PHYSIOLOGICAL STUDIES OF THE CHINOOK SALMON.

By CHARLES WILSON GREENE,
Professor of Physiology, University of Missouri.

I. Relation of the Blood Pressure to the Functional Activity.
II. A Study of the Blood and Serous Liquids by the Freezing-Point Method.

PHYSIOLOGICAL STUDIES OF THE CHINOOK SALMON.

By CHARLES WILSON GREENE,
Professor of Physiology, University of Missouri.

I. RELATION OF THE BLOOD PRESSURE TO THE FUNCTIONAL ACTIVITY.

INTRODUCTORY.

The salmon is an anadromous fish. Its natural spawning beds are in the cold waters of the mountain streams. When the eggs are hatched and the young are able to swim, they proceed down the streams and out into the open ocean, where they feed and grow for a period of two to four years. On the approach of maturity they reenter the mouths of the rivers and make the long journey back to the spawning grounds in the mountain waters, a distance sometimes of hundreds of miles. The mature salmon as they approach the mouths of the rivers are strong and vigorous and in the very prime of condition. They have been feeding voraciously on the abundant ocean fauna and their tissues are loaded with the supply of fats and oils and other constituents which make the flesh so much sought after because of its delicious flavor and nutritious excellence.

The fact which presents so peculiar and interesting a problem, or series of problems, in fact, to the physiologist is this: The salmon takes no food after it leaves the ocean and enters fresh water.[a] The journey, it may be of hundreds of miles, is made against the swift currents, rapids, and waterfalls of the mountain streams. It matters not how long the distance nor how great the exertion that is required, all the energy must be supplied from the store of material accumulated while the fish is feeding in the ocean, material present in its body when it enters the fresh-water stream.

A prolonged fast is always of especial physiological interest. The winter sleep or hibernation of the bats, dormice, and the bears, while it is a period of fasting, is also a period of inactivity. All the vital processes are reduced to a minimum and little energy is liberated. In the salmon, on the contrary, the fasting period is the period of the greatest activity of the fish's life. The changes and reactions within the body of an animal that is giving off daily a large amount of energy, and at the same time is taking in no food to renew its vitality, present peculiar physiological phenomena. Nature herself performs the experiment of inanition in the salmon and it remains for science to unravel the details. The main question is how long and through

---

[a] This statement is borne out by the researches of the Bureau of Fisheries, and investigations by Miescher-Ruesch and Noel Paton on the Atlantic salmon in Europe show the same to be true of that species also.

what stages this one-sided process can advance before disintegration reaches the point at which the organized life of the individual animal must come to an end.

The numerous investigations of the U. S. Bureau of Fisheries into the natural history of the salmon—especially the migration, feeding, and spawning habits—have firmly established the facts upon which the general statements made above are based. Of the numerous workers we may especially mention the recent investigations of Mr. Cloudsley Rutter, late naturalist of the Bureau's steamer *Albatross*, who was one of the best informed men on all scientific questions that pertain to the Pacific salmon. It is to his energy and skilled insight that we are indebted for the more accurate details of the conditions under which the young make the journey from the headwaters of the rivers to the sea, also for details as to the progress of the adults to the spawning grounds, as well as for saving improvements in the methods of propagation. Mr. Rutter was at the time of his death in the midst of an exhaustive study of the embryology of the salmon.

In the solution of the problem of the changes that occur in the salmon during the run to the spawning beds, there are three general courses open, in addition to the natural-history methods of observation. One is a study of the anatomy, by which may be followed the structural changes in the salmon after it reenters the rivers. Little has been done with this method except upon the alimentary canal. A second course is through the methods of physiological chemistry. These have been applied particularly by Miescher-Ruesch in Germany, and Paton and his coworkers in Scotland. The latter especially have published some instructive and interesting studies of the chemical changes in the tissues and organs of the Atlantic salmon (*Salmo salar*). No work has been published presenting the results of a chemical study of the salmon of our west coast, though the opportunities for observation are far more numerous and the natural setting of the problem is infinitely superior to that for the study of the Atlantic salmon either on the continent of Europe or in Scotland. The European species of salmon which spawns in the rivers of Scotland (*Salmo salar*), like our American steelhead (*Salmo gairdneri*), for example, returns to the sea for another period of feeding, thus spawning more than one season. *Oncorhynchus tschawytscha*, unlike the species of *Salmo*, does not return to the sea, but spawns once and dies, as was first conclusively proved by the investigations carried on by Doctor Evermann in Idaho in 1895 and 1896.[a] It therefore presents a peculiarly favorable opportunity for the chemical study of starvation.

The third and last line of observation seeks to trace the changes in the functional activity of the salmon by the methods of experimental physiology. These methods have never been applied to the study of this species—have been applied, indeed, in only a limited number of studies on fishes of any kind. The present investigation had its origin in the belief that good and fruitful results would be yielded by such an experimental study. Under the auspices of the U. S. Bureau of Fisheries, field work was begun during the summer of 1901. Only a small portion of the total results of the physiological investigations in progress will be reported in the following pages.

---

[a] Bull. U. S. Fish Comm. for 1896, pp. 151-202, and 1897, pp. 15-84.

METHODS.

The size of *Oncorhynchus tschawytscha*, individuals of which often weigh as much as 40 and 50 pounds, makes this a fish difficult to handle, especially since it succumbs rather quickly to artificial conditions. To take a salmon out of the water in which it lives and to keep it alive under conditions which will permit of physiological measurements is indeed about as difficult as putting a mammal under the water for such a purpose, and for much the same reasons. I have been able, however, to keep salmon alive under fairly normal conditions for as much as twenty minutes—in one case, forty-two minutes. The procedure was as follows: A salmon holder was made of a broad board supplied at one end with a grooved block, which was fitted over the nose. A similar block for the back was also used for the smaller fishes. A narrow strip of sail canvas was tacked to one edge of the board from a point opposite the shoulder-girdle to some distance back toward the tail. The head end of the holder was cut out in such a way as to allow free movement of the operculum in respiration. The salmon was placed on its side on this board, quickly wrapped in the canvas, and tied to the board by stout twine bands around the nose, the shoulder-girdle, and at intervals along the body and around the tail. The gills were aerated by a stream of water from a garden hose which siphoned water from the hatchery flume, the surface of which was about 5 feet above the operating table. The hose was inserted into the mouth and the water was allowed to flow freely out over the gills and escape on the tables and floor. Especial care was taken to have both opercles free and to direct the stream of water so that it irrigated the gills freely on both sides. The lower jaw was left free to make its respiratory movements, though these movements, of course, did not affect the artificial respiration. Blood-pressure measurements and respiratory counts were the tests made upon the salmon under these conditions.

Blood-pressure measurements were desired both of the ventral and dorsal aortæ. The difficulties in the way of measuring the pressure in the ventral aorta proved exceptionally great. The pericardial cavity extends forward and includes the conus arteriosus and the origin of the short ventral aorta. The aorta almost immediately makes a sharp turn upward toward the base of the branchial apparatus, where the afferent branchial arteries have their origin. These branchial arteries pass at once to the gillarches and it is impossible to isolate any one of them without injuring the delicate gill structure. This difficulty, together with the great coagulability of the blood, was sufficient to invalidate all the efforts to lead off the blood pressure from these vessels. The ventral aorta itself, though deep-seated in these large fishes, is more easily exposed, and after considerable practice I was able to make the necessary dissection accurately and quickly. The pectoral arch which covers the pericardial region is cartilaginous in the salmon. In exposing the ventral aorta it was necessary to cut away the greater part of the muscles of the gular region, together with the anterior portion of the pectoral arch down to the wall of the pericardium, and to slit open the extreme anterior ventral portion of the pericardium. A slight loss of blood attends this operation, but all delicate vessels are quickly and effectively stopped by rapid blood-clotting. It is evident that one can not insert a cannula into the ventral aorta in the usual way—i. e., by ligation. The vessel is too easily torn to permit the use or insertion of the form of cannula such as Fick's, and the blood clots would quickly stop it were it inserted. Successful pressures were finally secured with a short-necked, wide-bulb, T-shaped washout cannula of the form in common use in physiological laboratories and figured in Stirling's Hand-book, page 306. This cannula, filled with saturated magnesium sulphate under the proper pressure to prevent the too great loss of blood into its bulb, gave tracings unobstructed by clots for as much as ten minutes at a time, and clots could be easily removed by taking the cannula from the artery, washing it out, and replacing it. The cannula was inserted through a small puncture or slit cut in the artery with a slender pointed scalpel and was held in place not by the usual ligatures but by the elasticity of the tissue around the constricted neck of the cannula. The end of the cannula extended freely into the blood stream within the artery but was not large enough to offer any serious obstruction to the flow of the blood stream past its point of insertion. The greatest care was taken to adjust the whole apparatus with reference to the position of the fish at the beginning of the experiment so that the cannula should not be drawn out or the artery torn, either of which accidents was almost sure to end the experiment through the too great loss of blood.

Measurements of the blood pressure of the dorsal aorta were made by cutting off the tail and quickly inserting the cannula into the open end of this vessel, and plugging the accompanying veins when necessary, i. e., when they were not completely compressed by the cannula in the aorta. A little bleeding takes place around the cut skin, but a true measurement of the pressure and its variations is obtained for a period of two or three minutes and even longer.

The blood pressure in all of the experiments reported in this paper was measured by means of a Ludwig's mercury manometer, and the pressures are measured to the maximal pressures of the heart beats.

BLOOD PRESSURE IN SALMON FROM THE SEA.

