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RESISTANCE TO LACK OF OXYGEN IN ANIMALS.

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ON RESISTANCE TO LACK OF OXYGEN AND ON A METHOD OF INCREASING THIS RESISTANCE.

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I. INTRODUCTION.

THE most generally accepted theory of oxidation has been up to the present time that of Hoppe-Seyler.[1] According to this theory processes of fermentation take place in the protoplasm which result in the formation of nascent hydrogen. This combines with atmospheric oxygen, if it is present, forming water and setting free atoms of oxygen ($H_2 + O_2 = H_2O + O$). The nascent oxygen thus formed attacks the protoplasm, producing the oxidations characteristic of living matter at comparatively low temperatures. If atmospheric oxygen is not present the nascent hydrogen reduces substances in the cell and forms entirely different products, which are more or less poisonous. This theory did not account for anaërobic respiration in which the protoplasm carries on oxidations in the complete absence of free oxygen, or in the presence of only traces of it.

Mathews[2] has recently brought forward an hypothesis which differs somewhat from that of Hoppe-Seyler, and which is based on the recent work of Armstrong, Nef, and others. Armstrong[3] has shown that in ordinary oxidations the oxygen of the air does not combine directly with carbon to form carbon dioxide, or with hydrogen to form water. The presence of a certain amount of water is essential in all processes of oxidation, and the substances undergoing oxidation are first hydroxylized. The function of the atmospheric oxygen

[1] HOPPE-SEYLER: Zeitschrift für physiologische Chemie, 1877, i, p. 126.

[2] MATHEWS: Biological Bulletin, 1905, viii, p. 331.

[3] ARMSTRONG: Chemical News, 1904, xc, p. 25; Transactions of the chemical society of London, 1903, lxxxiii, p. 1088.

is simply to act as a depolarizer, to unite with the nascent hydrogen formed from the water. According to Mathews a somewhat similar process takes place in respiration. "Certain active particles in the protoplasm attack the water which is decomposed into oxygen and hydrogen. The oxygen combines with substances of the protoplasm, thus oxidizing them; the hydrogen is either set free in gaseous form, or it is united with atmospheric oxygen to form water; or it combines with other substances in the protoplasm." In other words, respiration is the dissociation of water with the liberation of hydrogen, and the real respiration is brought about not by the oxygen of the air but by that of water. If atmospheric oxygen is present, it unites with the hydrogen set free from the water, thus acting as a depolarizer. According to this theory aërobic and anaërobic respiration are identical; the only difference is that the anaërobic protoplasm is a powerful enough reducing agent to drive the hydrogen out of the water and let it escape as free hydrogen. The aërobic protoplasm, on the other hand, is less powerful, and requires the presence of oxygen to combine with the hydrogen. Barnes[1] has also reached very similar conclusions. Dr. Mathews further suggests that "as atmospheric oxygen thus acts the part only of a depolarizer, any other oxidizing agent, that is, any other substance which unites readily with nascent hydrogen, can replace the atmospheric oxygen and permit oxidation to go on in the absence of air." Lævulose is perhaps such a depolarizing substance. Maze[2] found that if lævulose were present alcohol was oxidized to acetic acid by a certain bacterium in the absence of air. The lævulose in this case united with the hydrogen formed, being itself changed to mannite. It was with a view of testing this point and others that at the suggestion of Dr. Mathews the following experiments were undertaken. The general problem was, How long will certain animals resist a lack of oxygen, and how may this resistance be increased.

II. Resistance to Lack of Oxygen.

That animals show a very unequal resistance to lack of air was shown more than a century ago by Spallanzani, who experimented by introducing his organisms into hermetically sealed vessels. Further experiments have been made by Bunge, Newport, Weinland, and others.

[1] Barnes: Botanical Gazette, 1905, xxxix, p. 81.

[2] Maze: Annales de l'Institut Pasteur, 1904, xviii, p. 277.

Bunge,[1] in his work upon the respiration of intestinal parasites and mud-dwelling organisms, showed that parasites in the intestine of warm-blooded animals must live practically in the absence of oxygen, as the contents of the intestine contain no free oxygen. Worms living in mud are also subject to similar conditions, decomposition processes, with the formation of reducing substances, keeping the oxygen absent. The following list showing the time that different animals will live in oxygen-free fluids is taken from Bunge's work:

Parasitic nematodes	4–6 days	Snails	½ day.
Leeches	4 days	Crayfish . . .	few hours
Planarians . . .	3 "	Water beetles .	" "
Earth worms . .	1 "	Mites	" "

Weinland[2] found that Ascarus lumbricoides from the intestine of the hog would live in boiled 1 per cent sodium chloride solution from four to six days. Newport[3] states that butterfly larvæ will live in an atmosphere of hydrogen for twelve hours.

It seemed advisable to determine for some of the common animals at Woods Hole the length of time that they would live in sea-water which had been freed from oxygen. The following method was used in the experiments. The sea-water was boiled for an hour, the water lost in evaporation being replaced by boiled distilled water. For the experiment the previously boiled water was placed in a 250 c.c. flask and a stream of hydrogen passed through it. The hydrogen was generated in the ordinary manner in a Kipp apparatus, and was thoroughly washed by passing through potassium hydrate solution, potassium permanganate solution, and distilled water. In several test experiments a solution of hæmoglobin was introduced into the sea-water to determine whether or not the oxygen had been completely removed. It was found that the hydrogen must be passed through the water from one and a half to two hours before the oxy-hæmoglobin as shown by the spectroscope was completely reduced. The animals to be tested were introduced into the flask while a rapid stream of hydrogen was passing through, and the hydrogen was continually passing during the entire course of the experiment. Table I gives the results of the experiments.

[1] BUNGE: Zeitschrift für physiologische Chemie, 1883, viii, p. 48; 1888, xii, p. 565; 1889, xiv, p. 318.

[2] WEINLAND: Zeitschrift für Biologie, 1901, xlii, p. 348.

[3] NEWPORT: Philosophical transactions, 1836, cxxvi, p. 529.

Several experiments were made on each species, and in the table the minimum, maximum, and the average time of all the experiments are given. Considerable individual variation in each species will be noticed. This depends somewhat upon the temperature and the condition of the animal at the time of the experiment, and whether or not it had been kept in an aquarium for some time previous. No correction was made for temperature beyond repeating the experiment a number of times and taking the mean. In the later experiments only fresh material just brought into the laboratory was used. It will be noticed from the table that there is a marked difference in the resistance of two common fish — Fundulus heteroclitis and Ctenolabrus. Loeb[1] has reported a similar difference in the resistance of both the eggs and embryos of these animals. Loeb[2] also found that in Fundulus the embryo was more sensitive to lack of oxygen the older it was. The fertilized egg could continue its development after having lain in an oxygen vacuum for four days, while the young fish just hatched could resist less than forty-eight hours

TABLE I.

LENGTH OF LIFE IN COMPLETE LACK OF OXYGEN.

	Minimum.	Maximum.	Average of all experiments.
	hrs. min.	hrs. min.	hrs. min.
Fundulus magalis	40	1 05	51
Fundulus heteroclitis	1 30	4 55	3 34
Ctenolabrus	35	55	45
Apeletes (Stickleback)	22	32	28
Gelasimus (Fiddler crab)	12 00	24 30	18 11
Eupagurus (Hermit crab)	11 30	15 00	14 15
Panopæus (Mud crab)	22 00	26 15	24 35
Palæmonetes	10	24	15
Talorchestia	44	1 18	1 03
Nereis	22 00	35 00	27 00
Amphitrite	18 00	22 00	21 00

[1] LOEB: Archiv für die gesammte Physiologie, 1895, lxii, p. 249.

[2] LOEB: *Ibid.*, 1894, lv, p. 530.

in the oxygen vacuum. This power of resistance evidently decreases with the growth of the animal, until in the adult stage it is three or four hours only.

The very high resistance of the worms and some of the mud-dwelling crustacea noted in Table I coincides with the observation of Bunge. On the other hand the hermit crab lives normally in the presence of abundant oxygen.

III. Effect of Increased Alkalinity of the Blood on Resistance to Lack of Oxygen.

It has been known for some time that an increase in the alkalinity of the protoplasm favored oxidation while a decrease caused it to cease. Zoethout[1] has shown that in paramœcium the resistance to lack of oxygen, or to poisons which prevent oxidation, may be increased by a very small percentage of alkali ($\frac{1}{400}-\frac{1}{2000}$ per cent KOH). The result is attributed to the antagonistic effect which the alkali has upon the poisons produced in the protoplasm by lack of oxygen. It seemed advisable to extend these experiments to some of the higher animals to test the effect of alkali on their resistance to lack of oxygen, by increasing the alkalinity of the blood. It is evident from the very great individual variation in resistance to lack of oxygen in any one species (see Table I) that any effect of the injection of alkali can be demonstrated only by the use of large numbers of individuals. Fundulus heteroclitis was selected for experiment as being easily obtained in sufficient quantities. Sodium bicarbonate was the alkali chosen. As is well known, dilute solutions of the acid sodium carbonate react alkaline. This is due to the hydrolysis of the acid salt by water setting free some hydroxyl ions: $NaHCO_3 + H_2O = \overset{+}{Na}, \overset{-}{OH} + H_2CO_3$. The amount of hydrolysis is slight and the carbonates and bicarbonates are much less irritating to animal tissues than the stronger hydrates. In order to avoid osmotic effects with the blood the bicarbonate was used in the strength of a $\frac{5}{16}$ *m* solution as being approximately isotonic with the blood of marine teleosts.[2] The method of the experiment was as follows: The fish were injected in the body cavity with three to eight drops (according to the size of the animal) of the sodium bicarbonate solution by means of a hypo-

[1] Zoethout: This journal, 1899, ii, p. 220.

[2] Garrey: Biological bulletin, 1905, viii, p. 257.

dermic syringe. They were then left for some time that the alkali might be absorbed. This was evidently very quickly done, as the length of time seems to make little difference in the experiment. A number of fish (usually 10 injected and 10 controls) were placed in a litre flask which was then completely filled with ordinary sea-water and tightly stoppered for the exclusion of all air. The animals very quickly exhausted the supply of oxygen in the water and thus were under conditions of lack of oxygen. A comparison of the tables will show that animals lived under these conditions but slightly longer than when elaborate methods were used to remove the oxygen from the water. That we are dealing here with phenomena of lack of oxygen and not with poisoning effects of the accumulation of carbon dioxide has been demonstrated by Jolyet and Regnard.[1] According to these authors a litre of sea-water contains 7.9 c.c. O; 15 c.c. N; 23.8 c.c. CO_2 (3.8 c.c. free: 20 c.c. in combination). If oxygen is supplied to fish in a closed vessel they will live in water in which the amount of carbon dioxide has increased to 211 c.c. per litre. Under the conditions of the experiment this accumulation would not take place until long after the relatively small amount of oxygen in sea-water had been entirely exhausted. The fish were left in the closed flask from half an hour to an hour after all movements of the animals had ceased. They were then removed to fresh, running sea-water and left from three to four hours for reviving. The length of time the animals should be left in the flask could not always be accurately determined. It depends a great deal upon the condition of the animals and the temperature; but as the controls and injected animals were subjected to the same conditions, the absolute length of time does not enter into the experiment. If they were not left long enough all the animals would revive, and in a few cases they were left too long and all were dead. Table II shows the results of the experiments.

It will be seen from the summary that out of the 183 individuals alive at the end of the experiments 55 or 30 per cent were controls and 128 or 70 per cent were injected; while out of 197 dead, 135 or 69 per cent were controls and only 62 or 31 per cent were injected. The 30 per cent alive controls are easily accounted for if we consider the variation in individual resistance. In every lot of animals there are always a few whose individual resistance is so great that they

[1] JOLYET et REGNARD: Archives de physiologie, 1877, pp. 44 and 584.

