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THE BLOOD OF NORTH AMERICAN FRESH-WATER MUSSELS UNDER NORMAL AND ADVERSE CONDITIONS [1]

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INTRODUCTION

In the course of field studies on the mussel beds of the upper Mississippi, conducted during the past three years, considerable numbers of dead and dying mussels have been found in various localities once very productive of commercial shells. In addition, a large per cent of the glochidia, taken from female mussels living in the same areas, have been dead or diseased, indicating the existence of conditions which are reducing the natural reproduction of even such adult mussels as are able to withstand the environment. It is well established that progressive changes in stream conditions, resulting from the needs of navigation and from contamination through municipal and industrial wastes, have materially altered the natural habitats of the fresh-water mussels at many points in the Mississippi drainage. In order to evaluate the effects of these changes on the mussel fauna, particularly the effects of municipal and industrial wastes on the mussels themselves, a series of physiological studies on fresh-water mussels has been undertaken.

In the fresh-water mussels the blood is associated not only with nutrition, respiration, excretion, and the general well-being of the individual as in the higher animals, but the blood also has a special mechanical function in connection with the peculiar locomotion of fresh-water mussels. The "foot," the muscular organ of locomotion

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which is extruded between the valves of the shell, may be expanded during activity to many times its retracted volume by an inflowing of blood which can be held in the foot. A considerable volume of blood is required for this procedure and provision must be made for this fluid when the foot is retracted into the shell. Consequently the volume of blood in proportion to the size of the animal is large, and there are numerous sinuses—that is, reservoirs for blood—in various parts of the body. (See figs. 1, 2, 6, 7, and 8.)

Although no data are available for the exact total volume of the blood of fresh-water mussels, Weinland (1919) gives some figures on the relative weights of the shell, the soft parts, and the body fluid of the European fresh-water mussel, *Anodonta cygnea*, which are suggestive in this connection. He describes the fluid as that which could be drained or easily pressed from the soft parts of the animal, so his figures do not apply to blood alone, nor do they necessarily represent all of the blood; but the major portion of this fluid was blood. He found this body fluid as described to constitute from 46 to 57 per cent of the total weight of the animal, including the shell, or almost two and one-half times the weight of the drained soft parts.

In view of the large volume of blood in a fresh-water mussel and the importance of this fluid not only in the general metabolism but in the locomotion of the animal, the blood has been used as a starting point for the physiological studies of fresh-water mussels, as the blood is known to reflect the condition of the individual in both health and disease in the higher animals.

NORMAL BLOOD OF FRESH-WATER MUSSELS

METHOD OF TAKING SAMPLES

As the first task in these studies was the determination of the normal values for fresh-water mussel blood from which deviation as produced by various factors could be observed, only vigorous individuals taken directly from the water were used unless otherwise stated. All animals failing to give prompt and strong contractions of the pedal muscle and mantle margin, and in which the heart was not beating regularly when the valves were opened, were rejected from these groups of normals.

In taking blood for analyses care was used to avoid dilution with the water contained in the gill cavities of the animal. The two shells were opened gently with mussel tongs (Coker, Shira, Clark, and Howard, 1921), and the water allowed to drain out of the gill and mantle cavities. The two large muscles holding the valves together were then cut and one valve turned back, the exposed gill and mantle on that side being cut away. This procedure left the pericardial cavity intact but easily accessible. The animal was again drained free of any water or blood which might have accumulated in the mantle cavity during the operation. The pericardial cavity was then opened with a pair of small iridectomy scissors and the blood taken directly from the pulsating heart. It was found in actual practice that in an animal opened in this manner blood rapidly accumulated between the uninjured mantle and the shell, and several cubic centimeters of blood could be extracted by making a small opening near the center of the mantle on the uninjured side. Tests of the blood accumulating in this mantle pocket showed that, if taken immediately, this blood did not differ in composition from blood drawn directly from the heart, and consequently this blood could be used when large samples were desired. However, as most of the determinations required only small samples of blood, the blood was usually taken directly from the heart as described above.

If blood samples were to be taken from a single mussel over a period of days the procedure was modified. The outside of the shell was ground away on an emery wheel until the portion directly over the pericardial cavity was quite thin. This grinding was done little at a time, the mussel being immersed frequently in water to prevent heating of the shell. When the shell had been ground to a suitable thinness in the region desired a "window" was opened in the shell by means of small bone forceps and the pericardium exposed. The mussel did not seem to be greatly disturbed by this operation, and the heartbeats could be counted readily through the pericardium. Using a fine dental needle on a Leur syringe the pericardium was punctured and blood drawn directly from the heart. After removing the needle the heart continued to beat regularly and animals so prepared were kept alive for a period of days, although samples of blood were drawn daily or at even shorter intervals.

GENERAL PHYSICAL PROPERTIES

The blood of the species of North American fresh-water mussels studied is a mobile, limpid, lusterless fluid, clear and colorless when first drawn from the heart or sinuses, but soon becoming slightly turbid. Drawn blood does not clot into a solid mass, but in from one to five minutes after the blood is removed from the body of the mussel, particles of a whitish, opaque coagulum appear, suspended like bits of curd in the more watery, uncoagulated fluid. These pieces of coagulum which agglutinate to some extent form only a small portion of the total volume. On heating to 50° C. or above, the separation of the coagulum proceeds more rapidly and as this albuminous precipitate settles to the bottom of the container the supernatant fluid becomes clear and sparkling. Dried mussel blood has a very faint, yellowish-brown color, which deepens on heating or on prolonged exposure to the air.

SPECIFIC GRAVITY

The specific gravity of the blood was determined by the Barbour and Hamilton (1926) falling-drop method. As this procedure requires only 0.01 cubic centimeter of blood, usually three or more determinations were made each time a sample was taken, and the values averaged.

In Table 1 the summarized data on specific gravity of the blood from 145 individual mussels, representing 19 species, are given. Only animals which appeared to be in good condition and which had not been subjected to experimentation were incorporated in this series. The determinations grouped in Table 1 include readings made in every month of the year and from both male and female mussels (see Table 2 for individual data) in order to obtain the average normal limits of variation in blood values.

The average specific gravity was 1.0026—a very low value for blood when compared with the specific gravity of the blood of man, the pigeon, and common fresh-water animals (see Table 3). Even the maximum specific gravity given in Table 1, 1.0078, and the maximum specific gravity of the blood from any mussel under experimental conditions in these studies, 1.0099 (from a moribund specimen of *Quadrula metanevra;* see Table 17), are well below the values commonly recorded for the blood specific gravity of animals other than fresh-water mussels.

TABLE 1.—*Specific gravity of blood of fresh-water mussels*

Scientific name	Common name	Number of individuals	Blood—specific gravity										
			1.0000–1.0010	1.0011–1.0020	1.0021–1.0030	1.0031–1.0040	1.0041–1.0050	1.0051–1.0060	1.0061–1.0070	1.0071–1.0080	Minimum	Average	Maximum
Subfamily Unioninæ:													
Fusconaia ebena	Niggerhead	4					3	1			1.0043	1.0048	1.0057
Fusconaia undata	Pig toe	7		1	1	3	1		1		1.0018	1.0036	1.0052
Tritogonia verrucosa	Buckhorn	7		2	4	1					1.0011	1.0023	1.0031
Amblema costata	Three-ridge	6			2	2	1	1			1.0022	1.0034	1.0042
Quadrula pustulosa	Pimple back	1					1					1.0043	
Quadrula metanevra	Monkey face	1					1					1.0043	
Unio popei	Pope's purple	2		2							1.0016	1.0017	1.0019
Subfamily Anodontinæ:													
Anodonta limnæana	Southern floater	3		1	1	1					1.0016	1.0027	1.0034
Lasmigona compressa	Heel splitter	1				1						1.0032	
Subfamily Lampsilinæ:													
Obliquaria reflexa	Three-horned warty-back.	6		2	1	2	1				1.0017	1.0027	1.0043
Proptera alata	Pink heel splitter	12		1	10	1					1.0011	1.0024	1.0031
Proptera laevissima	Paper shell	1				1						1.0036	
Plagiola lineolata	Butterfly	2		1	1						1.0013	1.0021	1.0028
Ligumia recta latissima	Black sand-shell	1			1							1.0026	
Lampsilis anodontoides	Yellow sand-shell	17	2	4	6	2	1	1	1		1.0007	1.0027	1.0063
Lampsilis fallaciosa	Slough sand-shell	29	1	12	11	4		1			1.0007	1.0024	1.0055
Lampsilis siliquoidea pepinensis	Lake Pepin mucket	29	5	8	10	3	1	1		1	1.0004	1.0024	1.0078
Lampsilis ventricosa	Pocketbook	1			1							1.0029	
Actinonaias carinata	River mucket	15	1	4	5	5					1.0003	1.0025	1.0043
Total	19 species	145	9	38	54	26	10	5	2	1	1.0003	[1] 1.0026	1.0078

[1] Average of all individuals.

TABLE 2.—*Individual specific gravity and pH data*

Date, species, and location	Specific gravity	pH
Niggerhead (Fusconaia ebena), Mississippi River, Nahant, Iowa:		
July 19, 1929	1.0043	7.6
Do	1.0044	7.7
Do	1.0049	7.5
Do	1.0057	------
Pig toe (Fusconaia undata), Mississippi River, Fairport, Iowa:		
July 15, 1929	1.0018	7.8
July 18, 1929	1.0026	7.7
July 17, 1929	1.0034	7.7
July 19, 1929	1.0037	7.6
July 13, 1929	1.0040	7.8
July 19, 1929	1.0043	7.7
Do	1.0052	------
Buckhorn (Tritogonia verrucosa), Mississippi River, Fairport, Iowa:		
Aug. 22, 1929	1.0011	7.5
July 23, 1929	1.0015	7.9
July 12, 1929	1.0021	7.8
July 25, 1929	1.0029	7.8
July 27, 1929	1.0029	8.1
Do	1.0029	7.9
Mar. 7, 1930	1.0031	------
Three-ridge (Amblema costata), Mississippi River, Fairport, Iowa:		
July 19, 1929	1.0022	7.5
Nov. 7, 1929	1.0027	7.7
July 19, 1929	1.0035	7.6
July 8, 1929	1.0036	7.9
July 19, 1929	1.0042	7.8
Do	1.0042	7.4
Pimple back (Quadrula pustulosa), Mississippi River, Nahant, Iowa:		
July 19, 1929	1.0043	7.6
Monkey face (Quadrula metanevra), Mississippi River, Nahant, Iowa:		
July 19, 1929	1.0043	7.7
Pope's purple (Unio popei), canals from Rio Grande, Mercedes, Tex.:		
May 21, 1929	1.0016	8.3
May 22, 1929	1.0019	8.1
Apr. 25, 1929	------	7.7
June 3, 1929	------	8.1
Southern floater (Anodonta limnæana), canals from Rio Grande, Mercedes, Tex.:		
Mar. 24, 1930	1.0016	7.8
Mar. 10, 1930	1.0021	8.0
Mar. 27, 1930	1.0034	8.0
Squaw foot (Strophitus rugosus), Mississippi River, Fairport, Iowa:		
June 27, 1929	------	8.3
Heel splitter (Lasmigona compressa), Mississippi River, Fairport, Iowa:		
July 19, 1929	1.0032	7.9
Three-horned warty-back (Obliquaria reflexa), Mississippi River, Fairport, Iowa:		
Aug. 28, 1929	1.0017	8.0
Aug. 27, 1929	1.0018	7.9
Aug. 26, 1929	1.0021	7.9
Do	1.0031	7.8
Aug. 27, 1929	1.0032	8.0
Do	1.0043	7.8
Pink heel splitter (Proptera alata), Mississippi River, Fairport, Iowa:		
Mar. 4, 1930	1.0011	8.0
Do	1.0021	7.9
Aug. 24, 1929	1.0021	7.7
Do	1.0022	8.0
Do	1.0022	8.0
Aug. 23, 1929	1.0025	7.9
Do	1.0026	8.0
Do	1.0026	7.9
Do	1.0027	7.8
Aug. 24, 1929	1.0028	7.9
Aug. 26, 1929	1.0029	8.0
Aug. 22, 1929	1.0031	8.0
Paper shell (Proptera laevissima), Mississippi River, Nahant, Iowa:		
July 19, 1929	1.0036	7.7
Butterfly (Plagiola lineolata), White River, Newport, Ark.:		
Mar. 29, 1930	1.0013	7.9
Mar. 27, 1930	1.0028	7.9
Black sand-shell (Ligumia recta latissima), Mississippi River, Fairport, Iowa:		
Mar. 4, 1930	1.0026	7.7