A vigorous effort was made during the summers of 1901 and 1902 to secure measurements of blood pressure from salmon taken directly from the sea. The fishing grounds at Monterey Bay, the point visited, are so far out that it is difficult to bring the fish to the shore in good condition. July 26, 1901, a single live male, 92 centimeters in length, was brought into the laboratory in poor condition, having lost some blood from a gaff wound and being considerably asphyxiated by the trip in from the fishing banks. The blood pressure measured in the ventral aorta was found to be 49 millimeters of mercury. This result can not be taken as normal, since it is vitiated both by loss of blood and by insufficient aeration of the gills during the experiment. It is of interest chiefly as the first attempt to make the difficult blood-pressure measurement on the salmon.

BLOOD PRESSURE IN SALMON FROM TIDE WATER.

Black Diamond, California, at the head of Suisun Bay, just where the Sacramento and San Joaquin rivers enter the bay, was visited in the first two weeks in August, 1902. Two live salmon, caught in nets about 3 miles distant, were brought in a small float to the wharf of the Sacramento River Packers' Association, to whom we are indebted for quarters as well as for many special favors facilitating our investigations.

As in the case of the marine salmon, it was found impossible to transport these fish without considerable asphyxiation, and they did not revive as well as could be wished. While the pressures given in Table I, below, are strong, still they must be considered somewhat below the normal for the fish in its native waters. The heart beat is obviously far below the normal, a fact which is due to the failure of the ventricle to follow each contraction of the auricle.

TABLE I.—*Blood pressure from the ventral aorta of salmon taken from Suisun Bay at the mouth of the Sacramento River.*

Date.	No.	Sex.	Length in millimeters.	Blood pressure in the ventral aorta in millimeters of mercury.	Heart rate per minute.
1902.					
July 10	197	....	756	78	15
July 10	198	Female	859	63	25

BLOOD PRESSURE IN SALMON FROM THE SPAWNING BEDS.

Blood-pressure experiments were performed on some forty different salmon taken from the McCloud River at Baird, Cal.[a] Twenty-one of these experiments were made in the summer of 1901 and the remaining nineteen in 1902. The majority of the fish used were young males or females that had been artificially spawned. A few were old exhausted specimens, males and females that had come down the river from the spawning beds above. Two or three were prime, large, unripe males and females. The great intrinsic value for propagation purposes of these prime fish at the hatchery deters one from using more than are absolutely necessary for experimental purposes. Figures 1 and 2 give typical ventral aortic blood-pressure tracings. Table II represents the total set of blood-pressure measurements. The maximal blood pressure, the heart rate, and the respiratory rates in all the blood-pressure experiments are brought together in the table following for convenience in reference.

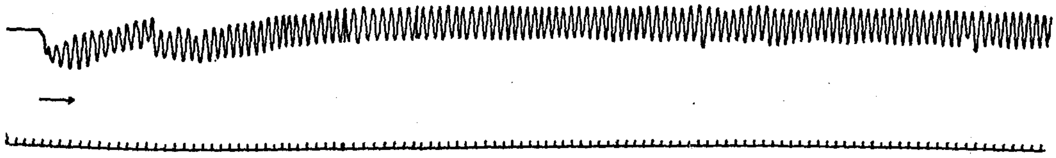


FIG. 1.—Experiment Aug. 20, 1902. Salmon, No. 207, female; length, 823 millimeters. This tracing gives a normal blood pressure from the ventral aorta. Maximal pressure, 75 millimeters of mercury; heart rate, 68 per minute; pulse pressure, 16 millimeters of mercury. In this and the following figures the tracings were recorded by means of a Ludwig's mercury manometer. The time is in seconds and the time line represents the zero pressure. All figures are reduced one-half.

---

[a] The United States fish hatchery at Baird furnishes an ideal spot for the study of the salmon on the spawning beds. An abundance of live specimens can be obtained at the very door of the hatchery, and the station is provided with the necessary equipment for handling the fish. I wish here to thank especially the superintendent, Mr. G. H. Lambson, and the accommodating hatchery force for numerous courtesies during the progress of the work.

TABLE II.—*The blood pressure and heart rate, together with the respiratory rate, of salmon from the spawning beds.*

[Experiments performed at Baird, Cal.]

Date.	No.	Sex.	Length in millimeters.	Ventral aorta.		Dorsal aorta.		Respiratory rate per minute.	Remarks.
				Blood pressure in millimeters of mercury.	Heart rate per minute.	Blood pressure in millimeters of mercury.	Heart rate per minute.		
1901.									
Aug. 8	7	Male	520	60	45	....	....	....	Beginning pressure 45, rate 24.
Aug. 8	9	Male	540	62	72	....	....	75	
Aug. 10	10	Male	....	40	80	....	....	96	Poor, irregular.
Aug. 12	14	Male	530	....	....	58	28	90	
Aug. 13	15	Male	530	....	....	39	(?)	....	Rate can not be determined.
Aug. 14	16	Male	440	33	34	....	....	72	Poor.
Aug. 14	17	Male	1,020	44	21	....	....	70	Old male off retaining rack; died in a few minutes.
Aug. 14	18	Male	530	....	....	58	72	....	
Aug. 15	19	Male	....	38	40	....	....	....	Poor.
Aug. 15	20	Male	520	....	....	30.4	(?)	....	
Aug. 16	22	Female...	850	86	60	....	....	64	Spawned naturally.
Aug. 17	23	Male	540	47	86	....	....	....	
Aug. 17	24	Female...	900	80	58	....	....	78	Spawned artificially.
Aug. 28	48	Male	650	64	52	....	....	75	After 6 minutes' pressure 66, rate 51.
Aug. 28	52	Female...	850	100	57	....	....	63	Spawned artificially.
Aug. 28	53	Female...	730	57	69	....	....	....	Do.
Aug. 28	54	Female...	890	120	60	....	....	63	Do.
Aug. 28	55	Female...	900	67	63	....	....	40	Spawned artificially; experiment continued for 40 minutes.
Aug. 29	59	Female...	1,160	70	63	....	....	62	Spawned naturally; after 28 minutes' pressure 67, rate 56; experiment continued 42 minutes.
Sept. 1	60	Female...	870	70	52	....	....	65	Spawned artificially; after 23 minutes' pressure 70, rate 50.
Aug. 31	61	Male	920	108	34	....	....	....	Rate and pressure very irregular.
1902.									
Aug. 18	201	....	....	59	66	....	....	102	
Aug. 18	202	....	....	28	66	....	....	75	Poor.
Aug. 18	203	Male	428	69	76	....	....	....	
Aug. 19	204	Male	459	45	54	....	....	72	
Aug. 19	206	Male	935	94	66	....	....	50	Old male off retaining rack.
Aug. 20	207	Female...	823	75	68	....	....	54	Spawned artificially.
Aug. 21	208	Male	413	75	60	....	....	79	
Aug. 22	209	Male	918	99	44	....	....	60	
Aug. 23	210	Female...	672	66	64	....	....	60	Unripe; after 10 minutes' pressure 64, rate 72.
Aug. 23	212	Female...	777	66	76	....	....	60	Spawned artificially.
Aug. 25	213	Male	752	94	56	....	....	71	Just ripening.
Aug. 26	214	Female...	829	50	48	....	....	61	
Aug. 26	215	Female...	866	73	66	....	....	52	
Aug. 26	216	Male	510	....	....	51	(?)	78	Pressure fell rapidly.
Aug. 27	217	....	590	85	56	....	....	0	Cocainized, no respiratory movements.
Aug. 27	218	Male	600	43	68	44	60	60	Dorsal pressure measured first.
Aug. 27	219	Male	860	72	20	....	....	....	Fresh male in fine flesh and color.
Aug. 28	220	Male	490	33	90	57	48	80	Dorsal pressure measured first.
Aug. 28	221	Male	440	56	....	52	38	....	Do.

A comparison of the experiments in this list, which may be considered as representing the results in normal animals, is interesting in several important respects. The experiments of this class showing the blood pressure in the ventral aorta have been selected and are presented below in Table III, from which it will be seen that the mean pressure for the 26 examples is 74.6 millimeters of mercury.

TABLE III.—*Showing the blood pressure in the ventral aorta, the heart rate, and the pulse pressure of all the examples that were considered normal under the conditions of experimentation.*

Date.	No.	Sex.	Length in millimeters.	Pressure in the ventral aorta.	Heart rate per minute.	Pulse pressure in millimeters.
1901.						
Aug. 8	7	Male	520	60	45	40
Aug. 9	9	Male	540	62	72	3
Aug. 16	22	Female.....	850	86	60	14
Aug. 17	23	Male	540	47	86	6
Aug. 17	24	Female.....	900	80	58	12
Aug. 28	48	Male	650	64	52	18
Aug. 28	52	Female.....	850	100	57	18
Aug. 28	53	Female.....	730	57	69	8
Aug. 28	54	Female.....	890	120	60	16
Aug. 28	55	Female.....	900	67	63	16
Aug. 29	59	Female.....	1,160	70	63	14
Sept. 1	60	Female.....	870	70	52	22
Aug. 31	61	Male	920	108	34	36
1902.						
Aug. 18	201	....	....	59	66	6
Aug. 18	203	Male	428	69	76	10
Aug. 19	204	Male	459	45	54	6
Aug. 19	206	Male	935	94	66	8
Aug. 20	207	Female.....	823	75	68	16
Aug. 21	208	Male	413	75	60	8
Aug. 22	209	Male	918	99	44	18
Aug. 23	210	Female.....	672	66	64	14
Aug. 23	212	Female.....	777	66	76	18
Aug. 25	213	Male	752	94	56	20
Aug. 26	214	Female.....	829	50	48	....
Aug. 26	215	Female.....	866	73	66	14
Aug. 27	217	....	....	85	56	16
Mean for 26 specimens..................				74.6	58.9	....
Mean for 11 males				74.3	58.6	....
Mean for 13 females				75.4	61.8	....