TABLE II.

FUNDULUS INJECTED WITH $\frac{5}{16}m$ $NaHCO_3$.

No. of experiment.	Time between injection and placing in flask.	Time in flask.	Controls.		Injected.	
			Alive.	Dead.	Alive.	Dead.
	hrs. min.	hrs. min.				
61	10	2 30	2	3	5	0
62	15	2 40	2	4	4	2
65	20	2 50	3	6	8	1
66	10	2 40	3	3	5	1
67	4 40	2 20	1	4	3	2
69	20	2 10	2	8	4	6
70	30	2 05	3	5	2	6
75	4 25	2 15	3	5	5	3
76	3 40	2 20	0	5	3	2
77	3 15	2 25	2	8	4	6
79	29 15	3 25	6	4	9	1
80	44 30	4 40	3	7	5	5
81	30	3 25	0	10	8	2
84	16 40	4 15	4	6	7	3
85	7 20	4 00	2	8	6	4
86	40	4 30	5	2	7	0
87	40	4 15	2	8	10	0
88	35	4 40	2	8	4	6
96	16 40	4 40	0	5	1	4
103	40	3 20	3	7	8	2
113	35	2 30	3	12	10	5
114	25	2 15	4	7	10	1
Total			55	135	128	62

SUMMARY.

Alive, 183 . . . . Controls, 55 (30%) Injected, 128 (70%)
Dead, 197 . . . . " 135 (69%) " 62 (31%)

will remain alive for the length of time of the experiment. The same reason also accounts for the 31 per cent dead animals which were injected. These are the animals whose individual resistance is so slight that even with the increased resistance given by the alkali, they are killed in the length of time of the experiment. The effect of the alkali lasts at least from one to two days. Evidently the alkali is not removed from the system very quickly.

In order to avoid the objection that perhaps the injection of a large amount of fluid may render the animals more resistant to lack of oxygen, the following two experiments are given. The animals were

TABLE III.

FUNDULUS INJECTED WITH DISTILLED WATER.

Time between injection and placing in flask.	Time in flask.	Controls.		Injected.	
		Alive.	Dead.	Alive.	Dead.
hrs. min.	hrs. min.				
2 00	2 10	7	3	7	3
2 05	2 15	6	4	6	4

injected with five drops of distilled water. In each case an equal number of controls and injected died, showing that a mere increase of fluid had no effect either favorable or detrimental.

IV. EFFECT OF DECREASED ALKALINITY OF THE BLOOD ON RESISTANCE TO LACK OF OXYGEN.

Table IV gives the result of the experiments to determine the effect of the injection of acid on the resistance to lack of oxygen. The animals were injected with $\frac{m}{250}$ or $\frac{m}{100}$ solution of acetic acid.

Experiments with $\frac{m}{500}$ acid showed that acid of that strength had no appreciable effect on the animals. A control experiment in which 50 animals were injected with $\frac{m}{100}$ acetic acid showed that the animals would endure acid of that strength. The animals lived in the aquarium with only the few deaths that would normally occur. From the summary it will be seen that there is exactly the reverse effect of the injection of the alkali. Of the 88 alive animals 59 (67 per cent) were controls and 29 (33 per cent) were injected and of the 108 dead 39 (36 per cent) were controls and 69 (64 per cent)

were injected. The detrimental effect of the acid is not quite as marked as the favorable effect of the alkali.

A comparison of the average length of life after injection of acid with that after the injection of alkali is instructive. Taking ten experiments which were carried out under conditions as near alike as

TABLE IV.

FUNDULUS INJECTED WITH $\frac{m}{100} - \frac{m}{250}$ ACETIC ACID.

No. of experiment.	Time between injection and placing in flask.	Time in flask.	Controls.		Injected.	
			Alive.	Dead.	Alive.	Dead.
	min.	hrs. min.				
92	10	3 40	6	4	4	6
94	10	3 30	7	3	5	5
98	15	4 00	5	5	4	6
99	5	4 00	9	1	5	5
102	20	3 20	3	7	0	10
107	20	2 25	4	6	1	9
110	35	2 40	10	0	4	6
111	5	2 05	3	5	1	7
112	10	1 55	6	4	2	8
115	15	2 15	6	4	3	7
Total			59	39	29	69

SUMMARY.

Alive, 88 . . . . Controls, 59 (67%) Injected, 29 (33%)
Dead, 108 . . . . " 39 (36%) " 69 (64%)

possible as regards temperature and freshness of the animals, we find that the average time in the flask for the animals injected with acid was two hours, fifty-nine minutes; for the animals injected with alkali, three hours, fifty-five minutes, an increase of fifty-six minutes in favor of the latter. It is possible that under the confined conditions in the flask the acid or alkali diffused into the water and affected the controls as well as those injected. Thus the length of life of the entire number in the flask with the acid would be shortened, while that of those with the alkali would be lengthened.

Rosenow[1] in some studies on pneumonia and pneumococcus infections reports the formation of acids by the growth of the pneumococcus in the consolidated lung and blood of pneumonic patients, and suggests that some of the symptoms of pneumonia are due to an acid intoxication. In the service of Dr. Frank Billings[2] at the Presbyterian Hospital, Chicago, sodium bicarbonate has been used clinically in the treatment of pneumonia for the past two years with favorable results. It is possible that these are conditions similar to those in our experiments. The effect of the acid in the blood is partly, at least, an interference with the processes of oxidation in the tissues which is relieved by the large doses of alkali with its favorable action on oxidation.

Loeb[3] has found that the addition of acids to sea-water delayed the development of the larvæ of sea-urchins, while the addition of alkalies accelerated the rate of development and growth. Loeb[4] also reports that the addition of alkalies to Van't Hoff's artificial sea-water favored the regeneration and growth of Tubularians. These facts may be interpreted as being the result of the favorable action of alkalies on processes of oxidation in the protoplasm.

Mathews[5] has given a possible explanation of the effect of acids and alkalies upon oxidation in terms of the solution-tension of the elements. "Respiration is carried on chiefly by the oxygen ions. When these are increased in number, as they are increased by making the protoplasm more alkaline, their solution-tension falls. They give up their negative charges so much the more rapidly and easily. When on the other hand we increase the acidity or reduce the alkalinity, there is a reduction of the hydroxyl ions, their solution-tension thereby increases, they no longer give up negative charges to protoplasm, and respiration is brought to a stop. Life and respiration of any cell is checked, as soon as the number of the hydroxyl ions is reduced so far that the solution-tension of the ion is greater than the solution-tension of the protoplasm." Whatever the explanation may be there is no doubt of the fact that many oxidations similar to those occurring in protoplasm are assisted by alkalies and retarded by acids.

[1] ROSENOW: Journal of infectious diseases, 1904, i, p. 280; Journal of the American Medical Association, 1905, xliv, p. 871.

[2] Cited from ROSENOW: *Loc. cit.*

[3] LOEB: Archiv für Entwickelungsmechanik, 1898, vii, p. 631.

[4] LOEB: Archiv für die gesammte Physiologie, 1904, ci, p. 340.

[5] MATHEWS: This journal, 1903–4, x, p. 319.

V. Effect of Lævulose.

It is to be assumed from the work of Maze[1] that lævulose may act as a depolarizer in the process of respiration, and permit oxidation to

TABLE V.

Fundulus Injected with $\frac{5}{8}m$ Laevulose.

No. of experiment.	Time between injection and placing in flask.	Time in flask.	Controls.		Injected.	
			Alive.	Dead.	Alive.	Dead.
	hrs. min.	hrs. min.				
71	10	1 45	4	3	5	2
72	15	1 28	6	3	7	2
73	10	2 35	1	3	2	2
116	1 20	2 40	6	3	7	2
117	10	2 30	7	3	2	8
120	5	3 15	7	3	7	3
121	28 00	5 00	3	6	3	6
125	50	3 30	9	1	6	4
127	1 40	4 00	10	5	9	6
128	19 10	4 20	2	2	2	2
129	28 00	3 25	4	6	3	7
130	4 00	3 15	3	3	4	2
131	3 45	3 15	6	4	5	5
132	3 45	3 15	6	4	5	5
133	28 55	4 00	1	9	9	1
Total			75	58	76	75

Summary.

Alive, 151	Controls, 75 (50%)	Injected, 76 (50%)
Dead, 115	" 58 (50%)	" 57 (50%)

go on in the absence of atmospheric oxygen. It was hoped that increasing the lævulose content of the blood of Fundulus would render them more resistant to lack of oxygen. The results of the experi-

[1] Maze: *Loc. cit.*

ments so far do not seem to bear out this conclusion. These results are given in Table IV.

The animals were injected with a $\frac{5}{8}m$ solution of lævulose as being approximately isotonic with the blood. From the summary it will be seen that approximately the same number of controls and injected animals remained alive and died. Lævulose is thus apparently indifferent in its action in regard to lack of oxygen. The experiments were necessarily brought to a close before the author was satisfied in regard to this, and further experiments will be taken up another summer. Varying strengths of lævulose should be tried. It may be also that not enough time is allowed for the absorption of the lævulose into the blood stream from the body cavity. Experiment No. 133, which was the last experiment tried, and which taken by itself is a very striking result, seems to indicate such a possibility. In this experiment 29 hours elapsed between the time of injection and the time of placing in the experiment.

Summary.

1. Increasing the alkalinity of the blood of Fundulus heteroclitis by the injection of three to eight drops of $\frac{5}{16}m$ solution of sodium bicarbonate increases their power of resistance to lack of oxygen. Decreasing the alkalinity by the injection of $\frac{m}{250}$ — $\frac{m}{500}$ solution of acetic acid decreases their power of resistance.

2. Increasing the lævulose content of the blood seems to have no effect on the power of resistance to lack of oxygen.

These observations were made in the Marine Biological Laboratory at Woods Hole, in a room provided by a grant from the Carnegie Institution, to whom and to the Assistant Director, Dr. F. R. Lillie, my thanks are due.

I wish also to thank Professor E. P. Lyon for suggestions and criticism, and I am especially indebted to Professor A. P. Mathews, under whose direction the work was done.

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THE EFFECT OF CARBOHYDRATES ON RESISTANCE TO LACK OF OXYGEN.

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I. INTRODUCTION.

IN a theory of the nature of protoplasmic respiration Mathews[1] has recently brought forward the hypothesis that respiration is the dissociation of water with the liberation of hydrogen. In the protoplasm there is some substance (or substances) of unknown nature which splits off water from itself and sets free active particles. These active particles attack the water of the protoplasm, decomposing it into oxygen and hydrogen. The oxygen combines with the substances of the protoplasm, thus oxidizing them; the hydrogen is either united with atmospheric oxygen to form water (aerobic respiration), or is set free in gaseous form, or it combines with other substances in the protoplasm (anaerobic respiration). According to this hypothesis the atmospheric oxygen acts the part only of a depolarizer to take care of the nascent hydrogen formed from the water, and any other substance which unites readily with nascent hydrogen can replace atmospheric oxygen and permit respiration to go on in the absence of air. Maze[2] found that acetic acid could be formed by certain bacteria from alcohol in the absence of air if lævulose were present; the lævulose at the same time being converted into mannite.

In the presence of air alcohol is oxidized to acetic acid as follows:

$$C_2H_5OH + O_2 = CH_3COOH + H_2O$$

In the absence of air and in the presence of lævulose the oxygen is derived from the water, and the hydrogen thus set free joins itself to the lævulose and forms mannite.

[1] MATHEWS: Biological bulletin, 1905, viii, p. 331.
[2] MAZE: Annales de l'Institut Pasteur, 1904, xviii, p. 277.

$$C_2H_5OH + H_2O = CH_3COOH + 2H_2$$
$$2H_2 + 2C_6H_{12}O_6 = 2C_6H_{14}O_6$$

In this case the lævulose acts as a depolarizer by uniting with the nascent hydrogen and permits oxidation to go on in the absence of air.

