

Experiment 11. B. Coli.

Oxygen admitted	4.59 cc.
Incubation at 37°	108. hours.
Total gas	10.56 cc.
CO_2	5.18 cc.
O_2	2.58 cc.
H_2	0.96 cc.
CO_2 per gram of dextrose	10.36 cc.
Ratio, CO_2:H_2	5.4
Oxygen retained	2.01 cc.

Experiments 3, 4, 5 and 10 show that it is possible to find conditions such that nearly equal volumes of CO_2 and H_2 are obtained but that the CO_2 is always in slight excess. Evidently the presence of oxygen, of nitrates or of sodium phosphate, since all these substances tend to increase the ratio of carbon dioxide to hydrogen, prevents the realization of the value unity for this ratio. Dolt[18] found that *B. coli* required either phosphates or nitrates for its growth. We have found that in the absence of nitrates anaërobic growth vanishes if the phosphate content is sensibly reduced below the lowest concentration used in the experiments given above, so that it appears that, in spite of a systematic error due to the greater solubility of carbon dioxide in the culture liquid, it is not possible to collect equal volumes of carbon dioxide and hydrogen. This means that if, as Harden concluded, the decomposition of dextrose by the action of *B. coli* results in the formation of an equal number of molecules of carbon dioxide and hydrogen, according to the "classical" fermentation of Duclaux,[19] there occurs also a process which either uses up hydrogen or produces carbon dioxide and which is therefore oxidational. This process seems to be necessary for growth.

Experiments with B. typhosus.

A few experiments were made with two strains of the typhoid bacillus. Neither strain could be grown on Uschinski's solution, on Fraenkel's modification or on various other simple media.

[18] Dolt: *Journ. Inf. Dis.*, v, p. 616, 1908.

[19] Duclaux: *Traité de microbiologie*, iv, p. 49, 1901.

The culture fluid consisted of 1.00 per cent each of Witte's peptone and dextrose in distilled water, prepared hot and filtered. In the first four experiments Merck's "Highest Purity" dextrose was used and in the fifth a preparation made by recrystallizing Merck's "Pure" (nitrate-free) dextrose from alcohol.

The following analysis for experiment 5 typifies the procedure followed in the analysis of small quantities of gases. The explo-

Analysis of experiment 5.

	OBSERVED VOLUME	MANOMETER READINGS		PRESSURE	TEMPERATURE	VOLUME AT 760 MM.	MEAN VOLUME
					°C.		
Total gas.....	4.20	523	124	399	22.0	2.150	2.183
	6.80	357	114	243		2.180	
	5.70	413	118	295	22.9	2.220	
After KOH...	1.00	264	138	126	22.5	0.166	0.171
	5.00	147	121	26	23.0	0.171	
	2.35	187	132	55		0.170	
	1.80	204	134	70	22.9	0.166	
	1.40	234	135	99		0.182	
Air added....	2.60	544	130	414	23.1	1.420	1.458
	3.75	425	126	299		1.470	
	5.15	339	120	219		1.480	
	5.80	309	118	191	23.0	1.460	
	4.60	365	123	242		1.460	
Pass spark....	4.60	363	123	244		1.460	
Added electrolytic gas in small quantities until mixture exploded on passing spark.	3.20	442	129	313		1.310	1.298
	5.60	296	119	177		1.300	
	6.80	258	114	144		1.290	
	3.40	410	128	282	23.0	1.290	

The result (volumes at room temperature, but dry without correction because the barometer space was saturated with moisture) is therefore

CO_2	2.012	Residual	0.054
H_2	0.107	Sum	2.173

All gas volumes were further reduced to 0°C.

sive gas was calculated as hydrogen, and the closeness with which the sums of the analytical percentages thus found approach 100 per cent indicates that the explosive gas was hydrogen. On so small a quantity of gas as that remaining for the hydrogen determination the non-production of carbon dioxide upon explosion could not be certainly proved.

Since the presence of dextrose is of great importance for growth of bacteria in the absence of oxygen, it is not possible to show by the omission of dextrose what is probably the truth: that the gases obtained came from the dextrose. Nevertheless a significant amount of fermentable sugar could not have been present in the peptone, since it was found that *B. coli* produced anaërobically on a 1 per cent solution of Witte's peptone in forty-eight hours at 37°C. only 0.095 cc. of CO_2 (38 per cent of the total gas) per gram of peptone, and *B. typhosus* produced under the same conditions less than 0.02 cc. of CO_2 per gram of peptone.

These results show that carbon dioxide and hydrogen are evolved by the action of *B. typhosus* on dextrose, but that the amounts produced are very much less than those produced by *B. coli*, and the ratio of carbon dioxide to hydrogen is many times higher in the case of *B. typhosus*. Upon the nitrate-free dextrose (experiment 5) considerably more hydrogen is produced. The increase per gram of dextrose is about 0.027 cc., whereas the volume of hydrogen which the amount of nitrate in question can use up in a *B. coli* fermentation we have found above to be 0.012 cc.

Just as the amount of nitrate present as impurity in the dextrose, while it introduced no significant error in the results for *B. coli*, made a large difference in the results for *B. typhosus* (where the total amount of gas was much smaller), so may the principles in the peptone which increase the ratio with *B. coli* make a greater difference with *B. typhosus*. It is possible that this is wholly responsible for the difference in the values of the ratio found for the two organisms.

Experiments with Bact. welchii.

Bact. welchii was grown anaërobically on a medium consisting of 1 per cent each of Witte's peptone and Merck's "Highest Purity" dextrose in distilled water. No alkali was added.

TABLE IV.

Bact. welchii in vacuo.

EXP. NO.	VOLUME OF MEDIUM	INCUBATION AT 37°	TOTAL GAS	CO_2	H_2	RESIDUAL GAS	CO_2 PER GRAM DEXTROSE	$\frac{CO_2}{H_2}$
	cc.	*hours*	*cc.*	*per cent*	*per cent*	*per cent*	*cc.*	
1	100	120	61.3	60.0	39.3	0.5	36.8	1.53
2	50	336	63.5	58.5	41.2	0.3	74.2	1.43

The results differ chiefly from those found for *B. coli* upon this medium in the larger volumes of gas found per gram of dextrose.

Comparison of the three microörganisms.

Some of the foregoing experiments give a basis for a comparison of the three organisms, namely, those anaërobic fermentations of a medium consisting of 1 per cent each of Witte's peptone and dextrose. In table V are given the maximum volumes of carbon dioxide evolved per gram of dextrose and the ratio CO_2:H_2. The lower value of the ratio given under *B. typhosus* is that obtained with nitrate-free dextrose.

TABLE V.

	B. TYPHOSUS	B. COLI	BACT. WELCHII
Carbon dioxide in cubic centimeters	2.1	30.7*	74.
Ratio, CO_2:H_2	from 19 to 44	1.31	1.48

*48.2 hours; possibly not maximum amount obtainable.

SUMMARY.

The gas evolution accompanying the growth of certain bacteria on culture media containing dextrose has been studied by an exact method.

I. The principal results for *B. coli* are:

A. Dextrose-peptone media yield considerably larger volumes of carbon dioxide than of hydrogen upon anaërobic fermentation. The volume-ratio CO_2:H_2 is 1.31.

B. A suitable "synthetic" medium (composed of ammonium lactate, disodium phosphate and dextrose) yields anaërobically nearly equal volumes of the two gases. The ratio CO_2:H_2 is always

greater than unity and has a mean value of 1.06 for a medium of given composition.

1. The presence of oxygen raises the value of this ratio.
2. Increase of phosphate content also raises the value.
3. The phosphate cannot be reduced sensibly in quantity, or substituted by a salt less objectionable.
4. The value 1.06 for the ratio CO_2:H_2 is minimal. This means that if the principal gas reaction consists of a liberation of an equal number of molecules of carbon dioxide and hydrogen from dextrose there also occurs an accompanying gas reaction of the nature of an oxidation.

II. *B. typhosus* produces anaërobically from a dextrose-peptone medium small volumes of carbon dioxide and an explosive gas, probably hydrogen. The ratio CO_2:H_2 is never lower than 19.

III. *Bact. welchii* produces anaërobically from a dextrose-peptone medium large volumes of carbon dioxide and hydrogen. The ratio CO_2:H_2 is 1.48.

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A CONTRIBUTION TO OUR KNOWLEDGE OF THE GAS METABOLISM OF BACTERIA.

SECOND PAPER.

THE ABSORPTION OF OXYGEN BY GROWING CULTURES OF B. COLI AND OF BACT. WELCHII.

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(Received for publication, October 9, 1912.)

We have studied the gas production of *B. coli* and of *Bact. welchii* for various incubation periods in the presence of oxygen admitted to the fermentation bulbs in known quantities after a vacuum had first been obtained. The technique employed has been described by us in the preceding paper.

From the data so obtained we can derive information concerning the rate at which oxygen is absorbed and concerning the relations existing among the quantities of oxygen absorbed and the quantities of carbon dioxide and hydrogen evolved.

We have not found in the literature any work on these points for any microörganism where the atmosphere over the culture was accurately controlled and the analyses were made on portions of gas accurately sampled.

Experiments with B. coli.

The culture medium consisted of 1.00 per cent each of Merck's "Highest Purity" dextrose and asparagine and 0.200 per cent of disodium phosphate and was made neutral to phenolphthalein with sodium hydrate. Corrections were made for the carbon dioxide thus introduced, as explained in the first paper, and the values of the corrections are given with the analyses.

The results of the gas analyses are given in table I.

The values of the ratio, CO_2:H_2, vary enormously, and are many times the value previously obtained by one of us[1] for anaërobic fermentations.

In an experiment described in the preceding paper (Exp. 11, p. 299) the retention of oxygen was found to be 2.0 cc., whereas the decrease in yield of hydrogen (*i.e.*, that amount which, together with the amount actually found, would make the ratio CO_2:H_2 equal to 1.06, the mean value for anaërobic fermentations on the same medium) is 3.9 cc. or only 0.1 cc. less than that which the oxygen could have oxidized. The medium for which this result was obtained was based on ammonium lactate. If this result were the rule, it would indicate that the oxygen was almost quantitatively taken up by nascent hydrogen from the dextrose. Similar calculations from the data here presented show, however, that this is not the rule (at least in the case of the asparagine medium) but that the missing volumes of hydrogen are sometimes greater and sometimes smaller than twice the volumes of absorbed oxygen.

The volume of oxygen absorbed per unit volume of carbon dioxide evolved (the "respiratory quotient") is given. The mean value is 0.135, with a probable error of $\pm$ 0.02.

The relation between the volume of oxygen admitted to the culture, the volume of oxygen not absorbed and the duration of incubation at 37°C. can well be seen from a calculation of the values of the expression $\frac{1}{t} \cdot \log \frac{V}{v}$; where t is the time in hours, V is the volume of oxygen admitted, and v is the volume of the unabsorbed oxygen. Since $\frac{V}{v}$ here equals the ratio of the corresponding pressures, the given expression is that for the constant of a monomolecular gas reaction. It is in fact nearly constant. The values are given in table III. We should note, however, that the fermentation bulbs were not shaken during incubation, so that although a certain degree of agitation was imparted to the medium by the brisk evolution of gas, the rate of oxygen absorption may, nevertheless (namely, if relatively fast), have been limited by the rate of distribution. In this case the given expression should be constant.

[1] Keyes: *Journ. of Med. Res.*, xxi (N.S., xvi), p. 69, 1909.

The data given in table I may properly be compared with those obtained for anaërobic fermentations induced by *B. coli* on the same medium, and given by one of us in a previous paper,[2] with the following results: (1) Smaller volumes of carbon dioxide are produced aërobically than anaërobically, for all periods of time. (2) For the same amount of carbon dioxide, less hydrogen is obtained aërobically than anaërobically. The presence of oxygen therefore appears to lessen the production of gases from dextrose and also either to cause some output of carbon dioxide by a respiratory process or to cause a disappearance of hydrogen (presumably) by oxidation.

Experiments with Bact. welchii.

Similar experiments were made with *Bact. welchii*. The medium consisted of 1.00 per cent each of Witte's peptone and Merck's "Highest Purity" dextrose in distilled water, prepared hot and filtered. No alkali was added. Other conditions were the same as before, except that all durations of incubation were much longer and the pressures of oxygen were much smaller.

The values of the expression $\frac{1}{t} \cdot \log \frac{V}{v}$ are given in table III. They are very nearly constant and are about one-third the value found for *B. coli*.

All other results are given in table II. The mean value for the respiratory quotient $\left(\frac{O_2}{CO_2}\right)$ is 0.014, *i.e.*, one-tenth the value found for *B. coli*, with a probable error of $\pm$ 0.002.

The ratio CO_2:H_2 is slightly raised by the presence of oxygen,[3] and does not vary for this organism in great degree as it does for *B. coli*.

As with *B. coli*, the "missing" volumes of hydrogen are not equal to twice the volumes of oxygen absorbed but are sometimes greater and sometimes smaller. It is perhaps of significance in this connection that both the media used for these organisms permit side-reactions which prevent the evolution of equal volumes of carbon dioxide and hydrogen by anaërobic fermentation of dextrose, as discussed in the first paper of this study.

[2] Keyes: *loc. cit.*

[3] Values for anaërobic fermentations are given in the first paper of this series.

TABLE I.

B. coli.

All gas volumes are reduced to standard conditions: 0°C., 760 mm. of mercury and to dryness.

NUMBER	WEIGHT MEDIUM	CORRECTION FOR CO_2 IN NaOH	SPACE ABOVE LIQUID	O_2 ADDED	INITIAL PRESSURE of O_2 in MM. Hg. (APPROX.)	INCUBATION AT 37°C.	TOTAL GAS FOUND	CO_2 FOUND	CO_2 CORR. FOR NaOH	O_2 FOUND	H_2 FOUND	N_2 FOUND	VOLUME-RATIO,* $\frac{CO_2}{H_2}$	O_2 ABSORBED	RESPIR. QUOTIENT $\frac{O_2}{CO_2}$	CO_2 PER GRAM DEXTROSE	CO_2 PER GRAM DEXTROSE ANAËROBICALLY (EXPERIMENTS OF KEYES, '00)
	grams	*cc.*	*cc.*	*cc.*		*hours*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*		*cc.*		*cc.*	*cc.*
1	200	0.78	50	5.27	80	24	28.61	24.52	23.74	3.81	0.04	0.24	590.0	1.46	0.062	11.87	16.9
2	135	0.00	115	5.34	35	66	20.06	14.79	14.79	3.40	1.74	N.D†	8.6	1.94	0.132	10.95	29.–63.
3	241	0.88	154	5.28	26	72	36.52	25.75	24.87	2.74	7.38	0.75	3.4	2.54	0.102	10.32	29.–63.
4	112	0.46	183	10.36	43	74	29.82	19.65	19.19	5.35	4.52	0.30	4.2	5.01	0.261	17.12	29.–63.
5	200	0.78	50	5.05	76	119	37.47	34.11	33.33	2.68	0.17	0.52	196.0	2.37	0.071	16.67	63.4+
6	180	0.57	70	7.38	80	167	48.97	45.94	45.37	2.29	0.47	0.33	96.5	5.09	0.112	25.20	63.4+
7	240	0.85	55	5.97	82	192	27.07	21.33	20.48	1.79	2.44	1.44	8.4	4.18	0.204	8.36	63.4+
															Mean = 0.135 ± 0.02		

* In anaërobic fermentations this ratio does not change with the time and has the mean value 1.77 (Keyes: *Journ. Amer. Chem. Soc.*, xxxi, p. 1271).
† N. D. = Not done.

TABLE II.

Bact. welchii.

All gas volumes are reduced to standard conditions.

NUMBER	WEIGHT MEDIUM	SPACE ABOVE LIQUID	O_2 ADDED	INITIAL PRESSURE of O_2 in MM. HG. (APPROX.)	INCUBATION AT 37°C.	TOTAL GAS FOUND	CO_2 FOUND	O_2 FOUND	H_2 FOUND	N_2 FOUND	VOLUME-RATIO, $\frac{CO_2}{H_2}$*	O_2 ABSORBED	RESPIR. QUOTIENT $\frac{O_2}{CO_2}$	CO_2 PER GRAM DEXTROSE	CO_2 PER GRAM DEXTROSE ANAËROBICALLY (FIRST PAPER OF THIS STUDY)
	grams	*cc.*	*cc.*		*hours*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*		*cc.*		*cc.*	*cc.*
1	63.0	237	0.38	0.12	240	73.28	43.35	0.18	28.38	0.42	1.53	0.19	0.0044	68.80	
2	57.5	242	0.97	3.05	240	57.07	36.06	0.55	19.55	0.90	1.85	0.42	0.0116	62.80	
3	42.5	253	0.94	2.85	336	54.36	33.20	0.65	19.84	0.61	1.67	0.29	0.0088	78.20	74.20
4	52.0	248	0.73	2.25	384	65.14	38.20	0.28	26.53	N.D.†	1.44	0.45	0.0118	73.50	
5	55.0	245	1.24	3.85	432	71.92	45.01	0.28	25.72	0.72	1.75	0.96	0.0214	81.90	
6	49.0	251	1.62	3.90	480	71.55	45.45	0.37	25.20	0.55	1.80	1.25	0.0275	92.80	
													Mean =0.014 ±0.002		

* For anaërobic fermentations, the values for this ratio were 1.53 and 1.42, as found in the preceding paper.
† N.D.—Not done.

TABLE III.

Values of $\frac{1}{t} \cdot \log \frac{\text{volume } O_2 \text{ admitted}}{\text{volume } O_2 \text{ recovered}}$.

t = time in hours; common logarithms.

HOURS	B. COLI	HOURS	BACT. WELCHII
24	0.0059	240	0.0013
66	0.0030	240	0.0010
72	0.0040	336	0.0005
74	0.0039	384	0.0011
119	0.0023	432	0.0015
167	0.0030	480	0.0013
192	0.0027		
Mean value	0.0035 ± 0.0003		0.0011 ± 0.0001

SUMMARY.

The absorption of oxygen by growing cultures on dextrose media of *B. coli* and of (the strict anaërobe) *Bact. welchii* has been studied by an exact method. Data afforded by complete gas analyses are given. The following comparisons are made possible:

1. For both microörganisms, the absorption of oxygen simulates a monomolecular reaction.

2. The mean values of the respiratory quotients, although the probable error of each is large, are widely different for the two microörganisms.

3. With varying pressures of oxygen, the ratio $CO_2 : H_2$ varies enormously in the case of *B. coli*, but varies only slightly in the case of *Bact. welchii*.

We think that the numerical differences in the results for the two microörganisms may *possibly* be referable to differences in experimental conditions; if so, probably to the differences in oxygen pressures.

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HOW DO ISOTONIC SODIUM CHLORIDE SOLUTION AND OTHER PARTHENOGENIC AGENTS INCREASE OXIDATION IN THE SEA URCHIN'S EGG?

BY J. F. McCLENDON AND PHILIP H. MITCHELL.

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Loeb has shown that ^{-}OH ions favor development.[1] Our own experiments (Table 6) as well as those of O. Warburg[2] demonstrate that increase in the alkalinity of the medium increases oxidation in fertilized eggs.

According to Warburg, the egg is impermeable to ^{-}OH ions or fixed alkalies, because, although eggs stained with neutral red are changed to yellow by NH_3 of a concentration which increases the oxidation only one-tenth, similar eggs are not changed to yellow by fixed alkalies of a concentration at which oxidation is greatly increased.

These facts are, however, capable of another interpretation. The egg is filled with lipoid particles which take up the neutral red to such an extent as to render the surrounding protoplasm and sea water colorless. The ^{-}OH ions cannot freely enter the lipoids in order to change the color of the neutral red. On the other hand ammonia is lipoid-soluble and can enter.

It might be objected that the ammonia cannot react with the dye in the non-aqueous medium owing to the suppression of ionization, but whether the dye is driven out of the lipoids or changed to yellow *in situ*, the fact remains that it does become yellow.

Harvey[3] observed that the addition of but a small quantity of alkali to sea water containing fertilized eggs stained with neutral

[1] Loeb: *Chemische Entwicklungserregung des tierischen Eies.* Berlin, 1909.
[2] Warburg: *Zeitschr. f. physiol. Chem.*, lx, p. 305, 1910.
[3] Harvey: *Journ. of Exp. Zoology*, x, p. 507, 1911.

red, changed the eggs to yellow. However, the eggs were injured by the alkali and probably their permeability was increased. It is possible that alkalies or ^{-}OH ions in any concentration enter the fertilized eggs but must be present in sufficient concentration to set free ammonia or change the lipoids in order to affect the dye.

We may assume, then, unless more conclusive evidence indicates the contrary, that the ^{-}OH ions increase oxidation after penetrating the egg.

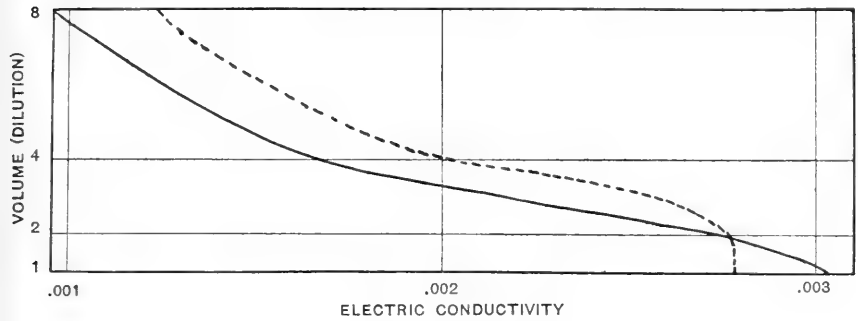
One of us has shown that unfertilized sea urchin's eggs are poorly permeable to salts and their ions, but become more permeable after fertilization or the initiation of parthenogenetic development.[1] Not only is the permeability increased but the oxidation rate is increased. Warburg observed that oxidation increases from five to seven times on fertilization (compare our Table 5, experiment III). He found also a large increase after the initiation of parthenogenetic development caused by hypertonic sea water,[2] fatty acid, alkalies or traces of the heavy metals,[3] silver or copper. Our own experiments, given below, confirm and extend these findings. There appears then to be some relation between permeability and oxidation, and the present paper is an attempt to determine what this relation is.

The living cell may be compared to a furnace, and R. Lillie[4] advanced the view that increase in permeability opens the draughts so to speak, allowing the escape of carbonic acid, and hence oxidation is increased. He supposes that the accumulation of carbonic acid and perhaps other end-products checks the oxidation, and increase in permeability to carbonic acid allows oxidation to proceed. The difficulty with this hypothesis lies in the fact that living cells have been shown by Overton and others to be freely permeable to substances which are easily soluble in fats and oils, or especially in lecithin and cholesterin. Carbon dioxide is soluble in oils and probably enters cells easily, at least there is evidence to show that red blood corpuscles are freely permeable to this gas. Fatty oils are permeable only to the undissociated molecules and not to the ions. Since the proportion of ions of carbonic

[1] McClendon: *Amer. Journ. of Physiol.*, xxvii, p. 240, 1910.
[2] Warburg: *Zeitschr. f. physiol. Chem.*, lxii, p. 1, 1908.
[3] Warburg: *Ibid.*, lxvi, p. 305, 1910.
[4] Lillie: *Biol. Bull.*, xvii, p. 188, 1909.

acid would ordinarily be small, what conditions in the egg might favor ionization of CO_2?

Not all of the alkali metals in the egg are combined with mineral acids; some are combined with proteins. This has been shown by the senior author with electric conductivity measurements of hens egg yolk given in the accompanying curves. The continuous line represents the conductivity of yolk freed from protein granules, and the dotted line, yolk containing an excess of protein



granules, separated by the centrifuge. The granules impede the current as shown by the fact that the granule-containing yolk is a poorer conductor than granule-free yolk. But on dilution, the granule-containing yolk becomes the better conductor. Therefore ions are set free from the granules on dilution.

The carbonic acid formed within the egg would react with the alkali albuminates with the formation of alkali carbonates and bicarbonates, which, notwithstanding hydrolysis, would liberate a considerable quantity of carbonic acid anions.[1] The dissociated carbonic acid, being unable to escape from the unfertilized egg would lower the ^{-}OH ion concentration and thus reduce oxidation. As the undissociated molecules of carbonic acid escape, more are formed by the slow oxidation in the egg.

On fertilization, the permeability to the anions of carbonic acid is greatly increased. They migrate out of the egg, and negative ions enter the egg to take their place. Since the ^{-}OH ions of the sea water are the fastest negative ions, they enter the egg and

[1] At about molecular concentration the equivalent electric conductivity of NaCl is 76; of Na_2CO_3, 45, at 18°.

increase oxidation. However, if some ^{-}Cl or $^{-}SO_4$ ions entered the egg they would tend to decrease the ^{-}OH ion concentration within the egg. But the senior author has shown that the fertilized *Fundulus* egg is impermeable to ^{-}Cl ions, for when placed in distilled water, or in solutions of nitrates or sulphates, practically no chlorine comes out of the eggs.[1] We may assume, therefore, that carbonic acid accumulates in the unfertilized egg until the reaction is neutral or slightly acid. But on fertilization, the permeability to carbonic acid anions is increased and the concentration of this acid is diminished so that the reaction is neutral or slightly alkaline. The increased alkalinity is the cause of the increased oxidation. We will now see to what extent our experiments bear out this assumption.

We observed that oxidation is about doubled when the egg is made parthenogenetic with carbonated sea water (Table 5, experiment II) or alkaline isotonic sodium chloride (Table 3, experiments I and II). In some cases the eggs, being physiologically different from those in other experiments, did not show the morphological signs of development in this solution, and oxidation was not doubled (Table 2: Table 3, experiment III; cf. Table 5, experiment I). The eggs begin development while in the alkaline solution, but eggs made parthenogenetic by treatment with neutral or acid solutions (neutral sodium chloride or carbonated sea water) begin development only when transferred to natural sea water or other alkaline solution. This bears out our hypothesis, for if the increased permeability remains after the egg is returned to an alkaline medium, a chance is given for an increase in alkalinity in the interior, which was lacking in the non-alkaline solution.

Certain facts may seem to contradict our assumption, but probably they merely limit its application:

1. A slight increase in ^{-}OH ions may cause even the unfertilized egg to absorb more oxygen (Table 2) and a greater increase causes it to develop. This does not necessarily show permeability of the unfertilized egg to hydroxyl ions. The increased alkalinity slowly causes an increased permeability of the egg and thus leads to parthenogenesis, but the degree of alkalinity of the medium necessary to induce development of the unfertilized egg is far greater

[1] These experiments will be published later in the *American Journal of Physiology*.

than that necessary for the development of the fertilized egg or the egg already made parthenogenetic.

2. The fertilized egg of *Arbacia punctulata* (but not of some other sea urchins) may develop in a natural medium, as Loeb observed and which we have confirmed. In other words, a hydroxyl ion concentration in the medium, greater than that of distilled water, is not necessary for development of this egg made freely permeable to carbonic acid. However this fact does not set a limit to the alkalinity of the egg interior. The egg probably contains more Na than Cl ions, and if it be impermeable to Na or Cl, the escape of carbonic acid might cause the egg interior to become alkaline or at least neutral. Eggs made parthenogenetic in some ways (neutral sodium chloride, for instance) do not develop unless transferred to an alkaline medium, but this may be due to the possibility that these parthenogenetic eggs are not quite as permeable as are fertilized eggs. The same may be inferred from oxidation measurements. Neutral sodium chloride causes the unfertilized egg to absorb more oxygen than it does in sea water (Table 4) but the increase is slight, and morphological development does not commence. If the eggs are then transferred to sea water or other alkaline solution, some of them may develop.

It appears therefore that increase in permeability is a gradual process. Although some eggs are so permeable as to be able to develop in an neutral medium others are less permeable and do not develop, or develop only in an alkaline medium. By treating eggs with parthenogenetic agents in various concentrations or for various lengths of time we may induce various degrees of permeability. Even fertilized eggs may be made more permeable by treatment with parthenogenic agents, and a corresponding increase in oxidation may be observed (Table 6). In these experiments the oxidation of the eggs in sea water was measured about ninety minutes after fertilization: they were then placed in isotonic, alkaline sodium chloride solution, in which the oxidation increased one-half, when returned to sea water the oxidation fell below its previous level in the same medium. According to Loeb, this indicates death of some of the eggs (20 per cent).

The experiments just described explain the discrepancy between the results of Warburg and those of Loeb. Warburg[1] found that

[1] Warburg: *loc. cit.*

the oxidation of the *fertilized egg* in isotonic sodium chloride solutions containing a trace of sodium cyanide, is much greater than in sea water containing the same concentration of sodium cyanide. Loeb[1] confirmed this determination, but observed further that if the cyanide is omitted (from both) no increased oxidation in sodium chloride solution occurs. The cyanogen in both sea water and sodium chloride solution depresses oxidation. Since sodium cyanide liberates ^{-}OH ions, we may conclude that the increase in oxidation in the sodium chloride solution used by Warburg was due to the increased penetration of hydroxyl ions, following increase of permeability.

In our experiments no cyanide was used, and the alkalinity of the sodium chloride solution was not greater than sea water, yet oxidation was increased. In Loeb's experiment the tendency of increased permeability to increase oxidation was counteracted by the effect of lower alkalinity, which decreases oxidations.

Alkaline sodium chloride solution also favors oxidation in eggs that have reached later stages of development, morula or blastula (Table 7). In this experiment, the rate of oxidation in sea water was rising gradually (see next section) before the eggs were placed in the alkaline soduim chloride solution, but in the latter a sudden increase of more than 50 per cent was observed.

MATERIALS AND METHODS.

The eggs of the sea urchins, *Arbacia punctulata*, were used. The animals were washed in a strong stream of fresh water and opened with precautions against introducing spermatozoa among the eggs. The ovaries were removed and placed in the first solution to be used, sea water or neutral van't Hoff's solution. The mass was strained through bolting cloth of such a grade as to allow but one egg to pass through one mesh at a time. The eggs were repeatedly precipitated by gravity in fresh portions of the solution in order to remove coelomic fluid cells (elaeocytes), and transferred with a small quantity of fluid to the determination flask.

The rate of oxidation in the various solutions was measured by comparison of the dissolved oxygen in the solution before and after the eggs had been suspended in it during a definite period.

[1] Loeb and Wasteneys: *Biochem. Zeitschr.*, xxviii, p. 340, 1910.

Winkler's thiosulphate method of oxygen determination (iodometric), as described in Treadwell's Quantitative Analysis, was used.

From 3 to 7 cc. of eggs were used in each experiment, but the actual volume of the eggs was not measured until after the oxygen determinations.

We tried a number of methods for filling the determination flask and sample bottles without an uncertain loss or gain of oxygen. Loeb collected the water in the sample bottle under petroleum. Although petroleum absorbs five times as much oxygen as water does, the oil would tend to reduce currents adjacent to the air-water surface, and thus reduce oxygen exchange. Using paraffin oil, we found that it was extremely difficult to prevent a little oil from sticking within the flask, and abandoned the method. Perhaps kerosene would have worked better, yet the quantity of kerosene that would dissolve in the water might vitiate the experiments.

Since the sea water and the solutions were shaken up and saturated with air at the given temperature before beginning the experiment, the control sample might have been taken under air, without change. But after loss of oxygen in the determination flask, a gain in oxygen would result from such treatment. By introducing the solution through a tube passing through a doubly perforated stopper, and extending to the bottom of the sample bottle, the exposed surface of the water was made as small and quiet as possible. By maintaining a constant rate of flow the error could be made to bear an approximately constant ratio to the oxidation, no matter whether the sample bottle was filled with air or some other gas. We tried the effect of introducing the sample under air, and also under hydrogen, and decided that the latter method was preferable for oxygen-low samples. In order to make all errors fall in the same direction we also collected the oxygen-high samples under hydrogen.

The experiments were so regulated that the oxygen content of the determination flask at the end of the exposure would not fall very low. However, Warburg failed to observe a decrease in the rate of oxidation in low oxygen concentration.

The water was forced out of the determination flask rapidly into the sample bottle by hydrogen under pressure. The determination

flask held 332 cc., the two sample bottles 152 and 142.6 cc. respectively.

The determination flask was placed in a thermostat which was kept 2° above the temperature which the air had reached at the beginning of the experiment. In most cases the time of exposure was one hour. During the first half-hour the eggs were distributed throughout the solution once every five minutes by rotating and rocking the flask, during the last half hour they were allowed to settle to the bottom.

Although the majority of the eggs settle to the bottom in ten minutes, at the end of one-half hour there are always a few which, on account of swelling or fragmentation, have failed to precipitate. To prevent these going over into the sample bottle and causing an error due to absorption of iodine, Loeb placed filter paper over the outlet. We found that a relatively hard filter paper was necessary to retain all fragments of eggs, and that this interfered with the rapid transfer of the solution. The error due to eggs in suspension is negligible, especially since it was practically constant in all of our experiments. For instance: a sample bottle filled with water contained 8.09 parts per million of oxygen, while water from the same jar run into a sample bottle in which had been placed about one-hundred times as many eggs as the water from the determination flask, contained 7.85 parts per million, an error of 0.24 parts per million due to eggs in suspension. As in our experiments, differences of 0.1—3.0 parts per million were obtained, an error of 0.024 parts per million would not reverse the results.

When the eggs were fertilized or placed in parthenogenic solutions they lost sufficient red pigment (McMunn's Echinochrome) to color the water a straw yellow. In order to ascertain whether this organic matter would vitiate the results, we took two sample bottles, into one of which was placed a mass of elaeocytes containing about fifty times as much echinochrome as is lost from the eggs in one experiment, and syphoned into each tap water from the same jar. Tap water causes these cells to liberate their pigment. The bottle containing water only, was found to hold 6.85 parts per million of oxygen, while that contaminated by elaeocytes was titrated as 6.36 parts per million of oxygen. We repeated this using three sample bottles. Two of these filled with water gave

6.86 and 6.91 parts per million respectively and the third contaminated with elaeocytes liberating about one hundred times as much pigment as is liberated in the experiments with eggs, gave 6.09 parts per million as the titration, showing an error of 0.8 parts per million. Probably this loss was due chiefly to the broken up cells, but if due entirely to the pigment, the error in our experiments would be only .008 parts per million.

In experiment 3, the eggs were divided into two equal portions and placed simultaneously in two determination flasks of equal capacity. Therefore the eggs in the two solutions were in the same stage of ripeness. In each of the other experiments the eggs were all placed in one flask and treated successively with the various solutions. In case of fertilized eggs Warburg observed[1] that the oxidation rate rose steeply from fertilization to the 2-cell stage then gradually to the 64-cell stage. In order to determine whether this would be a great source of error on our experiments, we measured the oxygen used by a mass of fertilized eggs in sea-water during successive periods in the first six hours of development, and found it to vary from 7.93 to 9.76 tenths of a milligram (Table 7). Within the duration of the majority of our experiments, however, the variation was only from 7.93 to 8.96 tenths of a milligram or 11.5 per cent, and would be less between successive exposures. This possible source of error would not reverse the results of the majority of our experiments.

In making solutions of the same alkalinity as the sea water, a colorimetric method was used, with phenolphthalein as indicator. At the end of the season, the sea water was diluted with heavy rains and failed to color phenolphthalein. The eggs behaved abnormally, though whether this was due to the decrease in alkalinity or salinity of the sea water or to some other cause was not determined.

In making the eggs parthenogenetic with carbon dioxide, they were placed with a small quantity of sea water in a "sparklet syphon" and charged under slight pressure for about one minute. At the end of five minutes they were poured into a large volume of sea water and this was syphoned off and fresh sea water added.

[1] Warburg: *Zeitschr. f. physiol. Chem.*, lx, p. 443, 1909 and *loc. cit.*

RESULTS OF EXPERIMENTS.

I. Experiments with Unfertilized Eggs.

TABLE 1.

Oxidation in neutral van't Hoff's solution contrasted with that in neutral NaCl solution.

EXPERIMENT	SOLUTION USED	VOLUME OF EGGS	TEMPERATURE THERMOSTAT	DURATION OF STIRRING	DURATION OF SETTLING	OXYGEN USED	REMARKS
		cc.	*°C*	*min.*	*min.*	$\frac{mg}{10}$	
First period..	Neutral van't Hoff	4	24	30—	30	2.49	
Second period	Neutral $\frac{M}{2}$ NaCl	4	24	30	30	5.47	No "fertilization membrane" formed.

TABLE 2.

Oxidation in neutral van't Hoff's solution contrasted with that in sea water and alkaline NaCl solution.

EXPERIMENT	SOLUTION USED	VOLUME OF EGGS	TEMPERATURE THERMOSTAT	DURATION OF STIRRING	DURATION OF SETTLING	OXYGEN USED	REMARKS
		cc.	*°C*	*min.*	*min.*	$\frac{mg}{10}$	
First period..	Neutral van't Hoff.		24	30	30	2.29	
Second period	Sea water		24	30	30	2.65	
Third period	$\frac{M}{2}$ NaCl +−OH ions		24	30	30	3.12	"Fertilization membranes" in very small per cent.
Fourth period	Sea water		24	30	30	3.48	

TABLE 3.

Oxidation in sea water contrasted with that in alkaline NaCl solution.

EXPERIMENT	SOLUTION USED	VOLUME OF EGGS	TEMPERATURE THERMOSTAT	DURATION OF STIRRING	DURATION OF SETTLING	OXYGEN USED	REMARKS
		cc.	*°C*	*min.*	*min.*	$\frac{mg}{10}$	
I. One-half of eggs.....	Sea water		23	30	30	4.80	
Second half of eggs.....	$\frac{M}{2}$ NaCl + $^{-}$OH ions		23	30	30	6.90	"Fertilization membrane" formed.
II. First period.....	Sea water		24.5	35	25	3.38	
Second period	$\frac{M}{2}$ NaCl + $^{-}$OH ions		24.5	35	25	7.80	"Fertilization membrane" formed.
III. First period.....	Sea water	4.5	24	30	30	6.04	
Second period	$\frac{M}{2}$ NaCl + $^{-}$OH ions	4.5	24	30	30	6.47	"Fertilization membranes" in very small per cent.

TABLE 4.

Oxidation in sea water contrasted with that in neutral NaCl.

EXPERIMENT	SOLUTION USED	VOLUME OF EGGS	TEMPERATURE THERMOSTAT	DURATION OF STIRRING	DURATION OF SETTLING	OXYGEN USED	REMARKS
		cc.	*°C*	*min.*	*min.*	$\frac{mg}{10}$	
I. First period.....	Sea water	5	23	30	30	2.65	
Second period	Neutral $\frac{M}{2}$ NaCl	5	23	30	30	3.60	No "fertilization membrane" formed.
II. First period....	Sea water	2.3	23.5	30	30	1.22	
Second period	Neutral $\frac{M}{2}$ NaCl	2.3	23.5	30	30	1.66	Ibid.

TABLE 5.

Effect of CO_2-parthenogenesis on oxidation. Effect of fertilization.

EXPERIMENT	SOLUTION USED	VOLUME OF EGGS	TEMPERATURE THERMOSTAT	DURATION OF STIRRING	DURATION OF SETTLING	OXYGEN USED	REMARKS
		cc.	*°C*	*min.*	*min.*	$\frac{mg}{10}$	
I. First period.....	Sea water	4.5	23.5	30	30	4.58	
After CO_2 treatment..	Sea water	4.5	23.5	30	30	6.00	"Fertilization membranes" in small per cent.
II. First period.....	Sea water	4.5	24	30	30	4.11	
After CO_2 treatment..	Sea water	4.5	24	30	30	9.91	Good membrane formed.
III. First period.....	Sea water		25	30	30	2.52	
After fertilization.....	Sea water	5	25	30	30	12.94	98 per cent of eggs segmented.

SUMMARY.

1. The presence of ^{-}OH ions in the medium, increases the rate of oxidation in fertilized eggs of the sea urchin.

2. The oxidation rate of unfertilized eggs is increased by fertilization or any treatment which causes them to develop parthenogenetically.

In 1 and 2 we merely confirm and extend the observations of Warburg.

3. Since it was shown by the senior author that fertilization or parthenogenesis means increased ionic permeability of this egg, and that the *Fundulus* egg, even after fertilization, is impermeable to ^{-}Cl ions, the increase in permeability probably applies so far as the anions are concerned, to ^{-}OH and $^{-}HCO_3$ or $^{-}CO_3$ ions. The carbonic acid anions are more concentrated within, and their outward diffusion would cause a potential gradient which would pull other

II. Experiments with Fertilized Eggs.

TABLE 6.

Oxidation in neutral van't Hoff's solution contrasted with that in sea water and alkaline NaCl.

EXPERIMENT	SOLUTION USED	VOLUME OF EGGS	TEMPERATURE THERMOSTAT	DURATION OF STIRRING	DURATION OF SETTLING	OXYGEN USED	REMARKS
		cc.	*°C*	*min.*	*min.*	*mgr./10*	
I. First period.....	Neutral van't Hoff	3.6	23	30	30	6.47	Contained some sea water.
Second period	Sea water	3.6	23	30	30	7.43	
Third period.....	$\frac{M}{2}$ NaCl + $^-$OH ions	3.6	23	30	30	12.28	
Fourth period	Sea water	3.6	23	30	30	5.97	Very few eggs dead.
II. First period.....	Neutral van't Hoff	4	23	30	30	3.91	Eggs washed in van't Hoff sol. and fertilized in it.
Second period	Sea water	4	23	30	30	7.17	
Third period	$\frac{M}{2}$ NaCl + $^-$OH ions	4	23	30	30	7.53	
Fourth period	Sea water	4	23	30	30	6.27	

anions, hence $^-$OH ions, into the egg, thereby increasing the internal concentration of $^-$OH ions. This increase in $^-$OH ions probably causes the increased oxidation.

4. The increase of $^-$OH ions in the medium causes even in unfertilized eggs, an increased oxidation. This is not interpreted as indicating that the unfertilized egg is normally permeable to $^-$OH ions, but that increased alkalinity causes increased permeability.

5. Increase in permeability is a gradual process. Beginning with the relatively impermeable unfertilized egg, and denoting degree of permeability by numerals, we have the following series,

TABLE 7.

Effect of alkaline NaCl on oxidation six hours after fertilization.

EXPERIMENT	SOLUTION USED	VOLUME OF EGGS	TEMPERATURE THERMOSTAT	DURATION OF STIRRING	DURATION OF SETTLING	OXYGEN USED	REMARKS
		cc.	*°C*	*min.*	*min.*	$\frac{mg}{10}$	
First period..	Sea water	7	22	20	30	8.90	Thirteen minutes after fertilization.
Second period	Sea water	7	22	20	30	8.96	2-cell stage.
Third period	Sea water	7	22	20	30	8.33	4-cell stage.
Fourth period	Sea water	7	22	20	30	7.93	16-cell stage.
Fifth period..	Sea water	7	22	20	30	8.13	32-cell stage.
Sixth period..	Sea water	7	22	20	30	9.76	Many retarded.
Seventh period.....	$\frac{M}{2}$ NaCl + $^{-}$OH ions	7	22	20	30	13.77	Many blastulae.
Eighth period	Sea water	7	22	20	30	8.98	All alive.

I. slightly increased oxidation, II. greater increase in oxidation rate, and imperfect "fertilization membrane" formation, III. oxidation still further increased, membrane formation perfect, followed by segmentation of the egg, IV, *ditto* except that oxidation is still further increased, and the eggs die sooner or later if oxidation is not reduced, V. oxidation enormous, membrane formation but no segmentation, premature death.

It is supposed that the primary effect of many toxic substances is an abnormal increase in the permeability of the egg, and fertilized eggs are more susceptible because they are already more permeable than unfertilized eggs.

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THE SIGNIFICANCE OF THE TIME AT WHICH GAS IS PRODUCED IN LACTOSE PEPTONE BILE.

BY WILLIAM W. BROWNE.

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THE SIGNIFICANCE OF THE TIME AT WHICH GAS IS PRODUCED IN LACTOSE PEPTONE BILE

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THE SIGNIFICANCE OF THE TIME AT WHICH GAS IS PRODUCED IN LACTOSE PEPTONE BILE.

WILLIAM W. BROWNE, PH.D.,
College of the City of New York.

During the summer of 1912, routine bacteriological examinations of the oysters of Narragansett Bay were made with the hope of determining the extent of the pollution of the oyster beds of Rhode Island by the sewage of cities and towns bordering on the bay. The examinations were conducted under the direction of Prof. F. P. Gorham of Brown University to whom the writer wishes to extend his thanks for the data here used. Throughout the entire series of experiments, the examinations of the oysters were made in general, according to the methods proposed in The Second Progress Report of the Committee on Standard Methods of Shell Fish Examination (December, 1911). In all cases, meat extract (3 grams to the liter) was used in the preparation of the media. Ease of preparation rendered its use invaluable in a work of this nature.

Lactose peptone bile was used as a presumptive test to indicate the presence of members of the Bacillus coli group and other lactose fermenters of intestinal origin. Inverted vials were used for the collection of the gas and the tubes were always incubated at 37° C. All inoculation into lactose peptone bile were made in duplicate and the gas was read and recorded at the end of 24-, 48- and 72-hour periods. In this paper are compiled the results obtained by comparing the number of tubes showing gas at the various periods.

During the period covered by this paper, 119 samples of oysters were examined. The beds from which these oysters were dredged varied greatly in character and position ranging from the highly polluted areas near the outlets of the sewers of the cities and towns through questionable areas which were sometimes coli-positive and again coli-negative to areas which were comparatively free from pollution near the ocean. During the examination of these 119 samples of oysters 3,570 tubes of lactose peptone bile were inoculated from which 1,929 produced gas distributed over the 24-, 48- and 72-hour periods as the following table will show:

TABLE I.

LACTOSE PEPTONE BILE TUBES SHOWING GAS.

Time in Hours	24	48	72
Number of Tubes Showing Gas	148	1361	420
Per cent	7.6	70.5	21.2

From the above table it can be seen that very few of the intestinal organisms, generally found in oysters, are able to produce gas in lactose

peptone bile by the end of the 24-hour period since out of 1,929 which showed gas only 148 or 7.6 per cent. produced gas at the end of that period. By the end of the 48th hour, however, 1,361 tubes or 70.5 per cent. produced gas showing that 48 hours represents the periods in which more than a majority of the intestinal organisms usually found in the oysters, produce their gas in lactose peptone bile. Gas was recorded at the end of the 72-hour period in 420 or 21.2 per cent. The gas produced by the end of the 72d hour probably represents the result of growth of attenuated forms and spore formers since we find more of this type of gas production in places which are distant from sources of pollution as will be explained in a later part of this paper.

An examination of the map of the State of Rhode Island reveals that Narragansett Bay is divided naturally into several well-defined areas by the presence of islands and peninsulas. The author has taken advantage of this fact and for the purposes of comparison, has divided that portion of the bay from which oysters for analysis were taken into eight distinct areas. Some of these areas include rivers which flow into the bay while others include large portions of the bay itself. Oysters were taken from the beds situated in almost every portion of these well-defined areas and at various intervals during the summer of 1912, so that any comparison that may be made between the various districts in the bay seem to the writer to be both reasonable and fair.

Plate I shows the divisions into which the author has divided the bay. A brief geographical description of each district will follow together with its likelihood of being polluted as might be judged both from bacteriological examinations and a superficial sanitary survey.

District I.

District I includes what is known as the Kickemuit River, a small shallow stream flowing into Mount Hope Bay from the north. Because of its shallowness and protection from winter storms, this river offers an ideal place for the cultivation of oysters. Extended bacteriological examinations have shown this river to be a variable locality. The examinations of one period showing the oysters to be of a high sanitary quality in so far as the presence of fecal organisms is concerned while only a few days later, examinations have revealed gross pollution. As there are no towns on its banks, this pollution must necessarily come from Mount Hope Bay. At the present time, it is thought that the wind and tide play a very important part in the varying conditions of this river.

District II.

This district includes Mount Hope Bay and extends from north to south from the state line to Bristol Ferry. The bay has two outlets one emptying into Narragansett Bay at Bristol Ferry while the other opens into the Sakonnet River. Bacteriological examinations reveal the oysters from Mount Hope Bay to be grossly polluted during the greater part of

the year. This condition might be expected since this bay receives the sewage of the City of Fall River and of the cities and towns along the Taunton River.

District III.

District III is known as the Sakonnet River which is nothing more than an outlet for Mount Hope Bay. Being very wide, however, and near the ocean, its waters are greatly diluted and as a result, we find the water of the Sakonnet River to be in a relatively high state of purity bacteriologically.

District IV.

District IV includes all the beds situated in Bristol Harbor and in the waters west of the Island of Rhode Island. Repeated bacteriological examinations have shown this region to be open to great pollution as it is the meeting place of the waters of Narragansett Bay, Bristol Harbor and Mount Hope Bay.

District V.

District V includes the beds in the Warren River which for the greater part of the year are subject to gross pollution.

District VI.

District VI may be designated as that region where the Providence River merges into Narragansett Bay. Bacteriological examinations show evidences of pollution.

District VII.

This district known as Upper Narragansett Bay although open to more or less pollution from the Providence River seems to be almost able to care for about all the pollution which reaches it. Examinations have revealed pollution but not in quantity.

District VIII.

District VIII includes that portion of the bay directly south of District VII and will be designated as Lower Narragansett Bay. Bacteriological examinations have failed to reveal evidences of much pollution.

In summary of the above description, the eight districts or areas may be roughly divided into three main classes as to their possibility of becoming polluted.

TABLE II.

CLASSIFICATION OF THE EIGHT DISTRICTS.

A. Open to Gross Pollution:	
Mount Hope Bay	District II.
Bristol Harbor	" IV.
Warren River	" V.
Providence River	" VI.
B. Variable Locality:	
Kickemuit River	District I.
C. Relatively Free from Pollution:	
Sakonnet River	District III.
Upper Narragansett Bay	" VII.
Lower Narragansett Bay	" VIII.

From the various beds which happened to be situated in the designated sections, oysters were taken to the laboratory and examined bacteriologically as outlined in the early part of the paper. In the following tables, are compiled the results obtained by recording the presence of gas in the lactose peptone bile tubes at the end of the 24-, 48- and 72-hour periods.

TABLE III.

NUMBER OF LACTOSE PEPTONE BILE TUBES SHOWING GAS FROM DISTRICT I.

KICKEMUIT RIVER.

Bed.	24 hours.	48 hours.	72 hours.
1	0	7	6
2	1	16	0
3	0	15	0
4	2	15	1
5	0	17	4
6	0	18	5
7	1	26	1
8	0	0	20
9	0	9	15
10	0	2	11
11	2	17	4
12	0	28	0
13	0	25	2
14	0	18	2
	6	213	71
Per cent.	2.0	73.4	24.4

TABLE IV

NUMBER OF LACTOSE PEPTONE BILE TUBES SHOWING GAS FROM DISTRICT II.

MOUNT HOPE BAY.

Bed.	24 hours.	48 hours.	72 hours.
1	4	10	2
2	1	21	1
3	0	10	0
4	0	10	2
5	1	6	1
6	1	6	0
7	9	6	0
8	1	10	2
9	0	16	1
10	6	6	0
11	0	12	1
12	12	3	1
13	3	18	1
14	5	24	0
15	0	3	3
	43	161	15
Per cent.	19.6	73.4	6.8

TABLE V.

NUMBER OF LACTOSE PEPTONE BILE TUBES SHOWING GAS FROM DISTRICT III.

SAKONNET RIVER.

Bed.	24 hours.	48 hours.	72 hours.
1	0	0	1
2	0	2	4
3	0	1	5
4	0	1	5
5	1	3	4
6	0	3	3
7	0	1	4
8	0	9	4
9	0	13	2
	1	33	32
Per cent.	1.5	50.0	48.4

TABLE VI.

NUMBER OF LACTOSE PEPTONE BILE TUBES SHOWING GAS FROM DISTRICT IV.

BRISTOL HARBOR.

Bed.	24 hours.	48 hours.	72 hours.
1	0	9	0
2	0	19	0
3	13	15	2
4	0	14	1
5	13	8	4
6	0	16	0
7	0	20	0
8	0	19	2
9	0	4	3
10	0	9	0
11	0	9	6
12	0	10	8
13	0	6	4
14	0	4	4
15	2	7	4
16	2	3	1
17	2	21	0
18	0	4	1
19	3	5	8
20	1	22	3
21	0	28	0
22	0	30	0
23	0	30	0
24	0	30	0
25	0	0	15
26	0	13	2
27	0	4	2
	36	349	70
Per cent.	7.9	76.7	15.3

A careful study of Plate II brings out some very interesting points concerning the production of gas in lactose peptone bile. As stated earlier in this paper, a very small percentage of the tubes show gas by the end of 24 hours. We would naturally expect those districts which are open

TABLE VII.

NUMBER OF LACTOSE PEPTONE BILE TUBES SHOWING GAS FROM DISTRICT V.

WARREN RIVER.

Bed.	24 hours.	48 hours.	72 hours.
1	0	26	0
2	1	17	2
3	13	11	3
4	5	10	0
5	0	11	3
6	0	25	1
7	0	18	2
8	0	23	0
9	0	11	3
	19	152	14
Per cent.	10.2	82.1	7.5

TABLE VIII.

NUMBER OF LACTOSE PEPTONE BILE TUBES SHOWING GAS FROM DISTRICT VI.

PROVIDENCE RIVER.

Bed.	24 hours.	48 hours.	72 hours.
1	2	26	0
2	2	12	5
3	1	13	4
4	0	19	2
5	2	17	1
6	1	8	4
7	8	21	1
8	0	13	7
9	0	17	1
10	0	10	4
11	0	13	2
12	0	5	3
13	0	21	0
14	0	30	0
	16	225	34
Per cent.	5.8	81.8	12.3

to gross pollution producing a larger percentage of tubes in 24 hours than those tubes inoculated with material from regions which are comparatively free from pollution. And such is the case. The arrangement of the districts beginning with the areas which show the largest percentage of tubes in 24 hours is as follows:

1. Mount Hope Bay ..... 19.6 per cent.
2. Lower Narragansett Bay 11.4

TABLE IX.

NUMBER OF LACTOSE PEPTONE BILE TUBES SHOWING GAS FROM DISTRICT VII.

UPPER NARRAGANSETT BAY.

Bed.	24 hours.	48 hours.	72 hours.
1	0	19	1
2	0	16	1
3	0	10	6
4	1	7	3
5	0	18	8
6	1	27	0
7	0	6	8
8	0	10	7
9	0	4	6
10	0	8	12
11	0	4	0
12	3	7	2
13	1	11	2
14	0	3	17
15	0	2	11
16	0	11	2
	6	163	86
Per cent.	2.3	63.9	33.7

TABLE X.

NUMBER OF LACTOSE PEPTONE BILE TUBES SHOWING GAS FROM DISTRICT VIII.

LOWER NARRAGANSETT BAY.

Bed.	24 hours.	48 hours.	72 hours.
1	1	15	12
2	3	12	1
3	0	5	6
4	0	5	5
5	4	5	0
6	0	5	2
7	10	1	0
8	0	9	17
9	0	4	5
10	0	0	10
11	0	4	12
12	0	0	7
13	1	1	6
14	0	0	5
15	2	0	9
	21	66	97
Per cent.	11.4	35.8	52.7

3. Warren River ..... 10.2 per cent.
4. Bristol Harbor 7.9

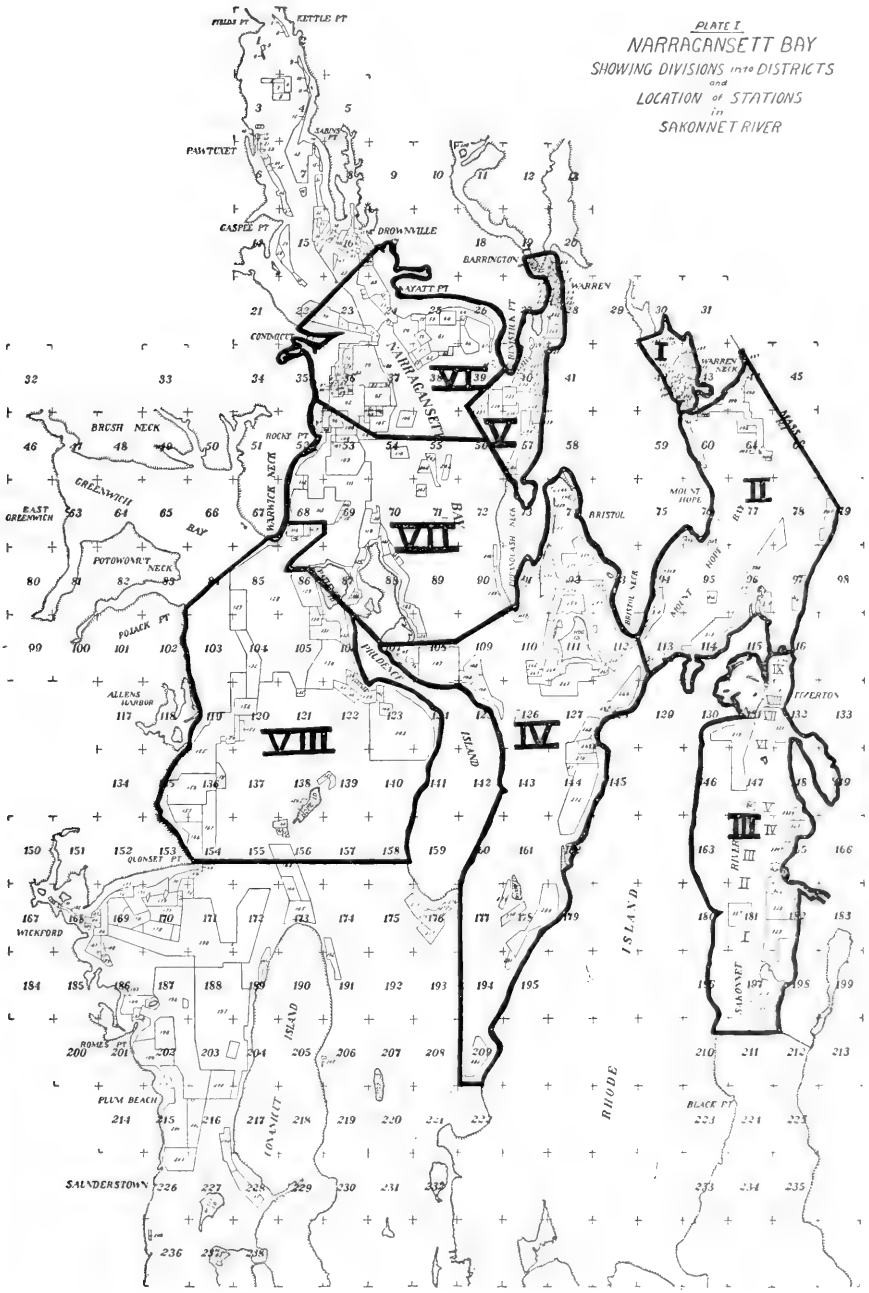
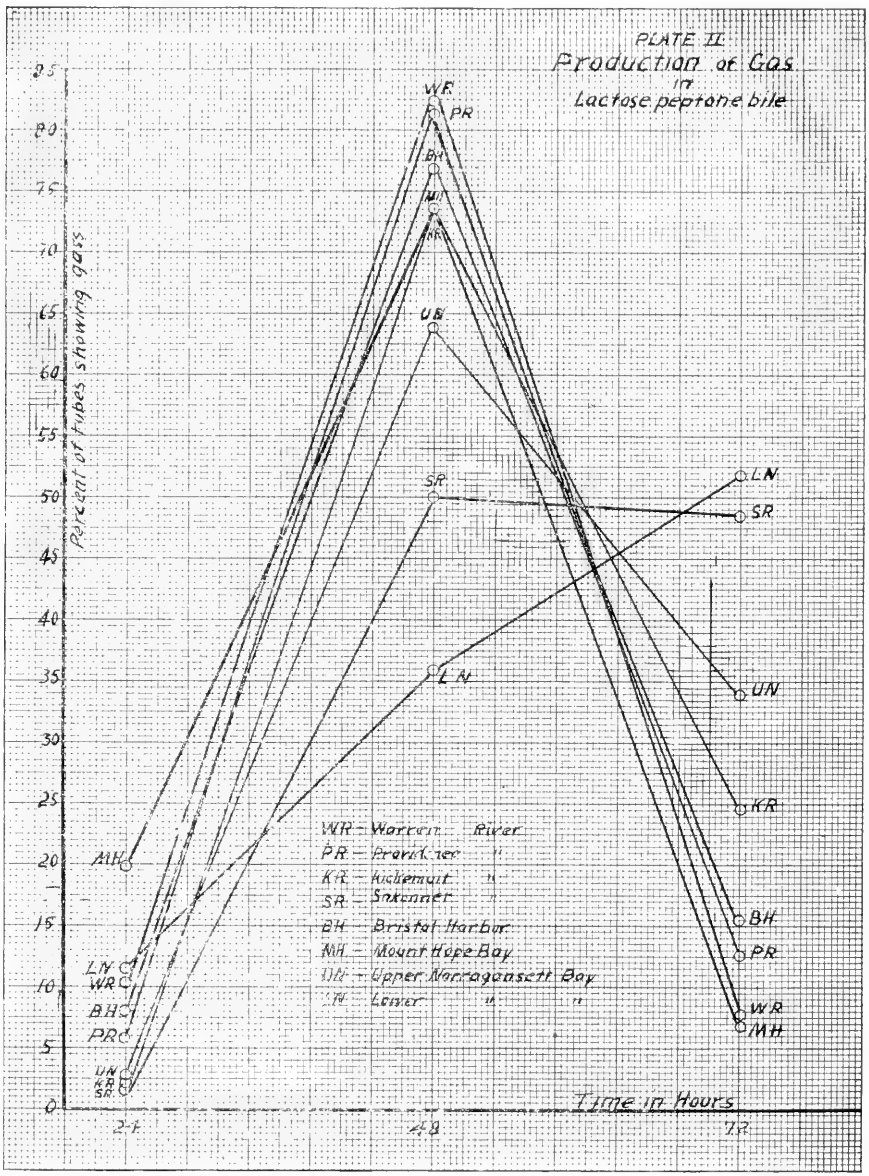


PLATE I
NARRAGANSETT BAY
SHOWING DIVISIONS into DISTRICTS
and
LOCATION of STATIONS
in
SAKONNET RIVER
PHILIPS PT
KETTLE PT
PAWTUCKET
GASPEE PT
DROWNVILLE
BARRINGTON
WARREN
LAYATT PT
CONIMICUT
NARRAGANSETT BAY
BUCKEIAH PT
WARREN NECK
BRUSH NECK
ROCKY PT
WARWICK NECK
GREENWICH BAY
EAST GREENWICH
POTOWOMUT NECK
POLLACK PT
BRISTOL
MOUNT HOPE
BAY
PHILIPSDALE
ALLENS HARBOR
TIVERTON
ISLAND
QUONSET PT
WICKFORD
ISLAND
ROMES PT
PLUM BEACH
CONANICUT
SAUNDERSTOWN
RHODE
ISLAND
BLACK PT
SAKONNET
I
II
III
IV
V
VI
VII
VIII
IX

5. Providence River ..... 5.8 per cent.
6. Upper Narragansett Bay 2.3
7. Kickemuit River 2.0



8. Sakonnet River ..... 1.5 per cent.

From this list it can be seen that districts like Mount Hope Bay, Bristol Harbor, Warren River and Providence River which are open to pollution

roughly group themselves at the top of the list while districts which are distant from sources of pollution like the Sakonnet River, Kickemuit River and Upper Narragansett are found at the bottom of the list. The district designated as Lower Narragansett Bay has cast its lot with the polluted areas as a result of a polluted sample from bed seven.

This division is still more apparent at the end of the 48th hour. The arrangement of the districts beginning with the districts having the largest percentage showing gas at the end of 48 hours is as follows:

1. Warren River.....82.1 per cent.
2. Providence River.....81.8
3. Bristol Harbor.....76.7
4. Mount Hope Bay.....73.4
5. Kickemuit River.....73.4
6. Upper Narragansett Bay.....63.9
7. Sakonnet River.....50.0
8. Lower Narragansett Bay.....35.8

Here we find those grossly polluted areas showing a larger percentage of gas tubes than those districts which are comparatively free from pollution. For instance, the percentage of tubes showing gas from the Warren River is over twice that percentage shown by the gas tubes from Lower Narragansett Bay which now has taken its rightful position among the less polluted areas. The Kickemuit River which has by extended bacteriological examinations been proved to be a very changeable district, occupies a position in the center of the list as we should expect.

