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On the Swim-bladder Gases.

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Abstract

The formation of the swim-bladder gases of some sea and fresh water fishes were investigated and the results may be summarized as follows : 1. As a rule, oxygen content in the swim-bladder is higher in a fish living at greater depth than at shallow, and sea water fishes, than fresh water ones. 2. Oxygen content in the swim-bladder of the fish living at great depth decreases after 1-2 days stay in the aquarium. 3. Carbon dioxide content in the swim-bladder of all fishes examined is very small. 4. Through the poisoning of carbon monoxide, the swim-bladder gas decreases in its oxygen content and increases slightly in its carbon dioxide. 5. Corresponding to the artificial increase or decrease of the external pressures influencing the body surface of the fish, oxygen content of the swim-bladder gases increases or decreases respectively. 6. After the evacuation of the swim-bladder gases, newly formed gases always contain high oxygen percentage. 7. When oxygen or carbon dioxide of high concentration are injected in the swim-bladder, these gases diffuse out easily through the wall of the swim-bladder during 1-2 days. 8. Oxygen dissociation curve of carp blood is remarkably steep compared with the human blood, and influenced very much with the presence of carbon dioxide so as to decrease the affinity of the blood to oxygen. 9. Histological examination of the swim-bladder of *Sebastiscus marmoratus* and *Carassius auratus* indicate characteristic structure of the blood capillaries which distributed in the internal layer of membranes and sinus-like dilated. 10. From the above experimental results, some considerations on the gas formation in the swim-bladder were offered.

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On the Swim-bladder Gases.

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Introduction.

Referring to the text-book of zoology, a great majority of the Teleostomi have the air-bladder or swim-bladder. In some cases it communicates with the pharynx by the duct, the pneumatic duct (physostomi) and others, it is a closed sack (physoclyst). This organ is embryologically developed from the excrescence of the digestive canal and morphologically synonymous with lung of higher animals. Among various opinions as to its physiological functions, the most predominant ones, initiated by *Moreau*¹⁾, appears to be hydrostatic, i. e., it serves to keep the specific gravity of the fish as same as the water, so that the variations in hydrostatic pressure as the fish ascends or descends are regulated by contraction or absorption or expansion or secretion of gas.

Apart from these functions the gaseous constituent of the swim-bladder shows more or less high percentage of oxygen which is believed to be produced by the secretory activity of the characteristic structure situated at the inner wall of the swim-bladder, termed as gas gland^{2), 3), 4), 5), 6), 7)}.

This opinion however is based on the assumption that (1) the gas gland is in its structure analogous with other secretory glands so that oxygen gas is secreted, (2) the higher oxygen content than the surrounding medium is not simply explained by any physical processes, such as gas diffusion. Thus the weakest point of the secretory theory is that it is wholly hypothetical and experimentally not proved.

The present investigation was undertaken to elucidate some physical processes involved in the secretory functions of gas gland of some marine and fresh water fishes.

Experimental Materials.

(1) Sea water fishes :

The experiments were carried out in the Summer of 1936, at the Honzima Marine Biological Station of Okayama Medical College. Several fishes, such as *Scomber tappeinocephalus*, *Halichores poecilopterus* and *Sebastiscus marmoratus*, were investigated. Among of them, *Halichores poecilopterus* were mostly used, because they were strong and able to survive for a long time in the aquarium. The other specimens, because they died shortly after the fishing, were afforded to the experiments directly after fishing on the boat only.

(2) Fresh water fishes :

After returning to our physiological laboratory, further experiments were performed on fresh water fishes, such as *Cyprinus carpio*, L., *Carassius auratus*, L., especially on the latter, because they were easily obtained for our disposals.

Experimental Methods and Apparatus.