The highest pressure recorded in any single instance was that of a female (No. 54) 89 centimeters in length, taken Aug. 28, 1901, which had been artificially spawned a few hours before. This female was in prime condition in so far as shown by external appearances. It gave a ventral aortic pressure of 120 millimeters of mercury. A male 92 centimeters long, taken Aug. 31, 1901, gave almost as great a maximal pressure, i. e., 108 millimeters of mercury. The minimal pressures included in the above table are 45 and 47 millimeters, respectively, given by Nos. 204 and 23. No. 23, taken Aug. 17, 1901, was marked in the notes as a "prime-condition fish," yet the pressure is very low, although the heart rate is considerably above the average.

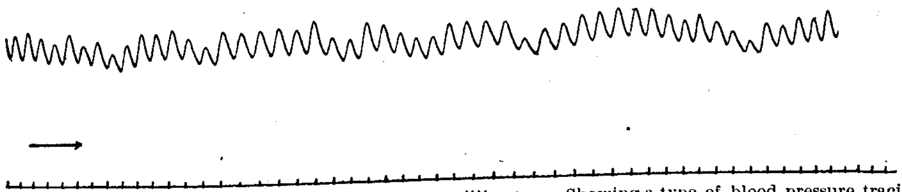


FIG 2.—Experiment Aug. 28, 1901, No. 48, male; length, 650 millimeters. Showing a type of blood pressure tracing from the ventral aorta in which the respiratory movements affect the pressure. See also fig. 3.

TABLE IV.—*Showing the range of variations in the ventral aortic blood pressure in normal salmon from the spawning beds.*

Number of specimens.	Ventral aortic pressure, in millimeters of mercury.
3	41 to 50
3	51 60
8	61 70
4	71 80
2	81 90
4	91 100
1	101 110
1	111 120
26 specimens	Mean, 74.6

It must be remembered, however, that in a series of experiments of this nature the artificial conditions tend to lower the natural or normal pressure which exists while the fish is in its natural habitat. Such events as loss of blood, inadequate respiratory arrangements, change in external pressure on the surface of the body exerted by the air as compared with that of the water, increase of body temperature in the air over that in the water out of which the specimen has just been taken, as well as the general indeterminate conditions that affect the vitality of these river salmon—all these act to minimize the observed pressures.

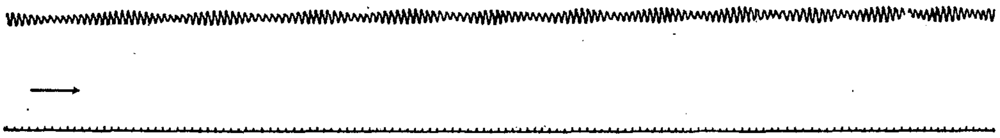
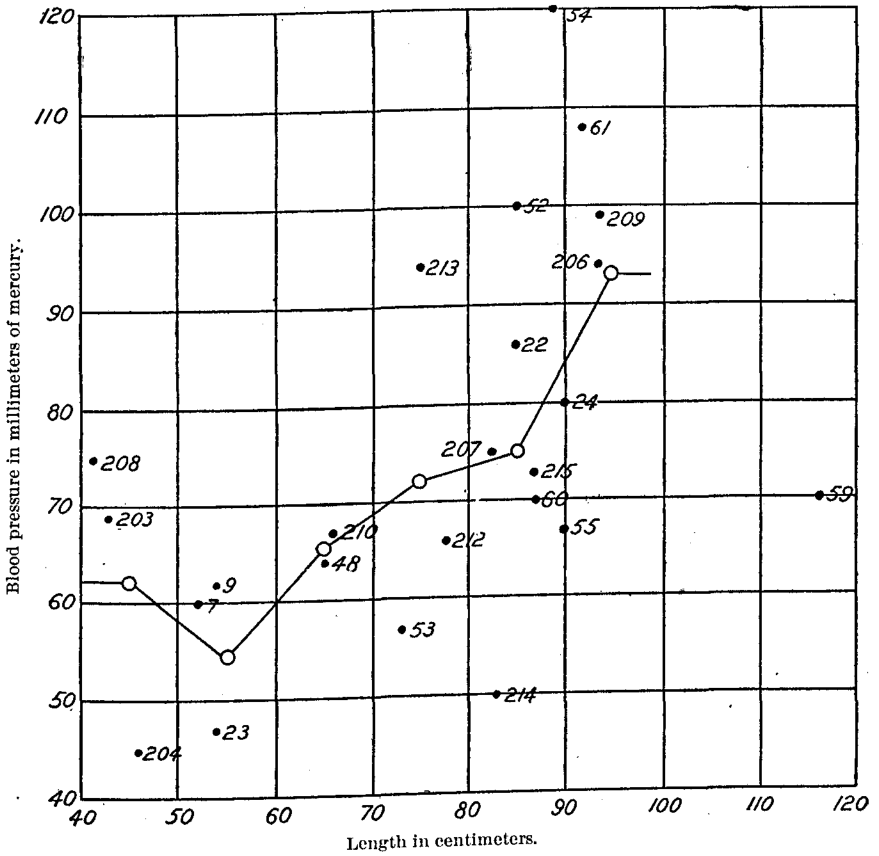


FIG. 3.—Experiment Aug 23, 1902, No. 210, female; length, 672 millimeters. Blood pressure from the ventral aorta, showing the rhythmic interference of the respiratory movements with the pulse pressure.

The 11 males used, varying from 428 to 935 millimeters in length, gave an average ventral aortic pressure of 74.3 millimeters of mercury. This is the equivalent of 101 centimeters of water. With the fishes, as with the higher animals, the larger specimens of otherwise equal physical condition may be expected to give slightly higher pressures. Indeed, a reference to Table V below will show at a glance that such is the case for the salmon. The mean or average pressure for all the specimens between 70 and 100 centimeters in length is 82.6 millimeters of mercury, the equivalent of 112.4 centimeters of water pressure. The pressure in the smaller specimens between 40 and 70 centimeters in length is only 61 millimeters of mercury or 83 centimeters of water. The relatively slight intrinsic value of the smaller fish, which are invariably males, and the greater facility in securing and manipulating them in experiments, leads to their use in larger numbers. When the mean pressure is compared in the males alone, the difference is much more striking. The 7 males between 40 and 70 centimeters in length gave an average pressure of 60 millimeters, while the 5 larger males between 70 and 100 centimeters long averaged 96 millimeters pressure. The pressure of 74.3 millimeters of mercury may, therefore, be considered somewhat lower than the average pressure of prime male salmon.

TABLE V.—*Showing the relation of the normal ventral aortic blood pressure to length in 24 specimens. The line is drawn through the mean or average. The figures refer to the specimen numbers.*



The mean blood pressure for the 13 females included in the table of relatively normal fish is 75.4 millimeters of mercury. These salmon vary in length from 67.2 to 116 centimeters, and the blood pressures obtained from 50 to 120 millimeters of mercury. The majority of these specimens were artificially spawned females, hence a question may be raised as to how far these represent the normal or average condition of the circulatory apparatus. The artificially spawned females that were used were allowed to recuperate for several hours or even for a day or two after the eggs were taken and before the blood pressure was measured. One would think that the artificial spawning process would leave the fish more or less completely exhausted, but this does not seem to be the case, barring the temporary asphyxia that comes from keeping the specimens out of the water for the time required to spawn them. When thrown back into the water these females become lively and vigorous in a very short time. An illustration will suffice to explain the situation. A series of four selected females from the ripe pen were artificially spawned on the morning of Aug. 28, 1901, then thrown back into a pen to recover for the experiments of blood pressure. The afternoon of the same day these females, Nos. 52, 53, 54, and 55, gave good strong pressures, as will be seen by a glance at the table below. One of them, No. 54, gave the highest blood pressure obtained during the whole series of experiments, namely, 120 millimeters of mercury, or 163 centimeters of water. These four fish to all external appearance seemed of equal vigor, and the pressures were taken under very favorable conditions, yet the results gave practically the extremes of the entire series.

TABLE VI.—*Showing blood pressure of four artificially spawned females taken under the same conditions.*

	Millimeters of mercury.
No. 52, ventral aortic pressure	100
No. 53, ventral aortic pressure	57
No. 54, ventral aortic pressure	120
No. 55, ventral aortic pressure	67

The vigor of the artificially spawned females is quite apparent. They do not succumb so quickly to the artificial aeration of the gills, and have more vitality on the experimental table than the prime conditioned fish which, presumably, have more recently arrived on the spawning grounds.

The exhausted fish which were secured off the retaining racks gave surprising blood pressures. No. 17, taken Aug. 14, 1901, was a ripe spent male, 102 centimeters long. This fish gave the ventral aortic pressure of 47 millimeters, though it quickly died on the table. A naturally spawned female, No. 59, taken Aug. 26, 1901, gave a pressure of 70 millimeters, which is almost as much as the average for all the females measured. An exhausted male taken Aug. 19, 1902, gave the stronger pressure of 94 millimeters for a short time, though the pressure was very irregular.

DORSAL AORTIC BLOOD PRESSURE.

The blood pressure measured in the ventral aorta being led off that vessel at a point anterior to the first afferent branchial vessel should represent the maximal pressure of the entire system. In order to determine the fall in pressure as the blood flows through the gills, it is necessary to measure the pressure in some one of the systemic vessels, the nearer the dorsal aortic trunk the better. Schoenlein measured the pressure from the afferent branchial artery and from one of the abdominal arteries in the torpedo and in sharks, and found a very decided fall in the latter as compared with the pressure measured in the first afferent branchial artery. He gave the pressure in the afferent branchial artery in the torpedo as 22 to 24, in no case over 30, centimeters of water (16 to 22 millimeters mercury), while in the branch of the dorsal aorta and in one of the abdominal arteries the pressure was only 10 to 12 centimeters of water as a maximum. This great difference secured by Schoenlein must have been due to the effect of the branchial resistance, which is presumably high in the Selachii.