If this principle is capable of a wide application, it ought to be possible by the injection of certain substances into the blood of animals which will thus act as depolarizers, to enable the tissues to carry on respiration in the absence of atmospheric oxygen, or, in other words, to greatly increase their power of resistance to a lack of oxygen.

In a previous paper [1] the author presented a series of experiments which showed that the power of resistance of the common Minnow (Fundulus heteroclitus) to lack of oxygen may be increased by increasing the alkalinity of the blood by the injection of sodium bicarbonate.

In the same paper was also reported the attempt to increase their power of resistance by the injection of lævulose, but without apparent success. It was, however, stated that the experiments were necessarily brought to a close before the author was satisfied in regard to this, and that further experiments would be taken up another summer. It was further suggested that perhaps enough time had not been allowed for the absorption of the lævulose from the body cavity into the blood stream. The experiments were repeated with the changes indicated during the past summer and were further extended to include other carbohydrates than lævulose.

II. Effect of the Injection of Carbohydrates on Resistance to Lack of Oxygen.

The experiments were performed in a manner similar to those described in the previous paper. The animals were first assorted according to size and sex, and in any one experiment only animals of the same sex and approximately the same size were used. This was afterwards proved to be an entirely unnecessary precaution, as experiments showed that there was no constant difference in resistance to lack of oxygen in males and females or in large and small animals. The fish were injected in the body cavity with from five to eight drops of a 0.5 molecular solution of the carbohydrate whose effect was to be tested. The above solution is approximately isotonic with the blood

[1] Packard: This journal, 1905, xv, p. 30.

of marine teleosts,[1] and was used in that strength to avoid osmotic effects with the blood.

For an experiment ten of the injected fish were placed in an aquarium jar with running water along with ten others properly marked to serve as controls. They were then left in the running water for about twenty-four hours to allow ample time for the absorption of the carbohydrate which previous experiments had shown to be quite slow. The effect was not shown if less than eighteen hours' time was allowed for absorption, nor did the effect seem to last beyond thirty-six hours after injection. The fish (10 injected and 10 controls) were then placed in a litre flask which was immediately filled with sea water and tightly stoppered so that all air was excluded. Under these conditions the animals quickly exhausted the available supply of air and were thus under conditions of lack of oxygen. In the previous paper[2] it was shown that this method was as effective as when elaborate methods were used to remove the oxygen from the water, and also that we are here dealing with phenomena of lack of oxygen and not with the effects of the accumulation of carbon dioxide. The length of time the animals were left in the flask is given in the tables in connection with each experiment. It was usually until all movements of the animals had ceased. The animals were then removed to fresh running sea water and left several hours for reviving. Enough time was allowed for this so that all animals not actually killed in the experiment were given a sufficient chance to recover.

Table I shows the results of the experiments with the injection of lævulose.

It will be seen from the summary that out of 194 live individuals 135, or 70 per cent, were those which had been injected ; while only 59, or 30 per cent, were controls. Of the 166 dead, only 45, or 27 per cent, had been injected, while 121, or 73 per cent, were the controls.

The very great individual variation in resistance to lack of oxygen in different individuals renders it necessary to use many experiments and large numbers of individuals. This individual variation also explains why some (27 per cent) of the injected animals were killed by the lack of oxygen and a similar number (30 per cent) of the controls were left alive.

In the table all the experiments which were made are given, and an examination of each will show that while the increase in resistance

[1] GARREY: Biological bulletin, 1905, viii, p. 257.

[2] PACKARD: *Loc. cit.*

conferred by the lævulose was in some cases not very marked, yet in every experiment the effect was shown, for in each case a greater number of the injected animals than of the controls were left alive, and a greater number of the controls than of the injected were dead.

TABLE I.

FUNDULUS INJECTED WITH 0.5 MOL. LÆVULOSE.

Time between injection and placing in flask.	Time in flask.	Injected.		Controls.	
		Alive.	Dead.	Alive.	Dead.
hrs. min.	hrs. min.				
22 50	2	9	1	7	3
22 40	3 30	7	3	2	8
23 40	3 30	2	8	0	10
23 20	3 35	4	6	2	8
22 30	3	10	0	4	6
21 20	3	9	1	0	10
21 35	3	9	1	4	6
22 20	3	7	3	4	6
17 10	3	6	4	1	9
16 25	3	10	0	7	3
15 55	3	10	0	6	4
18 35	3	6	4	0	10
35 50	3	10	0	4	6
35 40	3	7	3	4	6
26 10	3 15	4	6	1	9
23 30	3	9	1	5	5
20 35	3 15	8	2	4	6
19 55	3 15	8	2	4	6
Total		135	45	59	121

SUMMARY.

Alive, 194 . . . . . Injected 135 (70 per cent). Controls 59 (30 per cent).
Dead, 166 . . . . . Injected 45 (27 per cent). Controls 121 (73 per cent).

Table II shows the effect of the injection of glucose. The results are almost identical with those with lævulose. 71 per cent of the injected and only 29 per cent of the controls were alive, while only 27 per cent of the injected were dead as contrasted with 73 per cent controls.

TABLE II.

FUNDULUS INJECTED WITH 0.5 MOL. GLUCOSE.

Time between injection and placing in flask.	Time in flask.	Injected.		Controls.	
		Alive.	Dead.	Alive.	Dead.
hrs. min.	hrs. min.				
25 30	3 15	5	5	3	7
24 15	3 15	8	2	2	8
24 10	3 15	8	2	5	5
24 05	3 15	6	4	2	8
23 15	3	8	2	5	5
23 40	3 15	8	2	3	7
23 20	3 30	9	1	3	7
23 30	3 30	7	3	2	8
23 45	3	6	4	3	7
20 05	3 15	8	2	3	7
20 15	3 15	6	4	3	7
22 00	2 30	9	1	3	7
Total		88	32	37	83

SUMMARY.

Alive, 125 . . . . . Injected 88 (71 per cent). Controls 37 (29 per cent).
Dead, 115 . . . . . Injected 32 (27 per cent). Controls 83 (73 per cent).

Maze [1] was unable to obtain any results with glucose such as he obtained with lævulose. If glucose were used instead of lævulose, he found no production of mannite, and hence but a trifling oxidation of alcohol into acetic acid. Perhaps plant respiration may be slightly different in this respect from animal respiration.

Table III gives the results of the injection with maltose. The

[1] MAZE: *Loc. cit.*

effect of maltose is even greater than either of the monosaccharides used. Of those left alive 78 per cent were injected and 22 per cent were controls, while of the dead only 23 per cent were injected and 77 per cent were controls.

TABLE III.

FUNDULUS INJECTED WITH 0.5 MOL. MALTOSE.

Time between injection and placing in flask.	Time in flask.	Injected.		Controls.	
		Alive.	Dead.	Alive.	Dead.
hrs. min.	hrs. min.				
20 45	3 15	10	0	4	6
21 00	3 15	6	4	2	8
21 25	3 00	9	1	4	6
21 15	3 00	9	1	3	7
22 45	3 30	7	3	1	9
23 00	3 30	4	6	2	8
24 00	3 30	7	3	1	9
24 15	3 30	8	2	1	9
22 30	2 30	8	2	2	8
Total		68	22	20	70

SUMMARY.

Alive, 88	Injected 68 (78 per cent).	Controls 20 (22 per cent).
Dead, 92	Injected 22 (23 per cent).	Controls 70 (77 per cent).

Tables IV and V give the results of the injection with two other common disaccharides — cane sugar and lactose. It will be seen from the summary of each that approximately an equal number of controls and injected were alive. The difference in percentage between injected individuals and controls both left alive and dead is well within the limits of individual variation. Another fact is to be noted. The average length of time the animals injected with lactose were left in the flask is about the same as that in the experiments with lævulose, glucose, or maltose, and yet a much larger proportion of the animals died. The same is even more strikingly shown in the experiments with cane sugar, where the average length of time in the flask is much less than that in the other experiments, and still a much

larger proportion of the animals died. This fact adds evidence to the conclusion to be drawn from Tables IV and V, that the injection of cane sugar and lactose does not confer any increased power of resistance to lack of oxygen. The animals left alive were those

TABLE IV.

FUNDULUS INJECTED WITH 0.5 MOL. CANE SUGAR.

Time between injection and placing in flask.	Time in flask.	Injected.		Controls.	
		Alive.	Dead.	Alive.	Dead.
hrs. min.	hrs. min.				
27 10	2 45	5	5	4	6
27 30	2 45	0	10	1	9
27 15	2 45	5	5	5	5
27 30	2 45	4	6	4	6
24 45	2 30	5	5	2	8
24 30	2 30	3	7	4	6
24 15	2 30	3	7	1	9
24 15	2 30	3	7	1	9
22 30	2 00	9	1	5	5
22 30	2 00	6	4	7	3
22 30	2 00	5	5	7	2
Total		48	62	42	68

SUMMARY.

Alive, 90 . . . . . Injected 48 (53 per cent). Controls 42 (47 per cent).
Dead, 130 . . . . . Injected 62 (47 per cent). Controls 68 (53 per cent).

whose individual variation in resistance was great enough to enable them to live that length of time in lack of oxygen.

It is commonly accepted that carbohydrates cannot be absorbed and assimilated until they have been converted into monosaccharides.

If polysaccharides or disaccharides are introduced into the circulation they are immediately eliminated in the urine unless the blood contains the necessary enzymes to convert them into the simpler sugars.

Dastre, Voit, Blumenthal, and others have studied the assimilation of carbohydrates when introduced into the body with the avoidance of the alimentary tract.

TABLE V.

FUNDULUS INJECTED WITH 0.5 MOL. LACTOSE.

Time between injection and placing in flask.	Time in flask.	Injected.		Controls.	
		Alive.	Dead.	Alive.	Dead.
hrs. min.	hrs. min.				
20 00	3 15	4	6	1	9
20 00	3 15	3	7	5	5
22 45	3 15	2	8	3	7
23 00	3 15	0	10	2	8
24 15	2 45	1	9	2	8
24 30	2 45	3	7	3	7
21 00	3 15	2	8	0	10
21 00	3 15	1	9	1	9
22 10	3 15	3	7	4	6
22 15	3 15	4	6	4	6
23 15	3 15	1	9	1	9
22 55	2 15	2	8	2	8
23 10	2 15	2	8	3	7
Total		28	102	31	99

SUMMARY.

Alive, 59 . . . . . Injected, 28 (48 per cent). Controls, 31 (52 per cent).
Dead, 201 . . . . . Injected, 102 (51 per cent). Controls, 99 (49 per cent).

Dastre and Bourquelat [1] found that maltose when introduced subcutaneously and intravenously was absorbed nearly as well as dextrose and much better than cane sugar, nearly all of the cane sugar being recovered in the urine.

Dastre [2] later reported that lactose was scarcely absorbed at all.

[1] DASTRE and BOURQUELAT: Comptes rendus, 1884, xcviii, p. 1604.
[2] DASTRE: Archives de physiologie, 1889, p. 718; 1890, p. 103.

Voit,[1] in some studies on the excretion of various carbohydrates by the kidneys in man after subcutaneous injection, gives the same results. Practically no dextrose, lævulose, or maltose was recovered in the urine, while cane sugar and lactose were thrown out in almost the same quantities in which they were injected.

Our experiments plainly coincide with the above results. Dextrose, lævulose, and maltose were absorbed and produced an increased resistance to lack of oxygen; cane sugar and lactose were not absorbed, and hence produced no effect. The blood of Fundulus evidently contains a maltase, but no invertase or lactase.