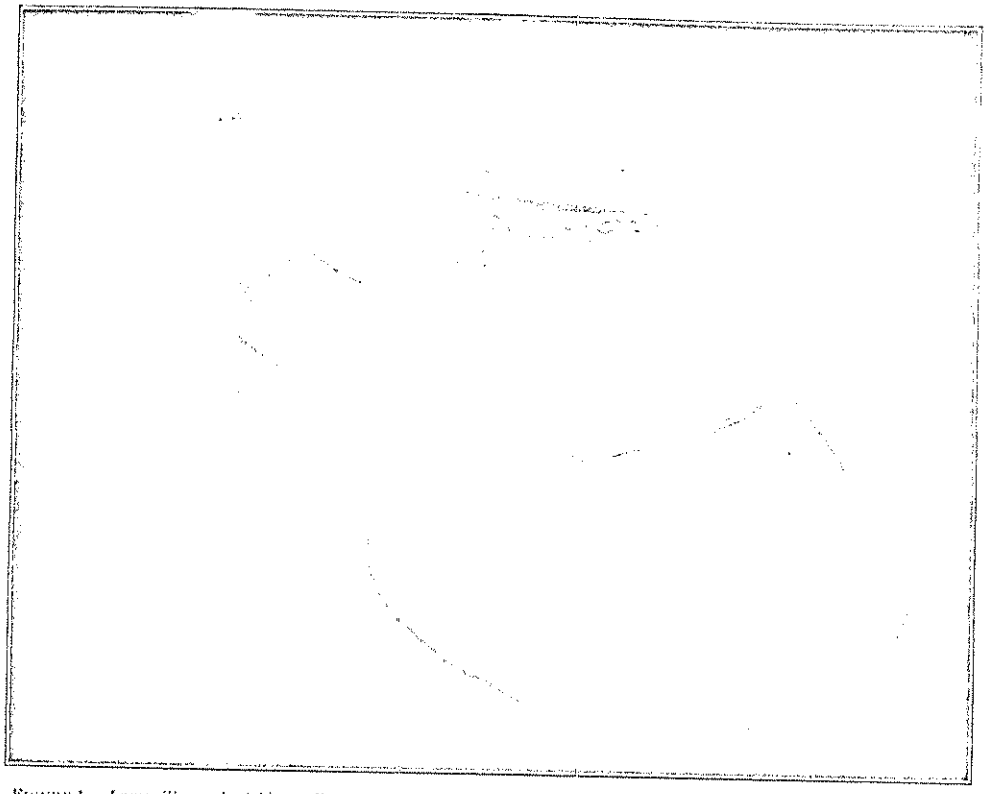

FIGURE 1.—*Lampsilis anodontoides*, yellow sand-shell, natural size. Living animal with foot completely extruded showing the relatively large volume of this organ and the demand such expansion makes on the blood and body fluids. The animal is supported on the far side by a glass rod attached to the valve of the shell by beeswax

FIGURE 2.—*Lampsilis anodontoides*, yellow sand-shell. Living animal (same as Fig. 1) with one valve of shell removed to show size and position of foot. Heart may be seen just below the open space near middle of the hinge

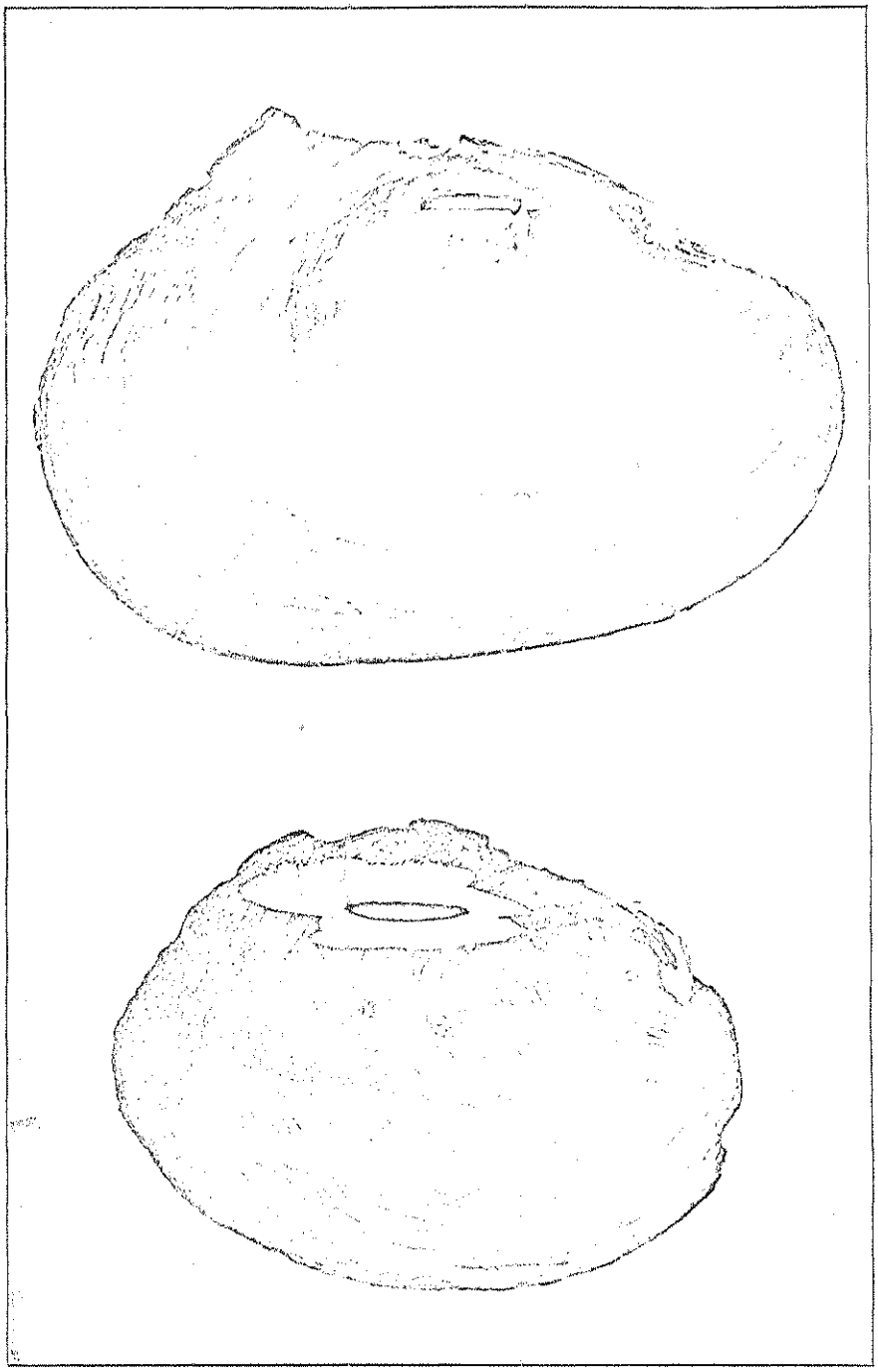

FIGURE 3.—Shells of *Proptera alata*, pink heelsplitter (upper), and *Amblema costata*, three-ridge (lower), with "windows" ground in the valves to expose pericardium and heart. Through such windows numerous blood samples could be drawn from a single individual, as animals so prepared often lived for a week or more