The dorsal aorta itself was used in my experiments on the salmon. In order to reach it, the tail of the salmon was cut off by a quick stroke and the arterial cannula inserted into the open end of the aortic trunk. The apparatus was all carefully adjusted with reference to the position of the fish before any cutting was done, and the time between the cutting of the artery and the insertion of the cannula was thus reduced to a minimum. The cannula itself usually compressed the vein in the hæmal arch enough to prevent bleeding, and the bleeding from the small cutaneous vessels, which may be considerable if left alone, was easily checked by a tight ligature thrown around the body just in front of the cut. This ligature should be laid during the preliminary preparations.

The blood pressure from the dorsal aorta is quite strong in the salmon, reaching as much as 58 millimeters of mercury in the maximal pressure measured (fig. 4). The average pressure for the six examples reported in Table VII is 53.3 millimeters of mercury, with the extremes of 44 and 58. The mean heart rate of this series is 49.2 per minute, while the average noted in connection with the measurements of ventral aortic pressure is 61 (for males alone 60). This lower average heart rate suggests the inference that the resulting mean blood pressure is below the normal average. This is no doubt possible on account of the slight loss of blood attendant on the operation, a loss which, though small, is felt all the more on account of the relatively small amount of blood in the salmon.

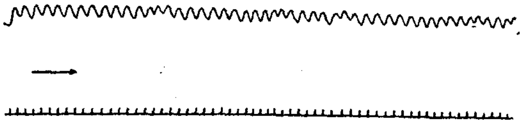


FIG. 4.—Experiment Aug. 28, 1902, No. 220, male; length, 490 millimeters. Blood pressure from the dorsal aorta. The strong pulse beat is noteworthy. Blood pressure, 57 millimeters of mercury; heart rate, 48 per minute.

TABLE VII.—*Blood pressure and heart rate measured from the dorsal aorta.*

Date.	No.	Sex.	Length in millimeters.	Pressure in the dorsal aorta in millimeters of mercury.	Heart rate per minute.
1901.					
Aug. 12	14	Male........	530	58	28
Aug. 14	18	Male........	530	58	72
1902.					
Aug. 26	216	Male........	590	51	(?)
Aug. 27	218	Male........	600	44	60
Aug. 28	220	Male........	490	57	48
Aug. 28	221	Male........	440	52.	38
Mean			....	53.3	49.2

The striking thing about the results of these measurements, notwithstanding the criticism offered above, is the fact that the pressure in the caudal portion of the dorsal aorta approaches so nearly that of the ventral aorta. It would seem that the resistance to the blood flow through the gills is comparatively slight, and the reduction of blood pressure correspondingly insignificant. This fact is further borne out by the presence of the dorsal aortic pulse, which is generally strong and of considerable amplitude. It would be of comparative interest if the pressure could be taken from one of the efferent branchial arteries, but in the salmon this would be extremely difficult, if not wholly impossible. The visceral branches of the aortic trunk are so much atrophied in salmon from the spawning beds that the method of measurement from these vessels, though very easy in the Selachii, is quite impracticable in the salmon.

No synchronous measurements of the pressure in the ventral and in the dorsal aortæ have been made, on account of lack of duplicate apparatus while in the field. Consecutive measurements are of little comparative value, owing to the necessary loss of blood in making the transfer of the cannula from one vessel to the other in the methods it was necessary to use. In the consecutive experiments reported it will be seen that the measurements taken second are considerably below the average, except in a single experiment, No. 221.

THE RATE AND FORCE OF THE HEART BEAT IN THE SALMON.

The tracings of the blood pressure taken from the ventral aorta at a point so near the heart, as was the case in the experiments reported in this paper, give considerable information about the heart itself. The 26 specimens given in Table III, page 437, give an average heart rate of 58.9—in round numbers 60—contractions per minute, or 1 per second. This rate seems rather rapid for so large a fish. A glance at the series of experiments shows a wide range of rates in different specimens, the extremes being 34 and 86 per minute, respectively. There seems no close correspondence between rate of heart beat and blood pressure. The three specimens giving the highest ventral aortic pressures, Nos. 52, 54, and 61, with pressures of 100, 120, and 108 millimeters of mercury, have heart rates of 57, 60, and 34 contractions per minute, respectively. It will be noticed, however, that the pulse pressure of No. 61 is very high, a fact which accounts for the maintenance of a strong blood pressure with a low heart rate. On the other hand, No. 23, which has a heart rate of 86 per minute, has a blood pressure in the ventral aorta of only 47 millimeters, while the average pressure of fish of this size is 60 millimeters.

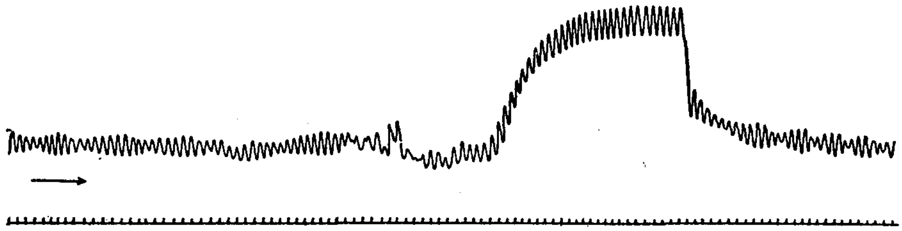


FIG. 5.—Experiment Aug. 23, 1902, No. 212, female; length, 777 millimeters. Showing the maximal blood pressure in the ventral aorta when that vessel is completely occluded. Pressure before occlusion of the aorta, 46 millimeters of mercury; after occlusion, 113 millimeters.

The pulse pressure, i. e., the increase of blood pressure accompanying each discharge of the heart into the aorta, varies exceedingly. In general the slower the rate the greater the amplitude of the pulse, though the exceptions are too numerous to conclude that the pulse pressure is an index of the rate; neither is it an index of the absolute pressure. Selected experiments indicate that there are factors to be determined which correlate the force and rate of the heart beat against the resistance to the discharge through the gills.

The possible force of the heart is indicated by the experiment of compressing the aorta, thus blocking the discharge of the blood and compelling the heart to contract to its fullest capacity. The maximal pressure is surprisingly great in these tests, as can be seen by reference to Table VIII.

TABLE VIII.—*Effects on the blood pressure and upon the heart rate produced by compressing the ventral aorta to the point of complete closure.*

Date.	No.	Sex.	Length in millimeters.	Closure of the ventral aorta.			
				Blood pressure in millimeters of mercury.		Heart rate per minute.	
				Before.	During.	Before.	During.
1902.							
Aug. 18 ..	203	Male	428	1st, 54	126	63	60
Aug. 18 ..	203	Male	428	2d, 53	137	66	66
Aug. 19 ..	204	Male	459	40	78	58	58
Aug. 20 ..	207	Female	823	50	100	66	60
Aug. 23 ..	212	Female	777	46	113	70	70
Aug. 25 ..	213	Male	752	67	172	64	68

In one case, No. 213, a pressure of 67 millimeters before compression of the aorta was increased to 172 millimeters during compression. During this high pressure the heart rate and the pulse pressure remain practically the same as before compression, the rate increasing only 4 beats per minute. These tests reveal a latent power of the heart quite enough to double the blood pressure, and, therefore, to double the efficiency of the circulation if there is any coordinating mechanism by which the salmon may call into activity this latent or potential heart energy, a fact which remains to be seen.

NERVOUS REGULATION OF THE HEART.

My experiments have proved that the heart responds to vagus stimulation and to reflexes. It was noticed over and over again that during the dissections made in the process of experiments the heart was often very irregular. An example is given in figure 6, where cutting away the margin of the opercle in the process of exposing the vagus nerves led to well-marked cardiac inhibitions, with fall of blood pressure. These inhibitions can be brought about reflexly by stimulating various parts of the skin. Direct stimulation of the vagus nerve with rapidly interrupted induction currents produced marked slowing of the heart rate with fall of blood pressure during the continuance of the stimulation. If the currents used were strong enough the slowing of the heart passed over into complete inhibition. The heart escaped from inhibition with single strong contractions, these contractions coming at long

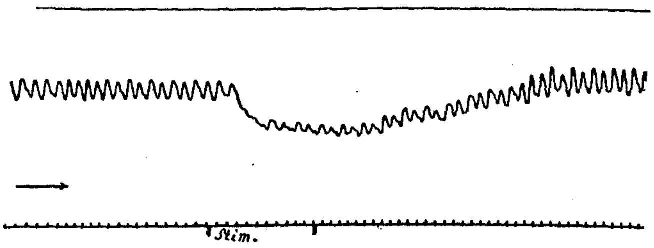


FIG. 6.—Experiment Aug. 22, 1902, No. 209, male; length, 918 millimeters. Showing the fall in ventral aortic pressure and the irregular heart rate following cutaneous stimulation.

intervals as the heart filled with blood, a phenomenon I have noticed in quite a number of different species of fishes. Figure 7 shows the effects of a series of short stimulations of the vagus in a large specimen, 116 centimeters in length.