At the end of the 72d hour the arrangement of the districts in the order of percentages of tubes showing gas is quite reversed as the the following list will show:

1. Lower Narragansett Bay.....52.7 per cent.
2. Sakonnet River.....48.4
3. Upper Narragansett Bay.....33.7
4. Kickemuit River.....24.4
5. Bristol Harbor.....15.3
6. Providence River.....12.3
7. Warren River..... 7.5
8. Mount Hope Bay..... 6.8

Those regions which are comparatively free from pollution produce much larger percentage than regions which are open to pollution. At the end of the 48-hour period, the percentages of tubes showing gas from the Warren River was over twice as large as that percentage from the Lower Narragansett Bay for the same period. At the end of the 72d hour, the positions are reversed and the percentage of showing gas from the Lower Narragansett Bay is over seven times as large. The same comparison may be made between the other districts with the same result. The Kickemuit

holds the same general position in the center of the list. Generally speaking those districts which were at the top of the list for the 24- and 48-hour periods are found at the bottom at the end of the 72d hour.

The only explanation of these results seems to the author to be that those districts which lie near the source of pollution must necessarily be exposed to vast numbers of healthy vigorous intestinal organisms which only recently have left their native haunts and when these organisms are placed in a suitable culture medium, like lactose peptone bile, a rapid growth with the production of gas must result. Hence, we find that the intestinal organisms isolated from oysters taken from polluted regions produce the greater part of their gas by the end of the 48th hour.

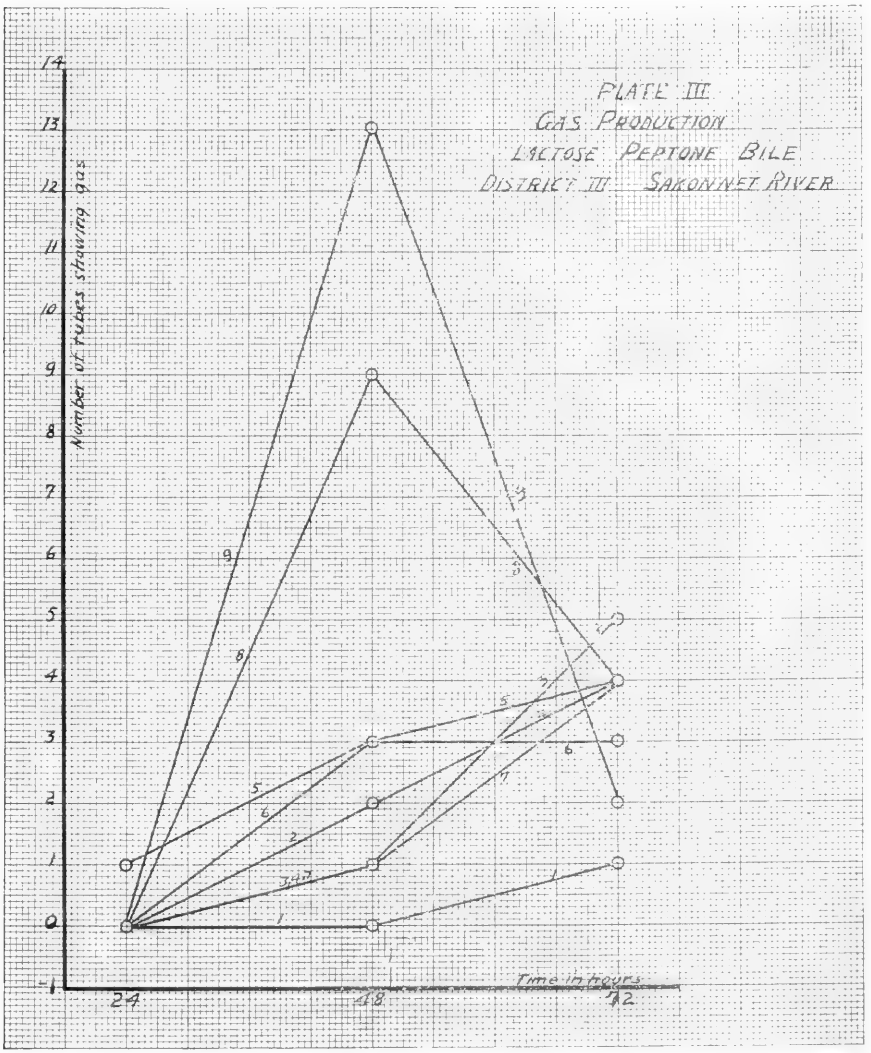
The beds which are situated in districts more remote from the sources of pollution present a different condition. The intestinal organisms which finally reach these beds, must certainly be in an attenuated condition and greatly reduced in numbers due to their long journey through the salt waters of the bay. Hence the growth of the organisms and with that, of course, the production of gas is retarded. The comparison between those districts near the source of pollution which produce the greater part of their gas by the 48th hour and those districts distant from the sources of pollution which produce the greater part of their gas after the 48th hour, may be seen in the following table:

District.	Gas produced at 48 hours in per cent.	Gas produced at 72 hours in per cent.
Mount Hope Bay	93.0	6.8
Warren River	92.3	7.5
Providence River	86.8	12.3
Bristol Harbor	84.6	15.3
Kickemuit River	75.4	24.4
Upper Narra. Bay	66.2	33.7
Sakonnet River	51.5	48.4
Lower Narra. Bay	47.2	52.7

From the above table it may be seen that the percentage of tubes showing gas by the end of the 48th hour decreases as we proceed from those districts open to gross pollution towards the districts which are free from pollution. At the end of the 72d hour things are just opposite. The percentage of tubes which show gas increase as we proceed from the greatly polluted towards the less polluted areas. With the exception of the Lower Narragansett Bay District over 50 per cent. of the tubes show their gas by the end of the 48th hour.

This same conditon was found to be true for the Sakonnet River which is really an outlet for the waters of Mount Hope Bay from which it receives the greater portion of its pollution. The town of Tiverton also con-

tributes a small part. During the summer nine samples of oysters were taken from different portions of the river ranging from the point of greatest pollution near Mount Hope Bay to those beds situated near the ocean. Those beds which were near Mount Hope Bay produced the greater part of their gas by the end of the 48th hour while those nearer the ocean showed



the greatest number at the end of the 72d hour. Plate III, Curve 1, represents the gas produced from oysters taken from beds nearest the ocean and so on up to Curve 9 which represents the gas produced by oysters taken from beds nearest the sources of pollution. The other districts of

the bay, on account of the complicated sources of their pollution, would not lend themselves to this treatment.

From the data gathered together in this paper, one cannot help but be impressed by the strict division obtainable by recording the number of tubes of lactose peptone bile showing gas at the end of the 24-, 48- and 72-hour periods. Those tubes inoculated from oysters taken from highly polluted areas produce almost all their gas by the end of the 48th hour while those tubes inoculated from oysters taken from regions more distant from the source of pollution produce a greater amount at the 72d hour. The writer is of the opinion that this method might be used as a supplement to the regular oyster analysis in the determination of whether the contamination is recent or remote by considering the temporal conditions of gas production in lactose peptone bile.

Summary.

1. Lactose peptone bile tubes inoculated with the shell liquor of oysters taken from 119 different beds of Narragansett Bay produce the greater part of their gas by the end of the 48th hour.

2. Tubes of lactose peptone bile inoculated with the shell liquor of oysters taken from polluted districts produce almost all their gas by the end of the 48th hour.

3. Tubes of lactose peptone bile inoculated with the shell liquor of oysters taken from regions which are comparatively free from pollution produce a larger part of their gas by the end of the 72d hour.

4. Consideration of this temporal factor in the production of gas in lactose peptone bile might aid in the determination of whether the contamination of oysters is recent or remote.

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A COMPARATIVE STUDY OF THE SMITH FERMENTATION TUBE AND THE INVERTED VIAL IN THE DETERMINATION OF SUGAR FERMENTATION.

BY WILLIAM W. BROWNE.

American Journal of Public Health, Vol. III, No. 7, 1913, pp. 701-704.

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WILLIAM W. BROWNE, PH.D.,
College of the City of New York.

During the summer of 1912, a sanitary survey of Narragansett Bay was made under the direction of Prof. F. P. Gorham of Brown University to determine the extent of the pollution of the oyster beds of the State of Rhode Island by sewage of the neighboring cities and towns.

Lactose peptone bile was used as a presumptive test to indicate the presence of the members of the Bacillus coli group and other lactose fermenters of intestinal origin. In the early part of the investigation, the regular Smith fermentation tube, as used at the Lawrence Station, was employed in determining the production of gas in lactose peptone bile, but later shell vials inverted in regular laboratory test tubes were substituted for the fermentation tube.

Immediately the question arose—which was the more efficient in a presumptive work of this kind, the fermentation tube or the inverted vial? This paper is the result of a brief comparative study of the two varieties.

The technic was very simple. Fifty-two oysters from a bed situated in a portion of the bay which was known to be polluted were taken to the laboratory and opened into sterile Petri dishes as proposed in the Second Progress Report of the Committee on Standard Methods of Shell Fish Analysis, Laboratory Section, American Public Health Association, 1911. From the Petri dishes, the shell liquor was inoculated directly into the lactose peptone bile tubes. The fermentation tubes were inoculated in the following dilutions:

1 tube 1 c. c. Shell liquor.
2 tube 0.1 c. c. Shell liquor.
2 tube 0.01 c. c. Shell liquor.

Scarcity of fermentation tubes forced us to economize by using only one tube in the cubic centimeter dilution.

The laboratory tubes containing the inverted vials were inoculated by Mr. George H. Smith, whom the writer wishes to thank for his labors, with the shell liquor from the same Petri dishes in the following dilutions:

2 tubes 1 c. c. Shell liquor.
2 " 0.1 c. c. Shell liquors.
2 " 0.01 c. c. Shell liquors.

The inoculated tubes were incubated at 37° C. and readings were made at the end of the twenty-fourth and forty-eighth hour. Pressure of outside work prevented further readings. No attempt was made to isolate specific organisms as the writer assumed that the production of gas in lactose peptone bile was indicative of the presence of some lactose fermenter of intestinal origin. The presence of gas was recorded by the plus (+) and (−) sign respectively since percentages of gas produced in fermentation tubes and inverted vial are of no quantitative value.

The following table will show the comparative gas production of lactose fermenters isolated from oysters taken from regions known to be polluted in fermentation tubes and inverted vials:

TABLE I.

PERCENTAGE OF EFFICIENCY.

CUBIC CENTIMETER.

	24 hours.		48 hours.	
	No. of Tubes Showing Gas.	Per cent.	No. of Tubes Showing Gas.	Per cent.
Ferment. Tube.....	44	84.6	49	94.3
Invert. Vial.....	49	92.3	50	96.1
ONE TENTH CUBIC CENTIMETER.				
Ferment. Tube.....	45	86.5	47	90.3
Invert. Vial.....	31	59.5	44	84.6
ONE HUNDREDTH CUBIC CENTIMETER.				
Ferment. Tube.....	17	32.6	29	55.7
Invert. Vial.....	12	23.0	22	42.3

An examination of the above table will show that in the cubic centimeter dilution the inverted vial was slightly more efficient than the fermentation. At twenty-four hours the inverted vial was 8 per cent. more efficient but at the end of the forty-eighth hour they were nearly equal. In the tenth cubic centimeter dilution, we find just the reverse to be true. The fermentation tube is much more efficient at both the twenty-fourth and forty-eighth hour periods. At the end of the twenty-fourth hour the difference of their percentage of efficiency is 27 per cent. while at the end of the forty-eighth hour period, as in the cubic centimeter dilution, the

difference is reduced to about 5 per cent. The same condition holds true in the hundredth dilution, except there is still a large difference in percentage of efficiency even at the end of the forty-eighth hour. From this little work, it would seem that the inverted vial is more efficacious in the low dilutions and the fermentation tube is more efficacious in the higher dilutions, as regards the production of gas in lactose peptone bile by lactose fermenters isolated from polluted oysters.

In a comparative work of this kind many irregularities in gas production have appeared which might be interesting to note. They have also appeared with great regularity in our main work on the Sanitary Survey. Carefully planned controls have failed to eliminate them from the work. Conclusions seem to point to some fault either in the method of making dilutions, since these irregularities were more apparent in the higher dilutions, or to the general inefficiency of lactose peptone bile. One of the most glaring irregularities which have appeared from time to time was the presence of gas in one of the duplicates and not in the other. The frequency of this condition may be noted in the following table:

TABLE II.

GAS PRESENT IN ONE DUPLICATE AND ABSENT IN THE OTHER.

Fermentation Tube.

	24 hours.		48 hours.	
	0.1 c. c.	0.01 c. c.	0.1 c. c.	0.01 c. c.
No. of tubes	22	13	22	21
Per cent	42.3	25.0	42.3	40.3

Inverted Vial.

	24 hours.			48 hours.		
	1 c. c.	0.1 c. c.	0.01 c c.	1 c. c.	0.1 c. c.	0.01 c. c.
No. of tubes	9	15	10	1	9	14
Per cent	17.6	28.8	19.2	1.9	17.6	26.9

From the above table it can be easily seen just how frequently these irregularities occur. They are more prevalent in the case of the fermentation tube than in the inverted vial. They also seem to occur in the higher dilution more frequently than in the lower dilution. The presence of

these irregularities certainly demonstrates the fact that in presumptive work with lactose peptone bile we should not depend entirely upon the gas production in a single tube but should use duplicates at least and even triplicates, if possible.

Summary.

As regards the production of gas in lactose peptone bile by intestinal organisms isolated from oysters:

1. Inverted vials seem to be more efficacious in the low dilutions than the fermentation tubes.

2. Fermentation tubes seem to be more efficacious in the higher dilutions than the inverted vials.

Irregularities show that it is not safe to depend upon a single tube to demonstrate fermentation but duplicates should always be used and, if possible, triplicates.

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REPORT OF MOSQUITO CONTROL FOR 1913.

BY FREDERIC P. GORHAM.

Report of Superintendent of Health for the year 1913.

REPORT ON MOSQUITO CONTROL FOR 1913.

BY FREDERIC P. GORHAM.

To the Superintendent of Health:

Dear Sir: The following is my report on mosquito work for the year 1913:

INTRODUCTION.

Previous Work. During the years 1900 and 1901, at the suggestion of Dr. C. V. Chapin, it was my privilege to devote considerable time to the study of mosquitoes in the city of Providence. These investigations were undertaken to determine the species present, their breeding places and their life histories. Primarily, of course, the object was to study the relation between the distribution of malaria and the breeding places of the malaria-carrying mosquitoes with the view of reducing malarial diseases by restricting the propagation of the mosquitoes. A second object was the determination of the breeding places of the common mosquitoes in order to determine whether it might not be possible to diminish or entirely abate the mosquito nuisance in the city. Some of the results of these investigations will be found in the 19th annual report of the Superintendent of Health for the year ending December 31, 1901.

From that time up to the past year little work along these lines has been done in the city although observations on the distribution of mosquitoes have been carried on by both Dr. Chapin and myself and a few experiments in oiling catch basins in certain sections of the city have been made by Drs. Chapin and King and by the Men's Club of the Free Evangelical Congregational Church.

Beginning of the work of the present year. During the later month of 1912, however, interest in the subject was renewed and under the leadership of Alderman John Kelso of the Second Ward, the Board of Aldermen began a consideration of the problem. At the request of the joint standing

committee on finance, Dr. Chapin, on November 29, 1912, presented a report on the feasibility and cost of controlling the mosquito nuisance in Providence as follows:

Health Department, City Hall,
Providence, R. I.,
November 29, 1912.

Henry A. Grimwood, Esq.

Chairman Joint Standing Committee on Finance,

Dear Sir:—

In accordance with your request I hereby present a report on the feasability and cost of controlling the mosquito nuisance in Providence.

Mosquitoes breed only in water.

If the breeding places be destroyed or the mosquitoes be prevented from breeding, they will be exterminated.

Complete extermination is almost impossible, but by persistent and intelligent effort their numbers can be reduced to a very small minimum. In Havana, after two years of work by Dr. Gorgas, the insects were no nuisance at all, and indeed it was not easy to find a single specimen at a season when they were formerly very abundant. But this work involves a very considerable expense. A good deal of effort has been made to get rid of mosquitoes in various parts of New Jersey, especially in Newark, and also on Staten Island, and in the Borough of Queens. The town of Brookline, Massachusetts has for the past ten years carried on a steady warfare against the pests. On a recent visit to Newark, New York and Brookline, I found that the men who have been carrying on this work are very enthusiastic about its success, but enquiry among other citizens indicates that while mosquitoes have been greatly reduced in numbers they have by no means been exterminated. Still, in Brookline, where conditions are most like those in Providence, people in whom I have every confidence tell me that they now sleep out-of-doors without any discomfort from mosquitoes, while formerly this was impossible. It was too late in the season for me to observe personally the presence of mosquitoes.

In Providence there are three classes of places in which mosquitoes breed.

1. Swamps, marshes, pond holes and the reedy banks of streams. A map has been prepared which shows these breeding places and it will be noticed that most of them are in the suburbs away from the centres of population. It will also be noticed that a number of important breeding places are in other cities and towns immediately adjoining Providence. Several breeding places are in the millponds along the Mosshassuck and Woonasquatucket Rivers and I am informed by the mill men, who use the water in finishing their goods, that they would consider the application of oil injurious. The only alternative would be to clear out the brush and weeds, but except in a few instances these areas are not near the centres of population and their eradication might be postponed for the present.

2. The catch basins along the streets, of which there are about 5,500. It is believed that most of the mosquitoes in the central part of the city are from this source.

3. Various receptacles on private property, such as catch basins, cisterns and old cellars, barrels, kegs, buckets, cans, fountains, aquaria, etc., in yards and receptacles on dumps.

The ultimate aim should be to permanently abolish as many of these breeding places as possible. A thorough inspection of private premises should result in the elimination of most of the smaller receptacles for water above referred to. The catch basins must be retained in substantially their present form. The swamps and marshes can be drained, filled or cleared of weeds and in the latter case fish will take care of the mosquitoes. The cost of draining and filling would be very considerable and for this and other reasons this part of the work had best be extended over several years.

The best way of immediately attacking the mosquito problem is by oiling the breeding places. This if properly done will in the catch basins and many pondholes and ditches effectually stop the breeding. It is much more difficult to successfully oil large swamps with bushes, grass and cat-tails, and it is often necessary to do some cutting of grass, and some ditching. Much encouragement was given to the oiling of swamps by the work of Prof. Gorham at Meshanticut last summer, where he succeeded in practically preventing the breeding of mosquitoes in several acres of thickly wooded swamp by oiling alone.

If it is proposed to undertake the work of mosquito eradication in Providence it is recommended:

1. That all breeding places be oiled at intervals of about two weeks from the first of April to the last of September.

2. That a systematic inspection be kept up during the greater part of the same period to discover minor breeding places on private property.

3. That legislation be secured to provide for the destruction of breeding places on private property.

4. That as many of the swampy places as possible be drained or filled thereby materially reducing the cost of oiling and more effectually preventing the breeding of mosquitoes.

It is estimated that for the oiling four gangs of three men and a horse and wagon in each gang would be necessary. At $2.00 per day for each man and for the horse and wagon this would for 160 working days amount to $1280 for each gang. It is estimated that 150 barrels of oil would be needed at about $3.50 per barrel.

Probably four inspectors would be required in April and May and two for the rest of the season. At $2.00 per day this would amount to about $1000.

The total cost of oiling and inspection for one season is therefore estimated at,

Four gangs of oilers	$5,120 00
150 barrels of oil at $3.50	525 00
Inspectors	1,000 00
Supervision	1,000 00
Contingencies	355 00
	$8,000 00

The above represents the entire cost of treatment for a single season, but does not include permanent drainage improvements. The latter are urged as the best means of combating the mosquito nuisance and as far as permanent drainage can be brought about by just so much will the cost of oiling in subsequent years be diminished. I have consulted with the City Engineer in regard to an estimate of the cost of draining or filling the various breeding places and with him made a tour of the city, pointing out the principal localities, and he informs me that he is at present at work upon such an estimate.

Respectfully submitted,

CHARLES V. CHAPIN,

Superintendent of Health.

This report was presented to the Board of Aldermen on January 9, 1913, by Alderman Kelso, together with a resolution appropriating $10,000, to be expended by the Superintendent of Health in a campaign of mosquito extermination. Soon after the resolution was passed in concurrence and approved by the mayor.

Later in the year, owing to lack of funds in the City treasury, $5,000 was withdrawn from the mosquito appropriation and still later on June 23, 1913, another appropriation of $2,500 was made. This gave a total of $7,500 to be expended for mosquito work.

ORGANIZATION AND EQUIPMENT.

In March, 1913, the writer was placed in charge of the work for the season. The information acquired in the earlier years was, of course, immediately available, and consequently there was but little investigation to be done before the actual organization of the work could be begun. Dr. Chapin made a trip to New York, Newark and Brookline, Mass., to study the methods of work there and the writer also visited Brookline for the same purpose.

Subdivision of the city and routing. First of all the map showing the location of all standing water where mosquitoes might breed which had been prepared in the earlier investigations was brought up to date and made as complete and accurate as possible. All the localities were then listed on separate cards, and divided into four sections, covering four parts of the city, with the idea that each section should be cared for by a separate gang. The cards in each section were then arranged in the order in which the locality could be visited in the least time and with the minimum amount of travel. This was possible only by actually going over the ground personally and studying the layout of each locality. Copies of the cards in this order for each section were then made and later given to the several gangs. These constituted their "route books" and were carefully followed throughout the year.

By the courtesy of the Sewer Department we were permitted to make copies of their route books, showing the location of all the sewer catch basins in the city. There were nine of these routes covering the 5,500 catch basins in the city. When we began the work of oiling the catch basins these routes were followed by the oiling gangs.

Equipment. Meanwhile bids were being secured for furnishing horses and wagons suitable for the work. These were finally secured from Mr. A. H. Barney at the rate of $2.00 per day.

Each wagon was equipped with cleats for supporting a barrel of oil in the rear, a lock box for holding tools, rubber boots, etc., under the seat in front, and an outfit of two galvanized iron pails, a shovel, an iron rake, two long-handled brooms, a whisk broom and an oil faucet.

The cost of this outfit was as follows:

Oil faucet	$ .31
2 pails at .17	.34
Shovel	.69
Rake	.35
2 brooms at .29	.58
Whisk broom	.09

At first ordinary watering pots were included in the outfit, but these were later discarded as they used too much oil and were not convenient. Later a cup was added for applying oil to the catch basins.

In addition to the outfit on each team a bush scythe was provided to be used in common by all the gangs as needed, and a flat-bottom punt which was small and light so that it could be carried on the wagon yet was strong enough to support a man and a pail of oil. This was used in common by the different gangs. The scythe, snath and stone, cost $1.60 and the boat $7.50.

Labor. Each of the four gangs consisted of two laborers and a foreman. The foremen were paid at the rate of $2.25

per day and the laborers $1.85, with the understanding that they were to furnish their own boots. Early in the work it was found that rubber boots became practically useless after a few weeks, so rapidly did the oil attack the rubber. Later the men adopted high leather boots which served very well. The men started at 7 A. M., or earlier if they preferred, and worked for 9 hours each day. During August they worked but one half day Saturdays.

Oil. Ordinary fuel oil was used, obtained from the Standard Oil Co. The price paid was 9 cents per gallon during April and part of May, 8 1-2 cents during the remainder of May, June and part of July, and 8 cents for the rest of July, August and September. This was the price paid per gallon delivered in barrels. There was a rebate for the barrels returned which made the net price paid per gallon range from 7.6 cents the highest, to 6.6 the lowest.

At first the oil was applied without dilution, but later it was found that an emulsion made by mixing one half oil and one half water, mixed thoroughly just before using, spread much more easily and the same amount of oil appeared to go farther than when the whole oil was used. This emulsion was particularly useful where the grass was high, as it seemed to reach the water much more easily than the clear oil.

As far as possible we tried to apply this oil at the rate of one gallon to 1200 square feet, although often it was necessary to use a greater amount where there was a current in the water or where the grass and reeds were thick.

Daily Report Cards. The following cards were provided on which the man in charge of each gang reported daily the work of his gang.

GANG NO..... DATE

LABORERS .... AND

LOCALITY	TIME STARTED	TIME FINISHED	AMOUNT OF OIL USED

IN CHARGE

METHODS OF WORK.

Applying the Oil. For petrolizing swamps and standing water each of the four gangs consisted of 3 men and a large wagon equipped as previously mentioned. On reaching the water to be oiled, the brush would be cut with the scythe whenever it was necessary in order to reach the water, and any grass, sticks or debris raked from the water as far as practicable. In applying the oil the boss would precede with a bucket of oil or the oil emulsion previously mentioned and with the small whisk broom scatter the oil as evenly as possible over the water surface.

The whisk broom was found fully as effective as any sprinkler on the market and far more economical. Spots twenty feet or more distant can easily be covered by dipping the broom in the bucket of oil and flicking the oil from the broom. The men soon become experts at this work. It may be that some form of knapsack sprayer would be more convenient and we shall make investigations along this line before another season. The two laborers would follow with their long handled brooms and thoroughly puddle the oil into all parts of the water, taking particular care to see that every little space among the grass, weeds or brush was covered with

the oil film. The puddling is a most essential part of the process and should not be omitted. One man could spread more than enough oil to keep the other two busy and many times the oiler had to stop and take a hand at the puddling. Usually about one barrel of oil could be spread in one day, but in certain localities where large areas were treated, two barrels would be used.

Oiling Catch Basins. In oiling the catch basins one man would remain in charge of the wagon and the laborers would walk along, one with a pail of oil and a tin cup for applying the oil, about 4 ounces to each basin, the other with the hook for lifting the covers and a pole with which to spread the oil in the basin. About 400 catch basins could be oiled in one day although at times as many as 800 have been oiled by one gang in one day.

Draining. In many localities, particularly in the early spring before any oil was applied, some intelligent ditching, even such as could be done by the three men in each gang, greatly reduced the area of the water to be oiled. Particularly after the first application of oil and after the men had become somewhat familiar with the territory, a very considerable amount of draining was accomplished thus greatly lessening the amount of oil and labor required in the subsequent treatment.

Probably the greatest amount of ditching was on the salt marshes, particularly in the neighborhood of Butler Hospital and Pitman street on the Seekonk, and Allen's Avenue at the City Dump and Old Maid's Cove. At the Butler Hospital we had the active cooperation of the Hospital authorities and much of the ditching was done by them.

Along the west side of the Blackstone Boulevard and between the Boulevard and Hope Street, much ditching was done by our own gangs. In this neighborhood the installation of the new boulevard sewer and the building of catch basins by the sewer department between the Boulevards, and by the Swan Point Cemetery owners on their land to the west of the Boulevard, will materially reduce the amount of standing water another year.

Frequency of Applying Oil. We endeavored as far as possible to keep a film of oil on all standing water throughout the season. In those localities where considerable current existed, a more frequent application of oil was necessary than in those cases where the water was quiet. There was always a tendency for the film of oil to be carried by the wind and in large open areas of standing water the film would shift about from shore to shore with changes in the wind. As far as possible we tried to visit every locality throughout the season at least once every ten days or two weeks. If a considerable film of oil remained, of course, no more was applied. In the catch basins regular application of oil was made once every two weeks irrespective of rain fall or cleaning of the basins.

Supervision of Gangs. At the beginning of the season and until the men in charge of the gangs became familiar with their work and with their several territories, it was necessary for the writer to be almost continually present to direct and instruct the men in their work. By the use of an automobile this was made possible so that each gang could be personally instructed as to the treatment of each locality. Later it became necessary simply to be present when the gangs started out each morning to see that they were fully equipped with men and materials and that they knew where to go and what to do. Then a single inspection each day sufficed to keep the work going.

Length of the Season. Oiling of the swamps and standing water was begun April 3, 1913 and was continued until September 13, 1913 when all mosquito work was suspended on account of lack of appropriation. Oiling of catch basins was begun on June 6, 1913 and continued until September 13, 1913.

Inspection of Dumps, Yards, Etc. Other communities which have attempted mosquito control work have laid particular stress on the necessity of careful inspection of the dumps and the destruction of bottles and cans which hold water and breed mosquitoes. We were prepared to begin such inspection

and to have our gangs visit the dumps and destroy all mosquito breeding receptacles. During the whole mosquito breeding season, however, frequent and thorough inspection failed to show that mosquitoes were breeding in these places. Consequently the gangs were not withdrawn from the oiling for work on the dumps.

The necessity of a very careful inspection of yards, on the other hand, was very early noted. Barrels and tubs and unused garbage cans and private catch basins which were breeding mosquitoes, were far more common than we had ever supposed. Owing to our limited funds but one inspector was employed in this work and only a portion of the city was covered. The number of mosquito breeding places which he located and abated, however, warrants us in planning for a very thorough inspection of this sort another season. About every greenhouse, stable, lumber yard, various sorts of receptacles were found holding water and breeding mosquitoes enough to disturb the whole neighborhood. More active cooperation on the part of householders would greatly help this part of the work.

In many yards and about stables and garages frequently private catch basins are located in which mosquitoes were found breeding. Whenever such basins were located they were added to our lists of city catch basins and were regularly oiled by the catch basin gangs.

Later in the season our attention was called to the traps on the sewer pipes leading from each house. These usually have an air vent which opens in the yard between the house and the street and is frequently open or covered with a perforated top. If the houses are closed for any length of time the water in these traps might breed mosquitoes, although a rain fall would flush them completely as the eaves of the houses are connected with these pipes. No mosquito larvæ were found in these during the past season, but they should be considered a possible breeding place.

RESULT OF THE WORK.

As was to be expected in the first year of such work complete success was not attained. While some parts of the city which had suffered severely from the pest in past years were practically free from mosquitoes throughout the season, other parts of the city were not. Careful records were made of the distribution of mosquitoes during the course of the work with the hope that the sources of the trouble might be discovered and abated. In most cases by the time the mosquitoes became abundant it was too late to accomplish much during the present year, but from the experience gained this year, more care can be taken in subsequent years.

The district in the neighborhood of the Blackstone Boulevard, Cole Avenue, Sessions Street and Morris Avenue has always been an abundant mosquito territory. Most of the mosquitoes in this section breed in the swamps between the Boulevards, between the Boulevard and Hope Street, and along Cole and Rochambeau Avenues. These swamps are always full of water in the spring and by the middle of summer are usually dry. It is the earlier varieties of mosquitoes which give the most trouble in this section. We were prepared to wage an active campaign in this part of the city from the very first of the season, but in spite of our efforts, even as early as the 18th of May, mosquitoes were quite abundant, particularly in the woods along Cole Avenue. The mosquitoes were of the striped leg variety of wood mosquito and were identified as *Ochlerotatus subcantans*.

The great extent of these swamps and the depth of the water made it almost impossible to keep a film of oil on all portions of them and undoubtedly some of the earlier broods of this species, which is our earliest of spring varieties, escaped our efforts to destroy them. The only possible way of dealing with this locality is to drain it and fortunately the construction of the new sewer along Hope Street and in the Boulevard will do this to a very considerable extent so that next season we may hope for better results in this part of the city.

As far as could be learned, other parts of the city were free from mosquitoes during all of May and June. There were no other localities within the territory which we were oiling which could not be thoroughly treated.

Such reports as the following were very gratifying to those in charge of the work.

"THE WAR ON MOSQUITOES.

Results So Far Show Wisdom of Further Appropriations.

To the Editor of the Sunday Journal:

The Health Department deserves the highest commendation for its effective work in its campaign against mosquitoes. It is little short of remarkable that certain sections of the city that were almost unhabitable a year ago are at this time, almost free of the pest.

Last summer the plague of mosquitoes in and about Blackstone Park was so great that it was impossible to make use of this playground with any comfort; and those living in the vicinity were driven indoors. No amount of the various smelly compounds or of tobacco smoke made it possible to sit out of doors in the evening; and the neighborhood was one to be shunned. This year there have been so few mosquitoes that they are almost unnoticeable.

Surely there has never been a better demonstration of efficient work for the public good. It is certainly appreciated by everyone who has been tormented in previous years; and it is to be hoped that the department will not be handicapped through a lack of means to carry on a fight which has so far been wonderfully successful.

Providence, June 30. Warren B. Lewis."

With the coming of July the common house mosquito, *Culex pipiens,* the mosquito which breeds in sewers, catch basins, rain water barrels and stagnant pools everywhere, was expected. In previous years the pest has been abundant quite generally throughout the city. On July 7, we recorded the capture of the first one of this species on Ivy Street, and on July 9 and 16

others were caught at Brown University, on July 21 and 22 several were taken in the neighborhood of Butler Avenue, and at Swan Point Cemetery. But during July these noted were isolated cases and the reports from nearly all sections of the city were that mosquitoes were either absent altogether or if a few were noted they were reported as nowhere nearly as abundant as in past seasons.

In August conditions were nearly the same as in July. Now and then reports would come in as to the abundance of mosquitoes in certain localities, most frequently from places near the outskirts, where a favorable wind might carry them in from outside the city. But on the whole more reports of the absence or scarcity of mosquitoes were received than of their abundance.

The same favorable conditions continued into September and numerous reports came to us of people returning to the city after the summer vacation who slept with wide open windows without screens, something entirely impossible in other years. Indeed we had reports of people who had slept without screens all summer without inconvenience.

In those places where mosquitoes were noted we tried to determine the source from whence they came. At this time mosquitoes were abundant in East Providence and over the city lines in Pawtucket and Cranston and along the banks of the Mosshassuck and Woonasquatuck Rivers, which were not being oiled on account of the objection of the mill owners, and we tried to find whether wind in certain directions brought these mosquitoes into the city. In certain sections of the city there was some evidence that this was the case. In parts of the northern section of the east side when the wind had been blowing from the north or northwest, mosquitoes were more abundant and in all probability were blown in from over the Pawtucket line or from the neighborhood of the Mosshassuck River swamps. In the Elmwood section, after a strong southwest wind, mosquitoes were apparently driven in from Cranston and the neighborhood of the Mashapaug region.

In all probability some mosquitoes came from breeding places which had escaped our attention within the city itself, such as water barrels, private catch basins, etc., which even the little inspection of private premises which we were able to do showed to be extremely common. During another season more thorough inspection will, we hope, exterminate even these few.

On the 12th and 23rd of July, when favorable reports were being received from practically all parts of the city, we visited the Olneyville district in the neighborhood of the swamp back of the Weybosset Mills. We had not oiled this swamp because of the objecton of the mill owners, who were using the water from the swamp. The water of the swamp was alive with mosquito larvæ. Adult mosquitoes were abundant in the whole neighborhood. The people interviewed reported mosquitoes as abundant as in past years. This comparision showed the effectiveness of the work in other sections of the city which in former years had been as badly infested as was the Olneyville section.

The thorough oiling of the catch basins also was certainly having its effect for no mosquito larvae were found in any of them, and yet a visit to Pawtucket on the 18th of August showed that every catch basin in the neighborhood examined, which was just over the city line from Providence, was swarming with larvæ and adults.

Only one salt marsh mosquito was captured in the city by our inspector during the season. This was *O. taeniorhynchus* and was taken on July 20 at Brown University. Even in the neighborhood of the salt marshes at Butler Hospital and on Allen's Avenue and at Field's Point the salt-marsh varieties were not troublesome.

The only observation of the malaria-carrying variety of mosquito made during the season was on August 4 when several larvæ were observed along the banks of the Mosshassuck River at Livingstone Street. At this time large numbers of

C. pipiens were found breeding along the banks of the river and from this time till the end of the season the breeding of mosquitoes along the banks of this river was prevented by assigning a man to patrol the banks and sweep the larvæ from the sheltered pools into the swift current where they were carried down stream. It was impossible to keep oil on these places even if the mill owners had permitted its use.

FINANCIAL STATEMENT.

Season of 1913.

Superintendent's Salary				$1000 00
Oiling Brigade—				
Foreman at $2.25 per day Laborers at $1.85 per day Inspectors at $2.25 per day				3143 69
Horses and Wagons at $2.00 per day				1054 00
Oil				1982 16
Tools—				
Brooms	at	$.29	$19.68	
Whisk broom	"	.09	2.11	
Galvanized iron pails	"	.17	2.16	
Watering pots	"	.39	2.75	
Oil faucets			1.86	
Rakes	"	.35	1.40	
Shovels	"	.69	2.76	
Scythe, snath and stone			1.60	34 32
Skiff				7 50
Miscellaneous—				
F. P. Gorham, trip to Brookline			$2.30	
9 Blank Books			4.28	
Ledger			.80	
Lock boxes for wagons			11.04	
Printing time cards			4.42	
Book, "The Mosquito in North and Central America			10.00	32 84
Total Expenses				$7254 51
Appropriation				7500 00
Balance				$245 49

Respectfully submitted,

F. P. GORHAM.

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HINTS ON THE HANDLING OF CLEAN OYSTERS.

BY LESTER A. ROUND.

Prepared for the Commissioners of Shell Fisheries of the State of Rhode Island, 1913.

State of Rhode Island and Providence Plantations.

HINTS ON THE HANDLING OF CLEAN OYSTERS

PREPARED FOR THE

Commissioners of Shell Fisheries

OF THE

STATE OF RHODE ISLAND

BY

LESTER A. ROUND, A. M.,

INSPECTOR OF OYSTER HOUSES

PROVIDENCE, R. I.

E. L. FREEMAN COMPANY, PRINTERS

1913

GENERAL CONSIDERATIONS.

In discussing oysters from a sanitary point of view, we must keep in mind always that oysters are a food product which to a large extent is consumed in a raw or uncooked form, and for this reason every precaution should be taken to keep them in as wholesome condition as possible. No one wants to eat food which is known to be contaminated with sewage or other filth, or which has been handled in such a manner as to allow contamination. Probably no other food resembles milk as much as oysters. Both are raw foods and in the preparation of the two for market, there are a great many parallels. These are readily understood by every oysterman who gives the subject thought. We all know of the dangers in the careless handling of milk; too often these dangers have proven themselves real in milk borne epidemics of scarlet fever, diphtheria, typhoid, etc. From these unfortunate experiences, of which nearly every city has too many instances, health officers and sanitarians in general have come to realize the dangers which are likely to follow careless and unsanitary methods in the milk industry. It is only quite recently, however, that sanitary experts have considered the danger which sometimes follows uncleanly methods in the handling of shellfish and the necessity of observing the same care in the preparation of oysters for the market that is observed in the handling of milk. It was not until these dangers had shown themselves in the form of typhoid fever epidemics and other intestinal disturbances, that this phase of the oyster industry began to receive serious consideration. It has now been proven on several occasions that these dangers do at times exist and that certain rules and regulations in the sanitation of oysters is just as necessary as in the milk industry.

Recently a great deal has been said and written regarding the danger of eating shellfish, especially oysters. Some of these statements have been just; but a great deal of this criticism has been unjust. Articles of a sensational nature have appeared in the newspapers and magazines, leading one to suppose that all oysters and other shellfish are a grave source of danger to the public health and that people are running a severe risk when they partake of a few oysters on the half-shell. A great deal of this sensational criticism is no doubt due

to the fact that the danger has been but recently recognized and for this reason has been over emphasized. When we consider the problem carefully, we find that the dangers from oysters and other shellfish are not to be compared with dangers existing in the public water supplies of many of our large cities or in the consumption of raw milk. Probably a great many cases, and whole epidemics of typhoid really due to shellfish, have been laid to the water or milk supply, because until recently the question of shellfish and disease had not been considered. Yet, allowing for all these instances the number of cases of disease due to eating shellfish is surprisingly small when compared to the number of cases definitely shown to be due to either an infected milk or water supply. Though this comparison shows the oyster to be the infecting cause in only a comparatively small number of cases, yet we must not forget that the oyster has caused some epidemics, sufficiently large to create national attention, and for this reason demands that certain regulations of a sanitary nature, both in the production and handling of our oysters and other shellfish must be observed in order to restore the public confidence in the consumption of one of our cheapest, most nutritious and most wholesome of raw food products. The purpose of this folder is to point out to the oystermen certain precautions which will enable them to produce a more cleanly and wholesome product and to restore to the oyster a confidence which has been too severely and too unjustly criticised.

The first consideration in the production of a clean product is of course to have a clean supply, one which is free from suspicion. If the oysters are already polluted as they are taken from the beds, no amount of care in their handling and preparation can free them from this condition. This is a matter which the Commissioners of Shell Fisheries regulate, through the bacteriological examination of all the oyster beds of the State. The exact sanitary condition of any bed is known at all times and this information is on file in the office of the Commission. No oysterman who has any regard for the public welfare or for his own reputation, will use oysters which are not free from suspicion. This information can be obtained from the Commission upon application and permission can be obtained to use the oysters from any bed which has been found to be of a sanitary condition sufficiently high to pass the standard set by the Commissioners and the Federal Government.

FLOATS.

When he is sure that he has a clean supply of oysters; that his oyster beds are free from objectionable sewage contamination, the real sanitary work of the oysterman begins. It used to be common practice on the part of the oystermen to remove oysters from the beds and place them in floats for a few days, under the pretense of allowing the oysters to cleanse themselves. These floats were almost always located in the mouth of a fresh water stream which was heavily polluted with sewage from the towns which lay along its banks. What was the result? To the oysterman the oysters became very plump and "fat" from the fresh water which they absorbed. But along with this water, they also became laden with sewage and the germs which sewage contain. If there were typhoid cases in the towns up-stream and the excreta from these patients was discharged into the river, there was great danger of the oysters taking up these organisms, for oysters act as filters and filter out all kinds of micro-organisms. Many cases and whole epidemics of typhoid have been traced to oysters which had been floated in such places. In New York, in 1910, over one hundred cases of typhoid were traced to oysters from Jamaica bay which had been stored in this manner. If space allowed, many other epidemics might be cited. The writer once had occasion to examine oysters kept in such a float and found that in spite of the fact that they were in a high state of purity when taken from the bed, when they were placed in such a float they very quickly became polluted.

From this it can be seen that the use of floats in general is a very bad practice and that floats should never be used, unless the oysterman is assured by a bacteriological and sanitary examination that his float is properly placed in water which is clean at all times, for if an epidemic of typhoid should be traced to his oysters, it would seriously affect, if not ruin his business.

CLEANLINESS OF OPENING HOUSES.

The point of next importance to the oysterman is the cleanliness of the oyster house, for if the opening house is unsanitary it is impossible to keep the oysters in a high state of purity. The walls and ceilings of many opening houses demand greater attention than is given them. Every year the walls and ceilings of all houses should be painted or

whitewashed. If they are already painted and in good condition they may be washed and thoroughly cleaned. Painting or whitewashing has several important features to recommend it. First; before painting or whitewashing it is necessary to clean the walls and ceiling thoroughly to remove dirt, cobwebs, etc., which would form a source of contamination. Second; both paint and whitewash are germicides and so kill the germs that are left on the walls and ceilings and form a smoother surface which prevents to some extent the lodging of bacteria. Third; a very important point concerns itself with the appearance of the house. If the walls are painted some light color it gives much better light to the operators and a much better appearance—a look of neatness to the house—provided it is kept clean. This latter effect, namely, the appearance, has a very marked effect upon visitors and this alone would influence a large percentage of people to give their trade to the better kept house. It is a pleasure to see that a great many of our oystermen take scrupulous pains to keep everything about their oyster houses in a clean and tidy manner.

The appearance and cleanliness of the open house necessarily has a marked effect upon the employees. If a house is kept in a neat and cleanly condition, instinctively the employees pay greater attention to their own personal cleanliness and appearance. The condition of the one necessarily reflects itself in the condition of the other and is worth the extra effort for this reason alone.

CONSTRUCTION OF HOUSE.

The nature of the material of which a house is constructed plays a very important part in the ease with which it can be cleaned. The use of cement instead of wood for benches and floors has a very important advantage in this respect and every oysterman should consider this matter seriously when about to reconstruct or build anew. Though the use of cement would entail a greater initial cost, yet the greater durability and ease of cleaning, together with the higher sanitary qualities, should be a good argument for cement construction.

CLEANING OF HOUSE AND BENCHES.

Great care should be exercised in keeping the bins and benches as clean as possible Every time a bin is emptied and before refilling, it should be cleaned out thoroughly with a hose, if running water is provided, or with a stiff broom and water in places where running

water cannot be obtained. This will remove mud and vegetable matter, which, if left upon the bench, would decay and produce ill-smelling results, to say nothing of the unsanitary aspects of such decomposing material. Every day the floor should be washed throughly in the same manner to remove any vegetable or animal matter which might otherwsie collect and decay with the same result as on the benches.

All the utensils, as opening cans, knives, colanders, etc., should be cleaned and rinsed as often as they are emptied. The mucus from the oyster contains a very large number of bacteria and this should be washed off from all the utensils with which it comes in contact.

TOILET FACILITIES.

The necessity of having a well arranged toilet, has been overlooked by a great many if not most people. The evils of an ill constructed toilet are fully appreciated but by a few. Records show that during the Spanish-American War more soldiers died from typhoid fever than were killed by Spanish bullets. A similar statement has been made regarding our Civil War. Surprising as it may seem to most people, the greatest factor in both these instances in the spread of this disease was the improper location and regulation of the toilets. In some cases perhaps the toilets were so located as to infect the drinking water, while in most cases, probably in all, the vaults were open to access by flies, which a few minutes later winged their way to the cook house or mess tent and proceeded at once to smear the victuals with typhoid bacilli from their germ laden feet and bodies. This last statement is no fairy tale, it is an established fact. When we realize how easily a fly might carry from the toilet to an oyster pail 1,000 to 100,000 colon bacilli, we can see how a contribution of this kind might seriously affect the sale of a shipment of oysters, if they should be examined by the Government. Add to this that wherever we find colon bacilli in large numbers we expect sooner or later to find typhoid bacilli, the matter takes on a serious aspect.

To overcome this danger of fly infection, the toilet should be built tightly and all openings from the vault, the windows, etc., should be screened to prevent flies from either entering or leaving. The door should be so constructed that it would shut tightly and should have a spring which would keep it shut at all times to prevent the entrance or egress of flies. Let it not be that this will prevent proper ventila-

tion. The idea is simply to have no cracks large enough to admit flies and to screen all windows, vents, etc., used for ventilation.

The arrangement of the toilet should be as convenient as possible, and every encouragement should be given the employees to exercise care, for we must realize that they return immediately to the handling of a raw food which someone will eat in a few hours. A supply of running water should be handy and the necessity of thoroughly and carefully washing the hands upon leaving the toilet must be emphatically impressed upon the employees.

The use of screens in the opening houses would prevent the entrance of flies and avoid contamination from this source. This is important, for in a thickly settled community, flies travel quite rapidly and may come from other peoples' premises over which the oysterman has no control.

HANDLING OF THE OPENED OYSTERS.

For washing the oysters as they come from the openers, several methods are in use. It is a difficult matter to say which is the best in all respects on account of the number of factors involved. The old process of washing with a stream of running water on a perforated bottom or colander, would appear to give satisfactory results and is the method used in most of our houses. There is no question, however, but that this method may be improved upon. The purpose of washing is of course to remove all kinds of foreign matter, as bits of shells, dirt, etc. But as much care should be used as possible to wash out the mucus from the gills of the oysters. This mucus contains more germs than any other part of the oyster. The purpose of this mucus is go catch and entangle the food of the oyster and together with the other constituents of the food, it also catches a large percentage of the bacteria contained in the water which is filtered through the gills. The mucus simply acts as a net and does its work quite efficiently. Now, the more of this mucus we can remove the fewer organisms our oysters will contain and the better our product will be from a sanitary point of view, and the better they will keep. Even though great precaution is used to keep the bacterial content of opened oysters at a very low point, yet the numbers will increase and the smaller number of bacteria we have in our oysters at the outset, the less danger there will be of large numbers developing some hours later. This is especially true of long shipments when the oysters are on the road for several days. The writer has seen oysters which were

reasonably low in bacterial content when shipped, reached their destination 48–72 hours later in such a condition that they were unsalable. This fact also brings up the important point of having the oysters properly iced at all times, both in the opening house before shipment and afterwards.

The government has fixed a standard for the B. coli content of opened oysters in interstate commerce. If oysters are found to contain more B. coli than this standard, they are seized and condemned. The oysters may have been in good condition when shipped, but either insufficient washing to remove the mucus and with it the bacteria, or improper refrigeration, or both, caused the bacteria to increase out of all proportion. This is a point which every oysterman should look after. Experiments are now being tried with rotary washers and the results are being watched with interest. The device which will wash oysters the most thoroughly—remove the most mucus—in the shortest possible time—without injuring the oysters, should prove the best method. We should be interested to have practical oystermen work out methods according to their own ideas. It is gratifying to see that this is being done in some places.

SOAKING OYSTERS.

On the other hand, oystermen should not go to the extreme and soak their oysters for long periods. This, as all oystermen are too well aware, produces the same effect as "floating" or "fattening" shell oysters in brackish water and constitutes an infringement of the Federal pure food law on account of the adulterant, *i. e.*, water which is added. The Government experts maintain that soaking more than half an hour shall not be allowed. In spite of this Government regulation the inspector has quite recently found instances where oysters were soaked over night and even from Saturday until Monday morning. So far the State has made no particular efforts to stop the practice and it is hoped such steps will not be necessary.

CLEANLINESS OF EMPLOYEES.

Cleanliness on the part of the employers is just as important as in any other part of the oyster industry. No person about the opening house should be allowed to touch opened oysters or vessels with which the oysters come in contact unless his hands are clean and free from contamination. It is always best to give the hands a thorough

washing before handling opened oysters or their containers. The hands of the openers, as well as their opening dishes, should be rinsed after each emptying of the opening cans.

Perhaps the most important precaution on the part of the people who handle the oysters and one which the oystermen should take the greatest care to see that it is fulfilled, is the thorough washing of the hands after the use of the toilet. This is a matter which cannot be too rigidly enforced. It is almost incredible how many oysters could be contaminated by a speck of fecal material too small to attract attention. When one stops to consider that a bit of fecal matter no larger than the head of a pin might contain several million germs, the fact becomes evident. In this respect the oysterman must think of the kitchen in his own house and the condition which he would tolerate there and apply this to his opening house, for both are places where food is prepared to be eaten raw to a large extent, the only difference being that the period before consumption on the part of the oysters is lengthened by a few hours.

THE WATER SUPPLY.

The quality of the water which is used to wash the oysters is a thing which should be watched carefully. In spite of the most careful vigilance in other parts of the industry, a polluted water supply used to wash the oysters might endanger the oysters to the same extent as "floating" them in polluted water. During the last year the inspector made examinations of all water supply when not derived from a regular town or city supply and reported the results to the oysterman whenever there was suspicion and made sanitary surveys and gave suggestions for its improvement whenever necessary. Whenever a new supply is contemplated, the sanitary quality of the new supply should be ascertained at once in order to save any unnecessary expense, if the supply proved to be unsafe.

STERILIZATION.

The matter of sterilization is comparatively new to oystermen and it is interesting to see how it has been treated. Usually the sterilizer is connected with the heating plant and so one furnace is made to perform the two-fold function. In the late summer and early fall the weather is still quite warm and there is no need of steam for warming the building. In this case, of course, there is no sterilization, for

It seems to the oysterman a needless waste of fuel to run the plant when the weather is still so warm. So many men have said when the subject of sterilization was mentioned: "It has been so warm that we haven't started our sterilizer yet. In the winter when we have steam we sterilize every night, but now we only sterilize once a week," or maybe not at all. Or perhaps they say: "We are not doing very much business now, it is so warm. We have only a few men working In the winter when we are doing a good business, we use our sterilizer every day." But the very time of year when people are inclined to neglect sterilization is the very time they should insist upon it most. The very purpose of sterilization is to kill bacteria which might multiply too rapidly and spoil the product. Under suitable conditions such as are furnished in warm weather, a single bacterium may multiply to thousands in a few hours and millions in a day, while in the winter time when the temperature is around freezing no growth would take place for weeks or even months. Every oysterman knows how much more important is frequent and careful icing in summer than in winter. Just so much more important is sterilization in warm weather than in cold. The purpose of icing is to keep the temperature so low that bacteria cannot grow; while the purpose of sterilization is to kill the bacteria so that there will be none or very few left to multiply when opportunity is given. From a practical standpoint, however, we have two questions to consider: (1) What shall be sterilized? (2) How shall sterilization be accomplished?

In answering the first question, namely, what shall be sterilized, we can consider as a general rule that every utensil that is used more than once in handling or holding oysters should be sterilized. This would include all returnable containers, opening pans and measures, knives, tubs, whether iron or wood, but especially the latter, washing basins, colanders, etc. There is no particular reason for sterilizing non-returnable containers, provided they are clean. Simply rinsing in clean pure water is all that is necessary. But, in the case of other utensils, sterilization should be effected every day.

There are two methods of sterilization that oystermen might use, both of which are feasible. The most reliable is the use of steam either as "live steam" or under pressure. If "live steam" is used it should be employed for one hour, but with a pressure of 15–20 pounds, 20–30 minutes would be sufficient. Whenever possible steam should be preferred.

The other method may be called chemical sterilization with calcium hypochlorite or, as it is familiarly known, bleaching powder. This is a very strong oxidizing agent and a small quantity will act very quickly and kill all the bacteria. In using bleaching powder one should use the proportion of one level teaspoonful to 30 gallons of water. This should not be mixed and kept on hand, but used *immediately* upon mixing because the sterilizing effect is soon lost when mixed with water. The utensils to be sterilized should be filled with water and the powder added in proper proportion and stirred in and dissolved. A large tank might be used into which all the utensils to be sterilized might be put and then filled with water. The bleaching powder can then be mixed in concentrated form in a pail of water and added to the tank. This should be allowed to act for one-half an hour and then draw off and the utensils rinsed in clean water and allowed to drain and dry.

(It must be understood that the commissioners do not recommend the use of bleaching powder instead of steam, but merely as an expedient when steam sterilization is not feasible.)

HEALTH OF THE EMPLOYEES.

The health of the employees and their families is a matter which should interest every oysterman. Frequent inquiry among the employees should be made for any cases of sickness—especially infectious diseases as typhoid, dysentery, scarlet fever, diphtheria, etc. The employer should make this a very important matter and should demand that every case of sickness of any kind among the employees or their families be reported to them in order that no case might go by undetected. A great many epidemics and isolated cases of typhoid and other intestinal disturbances have been traced to infected oysters, but so far as the writer is aware no cases of scarlet fever, diphtheria, measles or other infectious diseases have been traced to this source, and yet a great many epidemics of the latter diseases have been caused by an infected milk supply and there is reason to believe that oysters might carry such infection if the conditions were suitable. What person, oysterman or otherwise, would allow food prepared for him by a person in whose family there existed a case of scarlet fever, typhoid, diphtheria or any other infectious disease? And yet, the people who handle the oyster in an opening house are preparing a raw food, which will be consumed in a few hours.

When we consider that the opening house is but a kitchen for the preparation of food, we necessarily get a different point of view and different ideas concerning cleanliness. We firmly believe that many changes will be made when the oystermen consider their opening house from this point of view and compare certain processes and methods now existing with those of the well appointed home.

Within the last four years great advances have been made in the sanitation of oysters and oyster beds and in matters of cleanliness, both in the handling of oysters and the appointments of the house. The Commissioners appreciate and are deeply grateful for the attitude which the oystermen have taken in this matter. Almost to a man the oystermen of Rhode Island have entered into this phase of the subject zealously and with the firm resolution of making the Narragansett bay oyster the finest product in the country, both as regards wholesomeness and cleanliness. The efforts of our most progressive oystermen in this line are worthy of the truest admiration and highest respect and the Commissioners take this opportunity of extending to the oystermen of Rhode Island their sincerest appreciation for the work which they have done. Rhode Island was the first to take up this matter seriously and from the first has taken the lead in its campaign for clean oysters. But the work is only at its beginning; greater things are to be expected in the future than have been accomplished in the past and the Commissioners stand ready to aid the oystermen in every way possible in maintaining for the Narragansett Bay Oyster the superior reputation it at present enjoys.

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THE RATS OF PROVIDENCE AND THEIR PARASITES.

BY GEORGE H. ROBINSON.

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THE RATS OF PROVIDENCE AND THEIR PARASITES.

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That rats are a factor in the spread of bubonic plague is an established fact. It has been confirmed beyond the shadow of a doubt, in India, in California, in England, and in other regions where plague is endemic. Rats dead of the disease have been found time after time in houses where outbreaks have occurred and immediate deratisation has effectively stopped further spread of the epidemic. These facts and a great amount of experimental work show that the rodent disease and that of human beings are identical.

Bubonic plague is originally a disease of rodents. Its home is thought to be the region of Thibet,[1] its natural host being the Tarbagan of that region. Other rodents are easily susceptible to the disease, as the hares of England, the ground squirrels of California, and the various species of the Muridæ found in all temperate and tropical regions of the globe. Owing to their universal distribution and great abundance the rats are to be considered as the greatest factor in the spread of bubonic plague.

When rats were first connected with the spread of the disease it was thought that they distributed the germs by contact with human food. The plague bacilli can be found in the mucous secretions, feces and urine[2] of infected rats and so there is some evidence in support of this theory. In 1898 Simond working in India demonstrated that plague could be transmitted through the bites of fleas. Later work by the Indian Plague Commission[3] and others has shown the rat flea to be the probable distributor of the disease.

To the cities along the Altantic coast of this country the spectre of plague is not very real but with the outbreak of the disease in the West Indies in 1912 it took on a more than ghostly substance to those conversant with the situation. American cities have an abundance of rats, the rats are presumably well supplied with fleas and it seemed only necessary for the introduction of the germs in order to bring about an epidemic. The Federal authorities required that all ships running between the infected region and American ports be fumigated and advised the municipal health officers to conduct an examination of rats.

In accordance with this recommendation an examination of the rats of Providence was undertaken under the direction of Dr. Charles V. Chapin, Superintendent of Health. The object of the work was to determine if

[1] Preble, Paul, The Tarbagan (Arctomys bobac) and Plague, Public Health Reports XXVII, 1912, 31.
[2] Baxter-Tyrie, C. C., Plague in Queensland, Journal of Hygiene, V, 1905, 315.
[3] Report of Plague Investigations in India, Journal of Hygiene, VII, 1907, 395.

possible the presence of any epizoötic among rats, the kinds of rats and the number and kinds of fleas on the rats. At first the work was confined to the water front, but later its scope was extended to the entire city.

The work began on July 18, 1912, and continued with several short intervals, up to the last of December. From the beginning of the examination until the first of September the rats were taken in traps along the water front. A daily patrol of the shore was also made in order to pick up any which might have died. Only three were found dead. About the first of November a bounty of five cents was offered for every live rat brought to the laboratory and 142 were purchased.

The technic of the examination was as follows: the rats were caught alive in the French wire traps and carried to the laboratory; there they were placed in a reservoir of illuminating gas for several minutes killing the rats and stupefying the fleas; the rats were then immersed in a weak solution of corrosive sublimate or formalin and left over night; during this time the putrefactive bacteria entered the circulation but a shorter exposure to the fluid was not sufficient to penetrate the thick fur and kill the parasites; the wet fur was then turned aside with forceps, the parasites were removed and their position on the body was recorded; the lymph glands were then exposed, the abdomen was opened and the appearance of the organs especially the liver was noted.

During the months July, August and September, 118 rats, and from October to January, 223 were examined, making a total of 341. These were evenly divided as to sex: 333 were the brown rat (Mus norvegicus), 2 were the roof rat (Mus alexandrinus), 1 was the black rat (Mus rattus), 4 were apparently crosses between the brown and roof rats, and 1 appeared to be a cross between the black and brown rats. The two specimens of the roof rat were taken from the New York-Providence boat and so were doubtless New York rats.

The size ranged from 18 to 46 c. m. (7 to 18 inches). Studying the brown rats by the biometric method we find them grouped about two distinct modes, those of 24 and 36 c. m. in length. Thus we have a clear differentiation into the adults and those which are still immature. Of these 59 per cent. were adults and 41 per cent. were young.

The results of the examination for fleas were as follows:

Total number of rats examined	341
Total number of rats infected with fleas	195
Total number of fleas	2053
Average fleas per rat	6
Average fleas per flea-infected rat	11
Largest number of fleas from one rat	300

Per cent. of fleas:[1]

Xenopsylla cheopis Rothschild	75
Ceratophyllus fasciatus Bosc	22
Ctenopsyllus musculi Duges	2.5
Ctenocephalus canis Curtis	0.5

By way of comparison it is interesting to note that in California the average fleas per flea-infected rat was 3.5,[2] and a yearly average of 2.7 was found. In India the average fleas per rat was 4.0.[3] All who have experimented are agreed that the two most numerous species which we found, Xenopsylla cheopis and Ceratophyllus fasciatus, will bite man. Concerning the others there is some dispute but since they comprise only 3 per cent. of all they are of little consequence.

During the plague epidemics in India and California much importance was made of the location of the primary buboes on the rats as indicating the point of inoculation or the flea bite. In India[4] 74.3 per cent. of the buboes were located in the cervical region. No data on the regional distribution of fleas on rats in India is available but on guinea pigs artificially infected by the Commission, 88.9 per cent. of the fleas were found on the head and neck. Pound[5] in Australia reports fleas as most commonly occuring on the head and neck of rats. In San Francisco[6] 72.4 per cent. of the buboes were found in the groin and none in the cervical region while 73 per cent. of the fleas on the rats were found on the hind quarters.

With these results in view the location of the fleas on the Providence rats was noted and the following results obtained; 16 per cent. on the head and neck, 16 per cent. on the fore legs and shoulders, 41 per cent. on the body, and 27 per cent. on the hind legs and pelvic region. These results hardly coincide with those found in other places. If we compare the relative areas of the different regions we find that they stand in about the same ratio as the percentages of fleas found on them. So it seems quite just to believe that the fleas are distributed with comparative uniformity over the body of the rat. However, with the oncome of cold weather there was noticed a slight change in position from the exposed portions of the body to the more thickly furred pectoral and pelvic regions. The exact figures were as follows:

	July-September	October-December
Head and neck	15 per cent.	16 per cent.
Front legs and shoulders	15 per cent.	16 per cent.
Body	46 per cent.	37 per cent.
Hind legs and hips	24 per cent.	31 per cent.

[1] Acknowledgment is made of the assistance of Mr. Nathan Banks of the Bureau of Entomology in confirming the identification of the fleas.

[2] McCoy and Mitzmain, The Regional Distribution of Fleas on Rodents, Parasitology, II, 1909, 301.

[3] Seventh Report of Plague Investigations in India, Journal of Hygiene Plague Supplement, January, 1913, 217.

[4] Report of the Indian Plague Commission, Jour. of Hygiene, VII, 1907, 386.

[5] Pound, Report of Plague in Queensland, 1900–1907, 143.

[6] McCoy, G. W. A Report on Laboratory Work in Relation to the Examination of Rats for Plague at San Francisco, Cal. Public Health Reports, XXIII, 1908, 1051.

The average fleas per rat for the months July to September was 10.2 and that for October to December was 3.7, showing a marked reduction during the colder months.

Twenty-one per cent. of all the rats were found to be infected with mites and they became more numerous with the onset of cold weather. Lælaps echidninus Berlese was the species found. In one case, at the Providence Coal Co. stable, a specimen of Myonyssus decumani, Tiraboschi, said to occur on Mus decumanus in Italy, was found. It is interesting to note that a short while previous to the capture of its host an Italian barkentine had docked near that point. The common rat louse Polyplax spinulosus Burmeister was found on 24 per cent. of the specimens. On 12 per cent. were found open sores.

No numerical study of the occurrence of internal parasites was made except in cases where the liver was affected. This condition was found in 7 per cent. of the rats and was due to the encysted form of the cat tapeworm Tænia crassicollis and to the ova of some unknown parasite found also by McCoy[1] in California. One noticeable and possibly significant fact is that from two meat markets from which were obtained 15 and 22 rats, respectively, both these parasites were present in the liver in 25 per cent. of each lot. A bladder worm was present in all of six cases which were examined.

The question as to whether there is a relation between the filthiness of the home of the rat and the number of fleas is of interest. The dirty rats of the stables and docks might be expected to bear more fleas than would the cleaner rats of other localities. It was found in these investigations, however, that the average fleas per rat from the stables, and docks was especially low, in but few cases exceeding one flea. On the other hand the averages from the cleaner places were generally higher. The average from a restaurant from which 40 rats were taken was 36 fleas. That from a creamery was 22, from a dwelling house 10, from another 8, from a drygoods store 10, from a bar room 1, from two markets 1 each. The reason for this is doubtless that the fleas breed in the nests of the rats[2] and are only on the bodies of the rats to feed. So a damp, cold location of the nests would strongly inhibit the breeding of the fleas.