III. Effect of Feeding with Proteid Food on Resistance to Lack of Oxygen.

A possible objection to the conclusions drawn from our experiments with carbohydrates may be this; the effect of the injection of carbohydrates in increasing resistance to lack of oxygen is not due to the rôle they may play in respiration, but is merely the effect which any food would have in increasing general bodily strength and resistance to adverse conditions, especially when compared with animals used as controls which had been kept in an aquarium for some days, and hence in a condition of partial starvation.

In order to test this objection the following experiments were carried out. The Fundulus were kept in an aquarium without food for several days. For experimentation a series of animals were then placed in aquarium jars and fed with common mussels (Mytilus) for a period of about twenty-four hours. As they were in a partly starved condition, they were all seen to eat greedily, and they were kept supplied with food during the whole period. This diet of course consists very largely of proteids, with only a very small amount of carbohydrate material. Another series of animals were kept under similar conditions, but without food, for controls.

The fed animals and the controls were then placed in flasks, as in the other experiments, to test their resistance to lack of oxygen.

Table VI gives the results. From the summary it will be seen that approximately an equal percentage of the fed animals and the controls were left alive. These experiments prove that feeding with proteid food does not increase the power of resistance to lack of oxygen, and

[1] Voit: Münchener medicinische Wochenschrift, 1896, p. 717; Deutsches Archiv für klinische Medicin, 1897, lviii, p. 521.

hence answer the objection that carbohydrates act by merely increasing bodily strength.

The general conclusion to be drawn from all the experiments is that those carbohydrates which can be absorbed by the animal increase its

TABLE VI.

FUNDULUS FED WITH MUSSELS.

Time between feeding and placing in flask.	Time in flask.	Fed.		Controls.	
		Alive.	Dead.	Alive.	Dead.
hrs. min.	hrs. min.				
21 00	2 30	5	5	7	3
19 50	2 45	5	5	5	5
		4	6	5	5
		6	4	7	3
		7	3	7	3
19 15	2 45	4	6	5	5
19 15	2 45	3	7	4	6
20 00	2 45	4	6	4	6
		6	4	3	7
19 15	2 30	8	2	5	5
21 00	3 15	5	5	3	7
24 00	2 45	5	5	7	3
24 00	2 45	6	4	7	3
22 00	2 45	5	5	4	6
22 00	2 45	3	7	5	5
22 00	3 00	4	6	4	6
22 30	3 00	4	6	7	3
22 30	3 00	3	7	8	2
Total		87	93	97	83

SUMMARY.

Alive, 184 . . . . . Fed 87 (48 per cent). Controls 97 (52 per cent).
Dead, 176 . . . . . Fed 93 (52 per cent). Controls 83 (48 per cent).

resistance to lack of oxygen. The experiments also support Mathews' theory of protoplasmic respiration, that any substances which can readily unite with nascent hydrogen, and thus act as depolarizers, can replace atmospheric oxygen and permit respiration to go on in the absence of air.

Howell[1] has observed that cane sugar and dextrose will produce recovery of contractions after the so-called "sodium chloride arrest" in strips of heart muscle. He says: "After a heart strip has given its typical series of contractions in NaCl and the stage of the so-called exhaustion is reached, immersion in isotonic solutions of sugar gives usually a more or less definite series of contractions lasting for about an hour." The immersion of a fresh strip of heart muscle in the solutions of sugar gave no contractions whatever.

Lingle[2] believes that the "sodium chloride arrest is probably due to a lack of oxygen in the salt solutions," and finds that hydrogen peroxide and oxygen gas will produce recovery of contractions after sodium chloride exhaustion.

Martin[3] also mentions the fact of the recovery of muscle strips after sodium chloride arrest by the removal of the strip to an isotonic cane sugar solution, and also shows that the sugar solution has no effect on fresh muscle. Its effect is produced only after previous exhaustion.

In a more recent paper Martin[4] has carefully studied the influence of oxygen upon the activity of heart muscle strips. He finds that under conditions of deficiency of oxygen or in the presence of a moderate oxygen supply the sodium chloride arrest is probably chiefly due to lack of oxygen, and that "after exhaustion in an oxygen-free bath an excellent and long-continued recovery could be obtained by a thorough oxygenation of the solution or by transferring the strip to a moist chamber."

The above facts may be explained on the basis of Mathews' theory of respiration and in accordance with the experiments described in this paper. The sodium chloride arrest is acknowledged to be due in part at least to lack of oxygen, and the action of sugar solutions in the recovery from the exhaustion is simply their effect in acting as depolarizers and enabling the muscle to carry on respiration for a

[1] HOWELL: This journal, 1901, vi, p. 181.
[2] LINGLE: *Ibid.*, 1902, viii, p. 75.
[3] MARTIN: *Ibid.*, 1904, xi, p. 103.
[4] MARTIN: *Ibid.*, 1906, xv, p. 303.

time in the absence of oxygen. Placing the exhausted muscle in the sugar solutions has the same effect as placing them in a moist chamber in contact with the air or in an oxygenated solution. This explanation that carbohydrates act as depolarizers also indicates that the rôle of carbohydrates as the source of energy in muscular contraction may be somewhat different from that ordinarily accepted.

Among the plants, Diakonow[1] has found that "*Penicillum* and *Aspergillus* are killed or severely injured by the withdrawal of oxygen for a single hour, but both live a little longer when provided with sugar," which fact is also easily explained on the basis of the above theory.

IV. Resistance to Lack of Oxygen in Fundulus Embryos.

It is a commonly accepted fact that the embryo has a greater power of resistance in general than the fully developed animal. Little is known, however, as to whether this power of resistance decreases steadily with the progress of development of the embryo, or whether there are sudden changes in resistance in the transition from one stage of development to another. Loeb[2] has studied the relative sensitiveness of Fundulus embryos in various stages of development to lack of oxygen. He finds that the embryo is more sensitive to lack of oxygen the older it is, and that the sensitiveness increases more rapidly at first than later. Eggs just fertilized would continue their development after they had been in an oxygen vacuum for four days. Twenty-four hours after fertilization they would resist a lack of oxygen only forty-eight hours, while two-day-old and three-day-old embryos lost their power of development after thirty-two hours and twenty-two hours respectively. Young fish just hatched were still less resistant.

As it has been found that the average resistance to lack of oxygen in the adult Fundulus is about three and a half hours,[3] it seemed advisable to determine whether the decrease in power of resistance from twenty-four hours in the three-day-old embryo to three and a half hours in the adult was a constant decrease or not, and to determine if possible some of the causes of the decrease.

[1] Diakonow: The original papers were not available. Cited from Pfeffer's Physiology of plants, translated by A. J. Ewart, i, p. 536.

[2] Loeb: Archiv für die gesammte Physiologie, 1894, lv, p. 530.

[3] Packard: *Loc. cit.*

The results given in the following experiments show that the decrease in power of resistance to lack of oxygen is not continuous but exhibits sudden changes. The resistance of an embryo about ready to hatch (*i. e.*, twelve to fifteen days after fertilization) is practically the same as that of a three-day-old embryo. There is a sudden decrease in resistance of nearly one-half at the time of hatching and

TABLE VII.

LENGTH OF LIFE OF FUNDULUS EMBRYOS IN LACK OF OXYGEN.

Age of embryos.	Minimum.	Maximum.	Average of all experiments.
	hrs. min.	hrs. min.	hrs. min.
3–15 days after fertilization	12 30	17 30	16 33
Just hatched	7 45	9 30	8 15
Removed from egg membrane 12–16 days after fertilization.	7 15	11 00	8 52
12–24 hours after hatching	3 45	7 30	5 02
36–48 hours after hatching	2 45	4 30	3 25
4 days after hatching . .	2 15	2 45	2 30
7 days after hatching . .	50	2 10	1 14

following that a rather steady decrease until about forty-eight hours after hatching, when the power of resistance is no greater than that of the adult. This sudden increase in sensitiveness to lack of oxygen seems to be connected with the loss of the perivitelline fluid at the time of hatching, and the absorption of the yolk sac which takes place during the first thirty-six hours after hatching.

In these experiments the oxygen was removed by hydrogen gas, which was prepared in the usual manner in a Kipp apparatus. The gas was thoroughly washed through sodium hydrate solution, alkaline potassium permanganate solution, and distilled water. For observation the embryos were placed in paraffin cells in an Englemann gas chamber. As the circulation in the Fundulus embryo is already well established by the third day, the cessation of the heart contractions was in every case taken as the deathpoint. Table VII gives the results of these experiments.

No constant change in resistance to lack of oxygen could be detected in the embryos from the third day up to the time of hatching. All the differences shown in the different experiments were well

within the limits of individual variation in resistance. Loeb's observations showed that from after fertilization to the third day there was a constant decrease in power of resistance; but it seems that from this point on, after the circulation is well established in the embryo, there is practically no decrease. The general average of resistance to lack of oxygen during this period is given as about sixteen and a half hours.

At the time of hatching, however, a sudden change occurs. The resistance of the embryos just hatched is not more than half that of the embryo within the membrane. In many experiments with embryos twelve to sixteen days old and still within the egg membrane, the stimulus of the withdrawal of the oxygen was sufficient to cause a number of the embryos to break out of the egg membrane, and thus there were in the gas chamber both embryos still within the egg membrane and those just hatched out. In every case the resistance of those embryos just hatched was approximately one-half that of the embryos of the same age, but still within the egg membrane. The general average of resistance of such embryos just hatched is given in the table as eight hours and fifteen minutes.

It was also found that embryos from twelve to sixteen days old could be artificially hatched by pricking and tearing the egg membrane apart. The embryos thus removed from the membrane had approximately the same average of resistance as the embryos hatched under more normal conditions, and many experiments were made with embryos artificially hatched in this manner. Their general average of resistance is given as eight hours and fifty-two minutes, which is approximately the same as those which were more normally hatched.

From the time of hatching there is a constant and rapid decrease in power of resistance to lack of oxygen. In embryos from twelve to twenty-four hours after hatching the general average of resistance was about five hours, and for embryos thirty-six to forty-eight hours after hatching about three and a half hours, which is practically the general average of resistance for the adult individuals.

Thus within forty-eight hours the resistance of the embryos has decreased from sixteen and a half hours, the general average of the unhatched embryos, to three and a half hours, which is the general average of the adult. After this period of sudden change in power of resistance there follows slow and continuous decrease, which was followed in our experiments until the seventh day after hatching. The general average for the fourth day after hatching was two and a

half hours; for the seventh day one hour and fourteen minutes. At this time in the aquarium the embryos were dying rapidly. It seems difficult to keep them for a longer period under the artificial conditions of even a well-balanced aquarium.

In explanation of the relative increase in sensitiveness of the fish embryos to lack of oxygen Loeb[1] assumes "that the cells which are formed from the egg cell during the first stages of cleavage are different chemically from the cells which are formed later, so that the latter go to pieces more easily in lack of oxygen than the former." No indication, however, is given as to what constitutes this chemical difference.

On the basis of the experiments given in the first part of this paper the following explanation is offered.

It may be assumed that the Fundulus egg is well provided with some substance or substances, presumably of a carbohydrate nature, which can act as depolarizers in the process of respiration, and thus enable the egg to carry on respiration for a time in the absence of air. This would make the egg very resistant to the withdrawal of oxygen. It may further be assumed that these carbohydrate substances are used up in the processes of development, and the embryo thus becomes less and less resistant as development progresses. This material seems to be used up very rapidly during the first three days of development, and but very slowly if at all after the establishment of the circulation until the time of hatching. At the moment of hatching there is lost the fluid between the embryo and the egg membrane. In the embryos which were removed by pricking the egg membrane this fluid was always seen to exude through the puncture. Presumably this happens when the embryo itself breaks the membrane. The loss of this perivitelline fluid would account for the very sudden decrease of resistance at the time of hatching. After hatching the embryo enters upon a much more active life than when within the membrane. At the time of hatching the yolk sac attached to the embryo is large and rounded. It is so heavy that the animal swims about with difficulty. During the first forty-eight hours after hatching, however, this yolk sac is rapidly absorbed and the contour of the embryo becomes smooth.