TABLE 2.—*Individual specific gravity and pH data*—Continued

Date, species, and location	Specific gravity	pH
Yellow sand-shell (Lampsilis anodontoides), canals from Rio Grande, Mercedes, Tex.:		
Apr. 4, 1929	1.0007	8.0
Apr. 22, 1929	1.0010	8.0
Apr. 16, 1929	1.0011	8.3
Mar. 10, 1930	1.0016	8.0
Mar. 26, 1930	1.0018	.....
Mar. 27, 1930	1.0020	7.9
May 6, 1929	1.0024	7.9
Mar. 25, 1930	1.0024	.....
Mar. 10, 1930	1.0024	8.0
Feb. 21, 1930	1.0024	.....
Feb. 24, 1930	1.0026	7.7
May 9, 1929	1.0026	8.0
Apr. 30, 1929	1.0035	7.8
Mar. 17, 1930	1.0036	7.8
Apr. 18, 1929	1.0049	7.6
May 2, 1929	1.0053	7.7
Apr. 8, 1929	1.0063	8.1
Apr. 26, 1929	.....	7.9
Apr. 16, 1929	.....	7.9
May 29, 1929	.....	8.2
Slough sand-shell (Lampsilis fallaciosa), Mississippi River, Fairport, Iowa:		
July 2, 1929	1.0007	7.8
July 31, 1929	1.0015	7.9
Aug. 10, 1929	1.0015	8.1
Aug. 19, 1929	1.0015	8.2
Aug. 12, 1929	1.0017	8.1
Aug. 19, 1929	1.0017	7.7
Aug. 2, 1929	1.0017	.....
Aug. 7, 1929	1.0018	7.8
Aug. 19, 1929	1.0019	8.0
Aug. 8, 1929	1.0019	7.8
Aug. 4, 1929	1.0019	8.1
Aug. 13, 1929	1.0020	8.1
Aug. 5, 1929	1.0021	7.7
Aug. 9, 1929	1.0021	8.1
Aug. 3, 1929	1.0022	7.7
Aug. 15, 1929	1.0022	8.1
Aug. 9, 1929	1.0022	8.0
Aug. 29, 1929	1.0023	7.9
Aug. 16, 1929	1.0024	8.1
Aug. 21, 1929	1.0025	8.1
Aug. 22, 1929	1.0025	8.1
Aug. 12, 1929	1.0026	.....
Aug. 1, 1929	1.0028	8.0
Aug. 14, 1929	1.0029	8.0
July 30, 1929	1.0032	7.8
Aug. 16, 1929	1.0036	8.2
Aug. 6, 1929	1.0037	7.9
Aug. 21, 1929	1.0038	7.8
Aug. 17, 1929	1.0055	8.2
Lake Pepin mucket (Lampsilis siliquoidea pepinensis), Lake Pepin, Minn.:		
Jan. 5, 1930	1.0004	7.9
Do	1.0006	7.9
Jan. 5, 1930	1.0008	7.9
Jan. 6, 1930	1.0009	7.9
Apr. 1, 1929	1.0009	7.7
Dec. 17, 1929	1.0012	8.0
Nov. 18, 1929	1.0012	7.7
June 4, 1929	1.0015	8.1
Nov. 20, 1929	1.0016	.....
May 20, 1929	1.0019	8.3
Apr. 15, 1929	1.0020	8.0
Jan. 6, 1930	1.0020	7.9
Nov. 30, 1929	1.0020	.....
Dec. 16, 1929	1.0022	8.0
Do	1.0023	7.7
Oct. 4, 1929	1.0025	.....
Oct. 9, 1929	1.0026	7.8
Jan. 3, 1930	1.0026	7.6
May 23, 1929	1.0026	8.5
Oct. 9, 1929	1.0026	7.8
Jan. 3, 1930	1.0027	7.6
May 20, 1929	1.0029	8.2
June 7, 1929	1.0029	7.7
Oct. 3, 1929	1.0035	7.6
Oct. 1, 1929	1.0036	7.9
Oct. 9, 1929	1.0038	7.9
Do	1.0041	7.9
Apr. 29, 1929	1.0052	8.0
May 20, 1929	1.0078	8.1
Pocketbook (Lampsilis ventricosa), Mississippi River, Fairport, Iowa:		
July 5, 1929	1.0029	8.2
River mucket (Actinonaias carinata), Fox River, Millington, Ill.:		
May 7, 1929	1.0003	8.1
June 10, 1929	1.0015	7.8
Nov. 18, 1929	1.0018	8.0
May 3, 1929	1.0020	8.0
Oct. 30, 1929	1.0020	7.7
Apr. 1, 1929	1.0021	8.5
Apr. 4, 1929	1.0021	7.8
May 20, 1929	1.0022	8.3
Oct. 4, 1929	1.0023	7.9
Oct. 30, 1929	1.0024	7.9
Apr. 11, 1929	1.0032	7.9
Mar. 26, 1929	1.0034	7.9
Do	1.0035	8.4
Oct. 28, 1929	1.0039	7.7
Mar. 28, 1929	1.0043	8.2
Apr. 2, 1929	.....	8.4
Apr. 9, 1929	.....	7.8

TABLE 3.—*Comparison of specific gravity of the blood of fresh-water mussels with that of other bloods*

[All determinations made by the falling drop method]

Species	Common name	Specific gravity average	Number of cases	Locality
Homo sapiens	Man	1.0550	.....	Columbia, Mo.
Columba livia var	Street pigeon	1.0598	12	Do.
Chrysemys belli	Bell's painted turtle	1.0338	7	North Judson, Ind.
Rana pipiens	Leopard frog	1.0271	3	Do.
Ictiobus cyprinella	Largemouth buffalo fish	1.0317	1	Fairport, Iowa.
Ictalurus punctatus	Spotted catfish	1.0260	6	Do.
Lepisosteus platostomus	Short-nosed gar	1.0469	6	Do.
Cambarus virilis	Crawfish	1.0185	10	Hahatonka, Mo.
Unionidæ, 19 species	Fresh-water mussels	1.0026	145	Mississippi and Rio Grande drainages.

The corpuscles of the blood are of course an important factor in these comparisons of blood specific gravity. The blood of the fresh-water mussel does not contain pigmented, oxygen-carrying corpuscles of the red blood corpuscle type found in large numbers in the blood of vertebrates, although there are small numbers of amœbocytes or white blood corpuscles in the blood of fresh-water mussels. As the red blood

corpuscles have an average specific gravity of 1.0880 (Krüger, 1925), it would seem that a fairer comparison for mussel blood would be one with the plasma or serum of vertebrates. The average specific gravity values for the serum or plasma of various vertebrates lie between 1.0170 and 1.0309 (Krüger, 1925), and the specific gravity of the whole blood of the Japanese oyster, *Ostrea circumpicta* Pillsbury, which like the blood of the fresh-water mussels contains no red blood corpuscles, is between 1.0230 and 1.0280 according to Yazaki (1929). It is evident, therefore, that the low specific gravity of the blood of the fresh-water mussels is not due entirely to the absence of red blood corpuscles, since the specific gravity of the blood of the oyster is essentially the same as that of the serum or plasma of vertebrate blood.

From the distribution of the individual species in Table 1, and from the experimental tests (v. i.) the average range or normal variation in the specific gravity of the blood of the fresh-water mussels studied seems to lie between 1.0010 and 1.0050, a variation of 0.0040 and a deviation of −0.0016 to +0.0024 from the average of 1.0026. Stebbins and Leake (1927), using the Barbour method, report the diurnal variation in the specific gravity of the blood of dogs as 0.0044, of men as 0.0033, and of women as 0.0027. The actual variation in the specific gravity of the blood of fresh-water mussels is therefore of much the same magnitude as that of dogs and man, but the proportional change resulting from this variation is very much greater in the case of the mussel blood with an average specific gravity of 1.0026 than in human blood with an average specific gravity near 1.0554.

Although fewer specimens of *Unioninæ* than of *Lampsilinæ*, are included in Table 1, the grouping of the individual cases suggest that the *Unioninæ* have blood of a slightly higher specific gravity than the *Lampsilinæ*. Averaging the specific gravities from these two groups in Table 1 separately, the average specific gravity of the blood for all species of *Unioninæ* studied falls between 1.0030 and 1.0040 and that for all *Lampsilinæ* between 1.0020 and 1.0030. Differences found in experimental tests also show this same division of species on the basis of the specific gravity of the blood.

TOTAL SOLIDS AND ASH

The per cents of total solids and of ash contained in the blood were determined for four species of North American fresh-water mussels, the pink heelsplitter, *Proptera alata* (Say); the slop bucket, *Anodonta corpulenta* Cooper; the Lake Pepin mucket, *Lampsilis siliquoidea pepinensis* Baker; and the heelsplitter, *Lasmigona compressa* (Lea). For each species, the blood from several individuals was collected into a weighed pyrex beaker until a sample of 100 cubic centimeters or more was obtained. The beakers were then reweighed, and the blood slowly evaporated to dryness at temperatures below 60° C. The solid residues in the beakers were desiccated at 90°–105° C. in an electric drying oven, cooled over sulphuric acid, and weighed. Each residue was divided into convenient samples (0.3 to 0.5 gram), which were ignited in a platinum dish at a dull red heat and the ash brought to a constant weight. The per cent of total solids and of ash in the blood are given in Table 4.

TABLE 4.—*Per cent of total solids and ash in the blood of four species of North American fresh-water mussels, together with total solids and ash in the blood of other animals appended for comparisons*

Scientific name	Common name	Fluid used	Per cent of total solids	Per cent of ash	Ratio of total solids to ash	Locality or authority
Anodonta corpulenta	Slop bucket	Whole blood	0.3436	0.1256	100 : 37	Fairport, Iowa.[1]
Lasmigona compressa	Heel splitter	do	.4065	.1505	100 : 30	Do.
Proptera alata	Pink heel splitter	do	.4500	.1573	100 : 35	Do.
Lampsilis siliquoidea pepinensis	Lake Pepin mucket	do	.4440	.1820	100 : 44	Lynchville, Wis.[2]
Averages			.4260	.1539	100 : 36	
Anodonta cygnea	European fresh-water mussel.	do	.8540	.2600	100 : 30	Schmidt, 1845.
Anodonta and Unio	do	do	.3110	.1890	100 : 61	Voit, 1860.
Octopus macrops	Octopus	do	12.0300	2.9760	100 : 25	Bottazzi, 1911.
Saxidomus nuttali	Marine rock clam	do	4.3300	2.8[illegible]3	100 : 70	Myers, 1920.
Schizothaerus nuttali	Washington clam	do	4.2080	3.2560	100 : 80	Do.
Pecten sp	Scallop	do	1.7300	1.0100	100 : 58	Griffiths, 1892
Solen sp	Razor clam	do	1.7300	.9860	100 : 57	Do.
Mya sp	Soft-shell clam	do	1.6400	.9840	100 : 60	Do.
Helix pomatia	Edible snail	do	3.9000	.3000	100 : 8	Couvreur, 1900.
Astacus fluviatilis	European crayfish	do	4.8600	1.1360	100 : 23	Halliburton, 1885.
Cyprinus carpio	German carp	do	13.4300			Krüger, 1925.
Do	do	Serum	5.2000			Do.
Equus caballus	Horse	Whole blood	25.0080	1.0180	100 : 5	Abderhalden, 1911.
Do	do	Serum	9.7050	0.8890	100 : 90	Do.

[1] July 6, 1929. [2] Oct. 10, 1929.

The total solids found in these samples of fresh-water mussel blood varied from 0.344 to 0.497 per cent, around an average of 0.426 per cent of the weight of the blood. These values lie between the two determinations given for European fresh-water mussels—that of Schmidt (1845) being 0.845 per cent for *Anodonta cygnea* and that of Voit (1860) 0.311 per cent for both *Anodonta sp.* and *Unio sp.*—and if compared with the total solids found in the blood of various other animals, both vertebrate and invertebrate (see Table 4), show the fresh-water mussels to have a very dilute blood. In addition to the animals listed in Table 4, the writers in reviewing the existing literature on blood solids have checked some 60 species of animals in all without finding any one having as low total blood solids as the fresh-water mussels (for general lists see Winterstein, 1925; and Fürth, 1903). Relatively high total solids are the rule in vertebrate blood because of the large numbers of red blood corpuscles, not found in fresh-water mussel blood; but even the sera of vertebrate blood and the whole blood of invertebrates contain much larger amounts of solids than fresh-water mussel blood.

As the total solids contained in any blood determine the osmotic pressure of the fluid of the blood, and are of large importance therefore in regulating the water and salt balance in the living tissues of the animal, the very low total solids of fresh-water mussel blood suggest a rather close adjustment between the living tissue of the fresh-water mussel and the fluid environment in which these animals live. Such an adjustment, accomplished through the blood, was established (v. i.) in the experimental tests in fresh-water mussels.

Considering the low total solids of the fresh-water mussel blood in connection with their habitat of fresh-water—that is, a medium with a relatively low osmotic pressure—the low total solids of the soft-shell clam, *Mya sp.*, are of interest, both because the fresh-water mussels are regarded as having evolved from shore-dwelling marine forms and because *Mya* is an inhabitant of gravelly mud flats at the mouths of rivers (Rogers, 1913), where the salinity of the water would be subject to some modification by the outbound fresh-water.