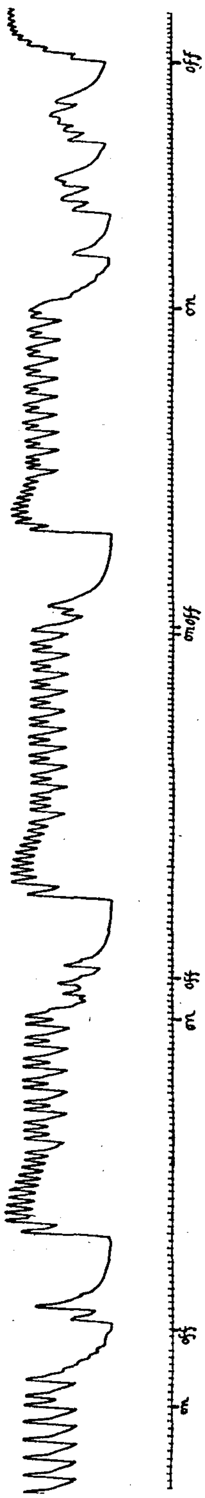


FIG. 7.—Experiment Aug. 29, 1901, No. 59, female; length, 1,160 millimeters. Showing the effect on the blood pressure in the ventral aorta and on the heart rate following stimulation of the vagus nerve. The stimulation in each instance was applied between the words "on" and "off." The inhibitory effects last a considerable time after the stimulation is removed.

No accelerator influences were noticed in any of the experiments of this series, though I ought to say that the work on the nervous coordination of the circulatory system has not been completed.

There is a well-defined respiratory influence on the circulation in the salmon. This finds expression in the tracings in a rhythmic variation in the pulse pressure and to a slight extent in the total blood pressure in the ventral aorta. The respiratory movements consist of alternate opening and closing of the inferior maxilla and the opercles. The total result of the movement is a rhythmical variation in the pressure of the pericardial sac exerted through that part of the wall formed by the branchial apparatus. This movement has been described in other fishes (*Leuciscus dobula*) as exerting an aspiratory function which materially assists in the return of blood to the heart.[a] Be this as it may, it seems clear from my experiments that the respiratory movements exert an influence which increases the pulse pressure when it falls together in time with the heart beat and decreases the pulse pressure when in opposite phase. The respiratory rate is so nearly synchronous with the heart rate that the resulting curve, illustrated by figure 3 (p. 438), often presents a very marked similarity to the vibrations of a reed or tuning fork showing beats. No respiratory influence was noticed on the blood pressure in the dorsal aorta.

SUMMARY.

In a review of these experiments on the circulatory apparatus, one can not but note the general vigor of the heart and circulation in the salmon. The main facts may be summarized as follows:

1. The heart rate is relatively high, the force of the beats as measured by the pulse pressure is strong.
2. The pressure in the ventral aorta is ample to maintain a good and efficient circulation even in old specimens that are otherwise too weak to keep themselves from being caught on the retaining racks.
3. This ventral aortic pressure is diminished comparatively little by the branchial circulation, with the net result that the dorsal aorta has a strong pressure to drive the blood into the muscles, a fact of vital significance when viewed in connection with the migration journey of the salmon.

---

[a] Brünnings, W., Zur Physiologie des Kreislaufes der Fische. Pflüger's Archiv f. d. ges. Physiologie, 75, 1899, 599.

REFERENCES.

RUTTER, CLOUDSLEY. Studies on the natural history of the Sacramento Salmon. Popular Science Monthly, July, 1902.

——— Natural history of the Quinnat Salmon. Bulletin U. S. Fish Commission, XXII, 1904, 65.

MIESCHER-RUESCH, FRIEDRICH. Statistische und Biologische Beiträge zur Kenntniss vom Leben des Rheinlachses im Süsswasser. Schweizerische Fischerei-Ausstellung zu Berlin, 1880, S. 154.

——— Histochemische und Physiologische Arbeiten. Leipzig, 1897.

HENDERSON, YANDELL. Contributions to biology from investigations on the breeding salmon. Popular Science Monthly, April, 1902. (This paper is a review of Miescher-Ruesch's contributions on the subject.)

PATON, D. NOEL. Report of investigations on the life history of the salmon in fresh water. Fishery Board for Scotland Reports, 1898.

SCHOENLEIN, K. Beobachtungen ueber Blutkreislauf und Respiration bei einigen Fischen. Zeitschrift fur Biologie, XXXII, 1895, S. 511-547.

BRÜNNINGS, W. Zur Physiologie des Kreislaufes der Fische. Pflüger's Archiv für die gesammte Physiologie, Bd. 75, 1899, S. 599.

II. A STUDY OF THE BLOOD AND SEROUS LIQUIDS BY THE FREEZING-POINT METHOD.

The chinook salmon, spending a greater part of the individual life in the sea, yet beginning that life and ending it in fresh water, would seem to furnish an ideal subject in which to study the osmotic balances which exist between the surrounding media and the living tissues.

It is known that among marine fishes the sharks and rays[a] have blood which has a general concentration as regards its physical characteristics about equivalent to that of the surrounding sea water. Further, it has been shown that these Selachii have a certain amount of power of adaptation to varying concentration of the surrounding water. The bony fishes, on the other hand, are said to have blood which varies very little from the concentration for mammalia and other higher vertebrates. Even marine bony fishes are supposed to possess this average concentration of the blood,[b] a concentration considerably below that of the water in which the animal lives. The salmon, like all anadromous fishes, must either possess the power of adapting itself to or is not susceptible to the change produced by running from fresh water to sea water when young, and to the reverse conditions when it runs up the rivers to the spawning beds, as already mentioned.

THE BLOOD OF SALMON FROM SALT WATER.

I collected samples of blood from 18 marine salmon during the month of July, 1902,[c] and measured the concentration of the same by the freezing-point method. The depression of the freezing point is a measure of the total molecular concentration, i. e., the concentration due to molecules and ions in solution. The conductivity was not measured in these experiments, hence I have no check on the proportionate quantity of nondissociated and of dissociated molecules. The rapid

---

[a] Rodier, E., Observations et expériences comparatives sur l'eau de mer, le sang et des liquides internes des animaux marins; Travaux des labor. de la stat. zool. d'Arcachon, 1899, 103-123. See also Compt. Rendus, 131, 1900, p. 1008.

[b] Griffiths, A. B., Physiology of the Invertebrata, New York, 1892, pp. 140-141, says that the blood of marine bony fishes does not contain more soluble salts than the blood of fresh-water fishes. Bottazzi, Archives italiennes de Biologie 28, p. 61, finds the concentration to be intermediate between that of the blood of sharks and of air-breathing animals.

[c] I have to thank the Sacramento River Packers' Association for the privilege of working at their Monterey wharf and for many courtesies extended.

decomposition of blood when drawn is a source of error which was guarded against. Determinations made on samples which had been kept too long are not included in this report. The table of determinations is given below.

TABLE IX.—*Showing the depression of the freezing point produced by blood from marine salmon taken at Monterey, Cal.*

Date.	No. of specimen.	Depression of the freezing point of blood.
1902.		° *C.*
July 15 ..	108	$\Delta = -0.792$
July 15 ..	109	−0.811
July 15 ..	111	−0.778
July 16 ..	112	−0.761
July 16 ..	113	−0.726
July 16 ..	114	−0.752
July 21 ..	131	−0.700
July 23 ..	135	−0.745
July 23 ..	141	−0.770
July 23 ..	144	−0.796
July 23 ..	148	−0.754
July 23 ..	149	−0.772
July 25 ..	160	−0.782
July 26 ..	170	−0.733
July 27 ..	173	−0.786
July 27 ..	174	−0.753
July 27 ..	175	−0.713
July 27 ..	179	−0.785
Mean for 18 specimens..................		−0.762

These samples were taken from selected fish as they came in to the Packers' wharf from the fishing grounds. All were medium and large fishes and were fresh, some still giving signs of life. The minimal depression of the freezing point was given by No. 31, −0.700° C.; the maximal depression by No. 9, −0.811° C. The mean depression of the series is −0.762° C., which represents a concentration of the blood equivalent to a 1.26 per cent solution of sodium chloride, assuming a dissociation of 86 per cent at this concentration.[a] The depression of the freezing point produced by the sea water taken from the fishing grounds of Monterey Bay is −1.924° C. Compared with sodium chloride this concentration is the equivalent of 3.20 per cent, or two and one-half times the concentration of the salmon's own blood. As a matter of fact the osmotic pressure of the sea water will be somewhat less, since the dissociation of the different salts will be not quite so great as sodium chloride. The fact I want to emphasize is obvious, namely, that the total osmotic pressure remains far above that of the salmon's blood.

The above showing justifies the conclusion that the thin epithelial layer of cells which separates the blood in the gills from the surrounding sea water does not admit of free diffusion of substances in solution in the blood on the one side and in the sea water on the other. Respiratory exchange of oxygen and carbon dioxide takes place through the gills between the blood and the sea water. The salts, however, can not freely pass. Even as diffusible a substance as sodium chloride is no exception to the rule, the gill epithelium being impermeable to it. In the absence of a chemical analysis of salmon blood, we may assume that the amount of sodium chloride present is considerably less than that necessary to produce a depression of the freezing point

---

[a] Interpolated from Ostwald's tables, Lehrbuch der Allgemeine Chemie, 2te Auf.

of −0.762° C., i. e., the total depression due to all the blood constituents and equivalent to 1.26 per cent[a] of sodium salt (see ante). The percentage of sodium chloride present in sea water, according to an analysis of the North Sea water by Backs,[b] is 2.358 per cent in a total of 3.046 per cent of salts. Applying this analysis to the sea water of Monterey, where the salmon are taken, it is obvious that there is a difference in concentration between the sea water and the salmon blood in sodium chloride alone of a little less than 2 per cent, a difference which ought to suffice on known laws of osmosis in establishing a strong current of water from the blood to the sea water.