Samples of gas were drawn from the swim-bladder with a trocar and an air-tight syringe of 5 cc. content, of which dead space was filled previously with liquid paraffin and punctured the swim-bladder through the body-wall or after opening the body cavity of the fish. The syringe was placed in a water trough at room temperature and the gases analysed by means of *Krogh's* micropipette, of which length is ca. 35 cm. and the diameter of its capillary tube 0.9 mm. It is graduated in 1 mm. at 25 cm. length and exactly the half of 1 division can be read at practical measurements, so that the results of the analyses lie within 0.2 p.c. error. The mean of 3 analyses of gas of the same specimens are presented in the following tables.

According to the experiments of *W. Jacobs*³⁾, who compared the results of the gas analyses of same specimens with *Krogh's* micropipette and *Haldane's* apparatus, carbon dioxide appeared to be slightly less from the first than the latter, but oxygen was exactly the same.

Experimental results.

I. Variations of oxygen content according to the specimens of fish and its relations to the depth of living places from the surface.

The experimental results on various marine and fresh water fishes are tabulated in Tab. 1. These results, seem to indicate the following facts :

(1) Sea water fishes (physoclyst) contain in general higher oxygen percentage than fresh water ones.

(2) Fishes at great depths contain in their swim-bladder much more oxygen than fishes near the surface.

(3) All fishes contain in their swim-bladder little carbon dioxide or none at all.

On the Swim-bladder Gases.

Table 1. Composition of the swim-bladder gases of various specimens of fishes.

Sea water fishes.		Fresh water fishes.	
<i>Sebastes marmoratus</i> (depth 30 meters, temp. 26.5–27°C.)		<i>Carassius auratus</i> (depth 50–60 cms., temp. 18–21°C.)	
CO ₂ (%)	O ₂ (%)	CO ₂ (%)	O ₂ (%)
1.9	14.3	0.4	7.0
1.5	40.3	0.3	9.1
0.7	54.3	0.6	6.9
2.3	27.9	0.1	5.5
3.9	24.5	0.6	5.6
Mean 2.1	32.3	0.5	6.6
<i>Halichores poecilopterus</i> (depth ca. 20 meters, temp. 27.5–28°C.)		Mean 0.5	6.6
0.0	30.2	<i>Carassius auratus</i> (Goldfish) (depth 20 cms., temp. 26–28°C.)	
0.0	19.4	1.0	5.3
1.4	29.9	0.8	3.7
4.3	27.6	1.1	4.8
2.4	20.0	1.7	6.2
4.1	25.4	0.7	6.2
1.3	23.6	1.3	3.5
0.0	17.9	Mean 1.1	5.0
Mean 2.6	24.2	<i>Cyprinus carpio</i> (depth ca. 50 cms.)	
<i>Scomber tappeinocephalus</i> (depth 5–10 meters, temp. 27–27.5°C.)		0.0	5.6
0.3	10.7	0.0	4.4
0.8	18.7	1.9	4.9
0.7	15.6	0.0	2.1
1.1	8.0	0.0	3.6
Mean 0.7	13.3	Mean 0.4	4.1

Biot (1807)⁸⁾ performed at first the analytical experiments on the gas of the swim-bladder and found that the composition of gas contained in the swim-bladder varies greatly by one species to other, even among the same species. He found also that the fishes living more deeply showed higher oxygen content and also the carbon dioxide content is negligible.

As a cause of high oxygen content, he assumed, after the opinion initiated by *Needham* (1667)⁹⁾, some sort of secretory mechanisms. *Delaroche*¹⁰⁾, *Configliachi*¹¹⁾, *Moreau*¹⁾ and later numerous observers

supported and further extended these experiments. Our above experiments indicate that the variation of the oxygen percentage in the swim-bladder of sea water fishes caught at various depths from the surface is due to the hydrostatic pressure influenced on the body-wall of the fishes.