The average values for the ash of the blood—that is, the inorganic constituents—of the four species of fresh-water mussels examined, were 0.1539 per cent of the weight of the blood, or a little more than one-third of the weight of the total solids. Because of the low values of the total solids the average per cent of ash in the blood of fresh-water mussels is of course much below the ash content of the blood of the vertebrates or of most invertebrates.

The ratio of ash to total solids in the blood, which is an expression of the relative amount of organic substances in the blood, varies widely in the several groups of animals. (See Table 4.) As the total solids of the fresh-water mussel blood are much lower than those of other bloods, the actual per cent of organic solids in the blood of the fresh-water mussels is of necessity also much lower than that of other bloods. Considering the relative amount of organic constituents, however, the fresh-water mussel blood falls about midway between vertebrate serum or plasma on the one hand and vertebrate whole blood on the other; that is, the fresh-water mussels are near the middle of the invertebrate group when this organic-inorganic solids ratio is considered.

The low total solids and the moderately high ratio of ash to total solids in the blood of the fresh-water mussels show this blood to be a very watery fluid, in which the low specific gravity indicates a small quantity of solids in solution or suspension rather than a mixture containing salts, in amounts comparable to those found in the blood of other animals, together with sufficient other substances lighter than water to produce the low specific gravity as found.

BLOOD SUGAR

Blood sugar, the only organic constituent of the blood considered separately, was determined by the Hagedorn-Jensen (1923) iodine titration method for 10 species of fresh-water mussels. (See Table 5.) The blood sugar averaged 31 milligrams per 100 cubic centimeters of blood, in these species, ranging from 7 to 93 milligrams per 100 cubic centimeters of blood. The average value and the range of variation are much the same as those for many other invertebrates, both marine and fresh water, in spite of the fact that the total solids and the inorganic salts are much lower in fresh-water mussel blood than in other invertebrates. Considering these 10 species of fresh-water mussels separately the variation between species was not considered significant as it was no greater than that between individuals of the same species in several cases. This wide variation in blood-sugar levels between individuals of the same species is perhaps the most striking feature in Table 5, but it is not without its parallel in other species of mollusks. Lang and Macleod (1920) report the blood sugar of the marine clam *Schizothaerus nuttali* as from 1.5 to 1.8 milligrams per 100 cubic centimeters, and Myers (1920) found 74 milligrams of blood sugar per 100 cubic centimeters in the same species; and according to the observations of Couvreur and Bellion (1907, 1908) and Sellier (1907, 1908), the amount and the type of sugar in the blood of the snail *Helix pomatia*, varies with the state of activity of the animal. It seems, therefore, that the blood sugar values of the fresh-water mussels do not differ materially either in average or in range from those of other mollusca, even though the blood of the fresh-water mussels is very dilute.

TABLE 5.—*Average blood sugar values for 10 species of fresh-water mussels*

Scientific name	Common name	Number of specimens	Number of determinations	Blood sugar in milligrams per 100 cubic centimeters of blood		
				Minimum	Average	Maximum
Tritogonia verrucosa	Buckhorn	2	6	34	58	74
Quadrula pustulosa	Pimple back	1	3	20	28	34
Quadrula quadrula	Maple leaf	1	3	20	23	25
Elliptio dilatatus	Lady finger	1	3	34	37	43
Anodonta limnæana	Southern floater	6	14	7	16	61
Strophitus rugosus	Squaw foot	1	3	8	16	20
Obliquaria reflexa	Three-horned warty-back	1	3	20	28	43
Proptera alata	Pink heel splitter	2	6	34	47	65
Lampsilis anodontoides	Yellow sand-shell	6	18	8	48	93
Lampsilis siliquoidea pepinensis	Lake Pepin mucket	1	3	10	17	22
Total		22	62		32	

INORGANIC SALTS

Since the pioneer work of Ringer (1882) on the inorganic salts of the blood, it has become well established that the chief inorganic salt of the blood of all animals is sodium chloride, and that in physiological balance with this salt are much smaller quantities of potassium and calcium salts, usually the chlorides. As qualitative tests on the blood of the species of North American mussels under consideration showed the presence of sodium, potassium, calcium, and magnesium, determinations of blood sodium by the pyroantimoniate method of Kramer and Tisdale (1921) and of the blood calcium by the oxalate method of Clark (1921) were made, the potassium-magnesium fraction being computed by difference. The data from these determinations are listed in Table 6.

The range between the maximum and minimum for both the sodium chloride and calcium chloride in the mussel blood was large when compared with the variation in these salts tolerated in dog blood or human blood, and it was only through the experimental tests (v. i.) that a satisfactory explanation of this variation in mussel blood was obtained. Without going into the experimental data here, it may be stated that it was found that the salt content of the blood of fresh-water mussels could be modified by the activity of the animal and by the environment in which the animal was held. The values in Table 6 represent, for the most part, blood from mussels just removed from the water; and as it was noted that a considerable concentration of the blood could be effected by the animal when the mussel was merely kept in the air for a time, the average values in Table 6 are lower than might be expected from the maximum values given there.

TABLE 6.—*Per cent of sodium, calcium, and other salts in the blood of fresh-water mussels*

Scientific name	Common name	Sodium as sodium chloride, per cent of whole blood			Calcium as calcium chloride, per cent of whole blood			Potassium, magnesium and other salts by difference, average	Total ash, averag
		Minimum	Average	Maximum	Minimum	Average	Maximum		
Anodonta corpulenta	Slop bucket	0.0310	0.1013	0.2210	0.0087	0.0187	0.0825	0.0056	0.1256
Lasmigona compressa	Heel splitter	.0505	.1092	.2289	.0103	.0404	.0859	.0089	.1496
Proptera alata	Pink heel splitter	.0469	.1144	.2778	.0130	.0225	.1060	.0204	.1573
Lampsilis siliquoidea pepinensis	Lake Pepin mucket	.0064	.1125	.2950					.1820
Average			.1093			.0272		.0090	.1539

NOTE.—Figures in this table are from 60 determinations.

The actual values of sodium chloride in the mussel blood are very low in comparison with the sodium chloride content of the blood of most animals, averaging between 0.1 and 0.2 per cent in the fresh-water mussels as against nearly 0.9 per cent in the blood of man and the mammals. The sodium chloride values for the blood of these North American species of mussels are comparable, however, with the salt values computed for the blood of European mussels. Philippson, Hannevart, and Thieren (1910) computed the sodium chloride content of the blood of *Anodonta cygnea* on the basis of the electroconductivity, and found it to represent about 0.2 per cent sodium chloride; and Fredericq (1899) and Monti (1914) give values for the depression of the freezing point of the blood of both *Unio* and *Anodonta* which would approximate 0.2 to 0.3 per cent sodium chloride.

The average calcium values in the fresh-water mussel blood are about the same as those for mammalian blood, in spite of the low salt content of the mussel blood; that is, the ratio of calcium to sodium is much higher in mussel blood than in mammalian blood. The maximum calcium per cent in mussel blood greatly exceed the calcium content of mammalian blood. This is not surprising, however, when it is considered that the calcium needs of the mussel are large in connection with the building and maintenance of a calcareous shell; and Collip (1920) has pointed out that, in the case of the marine clam *Mya arenaria*, the calcium of the shell can be used as a buffer to maintain the proper level of the alkalinity in the blood and body tissues in the face of various metabolic disturbances.

By difference the potassium-magnesium fraction was low. This has been confirmed by experimental studies in which it was found that the tissues of the fresh-water mussel are quite sensitive to slight changes in the potassium content of the medium surrounding them.

HYDROGEN-ION CONCENTRATION AND BUFFER VALUES

The pH of the blood of 20 species of fresh-water mussels was determined colorimetrically by the Gillaspie (1926) method, brom-thymol blue and cresol red being the dyes most frequently employed. Blood for this purpose was taken immediately after opening the animal to avoid changes due to loss of carbon dioxide on exposure to air. No determinations were made on less than 1 cubic centimeter of blood, and the turbidity factor was checked by carrying a control tube of blood behind the standard dye tubes in the comparometer block.

Readings, taken in all months of the year and from both sexes of the various species studied (see Table 7), show the blood of the fresh-water mussels to be definitely alkaline in reaction and with a more alkaline pH value than that of the blood of the higher animals. The average of 142 specimens of fresh-water mussels was pH 7.9, the individual readings ranging from pH 7.4 to pH 8.5, with the range pH 7.6 to pH 8.3 including 94 per cent of the cases. It is difficult to compare the pH of the mussel blood with that of man because of several factors, but VanSlyke (1921) lists the average pH value for man as 7.4, with a range of pH 7 to pH 7.8 as the limits compatible with life. The figures are subject to certain limitations, but greater alkalinity of the mussel blood is evident. (See Table 7 and fig. 4.)

TABLE 7.—*Hydrogen-ion concentration of the blood of fresh-water mussels*

Scientific name	Common name	Number of individuals	pH values								
			7.4	7.5–7.6	7.7–7.8	7.9–8.0	8.1–8.2	8.3–8.4	8.5–8.6	Minimum	Average	Maximum
Subfamily *Unioninæ*:												
Fusconaia ebena	Niggerhead	3		2	1					7.5	7.6	7.7
Fusconaia undata	Pig toe	6		1	5					7.6	7.7	7.8
Tritogonia verrucosa	Buckhorn	6		1	2	2	1			7.5	7.8	8.1
Amblema costata	Three-ridge	6	1	2	2	1				7.4	7.6	7.9
Quadrula pustulosa	Pimple back	1		1							7.6	
Quadrula metanevra	Monkey face	1			1						7.7	
Unio popei	Popo's purple	4			1		2	1		7.7	8.1	8.3
Subfamily Anodontinæ:												
Anodonta limnæana	Southern floater	3			1	2				7.8	7.9	8.0
Strophitus rugosus	Squaw foot	1						1			8.3	
Lasmigona compressa	Heel splitter	1				1					7.9	
Subfamily Lampsilinæ:												
Obliquaria reflexa	Three-horned warty-back.	6			2	4				7.8	7.9	8.0
Proptera alata	Pink heel splitter	12			2	10				7.7	7.9	8.0
Proptera laevissima	Paper shell	1			1						7.7	
Plagiola lineolata	Butterfly	2				2				7.9	7.9	7.9
Ligumia recta latissima	Black sand shell	1			1						7.7	
Lampsilis anodontoides	Yellow sand-shell	17		1	4	9	2	1		7.6	7.9	8.3
Lampsilis fallaciosa	Slough sand-shell	27			8	7	12			7.7	8.0	8.2
Lampsilis siliquoidea pepinensis	Lake Pepin mucket	26		3	6	12	3	1	1	7.6	7.9	8.3
Lampsilis ventricosa	Pocketbook	1					1				8.2	
Actinonaias carinata	River mucket	17			5	6	2	3	1	7.7	8.0	8.3
Total		142	1	11	42	56	23	7	2	7.4	[1] 7.9	8.3

[1] Average of all individuals.