The conclusions from our study of the rat situation in Providence are that rats are present in large numbers and cannot be exterminated until the city is rebuilt with ratproof construction; these rodents are supplied with as many fleas as are those in plague infected regions; opportunity for the fleas to bite human beings is always present as the rats are for the most part closely associated with man. It would seem, therefore, that only the germs of plague are lacking to complete the mechanism of an epidemic in Providence.

[1] McCoy, G. W. Pathological Conditions found in Rats, Public Health Reports, XXIII, 1908, 1365.
[2] Reports on Plague Investigation in India, Journal of Hygiene, VIII, 1908, 241.

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SIZE OF THE SAMPLE NECESSARY FOR THE ACCURATE DETERMINATION OF THE SANITARY QUALITY OF SHELL OYSTERS.

BY GEORGE H. SMITH.

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SIZE OF THE SAMPLE NECESSARY FOR THE ACCURATE DETERMINATION OF THE SANITARY QUALITY OF SHELL OYSTERS.

George H. Smith.

During the past three years while working at Brown University and for the Rhode Island Shell Fish Commission under the direction of Prof. F. P. Gorham, I have had occasion to witness and assist in the analysis of a large number of samples of oysters. Many times throughout this work the fact has been noted that in oysters taken from the same oyster-laying there was a very great variation in the colon content. Just how great this individual variation was or how important a part it played in oyster analysis has, so far as I know, never been determined. Thus, during the past winter and spring I have been conducting a series of experiments with a view to determining these facts.

It has generally been the custom, I believe, for a sample to consist of five oysters. In fact the Committee on Standard Methods of Shell-fish Examination of this Association in their "Second Progress Report" recommends that this number be used. Furthermore, I think that it is quite generally the practice to condemn as unfit for food those oysters which upon analysis and which according to the American Public Health Method of Rating Oysters for B. coli give a score of thirty-two or more, and to allow those oysters scoring twenty-three or less to be used as food, provided the sanitary survey does not show good reason why they should be condemned. Now in a sample composed of so few individuals as this, any one individual differing greatly from the others will influence the result very greatly, and the rating of the sample may be determined largely by this one oyster, whereas, if the sample were larger, the importance of one oyster would be greatly decreased. Thus it became apparent that the factor of chance might play quite an important part in the result of the analysis. When the analysis being made is for the purpose of determining whether these oysters are suitable for food or not this factor of chance assumes great weight. It is of vast importance to the oyster dealer in such cases just what oysters are chosen for the analysis. Also the health official should be concerned for it may very readily give him a false idea of the condition of the oysters which he is considering.

Not only in my own work has this variation been noticed but in examining the records of the analyses of some 230 oyster samples made by the Rhode Island Shell Fish Commission it was noted that in but seven of these did all of the five oysters comprising the sample show the same

degree of pollution, and of these seven, five were so badly contaminated that the colon bacillus was found in every dilution.

The factor of chance of which I have spoken is a variable quantity and is of far greater importance and value in some cases than in others. Obviously, in badly polluted beds and in layings which are free from contamination, it plays but a very small part. It is in the middle region, that is, in those layings that are questionable, intermediate between the good and bad, that it reaches its highest value.

Thus these experiments were with the view of determining how great this factor might be and if possible to devise some method which would eliminate it and give a more true index of pollution. To this end I have made analyses of six different oyster layings in Narragansett Bay whose locations were such that one would expect to find both extremely bad and good oysters and also some of an intermediate condition. From each bed at one time were taken at least 125 oysters. These oysters were transported to the laboratory and divided arbitrarily into samples of five oysters each. These samples were then subjected to the usual analysis in so far as time would permit. By this I mean that lactose-peptone-bile was inoculated with the shell-liquor in the cubic centimeter, tenth and hundredth cubic centimeter dilutions and gas production in this media was considered to indicate the presence of some member or members of the colon group. In but few cases was the colon isolated. The gas production was noted at 12 hours, 18, 24 and every 12 hours up to 96 hours. I may here say that by far the greater part (some 97 per cent.) occurred during the first 48 hours. The results of the analyses were as follows:

Of the first series of 25 samples, which were taken from the upper portion of the Bay where pollution was to be expected, all of the samples showed contamination to a considerable degree. None of the samples gave a score of less than 41. Some of them gave scores of 500.

Of the second series comprising 25 samples from a bed which from the sanitary survey would be classed as in the questionable area the scores ranged from 5 to 320. Six of the samples gave a score of 23 or less. The remaining 19 scoring higher than 32.

In the third series taken from a bed located in the same general region as the former, 23 of the 25 samples gave a score of 32 or higher. The remaining two samples scoring 23. The scores here ranged from 23 to 410.

The fourth series gave exactly the same results in so far as the scores were concerned. The individual content of the oysters, however, was somewhat different.

Series five, which represents oysters taken from a laying much farther down the Bay and which, from the sanitary survey would be judged favorably located, gave a result which was rather disappointing in that but one sample of the 25 gave a score of 23 while the remaining 24 samples scored from 32 to 410.

The sixth series of 25 samples was taken from that bed which, from the sanitary survey, should be the least likely of pollution in that it was the one farthest out to sea and the most distant from any sewerage outlets. This bed gave scores ranging from 2 to 320. Fifteen of the samples scored 23 or less, the remaining 10 being 32 or greater.

Thus it will be seen that in layings which are uniformly bad the part played by chance in the selection of the five oysters is of comparatively little importance. Also in layings which are good, although, unfortunately, I failed to find any, I think it is fair to draw a similar conclusion.

It is in the layings that are intermediate, where there is a wide variation among the individual oysters, that the importance of what oysters are chosen is manifest.

This is perhaps brought out the most clearly in series VI. These oysters were chosen at random and the result was that fifteen samples were comparatively good and ten were bad. If, however, by some power I had been able to choose these oysters with a previous knowledge of their colon content it would have been easily possible to have had all 25 samples score 23 or less. In fact, in this series, mathematically the chances were 2 to 1 that any given sample chosen at random would score less than 32.

Again in series II it is possible that 14 of the samples might have scored less than 32 while in fact only 6 did so. Here the mathematical chance of obtaining a sample at random which would be considered good is 1 in 8. For purposes of comparison I will say that while in series I, two samples scoring 23 could be derived yet the chance of obtaining such a sample is but 1 in 7,500. From a consideration of this work it would appear that in many cases at least the analysis of five oysters may fail to give a correct index of the degree of pollution present in the laying. The only remedy for this that I can see is to use a larger number of oysters for the analysis and perhaps place more weight on the number of bacillus coli present per cubic centimeter in a composite sample of the shell-liquor.

From the examination of my analyses it would appear that the choosing of any number of oysters less than 15 for a sample will not give consistent results.

As conclusions I may suggest,—

That the present method of analysis and scoring may work injustice and does not necessarily give a correct index of contamination.

That the sanitary survey, while undoubtedly of great value, is often misleading.

That a sample composed of five oysters analyzed individually is not sufficient.

That a standard should be adopted based on the number of bacteria of the colon group per cubic centimeter of a mixed sample of the shell liquor of at least fifteen oysters. That is, a standard for oyster-liquor should be established of a nature similar to that in use for water and milk.

In the following table is summarized the data derived from an examination of the work here reported:

THE SIZE OF THE OYSTER SAMPLE NECESSARY TO GIVE AN INDEX OF CONTAMINATION.

Series.	No. of Samples.	Sanitary Survey.	Oysters Showing no Colon.	Oysters Showing B. Coli in			No. of Samples Scoring Under 32	No. of Samples Scoring Above 23	Range of Scores.	Possible No. of Samples to Score Under 32.
				1 c.c.	0.1 c.c.	0.01 c.c.				
I	25	Very bad—Located near large sewage outlet.	0	6	55	64	0	25	40 to 500	2
II	25	Questionable area.	5	33	62	25	6	19	5 to 320	14
III	25	Questionable area.	1	11	59	54	2	23	23 to 410	4
IV	25	Questionable area.	5	13	62	45	2	23	23 to 410	8
V	25	Good.	1	13	69	42	1	24	23 to 410	5
VI	25	Excellent.	22	46	43	14	15	10	2 to 320	25

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SCHMUCKER'S "THE MEANING OF EVOLUTION."

BY HERBERT E. WALTER.

Science, N. S., Vol. XXXVIII, No. 987, p 779, November 28, 1913.

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SCHMUCKER'S "THE MEANING OF EVOLUTION"

The Meaning of Evolution. By SAMUEL CHRISTIAN SCHMUCKER, Ph.D. New York, The Macmillan Company. 1913. 12mo. Pp. 298.

This is a very readable book upon what is no longer a new theme. Following a literary "foreword" the pre-Darwinian history of evolution is sketched as a background for Darwin and Wallace. The historical chapter about Darwin presents the essentials of his career in a charmingly vivid and sympathetic manner. Then follows the "Underlying Idea" of natural selection as the method of evolution illustrated largely by means of the English sparrow, of which the author incidentally says (p. 84): "This pestiferous creature should be exterminated . . . but personally I am taking no share in his destruction . . . I confess that it would be with regret that I should see him disappear from the landscape."

Chapters IV. and V. deal with adaptation for the individual and for the species. The general attitude toward Lamarck is occasionally rather more conciliatory than the militant Weismannian would approve of, but this is not to be wondered at in one who is proud of having been a student of Professor Cope. It seems to be very easy to drop into Lamarckian explanations for adaptation. For instance (p. 89): "The modern scientist feels sure not only that the animal is fitted to his work, but that he has been so fitted by the work." It will probably always be a bone of contention whether the exercise of an organ determines its structure or the structure of an organ sets the limits to its exercise.

With respect to protective coloration and sexual selection the author proposes to retain the Darwinian interpretation until something better arises in spite of the recent loss of confidence in the adequacy of these explanations.

The three succeeding chapters upon "Life in the Past," "How the Mammals Developed," and "The Story of the Horse" marshal in review some of the classified evidence in support of animal evolution, while Chapter IX. takes up "Evolutionary Theories Since Darwin."

In this last chapter Weismann, whose name will doubtless be correctly spelled in subsequent editions, is justly given prominence because his "work has made us cautious and prevented our lightly accepting a belief in the influence of the environment." Moritz Wagner and Romanes with their isolation theories and the orthogenists receive attention, and finally Hugo deVries with mutation closes the chapter.

The book could have been written fifteen years ago so far as any analysis of the significant bearing which Mendelism or the pure-line theory of Johannsen has upon the question of evolution.

Chapter X. turns optimistically to the "Future Evolution of Man" and is sociological rather than biological in its treatment, while the final chapter, "Science and the Book" gives the impression that the professor has stepped out of the class room and is speaking to a church audience and speaking withal extremely well.

The word "Evolution" has lost most of its incendiary character of a generation ago yet there are no doubt many in whose minds it still stands contrasted with religion and the Bible as a faith-destroying invention of godless scientists. To all such persons this book is a welcome message of reassurance and peace while to others who no longer need to be convinced of the essential truth of the evolutionary processes, the pages will be turned with approving delight.

Dr. Schmucker has stated the facts of the case in clear non-technical language with much literary grace and with scientific accuracy, consequently the book is well adapted to a wide range of readers even outside the biologically initiated.

H. E. WALTER
BROWN UNIVERSITY

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GENETICS, AN INTRODUCTION TO THE STUDY OF HEREDITY.

BY HERBERT E. WALTER.

Published by the Macmillen Company.

GENETICS

AN INTRODUCTION TO THE STUDY OF HEREDITY

BY

HERBERT EUGENE WALTER

ASSISTANT PROFESSOR OF BIOLOGY
BROWN UNIVERSITY

WITH 72 FIGURES AND DIAGRAMS

Cloth, xiv+272 pp,, bibliogr., index, $1.50 net

PUBLISHED BY

THE MACMILLAN COMPANY
64-66 FIFTH AVENUE
NEW YORK

"THE subject of heredity," remarks Professor Walter, "concerns everyone, but many of those who wish to become better informed regarding it are either too busily engaged or lack the opportunity to study the matter out for themselves. The recent literature in this field is already very large, with every indication that much more is about to follow, which is a further discouragement to non-technical readers.

"It may not be a thankless task, therefore, out of the jargon of many tongues to raise a single voice to tell the tale of heredity. There may, too, be a certain advantage in having as spokesman one who is not at present immersed in the arduous technical investigations which are making the tale worth telling."

It is evident, therefore, that although the author is a trained scientist, he fully realizes the difficulties in understanding this complicated subject. This realization, combined with Professor Walter's mastery of the art of clear and interesting presentation of scientific truths, has enabled him to write a forceful, accurate and stimulating summary —for the intelligent, but uninitiated reader—of some of the more recent phases of the questions of heredity which are at present agitating the biological world.

The book is, therefore, well adapted for use as a text in college courses on breeding, heredity or evolution. It is also a volume from which the busy reader will find it a pleasure, rather than a task, to gain an acquaintance with this most vital and absorbing phase of the newer science which has a most direct bearing upon man and the betterment of the race.

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THE NEGRI BODIES IN RABIES.

BY ERNEST M. WATSON

Journal of Experimental Medicine, Vol. XVII, No. 1, 1913, pp 29-42.

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THE NEGRI BODIES IN RABIES.*

BY ERNEST M. WATSON.

(From the Bacteriological Laboratory of Brown University and the Hygienic Laboratory of the Johns Hopkins Medical School.)

PLATES 1 AND 2.

Since Negri, in 1903, discovered the bodies contained within the nerve cells of the central nervous system in cases of rabies, there has been much speculation as to their identity and importance. These structures, or Negri bodies, as they are now called, are constantly associated with rabies and we have come to regard them of prime importance from the standpoint of diagnosis. Their presence is demonstrated in the cerebral or cerebellar cortex, in the hippocampus major, or in the corpus striatum in over 98 per cent. of all cases with the clinical symptoms of rabies. The occasional failure to find them is, probably, due to our imperfect methods, to the fact that the bodies under certain conditions do not take the stain, or that in certain cases they occur in forms too small to be recognized by the one-twelfth inch oil immersion lens.

The nature of the Negri bodies is today a mooted question, and concerning them many and varied views have been advanced. The clinical history and symptoms of the disease, together with the nature of the infection and the properties of the virus, point toward a definite microörganism as the underlying cause. All observers now agree that the bodies described by Negri are definitely associated with rabies. One group of investigators believes the bodies to be definite parasitic organisms, and the cause of the disease. Another group considers them simply cell degenerations caused by the infecting agent, but incapable of producing the disease. Negri himself believed the bodies to be parasites of an animal nature and *ipso facto* the cause of rabies. Other Italian investigators including Volpino, Dominici, Marzocchi, d'Amato, Bandini, Fasoli, and others,

* Received for publication, September 5, 1912.

have corroborated Negri's work and with him agree that the bodies are of a parasitic nature. In this country Williams and Lowden, Calkins, and others believe that the Negri bodies are of protozoan nature. The former authors have worked out a tentative life history, illustrating undoubtedly a multiplicative cycle in the development of the organism and have classed it among the Microsporidia. Calkins believes it to be more closely allied to the ameba and has placed it under the classification of the Rhizopods. Other writers in this country who favor the protozoan identity of the Negri bodies are Poor, Stimson, Frothingham, Hadley, Harris, and others.

THE NEGRI BODIES.

In the following observations upon the nature of the Negri bodies the methods and technique employed have been those now generally used in most of the Board of Health Laboratories in this country. The examinations of rabic material were made in the regular smear preparations, and in paraffin sections of the cerebral and cerebellar cortex, and of the hippocampus major and corpus striatum. In general it was found that the smear preparation under proper manipulation gave the better results. The morphology of the bodies by this method can be brought out definitely and their internal structure shows clearly, much more so, in fact, than in the paraffin sections, while the possibility of changing the position of the bodies within the cell and of mutilating them in any way by pressure, is reduced practically to nil after a little experience. By the smear method the general contour and shape of the bodies is the same as that noted in the paraffin sections, and their arrangement in the cell shows no appreciable displacement of the cellular protoplasm. A further advantage in the smear method is that upon the slide the layer of brain material is of varying thickness, which fact, after staining, aids materially in the final conclusions which are based on a comparative study of very thin, medium, and thick areas.

The stains used have been the Van Gieson stain for rabies, which at times was modified by the substitution of basic fuchsin instead of the rose anilin violet, the other proportions remaining the same. Mann's method of staining was also used in attempts to bring out more definitely the inner structure of the bodies.

Shape.—In general it may be said that the Negri bodies tend to assume an oval shape. Sometimes, however, they appear round and occasionally rather irregular, appearing to be in the process of division. Usually the outline of the bodies is smooth. Less frequently the edges appear irregular.

At times forms resembling a process of budding are noted. There seems to be a pretty general distribution of the different shapes in a single animal. If anything the oval shape predominates, while the division forms are least frequently encountered.

Size.—The size of the bodies seems to vary within rather wide extremes, from 0.25 to 0.5 to 21 microns. Although in general the very small ones and the very large ones are met with less frequently than are those measuring two to nine microns across their longest diameter. All sizes may be present at the same time in one animal and frequently in one nerve cell (from Ammon's horn) bodies measuring one micron and others measuring ten to fourteen microns are present at the same time.

Number.—The number of bodies present in any individual case seems to be subject to considerable variation. Sometimes the large cells from the hippocampus and the cortex of the cerebrum and the cerebellum are literally filled with bodies, and at other times a search of several minutes is necessary to reveal a single body. In these latter cases, however, further search, in the writer's experience, has always been rewarded with the finding of an appreciable number of bodies, although they may be well scattered. When the bodies are few in number they seem to be uniformly small in size and almost without exception are quite regular in their morphology. For the most part the bodies are found with greater regularity in the horn of Ammon and in the corpus striatum than in either the cerebral cortex or the Purkinje cells of the cerebellum. A specimen brought into the laboratory some years ago from a case of human rabies showed nine well developed bodies in one of the Purkinje cells of the cerebellum.

Structure.—Concerning the structure of the Negri bodies there has been much speculation and much has been written in attempts to identify and determine the rôle of the various parts. With the Van Gieson stain, and also with the basic fuchsin modification of it,

the bodies take a characteristic pink stain, lighter and of a more delicate hue than the eosin red color imparted to the red blood cells which are frequently seen in the same section. With proper staining solutions, this typical color varies but little from day to day.

The Negri bodies are essentially intracellular parasites and we can speak of them with certainty only when they are found within the nerve cells. They never have been demonstrated along the longer and larger nerve trunks or along the fiber tracts in the cord. There is, however, good reason to believe that the infection reaches the central nervous system through the above mentioned channels. Within the nerve cells the pink color of the bodies is broken by the regular appearance within the body of the parasite itself of cellular inclusions termed chromatin granules. These granules are a constant part of the typical Negri body. They take a dark slate blue stain with all the stains which have methylene-blue or hematoxylin as a base. Their number in the body is variable, being dependent for the most part upon the size of the parasite. There is usually one slightly larger and more deeply stained than the rest which occupies a position more central than the others. This granule has been regarded as the nucleus and is present in practically all forms which are large enough to permit a structural examination of their inner cytoplasm. In the very small forms only the central chromatin granule or nucleus is discernible, while in the larger forms the granules vary from three or four to ten or twelve. In the larger forms the rather concentric arrangement of the chromatin granules around the parent nucleus appears like nuclear fragmentation preparatory to a subsequent division of the cytoplasm and these types have been believed by many to represent a preparatory stage to direct cell division by fission.

Williams and Lowden have described at length the succeeding stages in the growth and reproduction of this type of Negri body. According to them, the bodies are first found as definite tiny forms 0.5 to 1 micron in diameter, with one chromatin granule or nucleus taking a deep azure blue stain in the center and enclosed in a delicate pink staining cytoplasm, while around the whole and blending into the cell cytoplasm is a definite rim, or ectoplasm, staining a little deeper pink than the rest of the cell protoplasm. In addition to

these extremely tiny forms there are larger ones which are similar in shape and staining reactions, but which contain an extra chromatin granule or two, probably fragments of the nucleus. Still larger forms are also noticed which are, in general, similar to the bodies last described, but which begin to show evidences of division, *i. e.*, constrictions giving rise to hour glass forms. The various steps in the process of fission have been observed. In the last, only a fine filmy bit of protoplasm connects the two organisms. In the next stage, the two bodies have become entirely separate and lie side by side. In some cases the ectoplasm of the two bodies is in close proximity, but in others it is separated by a small space through which the protoplasm of the nerve cell shows as a blue staining line between the pink staining ectoplasmal rims of the two now separate individuals.

The above description outlines briefly one undoubted phase in the life history of the organism. It represents a stage of multiplicative reproduction,—a schizogonous life cycle, or, in speaking of the Myxosporidia, a stage of plasmotomy carried on within the cells of the host. This condition we expect on account of the vast numbers of parasites that are found in a given host and even in a single cell of a host, and the great difficulty of conceiving that each parasite could have arisen from a separate and individual spore infection. From the above commonly observed conditions, and from the nature and frequency of the findings, we have good reason to believe that this plasmotomous life cycle is supplemented, either within the body of the host or elsewhere, by another cycle of a different nature, this other cycle being concerned with a sexual reproduction.

Bodies corresponding to a sporogonous life cycle of this type have in two instances in the past few years come under the writer's observation, and a general description of these findings is reported at this time. The two cases and the specimens which showed these sporogonous types presented rather similar and singular histories. The dogs came from sections of the state some thirty miles apart and during life presented the usual clinical picture of uneasiness, restlessness, and signs of evident distress, and became very irritable. During this last stage both animals had bitten people. The dogs were consequently confined, and in approximately twenty days after

the initial signs of malaise both animals died, the reported cause of death being distemper. Unsuspecting the seriousness of the bites both dogs were buried, and several days elapsed before they were dug up in order that their heads might be sent to the laboratory for examination.

In these two cases the cells of the cerebral cortex, the Purkinje cells, and the giant cells of the horn of Ammon showed the presence of the typical Negri bodies with their characteristic nuclei and chromatin granules as described above under the usual cycle of an asexual intracellular development. In addition to these were certain forms quite different from any of the preceding, yet taking the stain readily and being closely associated with the former, many of them being in the same cells with the plasmotomous type of body. These new bodies were seen to vary in size. The smallest ones measured 0.25 to 0.5 of a micron in diameter (plate 1, figure 4, A). Some were possibly smaller than this, the difficulties of identification in these extremely small types being considerable. The smallest forms present are slightly round or oval, have a definite morphology, and are regular in outline. They take a deep pink, almost a crimson stain, and when treated with acid alcohol seem to hold the stain longer than those of the plasmotomous type. Their protoplasm stains with no indication of a nucleus or of chromatin granules. At one end, by careful focusing and with most of the light coming from the mirror, the condenser being cut out, a small refractive area is seen which takes the stain less readily than the other portions. As nearly as could be determined with the immersion lens this light spot presents an oval outline and gives every indication of being a capsule, and the whole body with its uniform and homogeneous consistency seems to have every appearance of a spore.

Bodies of this type are found scattered more or less diffusely throughout the slides, both inside and outside of the nerve cells. Forms similar to these in every way but slightly larger are also found, but are less plentiful than the very small ones (plate 1, figure 1, E, F; figure 2, E, F, H, J). The next larger body corresponding to these forms presents quite a different picture. They measure 1 to 1½ or 2 microns in diameter, stain a brilliant pink, are very regular in outline, and about them a definite membrane, or rim of

ectoplasm, can be made out, while their interior contains two, three, and sometimes four of the definite, tiny, regular oval spores described above. All these stain a brilliant pink and occupy completely the interior of the body, there being no nucleus and no chromatin granules, nothing in fact which takes the blue stain, and but little of the residual protoplasm of the parent body which takes a faint pink stain and shows up in marked contrast to the spores. Larger forms than this are also observed which include a definite cell membrane, within which are eight to ten, and sometimes twelve, of the spore-like bodies (plate 1, figure 2, H, E). They all stain similarly to the smaller forms and show only a little of the residual pink staining protoplasm of the original cell and no nucleus or chromatin granules. Larger forms than these show a broken ectoplasmal rim with the spores protruding and some are even quite outside the body itself and in the substance of the nerve cell (plate 1, figure 1, B; figure 2, E, H, I; figure 3, D). They present the same picture as the tiny round or oval spores described above. Some forms contain as many as twenty to thirty and these were observed with the ectoplasm intact and the spores completely filling the inside of the body. These forms measure eight to ten microns in diameter. In the still larger forms the membrane surrounding them becomes less distinct, until finally the definite structure of the rim-like band is lost completely. After losing the membrane the groups of spores in many cases are seen remaining close together as before (plate 1, figure 1, B). In general, however, after the disappearance of the cell membrane there is a marked outpushing of the spores from the center, making the body appear much larger than in any of the preceding forms and leaving in the center a small light pink staining area formerly occupied by the spores (plate 1, figure 2, I; figure 3, D). In these forms nothing which resembled a nucleus is seen nor are there any chromatin granules present. A later step in the development of the organism is seen when the spores become further removed from the center and occupy a much larger area. They form a circle with the pink staining residual protoplasmic remains of the original Negri body, possibly too a portion of the ectoplasm, on the inside and the blue staining cytoplasm of the nerve cell on the outside. The final step in the dissemination of the spores is seen when the circle of spores has

been broken up, and in certain nerve cells they are then seen scattered diffusely over the entire field (plate 1, figure 4, A).

This outlines briefly the successive stages seen in the growth, reproduction, and dissemination of this new type of Negri body. The succeeding steps in its growth and development, with the typical pictures presented in the different stages, and the multiplicative or schizogonous life cycle previously described, suggest immediately a sexual or sporogonous cycle for this new series of bodies described above.

Other writers have often referred to the possibility of finding a sexual cycle which in a measure would supplement the knowledge of the forms already found and do much toward increasing our knowledge of this protozoan parasite, which we now believe is the causative agent of rabies. The above findings, taken in the light of the multiplicative cycle already observed, are indeed strongly suggestive. Williams and Lowden, as has been stated, have placed the Negri bodies among the parasitic protozoa in the suborder Microsporidia of the Sporozoa. Organisms of this order in their sporogonous life cycle produce numerous oval or pear shaped spores corresponding closely to those described above, each of which possesses one polar capsule. This is not seen in the fresh specimen, but is brought out under suitable treatment with stains and reagents.

From the findings and observations recorded above the writer believes that we are now justified in placing the Negri bodies a little more definitely in the suborder of Microsporidia and that we may regard them as Oligosporogenea, in which forms the trophozoite produces a single pansporoblast. Its position is perhaps among these under the family Glugeidæ, when we bear in mind that in these the pansporoblast produces numerous spores.

The smallest forms observed with a single dark staining nucleus or chromatin granule are undoubtedly on the order of a trophozoite of a multiplicative cycle characterized ofttimes by an ameboid appearance and a dense rim of ectoplasm, with a granular endoplasm containing a nucleus and possibly chromatin granules. These may increase in size very rapidly until there are numbers of bodies of different sizes. During this growth they undoubtedly have the power of endogenous reproduction. This has been observed by several writers and may take place in different ways, all of which

are common to the members of this order. In the adult form (1) simple binary fission may take place, *i. e.,* an hour glass constriction, or (2) a simple budding may occur. This plasmotomous division, or breaking up of the multinucleate cell into numerous parts each having a nucleus with a bit of surrounding protoplasm has been termed, in the first case, a simple, and in the latter, a multiple type of division.

In the youngest stages of the organism there has been described nuclear fragmentation into several daughter nuclei, supposedly by a process of multiple amitosis, and this is similar to, if not identical with, that observed in the young forms of *Glugea lophii.* This type early spreads the infection through the tissues of the host and is probably the most common of all the methods of multiplicative growth. In the well known forms of Myxobolidæ and Glugeidæ it is undoubtedly the commonest type of growth. In considering the sporogonous or reproductive cycle, spores undoubtedly begin to develop at an early period in the life of the trophozoite. The finding of two, three, and four spores in a single small body, or pansporoblast, would seem to prove this. Among the better known and more closely studied members of the Microsporidia the first step in spore formation is the gathering of cell protoplasm around some of the nuclei of the mother endoplasm to form a primitive sphere (Gurley) or pansporoblast. Whether or not this has been observed in the case of the Negri bodies it is still difficult to say; stages which seemingly show a fragmentation of nuclei have been reported, but these may indicate a stage in the multiplicative, as well as a preparation for spore formation in the reproductive life cycle. The pansporoblasts as observed in the development of the Negri bodies have a definite membrane or envelope around them, and so far at least the spores they contain have been observed to vary considerably in number. Furthermore, when first observed they were rather completely formed so that all steps in their development, particularly the presence of a sporoblast stage, are not as yet completely understood. As to the origin of the polar capsules it has not as yet been observed often enough to state whether or not it arises like many other members of this order from a sphere-like vacuole.

ANALOGIES BETWEEN THE NEGRI BODIES AND THE BETTER KNOWN FORMS OF THE SUBORDER OF THE MICROSPORIDIA.

In general, the forms of the suborder of the Microsporidia show an extremely wide variation in their structure, habitat, and mode of life. The trophozoite has the following characteristic features which are well observed in the life cycle of the Negri bodies: (1) It is characteristically ameboid in shape. (2) Spore formation commences early and continues throughout life. (3) Spores are produced endogenously, *i. e.,* within the protoplasm of the trophozoite. (4) The spores have one or more polar capsules. While it is true that the Cryptocysts are found more often inhabiting invertebrate hosts, several forms are known which make their home exclusively in the tissues of the vertebrates. As parasites they are more destructive in their growth to the host cells than any other group of the Sporozoa. They are in every detail essentially intracellular organisms differing from the Phænocysts which are the intercellular forms, and only exceptionally at the very early stages are they found within the tissue cells.

The group in the main has two modes of life, one within the body cavities of the host, and the other within the cells and tissues of the host. The latter is clearly the manner of life of the Negri bodies. While muscle and connective tissue are the ones usually selected as the seat of growth of the organism, some forms attack the nervous tissue alone, as is seen in the *Glugea lophii* which has a special affinity for the ganglion cells of the central nervous system of *Lophius piscatorius.* The Cryptocysts may restrict their ravages to a single organ or tract, or they may attack all parts of the body. Naturally the method of growth may be of two kinds; that in which the growth is confined to definite foci, and that in which there is a diffuse infiltration of the tissues. The latter method is peculiar to the Negri bodies in which the body of the parasite and the protoplasm of the cell become closely intermingled, spores are formed, and the specific tissue becomes actually filled with young forms.

Trophozoite Stage.—This is characterized by its ameboid form in which an uncertain division can be made out between the coarser outer region and an inner granular center. The endoplasm has various inclusions; in the Negri bodies have been observed only the

nucleus, chromatin granules, and possibly occasionally a vacuole or two. In the tissue-infecting forms, the pseudopodia are usually small, of ectoplasmal origin, and cannot be observed in the stained preparations. In the Negri bodies no pseudopodia have been recognized. Here perhaps we have, as in other well known cases, the protective function of the ectoplasm developed at the expense of the motility. The youngest trophozoites have but one nucleus, the endoplasm increases continually by division until there are many present in the full grown forms, often ten or more, while definite nucleoli or karyosomes have not been recognized except in a few instances.

Spore Stage.—Spores are seen to develop in a very early stage of the trophozoite and sometimes they continue to be formed until the whole endoplasm of the trophozoite is used up in its production. In other cases, however, there is a small amount of residual protoplasm. All the stages of spore development are commonly found at the same time in a given individual. The first sign of spore formation is the gathering of protoplasm around one of the nuclei in the endoplasm. This comes to form the sporoblasts and later the spherical corpuscles, or pansporoblasts. All nuclei may not be used up in this manner, but may remain as residual nuclei (plate 2, figures i to xiii).

Among the Cryptocysts the pansporoblasts always give rise to more than two spores, *i. e.,* four in Gurleya, eight in Thelohania, and to a large number in Pleistophera and Glugea. One or more pansporoblasts may be formed from each trophozoite, the Oligosporogenea under this order produce but one, however, and here the condition simulates greatly the development of the Coccidia except that with them we have an exogenous development instead of an endogenous one. In general we may say that the Myxosporidia represent an alternation of generations between trophic and reproductive individuals.

The spores of the Myxosporidia are rather variable in their external structural details. The spores of the Cryptocysts are for the most part minute, measuring 3 to 4 microns in *Glugea anomala,* and 1.5 to 2.5 microns in *Glugea ovidea,* and are usually pear shaped or oval. The Phænocysts have much larger spores, some 100 by 12 microns (in *Cestomyxa spærulosa*).

The capsule of the spore is usually situated at one end and when this is the case, that end is termed the anterior pole. In the Glugeidæ the spore capsule is invisible in the fresh condition. These spore capsules are a distinctive feature of the Myxosporidia and by some it is believed that they represent sporozoites that have become specialized in nature. The developmental period which intervenes between the ripe spore and the youngest trophic stages is the least known period in the life cycle of this group of organisms. The microscopic parasite often migrates a great distance in the body of the host before it gets to the organ or tissue which is its final field for growth. This is well illustrated in the rabic infecting agent which, from a cutaneous bite or abrasion, must travel until it reaches the central nervous system before it finds a suitable field for growth. This stage shows that undoubtedly at one time in its life the parasite is able to select, and, furthermore, to seek out its specific tissue which may, in some instances, be at a great distance from the original seat of infection.

Schizogony, or Multiplicative Process.—When the amebula reaches its special tissue it can grow endogenously with great rapidity. The two processes of growth are called multiplicative, like schizogony, and reproductive, like sporogony. Multiplicative growth has always been attributed to the Negri bodies on account of the vast numbers of parasites that may be present in a given host, and the difficulty of supposing that each parasite could have originated from a separate and distinct spore infection. The multiplicative reproduction in the full grown trophozoite takes place in one of two ways, by simple fission, or by budding. This plasmotomous division has been termed, in the first case, simple, and in the latter, multiple amitosis (plate 2, figures 1 to 18).

The youngest forms divide by nuclear fragmentation and multiple amitosis following a breaking up of the protoplasm into minute uninucleate swarm spores, and in this way the tissue becomes diffusely infected.

CONCLUSION.

1. The Negri bodies, as the etiological agent in rabies, present two general types or phases in morphology, in growth, and in reproduction.

2. These two phases are constantly cyclic in their development and correspond (1) to a multiplicative, or schizogonous, and (2) to a reproductive, or sporogonous, life cycle.

3. By the detailed study of these forms and their succeeding stages we are inclined to believe that the Negri bodies are definite protozoan parasites, and from a study of their life history we are led to place them in the suborder of Cryptocysts, or Microsporidia, of the Sporozoa, and more definitely among the Oligosporogenea of the Glugeidæ family, which forms produce but one pansporoblast.

With the recent advances in the growth of tissues outside the body, particularly the work of Carrel, Burrows, Harrison, Lewis and Lewis, and Rous upon the infectiousness of tumor extracts, with the results of Noguchi in cultivating pathogenic *Treponema pallidum,* important discoveries concerning the Negri bodies will undoubtedly be made in the near future.

I wish to extend my thanks to Dr. Charles V. Chapin, Superintendent of Health of the City of Providence, for the material used in this work, and to Prof. F. P. Gorham, of Brown University, for his valuable suggestions.

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EXPLANATION OF PLATES.

Plate 1.

Fig. 1. Two nerve cells from hippocampus major in a smear preparation.
A. Deeper staining nuclei of the two nerve cells.
B. A large Negri body of the sexual type and pansporoblast stage, without a surrounding membrane and containing many individual spores.
C. Spores of the sexual type lying free in the protoplasm of the nerve cell.
D. A Negri body of the asexual type or multiplicative stage, containing the characteristic chromatin granules.
E. A small sexual pansporoblast containing several spores.
F. A small sexual pansporoblast surrounded by a membrane and containing several spores.

Fig. 2. Two nerve cells in a smear preparation.
A and B. Asexual type of Negri bodies with chromatin granules.
C and D. Nucleus of one of the nerve cells.
E. Sexual type of Negri body containing spores.
F. Smaller sexual type of a Negri body containing spores.
G. Proliferating endothelial cell.
H. A small sexual type of a Negri body containing spores.
I. A large pansporoblast containing many spores which have begun to leave the center of the Negri body preparatory to being disseminated through the protoplasm of the nerve cell. The membrane is lacking.
J. Small sexual type of a Negri body with membrane and spores.

Fig. 3. A. A small asexual trophozoite with chromatin granules.
B. Nucleus of the nerve cell.
C. A small asexual type of body.
D. A large pansporoblast pressed out of the nerve cell, showing absence of the membrane and the characteristic arrangement of the spores about the periphery.

Fig. 4. A. Spores liberated from the pansporoblast and lying free in the protoplasm of the nerve cell.
B. Nucleus of the cell.

Plate 2.

Sexual and asexual life cycle.

Figs. 1 to 18. Asexual or multiplicative cycle of the Negri body, showing the development from the spore and trophozoite through the various division forms, all containing the characteristic chromatin granules.

Figs. i to xiii. Sexual or sporogonous cycle, showing the development from the original spore to the pansporoblast with the surrounding membrane and endogenous spores; later, without the membrane and with the peripheral arrangement of the contained spores; and finally, the disseminated spores throughout the protoplasm of the nerve cell.

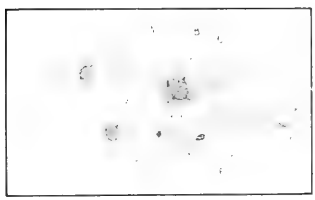


FIG. 1.

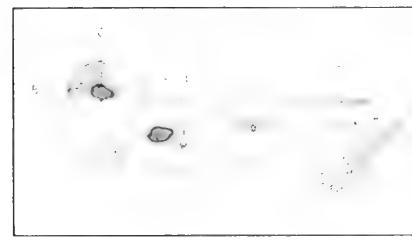


FIG. 2.

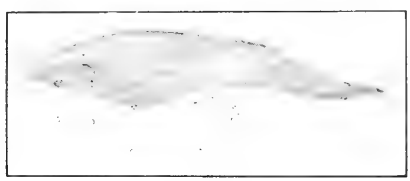


FIG. 3.

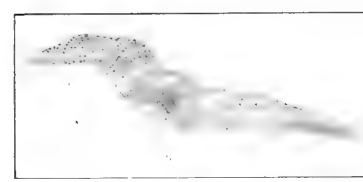
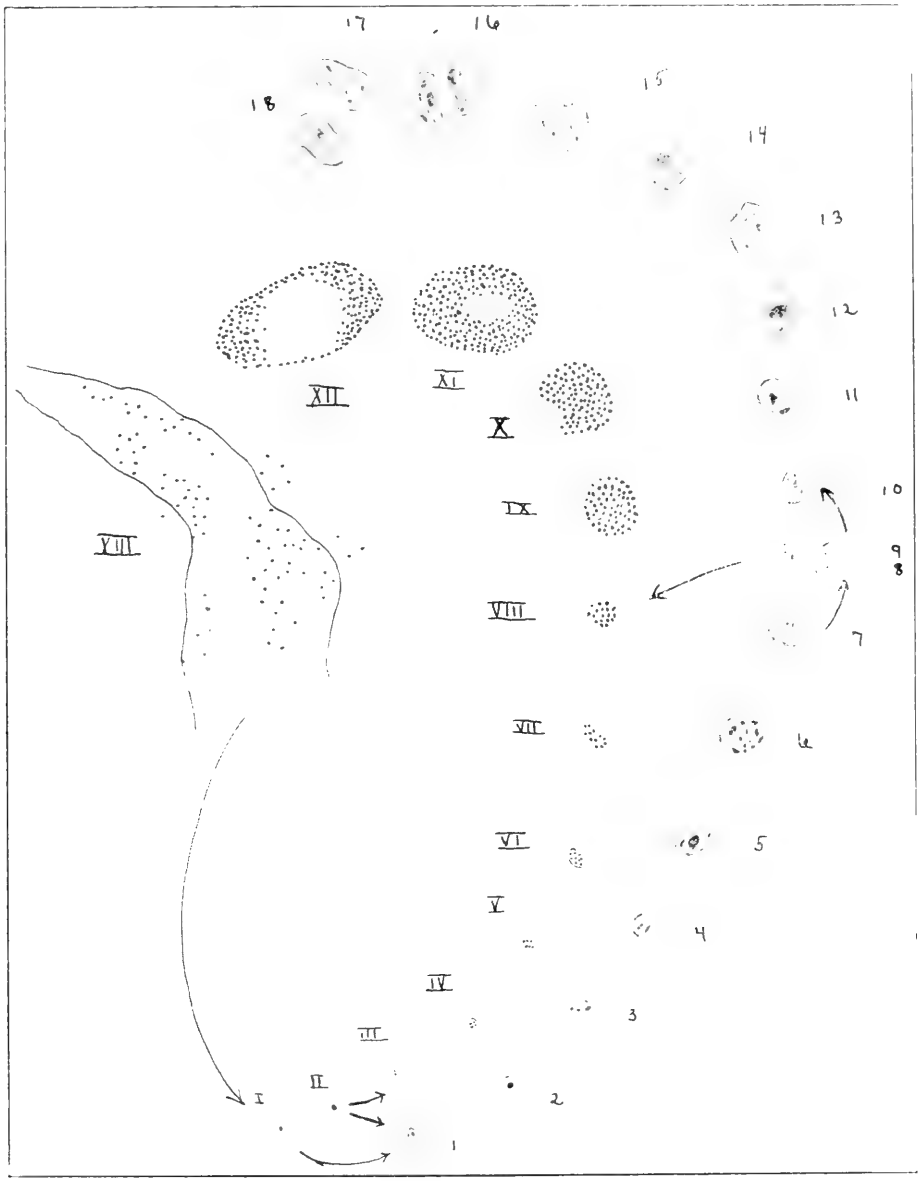


FIG. 4.

(WATSON: The Negri Bodies in Rabies.)



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THE PRODUCTION OF ACID BY THE BACILLUS COLI GROUP.

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THE PRODUCTION OF ACID BY THE BACILLUS COLI GROUP

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The year 1885 marks the beginning of the study of the bacteria of the intestinal tract in relation to their action on the various carbohydrates. In that year, Buchner, working with his "Darmbacillus G," which, in all probability, was a member of the bacillus coli group, found that growth in a medium, consisting of meat infusion, peptone, and sugar, was accompanied by the formation of acid and gas. The acid was demonstrated by the addition of litmus to the medium. The production of acid and gas, according to Buchner, was due to the breaking down, or decomposition, of the sugar brought about by the action of the bacteria. The evolved gas was found to be carbon dioxid, and the acids were defined as members of the fatty acid series. The work of Buchner may be considered as the starting point of all the work done on the relation of bacteria to the decomposition of carbohydrates with the production of acid and gas.

In connection with the production of acid in carbohydrate solutions by bacteria, progress has been particularly active along two distinct lines: (1) The differentiation of the colon group from the typhoid group and other alkali producers, and (2) the classification of the bacillus coli group, according to the ability of its members to ferment the various carbohydrates.

Petruschky's litmus whey medium, Beijerinck's calcium carbonate medium, Wurty's litmus lactose agar, Kaufmann's jequirity solution, and Hanna's proteid medium were important steps in the investigations along the former line. Among the workers of this period, who have contributed to our knowledge of carbohydrate fermentation, are Beginsky (1888), Lembke (1896), Von Sommoruga (1892), Capaldi and Proskauer (1897), Ziellecyky (1902) and Segin (1903). These results were augmented by Theobald Smith by use of the fermentation tube.

Beginning with the work of Durham in 1900, nearly all the investigations along the line of fermentation of carbohydrates by the bacillus coli group have been done with the idea of classifying the members of the group by means of their fermentative reactions on the various carbohydrates. The work of Dur-

ham has been extended and augmented by the notable researches of Houston (1902), MacConkey (1905), Winslow and Walker (1907), Graham Smith (1909), and Jackson (1911).

In spite of the importance of this fermentative reaction, however, there are many of the finer points of technic which have never been fully and conclusively worked out. Acid production, like all other biochemical reactions, is a function of the reacting organism, the substance decomposed, the end products formed, the time, the temperature, and all conditions which may check or favor the vital process. In the present investigation, I have attempted to isolate these various factors and study them, one by one, with the idea of gaining a clear idea of the quantitative significance of each. No startlingly novel conclusions could be expected, but it is hoped that the data obtained may furnish a surer basis for comparative studies of fermentative power in carbohydrate media than has been available in the past.

ORGANISMS USED IN THE INVESTIGATION

The organisms used in this work were all members of the bacillus coli group. Altho the identification of this group has been a subject of dispute for over a quarter of a century, it is now generally conceded that the following characteristics may be considered criteria of membership: (1) Short bacillus with rounded ends; (2) gram-negative; (3) non-liquefaction of gelatin in 16 days; (4) fermentation of dextrose and lactose with the production of acid and gas; (5) non-spore-forming; (6) facultative anaerobe; (7) gas in lactose-peptone-bile; (8) grayish white growth on agar at 20 C. and 37 C.

Other characteristic properties of this group, such as the reduction of nitrates to nitrites, production of indol, motility, and style of growth on various media, are valuable, if at all, merely for establishing minor subdivisions.

The organisms used in this investigation all conformed to the general characteristics of the bacillus coli group, as noted. In the early part of the work, no particular attention was paid to the subdivisions of the group, and, as a result, organisms were used which were identified only by the general group characteristics. As the work developed and the number of carbohydrates in use increased, the subdivisions of the group began to manifest themselves. A clean-cut differentiation into various species was obtainable by the production

or non-production of acid in the carbohydrate solutions. As the writer had no dulcite at hand, a subdivision of the group into species, as recommended by Jackson, was impossible, and, as a result, all the strains used throughout the work have been designated as the bacillus coli, altho it is certain that a number of species were present. As far as possible, those organisms which gave similar fermentative reactions have been grouped in the same table.

The cultures were obtained from two sources; either from oysters grown in contaminated water, or directly from feces. The oysters, from which the organisms were isolated, were taken from 242 different stations in Narragansett Bay during an investigation to determine the amount of pollution of the oyster-beds of the State of Rhode Island. The area from which the oysters were taken included localities, varying from those extremely polluted near sewer outlets to the less polluted areas, which were sometimes coli-positive and other times coli-negative.

One series of the cultures, isolated from feces, was obtained from normal stools of people in and about the laboratory at Brown University, while the other series was obtained from the stools of Italian immigrants, quarantined aboard the *S. S. Roma,* who were undergoing examinations for the cholera vibrio. These two series were kept entirely distinct and will be so designated throughout the work.

METHODS

Preparation of Culture Media.—The media were prepared after the methods proposed in the Report of the Committee on Standard Methods of Water Analysis to the Laboratory Section of the American Public Health Association, January 5, 1905, Liebig's meat extract was used, 3 grams to the liter. Ease of preparation rendered it more suitable for the work of this kind than a medium made up with meat. Great care was taken in the preparation of the media to make all the lots as uniform as possible. For this reason, all the constituents were taken from the same lot of materials during the entire course of the experiments.

The carbohydrate solutions were made by the addition of 1 percent of the various carbohydrates to neutral nutrient broth. Twenty-five cubic centimeters of the broth were placed in regular laboratory test tubes of large size, and sterilized on three successive days in streaming steam at 100 C.

During the course of the experiments, the production of acid was determined in the following carbohydrates:

I. I. Monosaccharids:

A. Hexoses.
1. Dextrose (Merck).
2. Galactose (Merck).
3. Levulose (Kahlbaum).

B. Pentoses.
1. Arabinose (Kahlbaum).
2. Xylose (Kahlbaum).

II. Disaccharids:
1. Lactose (Merck).
2. Maltose (Merck).
3. Saccharose (Merck).

III. Trisaccharid:
1. Raffinose (Kahlbaum).

IV. Hexatomic alcohols:
1. Iso-dulcite (Kahlbaum).
2. Mannite (Merck).

All cultures used in the determination of the production of acid in the carbohydrate solutions were grown at 37 C. for 24 hours, unless otherwise stated. The titrations were made with N/20 sodium hydroxid at the twenty-fourth hour, and results tabulated in direct percentages of normal sodium hydroxid.

Method of determining amount of acid produced.—From a twenty-four-hour agar slant culture of the organism, which had been identified as a member of the bacillus coli group, inoculations were made into peptone solutions, which were incubated for 24 hours at 37 C. At the end of 24 hours, the tubes of carbohydrate solution were inoculated by the addition of 0.5 c.c. of the twenty-four-hour peptone culture. By the addition of a definite amount of the twenty-four-hour culture, more consistent results were obtained than by the direct inoculation from the agar slant culture.

The inoculated carbohydrate solutions were incubated for 24 hours and were then ready for titration. The cultures were titrated as soon as possible after their removal from the incubator.[1]

Five cubic centimeters of the culture and 45 c.c. of distilled water were placed in a caserole and boiled briskly for one minute. One cubic centimeter of phenolphthalien, which consisted of 5 gm. of the commercial salt dissolved in one liter of 50 percent alcohol, was added as an indicator. Titrations were made into the hot solution until a faint, but permanent, pink color was obtained with N/20 sodium hydroxid solution.

THE RELATION OF TEMPERATURE TO THE AMOUNT OF ACID PRODUCED

Twenty-five cubic centimeters of the various sterilized carbohydrate solutions were inoculated with 0.5 c.c. of a twenty-four-hour peptone culture of the identified organisms. The cultures and their controls were kept for 24 hours at the following temperatures: Ice-box temperature, 3 C.; room temperature, 16 C.; incubator temperature, 28 C.; incubator temperature, 37 C.; incubator temperature, 45 C.

At the end of the twenty-fourth hour the cultures and their controls were titrated as soon as accuracy would allow. All titrations were made with N/20 sodium hydroxid, and all results are expressed in direct percentages of normal sodium hydroxid.

The results obtained in these experiments show that cultures of the bacillus coli group, grown at a temperature of 37 C., tend to produce more acid in carbohydrate solutions, in a given time, than cultures grown at other temperatures. Cultures grown at 3 C. show almost no acid production in 24 hours. As we approach 37 C., either from below

1. The method of titration followed was the one outlined in Standard Methods of Water Analysis, 1905, p. 106.

TABLE 1

AMOUNT OF ACID, IN PERCENT NORMAL, PRODUCED AT DIFFERENT TEMPERATURES BY BACILLUS COLI, ISOLATED FROM FECES

Temperature	Percentage of Acid with Dextrose	Percentage of Acid with Lactose	Percentage of Acid with Levulose	Percentage of Acid with Galactose	Percentage of Acid with Maltose	Percentage of Acid with Xylose	Percentage of Acid with Arabinose	Percentage of Acid with Mannite	Percentage of Acid with Isodulcite	Percentage of Acid with Control
3 C.	0	0	0	0	0	0	0	0	0	0
16 C.	0.6	0.3	0.7	0.3	0.3	0.3	0.3	0.3	0.2	0
28 C.	2.0	1.7	2.1	1.3	1.6	1.8	1.7	1.7	1.7	0
37 C.	2.3	1.9	2.3	1.9	1.9	2.0	2.0	2.0	2.2	0
45 C.	1.3	1.0	1.5	0.9	0.3	1.3	1.2	1.0	0.2	0

The results are the average of the titrations of two cultures.

TABLE 2

AMOUNT OF ACID, IN PERCENT NORMAL, PRODUCED AT DIFFERENT TEMPERATURES BY THE BACILLUS COLI, ISOLATED FROM OYSTERS

Temperature	Percentage of Acid with Dextrose	Percentage of Acid with Lactose	Percentage of Acid with Saccharose	Percentage of Acid with Levulose	Percentage of Acid with Galactose	Percentage of Acid with Maltose	Percentage of Acid with Raffinose	Percentage of Acid with Xylose	Percentage of Acid with Arabinose	Percentage of Acid with Mannite	Percentage of Acid with Isodulcite	Percentage of Acid with Control
3 C.	0	0	0	0	0	0	0	0	0	0	0	0
16 C.	0.4	0.1	0.1	0.6	0.2	0.3	0.2	0.3	0.3	0.3	0.2	0
28 C.	2.0	1.7	1.4	2.1	1.2	1.6	0.9	1.6	1.6	1.4	0.8	0
37 C.	2.2	2.0	1.4	2.3	1.8	1.9	1.5	1.9	2.0	1.9	2.0	0
45 C.	1.2	0.7	0.3	1.3	0.8	0.2	0.2	1.2	1.0	0.9	0.2	0

The results are the average of the titrations of two cultures.

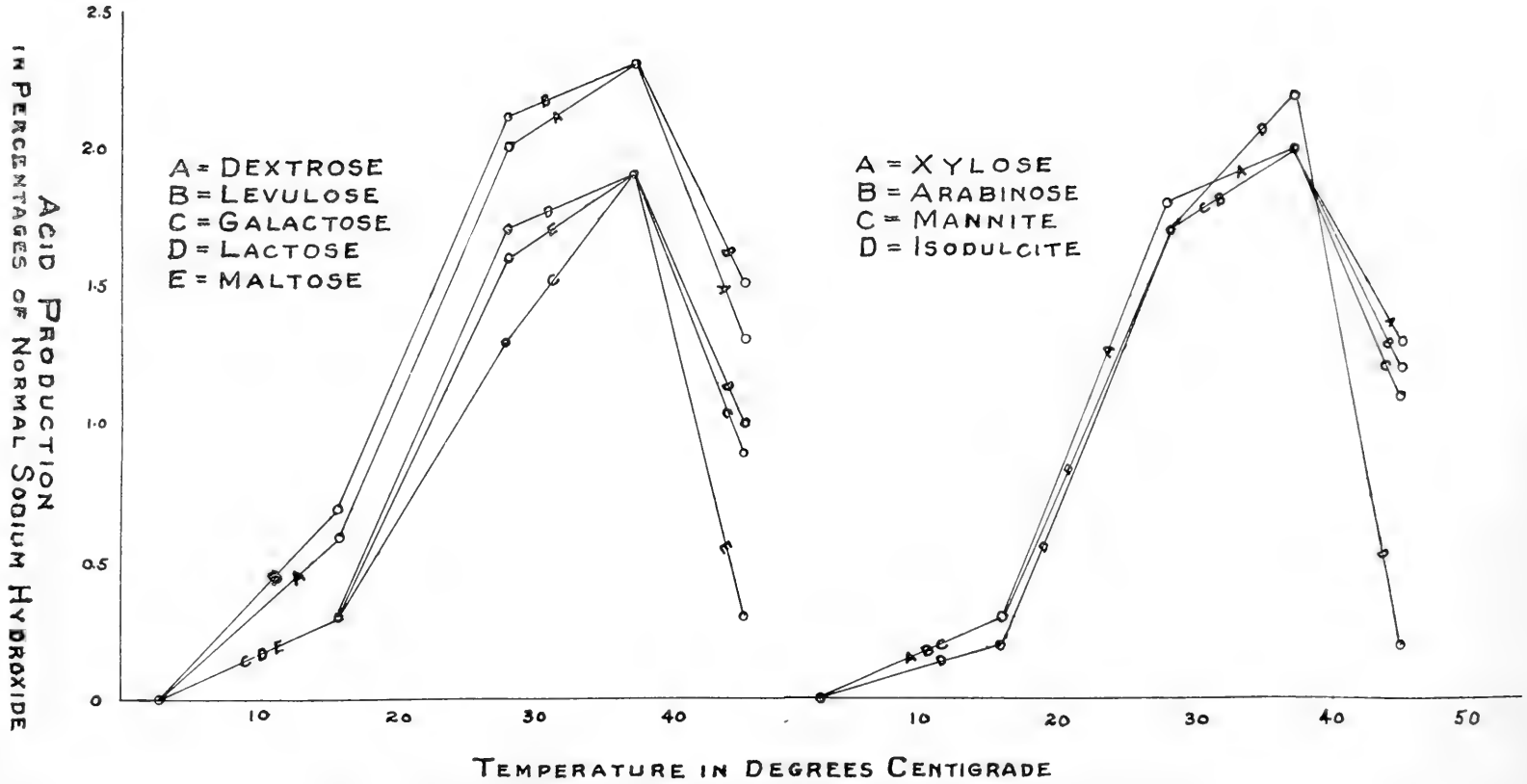

Chart 1.—The relation of temperature to the amount of acid produced by the bacillus coli, isolated from feces.

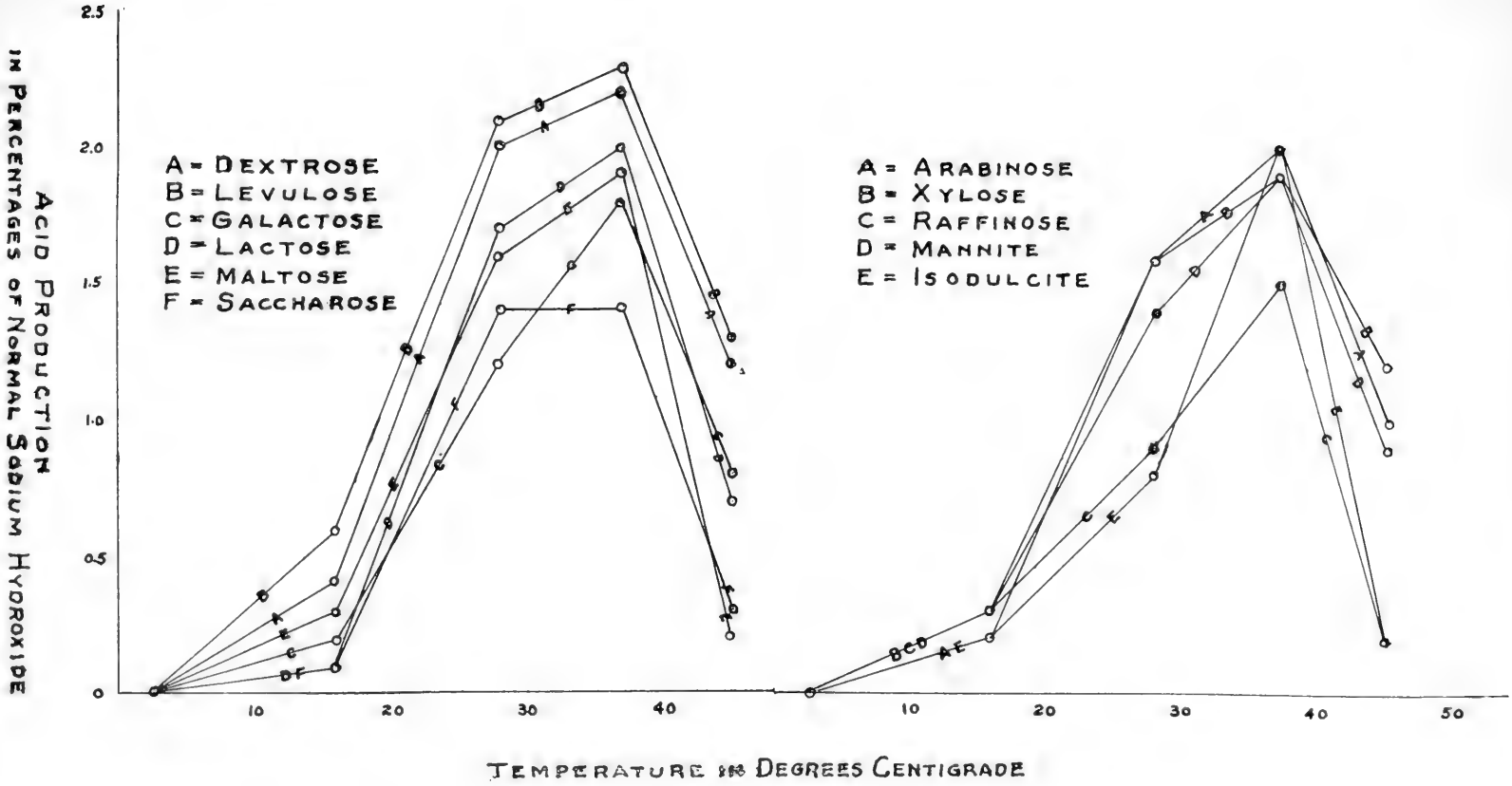

Chart 2.—The relation of temperature to the amount of acid produced by the bacillus coli, isolated from oysters.

or above, the amount of acid increases until the maximum amount of acid is produced at 37 C. We should expect this, since 37 C. is the optimum temperature for the growth of the bacillus coli group. If the temperature is too low or too high, we get less growth, and since acid production is dependent on the growth of the organism, we must surely find the optimum temperature for the production of acid identical with the optimum temperature for growth. This was found to be true in all the carbohydrate solutions used.

THE RELATION OF TIME TO THE AMOUNT OF ACID PRODUCED IN VARIOUS CARBOHYDRATE MEDIA

The general method of these experiments consisted in the inoculation of a large number of tubes of the 1 percent carbohydrate media with various members of the coli group. At definite intervals, a certain number of the tubes were removed from the incubator and titrated with N/20 sodium hydroxid. These experiments include 10 carbohydrates using various members of the bacillus coli group. Fifteen tubes of the 10 carbohydrates noted below, each containing 25 c.c. of the medium, were inoculated with 0.5 c.c. of a twenty-four-hour peptone culture of the bacillus coli. The tubes were incubated at 37 C., and were removed from the incubator and titrated at intervals of three hours.

From the results obtained in these experiments it is seen that, by the end of the twenty-fourth hour, the members of the bacillus coli group produce their maximum amount of acid when grown at 37 C., after the inoculation of a sugar medium with 0.5 c.c. of a twenty-four-hour peptone culture. In the case of some carbohydrates, such as dextrose or lactose, the maximum production of acid occurs in the eighteenth hour. The organisms used in this experiment produced their maximum amount of acid either before or at the twenty-fourth hour. Cultures, which were grown for 48 and 72 hours at 37 C., showed no increase over the amount of acid produced at the end of the twenty-fourth hour. The maximum amount of acid was produced by the organisms by the end of the twenty-fourth hour.

THE RELATION OF THE AMOUNT OF ACID PRODUCED TO THE AMOUNT OF MEDIUM INOCULATED

Erlenmeyer flasks, containing 25, 50, 100, 200, 300, 400, and 500 c.c. of dextrose and lactose broths, were inoculated with 0.5 c.c. of a twenty-four-hour culture of the coli bacillus. The flasks were incubated for 24 hours at 37 C., and then titrated with N/20 sodium hydroxid.

TABLE 3

ACID PRODUCTION, IN PERCENT NORMAL, AT INTERVALS OF THREE HOURS, BY THE BACILLUS COLI, ISOLATED FROM FECES

Hours	Percentage of Acid with Dextrose	Percentage of Acid with Lactose	Percentage of Acid with Saccharose	Percentage of Acid with Levulose	Percentage of Acid with Galactose	Percentage of Acid with Maltose	Percentage of Acid with Raffinose	Percentage of Acid with Arabinose	Percentage of Acid with Isodulcite	Percentage of Acid with Mannite
3	1.0	0.6	0.35	0.95	0.35	0.7	0.35	0.5	0.35	0.35
6	1.9	1.4	0.3	1.8	0.7	0.8	0.7	1.7	1.1	1.3
9	2.2	1.9	0.4	1.9	0.85	1.05	1.15	1.9	1.6	1.3
12	2.35	1.9	1.1	2.1	1.3	1.7	1.3	2.0	1.9	1.3
15	2.4	1.9	1.85	2.2	1.9	1.8	1.35	2.1	1.9	2.0
18	2.4	1.9	2.0	2.3	1.9	2.0	1.7	2.2	2.2	2.0
21	2.4	1.9	2.0	2.3	2.1	2.0	2.0	2.2	2.2	2.0
24	2.4	1.9	2.0	2.2	2.1	2.0	2.0	2.2	2.1	2.0
42	2.4	2.0	2.1	2.3	2.1	2.0	1.9	2.2	2.1	2.0

All titrations are the average of titrations of two cultures.
The controls titrated at the end of 24 hours gave a neutral reaction.
The temperature was 37 C.

TABLE 4

ACID PRODUCTION, IN PERCENT NORMAL, AT INTERVALS OF THREE HOURS, BY THE BACILLUS COLI, ISOLATED FROM OYSTERS

Hours	Percentage of Acid with Dextrose	Percentage of Acid with Lactose	Percentage of Acid with Saccharose	Percentage of Acid with Levulose	Percentage of Acid with Galactose	Percentage of Acid with Maltose	Percentage of Acid with Raffinose	Percentage of Acid with Arabinose	Percentage of Acid with Isodulcite	Percentage of Acid with Mannite
3	0.45	0.35	0.15	0.4	0.35	0.4	0.35	0.35	0.3	0.2
6	1.5	0.8	0.9	1.4	1.0	1.0	0.4	1.5	1.0	1.0
9	1.6	0.8	1.7	1.8	1.35	1.05	0.4	1.4	1.5	1.1
12	1.9	0.8	1.7	1.9	1.7	1.3	0.4	1.4	1.8	1.2
15	1.9	1.45	1.8	1.9	1.7	1.3	0.9	1.4	2.0	1.3
18	1.9	1.5	1.6	2.1	1.8	1.3	1.4	1.5	2.2	1.3
21	1.9	1.5	1.6	2.1	2.1	1.4	1.4	1.6	2.2	1.3
24	2.0	1.9	1.6	2.0	2.1	1.4	1.5	1.6	2.1	1.3
42	2.0	1.9	1.6	2.0	2.1	1.4	1.6	1.7	2.1	1.3

All titrations are the average of the titrations of two cultures.
The controls were titrated at the end of 24 hours and the reaction was neutral.
The temperature was 37 C.