Consequently we investigated further on *Halichores poecilopterus* as to the effect of the depth of living niveaus on oxygen percentage of the gas in the swim-bladder. The fishes were kept in the aquarium of which depth was 50 cm. for 2 or 3 days, and the gas analyses was performed. The comparison of these results with those of freshly caught fishes are shown in Tab. 2. These results show that the oxygen contents, even in the case of the same species, decreased about the half of those of the freshly caught fishes from 10-20 meters depth, and this difference may be due to the effect of hydrostatic pressure.

Table 2. Change of the oxygen percentage in the swim-bladder gas through the artificial change of the depth of living niveaus.

(*Halichores poecilopterus*)

No. of Exp.	Freshly caught fishes (depth ca. 20 meters)		Fishes surviving in the aquarium (depth 50 cms.) for 2-3 days after the catch	
	CO ₂ (%)	O ₂ (%)	CO ₂ (%)	O ₂ (%)
1	0.0	30.2	0.7	14.1
2	0.0	19.4	2.0	11.6
3	1.4	29.9	0.6	16.6
4	4.3	27.6	0.0	11.7
5	2.4	20.0	3.6	11.5
6	4.1	25.4	0.6	9.8
7	1.2	23.6	0.3	16.8
8	0.0	17.9	2.9	5.1
Mean	2.6	24.3	1.3	12.2

II. Variations of oxygen percentage due to the change of pressure applied on the body surface of fishes.

From the foregoing experiments it is thought that the variations of oxygen percentage in the swim-bladder of various fishes are due to the differences of hydrostatic pressure applied on the body surface of fish. In order to ratify further these facts the analytical experiments were carried out on the swim-bladder gas of *Carassius auratus*,

after the pressure applied on the body surfaces of fishes had been decreased or increased for several hours. Fishes of medium size were introduced in a glass bottle of 2 liters content, and equipped with air-tight strong stopper. By reducing or increasing the pressure, the bottle was attached to water pump or directly to tap-water pipe respectively. By reducing the pressure the fishes struggle to sink down to the bottom ejecting, from time to time, the bubbles from the gill or between the scales. By increasing the pressure, the fishes sunk down to the bottom and did not show movements. The results of gas analyses are shown in Tab. 3.

Table 3. Effect of the external pressure on the composition of gases in the swim-bladder.

No. of Exp.	Under 1 atm. pressure		Under 2 atm. pressure		Under low atm. pressure (From -300 to 360 mm. Hg.)	
	CO ₂ (%)	O ₂ (%)	CO ₂ (%)	O ₂ (%)	CO ₂ (%)	O ₂ (%)
1	0.4	7.0	0.3	7.5	4.7	3.5
2	0.3	9.1	0.2	9.6	2.8	6.0
3	0.6	6.9	0.0	6.8	5.5	5.5
4	0.1	5.5	0.0	6.9	3.5	4.1
5	0.6	5.6	0.2	6.1	3.4	3.0
6	0.5	6.6	0.0	7.9	2.9	8.2
7	1.6	5.2	0.2	7.6		
Mean	0.6	6.6	0.1	7.5	3.8	5.1

From these data it may be noted, even less in magnitude, that the oxygen percentage in the swim-bladder increases or decreases according to the change of the pressure, as in the case of the depth of living places. Further these results wholly coincide with those obtained by *Moreau*¹⁾, *W. Jacobs*³⁾, however, observed recently in some cases of his experiments on *Cyprinus carpio* that oxygen increased by lowering the pressure, but it may be considered as an experimental error, since the gaseous composition in the swim-bladder is very variable even in the members of the same specimens.

It can be understood from these experiments that the oxygen percentage of gas in the swim-bladder is totally a function of the hydrostatic pressure influencing the body surface of fishes.

III. Effect of the evacuation, oxygen or carbon dioxide injection on the gaseous constituents in the swim-bladder.

So far we have considered only the effect of external hydrostatic pressures on the variations of the swim-bladder gases. But it is also

important to know how much the internal conditions of the swim-bladder influence the gaseous compositions so that the swim-bladders of *Carassius auratus* of medium size were punctured in such a manner as described above, and the swim-bladder gas was evacuated as quickly as possible.