The water from which the animals used in making the determinations listed in Table 7 were taken varied in pH value from 7.4 to 7.9, with an occasional value of

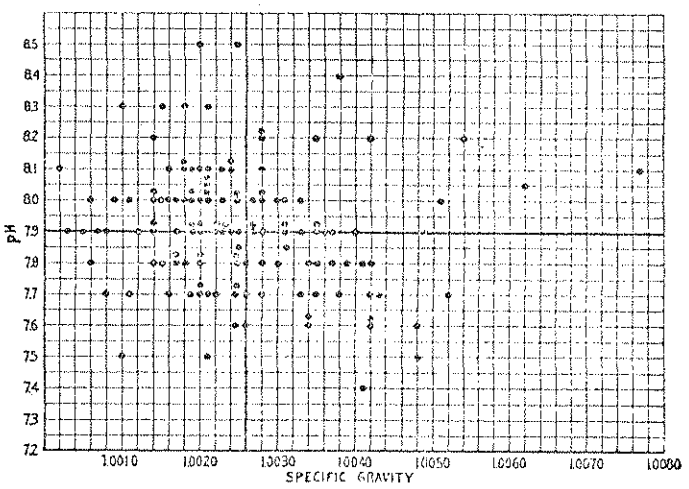

FIGURE 4.—Specific gravity plotted against pH, for normal blood of fresh-water mussels, showing trend away from alkalinity toward neutrality with an increase in specific gravity. This trend was also evident in blood of the mussels in the series exposed to air (v. i.). Average specific gravity (1.0026) and average pH (pH 7.9) are indicated by heavy lines

pH 8. The average pH value of the water in the Mississippi River at Fairport, Iowa, just above an apparently healthy bed of mussels was pH 7.65, while that of the tank water in which mussels at Columbia, Mo., were kept varied throughout the year from pH 7.5 to pH 7.8. When compared with the environment the pH of the mussel blood was consistently a little more alkaline than the surrounding water, if the animals were kept well aerated and in average normal condition.

This observation is of interest in connection with the fact that the calcium content of the mussel blood is high and that the mussel blood is a medium which must transport relatively large quantities of calcium in connection with the production of the shell.

Collip (1920) has stated that in the marine clam, *Mya arenaria*, the calcium carbonate of the shell is available for the animal as an almost unlimited source of buffer material, so that the carbon dioxide produced during the activity of the animal could unite with the calcium carbonate of the shell, forming a bicarbonate which is freely soluble and alkaline in reaction. In the event that the carbon dioxide could not be removed promptly from the body of the animal, as during periods when the animal is removed from the water or while it has its shell closed tightly even though still in the water, the carbon dioxide produced could be buffered down and the alkaline value of the blood maintained. This explains the absence of acid values for the pH of the blood of fresh-water mussels which were held in air in a closed condition for several hours (v. i.). There is evidence (v. i.) that the closed mussels continue to use the oxygen in the water contained in the shell when tightly closed, and that this buffering out of the carbon dioxide formed during the absence of fresh circulating water from the outside by the calcium carbonate of the shell, makes possible the utilization of the oxygen contained. (See salt experiments.)

Another check on this point of the buffering value of the shell in the closed animals was made by titration of the buffer value of the blood, in terms of N/44 hydrochloric acid, for mussels just removed from the water and in which the blood was presumably well aerated. These values given in Table 8 show that the buffer value of the blood is quite low, and as there are only small amounts of proteins and other organic buffers in the blood of the fresh-water mussels this buffer value must be due very largely to the inorganic carbonates present. It has been noted previously in this discussion that the salt-ash content of the blood of the fresh-water mussels just removed from the water was lower than that of those animals which had been held out of water for some time. Part of this rise in salt content is due to a concentration of the blood—that is, a water loss; but part of it may also be due to the addition of calcium carbonate to the blood, withdrawn from the shell to buffer down the carbon dioxide formed. Collip (loc. cit.) found an increase in the calcium content of his marine clams under such conditions.

TABLE 8.—*Buffer values of the blood of fresh-water mussels*

[Cubic centimeters of N/44 hydrochloric acid required to titrate 5 cubic centimeters of blood]

Scientific name	Common name	N/44 hydrochloric acid cubic centimeters	Scientific name	Common name	N/44 hydrochloric acid cubic centimeters
Lampsilis anodontoides	Yellow sand-shell	1.05	Actinonais carinata	River mucket	1.15
Do	do	1.05	Do	do	1.20
			Do	do	1.35
Average		1.05	Do	do	1.35
Actinonais carinata	River mucket	1.03	Average		1.18
Do	do	1.05			

BLOOD GASES

As the carbon dioxide of the blood seems so definitely tied up with the salt content of the blood, particularly the calcium content, analyses for blood gases were made with the Van Slyke apparatus (1917) immediately after the mussels were taken from the water. Determinations for oxygen, carbon dioxide, and nitrogen were made,

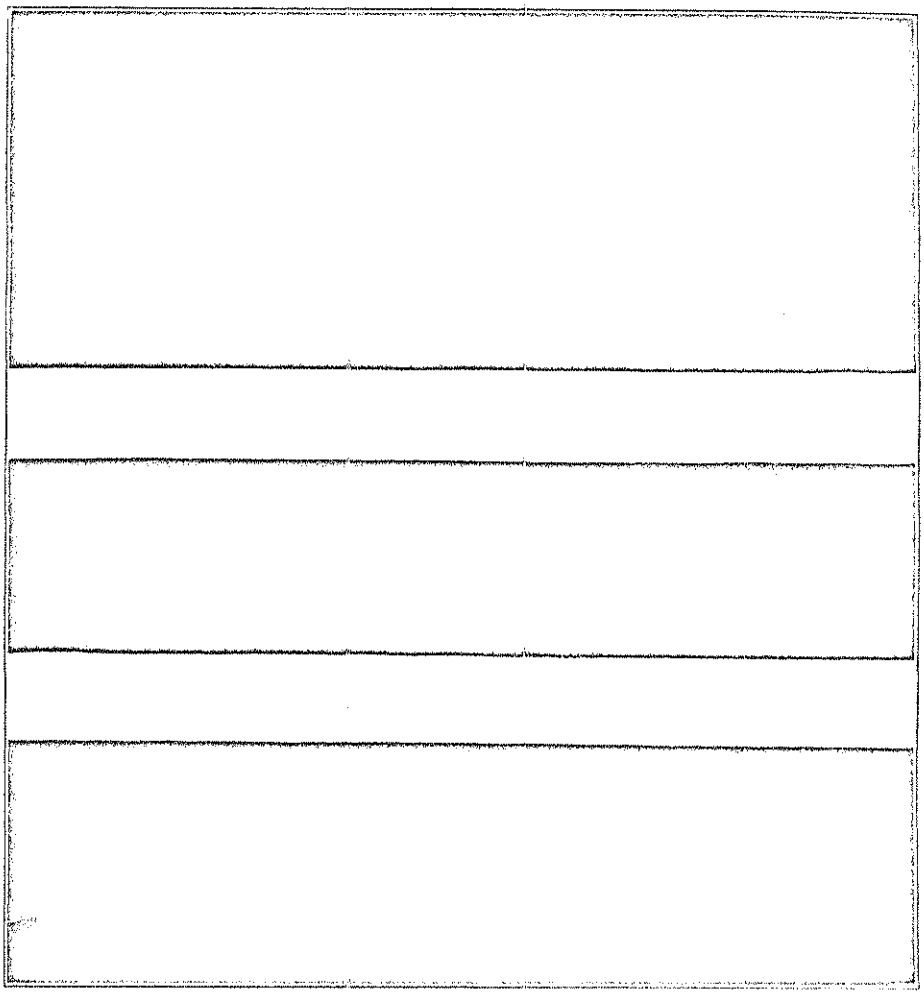

FIGURE 5.—Tracings of "foot strips" from fresh-water mussels showing the two types of contraction waves which pass over the foot continuously. Upper record from foot of *Tropiera alata*, the pink heelsplitter; middle record from foot of *Unio popeii*, Pope's purple; lower record from foot of *Lampsilis anodontoides*, the yellow sand-shell. Time at bottom of record; single strokes, five seconds each; double strokes, minutes

and the results are tabulated in Table 9. As might be expected of a blood without pigmented, oxygen-carrying corpuscles, and with a very low total solids content, the volume of blood gas was small. When compared with the blood of marine bivalves (Cuenot, 1901; Winterstein, 1909), the oxygen and nitrogen values of the blood of the fresh-water mussels and some of the marine clams are found to be much the same, but the carbon dioxide content of the blood of the fresh-water mussel is much lower than that of the marine clams cited above or of the Japanese oyster, as determined by Kokubo (1929). This fact again points to the adjustment of the buffer value and the carbon dioxide tension in the blood of the fresh-water mussels, for the blood gas determinations listed here for fresh-water mussels were made as soon as possible after the animals were removed from the water.

TABLE 9.—*Gas content of fresh-water mussel blood*

Scientific name	Common name	Total gas	Gases in 100 cc. of blood; that is, volumes per cent			
			O_2 plus CO_2	O_2	CO_2	N_2 and other gases
Tritogonia verrucosa	Buckhorn	2.33	1.08	0.71	0.36	1.25
Amblema costata	Three-ridge	2.36	.56			1.80
Do	do	1.86	.78			1.08
Do	do	2.27	.98	.33	.65	1.29
Do	do	2.09	.89	.32	.57	1.21
Do	do	2.23	.72	.36	.36	1.51
Andonta limneana	Southern floater	2.41	1.07	.89	.18	1.34
Do	do	2.16	.72			1.44
Lampsilis anodontoides	Yellow sand-shell	2.61	.99			1.62
Do	do	2.06	.86	.52	.34	1.20
Do	do	1.72	.52	.43	.09	1.20
Do	do	2.26	.89	.24	.65	1.37
Do	do	2.12	.97	.16	.81	1.15
Do	do	1.87	.72	.27	.45	1.15
Do	do	1.95	.80	.27	.53	1.15
Do	do	2.40	1.00			1.40
Lampsilis siliquoidea pepinensis	Lake Pepin mucket	1.91	.32	.18	.14	1.59
Actinonais carinata	River mucket					
Average		2.15	.82	.39	.43	1.34

VERIFICATION OF BLOOD-SALT VALUES BY THE FOOT-STRIP METHOD

It was observed early in the work that the fresh-water mussel maintains a rather constant and rhythmical motion of the free margin of the muscular foot. When the foot of the mussel is well filled with blood and extruded between the valves of the shell, these movements of the foot are of such magnitude that they are easily visible to the naked eye as undulating waves of contraction pass up and down the foot margin. By attaching a tiny steel hook to the margin of the foot and connecting the foot to a recording lever, by means of this hook and an attached thread a graphic record of these movements of the margin of the foot of the mussel was easily obtained. Placing a small piece of cork between the valves of the mussel when open, and allowing the animal to retract its foot with the tiny hook attached, the valves in closing on the bit of cork were held open far enough to permit the silk thread connecting the hook with the recording lever to move freely and operate the lever. In this way it was possible to study the movements of the foot margin when completely retracted within the shell as well as when expanded. The hook used was so small and light in weight that the animal apparently suffered no inconvenience from the presence of the hook, as mussels were kept under observation for days with the hook in place.