This period of absorption of the yolk sac and of greatly increased activity coincides with the period of rapid decrease in the resistance of the embryo to lack of oxygen. The greatly increased activity

[1] LOEB: *Loc. cit.*

rapidly uses up the supply of stored carbohydrate material, and the resistance to lack of oxygen is correspondingly decreased until it becomes no greater than in the adult. The slow decrease in resistance to lack of oxygen which follows this period, and which renders the embryo less resistant than the adult, is probably entirely due to the unfavorable conditions of aquarium life to which the embryos are subjected, in which the conditions of oxygen supply are very poor compared with natural conditions.

TABLE VIII.

LENGTH OF LIFE IN LACK OF OXYGEN OF FUNDULUS EMBRYOS IN SUGAR SOLUTIONS.

Solution of sugar used.	Length of life of controls in ordinary sea water.	Length of life in sugar solution.	Increase.
	hrs. min.	hrs. min.	per cent.
10 c.c. sea water + 2 c.c. of 0.95 mol. lævulose . . .	55	1 55	73
10 c.c. sea water + 2 c.c. of 0.95 mol. glucose	1 55	3 25	78
10 c.c. sea water + 2 c.c. of 0.95 mol. maltose. . . .	1 00	2 25	141
10 c.c. sea water + 2 c.c. of 0.95 mol. cane sugar. . .	1 05	2 30	130
10 c.c. sea water + 2 c.c. of sat. sol. lactose	55	58	0

V. EFFECT OF CARBOHYDRATES ON RESISTANCE TO LACK OF OXYGEN IN FUNDULUS EMBRYOS.

If the resistance to lack of oxygen of the Fundulus embryos is less in the later stages of development than in the earlier stages because of the loss of carbohydrate material, their resistance ought to be increased by supplying them with the necessary substances. Experiments show that this is the case.

Young Fundulus embryos seven and eight days old after hatching were placed in sea water to which a certain amount of isotonic (0.95 molecular) solution of the sugar whose effect was to be tested was added. They were left in this solution for several hours to allow time for the absorption of the sugar. The sugar could be absorbed either directly through the body wall or through the alimentary system after digestive processes. For experimentation three embryos were placed in an Englemann gas chamber along with three other embryos of the same age in ordinary sea water to serve as controls and the oxygen

replaced by hydrogen. The controls and the embryos in the sugar solution were placed in separate paraffin cells side by side, and hence were under the same conditions as regards temperature and lack of oxygen. As the heart beat in the embryos was plainly visible, the stoppage of the contractions was taken as the death point. Table VIII gives the length of life in the controls in ordinary sea water and the length of life in the different sugar solutions, together with the percentage of increase in each case.

The results amply confirm the experiments with the injection of these sugars given in the first part of this paper. Maltose again has a greater effect than either glucose or lævulose. Cane sugar is evidently inverted and absorbed through the digestive processes. Its effect is nearly as great as that of maltose. Lactose probably cannot be digested or absorbed.

It is to be noted in this connection that glucose and lævulose arising from splitting processes in digestion are much more effective in their action than the same sugars when chemically prepared outside the body.

Summary.

1. Those carbohydrates which can be absorbed when injected peritoneally into Fundulus heteroclitis (*i. e.*, maltose, glucose, and lævulose) greatly increase their resistance to lack of oxygen. This is due to the fact that simple sugars can act as depolarizers in the processes of protoplasmic respiration, and thus enable respiration to be carried on to a certain extent in the absence of oxygen.

2. The decrease in resistance to lack of oxygen shown by Fundulus embryos in successive stages of development is due to the using up of material (probably of carbohydrate nature) stored in the egg. When embryos in later stages of development are supplied with carbohydrates which can be digested and absorbed, their length of life in lack of oxygen is greatly increased.

My thanks are due to the Director and Assistant Director of. the Marine Biological Laboratory for the privilege of occupying a research room during the past summer.

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FURTHER STUDIES ON RESISTANCE TO LACK OF OXYGEN.

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I. Introduction.

IN a previous paper[1] it has been shown that the effect of those carbohydrates which can be absorbed when injected into the body cavity of the common minnow, Fundulus heteroclitis (*i. e.*, maltose, glucose, and lævulose), is to increase the resistance of these animals to lack of oxygen. This effect of these sugars was explained on the assumption that they act as depolarizers in the processes of protoplasmic respiration, uniting with the nascent hydrogen formed in these processes and thus permitting oxidation to go on in the absence of air.

The observations described in the following paper are an extension of the experiments already published. They were undertaken to test the effect of the other food substances and some drugs, when injected intraperitoneally, on the resistance to lack of oxygen.

The same animals (Fundulus heteroclitis) were used, and the experiments were performed in a similar manner to that described in the previous paper,[2] which may be briefly outlined as follows. The fish were first assorted according to size and sex and were then injected into the body cavity with three to eight drops, according to the size of the animal, of the solution whose effect was to be tested. After injection they were replaced in running water to allow time for absorption. The definite lengths of time in each experiment are stated in the tables. For an experiment, ten of the injected animals and ten others of the same size properly marked

[1] Packard: This journal, 1907, xviii, p. 164.

[2] Packard: *Loc. cit.*

to serve as controls were placed in a litre flask which was then completely filled with sea water and tightly stoppered. Under these conditions the available supply of oxygen was quickly exhausted and the animals were under conditions of lack of oxygen. The length of time the animals were left in the flask in each experiment is given in the tables. It varies somewhat with the temperature. If the animals were not left in the flask long enough, they would all revive, and if left too long, they would all be dead. The animals were then removed from the flask and placed in running water for several hours, until all those not actually killed in the experiment had revived.

In all experiments of this character the importance of large numbers of individuals is to be strongly emphasized. The individual variation in resistance to lack of oxygen is so great that one experiment or a few experiments may show nothing. It is only when large numbers are used that the effect sought for can be demonstrated. In the following tables all the experiments which were performed are given.

II. The Effect of Mannose and Galactose on Resistance to Lack of Oxygen.

The earlier observations on the effect of carbohydrates on resistance to lack of oxygen were extended to include two other monosaccharides, mannose and galactose. The results of the experiments on injection with mannose are shown in Table I.

From the summary it will be seen that out of 126 alive individuals 88, or 70 per cent, were those which had been injected, while only 38, or 30 per cent, were the controls. Of the 134 dead individuals only 42, or 31 per cent, were injected, while 92, or 69 per cent, were controls.

If the mannose had no effect on the resistance to lack of oxygen, an approximately equal number, or 50 per cent each, of injected and controls would have been left alive and an equal number would have been dead.

The results show that a much greater percentage of injected individuals than of the controls were left alive and a correspondingly smaller percentage of the injected than of the controls were dead. The effect of the injection of mannose is therefore to in-

crease the resistance to lack of oxygen and is similar to the effect of the glucose and lævulose.

Table II shows the results of injection with galactose. It will be seen from the summary that an approximately equal number of

TABLE I.

FUNDULUS INJECTED WITH 0.5 MOL. MANNOSE.

Time between injection and placing in flask.	Time in flask.	Injected.		Controls.	
		Alive.	Dead.	Alive.	Dead.
hrs. min.	hrs. min.				
28 00	2 45	7	3	3	7
27 45	2 45	6	4	3	7
27 30	2 45	7	3	6	4
27 20	2 45	6	4	4	6
18 25	2 45	8	2	2	8
18 20	2 45	5	5	2	8
18 10	2 45	9	1	3	7
23 30	2 45	6	4	2	8
23 15	2 45	9	1	3	7
22 25	2 30	6	4	2	8
22 10	2 30	5	5	3	7
22 05	2 30	8	2	3	7
22 00	2 30	6	4	2	8
Total		88	42	38	92

SUMMARY.

Alive, 126 . . . Injected, 88 (70 per cent). Controls, 38 (30 per cent).
Dead, 134 . . . Injected, 42 (31 per cent). Controls, 92 (69 per cent).

injected animals and controls were left alive and a correspondingly equal number were dead. The difference in percentage between the injected individuals and controls both alive and dead is well within the limits of individual variation and would probably disappear if larger numbers were used. The injection of galactose therefore has no effect on the resistance to lack of oxygen and is

similar to that found in previous experiments for cane sugar and lactose.

TABLE II.

FUNDULUS INJECTED WITH 0.5 MOL. GALACTOSE.

Time between injection and placing in flask.	Time in flask.	Injected.		Controls.	
		Alive.	Dead.	Alive.	Dead.
hrs. min.	hrs. min.				
26 50	2 45	6	4	4	6
26 35	2 45	3	7	3	7
26 25	2 25	10	0	5	5
26 10	2 45	6	4	8	2
17 25	2 45	9	1	4	6
22 45	2 45	4	6	6	4
16 20	2 45	3	7	3	7
15 35	2 45	5	5	3	7
20 55	2 30	5	5	8	2
20 50	2 30	7	3	4	6
23 15	2 30	2	8	1	9
22 15	2 30	2	8	3	7
16 5	2 45	1	9	3	7
22 50	2 30	0	10	2	8
15 50	2 45	1	9	1	9
23 00	2 30	0	10	0	10
22 30	2 30	1	9	1	9
Total		65	105	59	111

SUMMARY.

Alive, 124 . . . Injected, 65 (52 per cent). Controls, 59 (48 per cent).
Dead, 216 . . . Injected, 105 (48 per cent). Controls, 111 (52 per cent).

The fact that the disaccharides, cane sugar and lactose, did not increase the resistance to lack of oxygen, as did maltose, was explained [3] as probably due to the lack of the necessary enzymes for

[3] PACKARD: *Loc. cit.*

converting them into the simple sugars, as it is generally recognized that the disaccharides must be converted into monosaccharides before they can be absorbed and assimilated. These disaccharides would be found rarely if ever in the food of fishes, and the absence of the inverting enzymes would receive a teleological explanation in the statement that the specific enzymes are developed only in response to the requirements of the individual animal. Thus lactase has been found very generally wanting in animals lower than the mammals and also in the adults of many of the mammals.[4] The absence of a lactase would not, however, explain the fact that the injection of galactose does not produce any increase in resistance to lack of oxygen, for it is already a monosaccharide and does not require inversion before it is supposedly capable of absorption. There is, however, some evidence that galactose is not assimilated to any great extent. Thus Blumenthal[5] found that the limit of assimilation of galactose after intravenous injection in rabbits was very low, being but slightly above that of cane sugar or lactose and far below that of dextrose or lævulose. Pavy[6] also, in his studies on the intravenous injection of carbohydrates in rabbits, reports that while all the monosaccharides were utilized to a great extent (*i. e.*, not eliminated in the urine), yet galactose was utilized to a less extent than dextrose and lævulose.

On the other hand, Dastre[7] found that, after subcutaneous and intravenous injection, galactose appeared only as traces in the urine and was therefore utilized to almost as great an extent as was either dextrose or lævulose.

Fritz Voit[8] found that, after subcutaneous injection in the human subject, galactose was not recovered from the urine, but was absorbed similarly to dextrose, lævulose, and maltose. McGuigan,[9] who with Dr. S. A. Matthews determined the amount of various sugars it is necessary to inject intravenously in rabbits before the

[4] See MENDEL and MITCHELL: This journal, 1907, xx, p. 80, for a short summary of the literature on the presence and distribution of the inverting enzymes and a bibliography.