The results obtained show that no matter how much carbohydrate media is inoculated, up to 500 c.c., the same percentage of acidity is produced by the bacillus coli when grown at 37 C. for 24 hours after inoculation with 0.5 c.c. of a twenty-four-hour peptone culture.

THE RELATION OF THE CONCENTRATION OF THE CARBOHYDRATE MEDIUM TO THE AMOUNT OF ACID PRODUCED

Tubes of neutral nutrient broth, to which were added various percentages of dextrose and lactose, were inoculated with 0.5 c.c. of a twenty-four-hour peptone culture of the bacillus coli. The tubes were incubated for 24 hours at 37 C., and at the twenty-fourth hour the tubes were titrated with N/20 sodium hydroxid.

TABLE 5

AMOUNT OF ACID, IN PERCENT NORMAL, PRODUCED BY THE BACILLUS COLI ISOLATED FROM FECES, IN NEUTRAL BROTH CONTAINING VARYING PERCENTAGES OF DEXTROSE AND LACTOSE

Percentage of Dextrose	Amount of Acid Produced Percent	Percentage of Lactose	Amount of Acid Produced Percent
0.125	0.4	0.125	0.35
0.25	0.5	0.25	0.5
0.5	2.1	0.5	1.7
0.1	2.2	1	1.8
1.5	2.2	1.5	1.8
2	2.3	2	1.8
2.5	2.2	2.5	1.75
3	2.4	3	1.8
3.5	2.3	3.5	1.8
4	2.2	4	1.8
4.5	2.3	4.5	1.8
5	2.4	5	1.8
7.5	2.35	7.5	1.7
10	2.3	10	1.8
15	2.3		
20	2.3		
25	2.3		
30	1.8		
35	0.9		
40	0.8		
45	0.6		
50	0.3		

The results are the average of the titrations of two cultures. Temperature at 37 C.

From these experiments it appears that the concentration of the carbohydrates (dextrose and lactose) has little effect on the amount of acid produced, within certain limits, by the bacillus coli. Between 1 percent and 25 percent concentration, the amount of acid produced is nearly constant. Below 1 percent and above 25 percent there is less acid produced. A 1 percent solution gives the maximum amount of acid from the least amount of the carbohydrate.

THE RELATION OF THE INITIAL REACTION OF THE CARBOHYDRATE MEDIUM TO THE AMOUNT OF ACID PRODUCED

The purpose of these experiments was to determine whether or not the initial reaction of the carbohydrate medium had any influence upon the production of acid by the organisms.

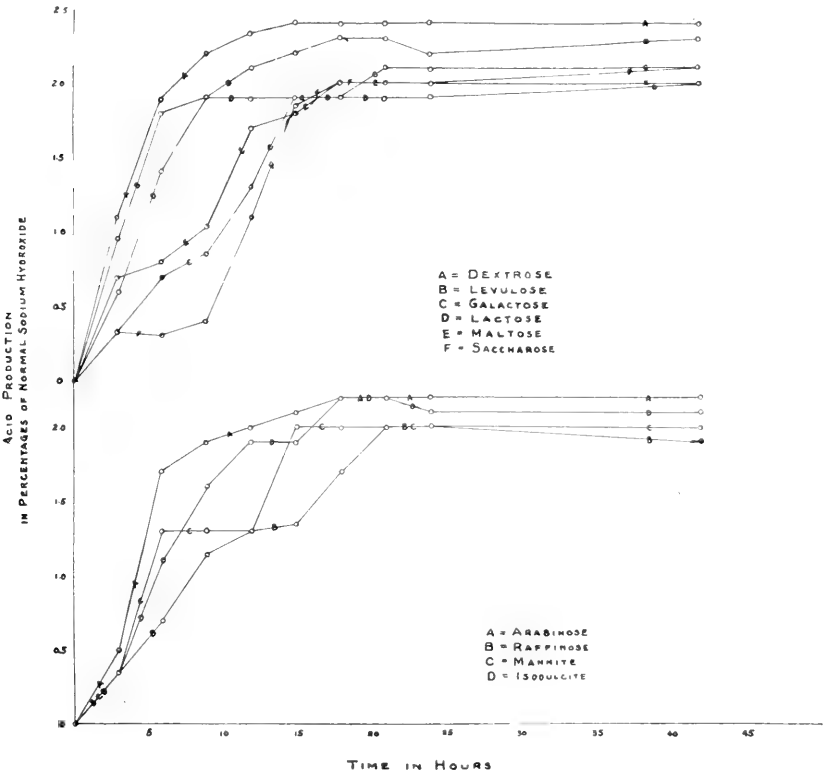

Chart 3.—The relation of time to the amount of acid produced by the bacillus coli, isolated from feces.

Tubes of dextrose broth, to which varying amounts of sterile acid and alkali were added after sterilization, were inoculated with 0.5 c.c. of a twenty-four-hour peptone culture of the bacillus coli. These tubes were incubated for 24 hours at 37 C. At the end of 24 hours the cultures were titrated with N/20 sodium hydroxid.

The amount of acid produced by the bacillus coli group, in various carbohydrate media, depends, in great part, upon the initial reaction of the medium. The maximum acid production of an organism is the

TABLE 6

AMOUNT OF ACID, IN PERCENT NORMAL, PRODUCED IN DEXTROSE BROTH, WITH VARYING INITIAL REACTIONS, BY THE BACILLUS COLI, ISOLATED FROM FECES AND FROM OYSTERS

Initial Reaction of Dextrose Broth	Amount of Acid Produced by Colon Bacilli from Feces					Amount of Acid Produced by Colon Bacilli from Oysters				
	Culture 1	Culture 2	Culture 3	Average	Control	Culture 1	Culture 2	Culture 3	Average	Control
+4.4	4.4	4.5	4.4	4.42	4.3	4.3	4.3	4.3	4.3	+4.4
+2.65	2.6	2.7	2.7	2.66	2.65	2.5	2.5	2.5	2.5	+2.6
+2.1	2.3	2.4	2.3	2.33	2.0	2.3	2.3	2.3	2.3	+2.1
+1.0	2.3	2.3	2.3	2.3	1.0	2.5	2.5	2.5	2.3	+1.0
+0.15	2.2	2.3	2.3	2.26	0.1	2.3	2.3	2.3	2.3	+0.15
—0.5	2.3	2.3	2.4	2.33	0.5	2.4	2.4	2.4	2.4	—0.5
—0.6	2.3	2.3	2.3	2.3	0.6	2.4	2.4	2.4	2.3	—0.6

Temperature was 37 C.

amount of acid necessary to prevent further growth. The farther away the initial reaction is from the maximum acid production, the more acid an organism can produce until it reaches the maximum. The limiting acidity of the bacillus coli group is about 2.4 percent normal sodium hydroxid. A medium with a slightly acid reaction offers a shorter path to travel to the maximum acidity than a medium

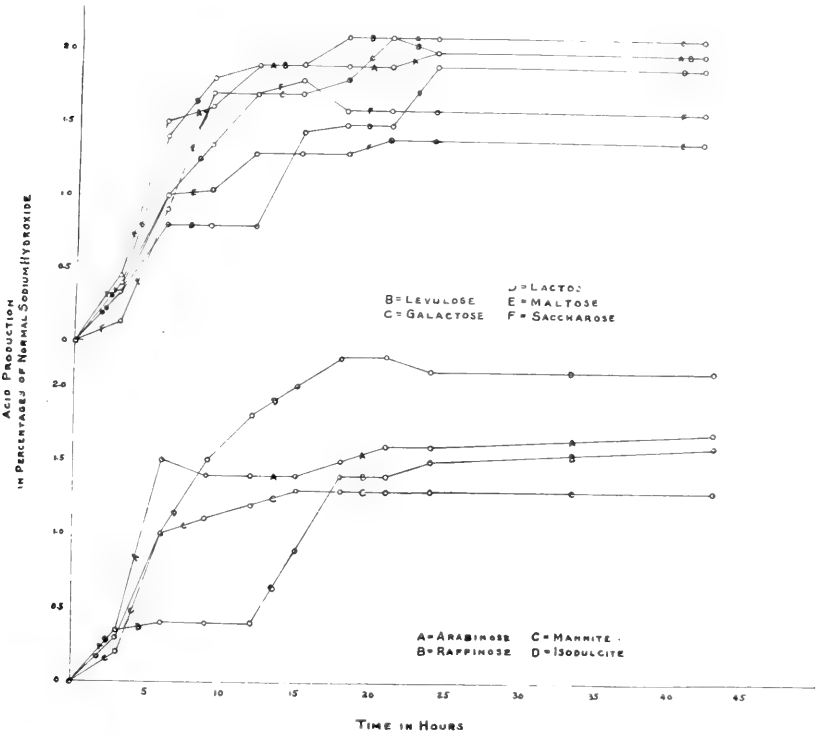

Chart 4.—The relation of time to the amount of acid produced by the bacillus coli, isolated from oysters.

with a neutral or slightly alkaline reaction. Hence, in a medium which is alkaline, the bacillus coli group will produce more acid than in a medium which is slightly acid, but in the end they will both reach the same goal — the maximum acidity of the organism. No acid is produced in a medium with over 2.4 percent acidity. The limit of alkalinity from which the organisms will produce acidity, was not determined.

TABLE 7

TOTAL AMOUNT ACID, IN PERCENT NORMAL, PRODUCED IN VARIOUS CARBOHYDRATES BY THE BACILLUS COLI, ISOLATED FROM FECES

Days	Percentage of Acid with Dextrose	Percentage of Acid with Levulose	Percentage of Acid with Galactose	Percentage of Acid with Lactose	Percentage of Acid with Maltose	Percentage of Acid with Saccharose	Percentage of Acid with Xylose	Percentage of Acid with Arabinose	Percentage of Acid with Raffinose	Percentage of Acid with Mannite	Percentage of Acid with Isodulcite
1	3.0	3.2	2.7	2.3	2.7	1.7	2.0	2.7	2.7	2.6	2.9
	2.9	2.9	2.1	2.5	2.3	2.7	2.4	2.8	1.4	2.7	2.6
2	3.0	3.7	2.5	2.7	2.3	2.2	2.4	2.5	2.9	1.3	2.9
	2.9	3.1	2.2	2.8	2.7	2.5	2.8	2.8	2.2	2.5	1.4
3	3.2	1.1	2.3	3.9	1.3	2.3	4.0	1.0	2.0	1.6	1.1
	2.6	2.1	2.5	2.5	0.6	3.7	2.8	2.0	2.0	1.0	1.2
4	0.3	0.3	0.3	0.4	0	1.6	0	0.25	1.0	0	0
	1.3	0.6	1.1	2.1	0.4	0.4	0	1.6	1.8	0	0.8
5	0.3	0.3	0.2	0.3	0	1.5	0	0.2	0.8	0	0
	0.3	0.3	0.5	0.8	0.5	0.3	0	0.4	0.5	0.2	0.6
6	0.2	0.2	0.3	0.3	0	1.35	0	0	0.4	0.4	0
	0.6	0.3	0.3	0.4	0.2	0.3	0	0	0.4	0.2	0.8
7	0.2	0	0	0.2	0	0.8	0	0	0.3	0	0
	0.4	0.2	0.3	0.4	0.7	0	0	0	0.3	0.2	0.8
8	0	0	0	0.4	0	0.5	0	0	0.2	0	0
	0	0	0.6	0	0	0	0	0	0	0	0.4
9	0	0	0	0	0	0.5	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0.1
10	0	0	0	0	0	0.2	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0
Total Average	10.6	9.15	8.95	11.0	6.85	11.7	8.2	7.62	9.45	6.35	7.3

TABLE 8

COMPARISON BETWEEN TOTAL ACID PRODUCTION AND THE MAXIMUM ACID PRODUCTION OF 24 HOURS INCUBATION

	Dextrose	Levulose	Galactose	Arabinose	Xylose	Mannite	Isodulcite	Lactose	Maltose	Saccharose	Raffinose
Total acid production	10.6	9.15	8.95	7.6	8.2	6.35	7.3	11.0	6.85	11.27	9.45
Maximum acid production of 24 hours	2.35	2.25	1.97	1.87	1.90	2.00	1.90	1.85	2.05	1.35	1.47

ACID PRODUCTION IN A MEDIUM PERIODICALLY NEUTRALIZED TO REMOVE THE ACIDS FORMED

Erlenmeyer flasks containing 100 c.c. of the various carbohydrates (1 percent concentration) were inoculated with 0.5 c.c. of a twenty-four-hour peptone culture of the different members of the bacillus coli group. Before inoculations were made, 0.5 c.c. of phenolphthalein (5 gm. of the commercial salt to one liter of 5 percent alcohol) was added as an indicator. The flasks were incubated at 37 C. and at intervals of 24 hours, the flasks were removed and sterile normal sodium hydroxid was added from a burette, until a faint pink color was obtained. The cultures were replaced in the incubator and the process was repeated at the end of every 24 hours, until no more acid was produced by the organisms.

Table 8 shows that the maximum acid production of 24 hours, which is, as previously proved, the greatest amount which the organism can produce at any one time, is limited by the excess of acid formed, and can be greatly increased by periodical neutralization.

The maximum amount of acid, which the bacillus coli group is able to produce in 1 percent carbohydrate solutions in 24 hours can be greatly increased if the excess of acid formed is neutralized. In all carbohydrates of 1 percent concentration, the total acid production is from 3 to 6 times as large as the maximum twenty-four-hour production which, as previously proved, is the same for 48 and 72 hours under ordinary conditions. From these experiments it may be assumed that acid production goes on until a maximum is reached (24 hours) and then ceases until some of the acid present is neutralized, when acid is again produced until the maximum is reached. This same thing occurs until all the carbohydrates are used. None of the cultures on the twelfth day gave a positive reaction for sugar, showing that the carbohydrates had been used entirely by the organisms in the production of acid. The total amount of acid an organism is able to produce, if the excess acid is neutralized, depends, in a large part, on how much fermentable carbohydrate there is present in the medium. The maximum twenty-four-hour production on the other hand, will use only that amount which is necessary to produce the maximum amount of acid which can be tolerated.

THE AMOUNT OF ACID PRODUCED IN VARIOUS CARBOHYDRATES

An attempt has been made to draw a comparison between the various carbohydrates by the amount of acid produced from them by different members of the bacillus coli group.

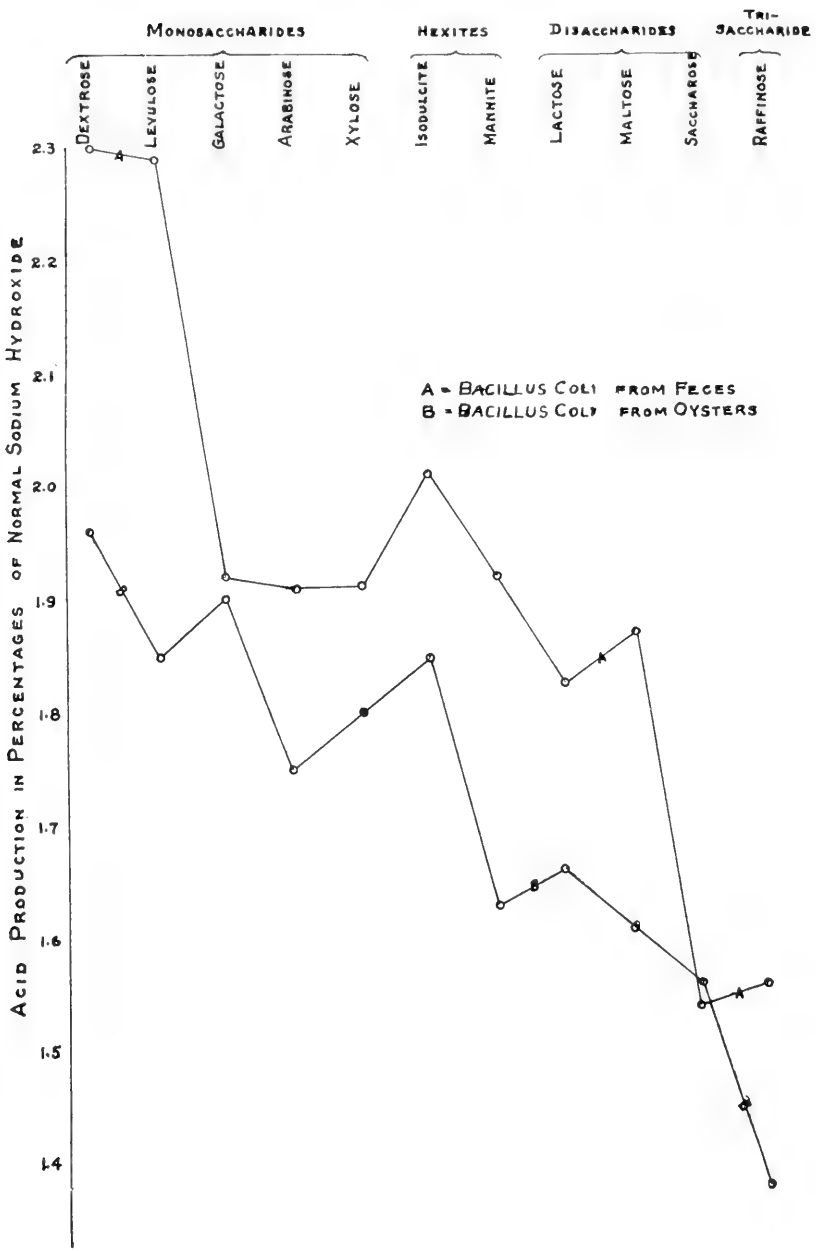

Chart 5.—The amount of acid produced in various carbohydrates by the bacillus coli, isolated from feces and from oysters.

Tubes containing 25 c.c., of the various carbohydrates, were inoculated with 0.5 c.c. of a twenty-four-hour culture of the different members of the bacillus coli group. After 24 hours incubation at 37 C., the cultures were titrated with N/20 sodium hydroxid. In each case strains were selected which could ferment the carbohyrate in question.

Tables 9 and 10 show that the colon bacillus, whether isolated from feces or oysters, produces the maximum amount of acid in the monosaccharids, less in the disaccharids, and the least in the trisaccharid.

The results obtained in this series of experiments show that the various members of the bacillus coli group are able to produce more acid in solutions containing carbohydrates of simple chemical structure than in solutions containing carbohydrates of complex chemical structure. For instance, more acid is produced in solutions containing the monosaccharids and hexites, such as dextrose, levulose, galactose, xylose, arabinose, mannite, and isodulcite than in solutions containing the disaccharids, lactose, maltose, and saccharose. The disaccharids are, in turn, more easily fermented by the bacillus coli group than the trisaccharid raffinose.

The polysaccharid starch is not fermented by any of the members of the coli group. The trisaccharid raffinose, while not so complex a molecule as starch, appears to offer chemically greater difficulties to the organisms in the carrying out of their oxidative processes in the formation of the various acids from the sugars than the organisms would encounter when fermenting, for instance, a disaccharid. In fact, there are members of this group which are unable to ferment raffinose at all.

The arrangement of the carbohydrates, according to the amounts of acid which the members of the bacillus coli group are able to produce from them, agrees somewhat with an arrangement made according to their chemical complexity of structure, as may be seen by the following:

Monosaccharids, 2.03, 1.82.
Disaccharids, 1.74, 1.61.
Trisaccharid, 1.56, 1.38.
Polysaccharid (Starch), 0, 0.

Among the monosaccharids, the hexoses, dextrose, and levulose, seem to be most easily oxidized by the organism with the formation of acid. No distinct difference could be noted in the amounts of acid produced in the levulose and dextrose by the methods used in this

TABLE 9

AMOUNT OF ACID, IN PERCENT NORMAL, PRODUCED IN VARIOUS CARBOHYDRATES BY BACILLUS COLI, ISOLATED FROM FECES

Carbohydrates	Cultures									
	1	2	3	4	5	6	7	8	9	Average
Dextrose	2.3	2.4	2.4	2.2	2.4	2.3	2.3	2.2	2.2	2.3
Levulose	2.25	2.3	2.4	2.4	2.4	2.1	2.4	2.1	2.3	2.29
Galactose	1.5	1.9	1.95	1.9	1.95	2.0	2.1	1.8	2.2	1.92
Arabinose	1.75	2.2	2.1	1.7	2.05	1.7	...	...	...	1.91
Xylose	1.79	1.9	2.1	2.0	2.0	1.8	...	...	...	1.91
Isodulcite	2.1	2.2	2.2	2.0	2.0	1.4	2.1	2.1	2.0	2.01
Mannite	1.7	2.0	1.9	1.7	2.0	2.0	2.0	2.0	2.0	1.92
Average of all Monosaccharids and Hexites				...	...	...	...	...	...	2.03
Lactose	1.7	1.9	1.9	2.0	1.8	1.9	1.7	1.7	1.89	1.82
Maltose	1.1	1.8	2.15	2.2	2.2	1.9	2.2	1.5	1.8	1.87
Saccharose	1.2	1.85	1.5	1.9	1.1	1.6	1.6	1.6	1.5	1.54
Average of all Dissaccharids			...	...	...	...	...	...	...	1.74
Raffinose	1.4	1.7	1.8	1.6	1.75	1.2	1.6	1.4	1.6	1.56
Average of Trisaccharid			...	...	...	...	...	...	...	1.56

investigation. The amount of acid produced for these sugars also was very constant. Galactose, while not forming so much acid as dextrose and levulose, showed a greater variance in the amount of acid produced. All organisms investigated produced acid in the monosaccharids mentioned.

TABLE 10

ACID, IN PERCENT NORMAL, PRODUCED IN VARIOUS CARBOHYDRATES BY BACILLUS COLI, ISOLATED FROM OYSTERS

Carbohydrates	Cultures						Average
	1	2	3	4	5	6	
Dextrose	2.0	1.9	1.8	2.0	2.2	1.9	1.96
Levulose	1.9	1.6	1.7	1.6	2.2	2.1	1.85
Galactose	2.0	1.9	1.8	2.0	1.9	1.8	1.9
Arabinose	...	...	1.8	1.8	1.9	1.5	1.75
Xylose	1.8	...	1.8	1.8	...	1.8	1.8
Isodulcite	1.7	1.9	1.9	1.6	1.8	2.2	1.85
Mannite	2.0	1.5	1.6	1.6	1.8	1.3	1.63
Average of all Monosaccharids and Hexites			...	...	...	...	1.82
Lactose	1.9	1.6	1.6	1.8	1.8	1.3	1.66
Maltose	1.9	1.5	2.0	1.4	1.6	1.3	1.61
Saccharose	1.7	1.2	1.6	1.5	1.8	1.6	1.56
Average of all Disaccharids			...	...	...		1.61
Raffinose	1.2	1.7	1.4	0	1.2	1.4	1.38
Average of Trisaccharid.			...	...	...		1.38

Tables 9 and 10 show that the colon bacillus, whether isolated from feces or oysters, produces the maximum amount of acid in the monosaccharids, less in the disaccharids, and least in the trisaccharid.

The pentoses, xylose and arabinose, seem to offer greater resistance to the bacterial oxidation processes, as we find less acid produced from the pentoses than from the hexoses. Throughout the work, these two sugars behaved exactly alike, and no differentiation was possible by the amount of acid produced from them by the group.

The hexites, mannite and isodulcite, showed less acid than the hexoses, and at times the amount of acid produced from them exceeded that produced from the pentoses. Many strains of the bacillus coli group were found, which were unable to ferment mannite and isodulcite.

Of the three disaccharids used, saccharose seems to offer greater resistance to the oxidative processes of the colon group than either lactose or maltose. In fact, many members of the group are unable to ferment it. This seems to be in accord with the chemical structure of the sugar, since we know that saccharose is the only one of the three mentioned disaccharids which cannot be oxidized. At times, the amount of acid produced in lactose and maltose equals or even exceeds the amount of acid produced in galactose, a monosaccharid.

The more complicated raffinose offers great difficulties to the fermentative processes of the bacillus coli group. Some strains failed to produce any acid at all from this sugar. As already mentioned, starch was not fermented by any strains used in this investigation.

It should not be understood, of course, that the size of the molecule is the only factor involved, for its configuration is also important. Winslow and Walker (1907) have shown that bacilli of the colon group, which ferment saccharose, usually ferment raffinose as well. The same thing appears in my results as to the amount of acid formed, saccharose results being lower than lactose results tho not quite so low as those obtained in raffinose.

There is an important distinction to be drawn between the power to ferment a given sugar and the amount of acid formed when it is fermented. In my work I have used only strains capable of attacking the sugar in question. When a low result is obtained it may be due to a slow action upon the sugar, or, in view of the evidence that it is the amount of end product formed which usually stops the reaction, it may be that the lower acidity produced in more complex sugars is the result of some other decomposition products, which accompany the acids, and, in connection with them, are able to inhibit growth.

A COMPARISON BETWEEN THE AMOUNT OF ACID PRODUCED BY VARIOUS MEMBERS OF THE BACILLUS COLI GROUP ISOLATED FROM DIFFERENT SOURCES

In this series of experiments, a comparison has been made between the amounts of acid produced in various carbohydrates by the different members of the bacillus coli isolated from three distinct sources: (1) from stools of healthy individuals in and about the laboratory; (2) from stools of Italian immigrants quarantined on board the *S. S. Roma;* (3) from oysters taken from different locations in Narragansett Bay, representing areas of widely diverse characters.

The organisms were inoculated into tubes of peptone broth which were incubated at 37 C. for 24 hours. The various carbohydrates were inoculated by the addition of 0.5 c.c. of this twenty-four-hour culture. At the end of 24 hours' incubation at 37 C. the cultures were titrated with N/20 sodium hydroxid.

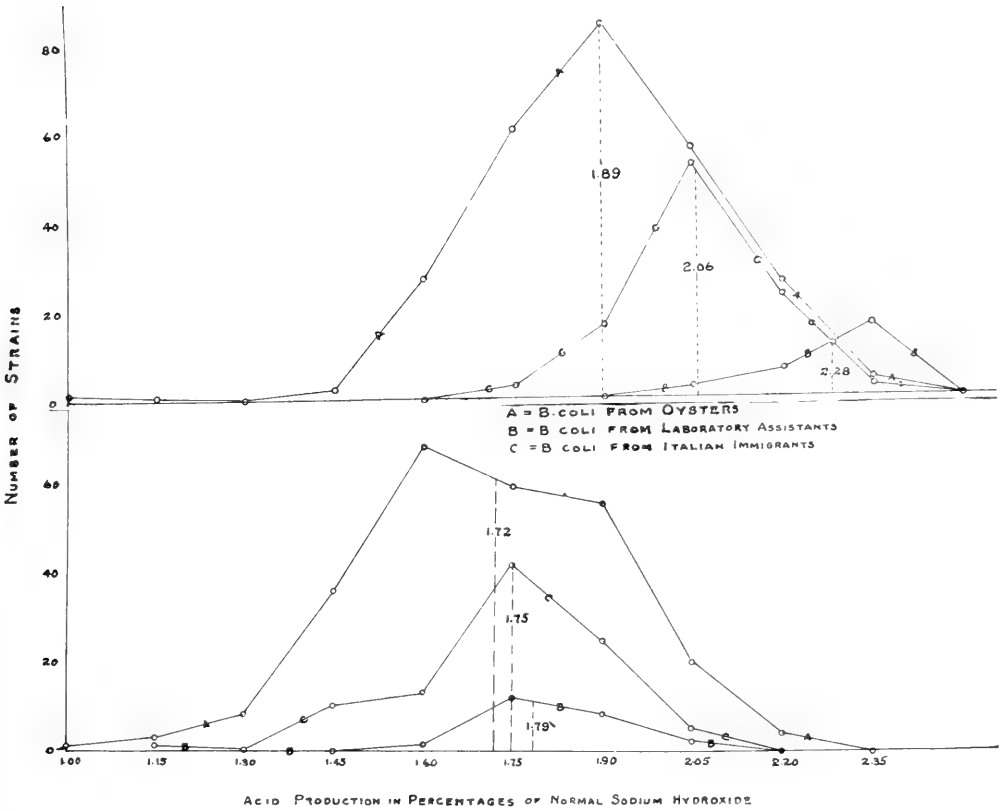

Chart 6.—The amount of acid produced by the bacillus coli in dextrose (upper curves) and lactose broth (lower curves).

The results obtained in this series of experiments seem to show that the source from which the members of the bacillus coli group were isolated has a direct effect upon the ability of the organisms to ferment carbohydrates with the production of acid.

A comparison between the amounts of acid produced in lactose and dextrose broths by members of the bacillus coli group gives the following results:

	From Laboratory Assistants	From Immigrants	From Oysters
Percentage in dextrose	2.28	2.06	1.89
Percentage in lactose	1.79	1.75	1.72

The bacillus coli isolated from feces, both from laboratory assistants and from the immigrants of the *S. S. Roma,* produced more acid in dextrose and lactose broth than the colon bacillus isolated from oysters. This seems to indicate that bacillus coli loses some of its ability to ferment carbohydrate with the production of acid during the journey from the intestinal tract to the oysters. Bacillus coli, isolated from the stools of laboratory assistants, produced more acid in dextrose and lactose broths than similar organisms isolated from the stools of Italian immigrants. The significance of this difference in the ability of the organisms, isolated from the above-named sources, to produce acid from carbohydrates means, is impossible to explain except that the general character of the diet may have had some effect on the ability of the organism to ferment carbohydrates with the production of acid.

EFFECT OF IMMERSION IN SEA WATER ON ABILITY TO PRODUCE ACID

The purpose of this experiment was to determine if the members of the bacillus coli group, after being kept for various periods in sea water, were affected in regard to their ability to produce acid in various carbohydrates.

In these experiments bottles of sea water, which gave two negative tests for the presence of the members of the bacillus coli group with lactose peptone bile, were inoculated with known cultures of the coli group and kept at the following temperatures: ice-box, 4 C.; room, 20 C.

At intervals of a week, portions of the sea water were inoculated into lactose peptone bile tubes, from which the members of the bacillus coli group were isolated and identified. From twenty-four-hour peptone cultures of these organisms, inoculations were made into the various carbohydrate media and incubated 24 hours at 37 C. At the end of that period titrations were made with N/20 sodium hydroxid.

TABLE 11

AMOUNT OF ACID, IN PERCENT NORMAL, PRODUCED IN DEXTROSE AND LACTOSE BROTH BY COLON BACILLI FROM DIFFERENT SOURCES

Source and Number of Cultures	Dextrose			Lactose		
	Lowest	Highest	Average	Lowest	Highest	Average
Italian immigrants, 95	1.75	2.30	2.06	1.40	1.95	1.75
Laboratory assistants, 24	2.10	2.40	2.28	1.20	2.0	1.79
Oysters, 260	1.00	2.40	1.89	1.10	2.25	1.72

TABLE 12

AMOUNT OF ACID PRODUCED IN VARIOUS CARBOHYDRATE MEDIA BY THE BACILLUS COLI RETAINED IN SEA WATER AT 4 C. AND 20 C.

Weeks	Dextrose		Levulose		Galactose		Arabinose		Xylose		Mannite		Lactose		Maltose	
	4 C.	20 C.	4 C.	20 C.	4 C.	20 C.	4 C.	20 C.	4 C.	20 C.	4 C.	20 C.	4 C.	20 C.	4 C.	20 C.
1	2.3	2.3	2.3	2.3	1.2	1.2	1.8	1.8	1.9	1.8	2.0	2.0	1.7	1.8	1.6	1.5
2	2.3	2.2	2.25	1.9	1.4	1.2	1.8	1.7	1.9	1.5	2.1	2.0	1.7	1.6	1.5	0.4
3	2.3	2.2	2.3	2.3	1.1	1.2	1.8	1.7	1.7	1.8	2.0	2.0	1.7	1.6	1.7	1.9
4	4.25	2.2	2.3	2.2	1.6	1.6	1.8	1.9	1.95	1.9	2.0	2.1	1.85	1.85	1.7	1.7
5	2.0	2.1	2.3	2.1	1.6	1.4	1.7	1.3	1.9	1.9	1.9	1.9	1.6	1.6	0.6	0.8
6	2.2	2.2	2.3	2.0	1.5	1.7	1.6	2.0	1.9	1.9	2.1	2.0	1.7	1.8	1.8	1.9
7	2.3	1.4	2.3	1.7	1.9	1.6	1.6	2.0	1.9	1.8	1.8	1.7	1.7	1.7	1.5	1.9
8	2.2	...	2.3	...	1.2	...	1.6	...	1.9	...	2.0	...	1.5	...	1.7	...

All results are the averages of two titrations.

These experiments show that the various members of the bacillus coli group, after remaining for 8 weeks in bottles of sea water kept at 4 C. and 20 C., were able to produce the same amount of acid in the various carbohydrates as when first added.

SUMMARY

The optimum temperature for the maximum production of acid in 24 hours by the members of the bacillus coli group, in a medium containing a fermentable carbohydrate, is 37 C. Acid production in this time is almost nil at 3 C., rises rapidly to 37 C., and falls as rapidly above that point, ceasing between 50 C. and 60 C.

Twenty-four hours' incubation at 37 C. is a sufficient period for the maximum production of acid by members of the coli group in a medium containing a fermentable carbohydrate, when 0.5 c.c. of a twenty-four-hour peptone culture is used as an inoculum. Under these conditions no further increase in acidity occurs after 20 hours.

The amount of medium, up to 500 c.c., inoculated with the members of the coli group, has no effect upon the percentage of acidity produced by the organisms, when incubated at 37 C. for 24 hours after inoculation with 0.5 c.c. of a twenty-four-hour peptone culture.

A medium containing 1 percent of fermentable carbohydrate offers a suitable amount of carbohydrate for the maximum acid production by members of the coli group. A medium of much less than 1 percent concentration does not contain enough carbohydrate to bring about the maximum reaction, while a medium of more than 1 percent concentration serves no useful purpose. A high concentration of carbohydrates (over 25 percent) will not only hinder but will prevent the production of acid, but between 1 and 25 percent the twenty-four-hour acid production is the same.

The more distant the initial reaction of the medium is from the maximum acidity necessary to inhibit production of acid, the greater amount of acid the organism can produce before that maximum is reached. The extreme limit of alkalinity, in which the organism will produce acid, was not determined.

The maximum amount of acid, which bacilli of the colon group will produce in ordinary carbohydrate media, is fixed by the tolerance of the bacteria to the acid itself. If the acid formed is neutralized daily by the addition of free alkali, the acid production will go on until all the carbohydrate present has been consumed. With 1 percent carbo-

hydrate in the medium, there will be 4 or 5 times as much acid formed as ordinarily.

The members of the coli group produce the greatest amount of acid in media containing the monosaccharids and hexites, dextrose, levulose, galactose, arabinose, xylose, mannite, and isodulcite; less in the disaccharids, maltose, lactose, and saccharose; and least in the trasaccharid, raffinose.

Members of the bacillus coli group, isolated from feces, produce more acid in media containing fermentable carbohydrates than strains of the bacillus coli group, isolated from oysters taken from different portions of Narragansett Bay. Of the members of the bacillus coli group isolated from feces, strains which were obtained from the stools of laboratory assistants produced more acid in media containing fermentable carbohydrates than strains isolated from the stools of Italian immigrants quarantined aboard the *S. S. Roma.*

The amount of acid produced in various carbohydrate media by the bacilli of the colon group is unaffected by storage in unsterilized sea water for a period of 8 weeks at temperatures of 20 C. and 40 C.

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ON THE CLASSIFICATION OF STREPTOCOCCI.

OBSERVATIONS ON HEMOLYSIN PRODUCTION BY THE STREPTOCOCCI.

Reprints Not Available.

BY HAROLD W. LYALL.

Journal of Medical Research. Vol. XXX, pp 487-532.

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THE STRUCTURE OF THE COTTON FIBRE.

BY BENJAMIN S. LEVINE.

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THE STRUCTURE OF THE COTTON FIBER

IN any kind of cotton the typical fiber, that is the one in which all the essential parts may be determined, can be found in rare cases. For this reason the structure of an ideal fiber can be inferred only from a series of studies of fibers in successive stages of development.

By subjecting such fibers to certain chemical and bacteriological treatments and then studying them under the microscope, we found that the typical cotton fiber consists of the following parts:

1. The outer layer or the integument.
2. The outer cellulose layer.
3. The layer of secondary deposits.
4. The walls of the lumen.
5. The substance in the lumen.

1. *The outer layer or the integument* is the incrusting layer and forms the cementing material of the fiber. Its chemical structure is not an homologous one, but is a mixture of components, some soluble in alcohol, some in ether, and some in water. The components are cutinous, pectinous, gummy, fatty and other unidentified bodies.

2. The *outer cellulose layer* is in its structure a distinct spiral, consisting of a limited number of component fibers, perhaps of one or of two. The structure of this layer is determined under the microscope from a longitudinal section of the fiber after the latter has been subjected to a series of chemical and bacteriological treatments. Careful treatment of some of the fibers by cuprammonia will show under the microscope this spiral. There

is some evidence to show that this spiral consists of impure cellulose.

3. The *layer of secondary deposits* seems to be made up of component fibers which in no case have shown a spiral structure. Unlike the fibers of the above described layer, these components are from about five to ten in number and run with some irregularities along the length of the fiber.

4. The structure of the layer forming the *walls of the lumen* is a spiral much the same as the outer spiral, but differs from it greatly in its chemical composition. This is determined from a microscopical study of the fiber while under a cuprammonia treatment.

5. *The substance in the lumen* is structureless and, as is proven by a microscopical test, is of a nitrogenous nature.

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THE EFFECT OF WATER-GAS TAR ON OYSTERS.

BY PHILIP H. MITCHELL.

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THE EFFECT OF WATER-GAS TAR ON OYSTERS

By Philip H. Mitchell

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THE EFFECT OF WATER-GAS TAR ON OYSTERS.

By PHILIP H. MITCHELL.

For a number of reasons it has become desirable to know the effects of oily and tar-like wastes on marine life of economic importance. Damages have been claimed for pollution of oyster beds by wastes from the manufacture of gas. A report to the Rhode Island shellfish commission has attributed to water-gas tar harmful effects to oysters in Narragansett Bay.[a] Tar of various sorts is used for coating piles or stakes, which might be in proximity to shellfish. Oily wastes are constantly escaping from passing craft in inland waters.

The present investigation does not cover the entire subject, but is confined to one important phase—the effect of water-gas tar on oysters. The experiments made during the summer of 1912 at the laboratory of the Bureau of Fisheries at Woods Hole could not then be carried further.

The tar was obtained from the separator at the works of the Providence Gas Co. on October 25, 1910, and was a mixture of the heavier and lighter tars as obtained in the manufacture of water gas, using an average temperature of 1,450° F. An analysis of tar taken under comparable conditions from the same separator at another time showed, according to the records of the Providence Gas Co., the following analysis:

Specific gravity at 65.5° F., 1.050.	Per cent by volume.
Water	7.65
Light oil at 200° F	13.95
Dark oil at 400° F	42.20
Medium pitch	36.20
Free carbon	2.70

Three series of experiments were made. In the first, oysters were exposed to water-gas tar in stagnant sea water; in the second series they were exposed to the tar in running sea water; while in the third small amounts of water-gas tar were introduced inside the shells of oysters.

SERIES I.

Experiment 1.—An oyster, marked with a file for identification, as were all the oysters used in these experiments, and weighing 83.2 grams, was put into a battery jar with 40 c. c. of water-gas tar and 2,500 c. c. of sea water. On the following day the water was changed by the method used in all the experiments of this series. A siphon delivered sea water from an aquarium to a point about 2 inches from the bottom of the jar, while another siphon at the same time drew off water from the middle of the jar. Water was allowed to run thus 10 to 15 minutes. As the tar stuck to the sides

[a] Field, G. W. In Annual Report of the Commissioners of Shell Fisheries, Rhode Island, 1906, appendix D, p. 46–64.

and bottom of the jar, or, in the case of the lighter more oily portions, floated on the surface of the water, none, or very little of it, was removed while the water flowed through the siphons. The water, therefore, was almost completely changed without removing the tar. Shortly after the water had been changed the oyster was seen to be slightly open, but could close at the slightest jar. The water was changed in this manner once or twice daily for 10 days, The oyster was often seen partly open during this time, but would always respond to a mechanical stimulus by closing. After 10 days the water was no longer changed, but was left entirely stagnant. At the end of two weeks under these conditions the oyster seemed to be affected. It now remained continuously open, and appeared unable to close when stimulated. A few days later it showed signs of putrefactive disintegration.

Experiment 2.—An oyster weighing 63.7 grams was treated exactly as in experiment 1. The results were quite the same, except that when the water was left entirely stagnant disintegration set in after five days.

Experiment 3.—An oyster weighing 71.6 grams was treated as in the preceding experiments, except that the tar was smeared all over the inside of the jar and on the oysters. After 24 hours the oyster was found wide open and unable to close when stimulated. It was removed from the jar, and was seen to have its mantle greatly retracted. It would spring open when closed by hand. It was washed and put into an aquarium in running sea water. Two days later it appeared to be entirely recovered, as it would close normally when stimulated. It was then put back into the jar of sea water and tar in which it had been at first. It was now kept in this jar during six days, with the water changed once or twice daily. At the end of this time it had again lost the ability to close normally, but when put in the aquarium once more it again apparently recovered. About three weeks later, however, it became disintegrated.

Experiment 4.—An oyster weighing 95 grams was arranged exactly as in experiment 3. The results were quite the same, that is, after 24 hours it refused to close, but recovered when put in the aquarium of running sea water. Some weeks later, however, it died. That its death was due to the tar is not certain, because at that time other oysters in the same aquarium died without any previous exposure to tar.

Experiment 5.—An oyster weighing 78.7 grams was treated exactly as in the preceding experiment. It, too, became unable to close after 24 hours, and when put in running sea water entirely recovered. At the end of the summer, nine weeks later, it seemed entirely normal and had a normal appearance when opened.

Experiment 6.—An oyster weighing 62 grams was treated exactly as in the preceding experiment. The result was slightly different in that the oyster did not begin to show a tendency to remain open until after three days, and became entirely unable to close after five days in the tarry water with daily changing of the water. It was then put in running sea water and began to disintegrate a few days later.

Experiment 7.—An oyster weighing 70.8 grams was put into a battery jar with 20 c. c. of the tar not in contact with it and 2,500 c. c. of sea water. The water was changed daily during the next 10 days. It was then left stagnant during 8 weeks. The oyster was sometimes observed to be open, but would then close if jarred. At the end of that

time it was cleaned and dried and found to weigh 71.6 grams. It had formed new shell all around the edge. Opened it gave no smell of tar, the heart was beating and the mantle was normally sensitive to mechanical stimuli. Part of the heart and portions of the gills were discolored.

Control experiment.—An oyster weighing 72.4 grams was put in a battery jar with 2,500 c. c. of sea water. The water was changed during the next 10 days as in the preceding experiment and was then left stagnant during 8 weeks. Examination then showed no noticeable new shell, but the heart, gills, and mantle were quite normal.

The experiments of this series indicate that when considerable quantities of water-gas tar are in intimate contact with oysters in stagnant water serious or fatal effects are produced. Under these circumstances the oyster can not use its natural defense against a relatively or entirely insoluble substance. When the water is stagnant, there is little opportunity to eject such substances and free the organism from them. As will be shown later, the oyster can rid itself of water-gas tar when in running sea water. When the tar can not be ejected it seems to produce an effect similar to paralysis, so that the initial symptom is a failure of the adductor muscle to respond to stimulation of the sensory nerves. No conclusions as to the structures specifically affected can be drawn from these experiments. Whether the fatal effects produced in five of the above experiments were due to a direct toxic effect of water-gas tar, or to some indirect effect also, does not appear from these experiments.

SERIES II.

Method.—Two oysters were put in each of four battery jars. Each jar was arranged with two siphons, one bringing sea water from an aquarium to the jar with the lower end of the siphon about 2 inches below the level of water maintained by the other siphon, which carried water from the jar to a sink. The running water therefore tended to carry off the light floating oils but left the heavier tar sticking to the bottom and sides of the jar. The siphons were so arranged that each jar contained constantly about 2,500 c. c. of sea water. Into each jar there were put 30 c. c. of water-gas tar mixed with sand and thoroughly smeared over the bottom and sides of the jar and on the shells of the two oysters. From time to time during the following weeks small amounts of tar were added to replace that carried away by the siphons. After remaining in the jars as described during nine weeks the oysters were cleaned, weighed, and examined. Comparison of their weights at the beginning and at the end of the experiments is given in tabular form:

Jar.	Initial weight.	Final weight.
	Grams.	*Grams.*
1	98.0	99.0
	47.8	47.8
2	92.0	92.0
	52.4	52.3
3	96.8	94.0
	61.8	62.5
4	101.1	100.7
	45.2	44.3

That these variations in weight have no special significance is indicated by comparison with the variations in the weights of oysters kept in the aquarium during the summer but not exposed to tar.

Initial weight.	Weight after five weeks.
Grams.	*Grams.*
66. 5	66. 0
76. 0	76. 5
244. 0	243. 0
147. 0	151. 0

When opened all were found to be normal in appearance and function. No odor of tar was detectable in the shell contents of the oysters, although an abundance of tar was left in the jars at the end of the experiment. Smears of tar were also still present on the outside of the oyster shells.

These experiments indicate that even intimate contact with water-gas tar does not injure oysters in the course of nine weeks, provided facilities for defense in the form of moving water frequently renewed are available.

SERIES III.

Experiment 1.—A small oyster was first pried open and injected with 0.5 c. c. of water-gas tar. It was then put in a jar of running sea water. It remained tightly closed during the next two hours. On the next day it was found to be quite normal. It was open and apparently feeding, but closed when stimulated. Drops of tar near it indicated that the foreign material had been ejected. One week later it still appeared entirely normal. It was then again injected with 0.5 c. c. of tar, and was now put in stagnant sea water. Four days later it did not, when stimulated, close as readily and tightly as a normal oyster. It was then opened and found normal in its heartbeat and in contractility of the bivalve muscle, but the mantle was not normally responsive to mechanical stimulus.

This experiment indicates that when an oyster ingests tar and can not get rid of it because the surrounding water is stagnant, some impairment of the sensory apparatus in the mantle results. This interferes with certain activities of the oyster, prevents normal closure, and eventually causes degeneration of muscular and other tissues.

Experiment 2.—Three medium-sized oysters were each injected with about 1.5 c. c. of water-gas tar and then put in separate jars of running sea water. Some time after they were seen in each case to open slightly and in a few minutes close violently so as to eject masses of tar. This process was repeated a number of times in the course of one to three hours after injection. They then remained constantly closed for some time, but were found normally open on the following day. They were left in the running sea water for a period of eight weeks and behaved throughout that time like control oysters in the aquarium. As it was then necessary to terminate the experiments, the oysters were

opened and carefully examined. They were found to be normal in color, odor, heartbeat, responsiveness of the mantle, ciliary movement, and in short in every respect.

This experiment distinctly indicates that water-gas tar in considerable doses is harmless to an oyster in running sea water. The conditions of this experiment more closely resemble those of the native habitat of the oyster than do those of the preceding experiment, because tides and other currents over oyster beds maintain a constant movement and a continuous changing of the surrounding medium.

Experiment 3.—A medium-sized oyster was injected with 1 c. c. of water-gas tar, which was distributed all around the mantle. It was then put into about 1,500 c. c. of sea water and carefully observed. During the next five hours it did not visibly open and no tar escaped from it. On the following day, however, a few drops of tar were floating on the surface of the water. During the next two days the oyster was only infrequently observed and was not seen open, but on the third day it was found normally opened and able to close when stimulated. It was left in the same sea water during the next two weeks. It had then developed the usual symptoms of imperfect closure and when opened did not show a normally beating heart or a responsive mantle. This experiment confirms the first one of this series.

EFFECT OF WATER-GAS TAR ON THE DISSOLVED OXYGEN OF SEA WATER.

It seemed possible that tar and similar substances might in a measure reduce the oxygen content of water so as to affect shellfish. Mixtures of tar and sea water were, therefore, allowed to stand for varying periods of time and then tested by Winkler's titration method to measure the quantity of dissolved oxygen in the water. The experiments are summarized in the following table. Three liters of sea water were used in each case.

Amount of water-gas tar used.	Time mixture was allowed to stand at room temperature.	Oxygen remaining in water at end of the time.
Cubic centimeters.	*Hours.*	*Parts per million.*
200	20	6.61
200	24	6.10
200	45	.00
200	130	.08
50	40	2.71
50	45	1.10
50	130	.08
None.	40	[a] 7.10
None.	45	[a] 8.09
None.	130	[a] 8.26

[a] Control, sea water alone.

These experiments show that the tar can cause the disappearance of dissolved oxygen in sea water. How potent a factor this may be in causing the effects of the tar on oysters in stagnant water it is not, however, safe to say. Oysters, as the author has shown, are remarkably resistant to lack of oxygen and do not when deprived of it

show inability to close except in the advanced stages of oxygen starvation. That oxygen consumption by tar may help to account for the fact that oysters are injured by stagnant tarry water, while they are uninjured by the tar in running sea water, is quite probable. In the natural habitat of the oyster, however, it seems quite impossible that the slight reduction of dissolved oxygen which small amounts of tar could effect would alter the results of oyster culture.

CONCLUSION.

These experiments show no noticeable effects of water-gas tar on oysters in constantly renewed sea water. This is true in spite of the fact that large amounts of tar mixed with stagnant sea water, or small amounts injected into oysters which are kept in stagnant water, do cause serious or fatal effects. Considerable quantities (1.5 c. c.) may be put inside the shell of an oyster kept under conditions resembling those of its natural habitat without causing any effect. The harmlessness of the tar under these circumstances is due apparently to the ability of the oyster to rid itself of such foreign matter. In stagnant water the organism can not be effectively washed out, and effects involving a loss of sensitiveness in the mantle result. That consumption of the dissolved oxygen in the stagnant water by tar may have some effect on oysters is a possibility.

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THE OXYGEN REQUIREMENTS OF SHELLFISH.

BY PHILIP H. MITCHELL.

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THE OXYGEN REQUIREMENTS OF SHELLFISH

By Philip H. Mitchell

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THE OXYGEN REQUIREMENTS OF SHELLFISH.

By PHILIP H. MITCHELL.

The respiratory exchanges in lamellibranchs seem not to have been investigated. Probably the most notable work related to it is that of Vernon.[a] He measured the oxygen utilization and carbon dioxide emission in a large number of marine forms, including certain Mollusca but no lamellibranchs. He showed that in the lower marine forms investigated, including Coelenterata, Tunicata, and Mollusca, the respiratory exchange was very small compared to the higher ones, for example, teleosts. There were, however, certain exceptions to this rule, notably the protozoan *Collozoum inerme*, which showed nearly as high a respiratory exchange as the fishes. He found also that, in general, the respiratory activity was more readily responsive to temperature in the lower than in the higher forms. He further showed that the gaseous exchange was relatively greater in the small than in the large individuals of the same species and found that, in general, the same distinction held between small and large species. The transparent pelagic animals were shown to have a very small proportion of solid organic matter in their tissues, so that calculated on that basis their respiratory activity was very large, greater indeed than fishes, amphibians, or even mammals.

In the present work some of those findings have been confirmed for the lamellibranchs. They show a ready responsiveness to temperature changes, a smaller utilization of oxygen in proportion to the body weight with increase in size, and those forms which showed a low oxygen requirement in relation to their entire weight showed a higher utilization in proportion to their dried weight.

The resistance to lack of oxygen in forms which have no power of locomotion is an important factor in adverse conditions. This is especially true of the edible shellfish, which, because of enforced closure during cold weather, or in the presence of polluted or roily water, or in water whose oxygen has been lowered by the presence of certain wastes or an abundance of life, must at times be deprived of their normal supply of oxygen. The subject therefore possesses an economic significance, and it was, in fact, the possibility that certain manufacturing wastes, removing oxygen from sea water, might therefore cause the death of oysters and clams which first directed the writer's attention to the subject. The particular wastes involved were those of gas works con-

[a] Vernon, H. M.: Respiration in marine forms, Journal of Physiology, vol. XIX, 1895, p. 18.

taining at least traces and sometimes significant quantities of water-gas tar and other oily matter. The results of this investigation indicate, in a word, that the tar or oily wastes could have no effect on shellfish in this way. The details of this part of the work, however, are reserved for a report dealing specifically with the effect of water-gas tar on oysters. This paper is confined to oxygen requirements of shellfish and their resistance to lack of oxygen.

METHOD OF EXPERIMENT.

The method of manipulation was, in brief, to place the shellfish in a desiccator completely filled with sea water of known content of dissolved oxygen, leave the apparatus at some constant temperature during a definite period, and then to sample the water in the desiccator so as to compute its decrease in dissolved oxygen. Winkler's well-known titration method was used to measure the oxygen both at the beginning and end of each experiment. A vacuum desiccator was used for the containing vessel because it could easily be closed water-tight, could accommodate almost any size of shellfish, and enabled one to take through the side opening with glass stopcock a fair sample of the contents.

In practice a number of precautions were found necessary. The hollow dome of the desiccator cover was entirely filled with paraffin to exclude air from entrapment in it. A glass tube within the desiccator was connected to its side stopcock and reached nearly to the bottom. When, therefore, the filled desiccator was opened at the top water could be sucked off through the opened side cock into the sampling bottle so that the sample would come from a point well below the surface of the water, where the oxygen content would be fairly constant. The sea water [a] used in each experiment was brought to the required temperature and placed in a large reservoir jar on a shelf, from which it could be siphoned without bubbling into the desiccator. When it was thus nearly filled, an oyster was gently placed on a glass tripod, where it would rest near the center of the desiccator. With clams it was found necessary to leave them in the desiccator submerged in water for a few hours before the experiment, because, unlike oysters, they would not open quickly after they had been handled. In either case, though, water from the shelf reservoir was siphoned into the overflowing desiccator for sufficient time to bring the oxygen content to approximate constancy and then the cover was put on, leaving just opening enough to admit the siphon tube.

The side cock of the desiccator was then connected by rubber tubing to the sampling bottle, and after starting by suction the water was allowed to siphon through the bottle two to three minutes. This period was found quite sufficient to give reliable duplicate results for water containing any percentage of oxygen measured in these experiments. The water running into the desiccator meantime was in excess of that running into the bottle, so that an overflow was maintained from the top of the desiccator and the sample rendered as fair as possible. At the exact second recorded as the beginning of the experiment the side stopcock was closed, the siphon quickly withdrawn from the desiccator,

[a] From aquarium of running sea water, a part of the aquarium system of the laboratory.

and its lid put completely on, excluding bubbles. The entire apparatus was then kept in a bath of sea water whose temperature was maintained approximately the same as the water within the desiccator. The oxygen in the sample was meantime measured by titration.

After a period, usually about an hour in length, the desiccator was two or three times inverted to render its contents uniform and to stimulate the shellfish to close and thus stop using oxygen. The exact time of the first rough movement of the apparatus was recorded as the closing time of the experiment. The outside of the desiccator was then dried with a towel and the entire apparatus weighed. When currents in the water had come practically to rest, the side stopcock was again connected to the same sampling bottle used at the beginning of the experiment and a sample taken as before. Since the capacity of the desiccators was sufficient for 1,200 grams of water, even with the large shellfish, and as the sampling bottles held approximately 300 grams, it is plain that water could run through the sampling bottle some time without emptying the desiccator. The stream indeed was allowed to run at least two minutes. The capacity of the desiccators was not enough to permit of taking a duplicate sample that would be reliable, but this was not necessary because there is little chance of error in the Winkler's titration.

The last step in the process was to empty the desiccator completely, dry it and the shellfish, and weigh them together. This gave a means of determining by difference the weight of the water. It was probably accurate to within 2 grams. As the dissolved oxygen of the water both at the beginning and the end of the experiment was calculated in parts per million, it was only necessary to multiply the weight of water used by the decrease in its oxygen content expressed in parts per million and divide the result by 100 to find the decimilligrams of oxygen used by the shellfish. Experiment showed that no correction for change in the oxygen content of the water due to microorganisms or physical factors was, under the circumstances of constant temperature, etc., necessary. Results were expressed in decimilligrams of oxygen per hour and in most cases also computed in decimilligrams per hour per 100 grams of shellfish and in some cases in decimilligrams per hour per 1 gram of the dried weight of the total shell contents of the organism.

A formula for the first of these three computations might be expressed, therefore, as follows:

$$A = W\frac{a' - a''}{100} \cdot \frac{60}{t}$$

where A is the decimilligrams of O used per hour, W the weight, in grams, of water in the desiccator, a' the parts per million of oxygen in the first sample and a'' in the second sample, and t is the time of the experiment in minutes.

Throughout the experiments it was found necessary to observe the animal at frequent intervals, and if the shells closed up to discard the experiment, or if the shells closed only after a considerable number of minutes had elapsed to terminate the experiment immediately. This precaution was necessary because, as is well known, certain of the shellfish used in this work can close water tight and in that condition take from the

water, as will be shown below, no oxygen except the very small amount absorbed by their shells.

It appeared early in the work that constancy of results was exceedingly difficult to obtain. This was due to a variety of causes, chief of which was the great variability in the openness of the shells, for not only could the oyster or clam entirely or at least obviously close so that the experiment had to be terminated, but it could partially and unnoticeably close or indeed fail to open wide even from the beginning of the experiment. For the oyster, at least, the author succeeded in demonstrating this by graphic records. One shell of an oyster was connected by tying a string to the projections with a lightly balanced lever recording on a slow kymograph. As soon as the oyster in a water bath at a temperature between 18° and 20° C. had opened somewhat, the kymograph was started and the temperature of the bath raised at the rate of 1° C. in about eight minutes. The oysters remained fairly well open with brief periods of partial closure as the temperature increased to about 22° C. At that point there invariably appeared in the three individuals observed periods of maximum openness lasting as long as no disturbing factor intervened. Sufficient stimulus for partial closure, however, was likely to occur frequently. A light tap on the table or water bath, a heavy step in the room, the slamming of a door in a neighboring part of the building, or, indeed, any slight jar was surely registered by some movement of the shell. As the temperature increased up to about 26° or 27° C., the effect of these stimuli was much less marked. The oysters then maintained their maximum openness very persistently. Between 27° and 30° a tendency to very slow and incomplete opening after closure was noticeable, indeed no maximum openness was seen. At about 30° or 31° C., the oysters closed tightly, even if no mechanical stimulus was given.

As the kymograph method served to detect movements of the shell not noticeable to unaided observation and also slight openings of the shell not otherwise visible, these experiments were an aid to planning and interpreting measurements of oxygen utilization. They showed that below 19° C. and above 26° C. observations on the opened oyster were impossible, that temperature must be maintained constant throughout the experiment, and mechanical disturbances must be avoided as far as possible. A further source of difficulty had also to be overcome. When the oyster excreted it closed violently, to drive the fecal matter out of the shell. If the position of the animal rendered complete excretion difficult, closures were frequently repeated, and sometimes the oyster shut up tightly. It was necessary, therefore, to lay the oyster in the desiccator tipped so that the more concave side of the shell, where excretion occurs, would be lower than the other.

In spite of all precautions, however, perfectly consistent results could not always be obtained. Under the same or comparable conditions of temperature, oxygen content of the water, and physical conditions, the same individual would sometimes in different experiments give results disagreeing beyond the limits of the calculated, probable, experimental error. Various observations make it seem likely that the nutritive condition of the individual could account for some, at least, of these discrepancies. Thus, after

remaining at a high temperature (e. g., 24°–26° C.) for some time, the oxygen requirement at a slightly lower temperature would tend to be greater than if the shellfish had been at a lower temperature before the experiment. If an oyster had been out of water for some time before the measurement, more oxygen would be used, generally, than if the specimen were left in the aquarium until the time of the experiment. After the shellfish had been kept in the aquarium some weeks they tended to use less oxygen than when taken recently from their native environment. Exceptionally an individual would show a high utilization of oxygen out of proportion to previous measurements and lasting for several days. As many of the interfering conditions as possible were, of course, eliminated, but still it seemed necessary to make a considerable number of experiments and draw conclusions only from averages. More than 350 measurements were made under various conditions on three types of lamellibranchs—the oyster (*Ostrea virginica*), the soft-shell or long clam (*Mya arenaria*), and the quahog or round clam (*Venus mercenaria*).

RESULTS.

OXYGEN REQUIREMENTS OF THE OYSTER.

Three series of experiments, each made on a limited number of individuals, are submitted. Although the results can scarcely be taken to show any seasonal variation, they are grouped, for convenience, according to the time they were carried out. In table I are the results of measurements taken during July, 1911; in table II, those of July and August, 1912; and in table III are results obtained during the latter part of August, 1912.

In the experiments of table I definite temperatures were not previously chosen and carefully adhered to as in the later work. The results, therefore, are here grouped for comparison as follows: All measurements taken at temperatures between 20° and 21.3° C. appear in one column, those at 21.5° to 23.5° C. in another, and those between 26° and 28° C. in a third. Where two or more experiments were made with one oyster at temperatures within a given range the average of the results is placed in parentheses. In the last three columns are the averages of comparable experiments computed as the decimilligrams of oxygen used per hour per 100 grams of oyster. By weight of oyster is meant in this and other tables not otherwise specified the weight in grams taken after it was closed under water, wiped as dry as possible with a towel, and left to dry in the air not more than half an hour. Such weighings were shown by duplication to be accurate to within two-tenths of a gram.

In table II are the results of experiments performed at definite chosen temperatures so controlled that the variation was not more than half a degree centigrade in any single experiment. The first five columns of results show the decimilligrams of oxygen used per hour at five temperatures by nine oysters. The averages of all comparable experiments are put in parentheses. The last five columns contain the averages, expressed in decimilligrams, of the oxygen used per hour per 100 grams of oysters.

TABLE I.—OXYGEN USED BY OYSTERS.

NOTE.—Figures in parentheses are averages of experiments under approximately uniform conditions.

Weight of whole oyster.	Decimilligrams of oxygen used per hour.			Average decimilligrams of oxygen per hour per 100 grams of oyster.		
	At 20° to 21.3° C.	At 21.5° to 23.5° C.	At 26° to 28° C.	At 20° to 21.3° C.	At 21.5° to 23.5° C.	At 26° to 28° C.
Grams.						
42.0	.....	5.6 9.0 7.8 6.8 (7.3)	.....	.....	17.4	.....
56.6	.....	11.8 7.6 (9.7)	.....	.....	17.2	.....
85.0	9.4 14.7 14.0 (12.7)	.....	19.3 18.8 (19.1)	14.9	.....	22.4
106.0	10.1 15.6 (12.8)	19.4	22.9 22.9 26.2 (24.0)	12.1	18.3	22.6
113.0	14.6	18.2 16.1 (17.1)	.....	12.9	15.1	.....
127.0	12.7 13.6 15.7 (14.0)	21.8 21.5 19.0 21.2 (20.9)	.....	11.1	16.5	.....
141.0	.....	19.0 18.0 (18.5)	.....	.....	13.1	.....

TABLE II.—OXYGEN USED BY OYSTERS.

NOTE.—Figures in parentheses are averages of measurements under approximately uniform conditions.