For several hours after the puncture, the fish remained motionless on the bottom of water trough. After 12-24 hours, however, the fish recovered to the previous normal conditions. By this time the second puncture was performed. Thus the results of gas analyses in the samples obtained from the first and second punctures were compared (see Tab. 4). Tab. 4 shows that gases produced after the first manipulation always showed higher oxygen percentage. Therefore it may be concluded that the conditions of reducing the volume of the swim-bladder, whether introduced from outside or inside, lead to the gas production of higher oxygen percentage.

Table 4. Variations of the swim-bladder gas through the evacuation.

(*Carassius auratus*)

No. of Exp.	Samples before the evacuation		Samples after the evacuation	
	CO ₂ (%)	O ₂ (%)	CO ₂ (%)	O ₂ (%)
1	1.6	4.9	0.0	7.3
2	0.0	6.1	0.5	9.8
3	0.5	6.6	0.0	12.0
4	0.6	5.3	0.3	9.8
5	4.0	7.5	1.7	8.6
6	3.5	7.0	1.6	8.0
7	1.6	5.2	2.6	5.7
Mean	1.7	6.1	1.0	8.7

Moreau's observations¹⁾ that the evacuation of the swim-bladder gas leads to an increase of oxygen contents were acknowledged by numerous subsequent investigators, such as *Bohr*⁶⁾, *Baglioni*¹²⁾, *Hüfner*¹³⁾ and others. Quite recently *Jacobs*³⁾, *Meesters* and *Nagel*⁴⁾ investigated further in details, the time course of the restoration of gas after the evacuation. *Jacobs* found that in an early stage of compensation, carbon dioxide plays an important rôle (83-85% CO₂, 15% O₂), but in later stage carbon dioxide was reabsorbed and oxygen showed high percentage (about 80% O₂). *Von Ledeber*⁵⁾ injected *Ringer's* solution after artificial evacuation of the swim-bladder and pursued the formation of new gas produced. She observed that fishes re-

cover their normal conditions at 5-6 days after the operation and contained in their swim-bladder 23-32% oxygen by this time. She also observed a slight increase of carbon dioxide (3%) after the puncture. In our experiments we could not observe any increase of carbon dioxide in all stages after the evacuation.

The results of injection of oxygen or carbon dioxide after the evacuation were presented in Tab. 5, from which it may be concluded that the injected carbon dioxide was absorbed very rapidly and the oxygen, even its absorption is less in rapidity, contrary to the results obtained by previous authors, also diffusible through the wall of the swim-bladder. We could not find an increase of oxygen percentage of the swim-bladder gas through the injection of carbon dioxide, which may be expected from the hypothesis offered by *Jacobs*³⁾.

Table 5. Variations of the swim-bladder gas after oxygen or carbon dioxide injection.

(*Carassius auratus*)

No. of Exp.	1 day after CO ₂ -injection		1 day after O ₂ -injection		2 days after O ₂ -injection		3 days after O ₂ -injection	
	CO ₂ (%)	O ₂ (%)	CO ₂ (%)	O ₂ (%)	CO ₂ (%)	O ₂ (%)	CO ₂ (%)	O ₂ (%)
1	0.3	2.3	4.2	46.1	0.0	5.5	0.0	3.8
2	0.3	1.7	4.5	29.6	0.7	11.9	0.0	2.1
3	0.3	3.9	3.6	37.4	0.3	3.8	0.0	3.0
4	1.4	4.2	5.4	38.4	0.0	4.8	0.0	5.6
5	0.0	3.9	0.0	25.0	0.0	5.4		
6	0.0	2.8	0.0	11.0	0.0	4.0		
7			0.9	29.7	0.0	10.5		
8			1.7	16.7				
9			0.6	13.8				
10			0.9	9.3				
Mean	0.4	3.1	2.2	25.7	0.1	6.6	0.0	3.6