To prevent the mussel from moving away from the connected apparatus, a block of beeswax was first attached to a glass rod and then to one valve of the mussel by

melting the surface of the wax and pressing the shell, which had previously been wiped dry with a cloth, into the beeswax just before it hardened. In this way it was possible to suspend the mussel in the water at any desired depth or angle and hold the animal stationary; but at the same time the movements of the foot, gills, and other soft parts were not interfered with in the least, as the mussel could open and close its valves at will. (It may be noted here that this method has proved very satisfactory for several types of experiments and individual mussels have been observed continuously for over three months while attached to beeswax blocks as described.)

From the experiments on the activity of the foot-margin (to be reported in detail elsewhere) it was found that the margin of the foot is kept in constant motion regardless of the position of the foot and whether it be expanded or retracted, and that two rhythms are maintained—that is, there are large contraction waves on which smaller or faster contraction waves are superimposed. These movements of the foot serve not only as part of the locomotion complex, but they produce currents in the water in the vicinity of the foot and in that way aid both respiration and the taking of food.

As these movements continue so regularly and are associated with vital activities of the mussel, they were used to verify the salt concentrations of the blood as determined by the analyses.

It is well established that strips of muscular organs may be removed from the bodies of various animals, and, when mounted properly, these pieces of organs will continue to display normal activity for many hours. As the muscular foot of the fresh-water mussel forms the outer portion of the visceral mass of the animal (see fig. 7) almost the entire foot may be removed by cutting along the line of junction of the foot and viscera. In this way a "foot strip" could be prepared free from all other organs and including almost all of the foot muscle.

Such strips of living tissue from other animals are customarily mounted in the blood serum of the animal from which they were taken or in some fluid containing the principal salts of the blood in the proper proportions, so that strips which have been separated from their connections with the circulatory system may obtain from the fluid in which they are immersed the essential salts or other substances which they would have received from the blood.

In order to test the validity of the normal values which were obtained from the various analyses of mussel blood, such a fluid was compounded containing the principal salts found in mussel blood in the proportions determined by these analyses, and the whole adjusted to the average pH value for mussel blood. This fluid because of its similarity to the "Ringer's fluid" commonly used for studies of vertebrate tissues, was designated "unionid ringers," and the formula is given in the following table:

TABLE 10.—*Composition of unionid ringers fluid in which mussel tissues maintain normal activity*

	Per cent
Sodium chloride	0. 153
Calcium chloride	. 012
Potassium chloride	. 015
Magnesium chloride	. 010
Di-basic sodium phosphate	. 009
Sodium bicarbonate, to adjust the pH value to pH 7.9.	

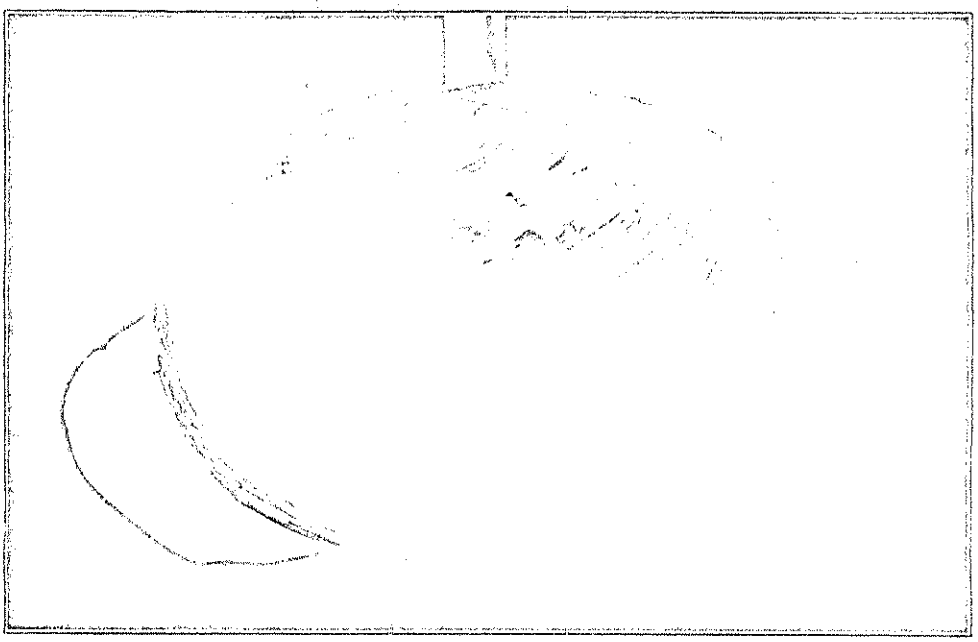

FIGURE 6.—*Actinonais carinata*, river mucket, natural size. Living animal with foot partly extended

FIGURE 7.—Living animal (same as Fig. 6) a few minutes later, with one valve of shell removed to show volume of foot. The heart may be seen lying along inside of shell just below hinge. The line of demarcation between foot and visceral mass may be followed as a shallow groove separating the rather rugose foot from the smooth visceral mass. This groove was followed when cutting off the foot strips

FIGURE 8.—*Actinonaias carinata*, river mucket, natural size. Living animal, with one valve of the shell removed to show foot in a completely retracted condition. Compare volume of foot in this with the expanded foot in preceding figures. The line of demarcation between foot and visceral mass is clearly shown

When foot strips were mounted in glass containers through which well aerated unionid ringers flowed continuously, they maintained the typical rhythmical contractions characteristic of the foot muscle when in place in the body of the mussel. These contractions continued for hours and gave ample opportunity to observe the action of the various blood salts of the mussel blood. (See figs. 5, 6, 7, and 8.)

A summary of these tests will suffice here. The rhythmic activity of the foot strip stopped very quickly when the strip was transferred to distilled water, to tap water, or even river water; that is, the small quantities of salts present in the mussel blood are essential to the activity of the foot tissue. Activity of the foot strips also failed rapidly and finally ceased if the strips were placed in a solution of sodium chloride of the same strength as the sodium chloride in the blood or the unionid ringers, but without the small quantities of potassium, calcium, and magnesium salts found in the mussel blood. It was evident that these other salts, even though present in very small amounts, exercise a regulatory action over the activities of the foot muscle and that sodium chloride alone will not maintain life activities in the mussel. This is similar to the results obtained in experimental studies of other animals. Potassium, calcium, and magnesium salts alone, and in concentrations found the mussel blood, also failed to maintain foot-strip activity. If the balance between calcium and potassium were disturbed—that is, if more or less potassium or calcium were used in proportion to the opposing salt—the activity of the foot strip was quickly disturbed. Excess or unbalanced potassium caused cessation of activity, the strip passing into a condition of rigor; and excess or unbalanced calcium causing cessation of activity accompanied by great loss of tone and relaxation. The limits between which the potassium content of the fluid could be varied were much narrower than those through which calcium variation was tolerated. This is in accord both with the analyses, and with the findings connected with the use of calcium as a buffer by the mussel.

Taken collectively, these tests with the foot strips show that the mussel is dependent upon the salts in the blood for the same types of activity regulation as those found in the higher animals and that although these salts are in very low concentrations in the mussel blood, these concentrations and the balances between the several salts can not be greatly changed without the cessation of activity and other serious consequences. These activity experiments, therefore, validate the analyses of the blood by giving physiological proof that salts are required in the concentrations and proportions determined. As a supplementary check to these tests, the entire heart of the mussel was carefully removed in several cases and mounted in unionid ringers, in which fluid it continued to beat regularly for several hours. Both heart and foot strips were very sensitive to oxygen want and activity ceased almost immediately, no matter what the concentration of the surrounding fluid, if the oxygen supply were shut off.

CHANGES IN BLOOD OF LIVING MUSSELS INDUCED BY ENVIRONMENTAL FACTORS

It was noted repeatedly while collecting the data for the normal values of the mussel blood that the physical factors of the environment influenced to some extent the values obtained. It was necessary, therefore, to select as normals only those animals which had just been removed from the water and which seemed to be in good condition. Nevertheless, even with these precautions, the constituents and char-

acteristics of the mussel blood varied over wider ranges than those for dog or human blood. The fresh-water mussel, however, in a physical sense at least, is in more intimate contact with its environment than many other animals, and the opportunities for the modification of the blood of the mussel are perhaps correspondingly greater.

When the shell of the mussel is open and the foot extruded, the water in which the animal is living has free access to a relatively large surface of soft tissue, and even if the foot be retracted and the shell closed a considerable volume of this same water is retained between the valves within the shell, where this water still bathes the soft parts of the animal. In addition, the mussel pumps through its gill system many times its own volume of water in the course of an active day, and although there is some opportunity to reject suspended objects of unsuitable size or quality at the siphon, because of the innervations of the siphon margins, substances in solution and fine material in suspension have full contact with the large surface of the soft parts and with the delicate structures of the gill system, as long as the animal pumps the water required for respiration and from which it takes its food. The mussel may avoid polluted water temporarily by closing its shell, and preliminary experiments completed by the writers show that these fresh-water mussels can remain closed, at 25° C., for 48 hours or more at a time, if the water included in the shell at the time of closure were well aerated. However, during the period that the mussel is closed it can not move, and therefore it is not able to leave the region of polluted water. Even if the polluted water can be tolerated for a time by the open mussel, the locomotion of the fresh-water mussel is so laborious and slow that the mussel has a much smaller chance of escape than a fish which can swim rapidly to other water. The fresh-water mussel therefore both because of the large amount of soft tissue in contact with the water, and because of its limited locomotion, is particularly exposed to the action of substances in the water.

From the normals obtained for the blood of the fresh-water mussel, the adaptation of these animals to the low osmotic pressure of the fresh-water in which they live is evident by comparison with the blood values of the marine clams—the nearest related forms. In the marine bivalves, the salt balance and adjustments of the blood are in accord with the salt content and the osmotic pressure of the sea water, but when these marine animals are placed in water of higher or lower salt content than that of the sea water in which they normally live (see Kokubo, 1929, on the Japanese oyster), changes in the blood follow shortly, and these changes tend to move the pH, specific gravity, and salt balance toward the level of the new environment, thus tending to equalize the osmotic balance between the animal and its environment.

In view of these observations on marine forms and the known activities of the fresh-water mussels, correlations have been made between the environment, both natural and modified, and the condition of the blood of the fresh-water mussels.

EFFECTS OF CHANGES IN SALT CONTENT OF WATER

To test the effect of changes in the salt content of the water in which the fresh-water mussels were living, both as regards the changes in osmotic pressure and the specific action of the salts themselves, on the blood of the mussels, these animals were placed in glass jars of about 8 liters capacity, containing solutions of various inorganic salts. Fresh-water mussels usually close for a considerable time after being disturbed, particularly if transferred to a new environment, and when they open again the

siphons are protruded cautiously so that little of the water in the new location is taken inside of the shell for some time. In order, therefore, that the test solution might have access to the soft parts of the animal at once, the mussels were allowed to close on small bits of cork (as described in the section on foot strips) before the transfer to the test solution. Unless otherwise specified, the valves of all mussels in the following series dealing with the effects of salts were propped open slightly with small pieces of cork.