Weight of whole oyster.	Decimilligrams of oxygen used per hour.					Decimilligrams of oxygen used per hour per 100 grams.				
	At 19.5° to 20° C.	At 21° to 21.5° C.	At 22° to 22.5° C.	At 24° to 24.5° C.	At 26° to 26.5° C.	At 19.5° to 20° C.	At 21° to 21.5° C.	At 22° to 22.5° C.	At 24° to 24.5° C.	At 26° to 26.5° C.
Grams.										
42.0	7.6 9.8 (8.7)	12.3 13.8 (13.1)	10.3 13.9 (12.2)	14.6	13.4 14.9 11.2 (13.1)	20.7	31.0	29.1	34.8	31.3
51.0	11.6 10.7 11.8 (11.3)	9.3 11.4 9.6 6.2 (9.6)	11.7 11.5 (11.6)	12.6 12.7 (12.65)	13.6	22.2	17.9	22.8	24.8	26.7
66.5	15.1 9.4 10.5 9.1 (11.2)	16.0 9.9 16.1 14.0 (14.0)	15.5 11.8 (13.7)	16.0	18.6 17.2 (17.9)	16.9	21.0	20.6	24.1	26.9
76.0	11.5 14.0 (12.7)	13.9 15.3 14.2 (14.5)	15.3 12.8 (14.05)	15.8 20.5 16.2 (17.5)	23.5 20.9 24.5 20.7 (22.4)	16.7	19.0	18.5	23.0	29.5
97.4	13.4	14.6	.....	18.0	22.1	13.8	15.0	.....	18.5	22.7
128.0	13.7 17.8 (15.75)	.....	17.9 25.9 18.6 18.4 (20.2)	25.9 19.8 (22.8)	24.8	12.3	.....	15.7	17.8	19.4
147.0	16.0 19.4 (17.7)	18.4 18.2 (18.3)	20.4 19.3 (19.8)	20.8 21.9 (21.3)	26.6 26.0 (26.3)	12.1	12.5	13.5	14.5	17.9
244.0	18.1 22.3 (20.2)	23.5 25.7 (24.6)	34.6 25.7 (30.1)	30.0 26.1 24.0 (26.7)	35.1	8.3	10.1	12.3	11.0	14.4
262.0	19.0	29.1 29.5 21.4 20.8 (25.2)	27.4 24.8 (26.1)	28.7 30.7 (29.7)	37.0	7.3	9.6	10.0	11.3	14.1
Average..	.....	.....	.....	.....	.....	14.5	17.0	17.8	20.0	22.5

In table III are the results of measurements at four chosen temperatures on four oysters. These experiments were all done within a few days after the oysters were taken from the beds and therefore serve to some extent as a control on the condition of the oysters used in the other series. The first columns of results show the decimilligrams of oxygen used per hour at the designated temperatures. The averages of comparable experiments are put in parentheses. The figures in the next four columns are obtained by computing the averages of the oxygen utilization per 100 grams of oyster. In the next column is the weight, in grams, of the shell contents of each oyster when dried to constant weight. The last four columns show average decimilligrams of oxygen used per gram of dried substance. The figures in these columns were corrected for the

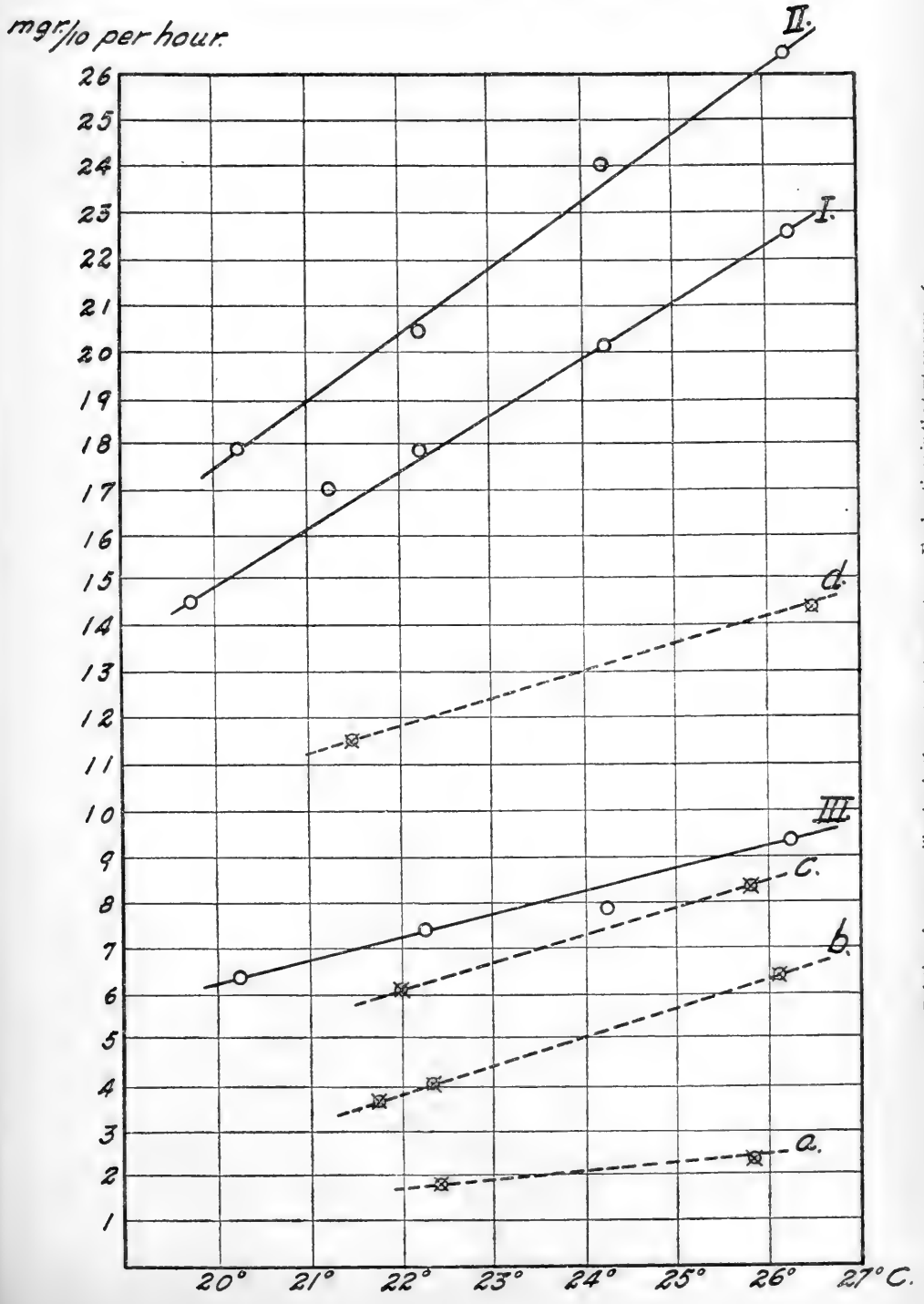
oxygen used by the shells. The shells of each oyster were at the end of the observations carefully cleaned in sea water and then used for two or three measurements of their oxygen-absorbing power at different temperatures. As these results were obtained under the same conditions as those expressed in table III, they represent a fair estimate of the oxygen absorbed by the shells during measurements with the intact oyster. There is reason, as will be shown below, to believe that oxygen removed from the water in this way is not utilized by the active tissues of the oyster. It seems reasonable, therefore, to subtract the amount (measured or computed) of oxygen utilized by the shells from that used by the whole oyster before computing the oxygen requirements per gram of dried tissue.

TABLE III.—OXYGEN USED BY OYSTERS.

NOTE.—Figures in parentheses are averages of measurements under approximately uniform conditions.

Weight, whole oyster.	Decimilligrams of oxygen used per hour.				Averages of same per hour per 100 grams.				Weight, dried oyster.	Average decimilligrams used per hour per gram of dried weight.			
	At 20° to 20.5° C.	At 22° to 22.5° C.	At 24° to 24.5° C.	At 26° to 26.5° C.	At 20° to 20.5° C.	At 22° to 22.5° C.	At 24° to 24.5° C.	At 26° to 26.5° C.		At 20° to 20.5° C.	At 22° to 22.5° C.	At 24° to 24.5° C.	At 26° to 26.5° C.
Grams.									*Grams.*				
47.8	10.6	12.2 14.1 (13.1)	14.2	17.1	22.2	27.4	29.7	35.7	1.15	7.92	9.74	10.40	12.90
73.5	16.6	20.0 17.5 (18.3)	23.1 18.3 (20.7)	22.0	22.6	25.0	28.2	30.0	1.66	8.20	8.61	9.43	9.56
153.0	19.4	22.4	21.4 21.7 24.5 (22.5)	25.2 25.9 (25.5)	12.7	14.6	14.7	16.6	2.36	5.98	6.78	6.56	7.15
173.0	23.5	24.5 24.7 (24.6)	29.2 30.8 (30.0)	39.0 34.3 (36.6)	13.6	14.2	17.3	21.2	3.64	3.49	4.00	4.53	6.10
Average.	17.5	19.7	21.9	25.3	17.8	20.3	23.5	25.9	.....	6.41	7.28	7.73	8.94

A graphic representation of the oxygen utilization of oysters at different temperatures is given on page 217. The ordinates represent decimilligrams of oxygen used per hour and the abscissæ are degrees Centigrade. The full lines represent measurements on intact oysters. Curve I is constructed from the averages of the oxygen utilization per hour per 100 grams in experiments on nine oysters as summarized in table II. Curve II is constructed from similar averages of experimental results on four oysters as shown in table III. Curve III is based on the same results used for curve II, but represents the averages of oxygen used per hour per gram of dried shell contents. The relationship between oxygen and temperature is apparently a simple one. The interrupted lines represent measurements on empty oyster shells showing the decimilligrams of oxygen used per hour by shells as recorded in table IV. Curves *a*, *b*, *c*, and *d* refer, respectively, to shells weighing 47.8, 73.4, 153, and 161 grams. The effect of temperature on the three larger shells seems to be fairly constant; their curves are parallel. The discrepancy in the smallest shell may be due to experimental error as so few determinations were made.



Relation of oxygen utilization in the oyster to temperature. Explanations in the text on page 216.

NONUTILIZATION OF OXYGEN BY CLOSED OYSTERS.

Since oysters can close absolutely water tight, it seemed possible that under such conditions they would take in no oxygen. The very small oxygen requirements of voluntarily closed individuals could not, however, be interpreted as proof of this because when the oyster appears to be closed it may be slightly and invisibly open. This was proved by graphic records. To insure closure throughout an observation it was necessary, therefore, to use some artificial closing device. Several clamps were tried. An ordinary surgeon's artery clamp was found to be most satisfactory. If clamps without imperfections, carefully vaselined, were used, they did not appreciably rust during the experiment and withdrew from the water only a small and constant quantity of oxygen. As control experiments the oxygen absorption of empty shells of oysters about the same size as the one observed was measured. The control shells were fastened together by a clamp duplicating the one used on the oyster. Water had some access to the interior of the empty shells through nicks in their edges. On their immersion in water all air was driven from them. In many experiments controls were deferred until the same oyster could be emptied and its shells used for control measurements.

The results of a number of determinations are given in table v. It is seen that in every case the experiment and its control are in close agreement, as the difference is within the limit of experimental error except in the case of the largest oyster used. Even these differences, however, show a larger oxygen absorption by the empty shells than by the closed intact oysters. That the slight oxygen disappearance does not represent a respiratory intake by diffusion through the shells is indicated not only by the controls but by the observation that a two hours' exposure of the clamped oyster does not cause twice the oxygen absorption observed during one hour. Thus in one experiment a clamped oyster used 1.83 decimilligrams of oxygen in one hour, but 3.01 in two hours, while another, which used 1.70 decimilligrams in one hour, required 2.80 for two hours.

The explanation of the constant slight absorption of oxygen by clamped oysters and empty shells was not positively determined, but would seem to lie in several causes. Bacteria and various marine vegetative growths on the shells were first considered as possible oxygen users. Oyster shells that had been soaked 16 hours in 80 per cent alcohol and then carefully scrubbed and dried, did, indeed, show a diminished oxygen absorbing capacity, in one case lowered from 5.25 decimilligrams per hour before the alcohol treatment to 4.68 after it, and in another, from 3.08 to 2.34. This indicates that foreign growths do not account for all the oxygen absorption, and, indeed, it was found that the cleanest and most carefully sterilized shells took oxygen from the water.

An adsorption effect of porous substances on dissolved oxygen suggested itself as another possibility. It was found that porcelain evaporating dishes about the size of oysters showed equivalent oxygen absorbing powers. Corks had the same capacity. Water containing medium sized corks lost 2.34 decimilligrams of oxygen per hour, while in a control experiment water alone lost only 0.26 decimilligram, which is within the limit of experimental error.

TABLE IV.—OXYGEN ABSORBED BY EMPTY OYSTER SHELLS.

Weight of whole oyster in grams	47.8	47.8	73.4	73.4	73.4	153	153	161	161
Temperature of experiment in degrees centigrade	22.4	25.8	21.7	22.3	26.1	22.0	25.8	21.5	26.5
Oxygen absorbed per hour in mgr/10	1.9	2.4	3.6	4.1	6.3	6.1	8.3	11.5	14.4

TABLE V.—OXYGEN ABSORBED BY CLOSED OYSTERS.

Weight of oyster.	Temperature of experiment.	Oxygen used per hour.	Description of control experiment.
	°C.	*Mgr/10.*	
45 grams...	22.0	1.70	
Control....	22.0	1.70	Empty shells of same oyster with artery clamp.
50 grams...	22.0	1.83	
Control.....	22.0	1.83	Do.
66.5 grams..	23.2	2.04	
Control.....	21.0	1.92	Do.
Control.....	23.2	2.58	Empty shells of oyster weighing 45 grams with clamp.
66.5 grams..	20.5	1.41	
Control.....	20.5	1.48	Empty shells of oyster weighing 51 grams with clamp.
128 grams..	20.5	1.79	
Control.....	20.5	1.92	Empty shells of same oyster with artery clamp.
147 grams..	20.5	1.74	
Control.....	20.5	1.62	Do.
244 grams..	20.5	1.70	
Control.....	21.0	2.34	Do.
262 grams..	20.5	3.39	
Control.....	21.0	4.68	Do.
244 grams..	27.5	3.47	
128 grams..	27.5	2.36	Control experiments not made.
66.5 grams..	27.5	2.49	
76 grams...	27.5	2.80	

RESISTANCE OF OYSTERS TO LACK OF OXYGEN.

A series of experiments was undertaken to find out the minimum oxygen supply that would maintain an oyster alive. The sea water surrounding an oyster in a vacuum desiccator was as far as possible rendered oxygen free by boiling at room temperature under diminished pressure for half an hour. It was found in two trials that sea water alone when so treated and kept twelve hours in the closed desiccator still had an oxygen content less than 0.5 of a part per million. An oyster kept under these circumstances four days showed no ill effects. Another was kept thus three days, transferred to a second desiccator of exhausted sea water, which was quickly again pumped out, and was then kept four days further in the oxygen poor medium. The shells then had some black deposits on them indicative of incipient anaerobic putrefaction. The sea water on opening the desiccator was found absolutely oxygen free. The oyster, however, seemed unimpaired, and after remaining in the aquarium some time was opened and found apparently entirely normal. As each desiccator held about 1,200 c. c. and the oxygen content of the water in each case was at the most 0.5 of a part per million, the oxygen available to the oyster might be estimated as 1.2 milligrams plus the small amount obtained during the transfer from one desiccator to the other. If we disregard the latter source because the oyster was tight closed at the time, only 1.2 milligrams of oxygen were used during seven days.

In another experiment an oyster was kept three days in the first desiccator full of pumped-out water, three days in the second, two days in the third, two days in the fourth, and two days in the fifth. By that time it was black and unable to close properly. After a few hours in the aquarium it showed disintegration, so that twelve days of life in oxygen-poor water proved fatal. Time and facilities for carrying out many of these experiments were missing, although the seven-day experiment was practically duplicated by one in which an oyster was kept in the same desiccator full of water six days with no ill effects. That the fatal effect of a twelve days' exposure was not due to insufficient renewal of the water was shown by control experiments in which several oysters were kept in the same water during fourteen days while air was bubbled through it. The oysters survived this treatment uninjured.

OXYGEN REQUIREMENTS OF CLAMS.

Measurements on clams were made by the same method used for oysters. The results of successful experiments are embodied in table VI. They show a general agreement with measurements on oysters. The amount of oxygen used by clams weighing about 60 grams is somewhat higher per 100 grams than that used by an oyster of about the same weight and observed at the same temperature. Computed per gram of dried shell contents, however, the oxygen requirements of the clam seem somewhat smaller than those of the oyster. Here, as with oysters, the amount of oxygen used is more or less proportional to increase in the temperature, though sufficient data to show a simple relationship were not obtained. In this case, also, the oxygen requirement per gram is less in the larger individual than in the smaller, again like the oyster.

TABLE VI.—OXYGEN USED BY CLAMS.

NOTE.—Figures in parentheses are averages of measurements under approximately uniform conditions.

Weight whole clam.	Decimilligrams of oxygen used per hour.			Averages of same per hour per 100 grams.			Weight dried clam.	Average mgr/10 used per hour per gram, dried weight.		
	At 20° to 21° C.	At 22° to 22.5° C.	At 24° to 24.5° C.	At 20° to 21° C.	At 22° to 22.5° C.	At 24° to 24.5° C.		At 20° to 21° C.	At 22° to 22.5° C.	At 24° to 24.5° C.
Grams.							*Grams.*			
7.0	1.2	.....	.....	29.6	.....	.....	.....	.....	.....	.....
	3.0									
	(2.1)									
19.5	5.19	9.7	15.1	26.7	49.7	77.4	1.32	3.93	7.35	11.4
21.0	9.2	.....	.....	26.9	.....	.....	.....	.....	.....	.....
	6.7									
	(7.9)									
28.0	8.2	.....	.....	29.3	.....	.....	.....	.....	.....	.....
57.0	11.0	.....	.....	20.7	.....	.....	.....	.....	.....	.....
	12.4									
	(11.7)									
57.5	.....	17.2	30.0	.....	30.0	52.2	3.97	.....	4.34	7.55
60.0	.....	19.1	.....	.....	31.9	.....	.....	.....	.....	.....
63.0	17.7	.....	.....	25.8	.....	.....	.....	.....	.....	.....
	15.2									
	(16.4)									

TABLE VII.—OXYGEN ABSORBED BY CLOSED QUAHOGS.

Weight of quahog in grams	150	150	91	91	246	246 [a]	150 [a]	150 [b]	91 [b]
Temperature in degrees centigrade	24.0	24.0	24.0	23.0	24.3	23.0	23.0	23.6	23.5
Oxygen per hour in mgr/10	.37	.25	.38	.25	.23	4.60	4.30	2.43	2.95

[a] Voluntarily closed but not clamped. [b] Measurements on empty shells alone.

OXYGEN ABSORPTION OF CLOSED CLAMS.

Only one experiment was made in this connection. A medium-sized clam was closed by an artery clamp over the siphon end of the shells. As a control, empty shells of an oyster of the same size similarly clamped were used. The live clam absorbed 2.68 decimilligrams of oxygen per hour at a temperature of 23.5° C., while the shell took 2.58. This indicates that the clam, like the oyster, has little or no opportunity to obtain oxygen when the shell is not open. Further observations incidentally made confirm this conclusion. A medium-sized clam which visibly failed to open throughout an experiment to determine its oxygen requirements took only 2.44 decimilligrams of oxygen from the water in an hour. Other similar results were obtained.

OXYGEN REQUIREMENTS OF QUAHOGS.

Great difficulty was experienced in measuring the oxygen utilization of quahogs, because they seldom opened for any length of time under the conditions of experimentation. As a result of this, only a few measurements that could be considered reliable were obtained. It was found necessary to place the specimen in sea water in the desiccator a long time before the experiment, usually the night before, in order to have it open at the time of observation. Handling or moving caused it to close and stay closed for some hours.

The few successful observations showed a rather low oxygen utilization. One specimen weighing 91 grams used, at 24° C., 10.1 decimilligrams of oxygen in one hour, 10.8 in another measurement, and 6.4 in a third. Another quahog, weighing 150 grams, used, at 24° C., 7.8 decimilligrams per hour, and a large one (470 grams) used 22.4 decimilligrams per hour. Many other measurements were attempted, but owing to closure soon after the beginning of observation were unreliable. The oxygen requirements in proportion to the dried weight showed a still greater discrepancy in comparison with similar computations for the oyster. The dried weights of the shell contents of the first two quahogs observed were, respectively, 2.22 grams and 3.86 grams. The oxygen used per hour and per gram of dried weight, then, was 4.10 decimilligrams for the first and 2.21 for the second. This is less than half the amount of oxygen used by oysters of comparable weight observed at the same temperature. To prop the shells open seemed hardly worth while, because the oxygen utilization under such circumstances would be abnormal on account of the resulting violent contractions of adductor muscles. It seemed best, therefore, to be content with the conclusion that under the circumstances of these experiments quahogs used only a small quantity of oxygen.

UTILIZATION OF OXYGEN BY CLOSED QUAHOGS.

Clamping these shells as in the experiments described for oysters showed that closed quahogs used no oxygen. Their very smooth shells apparently took almost no oxygen from the water. With various sizes of quahogs clamped and observed at 24° C., results were obtained as follows: 0.37, 0.25, 0.38, 0.23, and 0.25 decimilligram of oxygen per hour. The empty shells, considerably broken in the process of opening, used a distinctly larger amount of oxygen than did the closed intact animal. The small amounts of oxygen taken up under these conditions are no more than would disappear from the sea water with a clamp in it.

It was clearly shown, however, that voluntarily closed quahogs did take up appreciable quantities of oxygen. In observations where the shells were apparently quite closed, various medium and large sized specimens took up, at 24° C., 2.9, 6.1, 4.6, and 4.3 decimilligrams of oxygen per hour. It would seem, then, that when voluntarily closed they do not remain shut absolutely tight, but take in small amounts of water through an aperture too narrow to be visible to the naked eye. The results are summarized in table VII.

CONCLUSIONS.

1. Oysters of medium sizes, at temperatures between 19° and 28° C., used from 7 to 35 decimilligrams of oxygen per hour per 100 grams of entire weight. The amount varies with the temperature, so far as experiments show, according to simple relationship, so that the curve approximates a straight line. It is proportionally less for larger specimens. The oxygen utilization is, however, exceedingly variable, depending on a variety of conditions, most of which probably affect the openness of the shell.

2. Oysters when tightly closed take no oxygen from the surrounding water; at least, no more than is taken by empty shells.

3. Oysters show considerable resistance to lack of oxygen. Only exposure for more than a week to water containing very small quantities of oxygen proved fatal. This indicates that any conditions causing temporary decrease in the available oxygen are not a significant factor in oyster culture.

4. The common clam (*Mya arenaria*) shows a higher oxygen requirement than the oyster. This seems surprising, in view of the fact that clams so often exist in muds where oxygen-consuming putrefactions are going on. The oxygen requirements of clams and oysters in proportion to their dried weights are about equal.

5. Both clams and quahogs (*Venus mercenaria*) use no oxygen from the water when tightly closed, but the quahog takes up oxygen while slightly and invisibly open.

6. The oxygen requirements of the quahog are, under the conditions of these experiments, conspicuously low.

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CONTRIBUTIONS TO THE BACTERIOLOGY OF THE OYSTER.

BY LESTER A. ROUND.

The Results of Experiments and Observations made while Conducting an Investigation Directed and Authorized by the Commissioners of Shell Fisheries of the State of Rhode Island, 1914.

State of Rhode Island and Providence Plantations

CONTRIBUTIONS

TO THE

Bacteriology of the Oyster

THE RESULTS OF EXPERIMENTS AND OBSERVATIONS MADE WHILE CONDUCTING AN INVESTIGATION DIRECTED AND AUTHORIZED BY THE COMMISSIONERS OF SHELL FISHERIES OF THE STATE OF RHODE ISLAND.

BY

LESTER A. ROUND, PH. D.

PROVIDENCE:
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PREFACE.

In March, 1910, the Commissioners of Shell Fisheries authorized an examination of the sanitary condition of the waters of Narragansett Bay and its tributaries, relative to the growing of oysters. They placed Prof. F. P. Gorham, head of the Department of Bacteriology of Brown University, in charge of this work, with the writer as an assistant.

On beginning this work it was found that there were many problems that would require more study than could be given while performing the routine bacteriological examinations of water, mud, shellfish, etc. To a great extent this work was new and the method of procedure had to be worked out as the investigation progressed. While there had been much work performed upon shellfish examinations, both abroad and in this country, there were still many problems which had not been solved and it was deemed advisable by the Commission that some of these problems which were of great importance to the shellfish industry should be given special attention.

The writer was early assigned to conduct a series of experiments and investigations along the lines that had been found would apparently prove of the greatest advantage to the oyster industry. The results of some of these investigations is published in this booklet.

The writer wishes to take this opportunity to express his sincerest thanks to Prof. F. P. Gorham, head of the Department of Bacteriology of Brown University, whose valuable advice and criticisms have been exceedingly helpful and under whose direction the work herein reported has been done; to Drs. A. D. Mead, H. E. Walter and P. H. Mitchell, who have made valuable suggestions and criticisms on different points in the work; to the members of the Narragansett Bay Oyster Company, the American Oyster Company, the Wickford Oyster Company and the Beacon Oyster Company, and to Captain William B. Welden, all of whom have rendered valuable aid in carrying out many of the experiments; also to Mr. W. B. Mason of The Merchants' Cold Storage and Warehouse Company who has given free use of the company's cold storage rooms for the experiments on hibernation.

L. A. R.

Brown University,
May 1, 1914.

THE BACTERIOLOGY OF THE CLOACAL AND GILLS CHAMBERS OF THE OYSTER.

In describing the anatomy of the gills of the oyster, Kellogg in his book on "Shellfish Industries" makes the following statement: "Behind the body the four gills unite so as to separate a space above the cloacal chamber, from the large mantle chamber below." From this statement it has been assumed at times that the two chambers were entirely distinct and so constructed that bacteria could not pass from one chamber to the other, and that for this reason the bacterial content of the two chambers would differ. Anyone familar with the anatomy of the oyster knows that every day several gallons of water are filtered through the gills into the cloacal chamber. While it is probable that most of the protozoa and algæ are caught in the mucus which the gills secrete, it is also probable that a great many of the bacteria escape, being entrapped by the mucus and pass on into the cloacal chamber. But even though the gill-filter were proven to be bacteria-proof no one has demonstrated that bacteria cannot pass along the space between the mantle and the shell, or around the edge of the shell, between the flaps of the mantle and so pass from one chamber to the other. While it seems very probable from the structure of the oyster that bacteria can pass fron one chamber to the other without difficulty, properly conducted experiments are necessary to prove it. In order thus to prove that bacteria can and do pass, from one chamber to the other, the following experiments were tried.

Series 1.

Exp. 1. A well shaped mature oyster about four inches long and two broad was selected. Care was taken to obtain an oyster with a flat right valve. The oyster was placed in a frame with the left valve down so that the right valve was level and was then clamped firmly to the table. A hole was bored through the right valve into the gill chamber quite close to the edge and another into the cloacal

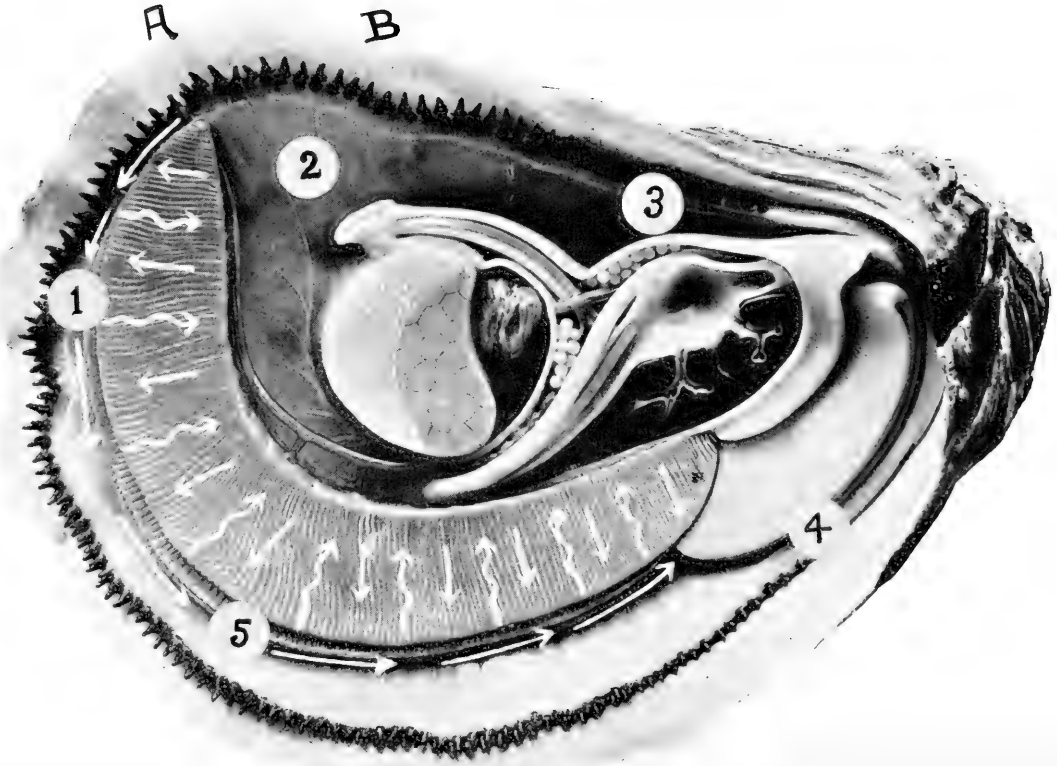

Plate Showing Position of Holes Bored in Shell of Oyster and Arrows Showing Direction of Ciliary Currents.

Photo by kindness of Mass. Fish and Game Commission.

chamber, at the anal orifice. This latter opening was ½ to ¾ of an inch above the lower end of the partition which separated the two chambers. A loopful of B. prodigiosus was then placed in the gill chamber, and loopfuls were removed from the cloacal chamber and plated at intervals of ten minutes for one hour, and then at the end of two hours and three hours. B. prodigiosus is a non-motile organism and was chosen because of its ease of identification and because in all our work extending over four years we have never isolated it from oysters.

No red colonies were found in the two control samples from the two chambers, but every plate made from the cloacal chamber after the introduction of the B. prodigiosus into the gill chamber showed colonies of B. prodigiosus.

Exp. 2. The above experiment was repeated with another oyster and B. prodigiosus was again found in the cloacal chamber ten minutes after its introduction into the gill chamber.

Series II.

Exp. 1. In another set of experiments four oysters were used. In these oysters five holes were bored as indicated in the plate shown on opposite page. Three of these holes opened into the gill chamber (1, 4, 5). Another hole (2) was made into the cloacal chamber near the anal orifice, and the last hole (3) opened on the edge of the mantle about an inch above the anal orifice.

All four of these oysters were inoculated in hole No. 5 with a loopful of B. prodigiosus. Loopfuls from the other holes were inoculated upon agar slants at two minute intervals, for ten minutes and then every five minutes, for twenty minutes, making a total of 30 minutes in each case. The result is seen in the following table:

TABLE NO. 1.

Showing the time at which B. prodigiosus was isolated from the different holes after inoculation of the gill chamber at hole No. 5.

Oyster No.	1				2				3				4			
Hole No.	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2 min	0	+	0	+	0	0	0	+	0	+	0	0	0	0	0	+
4 "	+	+	+	+	+	+	+	+	0	+	+	+	+	+	0	+
6 "	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+
8 "	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+
10 "	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+
15 "	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
20 "	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+
25 "	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
30 "	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+

+ = presence of B. prodigiosus. 0 = absence of B. prodigiosus.

From this table it is seen that in oysters Nos. 1 and 2 B. prodigiosus was isolated from all the holes at the end of four minutes; in oyster No. 3 at the end of six minutes, and in oyster No. 4 not until the end of fifteen minutes. Oyster No. 4 was a long narrow oyster and hole No. 3 in this case was necessarily moved further into the middle of the oysters, so that the opening came nearly over the stomach, and so did not reach the cloacal chamber. In the other cases, hole No 3 was made nearer the edge of the oyster and close to the free edge of the mantle, so that there was much greater chance of bacteria reaching the hole from the liquor between the free edges of the mantle, for the edges of the mantle are everywhere free except, at the "head" end, where the the edges fuse and form a hood, which is attached to the body by a flap of tissue. Between the free edges of the mantle and between the hood and the body there is a space which extends around the whole oyster and forms a kind of moat or trench filled with the liquor in which bacteria can and do move by the currents set up by the ciliary mechanism of the oyster, which will be described a little later. It will also be noticed that in every oyster of this series B. prodigiosus was isolated from hole No. 4 before they were from hole No. 1. although the distance between holes Nos. 4 and 5 were nearly twice as far as

between Nos. 1 and 5. It will further be noted that in oysters Nos. 1 and 3 B. prodigiosus was isolated from the cloacal chamber (hole No. 2) before it was found in hole No. 1, although the distance between holes Nos. 5 and 2 was also about twice as far as between 5 and 1. In no case did B. prodigiosus appear at hole No. 1 before it did at No. 2.

SERIES III.

A third set of experiments were now performed in order to show that bacteria can pass from the cloacal chamber to the gill chamber and to ascertain, if possible, the avenue through which this takes place. In this experiment two oysters were used and secured to the bench in the same manner as in the previous experiments. Three holes were bored into the branchial chamber as indicated by the Nos. 1, 5 and 4 in the plate. One hole was bored into the cloacal chamber as indicated by No. 2 in the plate. Control inoculations were made as before from these four holes. These showed no colonies of B. prodigiosus. A loopful of B. prodigiosus was placed in hole No. 2, and loopfuls were taken from holes Nos. 1, 5 and 4 at intervals of two minutes for fourteen minutes. The results are shown in table No. 2.

TABLE NO. 2.

Showing the time at which B. prodigiosus was recovered from holes Nos. 1, 5 and 4 after inoculation of the cloacal chamber at hole No. 2.

Oyster No.	1			2		
Hole No	1	5	4	1	5	4
Control	0	0	0	0	0	0
2 minutes	0	0	0	+	0	0
4 "	0	0	0	+	+	+
6 "	+	0	0	+	+	+
8 "	+	+	0	+	+	+
10 "	+	+	+	+	+	+
12 "	+	+	+	+	+	+
14 "	+	+	+	+	+	+

+ = presence of B. prodigiosus. 0 = absence of B. prodigiosus.

From this table it can be seen that B. prodigiosus was isolated from all the holes in oyster No. 1 at the end of ten minutes and in oyster No. 2 at the end of four minutes. It is further seen that in oyster No. 1 B. prodigiosus appeared first at hole No. 1, two minutes later at hole No. 5, and after another interval of two minutes at hole No. 4. In oyster No. 2 the bacillus appeared first at hole No. 1 and two minutes later at holes Nos. 5 and 4. In neither case did B. prodigiosus appear at holes 5 or 4 before it appeared at hole No. 1, nor in either case at hole No. 4 before hole No. 5.

To understand the reason for these results a description of the ciliary mechanism of the oyster is necessary.

When one opens an oyster without mutilating it, there is found between the two flaps of the mantle four folds of tissue which are the gills. These folds appear solid, but are really flaps folded back upon themselves and attached by the edges to the body so that really each gill is V shaped in cross section and the four gills form a double W (WW). With the unaided eye it can be seen that there are fine striations running vertically across each gill. These are the gill filaments. If we examine these filaments with a microscope we will see innumerable hairs or cilia about 1–500th of an inch long or less, waving vigorously back and forth. If we examine the cilia closely we find that they lash vigorously in one direction, recover themselves slowly and repeat the vigorous stroke. The movement is quite comparable to a man rowing a boat. He pulls vigorously in one direction, recovers himself and repeats the stroke. Now if we consider the boat fastened so that it could not move, the oarsman's efforts would send the water past the boat instead of propelling the boat through the water. Here we have the exact condition in the oyster. As Brooks (The Oyster, 1906) says, these little hairs "set up a current in the water. Each one is so small that its individual effect is inconceivably minute, but the innumerable multitude causes a vigorous circulation and each one is set at such a position that it drives the water before it from the gill chamber into one of the water pores and so into one of the water tubes inside the gill. As these are filled they overflow into the cloacal chamber and fill that." This set of cilia are located on the edges of the filaments and force the water through the gills from the branchial into the cloacal chamber. There is another set of cilia which wave in the opposite direction and by means of the mucus which is secreted by the mucus cells, they collect and entangle the micro-organisms and carry them over to the free edge of the gill

where a third set of cilia located on the very edge of the gill conveys the entangled organisms on the mouth. The arrangement of these last two sets of cilia can be seen in the plate. In this diagram the bent arrows show the course of the water through the gills into the cloacal chamber. The straight arrows indicate the course of the mucus and the entangled micro-organisms to the mouth.

When the valves of the oyster are open the current induced by the cilia is carried out of the oyster between the points "A" and "B." When the valves of the oyster are closed, however, the cilia keep waving as vigorously as before, because the oyster has no control over their movement, but in this case the current cannot pass out between the valves and we have what might be called a closed circulation. Instead of going out between the points "A" and "B," as is the case when the valves are open, the current must neccessarily return to the gill chamber around point "A," for a study of the currents induced by the cilia and taking the direction indicated by the arrows shows that no other course is possible. All the cilia of the cloacal chamber direct their motion towards point "A" and "B." All the currents in the branchial chamber are either through the gills into the cloacal chamber or along the edge of the gills to the mouth. As water is driven through the gills to the cloacal chamber water from the cloacal chamber must necessarily take its place. As point "A" is the point of least resistance the water necessarily passes from the cloacal chamber to the gill chamber around that point and further not only is there nothing to obstruct this current, but the current induced by the cilia on the edge of the gills is such that it would draw the water from the cloacal chamber into the gill chamber around this point. Hence we see that in the oyster we have a complete cycle of currents induced by ciliary motion. The result is that all the water in the oyster is filtered through the gills many times in an hour and the process is repeated every few minutes.

It happens that when bacteria enter the gill or branchial chamber, two courses are open. They may follow the currents through the gills into the cloacal chamber or they may become entangled in the mucus of the gills and be conveyed along the edge of the gills to the mouth. The chances of a bacterium going in either of these courses are about equal and if many bacteria are present some may go by one course and some by the other.

A study of table No. 1 will show that the B. prodigiosus followed both of these courses, some were entangled in the mucus and were

carried to the mouth (hole No. 4) while others escaped the mucus and passed through the gills into the cloacal chamber (hole No. 2). A further study of table No. 1 will show that the bacteria passed with the currents for this particular bacterium was non-motile and so could not have reached the different points by its own activity. Moreover, the interval of time which separated the inoculation of the branchial chamber and the subsequent recovery of the bacterium from the different holes in series II was only 4 minutes in all, except two cases when it was six and fifteen minutes. The distance between holes 5 and 4, 5 and 2, and 5 and 3, in all cases was at least an inch, in most cases, more. In series III the bacterium was recovered from all the holes in four minutes in one case and ten minutes in the other. The rate of travel of bacteria varies with the species, temperature, etc., but it is inconceivable that a bacterium of the speediest variety could move a distance of over an inch in four minutes by its own activity. In the case in hand, *i. e.* a non-motile bacterium, it is out of the question.

It is also seen in table No. 1 that in two cases B. prodigiosus was isolated from hole No. 2 before it was recovered from hole No. 1. In the two other cases they were recovered at the same time. While this is not conclusive it leads the writer to believe that the bacteria isolated at hole No. 1 had previously passed through the gills and the cloacal chamber and back into the branchial chamber by the return current. The results of the experiments in series III lend support to this view. The bacteria did not go directly from hole 5 to hole 1 because the currents along the edge of the gills is too strong to allow a bacterium to pass in that direction. An examination of this current under the microscope will convince anyone that a bacterium could not travel in that direction.

A study of table No. 2, which shows the appearance of B. prodigiosus in the gill chamber after the inoculation of the cloacal chamber, shows that the organisms appeared at hole No. 1 and later at hole Nos. 5 and 4. This is the order of time in which a current from the cloacal chamber and taking the direction of the arrows of the edges of the gills would appear at holes Nos. 1, 5 and 4, in the branchial chamber.

From the foregoing facts it is plain that the gills are not bacteria proof; that bacteria can and do pass from the gill chamber to the cloacal chamber through the gills and moreover, that bacteria may pass from the cloacal chamber to the gill chamber without passing through the gills. It is seen that we have a complete circle of currents

within the closed shell of the oyster which, under the conditions of the experiments, makes a complete circuit several times in an hour, and thus ensures a thorough mixing of the water and the bacterial content of the two chambers. In the conditions of the experiments the complete circuit was made in at least six minutes and in three cases in so short a period as four minutes. It naturally follows that any difference of bacterial count between the two chambers is not to be expected and such differences as are observed are within the limits of experimental error.

METHODS OF SHELLFISH EXAMINATION.

As soon as sufficient epidemiological evidence had been accumulated to show conclusively that oysters are under certain circumstances contributing factors in the spread of typhoid, Asiatic cholera and other gastro-enteric disturbances, it was but natural that bacteriologists should look for the specific cause of these diseases in the oysters themselves. If the typhoid bacillus and the spirillum of Asiatic cholera could be found in oysters, that would be evidence which no one could dispute. Although diligent search has been made for the typhoid bacillus in oysters on numerous occasions since 1893, it is interesting to note that there are on record four instances only in which B. typhosus has been reported to have been isolated from oysters. The first instance was reported by Klein.[1] Regarding this finding Klein says:

"In view of the importance likely to be attached to the finding of this bacillus in such numbers in one of these East Coast oysters, particular care has been exercised in subjecting it to every possible test . . . As a result, in all and every one of its characters it coincides with the typhoid bacillus obtained from the spleen of a typical case of typhoid fever, and for this reason I am prepared to affirm that this bacillus obtained from the "Deep Sea" oyster is the typhoid bacillus." Besides the cultural tests used, the Bordet-Durham reaction (macroscopic agglutination with immune serum 1:100) and Pfeiffer's phenomenon were also used and both proved positive while the controls in both instances were negative. In this instance the evidence seems quite sufficient to support Klein's assertion.

[1]Report of the Medical Officer to the Local Government Board, 1894-5. Supplement, Appendix No. 2, p. 115.

The second instance is cited by Fuller:[1] In 1902 at a meeting of physicians at Pera, Turkey, it was reported that a large percentage of the typhoid cases occurring in Constantinople could be traced to the consumption of polluted oysters and an examination of the oysters in a few instances showed the presence of B. typhosus. The writer has not been able to obtain the reference to this paper and the characteristics of the species have not been studied. As a result no definite comment can be made upon the findings in this instance.

The third instance is reported by Johnstone.[2] There is not so good evidence to support the identity of this bacillus as in the case reported by Klein. Johnstone's bacillus "formed acid and gas in bile salt glucose broth" and a "a slight discoloration in lactose litmus broth" and "agglutinated—in a dilution of one to thirty—in a serum which gave a positive reaction with a known strain of bacillus typhosus." All authorities are agreed that the typhoid bacillus produces no gas in any sugar medium. In regard to the agglutination in a dilution of one to thirty, the writer is inclined to question the specificity of so low a dilution. The report referred to above does not say what the titre of the serum was with any known strain of typhoid, nor whether one to thirty was the highest dilution that would give a positive reaction, though we are led to suspect that this was the case. A dilution of one to thirty cannot be relied upon explicitly, for other organisms closely related to typhoid as some strains of B. coli will agglutinate in a dilution of one to thirty and in the case of a strong serum in one to one hundred.[3]

In 1908 Stiles[4] isolated four organisms from oysters obtained from Jamaica Bay, Long Island which "resembled B. typhosus biologically, but did not agglutinate typhoid immune serum." In 1911, while investigating an epidemic of typhoid following a banquet given October 5, 1911, at the Music Hall, Goshen, N. Y., Stiles again examined oysters from Jamaica Bay, where the oysters were obtained for the banquet and in this instance he was able to isolate two strains of B. typhosus from oysters "which had been allowed to 'drink' under an oyster house at Inwood, Long Island." Besides

[1]The Distribution of Sewage in the waters of Narragansett Bay, with Especial Reference to the Contamination of the Oyster Beds, App. to Rep. of Commissioner of Fisheries for year ending June 30, 1904.

[2]Routine methods of Shellfish Examination with Reference to Sewage Pollution, Journal of Hygiene, IX. 1909, 433.

[3]Hiss & Zinsser; Text Book of Bacteriology, 1912, p. 42.

[4]Bureau of Chemistry, Bulletin No. 136.

showing all the cultural characteristics of the typhoid bacillus, it also agglutinated in five minutes in a 1:1000 dilution of typhoid immune serum. This organism was isolated from the oysters seven days after they were taken from the water. Later oysters from the same lot were examined after they had been out of the water twenty-one days and kept at 39° F. An organism was isolated which resembled typhoid in all its cultural characteristics and agglutinated macroscopically in a dilution of 1:1000. This test was confirmed by hanging drop preparations in dilutions of 1:200.

There can be no possible doubt that the organisms isolated by Stiles are true typhoid bacilli, while little can be desired to confirm the identity of the organism isolated by Klein.

An interesting feature of the work of Stiles is that he demonstrated the typhoid bacillus in oysters which had been infected under natural condition and which had been kept out of water for three weeks. Klein,[1] Foote[2] Herdman and Boyce,[3] and others have reported instances in which typhoid bacilli have been isolated after varying lengths of time up to 18 days after infection from oysters artificially infected with large numbers of typhoid bacilli in pure cultures or from typhoid stools and kept in sea water in the laboratory. So far as the writer is aware Stiles is the first one to show that oysters infected under normal circumstances with sewage containing typhoid bacilli and kept under favorable conditions can still harbor B. typhosus after 21 days. The condition here are somewhat different from laboratory experiments in that in sewage along with the typhoid bacilli are other bacteria whose influence is exceedingly hostile to the growth of B. typhosus.[4]

It is interesting to see that this organism has been isolated so few times, in spite of the abundant epidemological evidence in so many instances which points conclusively to the infection of oysters and other shellfish with typhoid bacilli. The reason for this, however, is quite readily understandable when we consider the number of typhoid bacilli which could be found in the sewage of any town or city in comparison with the number of other organisms found in that same sewage. It would be a case of searching for the proverbial

[1]Loc. cit.

[2]A Bacteriological study of Oysters, with Special Reference to them as a source of Typhoid Infection," 18th Ann. Report Com. State Board of Health.

[3]Oysters and Disease, Thompson Yates Laboratory Report, 1–2.

[4]Jordan, Russell & Zeit, Journal of Infectious Diseases 1, 1904, 641.

needle in the hay stack. Moreover the incubation period of typhoid varies from two to three weeks and this would make the period of infection two or three weeks before suspicion would be thrown on the oysters. That is, two or three weeks would elapse after infection, before we began to look for the organism. During this space of time the other oysters of the same laying would, in all probability, have time to rid themselves of the organisms, provided they too were infected. In the case of an epidemic of typhoid due to eating raw oysters, the time to examine the oysters for typhoid bacilli would be at the moment they were eaten. We may be quite sure from the history of the cases that the oysters which were consumed did contain B. typhosus, but we have no assurance that all the oysters of that particular bed contained the organism. There is great variation in the number of sewage organisms contained in the individual oysters of the same bed. This individual variation will be still greater if the bed is large and the amount of sewage small, tho highly infected with B. typhosus and other sewage organisms. Sewage does not ordinarily contain typhoid bacilli in constant numbers at any time and unless there is an extensive epidemic, B. typhosus would appear only intermittently and then in comparatively small numbers. In view of these facts the wonder is, considering that B. typhosus die off rapidly, both in sea water and in oysters, that typhoid bacilli have ever been found at all.

The spread of cholera through infected oysters has not attached so much attention as the transmission of typhoid. The latter is distributed much more widely throughout the world and the opportunity for such transmission is much greater. Occasionally, however, there has appeared references to the spread of cholera through infected oysters. In 1849 there was a small epidemic of cholera in England which was attributed to eating oysters. In 1893 Sir Richard Thorne attributed a number of scattered cases of cholera in England to the consumption of oysters. Recently it has been reported that a large extent of oyster beds in Italy have been destroyed because they were thought to be a menace to the public health on account of the danger of the cholera infection.

In most, if not all epidemics of typhoid from infected oysters or other articles of food, there have been a greater number of cases of gastro intestinal disturbances which have not developed into

typhoid.[1] We cannot tell the exact cause of these intestinal upsets. It may be due to bacteria other than typhoid or it may be due to chemical or ptomaine poisons which appear in the sewage as the end products of bacterial metabolism. Whatever the cause we are led to expect these disturbances as concomitants of any outbreak of typhoid due to an infected food.

Since one can rely so little upon the finding of the specific disease organism in sewage and in oysters, it was but natural that an index of greater reliability should be sought. Klein[2] at the beginning of his experimental work as well as in some previous investigations ascertained that B. coli and other intestinal bacteria form no part of the flora of oysters grown in non-polluted water and for this reason used B. coli as an index of pollution. Klein's observations in regard to the bacterial content of oysters grown in water free from sewage has been confirmed by Houston,[3] Ferguson[4] Fuller[5] and others. The presence of B. coli as an indication of sewage pollution has been adopted by all workers in this field and is the index used to-day to determine bacteriologically the presence of fecal matter.

In examining oysters, however, we have quite a different problem from the examination of water, for we have not only the juice, but the body of the oyster, the mucus covering the body, the alimentary canal, etc., to consider. It is interesting to see how the methods of examination have changed as our knowledge of the bacteriology of the different parts of the oyster has increased.

Perhaps the first person to make an extended study of the bacteriology of the oyster was Klein, who in 1893,[6] made a study of the "Relation of Oysters and Disease" for the Local Government Board. Klein describes his method of analysis as follows:—

"Each oyster was carefully washed and brushed in a small quantity of sterile water, with a view to collect therein any microbes adhering to its shell. Next, the oyster, after a further cleansing under a water tap and drying with a clean cloth was opened with a sterile knife.

[1]As an illustration, the reader is referred to the following reports: H. T. Bulstrode, in local Government Board, 32d Annual Report, 1902-1903, Suppl. App. A. pp. 129-189; H. W. Conn, The "Oyster Epidemic" of typhoid fever at Wesleyan University, Medical Record, 46,1894, 743-6; G. W. Stiles, Sewage Polluted Oysters as a Cause of Typhoid and other Gastro-intestinal Disturbances, Bureau of Chemistry, Bulletin 136, 1912.

[2]Loc. cit.

[3]4th Rep. Royal Sewage Commission, 1904.

[4]Bull. Virginia State Board of Health, May, 1909.

[5]Loc. cit.

[6]Supplement to Report of Medical Officer to Local Government Board, Appendix No. 2, pp. 109 and 117.

and its body mashed up with the liquor contained in the shell . . . and about ¼ to ½ c.c. of the liquor and the oyster tissue was removed by means of a freshly made capillary pipette and introduced into a phenolated broth tube which was incubated at 37° C for 24 hours." If growth occurred the culture was plated and the suspicious colonies fished and studied in pure culture. This method allowed no comparison between the bacterial content of the shell liquor and the "oyster tissue." Besides it did not allow a determination of the number of colon bacilli in the whole oyster nor per unit volume. Moreover, we have no evidence that any part of the oyster tissue except the epithelium of the outside of the body and the lining of the alimentary tract contain bacteria and this large amount (in comparison to the amount of shell liquor) of finely divided tissue—for it must have been finely divided to have been taken up in a capillary pipette—would interfere greatly, if one tried to obtain an accurate count.

Chantemesse, in June, 1896, reported to the Académie de Medicine, Paris, his observations on the relation of oysters to disease. In the article presented at this meeting he does not give the details of his technique, but says the shell liquor and the bodies of the oysters were submitted to a bacteriological examination and B. coli were found.

The next important investigation after that of Klein is the work of Herdmann and Boyce.[1] A great number of experiments were performed on the chemistry and biology and also on the bacteriology of the oyster. Only a small part of their work related to the presence of B. coli in normal oysters. For this work the stomach contents were used. The following is quoted from their report:—

"The method of analysis consisted in first cauterizing the mantle over the region of the stomach and then inserting a fine sterilized glass pipette, the pipette was moved about and when sufficient of the contents of the stomach and the juice had risen in the pipette, the latter was removed and its contents transferred to liquified agar, ordinary gelatine or sea-water gelatine and plate cultivations made."

Apparently no attempt was made to determine the number of colon bacilli either per unit quantity or in the contents of the stomach as a whole.

The next important investigation we have noted is the work of Dr. Houston.[2] Dr. Houston's method of analysis is as follows:—

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[1](1) Lancashire Sea Fisheries Memoir No. 1. (2) Proceedings of Royal Society, 1899. (3) Thompson Yates Lab. Rpt. 1-2.

[2]Fourth Report of Royal Sewage Commission. Vol. III, 1904

Cleaning of Oysters:—

"The outside of the oyster shells was well scrubbed with soap and water, and cleansed as thoroughly as possible under running water; the shells were then well washed in running main water, and finally with sterile water.

Cleansing of the Hands:—

"The hands of the experimenter were thoroughly cleansed with a hard scrubbing brush, soap and water, then rinsed first with 1:1000 corrosive sublimate solution, and finally with sterile water.

Subsequent Procedure:—

"The oysters were laid out upon a sterile towel, the flat shell uppermost. They were opened in this position with a sterile knife, held in the right hand, while they were held in this position with a corner of the sterile towel grasped in the left hand. Great care was taken to avoid any loss of liquor in the shell. This liquor was poured into a sterile 100 c. c. cylinder, the oyster was then partly cut with sterile scissors and the liquor thus freed allowed to run into the cylinder. Ten oysters were thus treated in each experiment. The volume of the oyster plus the oyster liquor was read off, and usually varied bewteen 80 and 120 c.c.. so that the oysters, being of medium size and containing a medium amount of liquor, 100 c.c. might be considered a fair average of the total shell contents of the ten oysters. Sterile water was then poured into the cylinder up to the 1,000 c.c. mark, and the whole well stirred with a sterile rod.

"An Alternative Quantative Method for the Bacteriological Examination of Oysters.

"An alternative method for the bacteriological examination of oysters may be given here, although the routine work, except where otherwise stated, has been carried out by the foregoing method.

"The oysters are cleansed and opened, with the same precautions already noted. Then the body of the oyster is cut into small pieces with sterile scissors: this process should be carried out in such a way as to insure the thorough mixture of the gastric juice of the oyster and the liquor. The oyster, meanwhile, is carefully held with the concave shell downwards and the flat shell bent back or altogether removed. To examine the liquid contents of the shell without this

preliminary step may partake of the nature of the examination of the last sample of sea water imbibed by the oyster before finally closing the shell. Indeed, the experiments detailed elsewhere seem to indicate that per unit volume the gastric juice of the oyster may be more impure bacteriologically than the oyster liquor.

"For cultural purposes the following quantities were made by proper dilutions:—100 c.c., 10 c.c., 1 c.c. 1–10 c.c., 1–100 c.c., 1–1000 c.c."

It appears that this was the first attempt to determine the number of B. coil or coli-like organisms within the oyster. The supposition was that the supernatant liquid above the oysters contained in an even distribution all the bacteria that were present in the shell liquor, the juices of the body, and on the outside of the oyster. Whether this assumption is true or not will be discussed later when the writer takes up his own experiments. Houston also performed "a series of experiments to ascertain the relation between the biological (bacteriological L. A. R.) composition of (1) the shell liquor and surface "washings" of the oyster, and (2) the "washed bodies of the oysters." In this series of experiments, four in number, by rapid fire calculation and assumptions, Houston arrives at some very startling conclusions.[1] From these experiments he states that volume for volume the stomach of the oyster contains more bacteria than any other part of the oyster. The method of conducting the experiments and the premises assumed and conclusions drawn will be discussed more at length when the writer takes up similar experiments of his own.

Fuller in the article cited above describes his method as follows:—

"In the examination, inoculations were made from the liquor contained between the shells, from the contents of the intestines, stomach, and rectum, and in some cases from portions of the visceral mass. In order to obtain samples of the juice from an oyster under aseptic conditions, the speciments to be examined were scrubbed thoroughly in tap water with a stiff brush, washed off in running sterile water, and dried on a sterile towel, after which they were opened with a sterile knife. To obtain cultures from the stomach, the top of the mantle covering the interior end of the oyster was slit open and the large palps on either dide of the mouth pushed aside; the mouth region was sterilized by passing a hot scalpel over these parts and a portion of the stomach contents was drawn out by means of a fine pipette or platinum loop introduced through the mouth opening. Cultures from the intestines were made in the following

[1]See page 27.

manner: After opening the shell, the oyster was removed from the shell and dried between filter papers. A hot spatula was then passed upon the surface of the mollusk directly over that portion of the intestine which it was desired to reach, and the tube was then opened with a sterile scalpel. Through this opening a portion of the contents was drawn out by means of a pipette or platinum loop. Portions of the visceral mass were obtained by cutting out cubes of flesh from that portion of the body after sterilizing the surface with a hot scalpel."

McWeeney[1] in his examination of oysters on the Irish Coast used the shell liquor alone, if abundant. But in cases where the amount of shell liquor was small he supplemented the small quantity of liquid "with a block of tissue cut from the animal itself so as to include portion of the alimentary canal."

The next worker to do a great deal of routine and experimental work in the examination of shellfish was H. W. Clark. In a preliminary report published in 1902[2] Clark describes his method of analysis as follows:—

"To determine the presence of B. coli in the juice on the shell, the clams, oysters, etc., were washed with sterilie water, then opened, and this juice inoculated into bouillon."

"To determine whether the germ was present in the bodies of the clams, oysters, etc., they were opened after washing with sterile water, and the intestine, after maceration with sterile water, was inoculated into phenol dextrose bouillon.

In 1905, Clark[3] in a report covering his experimental work for the previous five and one-half years makes the following statement in regard to the "Examination of Raw Oysters:"—"The shell liquor and the crushed body of the oyster were examined together by inserting the entire mass in a fermentation tube, and if fermentation was obtained, carrying out the cultural tests."

In determining the presence of B. coli in the body of the oyster as detailed in his first report it appears that Clark disected out the alimentary tract. This is not stated as part of the procedure, but it is implied from the above quotation. This procedure would be rather cumbersome if one attempted to use it on a large scale in routine exam-

[1]Report on the Bacterioscopic Examination of Samples taken from Shellfish Layings on the Irish Coast, Local Government Board for Ireland, 1904

[2]Senate Document 336, State of Mass., 1902.

[3]Report Mass. State Board of Health, 1905, 427.

inations. Moreover, Clark apparently assumes that the bacteria isolated in this manner all came from the intestinal tract and that no contaminating organisms from the mucus on the outside of the body entered into the bacterial flora of the macerated intestine. The writer in some experiments to be given in detail later has shown that on the average there are more—often many times more—bacteria in the mucus on the body of the oyster than in the total amount of shell liquor and further that volume for volume the contents of the stomach do not contain so many bacteria as the shell liquor. Since the stomach contains more liquid on the whole than the rest of the intestinal tract, it is but natural that it should contain more B. coli than the remainder of the intestinal tract. This would be all the more evident when it is understood that B. coli do not grow in oysters, but probably diminish as they pass through the intestinal tract.[1]

In his second article cited above, the whole contents of the oyster shell, "the shell water and the crushed body of the oyster were examined together by inserting the entire mass in a fermentation tube." Obviously this would allow of no comparison between the bacterial flora of the shell liquor and the body of the oyster. Yet in a following paragraph and also in a table he gives the results of the analysis in "Per cent. of Samples Giving Positive Tests," in "Shell Water, Intestine" and "Mash." Obviously there is some discrepancy, for if he followed out the method described it would be impossible to make such a differentiation. It is possible, however, that Clark was using a combination of the technique as stated in the two reports. The shell water and the "intestinal content" were examined as stated in his report of 1902, and his "mash" consisted of the shell liquor and crushed body, the entire mass of which was inserted into the fermentation tube. It would appear, however, that in order to carry out a combination of these two pieces of technique, two oysters would be necessary, one for the shell liquor and intestine and another for the "shell water and the crushed body." If this were true the individual variation of course, would allow of no definite comparison between all the parts tested. It may mean that the remains of the body tissue after dissecting out the intestine and the unused portion of the shell liquor were mixed and constituted the shell water and crushed body. But, in whatever manner we try to explain the matter, the fact remains that the method as described is insufficient to account

[1]Hardman & Boyce, loc. cit.

for the results obtained. But, as the results are expressed in the text and again in more detail in a table, we can feel quite certain that the method of analysis in the second report is not given in sufficient detail and the results expressed in the table are accurate so far as his methods would allow.

From the table referred to above it is seen that in the examination of one hundred and forty-five oysters approximately fifty per. cent of them gave positive tests for B. coli in the shell liquor, seventeen per cent in the "mash" and between seven and eight per cent. in intestine.

In three following tables is given the results of the analysis of shell liquor and intestine of 265 other oysters, making a total of 410 oysters examined in all. A comparison of the percentage of positive results in the shell liquor and intestine shows that B. coli were found nearly four times—50 to 14— as often in the shell liquor as in the intestine. From these experiments it seems apparently beyond question that the greatest number of B. coli are in the shell liquor of the oyster and that the body of the oyster should be disregarded in our search for the colon bacillus.

Stiles[1] describes his method of analysis as follows:

"The examination of composite samples of five or more oysters was supplemented by inoculating media with the liquor from single oysters to determine the presence of Bacillus coli in each. It was also decided to use only the liquor bathing the oysters, instead of both meat and liquor, as the latter represents the character of the whole contents of the shell sufficiently well to determine the presence of pollution."

Gage[2] discribes his methods as follows:—

"The upper shell being removed, a portion of the liquor in the lower shell is now transferred to a fermentation tube with a sterile pipette, or a portion of this shell-water may be carefully poured directly from the shell into the tube. The latter method is much simpler than the use of pipettes, but requires that the shell be so handled in the previous operation that the lip over which the liquor is poured has not been contaminated. The body is now washed with sterile water, then while held with the fingers of the left hand, an incision is made with a sterile scalpel and a portion of the intestine

[1]Shellfish Contamination from Sewage-Polluted Waters and from other Sources, Bureau of Chemistry, Bulletin 136, April 11, 1911.

[2]Methods of Testing Shellfish for Pollution, Jour. or Infectious Deseases, 1910, VII, 78.

transferred with sterile forceps to another fermentation tube, care being taken not to touch the parts where the incision is made with the fingers or to contaminate it in any way. This procedure is repeated until 10 individuals have been tested from each sample jar."

It would appear that the work of Clark has had wide influence in determining the method of shellfish analysis now in use in this country. So far as the writer is aware and so far as the literature at hand shows, the only part of the oyster used for bacteriological analysis for some years has been the shell liquor. The "Committee on Standard Methods of Shellfish examination" appointed by the American Public Health Association has recommended the use of the shell liquor only. So far as a perusal of the recent literature is concerned no one has questioned the advisability and propriety of using the shell liquor alone for analytical purposes except Gorham[1] upon results obtained by the writer in the laboratory of Brown University.

It will be noticed in all the work cited in which parts of the intestine have been used for analysis, except in the case of Fuller, no mention has been made of trying to avoid taking bacteria from the outside of the oyster as well. In the writer's opinion a great many of the bacteria alleged to have been found in the intestinal tract have come from the mucus on the outside of the body. There is no doubt that the intestine of the oyster does contain bacteria of sewage origin, but the mucus on the outside of the body is much more likely to contain such bacteria.

BACTERIOLOGY OF THE SHELL LIQUOR AND "WASHINGS" FROM THE BODY OF THE OYSTER.

A matter of great interest to the writer is that in all the work done upon oysters experimentally and otherwise no one has mentioned the mucus of the oyster or apparently realized that it plays any part in the bacteriology of the oyster.

The matter of the mucus in the oyster juice and on the oyster's body appears so self-evident that it seems impossible that it should have been entirely neglected. This mucus serves at least two purposes. (1) It acts as a protection to the body of the oyster and protects it from the deleterious effects of sea water in just the same way as the mucus of the dog fish and other selachians protects their skin from

[1]Report of Commissioners of Shell Fisheries, State of R. I., 1914.

the action of the sea water. (2) The other and more important function from the bacteriological point of view is that it serves as a net for the entrapping of the food of the oyster which consists largely of diatoms and algæ, but is made up of all sorts of microscopic particles, living or dead, organic or inorganic. As a consequence, the bacteria as well as the other microscopic organisms get entangled in this mucus.

When one opens an oyster and collects the juice, usually a great many particles of mucus, some particles very large comparatively speaking, are seen in the liquid. If one handles an oyster after opening, it is found covered with a vicid, slimy substance which does not wash off the hands easily If the bodies of the opened oysters are allowed to stand for sometime there rises to the surface long strings and flakes of this greenish yellow mucus. In shucking houses it is customary to allow opened oysters to lie for some time in large vats filled with water and with occasional stirring allow the mucus to rise to the surface of the water and run over the edge, if running water is used, or if not, it is skimmed off with a perforated dipper. This mucus often collects in "ropes" two, three, or more inches long and sometimes in large flakes the size of a half dollar.