IV. Effect of asphyxiation with carbon monoxide on the swim-bladder gas.

Some authors believe that the swim-bladder works as an accessory respiratory organ, providing for oxygen in the case of asphyxiation. If it is true, the oxygen contents must be decreased thereby. In order these processes to occur, it must be assumed that the wall of swim-bladder is able to pass oxygen gas with considerable easiness and the following experiment was undertaken:

Carassius auratus was brought into a water trough containing carbon monoxide haemoglobin in 5-10%, prepared from cow's blood. After 2-3 min. the fish begun to anguish and ceased the respiratory movement of gills, lying at the bottom of the water trough. The fish was brought out by this time, measuring the gaseous compositions in the swim-bladder, and at the sometime the percentage saturation of carbon monoxide haemoglobin with *Hartridge's* reversion spectroscope on the blood obtained from heart puncture of the asphyxiated fish. (See Tab. 6.)

Table 6. Variations of the swim-bladder gas due to asphyxiation with carbon monoxide.

(*Carassius auratus*)

No. of Exp.	Percentage saturation with carbon monoxide	Swim-bladder gas		Remarks
		CO ₂ (%)	O ₂ (%)	
1	50	2.0	4.9	←Compositions of the swim-bladder gas of the control.
2	50	1.9	7.1	
3	53	2.7	8.5	
4	56	1.8	7.1	←15-21% O ₂ 0.0% CO ₂
5	56	1.6	7.5	
6	50	1.2	6.2	
7	53	2.1	6.6	
8	50	2.4	8.1	
9	50	1.4	9.0	

These experiments were performed in summer. The oxygen contents in the swim-bladder of the control fishes were generally higher in summer (15-21% O₂), and the carbon dioxide lies under the limit of measurements. Comparing those of asphyxiated with the control fishes, the oxygen percentage decreased and the carbon dioxide increased in the asphyxiated ones within only about 10 min. The percentage saturation of carbon monoxide haemoglobin of the asphyxiated blood was 50-56%.

According to *Kunzel* and *Paasch*¹⁴⁾, fishes can survive for a long time at the cost of oxygen, which dissolved physically in the blood and the ability of the fish blood of combining with oxygen is not interfered by the presence of carbon monoxide. Our results, however, indicate that it does not hold true in our case. By half saturation of the blood with carbon monoxide, the fishes cease their respiratory movements and die after a short time. The above results indicate emphatically that the wall of the swim-bladder is,

contrary to the old opinions, permeable either from internal to external or in the reverse directions.

V. Characteristic properties of the fish blood.

Most investigators tried to bring about the direct relations between the swim-bladder gases and the partial pressure of gases contained in the surrounding media. But it is more reasonable to search about the correlation between the swim-bladder gases and the gases contained in the blood, because the swim-bladder has no direct contact with surrounding water, but with blood, though not directly, which is supplied richly with meshes of blood vessels, termed "Wundernetz", distributed in the wall of the swim-bladder. Therefore it is reasonable to consider that the swim-bladder gases originate from the gases of the fish blood. Under such suppositions the following experiments were undertaken.