Furthermore, the salmon are feeding constantly and voraciously at this time, a process which may introduce considerable sea water into the stomach. This is notably true at Monterey, where the chief food is the squid, the blood and body fluids of which have the concentration of sea water.[c] The skin, also, is a possible region of exchange of constituents between the blood and the sea water. Neither of these regions provides a channel by which the salts of the sea water may permanently enter the blood.

Enough has been given here to demonstrate that we do not have to deal with a question of simple osmotic balance between the sea water and the salmon's blood. Such a balance does not exist. The living epithelium of the gills does not act like an ordinary dead animal membrane; it is, in fact, a membrane impermeable, or at most permeable with great difficulty, to both water and salts. This question will be reviewed after presentation of the facts concerning the relations of the blood in fresh-water salmon from the upper streams.

THE BLOOD OF SALMON FROM TIDE WATER.

The blood was collected from seven different specimens of salmon taken near Black Diamond, California, in August, 1902, all from the main channel off Collinsville. The water in this region at the time of my examination lowered the freezing point by only −0.020° to −0.022° C., an amount that is insignificant as compared with the depression of the freezing point of sea water.

---

[a] The percentage obtained by interpolation from Hedin's tables (Skand. Arch., Bd. 5, 1895, S. 381) is somewhat less, i. e., 1.22 per cent.

[b] Quoted from Griffith's Physiology of Invertebrata, p. 137, New York, 1892.

[c] Bottazzi, La pression osmotique du sang des animaux marins. Archives italiennes de Biologie, T. 28, 1897, p. 61.

TABLE X.—*Depression of the freezing point of blood from salmon taken in the main channel of Suisun Bay at the head of brackish water of the San Francisco Bay region.*

Date.	No. of specimen.	Depression of freezing point of whole blood.
1902.		°C.
Aug. 7	191	$\Delta = -0.780$
Aug. 10	194	−0.772
Aug. 10	195	−0.691
Aug. 10	196	−0.679
Aug. 10	197	−0.744
Aug. 10	198	−0.766
Aug. 10	199	−0.727
Mean		−0.737

The mean depression in these seven examples is −0.737° C., a figure which differs from the mean of the sea salmon by 0.025° C. In making the run from salt water to the head of brackish water there is therefore a small fall in the osmotic tension of the blood.

In the meantime the salmon have ceased to feed. The stomachs are empty save for a thick mass of tough mucous, but no obvious changes have occurred in the gross structure of the alimentary canal. It is unfortunate that there is no definite information as to the time consumed by the fish in adapting itself to the change from salt to fresh water. It has not yet been demonstrated whether the run is direct and continuous or more slow and gradual. The most plausible explanation of the directive influences determining the rapidity with which salmon make the journey through lower tide water is given by Rutter,[a] though he ventures no conclusion as to the rapidity with which the passage through lower tide water is made. He has determined that the progress through upper tide water is comparatively rapid, the salmon making the journey from Vallejo to Sacramento in about four days. There is a belief current among the fishermen at Black Diamond that salmon spend considerable time on the Flats along the upper bay, and the muddy condition of the skin and gills of fish caught in this region would to some degree bear out this view.

Table X shows that a fall of the osmotic pressure of the blood has already begun by the time the fish have reached upper tide water, enough to depress the freezing point by −0.025° C.

THE CONDITION OF THE BLOOD IN SALMON ON THE SPAWNING BEDS.

Salmon make the run from upper tide water to the spawning grounds at a comparatively rapid rate. Rutter[b] found that three specimens branded in the lower river at Rio Vista made the run, a distance of about 300 miles, in 65 days in two cases and in 61 days in one case. Rutter states, further, that the average duration of the spring run is six weeks, but that the fish arrive on the spawning beds from two to six weeks before spawning. From these observations it will appear that the spring run of salmon at spawning time have been in fresh water from eight to twelve weeks. I have made a large number of determinations on the blood of salmon of this class taken from the spawning beds at the United States fish hatchery at Baird, Cal., the results of which are given in Table XI.

---

[a] Rutter, Popular Science Monthly, July, 1902, p. 207.

[b] Ibid., p. 209.

TABLE XI.—*Depression of the freezing point produced by blood and serum from salmon taken on the McCloud River, Baird, Cal.*

Date.	No.	Sex.	Length in centimeters.	Blood.	Serum.
				°C.	°C.
1901.					
Aug. 19	25	Female			Δ=−0.555
Aug. 19	26	Female			−0.570
Aug. 19	27	Female		Δ=−0.621	−0.580
Aug. 20	28	Male	53.0	−0.673	−0.662
Aug. 20	29	Male	54.0	−0.657	−0.650
Aug. 20	30	Male	55.0	−0.663	−0.663
Aug. 20	31	Male	60.0		−0.690
Aug. 21	32	Female	86.0		−0.613
Aug. 21	33	Female	93.0		−0.629
Aug. 21	34	Female	92.0		−0.626
Aug. 22	35	Female			−0.628
Aug. 23	39	Female		−0.586	−0.577
Aug. 24	40	Female	79.0	−0.587	−0.590
Aug. 24	41	Female	79.0	−0.583	−0.588
Aug. 28	52	Female	85.0	−0.628	−0.625
Aug. 28	53	Female	73.0		−0.626
Aug. 28	54	Female	89.0		−0.617
Aug. 28	55	Female	90.0		−0.657
Aug. 29	58	Female		−0.690	
Sept. 2	61	Male	92.0	−0.705	
Sept. 2	64	Female			−0.600
1902.					
Aug. 19	203	Male	42.8	−0.674	
Aug. 19	204	Male	45.9	−0.690	
Aug. 20	206	Male	93.5		−0.518
Aug. 20	207	Female	82.3	−0.576	−0.579
Aug. 22	209	Male	91.8	−0.613	−0.556
Aug. 23	210	Female	67.2	−0.635	
Aug. 23	211	Female		−0.614	−0.596
Aug. 23	212	Female	77.7	−0.557	
Aug. 25	213	Male	75.2	−0.619	−0.607
Aug. 26	214	Female	82.9	−0.634	
Aug. 26	215	Female	86.6	−0.614	
Aug. 27	219	Male	86.0	−0.617	−0.602
Aug. 28	220	Male	49.0	−0.593	
Aug. 28	222			−0.602	
Aug. 29	225	Female		−0.638	−0.635
Aug. 29	226	Female			−0.605
Mean of all determinations				−0.628	−0.613
Mean for males				−0.668	−0.653
Mean for females				−0.610	−0.605

The mean or average depression of the freezing point produced by the blood of salmon from the spawning beds, as shown by the above table, is −0.628° C., a figure that shows a marked falling off in the concentration of the blood when spawning salmon are compared with salt-water and tide-water salmon. The variation in the spawning-ground salmon is very great, as is to be expected when the probable great difference in time spent in fresh water is taken into consideration. Many salmon arrive on the beds during the spawning time in fine flesh and full vigor, while others which have been on the grounds for a longer time show unmistakable external signs of decreased energy and vitality. Such fish have a less concentrated blood and their tissues are notably less firm than is the case with fresh arrivals. The extremes of concentration of whole blood measured at Baird hatchery are Nos. 212 and 61, with a depression of the freezing point of −0.557° and −0.705° C., respectively. No. 212 was an artificially spawned female that was in thin flesh; No. 61 was a large prime male whose muscles were still pink and firm, a fish in full vigor. The larger number of samples of serum show an average depression of the freezing point of −0.613° C., which varies from the mean taken from whole blood determinations by about 2½ per cent only. If we consider only the 14 fish in which measurements were made on blood and on serum from the same individual, then the mean depression produced by the serum is only 1.7 per cent below that of whole blood. This difference appears almost wholly in two observations, Nos. 27 and 209, where the difference is considerable. The presence of the corpuscles in suspension in the serum in whole blood ought not to change the depression of the freezing point unless the corpuscles are disintegrating. Observations of Hamburger[a], Roth[b], Bugarsky and Tangl[c], and Stewart[d] all indicate that the corpuscles are inert in freezing determinations and nonconductors in electrical conductivity determinations. Considering the fact that the serum has essentially the same concentration as blood, then No. 206, with a serum that depresses the freezing point, −0.518° C., is the most dilute blood examined. This specimen was an old male off the retaining rack and in my list represents the specimen nearest death and disintegration. The sides of this specimen were covered with fungus patches, and the skin was broken on an area over the back and on the nose. These pathological conditions would tend to break down the general osmotic resistance of the skin, just as erosion of the skin permits free absorption of materials in man or the higher animals. On the other hand, one must not draw the conclusion that this is the only or even the primary factor leading to the dilution of salmon blood in fresh water. In specimen 212 the skin was clean and perfect, and the fish seemed externally in perfect condition as far as abrasions and general appearance of the skin indicate. Yet the blood gave only −0.557° C. and the ovarian fluid the remarkably low figure of −0.429° C., some 0.12° below the general average of the series. The lower the vitality of the tissues for whatever cause the more dilute the blood was found to be.

If salmon blood be allowed to stand for a day in a warm room, then the corpuscles break up in large quantities and the increased number of ions and molecules set free will of course increase the depression of the freezing point. Experiments in which there was evidence of such change were discarded and do not appear in the above considerations, as stated before. On the whole, the variations in the above table are to be explained as due to the different condition of the individuals studied, differences due to different times of sojourn in fresh water, differences in sex, vitality, etc.