[5] BLUMENTHAL: Beiträge zur chemischen Physiologie, 1905, vi, p. 829. The original paper was not available. Cited from MENDEL and MITCHELL: This journal, 1905, xiv, p. 239.

[6] PAVY: Journal of physiology, 1899, xxiv, p. 479.

[7] DASTRE: Centralblatt für Physiologie, 1889, p. 133.

[8] VOIT, F.: Deutsches Archiv für klinische Medicin, 1897, lviii, p. 523.

[9] MCGUIGAN: This journal, 1907, xix, p. 175.

appearance of sugar in the urine, reports that galactose was more readily oxidized than either lævulose or dextrose.

While the evidence in mammals is thus conflicting, there is no doubt, from the experiments given in this paper, that in fishes at least galactose is not absorbed to any great extent when injected intraperitoneally, for if the galactose had been absorbed it would probably have increased the resistance to lack of oxygen, since McGuigan [10] has found that, outside the body, galactose is oxidized by cupric acetate more easily than dextrose and only slightly less easily than lævulose.

This failure to absorb galactose in fishes might receive the same teleological explanation given above in the fact that lactose or its splitting products would ordinarily never occur in their food.

III. The Effect of Fats on Resistance to Lack of Oxygen.

Since it has been shown that carbohydrates when they can be absorbed increase the resistance of fishes to lack of oxygen, and also that proteids [11] when fed to the fish have no such effect, there is left yet to be considered the third group of food substances, the fats, which were next tested as to their effect on resistance to lack of oxygen. With this end in view the Fundulus were fed into the stomach by means of a pipette with 2–3 c.c. of oil and then placed in running water until the next day, or even longer (the definite lengths of time are shown in the tables), to allow time for the digestion and absorption of the oil, which would probably be rather slow. Both olive oil and linseed oil were used, and the results are given in Tables III and IV.

It will be seen from the summaries that in each case an approximately equal number of controls and fed individuals were left alive and an equal number were dead, thus showing that under the conditions of the experiment no effect on resistance to lack of oxygen was obtained. It was seen, on dissecting some of the fed individuals, that the oil had disappeared from the stomach and intestines, but whether it had been digested and absorbed or whether it had been passed out as excreta could not be determined, as the animals were necessarily left in running water and any oil excreted would have been immediately washed away.

[10] McGuigan: *Loc. cit.*

[11] Packard: *Loc. cit.*

The experiment was then tried of injecting linseed oil into the body cavity of the fish. The animals were left as before until the next day to allow time for absorption. The results are shown in Table V.

TABLE III.

FUNDULUS FED WITH OLIVE OIL.

Time between feeding and placing in flask.	Time in flask.	Fed.		Controls.	
		Alive.	Dead.	Alive.	Dead.
hrs. min.	hrs. min.				
20 10	2 30	3	7	3	7
24 00	2 30	2	8	2	8
22 45	2 30	5	5	4	6
39 20	2 40	4	6	5	5
39 00	2 40	5	5	2	8
24 00	2 30	3	7	6	4
23 50	2 30	4	6	4	6
23 35	2 30	8	2	6	4
23 20	2 30	3	7	3	7
38 20	2 40	1	9	4	6
Total		38	62	39	61

SUMMARY.

Alive, 77 . . . Fed, 38 (50 per cent). Controls, 39 (50 per cent).
Dead, 123 . . . Fed, 62 (50 per cent). Controls, 61 (50 per cent).

It will be seen from the summary that under the conditions of the experiment the resistance to lack of oxygen was greatly decreased. A much larger percentage of the injected animals than of the controls were dead, while a smaller percentage of the injected than of the controls were left alive.

An explanation of this result has not yet been arrived at. A series of fish were injected with linseed oil in a manner similar to that in the experiment and were then left in an aquarium for more than two weeks. During that time none of the fish showed any deleterious effect of the injected oil. At the end of that time all

the fish were alive and active and apparently normal. On opening the body cavity of the fish at the end of the two weeks it was found that it was still filled with the injected oil, and it could not be easily determined by comparing the amount with that previously injected whether any oil had been absorbed during all that time or not. It

TABLE IV.

FUNDULUS FED WITH LINSEED OIL.

Time between feeding and placing in flask.	Time in flask.	Fed.		Controls.	
		Alive.	Dead.	Alive.	Dead.
hrs. min.	hrs. min.				
8 15	2 45	4	6	3	7
8 00	2 25	5	5	5	5
21 15	2 45	5	5	3	7
20 45	2 45	5	5	3	7
22 30	2 30	4	6	5	5
22 15	2 30	5	5	4	6
18 35	2 30	5	5	6	4
18 20	2 30	6	4	9	1
18 00	2 30	4	6	6	4
Total		43	47	45	45

SUMMARY.

Alive, 88 . . . Fed, 43 (49 per cent). Controls, 45 (51 per cent).
Dead, 92 . . . Fed, 47 (51 per cent). Controls, 45 (49 per cent).

was judged that it had not. Since, then, the oil is probably not absorbed from the body cavity, no importance with reference to its effect on respiration can be attached to this experiment.

IV. THE EFFECT OF ALCOHOL ON RESISTANCE TO LACK OF OXYGEN.

The extensive literature on the physiological effects of alcohol both as a drug and as a food was thoroughly reviewed a few

years ago.[12] The earlier experiments on the influence of alcohol, considered as a drug, on respiration are concerned principally with the effect on the frequency and depth of the respiratory movements and with the influence on the oxygen intake and carbon-dioxide output. Although the earlier data are somewhat conflict-

TABLE V.

FUNDULUS INJECTED WITH LINSEED OIL.

Time between injection and placing in flask.	Time in flask.	Injected.		Controls.	
		Alive.	Dead.	Alive.	Dead.
hrs. min.	hrs. min.				
24 30	3 00	4	6	3	7
24 00	3 00	1	9	5	5
23 45	3 00	1	9	7	3
23 30	3 00	5	5	5	5
22 30	3 00	3	7	5	5
22 00	3 00	4	6	7	3
24 10	2 15	3	7	8	2
24 40	2 30	4	6	6	4
24 25	2 30	0	10	3	7
Total		25	65	49	41

SUMMARY.

Alive, 74 . . . . Injected, 25 (33 per cent). Controls, 49 (67 per cent).
Dead, 106 . . . . Injected, 65 (62 per cent). Controls, 41 (38 per cent).

ing, yet the later results, as summarized by Abel,[13] seem to indicate that in the higher animals, including man, alcohol in moderate doses acts as a respiratory stimulant for an hour or more after administration. This stimulating effect is seen in a slight increase in the volume of air passing through the lungs and in an increase in the absorption of oxygen (3.5 per cent). The stimulating effect,

[12] J. S. BILLINGS, Ed.: Physiological aspects of the liquor problem, 1903.

[13] ABEL: Pharmacological action of ethyl alcohol; Physiological aspects of the liquor problem, ii, p. 116.

however, is very transitory and is soon followed by a depressant action on both the rate and depth of the respiratory movements, an effect which is always obtained with the use of larger doses of alcohol. The explanation given by Singer[14] is adopted as the most probable one: "It is well known that alcohol dilates the superficial blood vessels and thus leads to an increase in heat dissipation. The animal organism counteracts this loss by an increase in heat production. In other words, alcohol induces loss of heat from the body and at the same time causes a compensatory increase in the oxygen intake in order that this loss may be made good by an increased combustion." Alcohol is thus not a direct respiratory stimulant, but acts only indirectly through an increased demand for oxygen required by an increase in the oxidative processes in the tissues which in turn was brought about by an increase in heat dissipation.

The question whether or not alcohol had a direct effect on protoplasmic respiration was not considered.

In summing up the literature on alcohol considered as a food in its relation to respiration, Atwood[15] gives the following:

1. "There is no evidence that alcohol, in moderate quantities, has any effect upon oxygen absorption different from that of ordinary nutrients.

2. "There is no evidence that alcohol in moderate quantities has any effect upon the oxidation of carbon or upon the elimination of carbon-dioxide, different from that of ordinary nutrients."

Atwater also calls attention to the fact that a clear distinction must be drawn between alcohol as a food and alcohol as a drug.

In his own experiments on the nutritive value of alcohol in which the "respiration calorimeter" was used, and which were far more accurate in plan and execution than any others which had preceded, Atwater[16] reaches the same conclusion, namely, that alcohol in moderate quantities (72 gm. of absolute alcohol per day) is oxidized in the body like sugar, starch, and fat and thus serves as a fuel, and that the body burns the alcohol at its disposal in the same way as any other food in sufficient quantity to supply the energy it needs for warmth and work. In other words,

[14] SINGER: Physiological aspects of the liquor problem, ii, pp. 112 *et seq.*

[15] ATWOOD: The nutritive value of alcohol; Physiological aspects of the liquor problem, ii, p. 190.

[16] ATWATER: Physiological aspects of the liquor problem, ii, p. 292.

alcohol in this quantity acts as a food and interferes in no way with the consumption of oxygen and the production of carbon-dioxide.

Since in general the experiments already mentioned do not touch directly the question as to the effect of alcohol on protoplasmic respiration, it was determined to test the effect of alcohol on resistance to lack of oxygen as bearing more directly on this point.

In the first few preliminary experiments the results obtained were very irregular, a fact which has been noted by all investigators on the effects of alcohol. There is evidently a great individual variation in the sensitiveness of different individuals to the effects of alcohol, and consistent results can be obtained only by the use of many experiments and large numbers of individuals. It was determined, therefore, to make a series of experiments with gradually increasing strengths of alcohol; commencing with 1 per cent and increasing until a strength was reached which was as much as the animals would stand. With each per cent of alcohol at least eight experiments were made involving the use of two hundred animals. In all about one hundred experiments were performed with more than two thousand fish, and the results showed a gratifying consistency. The alcohol used was the ordinary commercial alcohol (96 per cent), and was diluted with distilled water to make the proper per cent. The animals were injected with from three to eight drops of the alcohol according to the size of the fish. In each experiment only animals of approximately the same size were used, and an effort was made in the different experiments to inject an amount proportional to the size of the animals used, so that in all the experiments on each strength of alcohol, as near as possible, approximately the same ratio of alcohol to body weight was used. From ten to fifteen minutes were allowed after the injection of the alcohol for absorption before the animals were placed in the flask.

The results of all the experiments with the injection of alcohol are summarized in Table VI. In this table are collected together the summaries of individual tables similar in form to Tables VII–IX, but which are not given here.

The distilled water used in the dilution of the alcohol was first injected in a series of experiments as a control, and a summary of the results is included in the table with the results of the alcohol. The distilled water is seen to be without any effect on resistance to lack of oxygen, as an equal number (50 per cent)

of both controls and injected were left alive and an equal number (50 per cent) were dead. Hence, whatever effect on resistance to lack of oxygen was obtained with the diluted alcohol may be attributed to the alcohol itself, and not to the water used in dilution. From the remainder of the table it will be seen at a glance

TABLE VI.