The solutions used were made up in water from the same source as that supplying the water in which the animals were living, so that they would be subject to no change in salt balance, excepting that change produced by the substance added. The analysis of the water with which the solutions were prepared is given in Table 11.

TABLE 11.—*Analysis*[1] *of the water in which mussels live at Columbia, Mo.*

	Per cent
Calcium oxide	0. 00832
Magnesium oxide	. 00472
Ferrous oxide	. 00022
Aluminum oxide	. 00001
Silicon dioxide	. 00170
Sulphur (computed as SO_3)	. 00552
Chlorine	. 00194

Each solution jar was constantly aerated by a stream of air bubbles from a compressed-air line, the air passing through water before entering the test solution. The mussels were changed to fresh solution every 24 hours, unless otherwise noted, to avoid the complications resulting from the accumulation of waste products in the solutions. Specific gravity and pH determinations were made regularly on the test solutions themselves as well as on the blood of the mussels in the solutions, to check against unexpected changes in the medium. As the test solutions, with the exception of the distilled-water series, did not show any significant change in either pH or specific gravity values, these determinations for the test solutions have not been included in the tables. Oxygen determinations by the standard Winkler method were also made on the various test solutions to ascertain if the aeration were adequate, and as the oxygen values were all satisfactory they have not been listed.

DISTILLED WATER

For comparison with the responses to the various solutions of salts, several series of mussels were carried in distilled water, but otherwise under the same conditions as the animals in the salt-solution series. The data for these distilled-water tests are given in Table 12 and Figure 9.

Various species of mussels were able to live in aerated distilled water for several days, but all showed a decline in sensitivity, and moribund individuals appeared during the first 24 hours. (Throughout the discussion of these experimental results "moribund" designates a mussel in which the heart had stopped beating when the shell was opened, but the animal was still responsive to tactile stimulation of the mantle margin or the foot.)

It may readily be seen in Figure 9 that the specific gravity of the blood of all mussels living in distilled water was low, as nearly two-thirds of the cases lie below the line of average specific gravity (1.0026) for the blood of fresh-water mussels. This change in specific gravity came quickly, for at the end of the first 48 hours all

[1] Figures from analyses made by department of physical chemistry, University of Missouri.

values were below the average normal. After 96 hours, although still below the average normal specific gravity for mussel blood, the specific gravity of the blood of these individuals in distilled water tended to rise, judging from the few observations beyond 96 hours. This rise in specific gravity is suggestive in connection with the pH values of the blood.

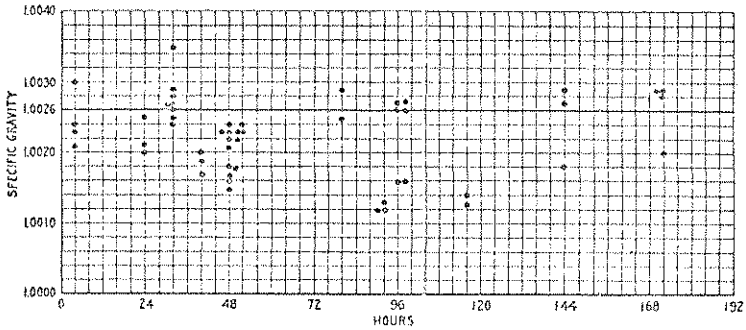

FIGURE 9.—Specific gravity of the blood of fresh-water mussels in distilled water

TABLE 12.—*Changes in specific gravity and pH of the blood of fresh-water mussels in distilled water*

Scientific name	Common name	Hours of exposure	Condition of animal	pH	Specific gravity
Quadrula trapezoides	Washboard	4	Heart beating	7.7	1.0021
Do	do	4	do	7.7	1.0023
Do	do	4	do	7.7	1.0024
Do	do	4	do	7.7	1.0030
Do	do	24	Moribund	8.1	1.0020
Do	do	24	Heart beating	8.3	1.0021
Do	do	24	Moribund	8.9	1.0025
Do	do	32	do	7.6	1.0024
Do	do	32	Heart beating	7.7	1.0025
Do	do	32	Moribund	7.6	1.0026
Do	do	32	Heart beating	7.7	1.0027
Do	do	32	Moribund	7.7	1.0027
Do	do	32	do	7.5	1.0028
Do	do	32	Heart beating	7.7	1.0029
Do	do	32	do	7.7	1.0035
Actinonaïs carinata	River mucket	40	do	8.1	1.0017
Do	do	40	do	8.0	1.0019
Do	do	40	do	8.0	1.0020
Quadrula trapezoides	Washboard	48	do	7.6	1.0015
Do	do	48	do	7.3	1.0016
Do	do	48	Moribund	7.6	1.0017
Do	do	48	Heart beating	8.0	1.0018
Do	do	48	do	7.4	1.0021
Do	do	48	do	7.9	1.0022
Do	do	48	do	7.9	1.0023
Do	do	48	Moribund	7.3	1.0024
Do	do	52	Heart beating	7.7	1.0023
Do	do	52	do	7.9	1.0023
Do	do	52	do	7.9	1.0024
Do	do	76	do	8.1	1.0025
Do	do	76	Moribund	8.2	1.0029
Actinonaïs carinata	River mucket	92	Heart beating	7.6	1.0012
Do	do	92	do	7.7	1.0012
Do	do	92	do	7.6	1.0013
Quadrula trapezoides	Washboard	96	do	7.5	1.0016
Do	do	96	do	7.5	1.0026
Do	do	96	do	7.5	1.0027
Unio popeii	Pope's purple	96	do	7.5	1.0026
Do	do	96	do	7.5	1.0027
Proptera laevissima	Paper shell	96	do	7.5	1.0016
Quadrula trapezoides	Washboard	116	Moribund	7.8	1.0013
Do	do	116	Heart beating	8.1	1.0014
Unio popeii	Pope's purple	144	do	7.5	1.0027
Do	do	144	do	7.5	1.0029
Proptera laevissima	Paper shell	144	do	7.7	1.0018
Anodonta limnetica	Southern floater	172	Dead	7.1	1.0020
Unio popeii	Pope's purple	172	Heart beating	8.4	1.0028
Do	do	172	do	8.3	1.0029

NOTE.—"Moribund," under condition of animal, designates a mussel in which the heart was not beating when shell was opened but an animal still responding to tactile stimulation of the foot or mantle.

In Figure 4 a relation between the specific gravity and the pH of the blood of fresh-water mussels was pointed out; namely, that as the blood approached neutrality the specific gravity rose—that is, the more alkaline bloods were usually those with the lower specific gravities. Under both the discussion of blood salts and of blood gases it was also noted that during exposures to conditions causing retention of carbon dioxide in the body of the mussel, the animal made more or less compensation by buffering down the carbon dioxide with calcium carbonate withdrawn from the shell. This addition of calcium salts to the blood, of course, affects the specific gravity of that fluid, not only through the actual addition of calcium salts and the retention of carbonates in the blood but also through the effects on various other constituents of the blood.

Throughout the several series of experimental tests, both with salts and with exposures to air, it was observed that as the mussel became moribund the specific gravity of the blood usually rose, and with this rise in specific gravity the pH value of the same blood approached neutrality; that is, became less alkaline. In animals which had been moribund or dead for several hours (but before decomposition changes set in) the blood frequently became quite alkaline again. This sequence of specific gravity and pH changes in the blood of living mussels and those which had just died was interpretated as showing the buffering action of the calcium carbonate of the shell on the acid products which are known to form in the tissues of moribund animals. It is also possible that changes in permeability develop under these conditions.

Applying these observations to the distilled water cases, the specific gravity of the blood of these mussels might be expected to rise slightly as the animal became moribund from the effects of the distilled water, but was still tending to buffer down the accumulating acid products.

Three checks were made. Mussels were placed in distilled water pH 5.3, and they were found to die more rapidly than mussels in the same distilled water adjusted to pH 6.8, particularly if the acid distilled water were changed frequently. If mussels were placed in distilled water pH 5.3 the pH of the water rose to pH 6.8 in 24 hours whereas control jars without mussels showed no change in pH. In order to evaluate this change in pH in the water around the mussels, fresh, empty mussel shells from which the living animals had just been removed were wiped dry and the inner surfaces completely coated with paraffin; that is only those parts of the shell which would be in contact with the water, were the shell occupied by the living mussel, were left uncoated with paraffin. The paraffined shells were then placed in acid distilled water pH 5.3 and treated as if the shells contained living mussels. After about 30 hours the pH of the water around these paraffined shells had risen to pH 6.8, where it remained. In the third check, mussels were placed in distilled water pH 5.3 and no change of fluid made, but the usual aeration was maintained. After 24 hours the water around these mussels had a pH of 6.8 and 24 hours later it had risen to pH 7.3. The blood of these mussels was near but still below average normal at the end of 144 hours. The distilled water even under these conditions continued to be toxic, and the animals died after about 200 hours.

Considering all of the distilled water data collectively the fresh-water mussels were found to be very sensitive to the hypotonic and unbuffered conditions of the environment offered by distilled water, in spite of the fact that the blood of the fresh-water mussels is much more dilute than that of the higher animals, and the correspond-

ing difference in tonicity between the blood and the fluid surrounding the animal much less. The specific gravity of the blood was definitely reduced during the first 24 hours of exposure to distilled water and remained low throughout the tests (nearly 200 hours in some cases). The rapidity of these changes in specific gravity of the blood suggests that the osmotic barrier between the blood of the fresh-water mussels and the fluid of their environment is not great, the fluid of the body tending to come to equilibrium with the environment rather promptly. The pH value of the blood was maintained fairly well as long as the animal was reasonably active, but the alkalinity of the blood fell as the mussel became moribund or as the exposure to distilled water lengthened.

SODIUM SALTS

As the fresh-water mussels are supposed to have evolved from marine clams and as sodium chloride is the major inorganic salt found in all living animals, sodium chloride was chosen as the standard with which to compare the action of the various hypertonic solutions used. Besides, as this salt passes through animal membranes easily under most conditions, the toxic action of sodium chloride to living tissues, in concentrations greater than the so-called physiological solutions, is well known.

Three solutions of sodium chloride were used in the tests on fresh-water mussels: A 0.25 per cent solution, which is roughly isotonic with the blood of fresh-water mussels; a 0.50 per cent solution, comparable with the blood of most cold-blooded vertebrates, and that of many invertebrates; and a 1.00 per cent solution, which is slightly more concentrated than mammalian or avian blood. The data from these series of tests are presented in Table 13 and Figure 10.