If one examines the liquor of the oyster he has just opened, it usually contains a great number of particles of mucus, some large, some small. If one collects the liquor in a bottle and allows it to stand over night it will be found to have separated into two distinct layers, a heavy, thick, viscous layer on the bottom and a clear, more limpid layer on the top. The bottom layer is the mucus which has precipitated out. Standard Methods of Water Analysis requires a water sample to be shaken twenty-five times before the analysis commences, in order to break up any clumps of bacteria. The second Progress Report of the Committee on Standard Methods of Shellfish Examination[1] recommends that "bacterial counts shall be made of a composite sample of each lot obtained by mixing the shell liquor of five oysters. Agar shall be used for the culture medium and in general the procedure shall be in accordance with the method recommended by the committee on Standard Methods of Water Analysis of the American Public Health Association." It can be inferred from the last sentence of the quotation that it includes shaking the sample. In draining the liquid from the oyster the water runs out of the shell not at a single point, but over a considerable part of the edge of the shell.

[1]Jour. Am. Pub. Health, II, 1912, 34.

For this reason the mouth of the ordinary water sample bottle is not large enough to collect all the juice and so in most laboratories a sterile petri dish is used for the purpose. This would preclude the possibility of shaking. Now if shaking of a water sample, which to the eye is perfectly clear, is advisable to break up the clumps of bacteria and give a more even distribution of bacteria, what can be said of the juice of the oyster which has a decided milky appearance and which usually contains strings and flakes of mucus large enough to be seen several feet away? If one plates a cubic centimeter of this mixture without shaking, the flakes will appear in the solid medium as irregular, opaque particles. The probabilities are from the writer's experience that the flakes of mucus carry a large number of bacteria and we have a large confluent mass of colonies developing around each mucus flake. Even if flakes are not present, large confluent masses of colonies from the size of a penny to the size of a quarter develop, which render counting impossible. Usually, however, only bile tubes are used for the presumptive test for B. coli and no plates made so that this clumping is not noticeable except where bile tubes do not duplicate or where one gets a positive test in the 1–10th c.c. or 1–100th c.c. dilution and not in the 1 c.c. or a positive presumptive test in the 1–100th c.c. dilution and not in the 1 c.c. or 1–10th c.c. dilution. In a study of about 2,000 tubes in the presumptive test the writer found that they duplicated only about two-thirds of the time and that in one set one might get a positive presumptive test in the 1–100th c.c. dilution and in the duplicate set only in the 1 c.c. dilution or not at all.

Aside from the part played by the mucus in oyster juice the part played by the mucus left upon the body of the oyster is, generally speaking, much more important. Often much more mucus is left upon the body of the oyster than is found in the oyster juice. As the mucus is the part which catches the bacteria and holds them, it follows that often more bacteria are left upon the oyster's body than are found in the oyster juice. Hence, it follows that, if we only examine the juice of the oyster we are only finding a fraction of the bacteria really present in the oyster. These facts will be brought out more clearly when the experimental work upon which these statements are based, is taken up.

The idea of comparing the number of bacteria found in the shell liquor with the number that can be "washed" from the body of the

oyster is not new. [1]Houston performed a series of experiments on this point and his technique and results are given in some detail.

EXPERIMENT "A."

September 9th, 1903.

"The oysters utilized for this experiment were gathered in the Helford River, at low tide, on September 8th. They were cleansed in the manner described elsewhere, before being opened with a sterile knife. Each oyster was carefully detached from the two valves of its shell, with as little injury as possible, and washed in the manner about to be described.

"A sterilized funnel was placed in a sterile, 1,000 c.c. measuring cylinder as shown in the accompanying figure. The liquor in the oyster shell was poured into the cylinder before the oyster was completely detached, and then the oyster was removed from the shell with sterile forceps, held over the funnel, well washed with sterile water, and allowed to rest in the funnel. Ten oysters were treated severally in this manner, and then allowed to drain in the funnel.

1. LIQUOR.

"The total amount of sterile water employed for washing purposes was..... 810 c.c.

The total volume of liquid (oyster liquor and "washings") in the measuring cylinder was..... 840 c.c.

Therefore the volume of oyster liquor for 10 oysters was..... 30 c.c.
or 3 c.c. liquor per oyster.

"The funnel containing the oysters was then lifted into a second sterile cylinder, and sterile water was poured into the first cylinder up to the 1,000 c.c. mark.

"The cultures were then carried out in the ordinary way described elsewhere.

Results of the Examination of the Liquor.

"Coli-like microbes were isolated in pure culture from 1 c.c. and 0.1 c.c. of the litre of mixed oyster liquor and sterile water.

[1]Loc. cit.

This result indicates that the litre consisting of oyster liquor + sterile water contained coli-like (apart from slow liquefaction of gelatine) microbes in amount corresponding to about 10 per c.c. The whole litre could thus be considered to contain about 10,000 coli-like microbes derived from 30 c.c. of oyster liquor.

Hence, if 10 oysters yield 30 c.c. of liquor containing 10,000 coli-like microbes, taking the average liquid contents of each oyster as 3 c.c., this works out at 1,000 coli-like microbes in the liquid contents of each oyster, or about 330 coli-like microbes per c.c. of oyster liquor.

II. OYSTERS.

"The oysters were one by one removed from the funnel, cut up with sterile scissors, and placed in the second sterile cylinder. A known quantity of sterile water (100 c.c.) was then added, the total volume read off, and hence after deducting 100 c.c. the volume of the oysters was obtained. It was found to be 90 c.c. Sterile water was then added to the cylinder until the volume of the liquid was equal to 1,000 c.c.

"The cultures were then carried out in the ordinary way described elsewhere.

Results of the Examinations of the Oysters' Bodies.

"Coli-like microbes were respectively isolated from 10 c.c. and 1 c.c. of the litre consisting of a mixture of washed oyster bodies and sterile water.

"The litre consisting of sterile water+macerated oysters might be considered to contain about 1,000 coli-like microbes derived from the bodies of 10 oysters. Therefore, each oyster body (deprived as far as possible of its natural liquor) would contain coli-like microbes corresponding in number to about 100.

"The total volume of oyster bodies being 90 c.c., the volume of each of the 10 oysters averaged 9 c.c.; each 9 c.c. of oyster body could be considered to contain 100 coli-like microbes, or, roughly speaking, 11 coli-like microbes per c.c. of body bulk. The contrast is very striking when this number is compared with that of 330 coli-like microbes per c.c. of oyster liquor, *i. e.*, volume for volume the oyster liquor contains about 30 times as many coli-like microbes as the oyster body.

"But the liquid contents of the oyster's stomach are certainly much less than 1 c.c., probably about 0.1 c.c. It is probably that the coli-like microbes isolated from the macerated oyster bodies in the foregoing experiment were totally, or in great part, derived from the contents of the stomach and intestinal tract. In fact, it is conceivable that the 100 coli-like microbes, which each washed oyster was found to contain, were all, or to a great extent, derived from the stomach juice which, for comparative purposes, may be assumed to be about 0.1 c.c. But if the body volume of each oyster be taken as 9 c.c., the volume of the stomach contents on the above assumption is only about one-ninetieth of the total bulk.

"This view alters considerably the complexion of affairs. For the ratio between the number, per unit of volume, of coli-like microbes present, respectively, in the oyster liquor and stomach juice, would then be 33:100. In other words, acting on this assumption the coli-like microbes were three times more numerous per unit of volume in the stomach or intestinal juice then in the oyster liquor.

1	2	3	4	5	6	7
Experiment.	Volume of oyster liquor per oyster in c.c.	Volume of oyster body per oyster in c.c.	Number of coli-like microbes per cc. of oyster liquor.	Number of coli-like microbes per cc. of oyster body.	Ratio of columns 4 and 5.	Ratio as regards coli-like microbes of oyster liquor to oyster body per unit of volume.*
A.	3	9	330	11	33:1	33:100
			about	about	about	about
B.	7.5	9.5	1.3	1	1.3:1	1:100
C.	3	8	330	125	2.64:1	3.3:100
D.	6	10	166	100	1.66:1	1.66:100

*On the assumption that all the coli-like microbes obtained from the macerated bodies of the oysters were derived from the stomach juice (taking the volume of the stomach juice as 0.1c.c. and the volume of the oyster apart from its liquor as 10c.c.)."

From these experiments and the conclusions drawn it is clear that Dr. Houston thought that all the bacteria on the outside of the oysters were washed off with the sterile water used to wash the bodies of the oysters and that all the organisms found in the minced oysters came from the stomach. Whether we can accept Dr. Houston's supposition or not will be discussed later under the writer's own experiments in this connection.

Stiles in the bulletin referred to above made some analyses showing the relative numbers of bacteria in the shell liquor and meat of oysters. He concludes: "The results show that the oyster liquor in these samples contained more than seven times as many organisms per given volume as did the minced meat and body contents of the same oysters. The results further show that the liquor contained eight times as many B. coli per cubid centimeter as the minced meat."

Stiles does not give his method of determining the number of bacteria in the minced body of the oyster. It may well be that his results actually do show the relative numbers of bacteria in the two parts of the oyster. His experiments included the results of only fifteen analyses, and the results uniformally show a greater number of bacteria in the shell liquor than in the minced body meat. In the light of the writer's results of similar analyses, however, we are led to believe that the method of analysis is not adequate to demonstrate the relative number of bacteria in the two parts of the oyster. It is conceded by all that the tissues of the oyster are sterile. It is only the outside of the body and the alimentary tract which normally harbor bacteria. It is easy to understand how so much minced tissue will interfere with accurate results. Secondly no mention is made of how the bacteria were separated from the minced meat. An immense amount of shaking would be necessary to make an even suspension of bacteria if one tried to wash them from the minced particles of the oyster meat. The bacteria are attached to the body of the oyster by the mucus which is not easily removed. Even though the minced oysters were shaken vigorously in water or salt solution, the particles would quickly settle out and being more or less entangled in the mucus a coagulum would be formed which settling out rapidly would take a great many if not most of the bacteria out of suspension. This is purely suppositional since the method of analysis is not given, but this is a perfectly logical method of procedure and a very probable explanation of the results. The temperature of the water from which the oysters were taken is not given. In the writer's opinion this is

an important matter, for the temperature of the water will influence the metabolism of the mucus secreting cells and will determine the amount of mucus present on the body of the oyster. This matter will be discussed further in another connection.

When the writer began his experiments, he did not know of Houston's work and so the experiments were not carried out in exactly the same manner, but, nevertheless, the experiments throw considerable light on the work just cited. The idea that the mucus of the oyster played a part as yet unappreciated led the writer to perform the following series of experiments.

Experiment I.

September 29, 1913, ten oysters were taken to the laboratory and analyzed as follows: The oysters were opened according to "Standard Methods" and the liquor drained into a small bottle graduated in two cubic centimeter divisions. The oysters were allowed to drain until a drop would not come away at least every five seconds. The amount of liquor was then read off and an equal volume of sterile salt solution added and the whole shaken vigorously one hundred times. The body of the oyster was removed from the shell and placed in a sterile jar and a quantity of sterile salt solution added equal to the volume of the shell liquor. The jars were covered and allowed to stand for a short time while the oyster juice was being inoculated into plates and bile tubes. The jars containing salt solution and oyster meat were then stirred vigorously with a sterile pipette and an attempt made to remove with the pipette as much mucus as possible from the body of the oyster. Then one cubic centimeter of the solution and dilutions thereof were inoculated into plain agar plates and lactose-peptone-bile in the same manner as in the case of oyster juice. A careful record was kept of the number of cubic centimeters of juice obtained from each oyster and the amount of salt solution used in washing each oyster in order to make a comparison of the bacterial content of all the shell liquor with the total number of bacteria washed from the oyster. This would show which part contained the greater number of bacteria.

Experiment II.

The above experiment was repeated on oysters obtained October 7, 1913. The total number of bacteria found in the shell liquor and the

washings from the bodies of the oysters in each of the two experiments is shown in the following table:

Table Showing the Total Number of Bacteria in the Shell Liquor of each Sample and the Total Number Washed from the Bodies of the Oysters Without Shaking.

Date.		20 Count.	B. coli Count.
Sept. 29.	Shell Liquor	330,000	7,400
	"Washings"	48,000	1,700
Oct. 7.	Shell Liquor	480,000	5,900
	"Washings"	50,000	850

The detailed results are shown in the two following tables:

Date.	No. of Oyster.	C.C. of Oyster Liquor.	20°C Count.		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liqour.	Based on Shell Liquor and Washings
Sept. 29, 1913	1	8	27,000	1,900	1,600	800	200	300
" "	2	4	4,000	1,600	80	400	20	120
" "	3	3	2,900	2,400	60	30	20	30
" "	4	10	51,000	2,400	200	10	20	21
" "	5	11	13,000	5,500	220	11	20	21
" "	6	10	14,000	4,800	200	100	20	30
" "	7	9	76,000	14,000	1,800	90	200	210
" "	8	13	14,000	4,700	260	130	20	30
" "	9	5	75,000	6,000	1,000	50	200	210
" "	10	10	58,000	4,600	2,000	100	200	210
Totals	...	83	334,900	47,900	7,420	1,721	920	1,182

Date.	No. of Oyster.	C.C. of Oyster Liquor.	20°C Count.		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor and Washings.
October 7, 1913 . .	1	10	40,000	1,000	200	100	20	30
" " . .	2	20	20,000	3,700	2,000	10	100	101
" " . .	3	12	54,000	20,000	240	10	20	21
" " . .	4	10	70,000	300	20	10	2	3
" " . .	5	18	13,500	1,200	18	100	1	61
" " . .	6	14	126,000	12,000	2,800	200	200	214
" " . .	7	18	73,800	5,000	180	200	10	21
" " . .	8	10	12,000	3,200	200	200	20	20
" " . .	9	12	72,100	3,400	240	0	20	20
" " . .	*10	...	.....	.....	.....	.....	.....	.....
Totals.....	...	...	481,300	49,800	5,898	830	393	511

*Not examined.

It will be noticed that in the last two columns of the table is given the score based upon the shell liquor alone and upon the shell liquor and the "washings" from the oyster combined. The method of scoring is based upon the same principal as the method of scoring recommended by "Standard Methods," but it works out a little differently for the method of analysis followed by the writer is not strictly in accordance with "Standard Methods." In the latter method 1 c.c., 1–10 c.c. and 1–100 c.c. quantities of the shell liquor are inoculated into lactose-peptone-bile. If the presumptive test shows B. coli in 1 c.c. dilution and not in the 1–10 c.c. and the 1–100 c.c. then the score of this oyster is one. If it shows B. coli in 1–10 c.c. and not in 1–100 c.c., the score is ten; if in 1–100 c.c. the score is 100. In other words, the score of the oyster equals the number of B. coli found in one cubic centimeter of the shell liquor. In the writer's experiments the shell liquor was carefully measured and diluted with an equal volume of one per cent. NaCl solution. One cubic centimeter of this mixture was used to make the various dilutions. The result is that the various dilutions contained ½ c.c., 1/20 c.c. and

1/200 c.c. of the shell liquor. Gas appearing in these respective dilutions would indicate two, twenty and two hundred B. coli per cubic centimeter instead of one, ten and one hundred as in the procedure of "Standard Methods."

The last column gives the combined score, in other words, the score based upon the number of B. coli in both the shell liquor and in the washings. The number of B. coli in each is added together and divided by the number of cubic centimeters of shell liquor. This method makes no allowance for the amount of mucus present on the body of the oyster. This quantity would not exceed one cubic centimeter on the average, for many of the oysters were small. It is more convenient and just as accurate for comparative purposes to ignore this quantity, while it is much more convenient in dividing the total number of B. coli found in the oyster by the quantity of shell liquor. It avoids fractions much more often than would be the case if we added one to the number of cubic centimeters of shell liquor. Occasionally, however, in the combined score, the quotient is not an even number and so the score is made the whole number next above or below depending whether the fraction was less than or more than one-half. Thus if the score came 20.4 it would be called 20; if the fraciton were .5 or more it would be called 21.

It would seem from these experiments that there is no question that the shell liquor contains many more bacteria than are left on the body of the oyster and that in analysis we could ignore entirely the bacteria left on the body of the oyster.

These results did not equal the writer's expectation and it was thought that perhaps the treatment of the oyster's body was not sufficient to remove all the mucus and bacteria present. Accordingly the following method of analysis was adopted for the subsequent experiments: The oyster liquor was collected and diluted in the same manner as before. It was shaken vigorously one hundred times before inoculating into agar and bile. The body of the oyster after draining was transferred to a sterile large mouthed, glass stoppered bottle and covered with twenty cubic centimeters of one per cent. NaCl solution. The oyster and salt solution were shaken fairly vigorously one hundred times and the solution of salt and mucus was removed by the pipette or poured into a smaller glass bottle and again shaken vigorously one hundred times. This mixture was then inoculated into the bile tubes and the agar plates. At first one per cent. sodium carbonate

solution was used with the hope that it would cut the mucus more readily, but later the salt solution was found just as effective. The shaking appeared to be the important feature.

It was found that a great deal of shaking was necessary to break up the clumps of bacteria and separate them from the mucus. If not thoroughly shaken the resulting plates would be found to contain large areas of confluent colonies which rendered counting impossible. Every bit of mucus would be found to be a nucleus around which would be a large confluent ring of colonies. After a thorough shaking, however, the flakes of mucus would in nearly all cases remain sterile and the bacteria would be found in well separated colonies evenly distributed in the medium.

Table Showing the Total Number of Bacteria in the Shell Liquor of Each Sample and the Total Number Washed from the Bodies of the Oysters by Shaking.

	No. of Experiment.		20°C. Count.	37°C. Count.	"Red" Count.	B. coli Count.	Score.
October 13	1	Shell Liquor	650,000	.....	.....	4,800	560
		"Washings"	500,000	.....	.....	14,000	*1,952
October 23	2	Shell Liquor	190,000	.....	.....	178	26
		"Washings"	45,000	.....	.....	500	101
October 27	3	Shell Liquor	200,000	.....	.....	113	8
		"Washings"	100,000	.....	.....	2,080	111
October 31	4	Shell Liquor	50,000	.....	.....	2,500	313
		"Washings"	24,000	.....	.....	3,200	720
November 3	5	Shell Liquor	78,000	.....	.....	2,000	248
		"Washings"	15,000	.....	.....	2,600	599
November 8	6	Shell Liquor	1,050,000	.....	.....	17,000	1,410
		"Washings"	140,000	.....	.....	8,800	2,327
December 6	7	Shell Liquor	100,000	39,000	10,000	4,000	284
		"Washings"	15,500	6,900	1,500	4,400	656
December 19	8	Shell Liquor	130,000	30,000	3,000	850	74
		"Washings"	35,000	3,000	800	275	84

*The score opposite the "washings" is the score based upon the number of B. coli in the shell liquor and in the "washings" from the body of the oysters as described in the text.

Table No. 1.—Showing Results of First Experiment.

Temperature of water 16° C.

Date.	No. of Oyster.	C.C. of Oyster Liquor.	20°C. Count.		37°C. Count.		Red Count.		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor and Washings.
October 13, 1913	1	13	130,000	47,000	.....	.....	.....	.....	260	1,000	20	97
" "	2	8	57,000	34,000	.....	.....	.....	.....	160	2,000	20	270
" "	3	9	22,000	53,000	.....	.....	.....	.....	180	200	20	42
" "	4	9	65,000	60,000	.....	.....	.....	.....	180	2,000	20	242
" "	5	8	22,000	29,000	.....	.....	.....	.....	160	200	20	45
" "	6	13	79,000	34,000	.....	.....	.....	.....	260	2,000	20	174
" "	7	12	77,000	40,000	.....	.....	.....	.....	240	2,000	20	187
" "	8	8	74,000	40,000	.....	.....	.....	.....	1,600	200	200	225
" "	9	9	88,000	136,000	.....	.....	.....	.....	1,600	2,000	200	450
" "	10	10	60,000	56,000	.....	.....	.....	.....	200	2,000	20	220
Totals	.....	.....	674,000	529,000	.....	.....	.....	.....	4,840	13,600	560	1,952

TABLE No. II.—*Showing Results of Second Experiment.*

Temperature of water 13.5°C.

DATE.	No. of Oyster.	C.C. of Oyster Liquor.	20°C. Count.		37°C. Count.*		Red Count.†		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor and Washings.
October 23, 1913	1	10	13,000	2,200	.....	.....	.....	.....	0	0	0	0
" "	2	7	13,000	4,200	.....	.....	.....	.....	14	200	2	31
" "	3	13	17,000	4,000	.....	.....	.....	.....	26	0	2	2
" "	4	6	35,000	2,500	.....	.....	.....	.....	120	20	20	24
" "	5	9	27,000	10,000	.....	.....	.....	.....	0	20	0	2
" "	6	9	21,000	5,200	.....	.....	.....	.....	18	20	2	4
" "	7	11	23,000	3,600	.....	.....	.....	.....	0	20	0	2
" "	8	8	10,000	5,000	.....	.....	.....	.....	0	0	0	0
" "	9	7	20,000	5,600	.....	.....	.....	.....	0	20	0	3
" "	10	6	7,000	3,800	.....	.....	.....	.....	0	200	0	33
Totals	...	...	186,000	46,100	.....	.....	.....	.....	178	500	26	101

*Not made. †Not made.

TABLE No. III.—*Showing Results of Third Experiment.*

Temperature of Water, 16°C.

DATE.	No. of Oyster.	C.C. of Oyster Liquor.	20°C. Count.		37°C. Count.*		Red Count.†		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor and Washings.
October 27, 1913	1	21	31,500	29,000	.....	.....	.....	.....	21	2,000	1	96
" "	2	20	8,000	9,000	.....	.....	.....	.....	20	20	1	2
" "	3	16	10,000	7,000	.....	.....	.....	.....	32	0	2	2
" "	4	12	19,200	10,000	.....	.....	.....	.....	24	20	2	4
" "	5	10	9,000	9,000	.....	.....	.....	.....	0	0	0	0
" "	6	12	64,000	11,000	.....	.....	.....	.....	0	20	0	2
" "	7	8	24,000	8,000	.....	.....	.....	.....	16	20	2	5
" "	8	14	15,000	9,000	.....	.....	.....	.....	0	0	0	0
" "	9	12	4,000	9,000	.....	.....	.....	.....	0	0	0	0
" "	10	13	12,000	2,400	.....	.....	.....	.....	0	0	0	0
Total	.....	.....	196,700	103,400	.....	.....	.....	.....	113	2,080	8	111

*Not made. †Not made.

TABLE No. IV.—*Showing Results of Fourth Experiment.*

Temperature of Water, 13°C.

DATE.	No. of Oyster.	C.C. of Oyster Liquor.	20°C. Count.		37°C. Count.		Red Count.		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor and Washings.
October 31, 1913	1	7	2,000	2,200	.....	.....	.....	.....	14	200	2	31
" "	2	8	2,400	1,400	.....	.....	.....	.....	160	20	20	23
" "	3	8	7,000	5,200	.....	.....	.....	.....	0	200	0	25
" "	4	20	12,000	1,800	.....	.....	.....	.....	200	200	10	20
" "	5	10	5,000	2,100	.....	.....	.....	.....	200	200	20	40
" "	6	19	5,500	1,200	.....	.....	.....	.....	19	0	1	1
" "	7	7	2,100	1,200	.....	.....	.....	.....	140	2,000	20	289
" "	8	8	5,500	4,400	.....	.....	.....	.....	160	0	20	20
" "	9	9	5,000	2,800	.....	.....	.....	.....	180	200	20	42
" "	10	7	1,700	1,200	.....	.....	.....	.....	1,400	200	200	229
Total			48,200	23,500	.....	.....	.....	.....	2,473	3,220	313	720

TABLE No. V.—*Showing Results of Fifth Experiment.*

Temperature of Water, 12°C.

DATE.	No. of Oyster.	C.C. of Oyster Liquor.	20°C. Count.		*37°C. Count.		†Red Count.		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor and Washings.
November 3, 1913	1	12	14,000	800	.....	.....	.....	.....	24	0	2	2
" "	2	8	2,500	1,200	.....	.....	.....	.....	1,600	0	200	200
" "	3	18	2,800	1,200	.....	.....	.....	.....	28	200	2	13
" "	4	7	11,500	5,600	.....	.....	.....	.....	140	2,000	20	306
" "	5	12	5,000	600	.....	.....	.....	.....	0	0	0	0
" "	6	11	900	1,600	.....	.....	.....	.....	220	0	20	22
" "	7	9	11,000	1,200	.....	.....	.....	.....	18	20	2	4
" "	8	11	25,000	800	.....	.....	.....	.....	0	0	0	0
" "	9	8	3,500	800	.....	.....	.....	.....	16	200	2	27
" "	10	8	2,000	800	.....	.....	.....	.....	0	200	0	25
Totals	.....	.....	78,200	14,600	.....	.....	.....	.....	.....	.....	.....	.....

*No count made. †No count made.

TABLE NO. VI.—*Showing Results of Sixth Experiment.*

Temperature of Water, 12°C.

DATE.	No. of Oyster.	C.C. of Oyster Liquor.	20°C. Count.		*37°C. Count.		†Red Count.		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor and Washings.
November 8, 1913	1	7	38,000	21,000	.....	.....	.....	.....	140	2,000	20	306
" "	2	12	216,000	8,000	.....	.....	.....	.....	2,400	0	220	200
" "	3	12	150,000	20,000	.....	.....	.....	.....	2,400	2,000	220	367
" "	4	12	60,000	1,600	.....	.....	.....	.....	2,400	200	220	217
" "	5	24	122,000	15,000	.....	.....	.....	.....	2,400	200	100	108
" "	6	8	320,000	46,000	.....	.....	.....	.....	1,600	2,000	250	450
" "	7	15	29,000	1,000	.....	.....	.....	.....	3,000	20	200	201
" "	8	11	10,000	14,000	.....	.....	.....	.....	220	220	20	38
" "	9	10	80,000	12,000	.....	.....	.....	.....	2,000	2,000	200	220
" "	10	10	15,000	2,800	.....	.....	.....	.....	200	200	20	220
Total	...	...	1,040,000	.....	.....	.....	.....	.....	16,760	8,820	1,410	2,327

*No count made. †No count made.

TABLE No. VII.—*Showing Results of Seventh Experiment.*

Temperature of Water, 8°C.

DATE.	No. of Oyster.	C.C. of Oyster Liquor.	20°C. Count.		37°C. Count.		Red Count.		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor. and Washings.
December 6, 1913	1	24	67,000	7,000	16,000	2,800	4,320	800	480	2,000	20	103
" "	2	10	3,200	300	3,000	0	400	0	20	0	2	2
" "	3	14	10,000	1,400	5,600	800	1,000	0	2,800	200	200	214
" "	4	8	1,400	4,200	1,600	2,000	500	200	160	2,000	20	270
" "	5	9	14,000	1,200	5,500	500	550	100	180	200	20	42
" "	6	17	3,700	800	5,000	600	2,500	0	340	20	20	21
" "	7	10	4,600	700	2,400	200	800	400	20	20	2	4
" "												
" "												
" "												
Total			103,900	15,600	39,100	6,900	10,070	1,500	4,000	4,140	284	656

TABLE No. VIII.—*Showing Results of Sample Number Eight.*

Temperature of Water, 6°C.

DATE.	No. of Oyster.	C.C. of Oyster Liquor.	20°C. Count.		37°C. Count.		Red Count.		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor and Washings.
December 19, 1913.....	1	18	25,000	1,600	6,300	220	550	80	36	20	2	3
" "	2	14	19,000	450	5,600	200	850	60	280	20	20	21
" "	3	7	17,000	1,110	7,000	240	1,800	40	14	0	2	2
" "	4	18	4,000	380	700	100	100	80	36	0	2	2
" "	5	9	4,500	1,000	1,500	160	150	0	180	200	20	24
" "	6	10	24,000	1,500	1,700	360	120	20	20	0	2	2
" "	7	8	4,500	500	200	60	120	20	16	0	2	2
" "	8	12	15,000	7,000	1,600	240	100	80	240	20	20	22
" "	9	12	12,000	7,000	550	200	50	60	24	0	2	2
" "	10	11	7,500	14,000	600	1,200	65	380	22	20	2	4
Total.....			132,500	34,530	25,750	2,980	3,905	820	868	280	74	84

In comparing the total number of bacteria in the shell liquor of all the oysters in each of the experiments with the total number washed from the bodies of these oysters it is seen that the total number of bacteria in the shell liquor of all the oysters was greater than the number washed from the bodies of the oysters. In the first experiment the numbers are nearly equal, but in the subsequent experiments there is a great difference. If we consider the individual oysters in all the experiments, we find that in only ten of the oysters out of seventy-seven was there a greater number of bacteria washed from the body than was found in the shell liquor of the corresponding oyster. In one instance the numbers were equal. In the remaining sixty-six oysters there were more bacteria in the shell liquor that were washed from the bodies of the oysters. The 37° C. count and the "red count" were made on only seventeen oysters and in only two instances did the number of bacteria washed from the bodies of the oysters exceed the number found in the shell liquor, while the total number from all the oysters of the two experiments showed that there were on the average a great many more in the shell liquor than were washed from the bodies of the oysters.

When we consider the number of B. coli found in the shell liquor and the number washed from the body of the same oyster we find the relative numbers quite different. It will be seen in six out of the eight experiments the total number of B. coli washed from the bodies of all the oysters of the experiment exceeded the total number in the shell liquor. In the first two experiments the difference is especially marked. If we consider the individual oysters we find that in thirty-three instances there were more B. coli on the body of the oyster than were in the shell liquor; in thirty oysters the number in the shell liquor exceeded the number washed from the body; in fourteen instances the numbers were equal. But if we consider the total number of B. coli found in the "washings" with the total number found in the shell liquor of all the oysters examined in this series of experiments we find there were on the average more B. coli in the "washings" than there were in the shell liquor.

We have no reason at present to suppose that B. coli should be distributed other than equally among the other bacteria in the oyster, yet there seems to be a concentration of B. coli in the mucus on the outside of the body of the oyster. The amount of shell liquor in the oysters averaged about ten cubic centimeters. If we consider that there was left upon the body of the oyster one cubic centimeter of

mucus, we find that there were volume for volume more than ten times as many B. coli on the body of the oyster as there were in the shell liquor. The question arises at once as to whether this unequal distribution of B. coli among the other bacteria in these two parts of the oyster is real or only apparent. It may be due to the difference in methods of analysis. With our present knowledge of the bacteriology of the oyster the writer is led to believe that this relation does not actually exist, but is due to the difference in methods used to determine the total number of bacteria and the number of B. coli.

Another point which appears interesting to the writer is that there is apparently a direct relation between the temperature of the water from which the oysters are taken and the relative number of B. coli found in the shell liquor and on the body of the oyster. It will be noticed that in the first three experiments there were a great many more B. coli on the body of the oyster than in the shell liquor, but this proportion is gradually reduced and in the sixth and eighth experiments there were more B. coli in the shell liquor than were found on the bodies of the oysters. The ratio of the total number of bacteria in the shell liquor to the total number washed from the bodies of the oysters in each sample is shown in the following table:

Table Arranged According to Temperature Showing the Approximate Ratio of the Total Number of Bacteria in the Shell Liquor to the Number in the Washings from the Bodies of the Oysters in each Sample.

Temperature.	Date.	20°C. Count.	37°C. Count.	"Red" Count.	B. coli Count.
16° C.	Oct. 13	1.3:1	.....	.....	1:3
16° C.	" 27	2:1	.....	.....	1:18
13.5° C.	" 23	4.2:1	.....	.....	1:2.8
13° C.	" 31	2:1	.....	.....	1:1.3
12° C.	Nov. 3	5.2:1	.....	.....	1.3:1
12° C.	" 8	7.5:1	.....	.....	2:1
8° C.	Dec. 6	6.5:1	6:1	7:1	1:1.1
6° C.	" 19	3.7:1	10:1	4.7:1	3:1

A long series of experiments necessitating the examination of a great number of oysters and extending over a whole year would be required to establish this relationship. However, this supposition is not so different from what we might expect when we consider the

biology of the oyster. The optimum temperature for the growth of the oyster is, probably between 20° C. and 25° C. At this temperature the cells of the oyster are most active. The mucus sells will secrete a larger amount of mucus than at decidedly lower temperatures. The more mucus secreted the more will remain clinging to the body of the oyster. Generally speaking the greater the amount of mucus the greater the number of bacteria we would expect to find in the mucus on the outside of the body. As the temperature of the water lowers, the metabolic processes of the oysters are correspondingly slowed and a smaller amount of mucus and for this reason fewer bacteria will be found on the body of the oyster. For this reason it seems fair to assume that the apparent relation between the temperature of the water and the proportion of B. coli on the outside of the oyster and the shell liquor is real and not accidental.

These two sets of experiments throw light on the findings of Houston cited above. It is easily seen that simply pouring water over the body of the oyster is not sufficient to remove all the bacteria. The experiments of the writer on the comparison of the bacterial content of the stomach and shell liquor shows that per unit volume the shell liquor contains on the average over twenty times as many bacteria as the stomach juices. Evidence from all sides shows that Houston's assumption that all the bacteria were washed from the body of the oyster by simply pouring water over the oyster and further that the bacteria found in the minced meat of the oysters so treated came entirely from the stomach are not in accordance with the facts.

These experiments show the necessity of examining not only the shell liquor, but also the mucus on the outside of the body of the oyster. This is especially true during the warmer months. At this time there are on the average many more B. coli on the body of the oyster than is contained in the shell liquor. It is perfectly legitimate to consider the mucus on the body of the oyster as part of the oyster juice. If we so consider the mucus, it makes a very decided difference in the score of the oyster. In one instance the combined score of one oyster was ninety-six times the score based upon the shell liquor alone. The combined score is never less and often many times more than the score based upon the shell liquor. If there were any constant relation between the B. coli content of the shell liquor and the mucus removed from the body of the oyster, the examination of the shell liquor alone would be sufficient. But as no such relation exists the necessity of examining both the shell liquor and the mucus is at once apparent.

COMPARISON OF THE BACTERIAL CONTENT OF THE STOMACH AND OF THE SHELL LIQUOR OF OYSTERS.

Houston in his report to the local Government Board, 1904, makes the following statement: "The experiments detailed elsewhere seem to indicate that per unit of volume the gastric juice of the oyster is more impure bacteriologically than the oyster liquor." The experiments upon which this statement is based are taken up in some detail under "Bacteriology of the Shell Liquor and 'Washings' from the Body of the Oyster," and so it is not necessary to take up these experiments in this connection. The writer has shown that these experiments and the conclusions drawn are not based upon sound assumptions and so these results are not to be relied upon.

Clark,[1] in a long series of experiments has shown that in both clams and oysters the shell liquor is much more likely to yield B. coli or sewage streptococci than either the stomach, intestine or rectum.

Both of these workers studied the B. coli content of the different parts of the oyster. The writer could not obtain any badly polluted oysters at the time of year during which the experiments were conducted and so he examined the shell liquor and stomach contents for the total number of bacteria which each part contained. It would have been possible to infect oysters artificially with the colon bacillus, but it is not certain that one could simulate natural conditions exactly and consequently wrong conclusions might be drawn. We have no reason to suppose that B. coli are distributed other than equally among the other bacteria in the oyster and so a comparison of the total quantity per unit volume ought to show the relative frequency with which one would expect to find any particular bacterium in either part of the oyster.

In this series of experiments forty-one oysters were used. The method of examination was as follows:—The juice of each oyster was collected in a small glass-stoppered bottle which was calibrated in two cubic centimeter divisions. The amount of shell liquor was read off in cubic centimeters and diluted with an equal amount of one per cent. sodium chloride solution. The shell liquor and the sodium chloride solution were shaken vigorously one hundred times and one cubic centimeter of this mixture was transferred to a tube containing nine cubic centimeters of one per cent. sodium chloride solution and

[1] Report State Board of Health of Mass. 1905, 428.

one cubic centimeter of this dilution was plated in agar. Also a cubic centimeter from this tube was transferred to another tube in nine cubic centimeters of salt solution. A cubic centimeter of this mixture was also plated. By this method of dilution the plates contained respectfully one twentieth and one two hundredth of a cubic centimeter of the original shell liquor. The plates were made in duplicate. After the oyster had been drained of its liquor the flat valve was removed and the other valve containing the body of the oyster was set on the edge and allowed to drain for several minutes. The excess of liquor was then removed with a piece of blotting paper and the region over the stomach was seared with a hot spatula and an incision made into the stomach with a sterile scalpel. With a graduated pipette one-twentieth of a cubic centimeter of the stomach contents was removed and plated another twentieth of a cubic centimeter was transferred to a tube containing nine cubic centimeters of salt solution and 1 cubic centimeter of this mixture plated. These plates contained respectfully one-twentieth and one-two hundredth of a cubic centimeter. The plates were also made in duplicate and in all cases the average of the two plates was taken as the count for each oyster. The counts given in the table below are for one-twentieth of a cubic centimeter of the oyster juice and the stomach contents.

Table Comparing the Number of Bacteria in One-Twentieth of a Cubic Centimeter of the Shell Liquor with the Number of an Equal Quantity of the Stomach Contents.

No. of Oyster.	No. of bacteria in shell liquor of oyster.	No. of bacteria in stomach contents of oyster.
1	20	1
2	220	5
3	8	1
4	130	6
5	16	1
6	1	11
7	50	3
8	90	2
9	20	8
10	110	13
11	3	9
12	85	6
13	12	0
14	17	4
15	430	3
16	55	4
17	1,000	1
18	95	1
19	500	2
20	260	1
21	340	1
22	120	1
23	340	1
24	155	0
25	1,000	2
26	725	1
27	38	6
28	2	2
29	1,000	20
30	1,000	3
31	90	12
32	19	14
33	50,000	470
34	10,000	280
35	100,000	150
36	15,000	10,000
37	10,000	10
38	24,000	30
39	10,000	10
40	50,000	2,500
41	50,000	25
Totals	304,351	13,620

From the table it is seen that in only two oysters, numbers six and eleven, out of the forty-one examined, was the number of bacteria per unit volume greater in the stomach contents than in the shell liquor. In oyster, twenty-eight the numbers were equal. In the remaining thirty-eight oysters there were more bacteria per unit volume in the shell liquor than in the stomach contents. In these thirty-eight oysters the ratio per unit quantity of the number of bacteria in the shell liquor to the number in the contents of the stomach varied from 3 to 2 in oyster Nos. 37 to 2000 to 1 in oyster No. 41. The ratio of the total number of bacteria per unit volume in the shell liquor of the forty-one oysters to the total number of bacteria in an equal quantity of the stomach contents was as 21.6 to 1. That is, a comparison of the average number of bacteria found in shell liquor with the number of bacteria in the stomach contents shows that per unit quantity there were more than twenty times as many bacteria in the shell liquor as in the stomach juice.

LENGTH OF TIME NECESSARY FOR BACTERIA TO PASS THROUGH THE INTESTINAL TRACT OF THE OYSTER.

So far as the writer is aware no one has ever made any determination of the rate at which food passess through the alimentary tract of the oyster. While it is difficult to determine this matter directly, it seemed possible to inoculate the shell liquor of oysters with some bacterium not found in oysters and trace its progress through the intestinal canal. B. prodigiosus was chosen because of its ease of identification and because the writer has never found it in oysters, and so far as he is aware it has never been reported as occurring in oysters.

In the first experiment twelve oysters were inoculated by sawing off a piece of the lip of the shell and inserting a loopful of a culture of B. prodigiosus into the branchial chamber. The oysters were layed very carefully upon cotton thoroughly saturated with water and covered with a glass dish to prevent evaporation. They were kept at the laboratory temperature which is about 20°C. At various intervals, as shown in the tables, three oysters were removed and examined. The examination was made as follows:—The right valve of the oyster was removed and the gills and mantle carefully dissected away. The remaining part of the body was then washed for several minutes in running tap water. The left valve containing the oyster was then

set on edge and allowed to drain thoroughly. The surplus water was removed with filter paper. The oyster was then seared with a hot spatula over the stomach, over the intestine, where it bends sharply upon itself on the ventral side and on the rectum just above the anus. An incision was then made into these three parts of the alimentary tract with sterile scalpels and a sterile capillary pipette inserted and a portion of the contents removed and plated upon agar which was grown at room temperature for two days and examined for red colonies. Control samples of the shell liquor were plated before the inoculation with B. prodigiosus and these were negative in all cases. In the first experiment the time of examination after inoculation ranged from thirteen hours to twenty-seven hours. In the second experiment the time varies from five hours to seventy-four hours.

Table Showing Length of Time at which B. Prodigiosus was Isolated from Different Parts of the Alimentary Tract after the Inoculation of the Gill Chamber.

No. of Oyster.	Hours after inoculation.	Stomach.	Intestine.	Rectum.
Experiment I.				
1	13	+	0	0
2	13	+	0	0
3	13	0	+	0
4	18	0	0	0
5	18	+	0	0
6	18	0	+	+
7	23	0	0	0
8	23	0	0	0
9	23	0	0	+
10	27	0	0	0
11	27	0	0	0
12	27	+	0	+
Experiment II.				
1	5	0	0	0
2	5	0	0	0
3	5	+	0	0
4	22	0	0	0
5	22	0	0	0
6	22	+	0	0
7	48	0	0	0
8	48	0	0	0
9	48	0	0	+
10	74	0	0	+
11	74	0	+	+
12	74	0	0	0

From these tables it can be seen that the first appearance of the bacteria in the intestine was thirteen hours after inoculation and in the rectum five hours later. We would expect to find the organisms in the stomach within a very short time after inoculation of the shell liquor. Since these experiments are few in number one must necessarily be conservative in the conclusion drawn.

THE BACTERIAL CONTENT OF OYSTERS DURING STORAGE.

The change in the bacterial content of oysters during storage at a temperature at which they are kept in oyster houses and during transportation is a matter of very great importance from the point of view of the public health. The oyster is a living organism capable of maintaining itself for a long period when removed from its natural element. It is possible that the digestive juices or the phagocytic cells of the oyster might materially decrease the number of bacteria in the oyster. On the other hand, even if the digestive secretions and the phagocytic cells were bactericidal, it is possible that the rapid multiplication of the bacteria in the shell liquor might be sufficient to maintain or increase the number of bacteria in the oyster as a whole. In order to observe the change in the bacterial content of oysters during storage the writer carried out the following experiment:

About a bushel of polluted oysters were taken from the Providence River December 5, 1913, and put into storage in the Laboratory at an average temperature of 10°C. The temperature was fairly constant and did not rise above 11°C., although for a short time during a period of exceptionally cold weather the temperature fell to 8°C., but it soon rose again to 10°C. The oysters were put into storage in the bag just as they were brought to the laboratory. No attempt was made to clean them in any way. As soon as they arrived a sample of ten oysters was taken from the bag and put on ice and examined the following day. At intervals other samples of ten oysters were removed and examined. The method of examination was the same as that described under "The Bacteriology of the Shell Liquors and the Washings from the Bodies of the Oysters." In all except two instances a 20°C. count, a 37°C. count, a "red" count, and a B. coli count were made. The detailed analysis of each oyster and the bacterial content of each sample as a whole is shown in the following table:

Table Showing Change in Bacterial Content of Oysters During Storage at 10°C.

Date.	Length of Storage.		20°C. Count.	37°C. Count.	"Red" Count.	Colon Count.	Total Colon Count.
December 6	0 Days	Shell Liquor	104,000	39,000	10,000	4,000	
		"Washings"	15,600	6,900	1,500	4,440	8,400
December 9	4 "	Shell Liquor	950,000	78,000	10,000	1,400	
		"Washings"	110,000	13,000	6,500	3,200	4,600
December 12	7 "	Shell Liquor	1,150,000	84,000	14,000	5,400	
		"Washings"	1,300,000	140,000	46,000	3,100	8,500
December 16	11 "	Shell Liquor	6,200,000	180,000	73,000	2,500	
		"Washings"	460,000	45,000	3,700	900	3,400
December 22	17 "	Shell Liquor	5,700,000	500,000	2,800	3,600	
		"Washings"	1,800,000	42,000	1,200	900	4,500
December 30	25 "	Shell Liquor	3,600,000	850,000	29,000	3,100	
		"Washings"	700,000	160,000	36,500	325	3,425
January 5	31 "	Shell Liquor	1,000,000	Not made	Not made	3,000	
		"Washings"	210,000			650	3,650
January 27	53 "	Shell Liquor	2,200,000	18,500	1,900	1,300	
		"Washings"	4,200,000	97,000	60,000	450	1,750
February 23	80 "	Shell Liquor	Not made	Not made	Not made	4,300	
		"Washings"				550	4,850

The Following Tables Show the Change in Bacterial Content of Oysters During Storage at 10°C.

TABLE No. I.—*Showing Bacterial Content at Beginning of Storage.*

DATE.	No. of Oyster.	C. C. of Oyster Liquor.	20°C. Count.		37°C. Count.		Red Count.		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor and Washings.
December 6, 1913	1	24	67,000	7,000	16,000	2,800	4,300	800	480	2,000	20	103
" "	2	10	3,200	300	3,000	0	400	0	20	0	2	2
" "	3	14	10,000	1,400	5,600	800	1,000	0	2,800	200	200	214
" "	4	8	1,500	4,200	1,600	2,000	500	200	160	2,000	20	270
" "	5	9	14,000	1,200	5,400	500	550	100	180	200	20	42
" "	6	17	3,700	800	5,100	600	2,600	0	340	20	20	21
" "	7	10	4,600	700	2,400	200	800	400	20	20	2	4
Total			104,000	15,600	39,100	6,900	10,150	1,500	4,000	4,440	284	656

TABLE No. II.—*Showing Bacterial Content After Four Days Storage.*

DATE.	No. of Oyster.	C.C. of Oyster Liquor.	20°C. Count.		37°C. Count.		Red Count.		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor and Washings.
December 9, 1913.....	1	7	112,000	8,500	1,550	1,450	770	1,000	140	200	20	49
" "	2	6	45,000	5,500	400	500	200	160	120	20	20	24
" "	3	10	100,000	2,000	2,000	1,200	500	600	200	0	20	20
" "	4	8	112,000	5,000	9,000	750	190	60	160	200	20	45
" "	5	9	125,000	6,000	3,500	750	160	20	180	200	20	42
" "	6	12	190,000	24,000	16,000	1,300	1,550	280	240	200	20	37
" "	7	7	105,000	7,000	13,000	800	125	200	14	200	2	31
" "	8	5	30,000	30,000	28,000	4,000	1,200	3,400	10	20	2	6
" "	9	8	80,000	2,400	2,700	1,300	3,800	450	160	200	20	45
" "	10	9	50,000	17,000	2,200	850	1,400	240	180	2,000	20	242
Total.....	...	...	949,000	107,000	78,350	12,900	9,895	6,510	1,404	3,240	164	541

TABLE No. III.—*Showing Bacterial Content After Seven Days Storage.*

DATE.	No. of Oyster.	C.C. of Oyster Liquor.	20°C. Count.		37°C. Count.		Red Count.		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor and Washings.
December 12, 1913	1	13	1,040,000	300,000	14,000	80,000	1,000	40,000	26	200	2	17
" "	2	6	210,000	240,000	3,800	1,200	50	20	120	20	20	23
" "	3	10	800,000	200,000	11,000	9,000	5,500	40	200	200	20	40
" "	4	11	880,000	160,000	15,000	11,000	700	500	2,200	2,000	200	382
" "	5	12	480,000	90,000	4,000	17,000	1,211	60	240	20	20	22
" "	6	14	84,000	8,500	1,700	600	700	40	280	200	20	34
" "	7	6	120,000	90,000	11,000	7,000	4,200	5,000	12	200	2	35
" "	8	7	210,000	16,000	5,000	2,600	70	40	140	20	20	23
" "	9	5	150,000	22,000	9,000	5,500	250	120	1,000	20	200	204
" "	10	6	180,000	200,000	9,500	4,500	480	320	1,200	200	200	233
Total			1,154,000	1,326,000	84,000	138,000	14,150	46,140	5,418	3,080	704	1,013

TABLE No. IV.—*Showing the Bacterial Content After Eleven Days Storage.*

DATE.	No. of Oyster.	C.C. of Oyster Liquor.	20°C. Count.		37°C. Count.		Red Count.		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor and Washings.
December 16, 1913....	1	11	180,000	20,000	8,500	3,000	250	200	280	200	20	34
" "	2	6	570,000	70,000	5,500	600	70	20	1,200	200	200	233
" "	3	9	110,000	28,000	8,000	12,500	2,700	1,300	18	200	2	24
" "	4	10	160,000	21,000	1,700	3,400	400	240	200	200	20	40
" "	5	10	2,800,000	160,000	9,000	800	300	20	200	0	20	20
" "	6	10	130,000	4,500	29,000	4,500	140	1,500	200	0	20	20
" "	7	10	180,000	45,000	300	160	40	20	20	20	2	4
" "	8	10	480,000	12,000	96,000	18,000	68,000	300	20	20	2	4
" "	9	10	80,000	23,000	5,000	1,200	800	60	200	20	20	22
" "	10	10	1,500,000	80,000	15,000	650	140	60	200	20	20	20
Total....	.....	...	6,190,000	463,500	178,000	44,810	72,840	3,720	2,538	880	326	423

TABLE No. V.—*Showing the Bacterial Content After Seventeen Days Storage.*

DATE.	No. of Oyster.	C.C. of Oyster Liquor.	20°C. Count.		37°C. Count.		Red Count.		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor and Washings.
December 22, 1913.....	1	8	74,000	13,000	4,000	1,200	65	0	16	0	2	2
" "	2	7	850,000	44,000	7,000	9,500	300	340	140	200	20	49
" "	3	14	25,000	22,000	1,500	2,000	110	100	280	20	20	22
" "	4	8	50,900	5,000	1,300	1,000	500	0	16	20	2	5
" "	5	6	21,000	230,000	1,400	2,600	12	100	0	200	0	33
" "	6	9	100,000	8,500	2,300	1,600	500	20	180	200	20	42
" "	7	1	2,055,000	300,000	36,000	1,400	24	0	2,400	20	200	202
" "	8	12	2,900,000	230,000	190,000	6,000	1,100	520	240	20	20	22
" "	9	6	395,000	400,000	9,000	11,000	180	20	12	20	2	5
" "	10	15	250,000	500,000	250,000	5,000	0	60	300	200	20	33
Total.....	...	...	5,720,000	1,752,500	502,500	41,300	2,791	1,160	3,584	900	306	415

TABLE No. VI.—*Showing Bacterial Content After Twenty-Five Days Storage.*

DATE.	No. of Oyster.	C.C. of Oyster Liquor.	20°C. Count.		37°C. Count.		Red Count.		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor and Washings.
December 30, 1913.....	1	17	1,000,000	14,000	140,000	5,000	7,500	140	34	20	2	3
" "	2	5	125,000	8,000	65,000	32,000	7,500	34,000	10	0	2	2
" "	3	13	100,000	3,600	155,000	2,200	2,100	900	260	200	20	35
" "	4	10	110,000	45,000	70,000	9,000	4,000	300	2,000	20	200	202
" "	5	11	120,000	230,000	175,000	2,800	900	240	220	20	20	22
" "	6	8	240,000	17,000	8,000	20,000	1,400	0	16	20	2	5
" "	7	12	575,000	70,000	85,000	40,000	1,700	0	240	20	20	22
" "	8	10	800,000	35,000	35,000	8,000	0	0	20	0	2	2
" "	9	13	26,000	8,500	14,000	2,4000	26	100	260	0	20	20
" "	10	13	450,000	270,000	90,000	7,500	3,600	800	26	20	2	4
Total.....	...	...	3,606,000	701,000	837,000	155,500	28,726	36,480	3,086	320	290	317

TABLE No. VII.—*Showing Bacterial Content After Thirty-One Days Storage.*

DATE.	No. of Oyster.	C.C. of Oyster Liquor.	20°C. Count.		37°C. Count.		Red Count.		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor. and Washings.
January 5, 1914	1	8	280,000	42,000	.....	.....	.....	.....	16	0	2	2
" "	2	9	34,000	20,000	.....	.....	.....	.....	360	20	40	42
" "	3	10	52,000	13,000	.....	.....	.....	.....	20	0	2	2
" "	4	12	145,000	22,000	.....	.....	.....	.....	2,400	0	200	200
" "	5	7	36,000	14,000	.....	.....	.....	.....	0	0	0	0
" "	6	8	140,000	48,000	.....	.....	.....	.....	160	20	20	23
" "	7	10	70,000	15,000	.....	.....	.....	.....	20	200	2	22
" "	8	16	160,000	20,000	.....	.....	.....	.....	0	200	0	13
" "	9	6	48,000	6,500	.....	.....	.....	.....	12	0	2	2
" "	10	11	50,000	13,000	.....	.....	.....	.....	22	200	2	20
Total	.....	.....	1,015,000	213,500	.....	.....	.....	.....	3,010	640	370	326

TABLE No. VIII.—*Showing Bacterial Content After Fifty-Three Days Storage.*

DATE.	No. of Oyster.	C.C. of Oyster Liquor.	20°C. Count.		37°C. Count.		Red Count.		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor and Washings.
January 27, 1914	1	14	450,000	37,000	4,800	1,500	550	300	28	0	2	2
" "	2	3	25,000	26,000	1,900	1,600	240	500	60	200	20	87
" "	3	11	400,000	36,000	2,400	42,000	110	30,000	220	20	20	22
" "	4	6	12,000	1,200	850	3,000	0	100	12	0	2	2
" "	5	10	160,000	11,000	4,000	0	100	0	200	0	20	20
" "	6	6	38,000	22,000	240	0	0	0	120	0	20	20
" "	7	11	310,000	15,000	1,300	1,200	660	200	220	20	20	22
" "	8	10	25,000	2,000	1,400	2,800	100	2,200	20	20	2	4
" "	9	9	320,000	4,000,000	90	40,000	0	24,000	180	0	20	20
" "	10	11	450,000	38,000	1,500	4,800	110	2,800	220	220	20	38
Total	...	...	2,190,000	4,188,200	18,480	96,900	1,870	60,100	1,280	460	146	237

TABLE No. IX.—*Showing Bacterial Content After Eighty Days.*

DATE.	No. of Oyster.	C.C. of Oyster Liquor.	*20°C. Count.		*37°C. Count.		*Red Count.		B. coli Count.		Score.	
			Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number of Bacteria in Shell Liquor.	Number of Bacteria Washed from Oyster.	Number in Shell Liquor.	Number Washed from Oyster.	Based on Shell Liquor.	Based on Shell Liquor and Washings.
February 23, 1914	1	6	.....	.....	.....	.....	.....	.....	120	20	20	23
" "	2	6	.....	.....	.....	.....	.....	.....	120	20	20	23
" "	3	8	.....	.....	.....	.....	.....	.....	16	200	2	27
" "	4	7	.....	.....	.....	.....	.....	.....	140	20	20	23
" "	5	11	.....	.....	.....	.....	.....	.....	22	20	2	4
" "	6	10	.....	.....	.....	.....	.....	.....	2,000	20	200	202
" "	7	9	.....	.....	.....	.....	.....	.....	1,800	0	200	200
" "	8	6	.....	.....	.....	.....	.....	.....	12	20	2	5
" "	9	4	.....	.....	.....	.....	.....	.....	80	20	20	24
" "	10	1	.....	.....	.....	.....	.....	.....	6	200	6	206
Totals	...	...	.....	.....	.....	.....	.....	.....	4,316	540	492	737

* Not made.

The results are so irregular that we can draw no very specific conclusions. It appears that in the first two weeks there is no initial decrease but rather a steady increase in the total number of bacteria present. This increase is also apparent in the 37°C. count and the "red" count. On January 27, fifty-three days after the beginning of the experiment there was a remarkable change in the proportion of bacteria in the "washing" as compared with the shell liquor in all except the B. coli count. The detailed analsyis for this date shows that oyster number nine is responsible for this marked change. It is very probably that this oyster had died and decomposition was taking place.

The B. coli count shows a decrease on the fourth day, but this decrease is not particularly marked and may well be due to variations in the oysters and not to an actual decrease. This is all the more likely when it is found that on the seventh day the number of B. coli is approximately the same as on the first day. The subsequent examinations show that the number of B. coli is about one-half the initial number and remains fairly constant throughout the experiment. In the last analysis made, eighty days after the beginning of the experiment, all the bile tubes showing gas after twenty-four hours incubation were tested for B. welchii by inoculating a cubic centimeter of the bile into freshly sterilized milk tubes and incubating anærobically. No visible change took place in the milk after eighteen hours incubation. It was a noticeable fact that not over ten per cent. of the tubes showing gas after twenty-four hours incubation had one hundred per cent. of gas, the amount said to be characteristic of B. welchii. Most of the tubes had about fifty per cent. gas. From these facts it appears that the fermentation was caused by some member of the B. coli group and not by B. welchii. It is not surprising to find that B. coli should live eighty days in oysters under such conditions for Clark[1] has shown that B. coli will live in ten per cent. sewage eighty-four days and in fifty per cent. sewage one hundred and sixty-six days. Unpublished results from this laboratory show that B. coli will live in sea water for one hundred and eighty days. The writer has shown in the experiments on the hibernation of the oyster that B. coli will live in oysters kept at 1.5°C. for at least one hundred days.

[1]Report of State Board of Health of Mass., 1905, p. 455.

The important conclusion to be drawn from this series of experiments is that under the conditions of the experiment bacteria of the B. coli group do not materially increase or decrease in oysters in the shell during storage.

CLEANSING OF POLLUTED OYSTERS.

As soon as the etiological connection between oysters and certain epidemics of typhoid and gastro-enteritis was firmly established, the question at once arose as to how long a time it would take oysters known to be polluted to free themselves from sewage organisms after they had been removed to water free from sewage contamination.

Klein[1] put oysters into tanks in the laboratory and infected them with B. typhosus. About one-third of the water was removed every day and replaced with clean sea water. Oysters were removed at various intervals and examined for B. typhosus. The experiments were repeated several times and B. typhosus was isolated at the end of the experiment in every case. The various experiments were concluded on the seventh, ninth, fourteenth, sixteenth, seventeenth and eighteenth day after infection. The bacilli were isolated from the sea water twenty-one days after the beginning of the experiment. Of course, these experiments did not approximate natural conditions and so we can draw no definite conclusions from them regarding the length of time necessary for oysters to rid themselves of these bacteria when taken from polluted areas and re-layed in water free from pollution.

Herdmann and Boyce[2] tried the experiment of infecting oysters artifically with large numbers of B. typhosus and then subjecting them "to a running stream of pure clean sea water." Eighteen oysters were infected and examined at different intervals varying from one to seven days. Only the stomach contents were examined and considerable allowance must be made for this, for the writer has shown in another part of this paper that the number of bacteria contained in the stomach are quite insignificant compared with the number in the shell liquor and on the body of the oyster.

In three of the eighteen oysters examined which had washed for three, five and seven days, respectfully, no typhoid bacilli were found. In the other fifteen oysters examined B. typhosus was

[1]Relation of Oysters and Disease, Supplement to the Report of the Medical Officer to the Local Government Board, 1893.

[2]Loc. cit.

found in varying numbers. Herdmann and Boyce sum up the matter as follows: "The result was definite and uniform; there was a great diminution or total disappearance of B. typhosus in from one to seven days."

Johnstone[1] took oysters known to be polluted and transferred to the purest water available. He found under the conditions of the experiment that four days was a sufficient period of quarantine, since after that time no further cleansing took place, because the water of the locality was not entirely free from sewage contamination.

Phelps in this country[2] found that only two to four days was necessary for polluted oysters to cleanse themselves when transferred to clean water.

In 1913, Fabre-Domergue[3] read a paper before the Académie de Medicine in which he recommended the placing of polluted oysters in basins fed by filtered water and removed often enough to insure complete evacuation of the liquid contained in the shells and in the digestive tract. From his results he considers it an established fact that this procedure eliminates all pathogenic bacteria from the molluscs in six or seven days.

Field[4] says: "These (oysters) get bacteria from the waters filled with waste and sewage, and it takes them at least seventy-two hours to free themselves from these impurities that they have taken in from the waters of the different harbors." Field does not say upon what evidence, if any, this statement is based. But he adds that in Massachusetts a law has been passed requiring such polluted oysters to be transferred to clean water and allowed to remain for four weeks before offered for sale.

The writer's own experiments on the cleansing of polluted oysters confirm in part the work cited above. It appears that the rapidity with which sewage bacteria are eliminated is influenced to quite a large extent by the temperature. If the water is warm, say around 20°—25 C. the oysters remain open probably most of the time. As this is about the optimum temperature for the most rapid growth and development of the oyster, it is also the temperature at which the oyster is most active. The ciliary motion is more rapid than at lower temperatures which would increase the amount of water

[1]Jour. of Hyg. IX, 1909.
[2]Jour. Am. Public Health Assn., Vol. 1, 1911, 305.
[3]Cited in Jour. Am. Med. Asso., LXI, 1913, 134.
[4]Report of Proceedings of 3rd Am. Convention of Nat. Asso. of Shellfish Commission, p. 34.

filtered through the gills and so increase the amount of "wash water" for carrying away the bacteria. Also the ciliary motion in the alimentary canal would be hastened and so the organisms contained therein would be more quickly disposed of. Further, the capacity of the oyster to digest and assimulate bacteria would be at its height at a temperature at which the cells are most active. Hence, the optimum temperature for the growth and development of the oyster we would expect to be the period at which all contaminating organisms would be eliminated most rapidly. As the temperature lowers the activities of the oyster lessen accordingly. Further, while above 20°C. the oyster has its valves open most of the time, as the temperature is lowered the oyster is more and more inclined to keep its valves closed for longer and longer periods. This would prevent the mechanical effect of the filtered water in carrying away the bacteria. This mechanical effect is very important for the writer has shown in another part of this paper that bacteria pass through the gills with the filtered water very rapidly. Further, the activity of the cells concerned in the digestion of bacteria would also be less active and also the antagonism between different species of bacteria would be lessened. So it is seen that at lower temperature the tendency would be for oysters to eliminate bacteria more slowly than at higher temperatures. Various opinions have been expressed regarding the temperature at which oysters "hibernate" or close their shells and remain closed due to the low temperature of the water. The theory of "hibernation" of the oyster was first proposed by Gorham[1] to explain the results obtained in his investigation of the sanitary conditions of the oyster beds of Narragansett Bay. The temperature at which this phenomenon is supposed to take place is a little above 0°C. So far as the writer is aware no experimental work of an exact nature has been done to substantiate or disprove this theory, but from personal observation the writer is led to suspect that the temperature at which the oyster closes its shell for a relatively long period is considerably higher as will appear from one of the experiments detailed below.

On the other hand, experiments to be detailed later under the hibernation of oysters seem to show that oysters do open and are active at temperatures only one to two degrees above 0°C. It appears that when the temperature is low oysters will close their shell

[1](1) Rep. of Commissioners of Shell Fisheries of R. I., 1910. (2) Seasonal Variation in the Bacterial Content of Oysters, Jour. Am. Pub. Health, Jan., 1912.

for sometime, but not for indefinite periods. It also appears that the closure of the shell is not due to cold rigor or loss of control of the adductor muscle, for at a temperature of 1.5°C. the oyster can open and close its shell with the same ease as at higher temperatures.

In the following experiments only the shell liquor was used. The method of examination of the oysters was the procedure recommended by the Second Progress Report of the Committee on Standard Methods of Shellfish Examination of the American Public Health Association. The medium used was lactose-peptone bile and the tubes were inoculated in duplicate. The tubes were examined every twenty-four hours for three days. If ten per cent. or more of gas appeared during this time, it was considered to show the presence of intestinal bacteria. Unfortunately in the first experiment the investigation had to be discontinued after November 29th, so that we have only the results extending over 12 days.

Experiment 1.

November 16, 1912, about a bushel of polluted oysters were taken from the Providence River and the following day were transferred to Wickford Harbor. They were laid upon clean sandy bottom on the edge of the channel and were well separated to allow free access of water. The temperature of the water at the time of taking the oysters was 14°C. The average of the maximum and minimum temperature at Wickford for November 16 and 17 was 6.5°C. A sample of the oysters was taken at the time they were placed in Wickford Harbor and the analysis showed a score on fifteen oysters of 870. Samples were shipped to the laboratory every day until November 29th. These were analyzed immediately so that only three or four hours elapsed between the time of collecting the sample and the time of analysis. The following table shows the results of the analysis of fifteen oysters on the different days. The temperature is the average of the maximum and minimum temperature as recorded at the lobster hatchery of the Inland Fish Commission which was located nearby.

Tables Showing the Results of Analysis of Fifteen Oysters Taken from a lot of Polluted Oysters which had been put into Unpolluted Water at Wickford on November 17, 1912.

Date, November 17, 1912.