FUNDULUS INJECTED WITH ALCOHOL.[1]

Strength of alcohol injected.	Alive.			Dead.		
	Total.	Injected.	Controls.	Total.	Injected.	Controls.
Dist. H_2O	69	34 (50%)	35 (50%)	71	36 (50%)	35 (50%)
1 per cent.	95	44 (47%)	51 (53%)	85	46 (54%)	39 (46%)
2 " "	85	40 (47%)	45 (53%)	55	30 (55%)	25 (45%)
3 " "	74	31 (42%)	43 (58%)	86	49 (57%)	37 (43%)
4 " "	71	28 (40%)	43 (60%)	89	52 (57%)	37 (43%)
5 " "	103	43 (42%)	60 (58%)	97	57 (58%)	40 (42%)
10 " "	99	37 (37%)	62 (63%)	141	83 (59%)	58 (41%)
15 " "	58	18 (31%)	40 (69%)	102	62 (60%)	40 (40%)
20 " "	96	28 (29%)	68 (71%)	64	52 (81%)	12 (19%)
25 " "	90	25 (27%)	65 (73%)	70	55 (79%)	15 (21%)
30 " "	93	25 (26%)	68 (74%)	67	55 (82%)	12 (18%)

[1] In these experiments with alcohol the summaries only of the tables, and not the individual tables, are given. With each per cent of alcohol at least eight experiments were made.

that with every strength of alcohol used there is a decrease in resistance to lack of oxygen, and that in general the resistance to lack of oxygen decreases in proportion as the strength of the alcohol used increases. The percentage of injected individuals left alive gradually and steadily decreases from 50 per cent when distilled water was used to 26 per cent when 30 per cent alcohol was used, and the percentage of injected individuals which were dead steadily increases from 50 per cent with distilled water to 82 per cent with 30 per cent alcohol, while there is a correspond-

ing increase and decrease in the percentage of controls left alive and dead. This decrease in resistance is very slight with 1 and 2 per cent alcohol, where it is almost within the limits of experimental error, but still it is a decrease and not an increase. With 3 per cent and higher strengths the resistance rapidly decreases.

There are certain small irregularities which a careful examina-

TABLE VII.

FUNDULUS INJECTED WITH 10 PER CENT ALCOHOL.

Time between injection and placing in flask.	Time in flask.	Injected.		Controls.	
		Alive.	Dead.	Alive.	Dead.
hrs. min.	hrs. min.				
2 30	2 05	5	5	7	3
2 30	2 05	2	8	3	7
1 40	2 00	7	3	8	2
2 30	2 00	6	4	5	5
2 45	1 30	5	5	7	3
2 40	1 30	8	2	10	0
2 15	1 45	6	4	7	3
2 10	1 45	8	2	8	2
Total		47	33	55	25

SUMMARY.

Alive, 102 . . . . Injected, 47 (46 per cent). Controls, 55 (54 per cent).
Dead, 58 . . . . Injected, 33 (57 per cent). Controls, 25 (43 per cent).

tion of the table will show. It will be noticed that the percentage of injected animals left alive does not in many cases exactly correspond to the percentage of injected animals which were dead. This, however, is easily explained as due to the fact that the total number of individuals, both injected and controls, left alive was not equal to the total number of injected and controls which were dead. This irregularity would disappear with the use of a still larger number of animals in the experiments when the number left alive and the number dead would become more nearly equal.

Other small irregularities in the figures may also be noticed as,

for instance, the percentage left alive after the injection of 5 per cent alcohol (42 per cent) is greater than the percentage left alive after the injection of 4 per cent (40 per cent), when it should have been less. Also the percentage left alive after the injection of 2 per cent (47 per cent) is the same as after the injection of 1 per cent, when it should have been less. These irregularities

TABLE VIII.

FUNDULUS INJECTED WITH 10 PER CENT ALCOHOL.

Time between injection and placing in flask.	Time in flask.	Injected.		Controls.	
		Alive.	Dead.	Alive.	Dead.
hrs. min.	hrs. min.				
20 45	2 30	4	6	4	6
20 45	2 30	4	6	6	4
18 00	2 30	5	5	4	6
17 00	2 30	8	2	9	1
17 45	2 30	8	2	6	4
17 45	2 30	8	2	10	0
Total		37	23	39	21

SUMMARY.

Alive, 76 . . . Injected, 37 (48 per cent). Controls, 39 (52 per cent).
Dead, 44 . . . Injected, 23 (53 per cent). Controls, 21 (47 per cent).

would undoubtedly disappear with the use of larger numbers of animals, and they do not in any way vitiate the general conclusion which may be drawn from the whole number of experiments; namely, that alcohol does not, in any strength, increase the resistance to lack of oxygen, but always causes a decrease, and in general this decrease is in proportion to the strength of the alcohol used.

Attention is to be called to the fact that, in general, Fundulus is very resistant to the ordinary effects of alcohol. At no time after the injection of alcohol in strengths up to 25 per cent were any symptoms of intoxication to be observed. An increased activity, loss of equilibrium, or narcosis were never noted. The

injected animals could not be seen to be any different from those used as controls.

With the injection of 30 per cent alcohol, however, effects began to be noticed. One or two in each experiment would show signs of narcosis, floating belly up at the surface of the water, with the respiratory movements much slower than normal. In most cases

TABLE IX.

FUNDULUS INJECTED WITH 25 PER CENT ALCOHOL.

Time between injection and placing in flask.	Time in flask.	Injected.		Controls.	
		Alive.	Dead.	Alive.	Dead.
hrs. min.	hrs. min.				
3 50	1 45	6	4	10	0
3 50	1 45	8	2	3	7
3 40	2 00	8	2	6	4
3 40	2 00	6	4	10	0
3 30	2 15	8	2	10	0
Total		36	14	39	11

SUMMARY.

Alive, 75 . . . . Injected, 36 (48 per cent). Controls, 39 (52 per cent).
Dead, 25 . . . . Injected, 14 (56 per cent). Controls, 11 (44 per cent).

recovery would take place within a few hours, when the animals would seem perfectly normal again. In a few cases, however, the animals would die. The abdomen in these cases was usually very red, showing an intense inflammation, brought about, no doubt, by the local irritant action of the alcohol on the peritoneum.

All these effects were more marked with the injection of 40 per cent alcohol. About five out of ten animals would be dead at the end of twenty-four hours after injection, with the same signs of inflammation in the abdomen. With the injection of 50 per cent, seven out of ten animals were dead, and with 60 per cent eight out of ten. Higher per cents of alcohol usually resulted in the death of all the animals, although occasionally one would survive the injection of even 80 per cent.

It has generally been recognized that alcohol when taken in

ordinary doses is very completely oxidized by the body, and that most of it disappears during the first ten or twelve hours. Atwater's[17] experiments indicate that fully 98 per cent is burned in the body, although the rate of oxidation is somewhat slower than had been ordinarily supposed, no evidence being found that it was burned any more rapidly than the ordinary nutrients of food.

In order to determine how quickly alcohol was oxidized by Fundulus a series of experiments were next undertaken to determine how quickly the effect of alcohol on resistance to lack of oxygen disappeared. In order to test this the fish were left several hours after injection before being placed under conditions of lack of oxygen; the definite length of time in each case is given in the tables. In the experiments given in Table VI from ten to fifteen minutes only had been allowed after injection before placing in the flask. Our results are shown in Tables VII–IX.

Table VII gives the results when between two and three hours' time after injection with 10 per cent alcohol was allowed before placing in the flask. The number of alive injected individuals, which was 37 per cent (Table VI) when only a few minutes elapsed between injection and placing in the flask, has now risen to 46 per cent. The number of dead injected animals, which was 59 per cent with the shorter time, has now decreased to 57 per cent. These results indicate that the effect of the injection of 10 per cent alcohol has decreased considerably by the end of two or three hours.

Table VIII gives the results when a still longer time (seventeen to twenty hours) was allowed between injection and placing in the flask. The number of alive injected individuals has now risen to 48 per cent, and the number of injected dead has fallen to 53 per cent, which is nearly within the limits of individual variation, and indicates that by the end of seventeen to twenty hours the effect of the alcohol had practically disappeared.

That a much shorter time even than that is needed before the effect disappears is shown by Table IX. The animals were here left not quite four hours between injecting with 25 per cent alcohol and placing in the flask. The number of alive injected animals, which was 27 per cent when only a few minutes had elapsed between injection and placing in the flask (Table VI), has

[17] ATWATER: *Loc. cit.*, ii, p. 243.

now risen to 48 per cent, and the number of injected dead individuals, which was 79 per cent with the shorter time, has now decreased to 56 per cent. Our results thus fall in line with the previous work in showing that during the first few hours alcohol is oxidized and disappears from the body.

In any discussion concerning the method of the action of alcohol in producing a decrease in the resistance to lack of oxygen there are several possibilities which must be considered.

1. A possible manner in which alcohol might decrease the resistance to lack of oxygen is suggested by some experiments on anærobic respiration in the higher plants. It was in fact a consideration of these experiments which led in the first place to the testing of the effect of alcohol on resistance to lack of oxygen. Stoklassa and his pupils in various papers[18] have shown that in anærobic respiration in seed plants, alcohol and carbon-dioxide are formed at the expense of the carbohydrates present and in the same proportion as in alcoholic fermentation by yeast. The conclusion is reached that the anærobic metabolism of the seed plants is essentially identical with alcoholic fermentation by yeast. The first step is a conversion of sugar to lactic acid. The lactic acid is then split into alcohol and carbon-dioxide. Similar conclusions have also been reached by Palladin and Kostytschew,[19] who have found that the anærobic respiration of etiolated bean and lupine leaves was very great after the addition of sugars, and also that the sugar-nourished leaves remained living a much longer time in lack of oxygen than leaves not so nourished. In these sugar-nourished leaves, in the absence of oxygen, alcohol and carbon-dioxide were found in the same proportion as in the fermentation of sugar by yeast, and therefore the anærobic respiration might be considered to be a true alcoholic fermentation.

Now, in accordance with the law of chemical equilibrium, the rate of any chemical action decreases as the products of the action accumulate, and the action finally ceases when the products have attained a definite concentration. The accumulation of alcohol in a sugar solution undergoing fermentation with yeast gradually

[18] STOKLASSA: Zeitschrift für die gesammte Biochemie, 1903, iii, Heft 11. Archiv für die gesammte Physiologie, 1904, ci. Berichte der deutschen botanischen Gesellschaft, 1906, xxiv, p. 542.

[19] PALLADIN und KOSTYTSCHEW: Berichte der deutschen botanischen Gesellschaft, 1906, xxiv, p. 273; 1907, xxv, p. 51.

causes the fermentative processes to cease, and the addition of alcohol to the fermentating solution greatly decreases the growth of the yeast.[20] Hence, if alcohol is a product formed during respiration in the absence of oxygen, the accumulation of alcohol or the addition of alcohol to the tissues would decrease the amount of respiration in proportion to its concentration. This would shorten the length of time in which respiratory processes could go on in the absence of oxygen, and by that means decrease the resistance to lack of oxygen.

Alcohol has been found in minute quantities in the muscles of the higher animals.[21] It is probably a partial decomposition product of metabolism, but it is not known, so far as could be determined, whether it increases in quantity or not under conditions of lack of oxygen; so no positive comparison can be made between anærobic respiration in animals and in plants, nor can a definite statement be made as to whether or not alcohol acts in the manner stated above in decreasing the resistance to lack of oxygen.

2. In the second place it may be possible that the products of oxidation of the alcohol in the body may interfere with the processes of protoplasmic respiration. Alcohol on oxidation outside the body would form acetic acid, and while it is possible that the oxidation in the body may not give rise to the same products, yet Thomas[22] has shown that a volatile fatty acid is present in the blood of rabbits during intoxication, and the alkalinity of the blood was much reduced. The nature of this fatty acid was not determined, but since it is a fact that formic acid is excreted after methyl alcohol,[23] there can be but little doubt that the acid is acetic acid. This lowering of the alkalinity of the blood by the presence of the volatile fatty acid arising from the oxidation of the alcohol would greatly decrease the resistance of the animals to lack of oxygen.

It is well known that acids greatly retard protoplasmic oxidations, and the author has shown[24] that the injection of *m*/200 solu-

[20] HODGES: Influence of alcohol in growth; Physiological aspects of liquor problem, i, p. 361.

[21] RAJEWSKY: Archiv für die gesammte Physiologie, 1875, xi, p. 122.

[22] THOMAS: Archiv für experimentelle Pathologie und Pharmakologie, xli, pp. 3 and 4.