The difference in the blood of males and of females is especially noticeable. The average depression for the whole blood of males is −0.668° C., and for serum −0.653°. The average for females is −0.610° and −0.605° C. for blood and for serum, respectively. This variation in the sexes amounts to about 8⅔ per cent—i. e., the female blood, as determined above, is 8⅔ per cent less concentrated than male blood and the serum 7⅓ per cent less. I believe this observation has its explanation in the more profound changes taking place in the development of the large mass of the ovary as compared with the relatively smaller mass of the testes, and in connection with the production of the large quantity of ovarian fluid at the time of the ripening of the eggs.

---

[a] Hamburger, H. J., Ueber die Regelung der osmotischen Spannkraft von Flüssigkeiten in Bauch- und Peritonealhöhle. Archiv f. Anat. u. Physiologie, Physiol. Abt., 1895, S. 281.

[b] Roth, Wm., Electrische Leitfähigkeit thierischer Flüssigkeiten. Virchow's Archiv, Bd. 154, 1899, S. 466.

[c] Bugarsky and Tangl, Untersuchungen über die molecularen Concentrations-Verhältnisse des Blutserums. Centralb. f. Physiologie, Bd. XI, 1897, S. 301.

[d] Stewart, G. N., Elektrische Leitfähigkeit thierischer Flüssigkeiten. Centralb. f. Physiologie, Bd. XI, 1897, S. 332.

THE OVARIAN LIQUID FROM MATURE FEMALE SALMON.

A quantity of serous liquid, the ovarian fluid of Miescher-Russch, is always found in the abdominal cavity of the ripe females, the liquid being extruded with the eggs. This liquid is easily collected during the artificial spawning. It amounts to from 100 to 150 cubic centimeters, but varies greatly in quantity in different individuals.

Determinations of the freezing point were made on series of samples of this ovarian liquid in the summers of 1901 and 1902. Females to be spawned were very carefully freed from excess of water by wiping with dry cloths and the eggs spawned into a clean dry spawning pan. The liquid extruded with the eggs was strained through dry cleansed cheese-cloth into prepared bottles. The method of collecting did not admit of contamination at any point. The depression of the freezing point produced by these samples is given in the table below:

Table XII.—*Depression of the freezing point produced by the ovarian liquid from ripe salmon from the spawning beds at Baird, Cal.*

Date.	No.	Depression of the freezing point.
1901.		°*C*.
Aug. 23 ..	36	Δ = −0. 553
Aug. 23 ..	37	−0. 560
Aug. 23 ..	38	−0. 563
Aug. 24 ..	40	−0. 536
Aug. 24 ..	41	−0. 552
Aug. 24 ..	42	−0. 555
Aug. 24 ..	43	−0. 533
Aug. 28 ..	51	−0. 545
Aug. 28 ..	52	−0. 546
Aug. 28 ..	53	−0. 563
Aug. 28 ..	54	−0. 562
Aug. 28 ..	55	−0. 599
Aug. 27 ..	56	−0. 528
Aug. 29 ..	58	−0. 531
Aug. 30 ..	60	−0. 562
Aug. 31 ..	62	−0. 571
Aug. 31 ..	63	−0. 575
1902.		
Aug. 20 ..	207	−0. 559
Aug. 23 ..	211	−0. 548
Aug. 23 ..	212	−0. 429
Aug. 26 ..	214	−0. 550
Aug. 26 ..	215	−0. 539
Aug. 29 ..	225	−0. 571
Aug. 29 ..	226	−0. 555
Mean of 24 specimens		−0. 549

The determinations reported in this table are remarkable for uniformity in result. There is only one noteworthy exception to the statement, viz., No. 212. In this fish the concentration of the ovarian liquid is exceptionally low. By reference to Table XI it will be seen that the blood of this fish also is very low, being −0.072° C. below the average in concentration. Leaving aside the ovarian fluid of No. 212, then the averages of determinations on the ovarian liquids for the two seasons agree within one point in one thousand, being −0.555 and −0.554 for 1901 and 1902, respectively. The concentration of the liquid from the abdominal cavity is less than that of the blood or the serum. The following tables give sets of determinations made on the ovarian fluid, the serum, and the whole blood from the same animal (Table XIII), and on the serum and ovarian liquid (Table XIV).

TABLE XIII.—*Depression of the freezing point produced by the blood, the serum, and the ovarian fluid from the same animal.*

Date.	No.	Blood.	Serum.	Ovarian fluid.
1901.		*° C.*	*° C.*	*° C.*
Aug. 24	40	Δ=−0.586	Δ=−0.590	Δ=−0.538
Aug. 24	41	−0.583	−0.588	−0.552
Aug. 28	52	−0.628	−0.625	−0.546
1902.				
Aug. 20	207	−0.576	−0.579	−0.559
Aug. 23	211	−0.614	−0.596	−0.548
Aug. 29	225	−0.638	−0.635	−0.571
Mean for 6	......	−0.604	−0.602	−0.552

TABLE XIV.—*Depression of the freezing point produced by the serum and by the ovarian fluid from the same fish.*

Date.	No.	Serum.	Ovarian fluid.
1901.		*° C.*	*° C.*
Aug. 24	40	Δ=−0.590	Δ=−0.538
Aug. 24	41	−0.588	−0.552
Aug. 28	52	−0.625	−0.546
Aug. 28	53	−0.626	−0.563
Aug. 28	54	−0.617	−0.562
Aug. 28	55	−0.651	−0.599
1902.			
Aug. 20	207	−0.579	−0.559
Aug. 23	211	−0.596	−0.548
Aug. 29	225	−0.635	−0.571
Aug. 29	226	−0.605	−0.555
Mean for 10	......	−0.611	−0.559

These tables, together with Tables XI and XII, demonstrate that the ovarian fluid has an average concentration less than that of the blood or serum of from 0.060° C. to 0.080° C. The ovarian fluid in those fish in which the serum also was measured has a mean or average depression of the freezing point less than that of the serum by 0.052° C. In the six instances in which the whole blood and the serum also were determined the difference between the serum and ovarian liquid is 0.054° C.; between the whole blood and ovarian fluid, 0.056° C.

Considering all the determinations on female salmon the mean difference becomes somewhat more, viz., 0.056° and 0.061° C., respectively. (See Tables XI and XII.) On the evidence at hand it would appear that the ovarian liquid bears a constant difference in concentration, represented by a difference in the freezing point, of from 0.050° to 0.060° C. less than that of the blood plasma of the same animal.

The origin of the ovarian fluid in the salmon needs further investigation. In the one unripe female which I used in experiments, in which the eggs were not yet set free in the body cavity, the eggs were free from excess moisture and there was no appreciable amount of liquid in the abdominal cavity. Mr. G. H. Lambson, the superintendent of Baird Station, writes me in response to my inquiry:

> We have opened many females before the eggs were ripe, but never have noticed any egg fluid in them. The eggs before they separate are rather dry and do not wet the fingers. When we open the green females it is to secure the eggs for fishing and we may have overlooked the fluid, but from the fact that the eggs are practically dry I should think none was present.

I have seen such females opened, but no liquid was present. Males do not contain more than a few drops of abdominal cavity liquid. In one of the largest male specimens used not enough liquid could be obtained from the body cavity for a freezing determination.

The ovarian liquid is a clear, limpid, slightly translucent fluid very like mammalian transudates, hydrocoele fluid for example, in appearance. It makes its appearance when the eggs are being set free from the ovary and following this event. Paton[a] gives a quotation from Miescher-Reusch containing the phrase "the ovarian fluid which readily exudes from the ripe ovary when broken down." Miescher-Ruesch[b] says that this ovarian fluid is a rich concentrated liquid to be regarded chemically as a "liquid caviar." He says that the fluid gives the proteid reactions, that it is rich in phosphorized fat (lecithin), containing as much as 20 per cent, and that on digestion with artificial gastric juice it gives a further yield of phosphorus derived from nucleo-proteid. The ovarian fluid in the chinook salmon is much more fluid and less concentrated than in *Salmo salar*, if one can rely on the observations referred to above.

At the time when the eggs are set free in the body cavity there is a very profound rupture of the surface of the ovary. There are from 4,000 to 6,000 and even more eggs in each ripe female, hence it is obvious that the entire surface of the ovary is involved in this morphological disturbance. So large a ruptured surface would furnish an ideal condition for the transudation of materials from the blood and the tissues. It is probable also that some of the liquid comes from liquefaction processes in the ovary itself antecedent to and resulting in the freeing of the eggs, though the quantitative relations do not justify the assumption that this latter source accounts for more than a fractional part of the total liquid. Hedin[c] calls attention to the close correspondence in osmotic tension of blood and transudates, a relation which does not exist between the blood and secretions, a correspondence close enough in this instance to class the ovarian fluid as a transudate. I have not seen indications of the presence of blood pigment in the ovarian liquid which would indicate rupture of blood vessels in the ovary. True, the blood is sometimes present in the artificially spawned mass of eggs and fluid, but always under conditions that point to artificial rupture of vessels (usually in the spleen) by the mechanical pressure of spawning. Occasionally the ovarian fluid has a slight yellow color the same as that of the eggs. This color is attributed to the rupture and disintegration of eggs in the body cavity itself.

COMPARISON OF THE FREEZING POINTS OF BLOOD OF SALMON FROM THE DIFFERENT REGIONS.

The average depression of the freezing point of the salmon blood is, for sea salmon, −0.762° C.; for brackish-water salmon, −0.737° C., and for spawning-ground salmon, −0.628° C. The decrease in concentration for the second and third groups, compared with the first as a standard, is represented by −0.025° C. and −0.134° C.,

---

[a] Paton, D. Noel, Report of the Fishery Board for Scotland, 1898, p. 143.

[b] Miescher-Ruesch, F. Statistische und Biologische Beiträge zur Kenntniss vom leben des Rheinlachses im Süsswasser. Schweizerischer Fischerei-Ausstellung zu Berlin, 1880, 154.