Dilution of Shell Liquor.	Average Temperature 14°C.																									
	No. of Oyster.																									
	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15	
1 c. c.	+	+	+	+	+	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1–10 c. c.	+	+	+	+	+	0	0	0	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	0	+	+	+	+	+	0
1–100 c. c.	+	0	0		+	0	0	0	0	0	+	0	+	0	0	0	+	0	+	+	+	0	0	0	+	0	+	0	0	0

Score, 870.

Date, November 21, 1912.

Dilution of Shell Liquor.	Average Temperature 5.2°C.																									
	No. of Oyster.																									
	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15	
1 c. c.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1–10 c. c.	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1–100 c. c.	0	0	+	+	+	0	+	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0	+	0	+	0	0	0

Score, 690

Date, November 22, 1912.

Dilution of Shell Liquor.	Average Temperature 6.4°C.														
	No. of Oyster.														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 c. c.	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
1–10 c. c.	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ 0
1–100 c. c.	+ +	+ +	+ 0	+ +	+ +	+ 0	+ +	+ +	+ +	+ +	+ +	+ 0	+ +	+ +	+ 0

Score, 1,500.

Date, November 23, 1912.

Dilution of Shell Liquor.	Average Temperature 7.2°C.														
	No. of Oyster.														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 c. c.	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
1–10 c. c.	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
1–100 c. c.	0 0	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +

Score, 1,410.

Date, November 24, 1912.

Dilution of Shell Liquor.	Average Temperature 6.1°C.																											
	No. of Oyster.																											
	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15	
1 c. c.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1–10 c. c.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1–100 c. c.	+	+	+	+	+	+	+	0	+	0	+	0	+	+	+	0	0	0	+	0	+	+	0	0	+	0	+	0	+	0

Score, 1,320.

Date, November 25, 1912.

Dilution of Shell Liquor.	Average Temperature 6.1°C.																											
	No. of Oyster.																											
	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15	
1 c. c.	+	0	+	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1–10 c. c.	+	0	+	0	0	0	+	+	+	0	+	+	+	+	0	0	+	+	+	0	+	+	+	+	+	+	+	+	+	+
1–100 c. c.	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	+	0	0	0	+	0	+	0

Score, 420.

Date, November 26, 1912.

Dilution of Shell Liquor.	Average Temperature 5°C.																											
	No. of Oyster.																											
	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15	
1 c. c.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1–10 c. c.	+	+	+	+	+	+	+	0	+	+	+	+	+	0	+	+	+	+	+	+	+	0	+	+	+	+	+	+	+	+
1–100 c. c.	+	+	+	0	+	0	0	0	+	+	0	0	0	0	+	+	+	0	+	+	0	0	+	+	+	+	+	0	+	+

Score, 1,140.

Date, November 28, 1912.

Dilution of Shell Liquor.	Average Temperature 6.1°C																											
	No. of Oyster.																											
	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15	
1 c. c.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	+	0	+	+	+	+	+	+	+	+	+	+	+	+
1–10 c. c.	0	0	+	+	+	0	+	+	+	+	+	+	+	0	+	+	+	0	+	0	+	+	+	+	+	+	+	+	+	+
1–100 c. c.	0	0	+	+	+	0	+	+	+	0	+	+	0	0	+	+	0	0	0	0	0	0	0	0	+	0	0	0	+	+

Score, 780.

Date, November 29, 1913.

Dilution of Shell Liquor	Average Temperature 4.4°C.																											
	No. of Oyster.																											
	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15	
1 c. c.	+	+	+	+	+	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0
1–10 c. c.	+	+	+	+	+	0	0	0	0	0	0	0	+	+	0	0	+	+	+	+	+	0	+	0	+	0	0	0	0	0
1–100 c. c.	+	0	+	0	0	0	0	0	0	0	0	0	+	0	+	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0

+ = positive presumptive test for B. coli.
0 = negative presumptive test for B. coli.

Score, 132.

Unfortunately the experiments could not be continued and so we cannot say whether the apparent cleansing, which appeared on the last two days, especially on the last day, was due to a fortunate selection of oysters or was the indication of a real elimination of the intestinal bacteria. The writer is led to believe that the oysters had just begun to open and so allowed the bacteria to be washed out. The low temperature of the water slowed the metabolic processes of the oyster and so, as food and oxygen were not needed in so great quantities, an oyster could maintain itself for sometime without renewing its supply. As soon as the supply was exhausted, however, the oyster opened its shell.

This investigation shows that under the conditions of the experiment with a temperature between 7.2°C. and 5°C. a period of twelve days is not sufficient to allow oysters to free themselves from intestinal bacteria.

Experiment II.

May 13, 1913, about a bushel of polluted oysters were taken from Providence River and transferred to the same location in Wickford Harbor as in the previous experiment.

The water of Wickford Harbor at the place where the oysters were put down was tested by the lactose-peptone-bile presumptive test and no sewage organisms were found. The methods and conditions of the experiment were the same as in the previous experiment except that ten oysters were used instead of fifteen. The sanitary condition of the oysters at the time of transplantation and on two subsequent occasions is shown in the following table:

Tables Showing Results of Analysis of Ten Oysters from a lot of Polluted Oysters which had been put into Relatively Unpolluted Water at Wickford, May 13, 1913.

Date, May 13, 1913.

Dilution of Shell Liquor.	Average Temperature.																	
	No. of Oyster.																	
	1		2		3		4		5		6		7		8		9		10	
1c.c.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1–10c.c.	+	+	+	+	+	+	0	0	+	+	+	+	+	+	+	+	+	0	0	0
1–100c.c.	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	0	0	0	0	0

Score, 460.

Date, May 17, 1913.

Dilution of Shell Liquor.	Average Temperature, 12.7°C.																	
	No. of Oyster.																	
	1		2		3		4		5		6		7		8		9		10	
1c.c.	+	+	+	+	+	+	+	+	+	+	+	0	+	0	+	+	+	+	+	+
1–10c.c.	+	0	0	0	+	+	+	+	0	0	+	0	0	0	+	0	+	+	0	0
1–100c.c.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0

Score, 73.

Date, May 22, 1913.

Dilution of Shell Liquor.	Average Temperature 14.7°C.																	
	No. of Oyster.																	
	1		2		3		4		5		6		7		8		9		10	
1c.c.	+	0	+	+	+	+	+	+	+	+	+	+	0	0	+	+	+	+	+	+
1–10c.c.	+	0	+	0	0	0	0	0	+	+	0	0	0	0	0	0	0	0	0	0
1–100c.c.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Score, 28.

From the table it is seen that at the beginning of the experiment there were on the average forty-six B. coli per cubic centimeter of oyster juice. After a period of four days this number had dropped to an average of 7.3 per cubic centimeter and after a period of nine days the number had still further decreased so that there were on the average only 2.8 B. coli per c.c. of the oyster juice. This shows that under the conditions of the experiment, oysters which contained 46 B. coli per cubic centimeter can in nine days free themselves from B. coli to such an extent that there remains only 2.8 B. coli pr cubic centimeter. This is well within the standard adopted by the Bureau of Chemistry which allows oysters to be shipped in interstate commerce which contain 4.6 B. coli per cubic centimeter of shell liquor.

Experiment III.

On November 8, 1913 a bushel of oysters were taken from Providence River and transplanted to Wickford. These oysters were put into two galvanized iron baskets and hung into the water from the floor of the Beacon Oyster Co. These oysters were suspended in the water near the edge of the channel and located only a few yards from the place where the oysters in the two previous experiments were placed. A sample of ten oysters was taken from this lot and carried to the laboratory for analysis. These ten oysters were found to be badly polluted and had a score of 640. Samples were sent to the laboratory and analyzed on November 10, 12, 14, 17, 19, 21 and 24. The methods of analysis were the same as in the previous experiments with two exceptions. The 1–10 c.c. and 1–100 c.c. dilutions were made in duplicate, while the one cubic centimeter samples were only inoculated singly. The oyster liquor was drained into glass-stoppered bottles which were graduated so that the amount of liquor could be read off in cubic centimeters. An equal amount of sterile one per cent. sodium chloride solution was added and the bottle shaken vigorously one hundred times. One cubic centimeter of this mixture was used for the first inoculation and to make the proper dilutions. As a result the quantities as given in the table are for the mixture of shell liquor and salt solution. The amount of shell liquor in the dilutions is not 1 c.c., 1–10 c.c. and 1–100 c.c., but ½ c.c., 1–20 c.c. and 1–200 c.c. But as ten oysters were used the result equals an analysis of five oysters where 1 c.c., 1–10 c.c. and 1–100 c.c. samples of the shell liquor were used. For comparative results,

however, it does not matter what quantity we use provided we use the same amount every time. The following table shows the results of the examination on the different days.

Table Showing the Results of Analysis of Polluted Oysters which were put into Comparatively Uncontaminated Water at Wickford, November 8, 1913.

Date, November 8, 1913.

Quantity of Shell Liquor and Salt Solution.	Average Temperature.									
	No. of Oyster.									
	1	2	3	4	5	6	7	8	9	10
1c.c.....	+	+	+	+	+	+	+	+	+	+
1–10c.c.....	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
1–100c.c.....	0 0	+ +	+ +	+ 0	+ 0	+ +	+ 0	0 0	+ 0	0 0

Score, 730.

Date, November 10, 1913.

Quantity of Shell Liquor and Salt Solution.	Average Temperature 11.1°C.									
	No. of Oyster.									
	1	2	3	4	5	6	7	8	9	10
1c.c.....	+	0	+	+	+	+	+	+	+	+
1–10c.c.....	+ 0	+ 0	+ 0	0 0	+ +	+ +	+ +	+ +	+ 0	+ 0
1–100c.c.....	0 0	0 0	0 0	0 0	+ 0	0 0	0 0	0 0	0 0	+ 0

Score, 190.

Date, November 12, 1913.

Quantity of Shell Liquor and Salt Solution.	Average Temperature 8.8°C.									
	No. of Oyster.									
	1	2	3	4	5	6	7	8	9	10
1c.c.....	0	0	0	0	+	+	+	+	+	+
1–10c.c.....	0 0	0 0	0 0	+ 0	0 0	0 0	+ 0	0 0	+ 0	+ 0
1–100c.c.....	0 0	+ 0	0 0	0 0	0 0	+ 0	0 0	0 0	+ 0	0 0

Score, 37.

Date, November 4, 1913.

Quantity of Shell Liquor and Salt Solution.	Average Temperature 9.7°C.									
	No. of Oyster.									
	1	2	3	4	5	6	7	8	9	10
1c.c.	+	+	+	0	0	+	+	+	+	+
1–10c.c.	0 0	+ 0	0 0	0 0	+ 0	0 0	0 0	0 0	0 0	0 0
1–100c.c.	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0

Score, 10.

Date, November 17, 1913.

Quantity of Shell Liquor and Salt Solution.	Average Temperature 7.7°C.									
	No. of Oyster.									
	1	2	3	4	5	5	7	8	9	10
1c.c.	+	+	+	+	+	+	+	+	+	+
1–10c.c.	0 0	0 0	0 0	+ 0	0 0	+ 0	0 0	0 0	0 0	0 0
1–100c.c.	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0

Score, 10.

Date, November 19, 1913.

Quantity of Shell Liquor and Salt Solution.	Average Temperature 7.3°C.									
	No. of Oyster.									
	1	2	3	4	5	6	7	8	9	10
1c.c.	+	+	+	+	0	+	+	0	+	0
1–10c.c.	+ 0	0 0	+ 0	0 0	0 0	+ +	0 0	0 0	0 0	+ 0
1–100c.c.	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0

Score, 19.

Date, November 21, 1913.

Quantity of Shell Liquor and Salt Solution.	Average Temperature 10.5°C.									
	No. of Oyster.									
	1	2	3	4	5	6	7	8	9	10
1c.c.....	+	+	+	+	0	+	+	+	+	+
1–10c.c.....	+ 0	0 0	0 0	0 0	0 0	+ 0	0 0	0 0	0 0	0 0
1–100c.c.....	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0

Score, 19.

Date, November 24, 1913.

Quantity of Shell Liquor and Salt Solution.	Average Temperature 10.5°C.									
	No. of Oyster.									
	1	2	3	4	5	6	7	8	9	10
1c.c.....	+	0	+	+	+	+	0	+	+	+
1–10c.c.....	0 0	0 0	+ +	0 0	+ 0	0 0	0 0	+ +	0 0	0 0
1–100c.c.....	0 0	0 0	0 0	0 0	0 0	0 0	0 0	+ 0	0 0	0 0

Score, 28.

+ = positive presumptive test for B. coli.
0 = negative presumptive test for B. coli.

It is seen from the tables that the oysters cleaned themselves as much in six days as at any time. The samples taken on the day of transporting showed on the average twenty-three B. coli per cubic centimeter of oyster juice. Six days later the sample showed only one B. coli per cubic centimeter and they showed no further cleansing after ten more days. The fact that the water at this place is not entirely free from sewage contamination probably explains why the oysters did not show any further elimination of B. coli, apparently there were enough B. coli in the water to maintain a small number in the oyster at all times.

Conclusions.

In one experiment with a temperature averaging 9.7°C. over the period of the investigation the oysters showed an elimination of B.

coli from 73 per cubic centimeter to one per cubic centimeter in six days. In another experiment with an average temperature of 13°C. during the period of investigation the oysters showed an elimination of B. coli from an average of 46 B. coli per cubic centimeter to 7.3 B. coli per cubic centimeter in four days and to 2.8 per cubic centimeter of shell liquor in nine days. As no examination was made between the fourth and ninth day, it is quite possible that the limit of possible elimination was reached sometime before the ninth day. No doubt an examination on the sixth or seventh day would have shown a B. coli content sufficiently low to pass the standard set by the Bureau of Chemistry of the Federal Government.

In another experiment in November, 1912, with an average temperature 5.4°C. twelve days was not sufficient to eliminate B. coli to any appreciable extent. The examination on the twelfth day showed a very marked decrease in the number of B. coli, but as no subsequent examinations were made it is not possible to say with authority whether this was the beginning of an elimination process or not, though the writer is led to believe such was the case. The interesting feature of this experiment is that no elimination took place in nine days, while in the other two experiments a very marked reduction took place in six days in one case and in five and nine days in the other case.

These sets of experiments seem to throw some light upon the so-called hibernation of the oyster. With an average temperature of 13°C. in one case and 9.7°C. in the other the oysters opened and began to eliminate B. coli almost immediately, but in the first experiment with an average temperature of 5.4°C. no reduction in B. coli was found until the twelfth day. These experiments lead the writer to believe that when the temperature of the water is somewhere between 9°C. and 5°C. oysters close their shells for a longer or shorter period. But from experiments detailed elsewhere, the writer believes that there is no time above 0°C. when oysters close their shells for an indefinite period. The length of time that oysters remain closed is in inverse proportion to the temperature which determines the rapidity of the metabolic processes going on within the oyster.

EXPERIMENTS ON THE HIBERNATION OF THE OYSTER.

The so-called hibernation of oysters has attracted much attention during the last four years. The theory that oysters close their shells

when the temperature of the water approaches 0°C. was first put forward by Gorham in 1910;[1] to explain certain bacteriological findings in Providence River oysters. It was found that during the warmer months the oysters in certain parts of the river were badly polluted, but in January, with the temperature of the water around 0°C., the oysters were found free from colon bacilli. In order to explain this phenomenon Gorham advanced the theory that when the temperature of the water approaches 0°C. the oyster closes its shell and remains closed until the temperature of the water begins to rise and then it opens its shell and resumes its normal activity. This period was called its "Hibernation Period." A little later Pease,[2] Field, of the Massachusetts Fish and Game Commission, and others advanced a similar idea. So far as the writer is aware, however, no experiments have been tried to confirm or deny this theory. The experiments of the writer cited elsewhere on the cleansing of polluted oysters seem to show that oysters do remain closed for several days with a temperature of about 5°C. But in order to throw further light upon the matter the following experiments were tried.

Experiment I.

January 12, fourteen oysters were placed in sea water which had been inoculated with a pure culture of B. coli. The oysters were left in the sea water a day and a night. They were removed January 13th, and the outside of the shells scrubbed thoroughly with a stiff brush and running tap water and were then put into a strong solution of calcium hypochlorite for one-half hour and stirred up about once a minute. They were then put into 7% formalin for the same length of time and stirred with a glass rod for a few seconds at about one minute intervals. They were then washed for a considerable time in fast running tap water, temperatures between 7°C. and 8°C., and stirred at intervals of two or three minutes. The oysters were then taken (Jan. 13), to a cold storage room of the Merchant's Cold Storage and Warehouse Co., Providence, and put into storage at 34°F. (about 1.1°C.) The temperature of the room is maintained constant throughout the year and is never allowed to vary more than .5°F. The next day sterile sea water which had been kept in the

[1](1) Report of Commissioners of Shell Fisheries of R. I., 1910. (2) Seasonal Variation in the Bacterial Content of Oysters, Am. Jour. Pub. Health, II, 1910, 24.

[2]Some Bacteriological Problems in the Oyster Industry, The Fishing Gazette, 28, 1911, 865. July 15.

room for several days was poured into the dishes until it covered the oysters. Immediately after the oysters were covered five samples of two cubic centimeters each were taken from each dish and inoculated into bile tubes and incubated at 37°C. for 18 hours. Every tube showed gas. January 22, the oysters were examined in the dishes and it was found that four were closed tightly, five were open widely enough to be seen as they lay in the dishes and the other seven were found to be slightly open. The opening of these last seven was not perceptible to the eye, but upon taking them out and squeezing them one could hear a "squashy" sound, showing that they were not firmly closed. Apparently the five oysters that were open had lost their sensitiveness, for they would not remain closed when the valves were pressed together. The mechanical stimulation of the gills and mantle was not tried. The oysters were observed on several days until February 2nd and it was found that some of the oysters that had been firmly closed at first had opened and vice versa.

The oysters were not observed again until March 23. It was found that two of the oysters in one dish were open and dead. Two others were wide open but closed immediately when touched. These two oysters were brought to the laboratory and put into a dish of sterile sea water and observed for several days. They were just as active as oysters freshly brought from the beds. They were then tested for B. coli. Both oysters showed gas in 1–100 c.c. of shell liquor. These tubes were plated in litmus-lactose-agar and typical colon colonies were found in the plates from one oyster, but not from the other. This showed that B. coli can live under such condition for at least sixty-nine days.

The remaining oysters were again examined April 24, one hundred days after they were put into storage. Five of the oysters were apparently living, while the others were dead. These five were brought to the laboratory and examined. It was found that three were closed tightly, while the other two appeared a little "weak." One of the tightly closed oysters was put into a dish of sea water and it soon opened like an oyster removed only recently from its natural element. When the shell was touched it would close immediately, though its movements were not so vigorous as those of an oyster taken directly from the water. When the gills and mantle were touched with a wire it did not respond readily. Apparently its tactile sensations were not very acute, although after repeated stimulations it closed and gripped the wire so that it took considerable

strength to pull it out. The writer has noticed that oysters which have been removed from sea water for some time require a great deal of stimulation to make them close again, though after they have been open for a time they react immediately. It may be that the tango-receptors are very much dulled or that the desire for oxygen is stronger than the sense of self-protection.

The other four oysters were opened with the proper precautions and the mixed shell liquor and the "washings" from the body were inoculated into bile tubes. Two of the oysters were normal in appearance and exceptionally plump. The other two showed slight evidences of decomposition. All the tubes from three of the oysters showed gas and typical B. coli colonies were isolated on litmus-lactose-agar plates. Further identification was not regarded as necessary. The tubes inoculated from the third oyster showed no gas after three days incubation.

Experiment II.

Seven oysters were obtained fresh from the water and impregnated with a solution of azolitmin in sea water. They were then washed thoroughly with a stiff brush in running water and immersed in chromic acid for a few seconds and then washed again. All the color was removed in this manner. January 29 they were placed in tumblers and put into cold storage at 34°F. They were left over night to acquire the same temperature as the room and then the tumblers were filled with sea water. The dishes were watched to see if any color had escaped from the oysters. February 2 a slight coloration was found in the bottom of two of the tumblers, but this did not appear to increase for several days. The oysters were not examined again until March 23rd. The color had disappeared from the two tumblers that had previously been discolored. It was observed, however, that the water in the tumblers was not entirely clear. There was a sediment in the bottom of the tumblers that resembled the bits of mucus thrown off by oysters. One of the oysters was taken to the laboratory and placed in sea water. It soon opened, but did not contain any color. It was as active as a normal oyster. Some of the mucus thrown out by the oyster had a purplish color which had been stained with azolitmin.

April 17 the remaining six oysters were examined. It was found that three of the oysters were open and the other three closed. Covers

were fitted to all the dishes and during the process two of the oysters closed. The other one remained open even after reaching the laboratory. After the cover was removed and the shell touched with a glass rod it closed immediately. A heavy precipitate was found on the bottom of each of the dishes and a great deal of mucus was seen in suspension. This matter could not have come from the outside of the oyster, because they were thoroughly cleaned before the experiment began. There is no question but what the oyster had opened; three were found open and two of them closed immediately upon being agitated. The other three must have opened in order to discharge so much mucus, but had closed again of their own accord at 34°F.

Two oysters were infected with B. coli and put into dishes in cold storage January 20. The dishes were later found to be cracked and the water leaked out. These two oysters were brought to the laboratory April 17. One was put into a dish of sea water, while the other was opened and two cubic centimeters of the juice was inoculated into each of four bile tubes. Gas appeared in each tube and typical B. coli was isolated on litmus-lactose-agar plates. This was eighty-seven days after infection. The other oyster opened before morning, but was apparently dead for it would not respond to a mechanical stimulation of its gills and mantle. When opened both oysters appeared plump and in prime condition. From their appearance they could not have been told from oysters freshly caught.

From these experiments the writer believes that oysters do close their shells for varying periods, depending upon the temperature. Whether they close their shells under natural conditions when the temperature falls around 0°C. no one has determined. That they do not lose control of their adductor muscles is demonstrated in both experiments. The writer is lead to believe that there is no definite period at which this phenomenon can be said to begin. Mitchell in an unpublished observation states that with a temperature below 20°C. oysters get "nervous" and will close upon the slightest provocation and remain closed for fairly long periods. It appears that at this temperature the irritability of the oyster is much increased.

These experiments lead one to conclude that the so-called period of hibernation of the oyster is a relative term. The length of time that they remain closed depends upon the temperature which determines the rapidity of the oxidative and other metabolic processes of the oyster. An oyster will remain closed as long as its supply of

food and oxygen remains sufficient and the lower the temperature the longer this period will be. The oyster does not close on account of "rigor frigoris," for the control of the adductor muscle is still very marked at a temperature of 1.1°C., and is scarcely distinguishable from normal.

Mitchell,[1] in an extended study of the oxygen requirements of shellfish states as one of his conclusions that "oysters of medium sizes, at temperatures between 19° and 28°C., used from 7 to 35 decimilligrams of oxygen per hour per 100 grams of entire weight. The amount varies with the temperature, so far as experiments show, according to simple relationship, so that the curve approximates a straight line." . . . "The common clam (Mya Arenaria) shows a higher oxygen requirement than the oyster."

The theory of hibernation which the writer has advanced appears to be in harmony with the experiments of Mitchell on the oxygen requirements of oysters. The lower the temperature the less the amount of oxygen used. But no matter what the temperature so long as the oyster is living it needs a certain amount of oxygen to carry on its oxidative processes. When the amount available within its shell is exhausted, it will open to renew its supply.

The statements of practical oyster growers also leads to the same conclusion. It is said that oysters from Narragansett Bay in February cannot be shipped very far in the shell, because, as the oyster men say, they will "cluck," that is, open their shells and allow the shell liquor to run out. The explanation no doubt is that during the "zero weather" of January, the oysters are closed and as their oxygen requirements under the circumstances are small they can remain closed for sometime without exhausting the supply available in the shell liquor. The period of cold weather, however, is sufficiently long perhaps to allow the oysters, even with their small requirements, to nearly, if not quite exhaust the available supply of oxygen within their closed shells. The result is that in February when they are removed to the opening house or express car which has relatively a much higher temperature than the water from which they were taken, the metabolic processes of the oyster are greatly increased and there is a demand for more oxygen. The supply within the shell, which has already been greatly reduced, is quickly used up, and consequently the oyster opens to renew its supply.

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[1]The Oxygen Requirements of Shellfish, Bull. U. S. Bureau of Fisheries, XXXII, 1912, 209.

It is said that the soft-shelled clam does not hibernate during the winter. The second quotation from Mitchell's paper, namely, that the oxygen requirement in the common clam is higher than in the oyster may account for this phenomenon. The sooner the available quantity of oxygen is used up, the more quickly will the mollusc open to renew its supply.

SUGGESTED CHANGES IN STANDARD METHODS OF SHELLFISH EXAMINATION.

The Second Progress Report of the Committee on Standard Methods of Shellfish Examination recommends that "twelve oysters of the average size of the lot under examination, with deep bowls, short lips and shells tightly closed, shall be picked out by hand and prepared for transportation to the laboratory." . . .

"Bacterial counts shall be made of a composite sample of each lot obtained by mixing the shell liquor of five oysters." . . .

Under the heading of "Methods of Rating Oysters for B. coli," the following statement is made: "The following values shall be assigned to the presence of bacteria of the B. coli group in each of the five oysters examined." Then follows a statement and illustration of the method of scoring as adopted by the American Public Health Association. It is clear at once that if we mixed the shell liquor of the five oysters and examined it as a composite sample, it would be impossible to assign values "to the presence of bacteria of the B. coli group in each of the five oysters examined," for the composite sample must be treated as the juice of a single oyster. It is evident that a composite sample is not what is intended, but rather that each oyster shall be examined separately.

Some workers have based their analysis upon several composite samples of five oysters each, while others have used five, ten or fifteen oysters separately. There is great variation in the bacterial content of oysters from the same lot. In one oyster there may be one hundred B. coli per cubic centimeter of the shell liquor, while in another oyster from the same sample they may be entirely absent. The important consideration in the examination of oysters is the average number of B. coli in the oysters as a whole and not the number in any individual oyster. For this reason the larger the sample, within reasonable limits, the more accurate the results as an indication

of the B. coli content of the oysters of any particular area. Smith,[1] in the analysis of one hundred and twenty-five oysters in each of a series of samples, came to the conclusion that not less than fifteen oysters should be used. The use of too small a sample may account in part for the wide variation in results obtained by different analysts in the examination of the same oyster bed at approximately the same time. In the writer's opinion twenty-five oysters is not too large a sample to be used in any analysis.

The changes which the writer would suggest in "Standard Methods of Shellfish Examination," are as follows:

The size of the sample should be at least twenty-five oysters. After reaching the laboratory the oysters should be scrubbed thoroughly with a stiff brush in water free from B. coli and dried. When ready for examination the oyster should be held between the thumb and the fore-finger and the lip of the shell flamed in the bunsen burner or burned off with alcohol. The opening should be done with an oyster knife which has previously been burned with alcohol. The method of drilling a hole through the shell and pipetting out the oyster juice should never be substituted as an alternative method.

The shell liquor of the five oysters of each of the five composite samples should be collected in sterile, graduated, glass-stoppered bottles and the bodies of the five oysters should be placed in a wide-mouth, glass-stoppered bottle. The amount of shell liquor should be read off and an equal amount of sterile one per cent. salt solution or sea water added to the bottle containing the bodies of the oysters. The stopper should be replaced and the bottle shaken at least one hundred times. (The writer's experience has been that, if the oysters are opened carefully so as to avoid mutilation, the bodies of the oysters are damaged but very little by this procedure unless the shaking is especially vigorous.) The salt solution and mucus should then be decanted into the bottle containing the shell liquor and the whole shaken vigorously one hundred times to break up any clumps of bacteria and to separate as far as possible the bacteria from the bits of mucus. The five sets of oysters should be treated in this manner, making five samples of five oysters each. If the operation is conducted properly there should be an equal quantity of shell liquor and salt solution in each of the five composite samples.

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[1]Size of the Sample Necessary for the Accurate Determination of the Sanitary Quality of Shell Oysters, American Journal of Public Health, III, 1913, 705.

The subsequent procedure should be the same as that recommended by "Standard Methods" and the method of scoring should be the same except that the score as obtained by this method should be multiplied by two, because we are using ½ c.c. 1–20 c.c. and 1–200 c.c. of the original shell liquor instead of 1 c.c., 1–10 c.c. and 1–100 c.c. as recommended by "Standard Methods."

The advantages of this method are that we are basing our examination upon twenty-five oysters instead of five and the result will be much nearer the true bacterial content of the sample.

Another point worthy of consideration by the Committee on "Standard Methods" is the number of bile tubes to be used in the different dilutions. The writer in all the work reported in this paper and for a long time previous has used duplicate tubes. An interesting feature of this method is that both tubes from each dilution show gas only approximately two-thirds of the time. The writer has regarded gas in either of the two duplicate tubes as positive for the dilution and has assigned it the value as recommended by "Standard Methods." By using this method approximately thirty-three per cent. more B. coli are found than would be the case if only one tube were used.

"Standard Methods" under "Illustration of the Application of the Method of Rating Oysters for B. coli" recommends the transferring of a positive result in a high dilution in one oyster to a lower dilution in another oyster, if in the latter oyster the B. coli test is negative in the lower dilution. Below is an illustrated case from "Standard Methods:"

Case C. Results of B. Coli Tests in Dilutions Indicated.

Oyster.	1.0c.c	0.1c.c	0.11c.c	Numerical value.
1	+	+	0	10 (not 100)
2	+	+	0	10
3	+	+	+	100
4	+	+	+	10 (not 100)
5	+	0	0	10 (not 1)

140 = rating.

But suppose in this sample the tubes have been inoculated in duplicate instead of one tube for each dilution. The following table shows a not unexpected result:

Results of B. Coli Test in Duplicate Tubes in Dilutions Indicated.

Oyster	1 c.c.		0.1c.c		0.001c.c	
1	+	+	+	0	0	0
2	+	+	+	0	0	0
3	+	+	+	+	+	0
4	+	+	+	0	+	0
5	+	0	0	0	0	0

The question now arises as to what numerical value we shall assign to the dilutions which are positive in one tube and not in the other. Is it proper to assign full value to these dilutions? Would it not be fairer to assign one-half the value recommended by "Standard Methods" to these dilutions, because basing our calculation upon both tubes there are only one-half the B. coli present that would be indicated by the positive result alone?

Suppose now we wanted to transfer the positive result in the 1–100 c.c. dilution of oyster No. 4. Should it be transferred to the negative tube in oyster No. 5, or to the 1–10 c.c. dilution of the same oyster? Further, what shall we do with the positive result in the 1–100 c.c. dilution of oyster No. 3. Shall it remain where it is or shall it be transferred to the negative tube in the 1–10 c.c. dilution of oyster No. 1 or 2? Again suppose in oyster No. 3 in the 1–100 c.c. dilution both the tubes should be positive and there were only one tube negative in the 1–10 c.c. dilution of any of the oysters, should these two positive tubes be separated and transferred to the negative tubes in two of the other oysters?

But whatever method we use for transferring, shall we assign the full value recommended by "Standard Methods" to the dilutions which are positive in one dilution and negative in the other, or shall we assign the better value, *i. e.*, one-half the value recommended by "Standard Methods?" By taking advantage of the various possibilities we can obtain ratings varying between thirty, the lowest possible, and one hundred and forty, the highest possible. In the first case the oysters would be very near the permissible standard, while in the other, the oysters would be considered badly polluted. If we use three tubes in each dilution the matter is still further complicated.

Another possibility would be to regard each set of tubes separately and average the results. This would be the simplest method, but it would not give so low a result as would be possible by one of the other methods. In the case in hand the rating would be seventy-seven as against thirty, the rating obtained by one of the other methods.

The writer has a case in mind in which the rating on one set of tubes was three, which showed the oysters to be in a high state of purity, while the duplicate set showed a rating of thirty-two, which would condemn the oysters on the strict application of the standard set by the Bureau of Chemistry. Obviously it would be unjust to base our rating on either of the two sets of tubes alone.

In the writer's opinion the standard set by the Bureau of Chemistry of twenty-three as the highest permissible rating is very stringent and every opportunity should be given the oyster growers to avail themselves of a method of oyster analysis which will be more accurate in its results and a method of rating that will more nearly represent the sanitary condition of their product.

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REPORT ON THE INSPECTION OF CAMPS, 1913.

BY LESTER A. ROUND.

Health Bulletin of the Rhode Island State Board of Health for February, 1914.

REPORT

ON THE

INSPECTION OF CAMPS

1913

LESTER A. ROUND

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REPORT ON THE INSPECTION OF CAMPS, 1913.

The following is a report of the work on the inspection of camps during the summer of 1913. The work occupied parts of four days and included the inspection of four camps, two camps on Narrow River, west of Saunderstown, Crescent Heights, near Riverside, and the camp ground at Rocky Point. Owing to the fact that the summer was far gone when the inspection was commenced and to the interruption during the epidemic of infantile paralysis the work was necessarily incomplete.

Trinity Church Club of Newport.

This camp was situated on Narrow River, back of Saunderstown, and was in charge of Mr. Newcome, a student at Brown University. The camp was located on a knoll on the west bank of the river on land belonging to Mr. A. F. Carson, and consisted of three tents and one building. The latter was divided into a cook house and a sort of club room. On the west side of the building was a wide piazza which protected the mess table. The tents were large and roomy and were used only for sleeping.

The camp was under the administration of the Trinity Church Club of Newport, and consisted of boys belonging to the club. At the time of inspection there were twenty-eight persons in the camp, twenty-two of whom were boys under the age of fifteen. The number varied from week to week as the boys came and went. So far as I could learn there were no cases of sickness among the boys before coming. Mr. Newcome's attention was called to the dangers which might arise upon admitting to the camp a typhoid convalescent or any one who had recently suffered from any infectious disease. He was

advised not to admit any such person without a physician's certificate. He promised to give the matter careful attention.

The water supply was derived from a spring nearby and judging from its physical qualities appeared to be of excellent character. The milk used in the camp came from Mr. Carson's farm, the owner of the land upon which the camp was located, and who has the reputation of having an excellent dairy.

The privy vault was located on the same lot about forty yards northwest of the camp and consisted of an unscreened pit-vault. An attempt was made to cover the fæces, but was unsuccessful. The copious use of bleaching powder, however, protected the fecal matter from flies which were not numerous in any part of the camp, not even in the cook house. All kitchen wastes were thrown into a pit and covered with earth. The bathing beach was located at the foot of the knoll on which the camp was located, and was free from any drains or sewage outfalls. Although marshy land was near, the campers were troubled with mosquitoes only when the wind was south or southeast, in which case the mosquitoes were brought from the marshes situated below on the river.

St. John's Church of Newport.

This camp was also located on Narrow River, about two hundred yards north of the camp of the Trinity Church Club.

It was situated on a knoll on the west bank of the river. The camp was in charge of Mr. J. A. McNulty, and consisted of four tents and a cook house. The location of the camp was new and the boys had put in most of their time clearing away the brush and making a clearing in the woods. At the time of visitation there were fifteen boys in the camp, eight being under fifteen years of age. As in the case of the Trinity Church Camp, however, the number varied, for some of the boys left each week while others came to take their places as the accommodations of the camp permitted. So far as I could ascertain, no one had suffered with any infectious disease while in camp or for a considerable time before coming.

The water supply was derived from the same spring as in the case of the Trinity Church Camp, and the milk supply was derived from the same dairy. All kitchen wastes, garbage, swill, etc., were disposed of by burial.

The privy was located about fifty yards northwest of the camp and consisted of a pit-vault which was tightly boarded. No screens were used, but a plentiful supply of bleaching powder prevented flies from breeding in the fecal matter or from being attracted to it. Almost no flies were seen in any part of the camp nor had the campers been troubled with mosquitoes.

The sanitary aspects of both the camps on Narrow River were very good and left but little to be suggested by the inspector.

Crescent Heights.

Crescent Heights is the name now given to what was formerly known as Camp White, and is situated at the south end of Riverside, just north of Crescent Park. The camp was situated on land owned by the Hope Land Co., and was in charge of Mr. Looff, manager of Crescent Park. At the time of inspection there were twenty-eight tents and seventy-four persons in camp, though the number fluctuated as campers were coming and going continuously. Some of the campers had tents on the grounds that were occupied only during holidays and week-ends. On the day of inspection there were thirty-five adults and thirty-nine children in the camp.

No cases of sickness of any kind were found on the grounds, nor had any of the people suffered from any infectious disease for some considerable time before coming to camp.

The water supply was derived from the East Providence system, and the milk from the Broadway Dairy Co., and from several farmers whose names were not known. The garbage and kitchen refuse was placed in kegs and these were emptied every day. Refuse matter, such as paper and other combustible material, was burned by the campers themselves, while tin cans, cornhusks, etc., were put into large bins which were emptied once a week.

The toilets were located at either end of the camp ground and were pit-vaults in which no attempt was made to screen or cover the fæces. Yet in spite of this fact there were very few flies around the buildings, though in other parts of the camp they were very numerous. So far as I could learn no disinfectant, such as bleaching powder or some coal-tar derivitive, was used in the privy vaults. Some method of protecting the fæces from flies should be provided, however, since many of the tents are located very near. As the inspection was made the Saturday before Labor Day, and most of the campers were intending to leave the following Monday or Tuesday, the matter was not brought to Mr. Looff's attention. This was the only glaring deficiency that I could find in the camp. This matter should be called to the attention of Mr. Looff at the opening of the next season, in order that proper protection be provided.

An inspection of the shore from the camp grounds north showed nothing unusual. Some seaweed and other marine growths were scattered along the beach. Evidently the condition which caused the complaint earlier in the season had been removed.

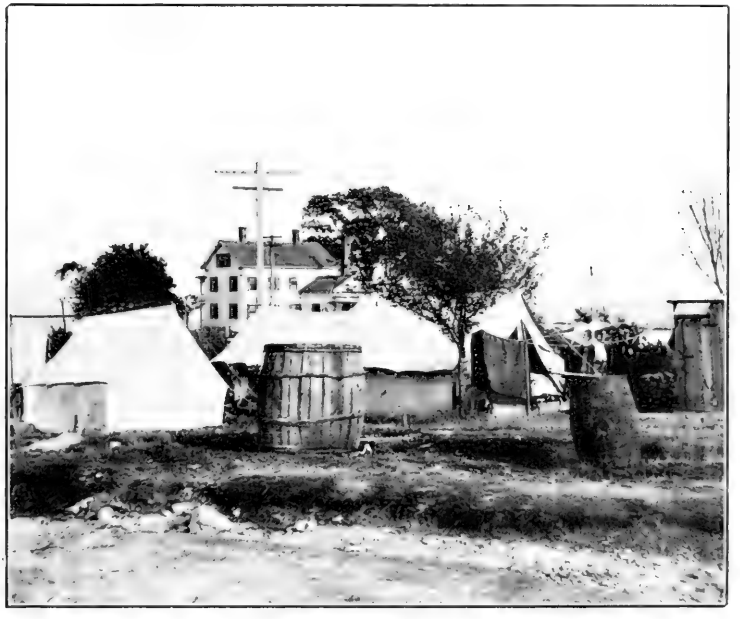
Rocky Point.

This camp was situated just north of the amusement park in the same place it has been for a number of years. At the time of inspection it consisted of sixty-eight cottages and forty-four tents. In the tents were found eighty-two persons, twenty-five of whom were children under fifteen years of age. The camp was in charge of Mr. R. A. Harrington, who also controls the amusement park.

So far as I could learn, no one had been sick in spite of the unsanitary conditions that existed. The water supply was derived from a spring on the shore some distance from the camp ground, and necessitated considerable inconvenience to the campers in obtaining their water supply. This spring is the only water supply available on the camp grounds and furnishes the water for from three hundred to five hundred persons throughout the season. The location of the spring

is very bad. It is just level with the beach and is wholly unprotected, while a quahaug shell or an old tin can serves as a "common drinking cup." The fact that the spring is perfectly unprotected in such an open spot gives abundant opportunity for gross pollution of all kinds. The milk supply is obtained from various farmers.

The privy system and the method of garbage disposal found on the camp grounds would be an abomination to any civilized community. Four barrels were placed at different corners of the lot and in these the swill, garbage and kitchen wastes were placed by the cottages and campers. It was the intention of the man in charge of this work to empty these barrels once every week. But I was informed by several campers independently



This picture shows two of the swill barrels and a privy and the proximity to some of the tents. These barrels were only emptied once each week and on one occasion were not emptied for three weeks. At this time the barrels were not only filled, but the garbage completely covered the barrels and filled the space between them.

that on one occasion, these barrels were not emptied for three weeks. At this time they were not only filled, but the garbage formed a pile around the barrels large enough to completely hide them, and in the picture shown on this page the space between the two was filled according to the statement of several of the campers. As is seen in the picture these barrels were sometimes located very close to the camps, only twenty-five or thirty feet in some cases. One can easily imagine the odor and stench arising from a pile of garbage of such dimensions which had been collecting for three weeks during the hottest days of our summer months. The description of the odors by the campers in all probability did not exaggerate the matter in the least. The number or flies attracted, plus the number bred during this time, would be almost inconceivable. But during the other periods when the garbage was collected every week the conditions must have been very bad, especially during a period of the warmest weather of the year. The amount of garbage collected from three to five hundred people during a week would be considerable, and would furnish one of the best baits for flies imaginable. This would be especially true when the garbage was unprotected. Not only that, but it would furnish an excellent breeding place for flies, certain species of which could pass through their laval and pupal stages during this short period. Moreover, it would give sufficient time for the ordinary house fly—musca domestica—to pass through its laval stage and then bore into the ground and complete its metamorphasis. At both visits the barrels had just been cleaned, but in no very cleanly manner, and swarms of pseudopyrellia cornicina were seen in and around the barrels. In a camp of the size of Rocky Point, with conditions such as they are, there can be no reasonable excuse for not collecting the swill and garbage every day, especially when we consider the revenue which is obtained from this small plot of ground. This revenue is estimated at $1,500 to $2,000 annually, with almost no cost of maintenance except taxes.

The toilets were arranged in long rows on the north and south ends of the lot. The construction of the houses and the

vaults was exceedingly flimsy. The latter were simply pits unprotected from flies. As can be seen from the picture on this page and the ones following, the buildings were well ventilated, both under the bottom—into the vaults—and over the doors or on the sides. The purpose of the holes no doubt was for light alone, but as they were unscreened they afforded an excellent avenue of entrance and exit for flies which were very numerous, not only around the privies, but in other parts of the camp as well. As far as I could learn no repellent as chloride of lime was used except in a few instances when it was furnished by the tenant. These buildings were often located within a few feet of the camps as can be seen from the pictures.

Showing the construction and "ventilation" of the privies. Note the wide open spaces under the bottom and over the tops of the doors.

Two more views showing the construction of the privies and the proximity to the tents and cottages. (The distance is much shorter than it would seem from the pictures.)

Another view showing the arrangement of swill barrels, privy and camps.

Before the opening of next season Mr. Harrington should be informed of the conditions which he allows to exist upon his camp ground and the remedies pointed out, and the State Board of Health should see to it that such improvements be made as to ensure the campers against a nuisance such as existed at Rocky Point during the last summer.

Respectfully submitted,

LESTER A. ROUND.

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THE IMPORTANCE OF A CONSIDERATION OF THE FIBER PROTEINS IN THE PROCESS OF BLEACHING COTTON.

BY BENJAMIN S. LEVINE.

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THE IMPORTANCE OF A CONSIDERATION OF THE FIBER PROTEINS IN THE PROCESS OF BLEACHING COTTON

THE nitrogen which is found in the ripe cotton fiber seems to have some bearing upon the yellowing of bleached cotton cloth, as was pointed out by J. C. Hebden in his paper read in Troy before the American Institute of Chemical Engineers.[1] He showed that in the process of bleaching cotton cloth after the first caustic boil 91.5 per cent. of the proteins were removed from the fiber, whereas of the fats and waxes only 20.4 per cent. were removed; and after the second caustic boil 91.7 per cent. of the proteins and only 64 per cent. of the fats and waxes were eliminated; the "chemick" and the "sour" together, he showed, removed 12.05 per cent. of the remaining protein impurities and 10.23 per cent. of the remaining fats and waxes. According to his analysis, after all the bleaching operations there were still left on the fiber 30.4 per cent. of the total fatty and waxy impurities, whereas of the total proteins there were left only 7.3 per cent., and as the cloth which he analyzed had undergone a "good bleach," he felt safe in inferring that it is the failure to remove the protein impurities from the cotton that results in a "bad bleach" or causes the yellowing of cloth in steaming or during storage.

So far as we know, the investigator above referred to was the first to point out the possibility that the proteins of the fiber played such a part in the bleaching of the cloth. Previous to this it has been believed that the fatty and waxy matters and especially the pectins were chiefly responsible for the yellowing of the fiber, since they formed water insoluble compounds, which remained on the fiber. The analysis of Hebden, however, showed that the calcium fixed on the fiber in the form of calcium salts was decomposed by the following acid treatments, and he explained the presence of the calcium on the fiber after the sour by the formation of a calcium cellulose similar to that of soda cellulose. The possibility of the formation of such a cellulose, he believed, was supported by the fact that cotton cloth which has been boiled and bleached did not produce as clear and as brilliant a turkey-red as cloth which had been simply boiled, because the former was not in a condition to fix calcium.

As the result of investigation in this line on cloth from different bleacheries, it occurred to us that an analysis of the growing cotton fiber with a view of determining the nitrogen and the fat and wax factors might reveal some points of importance. The determinations were carried out on Durango cotton raised on the United States Experiment Farm, San Antonio, Texas.[2]

The nitrogen factors were determined by the Kjeldhal-Gunning method, and the fat and wax factors by extracting samples of the fibers first by ether and then by alcohol. Some of the experiments were carried out in duplicates and some in triplicates, and the averages of the determinations were recorded as the final results. The figures given in the table below can only be regarded as approximating the absolute values of the nitrogenous and fatty and waxy constituents of the fiber; for the determination of exact values a much larger number of experiments should be performed. Nevertheless, they show the tendencies of the two factors and have, therefore, significance. The nitrogen determinations were made and recorded beginnings from the 14–16-day stage

[1] *Journal of Industrial and Engineering Chemistry*, September, 1914, Volume 6, No. 9, page 714.

[2] We wish to thank Mr. Rowland D. Mead, of the United States Department of Agriculture, for supplying us with the necessary samples of the cotton fibers.

up to the 36–38-day stage, whereas the ether and the alcohol extracts were recorded only beginning with the 22–24-day stage, because in the stages previous to this the amount of tannins extracted by both ether and alcohol were much higher than the fats and waxes.

Age in Days from Flowering	Nitrogen in Per Cent.	Protein N. × 6.25	Alcohol Extr. in Per Cent.	Ether Extr. in Per Cent.	Fat and Wax in Per Cent.
14–16	2.2300	13.938	....	....	....
16–18	1.9480	12.175	....	....	....
18–20	1.4250	8.907	....	....	....
20–22	1.1820	7.388	....	....	....
22–24	....	....	4.405	2.819	7.225
24–26	.3760	2.350	1.745	.775	2.418
26–28	.3195	1.997	1.398	.713	2.111
28–30	.3123	1.952	1.415	.800	2.215
30–32	.2657	1.661	1.522	.782	2.304
32–34	.2590	1.619	1.536	.709	2.245
34–36	.2503	1.564	1.403	.802	2.205
36–38	.1815	1.134	1.409	791	2.200

From the above table it may be seen that the fats and waxes showed neither a gradual increase nor a gradual decrease in their percentages, and in view of the fact that the fiber was growing, it seems reasonable to suppose that the fatty and waxy substances increased proportionally as did the fiber. We believe that were the numbers of the experiments large enough to give averages approximating the absolute values of the fatty and waxy factors, this point would have been brought out much clearer. But even from the determinations which we can report, it appears that the fats and waxes extended in an even and constant thickness over the fiber. If we accept the view that the function of these substances is to protect the fiber from external influences of weather and disease, that is that they are merely external coats of the fiber, the significance of such a proportional growth of these constituents becomes clear. If, however, the fats and waxes are phosphotides taking part in the growth, there would also be a proportional increase. The nitrogen figures, on the other hand, show gradual decrease in percentage with the increase of the age of the fiber. The sudden increase of the factor at the 20–22-day stage as compared with that of the 24–26-day stage may be due either to a rapid growth of the nitrogenous constituents of the fiber or to the adhering nitrogenous coloring matters of the parts of the boll which surrounded the fibers. If we limit ourselves to a consideration of the nitrogen figures of the samples representing only the higher stages of development of the cotton fiber even then we are permitted to assume that the nitrogen was deposited early in the lumen of the fiber and its absolute value remained constant. This assumption becomes more plausible when the nitrogen figures are multiplied by 6.25 to express the percentage of proteins present in the fiber. Most of this early and constant protein deposit remains in the lumen in the form of insoluble albuminoids and in the form of alcohol soluble proteins; some of it is utilized by the growing fiber, probably by the spiral forming the walls of the lumen. That the proteins of the fiber are of an insoluble nature is shown by the fact that the percentage of nitrogen of gray cloth as obtained by Hebden (0.191 per cent.) remained practically unchanged after the "steep" (0.192 per cent.), and that some of it exists in the fiber in the form of alcohol soluble proteins, is shown by the number which he obtained for nitrogen after extracting the cloth by ether and alcohol. The percentage, as shown in his table, was reduced from 0.191 per cent. to 0.161 per cent. The fact that the first caustic boil removed 91.5 per cent. of the protein content clearly points to the decomposing action of boiling alkali upon the albuminoids.

The 7.3 per cent. of total protein content remaining in the fiber after all the operations of the bleaching process can be considered as that part of the fiber proteins which has become an inseparable part of the wall of the lumen. The lowest percentage for fats and waxes (2.200 per cent.) obtained by us for the fiber taken directly from the field was considerable higher than that obtained by Hebden for fibers which were ginned, carded, spun and woven (1.405 per cent.). The removal of a large part of the fats and waxes by mechanical means during ginning, carding, spinning and weaving proves that these constituents form the outside cover of the fiber, and it is reasonable to suppose, therefore, that they do not

play as important a part in bleaching as is ascribed to them. The percentage of nitrogen in our experiment (0.1815 per cent.) was somewhat smaller than that obtained by Hebden for cotton in the form of cloth (0.191 per cent.) and points to the fact that, unlike the fats and waxes, the proteins of the fiber are not adventitious nor coating factors, but that they are within the lumen or are in part intimately bound to the fiber. As the proteins are of the insoluble kind, the above seems to justify the assumption of Hebden that in bleaching the removal of the proteins may be of more importance than that of the fats and waxes.

These results and the results of Hebden show the necessity of a careful investigation of the chemical nature of the fatty and waxy substance as well as of a further study of the effect of growth on these constituents of the cotton fiber.

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AN EPIDEMIC, SIMULATING TYPHOID, CAUSED BY A PARAGAERTNER ORGANISM.

BY GEORGE H. ROBINSON.

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AN EPIDEMIC, SIMULATING TYPHOID, CAUSED BY A PARAGAERTNER ORGANISM

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Epidemics of typhoid fever due to the drinking of sewage-polluted water show a marked similarity in the ratio of those affected with gastro-enteritis to the cases of clinical typhoid fever. Comparison of several epidemics of this nature indicate that 40-60 percent of those exposed to infection contract gastro-enteritis and 2-8 percent develop clinical typhoid. In epidemics in which food or milk, infected by typhoid carriers or incipient cases of typhoid, are the vehicles of infection, the incidence of typhoid is much higher and the accompanying gastro-enteritis is generally absent. During the investigation of the following epidemic a study was made of the cases of enteritis as well as of those pronounced as typhoid.

In the fall of 1913 there occurred an epidemic simulating typhoid among the Rhode Island party attending the Perry Centennial at Put-In-Bay, Lake Erie. The itinerary of the party and the sanitary aspect of the epidemic are thoroughly discussed in De Valin's report[1] and that of Swarts.[2] The party, consisting of 422 members, left Providence September 8 and upon reaching Buffalo was quartered on two steamers, the Rochester and the Greyhound, 300 being accommodated on the former and 122 on the latter. The courses of both boats were practically the same and both parties attended the same functions. The delegation returned to Providence on September 14.

Reports from 235 members of the party on the Rochester showed 122 cases, or 52 percent, of gastro-enteritis. Of the total 300 on board 42, or 14 percent, developed clinical typhoid, including 6 deaths. The party on the Greyhound yielded upon inquiry 12 mild and temporary attacks of diarrhea" such as any change of water or diet might cause. In many ways this epidemic resembled others of infected-water origin, except that the number of typhoid cases was larger than the average.

1. Publ. Health Rep., 1913, 28, p. 2761.
2. Health Bull. Rhode Island State Board of Health, 1914, 3, p. 1.

All possible sources of infection were carefully investigated by Dr. C. V. Chapin of the Providence Health Department, Dr. G. T. Swarts of the State Board of Health, and Dr. de Valin of the U. S. Public Health Service. The most salient points of the inquiry were: On September 11 the Rochester pumped water for drinking purposes from Lake Erie at points "considered decidedly unsafe;" during the trip a cook had performed his duties while suffering from typhoid fever; an electrician on the Rochester was taken sick with typhoid August 16; a maid and a water-tender both left the boat on September 20 feeling unwell and were later diagnosed as cases of typhoid. The maid died without giving a positive agglutination test with typhoid bacilli.

A check on the manner of infection is supplied in the case of members of the Newport Artillery, one of the organizations taking passage on the Rochester. The 83 members of this company were previously warned against the dangers of drinking water and so drank sparingly. None of them was affected with enteritis, but twenty-two of them developed typhoid symptoms about two weeks after their return. Eliminating those who drank little water, we find at least 122 cases of enteritis, or 80 percent, of the 152 members of the party who did drink freely of the water.

SYMPTOMS

The most common symptoms were enteritis, diarrhea, malaise, and fever. "Forty-three presented symptoms varying from a mild paratyphoid, to a perfect clinical, typhoid character." Four of the patients were admitted to the Rhode Island Hospital, extracts from whose records follow.

CASE 16.—Admitted Oct. 4. Diagnosis, typhoid fever with lobar pneumonia complication.

Patient began to complain of headache, abdominal distress, pain in back, and fever, September 25. Went to bed September 28 because he felt weak. Appetite poor, bowels regular. No nose bleed. No cough. Stated that he had had a similar attack six years before. Malaria four years before.

Well developed and well nourished. Face flushed, lips cracked. Tongue thickly coated on dorsum, red and moist at tips and edges. On chest and abdomen are a few circumscribed papules varying from the size of a pin to half the size of a pea, which disappear on pressure. No râles in chest. No murmurs of heart. Leukocytes 14,000, polymorphonuclears 81 percent. No agglutination of typhoid bacilli.

Oct. 6.—Irrational, tosses about in bed, gets up if not watched. Spleen not palpable.

Oct. 7.—More delirious, temperature 103 F.

Oct. 8.—Temperature 101 F. yesterday, during the night rose to 104 F.; pulse 130. Abdomen soft. No blood in stools. Impaired resonance in right lower lobe of lung. Died.

CASE 21.—Admitted Oct. 8. Diagnosis, typhoid fever.

Patient had headache and diarrhea with cramps, Sept. 14. This lasted four days after which he felt as well as ever except that he became tired easily. On Oct. 4, he went on a canoe trip and became thoroughly exhausted. Since then has had severe headache. No chills and no fever. Had typhoid fever with a relapse nine years ago.

No râles, no heart murmurs, no tenderness on abdomen, no glandular enlargement. Leukocytes 5,600, polymorphonuclears 63 percent, mononuclears 33.

Oct. 10.—Temperature reached highest point, 103.5 F.

Oct. 15.—Many typhoid bacilli in blood culture.

Oct. 20.—Temperature normal.

Nov. 4.—Discharged.

CASE F.—Admitted Oct. 4. Diagnosis, typhoid fever.

Felt sick on return trip Sept. 13. Headache, and later in the day watery and frequent bowel movements and vomiting. Had profuse diarrhea up to Oct. 4. Has cramp in abdomen before every bowel movement and pain in the middle of the back almost constantly. No blood in stools. Has lost about 8 pounds. No nose bleed. Bad taste, appetite poor. High fever.

No tenderness in abdomen. Spleen palpable. No rose spots. Leukocytes 6,400, polymorphonuclears 70.5 percent, mononuclears 28.

Oct. 7.—Only complaint is dizziness. Spleen greatly increased in size. Serum agglutinates typhoid bacilli.

Oct. 10.—General condition good. Fever declining.

Oct. 13.—Patient at times irrational and slightly delirious. Temperature again elevated. No sign of perforation or hemorrhage.

Oct. 16.—During last three days has been profoundly toxic. During last twenty-four hours heart has been failing. Died.

CASE 40.—Admitted Oct. 29. Diagnosis, typhoid fever.

Symptoms began Oct. 22 with headache and loss of appetite, slight cough, but no nose bleed; slight fever, no chills, no vomiting, no pain in abdomen or chest or joints or back, no night sweats; bowels moved frequently last four days. Headache and malaise. Took complete typhoid inoculation one year ago.

Abdomen has suggestive rose spots. No tenderness or rigidity or spasms. Lower end of spleen felt. Leukocytes 11,000. Serum agglutinates typhoid bacilli.

Nov. 2.—Blood culture negative. Comfortable. Diarrhea stopped.

Nov. 5.—Feeling well but temperature is again 103 F. at evening.

Nov. 8.—Is beginning to feel listless and a little weak; no headache and no abdominal pain.

Nov. 11.—Temperature up. Weak.

Nov. 21.—Temperature normal.

Dec. 11.—Uneventful recovery. Discharged.

AGGLUTINATION

The first agglutination of typhoid bacilli by the serum of any of the party was obtained September 28. This patient, however, had received prophylactic treatment for typhoid a few months previously, but during the investigation of his case it became evident that there was an infection of some kind among the members of the party. The last specimen of blood from this epidemic was sent in November 9. During this time 39 specimens of blood were received at the city and state health laboratories from those residing in or near Providence who attended the Perry Centennial.

These yielded the following results:

	City Laboratory	State Laboratory
Positive to B. typhosus alone	4	6
Partial to B. typhosus alone	2	0
Positive to B. paratyphosus (B) alone	0	1
Positive to both	2	4
Partial to B. typhosus and suspicious to B. paratyphosus (B)	0	1
Negative to both	8	11
Total	16	23

The large percentage of the cases which showed a reaction with B. paratyphosus (B) was noticeable throughout the epidemic.

EXAMINATIONS OF FECES

Specimens of feces were obtained from nineteen of the men in Providence. Through the efforts of Dr. C. V. Chapin specimens of feces and blood were obtained from the cook on the Rochester. About two months after the infection, through the kindness of Dr. Swarts, specimens were obtained from the convalescents in the Newport Hospital. A technic which had proven very successful was used in the examination of the feces. Small portions of the material were incubated in lactose peptone bile for six to twelve hours and then the bile culture was plated on Endo's medium. No typhoid organisms were isolated from any of the specimens. An organism was found in ten of the specimens, however, which resembled both typhoid and paratyphoid types in some respects, and differed in others.

This organism was obtained from feces of the cook of the Rochester and also from Cases 16 and 21.

It will be seen that the organism is similar to typhoid in that it does not ferment dulcite and does ferment dextrin. Like B. paratyphosus (B) it produces visible amounts of gas and ferments arabinose.

TABLE 1

RESULTS OF CARBOHYDRATE FERMENTATION TESTS OF CULTURES ISOLATED FROM FECES

Culture	Dextrose	Lactose	Gas in Dextrose	Maltose	Mannite	Saccharose	Raffinose	Dulcite	Arabinose	Galactose	Levulose	Dextrin	Glycerin	Inulin	Salicin	Gelatin	Indol	Milk
Typhoid.....	+	—	—	+	+	—	—	—	—	+	+	+	—	—	—	—	—	+
Paratyphoid (A)	+	—	+	+	+	—	—	+	+	+	+	+	—	—	—	—	—	+
Paratyphoid (B)	+	—	+	+	+	—	—	+	+	+	+	+		—	—	—	—	+
13.2.....	+	—	+	+	+	—	—	—	+	+	+	+	—	—	—	—	—	+
15.1.....	+	—	+	+	+	—	—	—	+	+	+	+	—	—	—	—	—	+
16.3.....	+	—	+	+	+	—	—	—	+	+	+	+	—	—	—	—	—	+
21.14.....	+	—	+	+	+	—	—	—	+	+	+	+	—	—	—	—	—	+
22.4.....	+	—	+	+	+	—	—	—	+	+	+	+	—	—	—	—	—	+
24.1.....	+	—	+	+	+	—	—	—	+	+	+	+	—	—	—	—	—	+
30.6.....	+	—	+	+	+	—	—	—	+	+	+	+	—	—	—	—	—	+
33.29.....	+	—	+	+	+	—	—	—	+	+	+	+	—	—	—	—	—	+
45.1.....	+	—	+	+	+	—	—	—	+	+	+	+	—	—	—	—	—	+
54.2.....	+	—	+	+	+	—	—	—	+	+	+	+	—	—	—	—	—	+
58.2.....	+	—	+	+	+	—	—	—	+	+	+	+	—	—	—	—	—	+

Integral numbers of the cultures refer to the number of the case; the decimal refers to the number of the culture from the case. The sign + indicates the production of acid except in the column indicating gas production. In the column under gelatin, — signifies no liquefaction during sixty-three days.

It is about the size of the typhoid bacillus, actively motile, and is gram-negative. According to its cultural characters and carbohydrate reactions, this organism would be classified as a member of the intermediate group. It could not be reckoned as a true gaertner organism on account of its production of permanent acidity in litmus milk. Evidently, it is one of the paragaertner organisms.

SERUM REACTIONS.—These cultures were tested with the serum of the patients from whom they were isolated, as well as with known typhoid and paratyphoid (A) and (B) sera. No reaction was obtained except in the case of the cook (Case 33). Cultures obtained from him agglutinated with his serum at a dilution of 1:60. Two rabbits were immunized against Culture 16.3 obtained from a fatal case. Five inoculations of 1 c.c. of killed twenty-four-hour broth cultures at intervals of 7 days in one rabbit and 5 days in the other failed to produce a serum reacting strongly with the organism injected or any of the other organisms isolated during the epidemic. One of the sera did produce an agglutination at a dilution of 1:10. Both sera, however, gave a positive reaction with B. typhosus at a dilution of 1:60. This would account for the positive agglutination tests among those of the party who were infected. The organism is evidently one which stimulates the formation of agglutinins slowly. Those patients who did not give positive agglutination tests had probably not been infected long enough.

PATHOGENICITY FOR LABORATORY ANIMALS.—The pathogenicity of the different strains evidently varied. Each strain was inoculated into a mouse but several of them did not succumb. Culture 16.3 isolated from one of the fatal cases seemed to be most pathogenic.

Mouse 2.—Weight, 20 gm. Inoculated subcutaneously with 0.2 c.c. twenty-four-hour broth culture of 16.3; death in 12-18 hours; congestion about point of inoculation, lungs somewhat congested. Organism isolated in pure culture from liver, lungs, heart, and kidneys.

Mouse 21.—Weight, 25 gm. Inoculated subcutaneously with 0.25 c.c. twenty-four-hour broth culture of 16.3; evidently sick and feverish after forty-eight hours; diarrhea; died after fifty-five hours; lymph glands hemorrhagic, congestion at point of inoculation. Isolated organism from heart, liver, lung, stomach, and feces.

Mouse 12.—Jan. 20, fed bread soaked in twenty-four-hour broth culture of 16.3; Jan. 26, fed bread soaked in twenty-four-hour broth culture 16.3; Jan. 30, drank about 3 c.c. of twelve-hour-broth culture; Feb. 3, drank about 1 c.c. of twenty-four-hour broth culture; Feb. 6, looked sick; Feb. 7, died, eight days after drinking broth culture. Axillary and lumbar lymph glands swollen and

congested, gall-bladder very full, spleen apparently normal, diarrhea. Serum would not agglutinate culture. Organisms isolated from gall-bladder, small intestine, urinary bladder, and kidney. Heart and liver sterile.

Mouse 15.—Drank 2 drops twenty-four-hour broth culture of 16.3; died ten days later; appearance of organs normal. Organism isolated from heart and liver.

Guinea-pig 15.—Drank 2 c.c. twelve-hour broth culture of 16.3; died six days later; gall-bladder much enlarged, suprarenals enlarged, abundant peritoneal fluid, spleen normal, other organs apparently normal. Organism isolated from heart, liver, kidney, peritoneal fluid, gall-bladder, urinary bladder, and small intestine.