[23] POHL: Archiv für experimentelle Pathologie und Pharmakologie, xxxi, p. 281.

[24] PACKARD: This journal, 1905, xv, p. 30.

tion of acetic decreased the resistance of Fundulus to lack of oxygen. So it is possible that the effect of the alcohol may be due to its products of oxidation in the body.

3. In the third place the alcohol may be conceived of as affecting directly the respiration of the protoplasm, lowering its power of oxidation. There can be no doubt that while alcohol at times and in small quantities may serve to a limited extent as a food, yet it acts at all times as a drug and in large quantities is positively toxic, acting directly upon cell tissue. It is clearly a poison, retarding or even preventing metabolism, and as such would interfere with the respiration of the protoplasm.

The amount of alcohol necessary to produce a poisonous effect varies greatly with different living organisms. It can only be considered as a weak poison. It has been shown that algæ can withstand the effects of a 2 per cent solution for twenty-four hours, and infusoria tolerate a 1 per cent solution for some time.[25] Martin and Stevens[26] have shown, in the case of the isolated heart of a dog, that if the blood supplied to the heart contains one half of 1 per cent alcohol, the heart is no longer able to do its work. If the quantity of alcohol is less (*e. g.*, one fourth of 1 per cent), the effect on the heart is less, while if the percentage is only one eighth of 1 per cent, the alcohol has no influence at all.

Grehant[27] has shown that when the dog is in a profound state of intoxication the blood contains only one half of 1 per cent alcohol. The presence of less than 1 per cent alcohol in the blood was sufficient to cause death from respiratory paralysis. Carlson[28] states that the heart of Limulus will continue to beat for several hours with the ganglion in one fifth of 1 per cent alcohol, but with 1 per cent the primary stimulating phase is soon followed by depression and ultimate paralysis. The heart muscle itself is a little less sensitive.

In our own experiments with Fundulus the injection of 10 per cent alcohol, as given in the experiments in Table VII, is equivalent to 0.5 gm. of absolute alcohol per 100 gm. of body weight, or one half per cent of alcohol per body weight. The injection of 25 per cent alcohol in the experiments given in Table IX is equiva-

[25] Physiological aspects of liquor problem, ii, p. 13.

[26] MARTIN and STEVENS: Studies from Johns Hopkins Biological Laboratory, ii, p. 477.

[27] GREHANT: Journal de l'anatomie, xxxvi, p. 143.

[28] CARLSON: This journal, xvii, p. 177.

lent to 1.5 gm. of alcohol per 100 gm. of body weight, or 1½ per cent alcohol per body weight. These results show that fish are much more resistant to the toxic action of alcohol than is the dog, for the injection of 1.5 gm. of alcohol per 100 gm. of body weight was not sufficient to cause the death of the animals. Although the quantity above mentioned was not sufficient to cause death, it may have been great enough to have exerted a poisonous action upon the cell tissue sufficient to have decreased the resistance to lack of oxygen to the extent obtained in the experiments, and thus the action of alcohol in decreasing resistance to lack of oxygen may be explained on the basis of its toxic action upon protoplasm. When one considers the amount of alcohol which it is necessary to inject before any great decrease in resistance to lack of oxygen is obtained, it seems as if the view that alcohol acts as a weak poison must be the more probable explanation as to the manner in which alcohol acts in decreasing resistance to lack of oxygen.

V. Effect of Acetone on Resistance to Lack of Oxygen.

Acetone was found by Palladin and Kostytschew[29] to be formed along with alcohol, under certain conditions, both in ærobic and anærobic respiration of living and frozen plants. Acetone is also found in the urine in diabetes mellitus and fevers,[30] where it is supposed to be the result of an abnormal metabolism of the body's organized proteid. It is also found in the urine in other diseases in which there is an abundant destruction of body proteid.

As very little seemed to be known in regard to the pharmacological action of acetone, it was determined to test its effect on resistance to lack of oxygen. Acetone when treated with hydrogen is resolved into secondary propyl alcohol, and it was hoped that it might perhaps be able to act as a depolarizer, as the sugars do, and increase the resistance to lack of oxygen. Our results as summarized in Table X show that this is not the case, for when acetone was injected in solutions strong enough to produce any effect on resistance to lack of oxygen it always caused a decrease and never an increase. The injection of 0.1 per cent acetone had no effect upon the resistance to lack of oxygen, as an equal number of injected and controls were left alive and an equal number were

[29] Palladin und Kostytschew: Berichte der deutschen botanischen Gesellschaft, 1906, xxiv, p. 273.

[30] Jaksch: Ueber Acetonurie und Diaceturia, 1885.

dead. 0.5 per cent acetone caused a decrease in the resistance to lack of oxygen. Only 40 per cent of the injected were left alive, while 68 per cent of the injected were dead.

There is a still further decrease in the resistance to lack of oxygen with the injection of 5 per cent, though the effect is not so great as might be supposed from the sudden increase in the strength of the acetone used. Thirty-eight per cent of the injected were

TABLE X.

FUNDULUS INJECTED ACETONE.[1]

Strength of acetone injected.	Alive.			Dead.		
	Total.	Injected.	Controls.	Total.	Injected.	Controls.
per cent.						
0.1	72	36 (50%)	36 (50%)	68	34 (50%)	34 (50%)
0.5	74	29 (40%)	45 (60%)	46	31 (68%)	15 (32%)
5.0	86	33 (38%)	53 (62%)	34	27 (79%)	7 (21%)

[1] In this table the summaries only of individual tables are used. With each per cent of acetone at least six experiments were made. In these experiments from ten to fifteen minutes were allowed for the absorption of the acetone after injection before placing in the flask.

left alive, and 79 per cent were dead. A series of control experiments were made to test any effect the acetone might have on the normal animals when they were not placed under conditions of lack of oxygen. The injection of 0.5 per cent acetone was followed by no effect that could be perceived. The animals seemed perfectly normal at all times. After the injection of 5 per cent acetone a few of the animals would show signs of narcosis within a few minutes, usually floating at the surface of the water, with the rate of respiratory movements much slower than normal. Recovery, however, took place after one to two hours, and the animals seemed perfectly normal again. The injection of 10 per cent acetone was followed by signs of narcosis in a greater number of animals, and usually at the end of twenty-four hours two or three individuals out of ten animals would be dead. It was therefore deemed useless to test the effect of this strength or any stronger solution on resistance to lack of oxygen.

Lack of time prevented the carrying out of experiments to determine how quickly the effect of the acetone disappeared. It may be judged, from the rather quick recovery that the animals made from the narcotic effects, that the acetone is oxidized and eliminated from the body more quickly than alcohol. Acetone produces narcosis more quickly and in weaker solutions than does alcohol, and its effects correspondingly more quickly disappear.

VI. The Effect of Pilocarpine on Resistance to Lack of Oxygen.

Mathews[31] has shown that the addition of small amounts of pilocarpine hydrochlorate to sea water (0.5 c.c. to 1 c.c. of one half per cent pilocarpine to 100 c.c. of sea water) hastens the development of embryos of starfish and sea-urchins and gives rise to abnormally large embryos. The explanation given is that the nature of the action suggests that pilocarpine increases the oxidations taking place in the cells. Sollmann[32] gives the same results. Pilocarpine added to sea water in concentration of 0.2 to 2.0 : 10,000 hastened the development of starfish to embryos. Larger doses produced the opposite effect.

If pilocarpine increases the oxidations of the protoplasm, there are two possibilities as to what its effect might be on resistance to lack of oxygen. The effect of the drug might be an increased power of oxidation, in which case the resistance to lack of oxygen would be increased. Or the effect might be an increased rapidity in the oxidative processes, in which case under condition of lack of oxygen the available supply of oxygen and oxidizable substances would be more quickly exhausted and the resistance to lack of oxygen would be decreased. The results of our experiments summarized in Table XI show that the latter is the case, for when pilocarpine was injected in solutions strong enough to have any effect on resistance to lack of oxygen it always caused a decrease and never an increase.

The injection of 0.01 per cent pilocarpine produced no effect on resistance to lack of oxygen, as exactly an equal number of injected animals (50 per cent) were left alive and were dead and a correspondingly equal number of controls were left alive and were dead. With the injection of 0.05 per cent the number of

[31] Mathews: This journal, 1901, vi, p. 207.

[32] Sollmann: This journal, 1904, x, p. 352.

alive injected animals has fallen to 27 per cent and the number of dead injected individuals has increased to 68 per cent. The effect is still more marked with the injection of 0.1 per cent. The number of alive injected animals is now only 23 per cent, while the number of dead injected is 72 per cent.

In control experiments where the animals were not placed under conditions of lack of oxygen but were left in running water for

TABLE XI.

FUNDULUS INJECTED WITH PILOCARPINE.[1]

Strength of pilocarpine injected.	Alive.			Dead.		
	Total.	Injected.	Controls.	Total.	Injected.	Controls.
per cent. 0.01	106	53 (50%)	53 (50%)	94	47 (50%)	47 (50%)
0.05	52	14 (27%)	38 (73%)	68	46 (68%)	22 (32%)
0.1	62	14 (23%)	48 (77%)	78	56 (72%)	22 (28%)

[1] In this table the summaries only of individual tables are given. With each per cent of pilocarpine at least eight experiments were made. From ten to fifteen minutes were allowed for absorption of the pilocarpine after injection before placing in the flask.

many hours after injection, the injection of 0.05 per cent pilocarpine produced no visible effect on the animals. Their activities, so far as could be seen, seemed perfectly normal. The injection of 0.1 per cent would generally cause the death of about one animal out of ten at the end of twenty-four hours. This effect was still greater with the injection of 0.5 per cent, which would cause the death of about three animals out of every ten. Doses of this strength were therefore poisonous, and could not be used in testing the effect on resistance to lack of oxygen.

No experiments were made to determine how long the effect of pilocarpine in decreasing resistance of lack of oxygen would last.

SUMMARY.

1. Mannose, when injected intraperitoneally into Fundulus heteroclitis, increases their resistance to lack of oxygen. Its effect is

the same as that found in previous work for maltose, glucose, and lævulose, and the same explanation of its action is given; namely, that it acts as a depolarizer in the processes of protoplasmic respiration. Galactose is apparently not absorbed from the body cavity and so cannot increase the resistance to lack of oxygen.

2. Linseed oil and olive oil when fed into the stomach by means of a pipette have no effect on resistance to lack of oxygen. It could not be determined whether the fats were absorbed or not. Linseed oil when injected intraperitoneally causes a decrease in the resistance to lack of oxygen. The oil was probably not absorbed from the body cavity, and no explanation of this effect is suggested. No importance is attached to this result from the standpoint of respiration.

3. Ethyl alcohol in concentration of 40 per cent and upward produces death in some of the animals injected. In all strengths below this it causes a uniform decrease in resistance to lack of oxygen. The decrease is slight with 1 per cent, but gradually grows greater in proportion as the strength of alcohol used is increased. The effect of the alcohol is probably due to its toxic action on the protoplasm, lowering its powers of respiration. The alcohol is oxidized within four or five hours after injection, and the effect on resistance to lack of oxygen disappears.

4. Acetone causes a decrease in resistance to lack of oxygen in all strengths from 0.5 per cent up to that which is poisonous and produces death (5.0 per cent). Weaker solutions have no effect. Acetone is thus more toxic than alcohol, but probably acts in the same manner as alcohol.

5. Pilocarpine in strengths from 0.05 per cent to 0.1 per cent causes a decrease in resistance to lack of oxygen in proportion to the concentration used. Weaker solutions have no effect, and stronger solutions are poisonous. Since pilocarpine is supposed to increase the oxidations of the protoplasm, this effect is probably due to the more rapid use of the available oxidizable substances, which shortens the length of life in lack of oxygen.

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