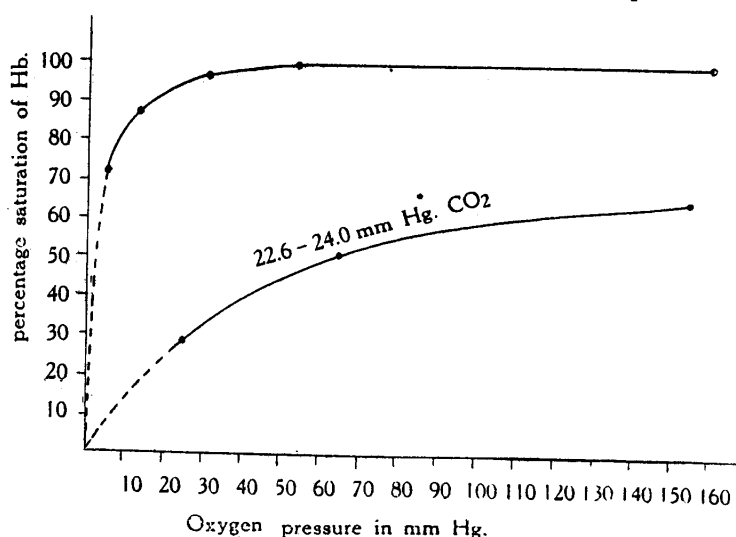
In order to get plenty of blood, a large fresh water fish, *Cyprinus carpio* was chosen. From the cut end of the caudal arteries blood was drawn in a beaker, which was paraffined at the bottom and contained liquid paraffin. This blood was promptly sucked in a air-tight syringe containing 10 p.c. sodium citrate in a proportion 10:1 for the prevention of clotting.

Samples of the blood were introduced and exposed in sampling tubes of *Barcroft* type to atmospheres of different composition of oxygen gas at constant temperature. When an equilibrium has been attained, a part of the blood was withdrawn and its percentage saturation with oxygen was determined with *van Slyke's* blood gas apparatus. On the other hand, the gaseous composition in the sampling tubes were determined with *Haldane's* apparatus. From the above two determinations the percentage saturation of the fish blood with oxygen was plotted to the partial pressure of oxygen. Thereby, the oxygen capacity was adopted at 150 mm. Hg. oxygen pressure as 100, and this was in mean 13.65 cc. per 100 cc. of the fish blood. As the maximum oxygen capacity is considerably variable from one to another individuals, we calculated, in such cases, the percentage saturation under the supposition that it is 100 at 150 mm. Hg. oxygen pressure, whatever the absolute value of the oxygen capacity may be.

The results obtained are given in Figure 1. It was investigated, moreover, on the effect of carbon dioxide (for example, 23 mm. Hg. partial pressure) on the oxygen dissociation curve. As may be seen in the Figure 1, the oxygen dissociation curve of the fish blood is very steep. In other words, it can be stated that the fish blood

attains its maximum oxygen capacity at considerable low oxygen pressure. Moreover, carbon dioxide, even at low partial pressure, influences greatly the oxygen dissociation curve in such a way as giving off oxygen easily from the blood at the same oxygen pressure. These characteristics of the fish blood is due to the adaptation of fishes living at low oxygen content and of carrying oxygen from the respiratory organ to the tissues. Our results on the carp blood coincide with those of *Krogh* and *Leitch*¹⁵⁾ on fresh water fishes.

Fig. 1. Oxygen dissociation curve of carp blood.



Considering the gas formation in the swim-bladder of fishes from the characteristic properties of the fish blood, it is reasonably supposed that some unknown substances, like carbon dioxide, which affect the affinity of blood to oxygen, are produced in the gas gland and that the oxygen gas coming from the blood can diffuse through the wall of the swim-bladder.

*Akita's*¹⁶⁾ experiments substantiate the supposition, who observed that the venous blood of *Monacanthus cirrhifer* coming from the swim-bladder tends to become more acid when the secretion is taking place, i. e., pH 7.71 at normal condition becomes pH 7.49 at the secretion time. He also observed that the oxygen content is increased through the injection of carbon dioxide.

From our assumptions it can be understood that the results obtained by *Jacobs*³⁾, who established the fact that the carbon dioxide in the swim-bladder increases remarkably at the early period of gas formation after the evacuation.