Guinea-pig 16.—Weight, 305 gm. Inoculated intraperitoneally, 1 c.c., twelve-hour broth culture of 16.3; died four days later; abscess at point of inoculation, peritoneal cavity full of a white fluid, intestine and mesenteries inflamed, spleen apparently normal. Organism isolated from heart, liver, and peritoneal fluid.

Mouse 7.—Weight, 22 gm. Inoculated subcutaneously with 0.25 c.c. twenty-four-hour culture of 54.2; died after five days; congestion at point of inoculation; lymph glands swollen. Organism isolated from heart, liver, lungs, stomach, large intestine, and point of inoculation.

Mouse 14.—Drank 0.5 c.c. twenty-four-hour broth culture of 54.2; a week later refused to eat, evidently sick; died next day; appearance of organs normal. Organism isolated from stomach, small intestine, and large intestine. Heart, liver, and kidneys sterile.

Mouse 6.—Weight 22 gm. Inoculated subcutaneously with 0.25 c.c. twenty-four-hour culture of 33.29; died after fifteen days; spleen swollen; point of inoculation congested. Organism could be isolated only from point of inoculation.

Mouse 19.—Weight 20 gm. Inoculated subcutaneously with 0.2 c.c. twenty-four-hour broth culture of B. paratyphosus (B); died in forty hours; appearance of organs normal; lymph nodes congested; mesenteries congested. Organism isolated from heart, liver, kidneys, small intestine, and large intestine.

Examination of the organs in fatal cases showed no marked pathological changes. When infection occurs through the digestive tract, it is capable of producing enteritis and diarrhea in mice and guinea-pigs.

Mouse 12 was apparently unaffected ten days after eating solid food infected with culture of 16.3 but died on the eighth day after taking the organism in a liquid. Two other mice (Mice 13 and 15) died seven and ten days, respectively, after drinking liquid culture. Infection through the intestine is best accomplished by means of liquid rather than solid food.

PROPHYLACTIC TREATMENT OF MEMBERS OF THE PARTY

Sixteen members of the party had taken typhoid immunizing treatment less than a year before this epidemic. Of these, five were affected with enteritis and diarrhea and one (Case 40) was considered at the

hospital as having typhoid. Two other cases considered as typhoid fever (one of them Case 21) gave definite histories of having had it before. In view of the opinion among medical men that one attack of typhoid protects against another and that the prophylactic treatment protects for at least two years, these data would confirm our findings that it was not the typhoid bacillus with which the members of the party were infected.

SUMMARY

An epidemic has occurred resembling in many ways a water-borne typhoid epidemic. A careful bacteriological examination of feces of patients was negative for typhoid, but an organism was found which seemed to have the cultural characteristics of the paragaertner group.

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ISOLATION, IDENTIFICATION, AND SERUM REACTIONS OF TYPHOID AND PARATYPHOID BACILLI.

BY GEORGE H. ROBINSON.

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ISOLATION, IDENTIFICATION, AND SERUM REACTIONS OF TYPHOID AND PARATYPHOID BACILLI.*

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Introduction: Statement of Problem.

I. Methods of isolation of typhoid and paratyphoid bacilli: (*a*) Enrichment media; (*b*) Differential media; (*c*) Comparison of different methods.

II. Methods of identification: (*a*) By carbohydrate media; Preparation of media. (*b*) By serum reactions: 1, Specific agglutinins; 2, Group agglutinins. (*c*) Classification of organisms.

III. Diagnosis by serum reactions: (*a*) Reaction of normal blood; (*b*) Partial reactions; (*c*) Mixed infections.

IV. Serum reactions during vaccine treatment.

It has been our experience in the routine diagnosis of typhoid fever that problems are constantly arising which need more experimental data. In order to gain more information on some of these questions the work dealt with in this paper was undertaken. The following subjects were considered:

I. The development of an accurate, quick, and easy method of isolating typhoid and paratyphoid organisms.

II. The value of cultural and serum reactions in the identification of these cultures.

III. The proper interpretation of serum reactions obtained in the routine examination of suspected blood specimens.

IV. A comparison between the serum reactions produced as the result of vaccination and those in clinical cases.

I. METHODS OF ISOLATION.

Considering the fact that both typhoid and intermediate organisms grow readily upon artificial media, methods devised for the isolation of typhoid are equally applicable to the intermediate group. Hurler (1912) has given a detailed

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description of the members of the intermediate group upon special media.

As any material, whether feces, water, sewage, or food likely to contain typhoid bacilli, also contains a vastly greater number of other organisms, the isolation of the desired organism much resembles the proverbial finding of a needle in a haystack. A great many methods and media have been devised, some of them giving fair results but more of them not attaining the degree of perfection desired. In general, the media prepared for the isolation of typhoid aims at one or both of two ends: isolation by inhibition of other organisms or isolation by favoring the typhoid in some cultural character.

(*a.*) Enrichment media. — By the first method, inhibition is brought about by means of some weakly bactericidal substance. Carbolic acid was first used but this was found to inhibit the typhoid bacilli almost as much as the others. A method suggested by Elsner (1889) was the addition of one per cent potassium iodide. This method has been discarded. Drigalski and Conradi (1902) prepared a medium containing crystal violet as an inhibiting agent. Loeffler (1906) devised a plate medium containing malachite green. Peabody and Pratt (1908) used malachite green in an enrichment broth before plating upon Conradi-Drigalski medium. Brilliant green was recently suggested by Browning, Gilmour, and Mackie (1913). Jackson and Melia (1909) recommend the use of lactose bile as an enrichment broth. The authors say that it will inhibit all organisms except B. coli and B. typhosus and that eventually the typhoid will overgrow the colon organisms.

(*b.*) Differential media. — Of the media differentiating typhoid by favoring some cultural characteristic, there are several worthy of note. Hiss (1897) advocated the use of a gelatin-agar medium upon which the typhoid grew in small colonies having fringes of thread-like projections. Endo's (1904) medium contains fuchsin and lactose. Fermentation

of the lactose turns the fuchsin red, thus distinguishing the colon from the non-fermenting typhoid. Hesse (1908), relying on the active motility of typhoid organisms, prepared a semi-liquid medium containing only .5 per cent agar. The typhoid present a large ringed colony; colon a smaller opaque appearance.

For confirming a suspicious colony, Hiss (1902) has also devised a tube medium containing .5 per cent agar, eight per cent gelatin and one per cent dextrose. Typhoid bacilli give a characteristic growth along the stab, whereas colon will form gas bubbles along the line of inoculation. Russell (1911.) has described a medium much better than that of Hiss for general work, containing one per cent litmus, one per cent lactose, and .1 per cent dextrose, added to 1.5 per cent agar. Typhoid organisms produce a characteristic coloring.

(*c.*) Comparison of different methods. — Most investigators agree that some of these media are no better than others, but that the advantage lies wholly in the familiarity of the worker with one particular medium. Thus, in selecting a method of procedure for a routine examination of feces regard should be taken for speed, accuracy and ease of preparation. Owing to the fact that the typhoid bacilli are few in number in comparison with other intestinal organisms, an enrichment medium seems desirable before plating.

Enrichment media: The brilliant green medium suggested by Browning, Gilmour and Mackie was tried but without success. A concentration of brilliant green sufficient to inhibit B. coli and other forms would also inhibit typhoid. Conversely, a dilution permitting the growth of typhoid also allowed B. coli to flourish.

ENRICHMENT IN BRILLIANT GREEN BROTH.

Amount of B.G. Solution.	Typhoid.	Para A.	Para B.	B. Coli.	Approximate Dilution of B.G.
.04 cc.....	O	O	Inf.	9	1 : 1,250,000
.08 "	O	O	Inf.	O	1 : 625,000
.12 "	O	O	Inf.	O	1 : 416,000
.16 "	O	O	Inf.	O	1 : 310,000
.22 "	O	O	100	O	1 : 230,000
.3 "	O	O	50	O	1 : 170,000

The brilliant green solution was made at a dilution of 1 : 10,000. Amounts indicated were added to broth (reaction + 0.5) tubes containing five cubic centimeters. A loopful of twenty-four-hour broth culture of the organisms to be tested was added to each tube. After twenty-four hours' incubation a loopful was removed from each of the tubes and plated on Endo's medium. When possible the number of colonies was indicated; otherwise the number was expressed as infinity (Inf.).

Amount of B.G. Solution.	Typhoid.	Para A.	Para B.	B. Coli.	Approximate Dilution of B.G.
.1 cc.	+	+	+ +	+ +	1 : 5,000,000
.2 "	+	+	+ +	+ +	1 : 2,500,000
.3 "	+	+	+ +	+ +	1 : 1,500,000
.4 "	−	+	+ +	+ +	1 : 1,250,000
.5 "	−	−	+ +	+	1 : 1,000,000
.6 "	+	−	+ +	−	1 : 700,000

The brilliant green solution was made at a dilution of 1 : 100,000. The amount of growth was judged by the clouding of the medium. Grubler's "Brilliantgrün" was used.

The lactose bile medium of Jackson and Melia was tried and gave very encouraging results, although nothing like the claims of the authors could be realized. A loopful of normal feces and a loopful of a twenty-four-hour broth culture of typhoid were inoculated into a lactose bile tube and thoroughly shaken. After different intervals of time a loopful of the bile culture was removed and plated on Endo's medium and litmus-lactose agar. The lactose agar was used in order to determine whether or not any of the constituents of the Endo's medium inhibited growth. Counts were practically the same on both media. In the first three readings the proportion of the lactose fermenting to the non-lactose fermenting organisms is given. Where the number of colonies was too great to count it is expressed as infinity (Inf.). The non-lactose fermenters were found by agglutination tests to be typhoid. Time is expressed as the number of hours from inoculation into bile to time of plating. Duplicate experiments (A and B) were recorded. Cultures were kept at 37° C.

ENRICHMENT IN LACTOSE BILE.

Medium.	A.		B.		Time.
	Lactose Fermenters.	Typhoid.	Lactose Fermenters.	Typhoid.	
Endo's	1	1	5	1	3 hours.
L.L.A.	1	1	5	1	3 "
Endo's	5	1	Inf.	0	6 "
L.L.A.	Inf.	0	Inf.	0	6 "
Endo's	Inf.	0	Inf.	0	12 "
L.L.A.	Inf.	0	Inf.	0	12 "
Endo's	Inf.	0	Inf.	0	18 "
L.L.A.	Inf.	0	Inf.	0	18 "
Endo's	100	0	100	0	24 "
L.L.A.	100	0	100	0	24 "
Endo's	30	3	100	1	36 "
Endo's	150	0	200	0	60 "
Endo's	200	0	200	15	84 "
Endo's	200	0	12	0	96 "
Endo's	150	0	50	0	108 "

It was found that in from three to twelve hours a loopful of the surface liquid contained a greater proportion of typhoid organisms than at any other time. After twenty-four hours the number of all organisms began to decrease and the typhoid also disappeared, contrary to the claim of the authors.

Plate media: The ideal plate medium is one which will permit the distinction of the greatest number of colonies and will not inhibit any of the desired organisms. Hiss' plate medium is undoubtedly excellent for growth, but various investigators have found that the distinguishing thread formation of typhoid is variable and careful search must be made

with a microscope for suspicious colonies. Hesse's medium is very favorable for growth of typhoid and was given an extensive trial in this work and previously. It is easy to prepare and easy to manipulate. It does not, however, seem to be particularly valuable in the isolation of typhoid from feces. Owing to its semi-liquid consistency all colonies are of large size, allowing in a thickly seeded culture only the last few dilutions to be clearly distinct. If the typhoid bacilli are greatly outnumbered the chances of finding them in the higher dilutions are small. When urine or blood containing a pure culture of typhoid was examined, Hesse's proved to be a most efficient and satisfactory medium. Litmus lactose agar gave poor results owing to the spread of the acidity throughout the medium. Endo's fuchsin agar, made according to the method described in Standard Methods of Water Analysis (1912), gave very satisfactory results. By the method of inoculating with the bent rod a large number of organisms can be spread upon a plate. The high percentage of agar (three per cent) prevents spread of the acid and the colorless typhoid colonies are easily distinguished by the naked eye. On this medium it is quite possible to detect typhoid colonies when outnumbered a thousand to one. Endo's medium used in connection with bile constituted the most efficient and expedient method of isolation. Enrichment in bile for six hours and plating on Endo's for eighteen to twenty-four hours was sufficient time to establish the presence or absence of typhoid. Inoculation of suspicious colonies into Russell's medium was found valuable as a check on the Endo's medium.

Eight specimens of feces from persons giving positive Widal reactions were inoculated into bile and after three, six, twelve, eighteen, and twenty-four hours portions of the bile culture were inoculated into Endo's medium, Hesse's medium, and litmus lactose agar. Three yielded cultures of typhoid on Endo's medium after six hours and were negative on the other media. One was positive on litmus lactose agar after six hours and on Endo's medium after twelve hours. The urine from one case gave a pure culture of typhoid bacilli,

while the feces were negative. In the other three cases no typhoid bacilli could be found.

II. METHODS OF IDENTIFICATION.

At present we have but two convenient methods of identifying isolated organisms of the typhoid-colon group; by means of their cultural characteristics in carbohydrate media and by their serum reactions. The acid agglutination method of Michaelis (1911) may have possibilities but at present it seems unsuited to routine work.

(*a*.) By carbohydrate media. — The power of carbohydrate fermentation is considered by some to be one of the most constant means of identification. Typhoid is characterized by acid production without visible evolution of gas in dextrose, mannite, maltose, levulose, galactose, dextrin, and sorbite, with no change in lactose, saccharose, dulcite, salicin, and arabinose. MacConkey (1905) states that typhoid ferments raffinose while others have found that there is no change. It has been our experience, however, that the sugar is not affected.

Penfold (1911) and Twort (1907) show that a typical strain of typhoid can be induced to produce variations under cultural conditions. Twort, after two years of cultivation on lactose media, was able to produce a lactose fermenting strain. Penfold with less difficulty was able to produce mutants toward dulcite, isodulcite, and arabinose. How frequently these variations occur in nature there is no record, but since strains differing in their reaction towards glycerin occur it is quite possible that variations in other directions exist. If a lactose fermenting strain did occur under natural conditions its presence would never be suspected from the isolation plates. The mere fact that these mutants are possible might tend to make one skeptical of slightly discordant results.

The intermediate group of organisms produce acid and gas from dextrose, mannite, dulcite, maltose, levulose, galactose,

and sorbite. There is no change in lactose, saccharose, raffinose, salicin, glycerin, erythrite, and inulin. These reactions refer only to the paratyphoid and Gaertner strains.

Paratyphoid organisms were induced by Penfold to form variants from some of the usual types of carbohydrate reactions. If these bacilli are, as some investigators believe, off-shoots of either the typhoid or colon types they should be particularly liable to mutation. The "gas-los" strain isolated by Wagner (1913) from the blood was no doubt a mutant occurring under natural conditions. Cultural characteristics alone indicated that this strain was neither typhoid nor paratyphoid. In spite of the fact that there are variations from the usual type of carbohydrate fermentation, these reactions must be relied upon to a great extent for want of a better method.

Preparation of sugar media: Whether or not sugars are broken down during sterilization under steam pressure has not been satisfactorily determined. In order to avoid any complications on this score the carbohydrate media employed in this work were filtered through Berkefeld candles. A two per cent solution of Witte's Peptone having a reaction of +0.6 to phenolphthalein was made and sterilized in the autoclave. A two per cent sugar solution was made and filtered into sterile flasks. The two solutions were mixed in equal parts and .05 per cent of azolitmin added. The resulting sugar broth was placed in sterile tubes (10 x 43 millimeters) and incubated twenty-four hours to insure sterility.

The organisms to be tested were inoculated into nutrient broth and a loopful of twenty-four-hour culture was transferred into the sugar broth tubes. This method insured the inoculation of an approximately equal number of organisms into each tube, which cannot be done when inoculating directly from solid media into broth. Growth in this medium took place readily and abundantly. Acid production could be easily detected by the reddening of the medium. Gas production was indicated by a ring of foam around the surface of the liquid if care was taken not to violently agitate the tubes. A test of all cultures for gas production was

made in dextrose broth with the inverted vials. The results with media prepared in this manner corresponded with the reactions obtained by others with the fractional sterilization which they probably used. Liquefaction of gelatin and indol production was tested with all cultures.

(*b.*) By serum reactions. — Specific agglutinins: The agglutination of typhoid bacilli is, theoretically, a specific reaction. The organism is, as a rule, very sensitive to agglutinin; the reaction with immune serum taking place often in a dilution of one to a hundred thousand and in some cases at one to several million. There is said to be but little difference in the agglutinability of different strains which agglutinate at all. Several hundred tests during the routine work in this laboratory showed no difference in two strains: one Worcester, the other Rawlings. In work to be referred to later no difference was noted in the agglutination of homologous and heterologous strains. Strains are frequently isolated which agglutinate only after repeated transfers on artificial media. Recently McIntosh and McQueen (1914) have reported work with a strain which was incapable of agglutination. When an animal was immunized with this strain the serum failed to react with it, yet a strong reaction was obtained with other typhoid cultures. Strains of the intermediate group have been found which failed to agglutinate. Torrey (1913) reports such strains. No specific agglutinins were found in the blood of the dogs from which the organisms were isolated. These mutants from the prevailing type seem to be observed only incidentally. Undoubtedly a great number of these atypical strains escape unnoticed.

Group agglutinins: In agglutinating powers the members of the intermediate group are similar to typhoid. The specificity of the reaction is not absolute with these organisms owing to the interaction of the group. Boycott (1906) reports that out of eighty-six typhoid immune sera fifty-nine per cent showed agglutinating power towards members of the intermediate group, fifty-five per cent toward Gaertner,

forty-one per cent toward B. paratyphosus B, and twelve per cent toward B. paratyphosus A. Ten of these (or nine per cent) showed a reaction toward all four strains. Sera immune to one of the members of the group often possess secondary agglutinins for several of the others. It was not usual, however, for the secondary agglutinins to reach more than fifty per cent of the primary.

Castellani (1902) has developed a method for separating the primary from the secondary agglutinins. He found that if a typhoid immune serum, which would agglutinate also a member of the intermediate group, was saturated with typhoid organisms, not only were the typhoid agglutinins removed but also the secondary ones. On the other hand, if the serum was made immune against two organisms, then absorption with one of them would not remove the agglutinins from the other. To express the reaction graphically in the terms of Durham (1901): let A + B + C + D + E represent the agglutinins for the typhoid bacillus and C + D + E + F that for an intermediate organism. Then after absorption with typhoid the mechanism for a reaction with an intermediate organism would be incomplete if the serum was immune to typhoid only. After the serum was immunized against two organisms as in a mixed infection then there would be a double quantity of C + D + E and, after absorption with typhoid, agglutinins for the intermediate organism would be intact. We might expect organisms culturally and morphologically similar to show inter-reactions, but we are at a loss to explain the inter-reactions of widely different organisms. Frost (1910) reports strains of Pseudomona proteus which will agglutinate at higher dilutions with typhoid immune serum than will the typhoid bacillus itself. Boycott (1906) describes an organism "Valerie 21" culturally quite distinct from any members of the typhoid-intermediate group and yet agglutinating at high dilutions with both typhoid and intermediate sera. Group agglutinations, therefore, although they may have some significance are uncertain and unreliable.

Cultures obtained in the routine examination of feces

which conform to the cultural reactions of typhoid or paratyphoid are tested for their agglutinating powers with a known serum. The serum used is of high titer obtained from the Mulford Company. The serum is dissolved in normal saline: one part of serum to fifty parts of saline. Tests are made microscopically using a dilution of 1:100. Results are observed at the end of one hour.

(*c.*) Classification of organisms. — With so many variations from the type organisms the question of differentiating species is an important one. The typhoid bacillus, as we know it, is characteristic. Its failure to produce gas during carbohydrate fermentation, the carbohydrates upon which it acts, and the specificity of the serum reactions, are distinctive enough for practical purposes. A strain varying in one respect is likely to conform to type in other respects. This assumption is made in spite of the fact that variability is one of the characteristics of bacteria and also ignoring the possibility of several mutants occurring at once. Few if any bacterial strains have been found closely resembling the typhoid type, hence cultural and serum reactions are of especial value in the classification of the typhoid organisms.

The classification of the intermediate group is more complex. The criteria for placing an organism in this group are vague and ill-defined. The cultural characteristics of the Gaertner organism are generally considered as the type for this group. Savage (1913) would divide the group into the true Gaertner and paragaertner types by an extended set of carbohydrate tests. He also states that the paragaertner organisms are for the most part non-pathogenic. Paratyphoid A would thus be considered in the paragaertner sub-group, while Paratyphoid B would be a species of true Gaertner. Bradley (1912) claims that "the sub-division of A and B types cannot be strictly maintained biochemically as linking types are found." Henderson Smith (1913) would classify the group primarily by cultural characteristics. The true type organisms are then divided by agglutination tests into three groups: one containing B. gaertner,

a second B. paratyphosus B, and a third B. suipestifer. Bainbridge (1911) would identify and classify the organisms conforming culturally to the Gaertner type by agglutination and absorption tests.

Since the agglutination reaction is not specific among the members of the intermediate group this test alone cannot be relied upon. Thus, in this group, classification must depend largely upon the carbohydrate reactions. It is the most practical method available at present. It need not be an arbitrary method but is elastic enough to allow the addition of new species which undoubtedly exist.

III. DIAGNOSIS BY SERUM REACTION.

In the routine diagnosis of suspected blood we use three strains of organisms, viz.: "Worcester" Typhoid, Paratyphoid A (Army), and Paratyphoid B (Army). All are actively motile and free from spontaneous agglutination. Three reports are given: positive, the complete, closely clumping, "windrow formation;" partial, in which the organisms show some tendency towards agglutination, and negative, being the same as the control test. Specimens to be tested are drops of patient's blood dried on a glass slide. The blood is suspended in normal saline and made up to match the color of a known suspension of normal blood. A dilution of 1 to 60 was employed in the tests and a test showing complete reaction within an hour was considered positive.

During the six months July–December, 1913, two hundred and forty-five specimens of blood from suspected typhoid patients were examined. These were reported as follows:

Positive to Bacillus typhosus alone	47	19%
Partial " " " "	40	15%
Positive to Bacillus paratyphosus B alone	5	2%
Partial " " " " "	4	2%
Positive to both	8	4%
Positive to B. typhosus and partial to B. paratyphosus B,	2	1%
Negative to both	139	57%

Most of the cases reported positive were confirmed by the physician from the clinical symptoms. Occasionally a complete or partial reaction would be obtained with blood from a person who was either not sick or developed some other disease. The specimens of blood were taken very early in the course of the disease in most cases, which seemed a plausible reason for getting partial or incomplete reactions.

A number of positive bloods sent in by physicians were tested as to the limits of the agglutinating power. It must be remembered that the majority of them were taken during the first week of illness. It was found from the history of the cases that those having the highest titer had been sick longest. The highest titer of those tested was 1200 and from that down to those which gave a partial reaction at 1 : 60. The greater number of those examined were found to have a titer between 100 and 200. These results would confirm the use of 1 : 100 as the most favorable dilution.

(*a.*) Reaction of normal blood. — In order to get a suitable check on the reaction it seemed best to examine specimens of normal blood. Specimens were obtained from University students according to Wright's method in capillary pipettes. The blood was allowed to stand until the clot formed. Then the clear serum was removed and accurate dilutions were made with capillary tubes. Tests were first made at a dilution of 1 : 10 and if positive the dilution was increased until the agglutinating limit was reached. All tests were made microscopically and results observed at the end of one hour. Results were as follows:

Number of specimens examined	103
Positive at 1 : 10 for B. typhosus	29
" " 1 : 20 " " "	11
" " 1 : 40 " " "	6
" " 1 : 60 " " "	0
" " 1 : 10 " B. paratyphosus B.	6
" " 1 : 20 " " " "	2
" " 1 : 40 " " " "	0

Only one reported a previous attack of typhoid fever five years ago and this blood was only positive at 1 : 10. Another had taken one inoculation of typhoid vaccine and his blood was negative. From these results it was evident that no test should be made at a dilution of less than 1 : 60 and that probably 1 : 100 would be less prone to complications with natural agglutinins. Park (1910) reports that in the New York Health Department a dilution of 1 : 10 is used and an immediate reaction only is considered positive. It has been our experience, however, that specimens of dried blood cannot be conveniently diagnosed at such a low dilution.

(*b.*) Partial reactions. — The partial reaction which it was found necessary to report at times is frequently questioned by the physician. Wilson (1901) in the examination of one thousand six hundred and fifty blood specimens found that one hundred and sixty or about ten per cent gave a partial reaction. He also had eight cases giving a positive reaction which were not diagnosed as typhoid. From his work he concludes that the partial reaction is valueless as far as a diagnosis is concerned. With such a report the physician should judge by the clinical symptoms during the course of the illness. Of the forty partial reactions reported, further inquiry elicited the following results:

Clinically typhoid	14
" not typhoid	13
Physician did not know	2
No report	5
Later found negative	2
" " positive	2
Counted twice	6

Two of these cases had suffered an attack of typhoid three or four years previously; one the physician considered a mild case at the time of the partial Widal. Two of those in which the partial reaction persisted were considered as positive cases and two were not typhoid. From these results it is evident that the final diagnosis of a partial Widal reaction must be made on the clinical symptoms of the patient.

(*c*.) Mixed infections. — Unfortunately it was not possible to make a thorough study of all of the eight cases showing a positive reaction to both B. typhosus and B. paratyphosus B. In only two of these specimens was the exact titer determined, and in both instances were positive to B. typhosus at 1 : 200 and B. paratyphosus B at 1 : 100. Specimens of feces yielded in one case typhoid and the other paratyphoid organisms. Whether or not these were mixed infections we do not know, yet it seems probable that they were.

IV. SERUM REACTIONS DURING VACCINE TREATMENT.

Just how far a comparison may be drawn between the serum reactions produced by vaccination and those in clinical cases of typhoid, there may be considerable doubt. In the case of infection the number of organisms probably increases gradually from comparatively few to a vast number. In vaccination a known number of dead organisms are given at definite intervals. There are several points upon which comparative data are of interest.

(1.) The length of time from infection to the appearance of agglutinins in the blood.
(2.) The rate of production of agglutinins.
(3.) Whether or not secondary agglutinins appear.
(4.) Whether there is variation in different individuals.

In order to study the serum reactions during typhoid vaccination fourteen of the students in the Brown University Biological Laboratory were given the treatment. The first four were given the typhoid vaccine and ten the mixed. Commercial bacterins were used. The pure typhoid bacterin contains five hundred million bacilli for the first dose and one billion for each of the second and third doses. The mixed bacterin contains the same number of typhoid bacilli with the addition of two hundred and fifty million each of paratyphoid A and B for the first dose and five hundred million for the second and third. Injections were made at intervals of a week except in case of five of those taking the mixed vaccine when the interval was ten days. Blood was

taken every other day as nearly as possible. The clear serum was used for tests made according to methods previously outlined. In each case the titer recorded was that at which a complete reaction took place.

SERUM REACTIONS DURING TYPHOID VACCINATION.

No.	Agglutinins Increase After —			Agglutination at 1 : 60 After —			Maximum Reaction After —			Titer of Maximum Reaction.		
	Typh.	A.	B.	Typh.	A.	B.	Typh.	A.	B.	Typh.	A.	B.
	Days.	*Days.*	*Days.*	*Days.*	*Days.*	*Days.*	*Days.*	*Days.*	*Days*			
1.	4	0	0	8	0	0	10	0	0	100	0	0
2.	18	0	0	18	0	0	30	0	0	1,000	0	0
3.	4	0	0	8	0	0	12	0	0	80	0	0
4.	4	0	0	6	0	0	12	0	0	80	0	0
5.	20	26	12	26	28	26	30	30	30	1,000	1,000	1,000
6.	8	8	8	14	14	14	20	18	18	6,000	4,000	4,000
7.	8	14	12	16	16	12	18	18	28	1,600	1,600	800
8.	10	18	18	18	18	18	18	18	18	2,000	800	600
9.	12	12	12	12	16	12	16	16	16	1,200	1,200	1,200
10.	20	20	20	20	20	20	22	22	22	600	600	600
11.	18	18	18	18	18	18	18	18	18	400	400	400
12.	20	20	20	20	20	20	24	24	24	1,000	1,000	1,000
13.	20	20	20	22	22	22	24	24	24	800	800	800
14.	16	16	16	24	24	24	26	26	26	400	400	400

It must be observed at first glance that there is a great individual variation in all respects. The period from the first inoculation to the appearance of an increase in agglutinins varied from four days to eighteen days. The average length of time from the first inoculation until the blood agglutinated in a dilution of 1 : 60 was sixteen days, and varied from six to twenty-eight days. Park (1910) states that twenty per cent of the clinical cases give positive results the first week, sixty per cent the second, eighty per cent the third, and ninety per cent the fourth. If ten to fourteen days are allowed for an incubation period in clinical cases, the average length of time from infection until a positive reaction is obtained is probably slightly longer than the time

from the first inoculation of a vaccine until a positive reaction appears.

The agglutinins are not produced in gradually increasing amounts. Castellani (1914) reports that, although the cases which he vaccinated showed no agglutinating power the first week after inoculation, at the end of the second the maximum agglutinating power was reached. The writer's results correspond very nearly to those of Castellani. There seemed to be a general tendency towards a marked rise in agglutinating power during the first part of the third week. That this also occurs in infection seems probable. One clinical case which came under our observation was negative twenty-four days after infection but was strongly positive on the twenty-seventh day. Another which was partial on the twenty-eighth was strongly positive on the twenty-ninth. After the maximum agglutinating power is reached, the amount may fluctuate but the tendency is to diminish.

The typhoid bacterin was used chiefly to observe whether or not secondary agglutinins were produced. The bacterin was used almost at the expiration of the period for which it is guaranteed, which probably accounts for the low titer of the sera. There were, however, no secondary agglutinins observed. In the sera of those immunized against three strains the agglutinins were practically the same for each one, although the number of paratyphoid organisms was only half that of the typhoid. Wherever there was a difference the typhoid antibodies exceeded those for paratyphoid. Two typhoid cultures were used in all of the tests, the Rawling's strain, of which the bacterin was made, and the Worcester strain, but no difference in agglutinating power was noted. The same strains of paratyphoid were used as were employed in the bacterin.

These observations upon the production of agglutinins during immunizing treatment confirm the experience of routine diagnosis. The physician should not consider a negative Widal as final. If the suspicious clinical symptoms persist the Widal should be repeated at intervals. Whereas some individuals would show a reaction early in the disease,

others would not show it for some time. The agglutinins should appear during the third week after infection although individual differences occur. A serum which reacts to both typhoid and paratyphoid seems to indicate an infection by both of these organisms.

SUMMARY.

1. The most satisfactory method of isolating typhoid and paratyphoid organisms from feces is by incubation in peptone lactose bile for six to twelve hours and then plating upon Endo's medium.

2. Identification of organisms is best accomplished by reactions in carbohydrate media. Serum reactions are of more value with typhoid than with intermediate group organisms.

3. Normal blood is frequently capable of agglutinating typhoid and paratyphoid bacilli.

4. A partial Widal reaction has no diagnostic significance. The test should be repeated at intervals until a positive or negative result is obtained or else the case should be diagnosed by its clinical symptoms.

5. A serum agglutinating both B. typhosus and B. paratyphosus A or B indicates a mixed infection.

6. A study of the serum reactions during typhoid immunization demonstrated that —

(*a*) Great individual differences occur.

(*b*) Agglutinins begin to increase in the blood from four to eighteen days after the first inoculation.

(*c*) The blood gives a positive agglutination at 1 : 60, on the average, sixteen days after the first inoculation.

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THE OLD MEDICAL SCHOOL IN BROWN UNIVERSITY.

BY FREDERIC P. GORHAM.

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The Old Medical School in Brown University*

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Medical instruction in colonial times consisted usually of an apprenticeship to some retired army surgeon or physician of renown for a term of three or four years, after which the student was certified for practice not by receiving the degree of M. D. but by a license to practice from some examining board. One of the first acts of the General Assembly of the Colony of Rhode Island after receiving the charter of 1663 was to confer upon Capt. John Cranston a license "to administer physic and practice chirurgery" and also the degree of "Doctor of Phissick and Chirurgery."

The first practical instruction in anatomy given in Providence was that imparted by Dr. David Vandelight to a few students in his own house. Dr. Vandelight was a graduate of Leyden. He was attracted to Providence in all probability by its commercial possibilities for besides his duties as physician and apothecary he was skilled in the chemistry of the day and introduced here the Dutch method of separating spermaceti from its oil, a process which brought much wealth to the doctor and to the Plantations. At his death on February 14, 1755, the value of his drugs and instruments was inventoried at over four thousand pounds. His house is still standing on South Main Street between College and Hopkins Streets. Dr. Vandelight died fifteen years before Rhode Island College moved to Providence and as far as we know he had no connection with that institution, though he was a brother-in-law of Nicolas, John and Moses Brown whose name was later to adorn the University.

Until 1811 only two medical schools existed in New England, that at Harvard, founded mainly through the efforts of Dr. John

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Warren about the close of the Revolutionary War; the other at Dartmouth, created in 1798 by the persistence of Dr. Nathan Smith, a native of our neighboring town of Rehoboth, "a great organizer and very eminent medical teacher and writer, who for some years was its only professor."

In September, 1811, three medical professors were appointed in Brown University. Dr. William Ingalls of anatomy and surgery, Dr. Solomon Drowne of materia medica and botany and Dr. William Corlis Bowen of "chymistry," and a committee of the Corporation was appointed to procure a suitable person to give lectures on the theory and practice of physic.

Previous to this time the degree of M. D. had been conferred by the university only as an honorary degree upon persons already eminent in the profession. With the appointment of these professors the university was now equipped to give the degree in course, and in the year 1814 we find the first graduates with the degree of M. D. appearing on the record. At the time of the appointment of these men the university was pursuing its ordinary courses of academic training with about one hundred students. It had moved from Warren in 1770, had changed its name from Rhode Island College to Brown University in 1804. Dr. Messer had been President since 1804. Dr. Solomon Drowne of the class of 1773 had become a Fellow of the college in 1783, and was given the honorary degree of M. D. in 1804, which he had previously earned in course at the University of Pennsylvania. As the only physician on the Board of Fellows when the Medical School was created, he probably had a large part to do in suggesting and promoting its establishment.

Dr. Solomon Drowne was an interesting character. Born in Providence, March 11, 1753, "he graduated in the fifth class at Rhode Island College in 1773. He studied medicine with Dr. William Bowen and at the University of Pennsylvania, then the only organized medical school in the country. He was in the service of the colonies as surgeon, was in General Sullivan's expedition on Rhode Island, served at different points in Connecticut and New York and witnessed the evacuation of New York City by the British troops. In the fall of 1780 he went on a cruise as surgeon in the private sloop-of-war Hope, his journal of which has been printed. He won the regard of Lafayette, the Counts de Rochambeau and d'Estaing, as well as of other French officers, to such a degree by his medical ability and skill

as a surgeon that the chief of the medical staff entrusted their invalid soldiers to his care when they left for home." In 1784 he went to Europe, attended hospitals and lectures in London and Paris. Later he moved to Ohio, removed to Virginia and then for several years lived in Pennsylvania. In 1801 he came back to Providence and bought an estate in the town of Foster, "where he could indulge his love for country life, his fondness for gardening, for botany and varied reading. He built his house on a hill, which he named Mount Hygeia. He founded a botanical garden, and from his professional tours, which extended to Providence, and not infrequently to other states, he always returned with seeds or plants to enrich his collection. He also sent abroad for plants and was the first to introduce many species into our country which have since become common. People came from long distances to see his famous garden. He is said to have raised and prepared his own opium. In medical practice he appears to have tended to simplicity and to a greater trust in the powers of nature than was common in his time. By simplicity he meant an avoidance of officious interference, and in particular of uniting many remedies in one prescription. Butternut pills, decoction of mallows and pussy-willow tea were among his favorite medicines." Last spring the graduate students and faculty of the Biological Department of the university made a pilgrimage to his old home in Foster, visited his grave, wandered through the remains of his botanic garden, where still many unusual herbs and shrubs run wild, examined his library, which is still intact, and saw many relics of the old doctor, such as wearing apparel, diplomas, foreign decorations, mortars and pestles, medicine chests, bottles and jars, many still containing the curious medicines which he compounded. In all probability the medical school in Brown University had its origin in the mind of Dr. Solomon Drowne.

Of the other members of this first medical faculty just a word. Dr. Ingalls was the least known in Rhode Island. He was born in Newburyport, Mass., May 3, 1769, graduated at Harvard in 1790 and took his degree of Doctor of Medicine at Harvard in 1801. He lived in Boston throughout his professional life and became prominent in practice, especially in surgery, and as an instructor in anatomy. He began to give lectures in Brown University soon after his appointment as Professor of Anatomy and Surgery, in 1811, and continued to lecture there until 1816, when he resigned. His resignation was

not accepted, however, although he did not thereafter teach in Providence, but students of the university continued to attend his lectures in Boston, at least until 1820, as a part of their course for the medical degree at Brown. Dr. Ingalls died in 1851.

In 1815, just before Dr. Ingalls resigned, Dr. John Mathewson Eddy of Providence was appointed adjunct or assistant professor to Dr. Ingalls. Dr. Eddy gave little instruction and died early in the year 1817. He was one of the forty-nine physicians who petitioned for an act of incorporation for the Rhode Island Medical Society and who were named in it as the original fellows in 1812.

Dr. William Corlis Bowen, professor of "chymistry," belonged to a family eminent in medical practice. Indeed, long before the town records of Providence mention the name of any physician, during the early decades of the eighteenth century, whoever desired the best medical or surgical aid sent to Rehoboth for Dr. Jabez Bowen. Later Dr. Bowen moved to Providence and was the first regularly educated practitioner of medicine and surgery who permanently resided in the town. Dr. William Corlis Bowen, the professor, was his son, born June 2, 1785, entered Rhode Island College, but went with President Maxcy to Union College, where he graduated in 1803. "After a few years of practice in Providence he went to Europe and studied at the University of Edinburgh, where he took his medical degree in 1809. He studied also at Paris and London, where he was a private pupil of Sir Astley Cooper." He resigned his professorship in 1813 and in 1817 his successor, Professor John DeWolf, Jr., of Bristol, was appointed.

In September, 1815, the chair of theory and practice of physic was filled by the appointment of Dr. Levi Wheaton, a native of Providence and a graduate of Rhode Island College in 1782. In 1778 he was attached to a military hospital in Providence; in the summer of 1779 he studied medicine with Dr. Joshua Babcock of Westerly. He was afterwards surgeon of a privateer and later had charge of the prison-ship Falmouth at New York. At the close of the war he settled in Hudson, N. Y., where he practiced ten years, and later removed to his native town, where he lived for over half a century, dying August 29, 1852.

In September, 1823, Dr. Usher Parsons of Providence was appointed adjunct professor of anatomy and surgery and one year later was chosen professor in place of Dr. Ingalls. Dr. Parsons was born

in Alfred, Maine, August 18, 1788. He studied medicine in his native town and afterwards at Boston under the tuition of Dr. John Warren. In July, 1812, he was commissioned surgeon's mate in the navy and served under Commodore Perry on the Great Lakes and in the battle of Lake Erie. He received the degree of M. D. at Harvard in 1818. During his service in the navy he visited the medical and scientific institutions of Naples, Palermo, Rome, and Florence and spent several months in Paris and London, attending the hospitals and gaining the acquaintance of eminent surgeons and naturalists. He was professor of anatomy and surgery at Dartmouth College in 1821 and took up his residence in Providence in 1822. He wrote that his "motive for engaging in the business of lecturing was a desire to establish a museum of anatomy, human and comparative, on the plan of the late John Hunter's." In Brown University he gave lectures both to the medical class and to the college students till 1826, and it is said that the lectures of Dr. Parsons gave new life to the institution. Dr. Parsons subsequently became very prominent in the practice of his profession, especially in surgery and as a consulting physician. He contributed largely to medical journals, he served as Vice-President and acting President of the American Medical Association and took an active part in the founding of the Rhode Island Hospital. He died in Providence, December 19, 1868.

This was the distinguished faculty of the Brown University Medical School. What of the students and graduates? During the period from 1814 to 1828, ninety-three men received the medical degree in course. It would take too long to mention even the names of these Brown doctors. Four of them became distinguished practitioners in this State, Dr. Lewis J. Miller of the class of 1820, Dr. George Capron of the class of 1823, Dr. Hiram Allen of Woonsocket of the class of 1825 and Dr. Francis L. Wheaton of the class of 1828.

Dr. Jerome V. C. Smith of the class of 1818 was a well-known author and for many years editor of the Boston Medical and Surgical Journal and Mayor of Boston in 1854. Dr. Alden March of the class of 1820 practiced many years in Albany, N. Y., going there directly after graduation, was founder and President and for many years leading instructor of the Albany Medical College, was President and one of the founders of the American Medical Association. Dr. Elisha Bartlett was a member of the class of 1826, a professor of Dartmouth College and later of the College of Physicians and Surgeons.

Besides the ninety-three graduates the names of twenty non-graduate medical students are known. Thacher's American Medical Biography, published in 1828, gives the number of students in the Medical Department of the university, 1825-'26, as forty. In the years 1818-'20 there were sixteen graduates in course; 1821-'23, twenty-two, and in 1824-'26, twenty-eight. The number appeared to be on the increase and promised well for the development of a strong Medical School at the university.

But its death blow fell suddenly and unexpectedly. At a special meeting of the Corporation held on December 13, 1826, Dr. Messer resigned the presidency and the Rev. Francis Wayland, Jr., of Boston was unanimously chosen to fill that office. Dr. Wayland had very definite and pronounced views as to the requirements of college discipline. Early in 1827 the Corporation voted "that salaries shall be paid only to such professors, tutors, or other officers as shall devote themselves during term-time exclusively to the instruction and discipline of the institution, and shall occupy rooms in college during study-hours, and attend in their several departments such recitations as the President may direct, not exceeding three recitations of one hour each in every day." Such regulations were impossible for a medical school. Physicians in active practice could not serve as officers of discipline as thus strictly defined.

The names of the medical professors appear in the catalog of 1827 with no special remark. But in 1828 an asterisk is prefixed to the names of Drowne, Wheaton, DeWolf and Parsons with the following note: "The gentlemen to whose names the asterisks are affixed are not of the immediate government and do not at present give any instruction in the University." In 1829 Dr. Wheaton's name is omitted, but the same asterisks and note mark the others. In the catalogs of 1830 and 1831 the note is changed to read thus: "The gentlemen to whose names the asterisk is prefixed give no instruction in the University and have no concern in its government." In succeeding issues the names of these eminent men drop out altogether.

The downfall of the medical school was not due to any hostile feeling on the part of the Corporation and President, but was apparently an incidental result of President Wayland's unswerving conviction and policy in regard to college discipline and government. Indeed, as far as we can learn, President Wayland was particularly interested in this department of the university, for he himself had

studied medicine in his youth, and for some years after the close of the medical school, he taught anatomy and physiology along with a host of other subjects.

But for fifty years after President Wayland came, not a man holding a medical degree taught upon the faculty. In 1834 Professor Chase took the title of professor of chemistry, physiology and geology, but it was not until 1867 that we find the name of Dr. Charles W. Parsons appearing, first as lecturer in 1874 and then as professor of physiology in 1878. In 1872 Dr. C. L. Nichols was assistant in analytical chemistry, in 1878 Dr. A. S. Packard became professor of zoology and geology and in 1882 Dr. C. V. Chapin became instructor in physiology and in 1886 professor of physiology. These are all the medical men who have served upon the faculty until quite recent years. Since the death of Dr. Packard in 1905 not a man holding the medical degree has served the university as professor.

But this does not mean that medical science has received no attention during this time. Physiology was regularly taught from 1867 to 1895 under the able instruction of Drs. Parsons and Chapin. But it was not until the advent of Dr. Bumpus in 1890 that other departments of medical instruction received adequate attention.

This date, 1890, marks the beginning of the present Biological Department of the university. Starting with a single course in comparative anatomy with ten or a dozen students, under Dr. Bumpus, this department now numbers six instructors and a total enrollment of 424 undergraduates and fifteen graduates. Courses in general biology, comparative anatomy, physiology, bacteriology, histology, pathology, neurology are regularly given. Although the names of these courses suggest the medical school, the work of the department is carried on with no idea of developing at some time into a new medical school at Brown University, but with three very definite objects in view.

First, to provide a broad biological training in the principles of life and healthful living for the young men and women of Brown. That this is being accomplished at the present time may best be attested by the fact that only recently the faculty and corporation have made the general biology course required for all candidates for the degree of Bachelor of Philosophy, and further by the fact that in this course at present there are registered over one hundred and fifty men and sixty women.

Second, to provide a sound preliminary training for those who intend to enter the medical profession, giving instruction in matters which in no way duplicate the work which they will later do in medical school, but giving them a broad foundation upon which to build their future medical education. That this is being done at present may be fully demonstrated by pointing to the records in medical schools and hospitals of the graduates of the department. At Harvard, Yale, Columbia, Cornell and Johns Hopkins, Brown men are winning honors for themselves and their Alma Mater. I have only to glance about me in this room to see men ranking high in the medical work of this city and state who are building on a foundation laid in the biological department of Brown University.

Third, and to this function of the university I wish to call your particular attention, as I believe it marks a new line of development for the future, one of great usefulness to the community which the university serves, and one which will require your active assistance and coöperation,—the development of a school of health officers.

We are at the present time in Brown University actively engaged in devoloping the instruction in sanitary science for the purpose of training men competent to serve their communities as health officers, sanitary officials, men in charge of health department laboratories, water works, sewage-disposal plants and men trained in the work of the sanitary engineer. I do not need to call your attention to the rapid development of the science of sanitation in the last few years. There was a time when the doctor of medicine was fully competent to serve as health officer in a community. That time has passed. The protection of the health of a community, the prevention of disease rather than its cure, has developed to a point at present where men equipped to serve as sanatarians must be especially trained along lines but little taught in medical schools. The science of bacteriology, the laboratory diagnosis of disease, the application of chemistry and bacteriology and engineering to the purification of water supplies, the disposal of garbage and sewage, the purity of foods, the bacteriological and chemical control of the milk supply, mosquito extermination, the preparation of vaccines and sera for the treatment of disease or for immunization, are becoming increasingly more important and are demanding specially trained workers.

Brown University is ideally situated for the training of such men. The present affiliations of the biological department of the university

with the Health Department of Providence, the State Board of Health, the Rhode Island Hospital, the Providence City Hospital, the State Sanatorium, the State and Federal Fish Commissions, the Federal Bureau of Chemistry, the Providence Milk Department, make our opportunities unique. After undergraduate training in biology, anatomy, physiology, histology, pathology and bacteriology our students are ready as graduate students to begin actual experience in practical work under the guidance of men of national reputation in sanitary matters. Graduate students in the department are at present and for sometime have been actively engaged in the work of the Health Department of Providence under Dr Chapin. Here they may learn the methods of laboratory diagnosis of diphtheria, typhoid, gonnorhea, syphilis, malaria and rabies, the methods of keeping vital statistics, the actual operation of a modern health department. At the Rhode Island Hospital, at the City Hospital and at the State Sanatorium, they may engage in a study of the diagnosis of contagious diseases, the methods of serum and vaccine treatment and such other activities as belong to the operation of such hospitals. In the milk department of Providence they may learn the chemical and bacteriological methods of the sanitary control of a milk supply. Through affiliation with the United States Bureau of Chemistry, (Dr. Mitchell of the department staff is collaborating chemist of the Bureau), they may learn the methods of sanitary food analysis and control. Through affiliation with the Rhode Island Shellfish Commission they may learn the methods of sanitary analysis and control of oysters and other shellfish. Through coöperation with the engineering department of the University they may become trained sanitary engineers, competent to serve as chemist, bacteriologist or engineer in charge of waterworks, sewage plants, etc. Two or three years of graduate work after preliminary undergraduate training will fit a man for any of these lines of work.

It is but necessary to mention some of the positions graduates of the department are filling at present to show that the training is efficient: Pathologist of Otisville Sanatorium, city of New York; bacteriologist, Hudson County, New Jersey; associates in Rockefeller Institute and Hospital; chemists and bacteriologists with various commercial houses, city departments and in Government positions; professor of bacteriology in the University of Wisconsin;

professor of sanitary science, College of the City of New York; instructor in sanitary science, Harvard University.

With a grand new laboratory just finished for the department through the generosity of Dr. Oliver Henry Arnold, completely equipped through the untiring efforts of certain members of this Society, to be dedicated on Tuesday, June 15, we have the material equipment necessary for this work. With such men as Dr. Chapin of the City Health Department, Dr. Swarts of the State Board of Health, Dr. Richardson of the City Hospital, Dr. Barnes of the State Sanatorium, the officers of the Rhode Island Hospital and the various health officials throughout the State, ready to coöperate, we could establish a school of health officers second to none in the country.

I believe it is time to resuscitate the old medical school, not for the purpose of providing medical instruction in competition with other well-founded and endowed medical schools, but for the purpose of providing adequate, modern instruction in preventive medicine.

Let us *not* look forward to the time when the degree of doctor of medicine shall be given again by the university on the hill, but rather, in answer to the insistent call from a new field of medical science, let us plan to give the new degree of doctor of public health.

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THE AIM AND CONTENT OF HIGH SCHOOL BIOLOGY.

BY HERBERT E. WALTER.

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THE AIM AND CONTENT OF HIGH-SCHOOL BIOLOGY[1]

IN reconstructing our educational machinery at least four variable factors must be considered, viz.: *equipment*, *pupil*, *teacher* and *plan*.

By *equipment* is meant all sorts of pedagogical material, both animate and inanimate, such as school buildings and furniture, library and laboratory facilities, janitors and superintendents.

Good educational results can be, and often are, attained in spite of a miserably poor outfit, and, on the other hand, it is possible to be so over-equipped that degeneration of initiative and resource is the result. In most instances there is provided for us an outfit to work with and it is our proper function to make the best of what we have rather than to attempt to change it in any fundamental way. At any rate there are more urgent and modifiable phases of our work than this. On a basis of 100 I should evaluate the equipment factor at about 10 per cent.

The *pupil* is at least four times as important as the schoolhouse with all its equipment. In the final issue the unfolding process of education can be successful only when there is something worth while to unfold.

Here again the teacher can not choose his material. He must take the raw human stuff as it comes to him to make the best of it. I thoroughly believe, however, that we shall soon come to recognize more than we do now the fact that the far-sighted way to get the best educational results is to provide the teacher with better pupils at the start, or, in other words, with pupils having greater inborn capacities. This, of course, is applied eugenics. At present the average educator is swamped so immediately with boys and girls already born and thus having their innate capacities irretrievably determined, that he hardly has time or opportunity to raise his eyes to that larger ideal of theoretical unborn children which so stirs the imagination of the eugenist. Granting that the ordinary pupil reckons at 40 per cent., surely the *teacher* should be valued at, at least, 30 per cent. of the educational complex, that is, at three times the value of the material equipment with its administration.

The value of the teacher is particularly emphasized in our American schools where educational schemes are so varied and empirical and where so much depends upon the execution of the scheme. A good teacher can make even a faulty scheme succeed, but a poor techer may confuse and upset things generally, although provided with the best of schemes.

It is not proposed to-day, however, to open the embarrassing personal question of how better teachers may be obtained, but, having recognized their importance in the educational system, to pass on to the fourth

[1] An address given before the science section of the Worcester County Teachers' Association at Worcester, November 5, 1915.

phase of our common problem, the *plan,* or content of our work. It is a good thing that our plans are not fixed forms from the outside and that we teachers repeatedly apply ourselves with diligence to their revision.

So far as the subject of biology in the high schools is concerned, in spite of its prime importance, its status, particularly in conservative New England, is by no means clear.

At the present time it is frequently true that the biological course of the average high school has a strong resemblance to hash. To speak plainly, it too often consists of warmed-up left-overs, for example, a little nature study from the grades, some large indigestible chunks of college zoology, a dash of elementary physics and chemistry, a little botany and hygiene, and, in certain states where required by law, an intemperate dose of temperance.

Now hash, correctly compounded and browned in the pan, requires no apology, but we all have a pardonable interest in what goes into it, hence this inquiry into the content of high-school biology.

At the July meeting of the National Education Association in 1913 with the cooperation of Mr. Claxton, the U. S. Commissioner of Education, an important national commission on the reorganization of secondary education was appointed, in the hope that it would, among other things, "formulate statements of valid aims, efficient methods and kinds of material whereby each subject may best serve the needs of high-school pupils." This commission was organized into 14 committees and numerous subcommittees. Mr. William Orr, the deputy state commissioner of education of Massachusetts, was made chairman of the committee on natural science and he very soon organized his department into five subcommittees, one of which, that of biology, had in its personnel ten high-school teachers, three university professors, three normal-school instructors and one physician. The seventeen members comprising this committee, coming as they do from New York and vicinity, from Chicago and the middle west, from the Pacific coast and from New England, are fairly representative of the country at large.

Under the leadership of Mr. James E. Peabody, chairman, of the Morris High School, New York City, this subcommittee on biology has had numerous conferences during the last two years and is still wrestling with its uncompleted task. A final report is not yet ready, but a preliminary draft has been published in which certain conclusions have been reached, and I wish to present to you some features of this tentative statement in the hope that constructive criticism may be called forth which may aid in the formulation of the final report as well as in clarifying our ideas generally about the aims and content of high-school biology.

First. *"Fundamental Principles."* To quote from the preliminary report,

> The committee maintains that *unity of subject matter in any course in science is of first importance,* by which is meant that the subject matter should be so organized that appreciation of *underlying principles* shall form the foundation of the student's knowledge, thus giving him a scientific basis for the organization of his knowledge.

This concept of *"unity of subject-matter"* immediately differentiates biological science of the secondary school from the nature-study which is supposed to precede it in the grades.

Hitherto the attention of the well-trained child has been engaged, and diverted with many varied aspects of the living things more or less unrelated as they may happen to be incidentally encountered. Now Mother Nature is an exceedingly entertaining, instructive and edifying companion, as Gilbert White well knew, but we must all confess that she is hopelessly illogical in

the way that her materials are distributed throughout the earth and that she almost entirely lacks any bump of order. When the pupil reaches high-school age, his mind begins to crave and appreciate the orderly sequence of facts and ideas which constitutes the science of biology as contrasted with the hit-or-miss art of nature study. It is a keen intellectual delight for him to create out of the scattered blocks of familiar facts a building of ideas whose architecture gives mental satisfaction. This is why "underlying principles" and "unity of subject-matter" begin to make their definite appeal to the unfolding mind at the high-school age. The psychological moment has arrived for replacing disconnected excursions and picnics in the pleasant fields of natural phenomena with the laborious straight furrows of scientific husbandry, hence the committee stands, first of all, for *fundamental principles* and for a *unity of subject-matter.*

It follows from the foregoing that courses in so-called "general science" for the first year in high school would not receive the indorsement of this committee since, in the very nature of the case, such courses can not deal with a unified subject-matter. They must consist necessarily of fragments of various sciences superficially treated, with the result that the furrows thus plowed in the field of knowledge are all so short, divergent and interrupted that they lead nowhere with definiteness. The committee's report formulates the aims of teaching biology as follows: "(1) to train the pupil in observation and reasoning; (2) to acquaint him with his environment and with common forms of plant and animal life, and especially with the structure, functions and care of his own body, together with the general biological principles derived from this study; and (3) to show him his place in nature and his share of responsibility for the present and future of human society."

These aims may be ticketed under five heads:

1. Research
2. Information
3. Prophylaxis
4. Orientation
5. Application

In review I wish to pass briefly these five aims for teaching high-school biology.

I. *Research,* or "training in observation and reasoning," is a method of procedure that has become one of the greatest instruments at the command of the human mind. President Bumpus, of Tufts College, has recently defined research as "finding out things for yourself at first hand." There is nothing obscure or uncanny about the scientific method of research. Anybody can engage in it, but not every one habitually does so. It is often easier, if one has a slothful mental attitude, to rely upon the authority of others in getting the answer to life's problems, than to replace the passive acceptance of ready-made ideas with independent and resourceful conclusions based upon research.

II. The second aim, *information,* is the necessary ballast in any course of study. I believe that young minds require a whole lot of it, just as young robins will thrive best when they have literally yards of worms inserted into their cavernous throats. What if the pupil does forget a large part of the information that comes his way? It is a good thing to do so and it may be reasonably expected to occur with the average pupil. The young robins just cited do not retain the great amount of worms they devour, but in the process of getting rid of them they grow visibly. So a pupil will grow in intellectual capacity and power in the process of passing information through his central nervous system, and it is well for him to cultivate the habit of doing so. A large part of the high-school course in biology, consequently, should be informa-

tional in character and administered in good, stiff, honest doses with a minimum penalty applied if inquisitorial tests and examinations reveal that much of the information engulfed refuses to emerge.

III. By *prophylaxis* is meant personal application to the pupil's own body and life of those hygienic measures taught by biology which will prevent inefficient living on his part. The importance of this aim is so obvious that it calls for no defence, but I wish to quote a well-expressed paragraph from Professor Winslow's Buffalo address given last year before the Fourth International Congress of School Hygiene.

> It is an obvious truism that education is meant to prepare for living; it seems clear that the most general and fundamental phases of the art of life should receive proportionate representation in the preparatory process. The average man uses his history once a day perhaps, his arithmetic somewhat oftener. Even his English grammar is on trial during a part of his working hours only, and his whole mental equipment is put by for a third of the twenty-four. He is *living* all the time, however, and is either well or ill, happy or miserable, efficient or useless, largely as a result of the conduct and management of the delicate physical machine which is in his charge. He may be innocent of historic fact, of the multiplication table and of syntax and yet be a useful and a contented citizen. He can not be either long without observing the laws of hygiene and sanitation. I fancy that any one with a child of his own will have no doubt that knowledge of what to eat and what not to eat and why, of the meaning and importance of fresh air, of the claims of exercise and rest, of the essential routine of body cleanliness, of how germ diseases are spread and how they may be controlled, of the methods of rendering water and milk safe and the reasons therefor, of the dangers of insect-borne diseases and of the essentials of public sanitation, these are of even greater moment than those which prepare for the intellectual and social life.

IV. *Orientation*, or a "knowledge of general biological principles that underlie these studies" is the fourth aim in teaching biology in secondary schools. Only when the pupil senses the larger values and has his imagination correspondingly stimulated will he enter upon detailed work with the blessed enthusiasm of the true scholar. By these larger values of orientation is meant something more than appreciation of the practical applications of biology to life. They include an unshakable confidence in underlying truth and in the orderly sequence of events, which unconsciously permeates and characterizes the scientific mind. It is the lack of appreciating these larger and underlying principles that renders any subject petty, stupid and "dry" so that aggressive interest gives way to neutral acquiescence. It is the unoriented pupil who asks the discouraged teacher that most exasperating of all questions: "What do you want me to do next?"

High-school pupils are old enough to appreciate being taken behind the scenes and shown what the whole thing is about. As soon as they once gain a panoramic view of the great field of biological science their study ceases to be a personally conducted excursion and resolves itself into a real adventure that is worth while.

V. Finally, the *application* of it all is "to show him his place in nature and his share and responsibility for the present and future of human society." The moral of the whole matter is to get rid of the rubbish in life so far as possible by rational behavior and effective conduct. Proper training in biology will do more than safeguard the individual against personal blunders in the conduct of his own life, because it stands for more than the simple acquisition of information and skill for selfish ends alone. It has to do with the discernment of truth and error, with the unconscious habit of correct judgment of cause and effect *independent of any personal bias.*

This unselfish, impersonal attitude of mind which true biological study engenders

is exactly what we want for making good citizens in the world. In such a scientific atmosphere prejudice, ignorance, greed and passion do not flourish, and it is in the development of this atmosphere rather than in the more obvious, utilitarian benefits of biological knowledge that the greatest application of our science to human welfare is to be found. So much for the aims and ideals in teaching biology in the secondary schools.

The report goes on to affirm:

We do not believe in rigidity of method in science instruction. Quizzes, conferences, experiments, individual reports, excursions, text-assignments are all good. They offer a rich and varied choice of pedagogical method, and each teacher should be given freedom to develop the methods best adapted to his own group of students and to the environment of the school in which he is teaching.

In laboratory work time should not be wasted in detailed microscopical work, in complicated experimentation, in useless attention to drawing or other "busy work." All laboratory work should be based on definite information ungrudgingly and interestingly furnished, and should be in the nature of a direct effort to acquire more knowledge at first hand. Experiments, results and conclusions should be accurately recorded. Neatness in notebook records is desirable but must not be exalted above thinking and understanding.

With respect to how the work should be planned the committee unanimously agrees that *two years of work in elementary science* should be the basis for more advanced courses in science. Such work should deal with physical environment (including a study of matter and forces), plants, animals (including man), and the general applications of scientific principles to human welfare. If, however, administrative conditions make a two-year course out of the question, the committee recommends that a first-year course be required of all, and that a second year be offered as an elective.

There are added in the preliminary report four appendices in which tentative outlines of the content of each of the four half years recommended are given.

In part these appendices are as follows:

APPENDIX A. PHYSICAL ENVIRONMENT

This study should acquaint the pupil with the nature of his environment and emphasize the necessity for his adjustment to it.

1. *States of Matter.*

2. *Water.*—Changes under different temperature; evaporation; condensation; distribution; the ocean as a modifier of climate; winds, currents, rainfall; solution, precipitation, filtration; carrying power; water power; steam power; action in modifying the earth's surface; ice, snow and hail; circulation of ground water; water in living things; osmosis.

3. *Air.*—Physical features; elements, compounds, chemical changes; oxidation; photosynthesis; indestructibility of matter; the CO_2 cycle; forces of heat, light and electricity.

4. *Soil.*—Origin; nature; its constant changes, soil bacteria; the materials for plant growth.

5. *The Past History of the Earth.*—Forces preparing an environment for mankind; geography of to-day; man's relation to it.

6. *Environment Extended to the Limits of Space.*—The heavenly bodies and their effect on the earth; fixed stars and the sources of our knowledge of them; the solar system.

7. *Summary of the Relation of the Physical Environment and Life to Show the Responsibility of Man.*—The conservation of natural resources. In this preliminary half year on the physical environment the stage is set for the biological studies proper that follow.

APPENDIX B. PLANTS

1. *Introductory.*—The general relations of plants to animals and man; a consideration of the familiar parts of a typical green plant with the functions of these parts.

2. *Nutrition.*—The essentials obtained through a study of roots, stem and leaves. Nutritive processes in plants that are not green.

3. *Reproduction,* the essentials obtained through a study of flowers, fruits and seeds.

4. *Applications* of plant biology to human welfare.

5. *Survey of plant groups,* and plant identifications.

APPENDIX C. ANIMALS

1. *Animals in General.*—Types, structure, distribution and relationships.

2. *Life Histories.*—Reproduction, growth, metamorphosis.

3. *Response to Physical Environment.*—Geographic distribution, reactions.

4. *Response to Organic Environment.*—Competition, parasitism, symbiosis.

5. *The Continuity of Life.*—Genetic relationship, the paleontological record.

6. *Man's Place in Nature.*—His rise and dominance; his distribution and variety.

7. *Man's Opportunity to Use His Biological Knowledge.*—Medicine, sanitation, agriculture; domestication and control of animal life.

APPENDIX D. MAN

1. *Structure of the Human Body.*—Similarity and contrasts with the lower animals. Characteristics of the most common tissues.

2. *Physiology.*—Nutrition, food and diet; digestion, absorption; distribution of food in the body; removal of waste; the part played by oxygen. Functions of the tissues.

3. *Hygienic Care of the Body.*—Healthful diets; drugs and stimulants; prevention of constipation; exercise and rest; clothing; cleanliness; first aid to the injured; special hygiene of eyes, ears, nose and throat.

4. *Bacteria and Sanitation.*—General characteristics of bacteria, distribution, form, size, reproduction and conditions of growth; food preservation, soil inoculation and disease prevention. Public health; care of streets, water supply, sewage disposal, milk and inspection of foods, quarantine, disinfection and methods of securing immunity.

It is hoped in the final report to include various syllabi of courses in biology which have proved successful, but the committee feels that at present it is more desirable to gain greater uniformity and agreement in the aims and purposes of biological study in the secondary schools than in the content of the courses themselves. Given a pupil of normal capacity, it is of greater moment to have a teacher imbued with high ideals and aims, with a reasonably good preliminary training and endowed with the indispensable gift of gumption, than it is to have a definite program of topics outlined in the curriculum. In fact it would be disastrous to all future educational growth if we should ever reach such a degree of standardized efficiency in our plans and programs of study that we could be content to rest upon our laurels.

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BROWN UNIVERSITY

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