[c] Hedin, S. G., Skand. Archiv f. Physiologie, 5, S. 277.

respectively, or a decrease of 3.3 per cent for brackish-water salmon and 17.6 per cent for spawning-ground salmon. This decrease of 17.6 per cent in blood concentration is very significant from two very different points of view. First, because the change is as small as it is, considered in relation to so profound a change in environment as that represented by a passage from sea water to fresh water. This change made suddenly is well known to be fatal to many sea forms. The cyclostome *Polistotrema stouti*,[a] when transferred to sea water diluted one-half with fresh water, dies in a few minutes with violent swimming and struggling. The blood of this cyclostome, however, has a concentration about the same as that of the sea water in which it lives. In three samples determined in July, 1901, the average depression of the freezing point for *Polistotrema stouti* serum is −1.966° C., as against −1.924° C. for the water of Monterey Bay, in which it lives. This form can not make the transition suddenly, at any rate. The related lampreys are anadromous, and it will be interesting to see what is the concentration of lamprey blood. Marine invertebrates can not safely be transferred to fresh water. In this group, however, as Bottazzi[b] and others have shown, the blood has the concentration of sea water. Quinton[c] found that the blood of marine invertebrates takes the concentration of the bathing liquid when placed in water of greater or less concentration than sea water—that is, that the skins are permeable to both salts and water. Garrey[d] has just shown that blood or body fluids of certain marine invertebrates varies not more than 0.02° C. in its freezing point from that of the water in which they live. He tested the following: *Thyone briareus*, *Arbacia punctulata*, *Asterias vulgaris*, *Sycotypus canaliculatus*, *Venus mercenaria*, *Mya arenaria*, *Homarus americanus*, and *Limulus polyphemus*. He also shows that the external tissues of these animals are permeable. Mosso[e] found that sharks (*Scyllium*) transferred to fresh water died within an hour. In fact, the circulation ceased at the end of one-half hour, though the heart still contracted, the blocking of the circulation being due to clogging of the vessels of the gills. Bottazzi also points out that in marine bony fishes the blood has a far less concentration than in invertebrates or in cartilaginous fishes, a fact which I can confirm for all three groups. He makes the point that bony fishes show an intermediate position in this regard between cartilaginous fishes and air-breathing vertebrates in that they have acquired a certain degree of independence of their surroundings. Garrey was unable to demonstrate permeability of the tissues of *Fundulus* when transferred from sea water to fresh water.

The salmon show a very decided independence in the relation between the composition of the blood and the surrounding water. Their gills and skin are impermeable—the exception, of course, being the permeability to oxygen and carbon dioxide. At first thought one would be inclined to ascribe the fall of concentration of the blood of 17.6 per cent to direct absorption of water in fresh water, but such

---

[a] This interesting species has been the subject of a number of valuable physiological and morphological papers. It was originally described under the name *Bdellostoma stouti* Cooper, and has been identified with *Bdellostoma dombeyi* by several authors.

[b] Bottazzi, La pression osmotique du sang des animaux marins, Arch. ital. de Biol. 29, 1897, 61.

[c] Quinton, M. R. Communication osmòtique chez l'invertebres marin normal, entre le milieu interieur l'animal et le milieu exterieur. Comptes Rendus, 131, 1900, 905.

[d] Garrey, Walter E. Osmotic pressure of sea water and of the blood of marine animals. Biol. Bull., VIII, 1905, 257.

[e] Mosso, Ueber verschiedene Resistenz der Blutkörperschen bei verschiedenen Fischarten, Biol. Centralb. Bd. 10, 1890, S. 570.

is not necessarily the inference. The absence of food and the important metabolisms occurring during the eight to twelve weeks' sojourn in fresh water are to be considered in this connection, and possibly are sufficient to account for the change.

The second consideration of the fall of concentration of the blood is in regard to its effect on tissue metabolism and tissue life. Observations on vertebrates have shown that while the concentration of blood may temporarily vary sharply, owing to the taking of water with the food or during abstinence from water and food, still on the whole the concentration is remarkably constant, as Roth[a] has already emphasized. This constancy in physical condition, or isotonicity, is in fact regarded as a prime physiological necessity for the normal life and activities of the tissues.

The salmon undergoes a permanent alteration of 17.6 per cent, almost one-fifth, in the concentration of the blood, yet it is able to carry on vigorous activities of the muscular and nervous systems, as well as those internal metabolisms which result in the growth and development of the ovaries and testes and which involve a transference of materials in large amount to these organs from other parts of the body, especially from the muscles. The question may be raised, Is this decrease in the proportion of solids in the blood really injurious; and if so, how far may it proceed before death takes place? An indication of the limits to this process is given by specimen No. 206, an old male too weak to keep off the retaining rack, from whence I removed it on Aug. 20, 1902. This salmon remained alive long enough to secure a blood-pressure measurement. The serum depressed the freezing point by only −0.518° C., representing a fall in concentration of 32 per cent in comparison with blood from the marine salmon. Basing judgment on this single case, one would say that a 32 per cent decrease in blood concentration represents the approximate limits of blood dilution which will support the organized life of the individual.

A reference table of the determinations of the freezing points made by different observers on the blood and serum of divers species of animals is here presented. It is not exhaustive, but represents the results given in the papers available, together with some determinations of my own not previously published. Among the Selachii and marine invertebrata, where the blood concentration follows closely that of the sea water in which they live, obviously one must in making comparisons take the environment into consideration.

---

[a] Roth, Virchow's Archiv, Bd. 154, 1899, S. 488.

TABLE XV.—*The depression of the freezing point produced by the blood and serum from different animals as presented by various observers.*

Animal.	Blood.	Serum.	Observer.
	°C.	*°C.*	
Man		Δ=−0.560	Dresser.[a]
		−0.560	Korányi.[b]
Horse	Δ=−0.580	−0.585	Hamburger.[c]
		−0.596	Do.[d]
		−0.565	Winter.[e]
		−0.530	Bugarsky and Tangl.[f]
Dog	−0.599	−0.605	Hamburger.[c]
		−0.565	Winter.[e]
		−0.587	Bugarsky and Tangl.[f]
Cat		−0.617	Do.[f]
Pig	−0.625	−0.621	Hamburger.[c]
		−0.550	Winter.[e]
		−0.598	Bugarsky and Tangl.[f]
Ox	−0.601	−0.600	Hamburger.[c]
		−0.647	Do.[d]
		−0.550	Winter.[e]
		−0.598	Bugarsky and Tangl.[f]
		−0.592	Hedin.[g]
		−0.57	Roth.[h]
Sheep	−0.662	−0.550	Winter.[e]
		−0.57	Roth.[h]
Rabbit	−0.578	−0.580	Hamburger.[c]
		−0.570	Winter.[e]
Thalassochelys caretta		−0.615	Bottazzi.[i]
Cerna gigas		−1.035	Do.
Charax puntazzo		−1.04	Do.
Scorpænichthys marmoratus		−1.053	Greene.
Oncorhynchus tschawytscha:			
Marine	−0.762		Do.
Fresh-water	−0.628	−0.613	Do.
Salmo irideus	−0.645	−0.647	Do.
Torpedo marmorata	−2.26	−2.29	Bottazzi.[i]
Mustelus vulgaris	−2.36		Do.
Trigon violacea	−2.44	−2.43	Do.
Raja occidentalis		−1.996	Greene.
Polistotrema stouti		−1.996	Do.
Tautoga onitis	0.86		Garrey.[k]
Cynoscion regalis	0.86		Do.
Leptocephalus conger	0.80		Do.
Anguilla chrisypa	0.90		Do.
Xiphias gladius	0.90		Do.
Mustelus canis	1.86		Do.
Carcharias littoralis	1.88		Do.
Octopus vulgaris		−2.29	Bottazzi.[i]
Octopus macropus		−2.314	Do.
Aplysia limacina		−2.34	Do.
Aplysia depilans		−2.32	Do.
Homarus vulgaris		−2.292	Do.
Maja squinado		−2.36	Do.
Sipunculus midus		−2.31	Do.
Holothuria tubulosa		−2.315	Do.
Asterias glacialis		−2.295	Do.
Astropecten auriantiacus		−2.312	Do.
Alcyonarium palmatum		−2.196	Do.
Sea water, Bay of Naples, Mediterranean.		−2.29	Do.
Sea water from Monterey Bay, Pacific Ocean.		−1.924	Greene.
Sodium chloride:			
1 per cent		−0.606	Hamburger.[d]
3.783 per cent		−2.29	Bottazzi.[i]

[a] Dresser, Arch. f. exp. Pathol. u. Pharm., Bd. XXIX, 1892, S. 306.

[b] Korányi, Centralb. f. Physiologie, Bd. VIII, 1894, S. 503.

[c] Hamburger, Archiv. f. Anat. u. Phys., Phys. Abt., 1895, S. 281.

[d] Hamburger, Centralb. f. Physiologie, Bd. VII, 1894, S. 758.

[e] Winter, Arch. de Physiol., 1895. (This reference and the figures quoted are taken from R chet's Dictionaire de Physiologie, T. 4, p. 595.)

[f] Bugarsky and Tangl, Centralb. f. Physiol., Bd. XI, 1897, S. 301.

[g] Hedin, Skand. Arch. f. Physiol., Bd. 5, 1895, S. 277.

[h] Roth, Virchow's Archiv., Bd. 154, 1899, S. 466. Centralb. f. Physiol., Bd. XI, 1897, S. 271.

[i] Bottazzi, Arch. italien de Biol., T. 28, 1897, p. 61.

[k] Garrey, Biol. Bull., VIII, 1905, 257.