VI. Histological structure of the swim-bladder.

The microscopical preparations were made by the authors on *Carassius auratus* and *Sebastisus marmoratus*. As described by *Jäger*⁷⁾, the wall of the swim-bladder is constructed from 3 layers of fibrous membrane, of which internal layer is covered by endothelium-like pavement epithelium and receive rich blood capillary supply. The blood capillaries are dilated there sinus-like and filled with red blood corpuscles. Around the blood capillaries, large oval-shaped epithelial-cells containing much chromatin are usually observed. But in these fishes the specific histological structure, the "red body" termed by *Delaroche*¹⁰⁾ and cited by many authors was not found. The outer and middle layers are composed of fibrous connective tissue. At any portion of the wall, Oval (*Jäger*) or non-striated muscles were not found (see Fig. 2).

Fig. 2. Microscopical preparation of the swim-bladder.
(*Carassius auratus*)

Sinus-like dilation
of blood capillaries



Discussion.

The compositions of the swim-bladder gases are influenced by many factors; (1) hydrostatic pressure influencing on the body wall of fishes, (2) internal pressure of the swim-bladder, (3) blood gas. In either cases, it is clear that they are derived from blood gases. It can not be assumed, from our experimental results, that the existence of specific functional and structural properties of the swim-bladder wall for a specific sort of gases. And the only peculiarity remained to be considered is the characteristic properties of the fish blood to oxygen. Since the purpose of our investigation is to elucidate physically the complicated biological phenomena, the vitalistic idea, such

as the secretion theory, concerning the gas formation in the swim-bladder, which has no direct experimental proof could not be considered.

The following points are emphasized from our results :

Fish blood has greater affinity to oxygen than mammalian blood, and this affinity to oxygen is moreover remarkably influenced with the presence of carbon dioxide even at low tension so as to raise the "tension of unloading" and give off oxygen from blood. This is the adaptation of fishes living in the environment of low oxygen tension. As supposed by *Krogh* and *Leitch*¹⁵⁾, this influence of carbon dioxide or like substances produced as a result of metabolic dissimilation is confined to the capillaries, in our cases to the capillaries of the swim-bladder wall, in which it will greatly facilitate the "unloading" of oxygen from the blood. Oxygen gas thus isolated diffuses through the wall of the swim-bladder and accumulates in it. The special distributions of blood capillaries of the swim-bladder termed "Wundernetz", which was described by many authors and sinus-like dilation of the capillaries as we observed, are favourable to take place in such processes.

So far only oxygen was considered as a principal constituent of the swim-bladder gases. But it is said in some fishes nitrogen constitutes the principal composition. As to the mechanisms of the nitrogen formation in such a case is left for future investigation.

Summary.

The formation of the swim-bladder gases of some sea and fresh water fishes were investigated and the results may be summarized as follows :

1. As a rule, oxygen content in the swim-bladder is higher in a fish living at greater depth than at shallow, and sea water fishes, than fresh water ones.
2. Oxygen content in the swim-bladder of the fish living at great depth decreases after 1-2 days stay in the aquarium.
3. Carbon dioxide content in the swim-bladder of all fishes examined is very small.
4. Through the poisoning of carbon monoxide, the swim-bladder gas decreases in its oxygen content and increases slightly in its carbon dioxide.
5. Corresponding to the artificial increase or decrease of the external pressures influencing the body surface of the fish, oxygen content of the swim-bladder gases increases or decreases respectively.

6. After the evacuation of the swim-bladder gases, newly formed gases always contain high oxygen percentage.

7. When oxygen or carbon dioxide of high concentration are injected in the swim-bladder, these gases diffuse out easily through the wall of the swim-bladder during 1-2 days.

8. Oxygen dissociation curve of carp blood is remarkably steep compared with the human blood, and influenced very much with the presence of carbon dioxide so as to decrease the affinity of the blood to oxygen.

9. Histological examination of the swim-bladder of *Sebastiscus marmoratus* and *Carassius auratus* indicate characteristic structure of the blood capillaries which distributed in the internal layer of membranes and sinus-like dilated.

10. From the above experimental results, some considerations on the gas formation in the swim-bladder were offered.

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