TABLE 13.—*Changes in specific gravity and pH of the blood of fresh-water mussels in solutions of sodium salts.*

Scientific name	Common name	Hours of exposure	Condition of animal	pH	Specific gravity
SODIUM CHLORIDE, 1.00 PER CENT					
Quadrula trapezoides	Washboard	4	Heart beating	8.0	1.0046
Do	do	4	do	7.3	1.0051
Do	do	4	do	7.6	1.0051
Do	do	4	do	7.6	1.0051
Do	do	12	do	7.4	1.0056
Do	do	12	do	7.4	1.0052
Do	do	12	do	7.5	1.0073
Do	do	24	do	7.0	1.0053
SODIUM CHLORIDE, 0.50 PER CENT					
Quadrula trapezoides	Washboard	4	Heart beating	7.9	1.0033
Do	do	4	do	7.8	1.0036
Do	do	4	do	7.8	
Do	do	12	do	7.5	1.0054
Do	do	12	do	7.8	1.0054
Do	do	12	do	7.8	1.0059
Do	do	24	Moribund	7.0	1.0049
Do	do	24	do	7.0	1.0050
Do	do	24	Heart beating	7.0	1.0050
Do	do	24	do	7.5	1.0050
SODIUM CHLORIDE, 0.25 PER CENT					
Quadrula trapezoides	Washboard	4	Heart beating	8.0	1.0029
Do	do	4	do	8.1	1.0036
Do	do	24	do	7.2	1.0030
Do	do	24	do	7.9	1.0030
Do	do	24	do	7.9	1.0032
Do	do	24	do	8.0	1.0041
Do	do	76	do	7.7	1.0034
Do	do	76	do	7.9	1.0034
Do	do	76	do	7.9	1.0030
Do	do	76	do	7.7	1.0037

NOTE.—"Moribund," under condition of animal, designates a mussel in which the heart was not beating when shell was opened, but an animal still responding to tactile stimulation of the foot or mantle.

The almost immediate rise in specific gravity of the blood of fresh-water mussels when placed in these solutions of sodium chloride is in sharp contrast with the results of the distilled water series, and confirms the statement made in the discussion of the distilled water series that the restriction to osmotic adjustment between the blood of the fresh-water mussel and its fluid environment is slight. Within four hours after placing the mussels in these salt solutions the specific gravity of the blood of all individuals exceeded the average normal specific gravity for the blood of fresh-water mussels excepting one. This mussel (see fig. 10) was placed in 0.50 per cent sodium chloride solution while closed, and without a cork between the valves. It is probable that very little of the salt solution penetrated to the soft parts of this animal as it was not observed to open its valves during this 4-hour period. Several other mussels were tested in the salt solutions in this same way; that is, they were closed in air and then placed in the solution while closed without cork between the valves. The data from these animals have been included in Figure 10 (not in Table 13) for comparison. For the most part these closed animals remained closed in the salt solutions and took little of the test solutions inside their shells, as the blood specific gravities attest.

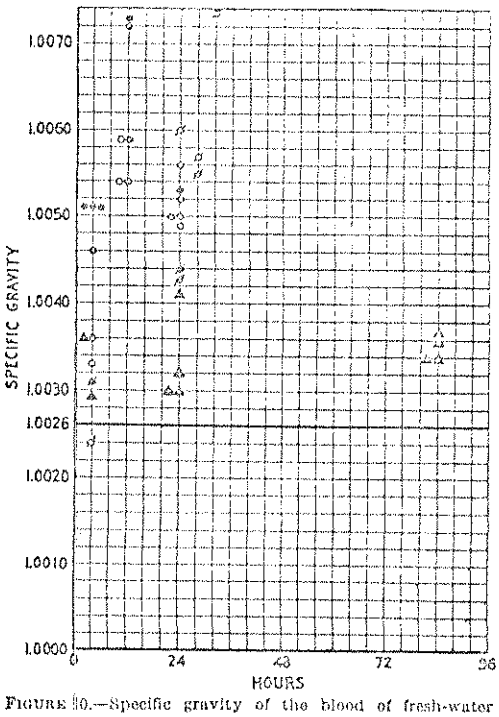

FIGURE 10.—Specific gravity of the blood of fresh-water mussels in tap water plus sodium chloride. Black circle, 1 per cent sodium chloride, valves propped open; scored black circle, 1 per cent sodium chloride, animal unrestricted; circle, 0.5 per cent sodium chloride, valves propped open; scored circle, 0.5 per cent sodium chloride, animal unrestricted; black triangle, 0.25 per cent sodium chloride, valves propped open

By graded additions of salt to the water in which European fresh-water mussels (Anodonta and Unio) were living, Beudant (1816), found that these animals could adapt themselves to almost 2 per cent salt solution. Similarly, Philippson, Hannevart, and Thieren (1910) were able to raise the salt content of the water in which specimens of the European fresh-water mussel, *Anodonta cygnea*, were living to 2 per cent if sodium chloride were used, or even higher if "sea salt" were added gradually. In this work they found, using the electroconductivity method, that the salt content of the blood of these fresh-water mussels rose, but that the salt value of the blood never equaled the salt content of the surrounding medium, presumably because of the presence of some colloidal material in the mussel blood. The return of salt-adapted mussels to fresh water was also followed by a drop in the salt content of the mussel blood as these animals readapted themselves to the fresh water. These observations on the European fresh-water mussel parallel the findings in the present series of North American fresh-water mussels.

The degree of adaptation which North American fresh-water mussels can make to solutions of sodium chloride, following the gradual addition of this compound to the water in which these mussels are found, is not to be discussed here; but the rapid changes in specific gravity of the blood of these mussels following abrupt changes

from fresh water to even dilute salt solutions show the potential dangers to the fresh-water mussels from the addition of salt to the water in which they are living, which may result from the introduction of certain types of industrial wastes.

The pH values of the blood of mussels from the sodium chloride series seemed to vary toward neutrality, the greater the concentration of the salt solution. This finding, in view of observations made in other series, suggests that the stronger solutions, as might be expected, were producing more extended disturbances of the body functions than the weaker solutions.

POTASSIUM SALTS

Various species of mussels were tested in solutions of potassium chloride, potassium

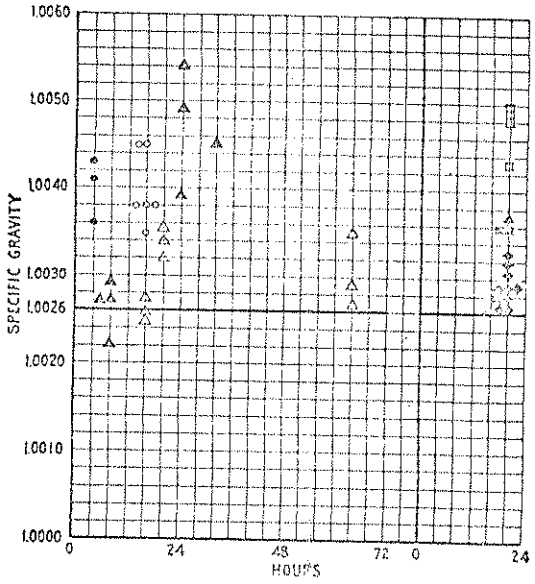

FIGURE 11.—Specific gravity of the blood of fresh-water mussels in tap water plus potassium salts. All animals with valves propped open. Black circle, 1 per cent potassium chloride; circle, 0.50 per cent potassium chloride; black triangle, 0.25 per cent potassium chloride; triangle, 0.10 per cent potassium chloride; square, 0.5 per cent carbonate; black square 0.25 per cent potassium carbonate; and black triangle, 0.25 per cent potassium sulphate

carbonate, potassium sulphate, and in a mixture of potassium chloride and calcium chloride. These series are summarized in Table 14 and Figure 11.

The toxic action of the potassium salts on fresh-water mussels is evident from these tests, and is in accord with the known toxic action of potassium compounds to other types of animals. The mussels rapidly became moribund in solutions of potassium salts, and as a result the potassium series were not carried beyond 24 hours in most cases.

The general effects of potassium salts on the specific gravity of the blood were the same as those noted for the sodium chloride series. The animals made rapid adjustments in the specific gravity of the blood, the actual values being well above the average normal specific gravity of fresh-water mussel blood.

Owing to the high toxicity of the potassium salts, the body condition declined rapidly and the alkalinity of the blood was lowered, as in other moribund mussels. Extreme retraction of the foot was evident in mussels dying in solutions of potassium salts.

TABLE 14.—*Changes in specific gravity and pH of the blood of fresh-water mussels in solutions of potassium salts*

POTASSIUM CHLORIDE 1.00 PER CENT

Scientific name	Common name	Hours of exposure	Condition of animal	pH	Specific gravity
Quadrula trapezoides	Washboard	4	Heart beating	7.9	1.0036
Do	do	4	Moribund	7.9	1.0041
Do	do	4	do	7.5	1.0043

POTASSIUM CHLORIDE, 0.50 PER CENT

Quadrula trapezoides	Washboard	16	Moribund	7.5	1.0035
Do	do	16	do	7.5	1.0038
Do	do	16	do	7.5	1.0038
Do	do	16	do	7.7	1.0038
Do	do	16	do	7.5	1.0045
Do	do	16	Dead		1.0045

POTASSIUM CHLORIDE, 0.25 PER CENT

Lampsilis anodontoides	Yellow sand-shell	8	Moribund	7.5	1.0022
Do	do	8	do	7.5	1.0027
Do	do	8	Heart beating	7.5	1.0027
Do	do	8	Moribund	7.5	1.0029
Do	do	24	Dead		
Do	do	24	Heart beating	7.3	1.0039
Do	do	24	do	7.3	1.0049
Do	do	24	do	7.5	1.0054
Do	do	32	Moribund	7.4	1.0045

POTASSIUM CHLORIDE, 0.10 PER CENT

Lampsilis anodontoides	Yellow sand-shell	16	Moribund	7.7	1.0025
Do	do	16	do	7.6	1.0026
Do	do	16	do	7.3	1.0027
Quadrula nodulata	Warty-back	16	do	7.1	1.0027
Lampsilis anodontoides	Yellow sand-shell	20	do	6.9	1.0032
Proptera laevissima	Paper shell	20	Heart beating	7.3	1.0034
Quadrula nodulata	Warty-back	20	do	7.1	1.0035
Actinonais carinata	River mucket	64	Moribund	7.1	1.0027
Proptera laevissima	Paper shell	64	do	7.3	1.0029
Quadrula nodulata	Warty-back	64	do	7.3	1.0035

POTASSIUM CARBONATE, 0.50 PER CENT

Unio popeli	Pope's purple	20	Moribund	7.1	1.0043
Do	do	20	Dead	7.1	1.0048
Do	do	20	Moribund	7.3	1.0050
Anodonta imbecillis	Pond paper shell	20	do	7.2	1.0049

POTASSIUM CARBONATE, 0.25 PER CENT

Quadrula trapezoides	Washboard	20	Moribund	7.5	1.0028
Do	do	20	do	7.7	1.0035
Do	do	20	Dead	8.0	1.0045

POTASSIUM SULPHATE, 0.25 PER CENT

Quadrula trapezoides	Washboard	20	Moribund	8.0	1.0027
Do	do	20	do	7.7	1.0028
Do	do	20	do	7.5	1.0029
Do	do	20	do	7.5	1.0029
Do	do	20	do	7.6	1.0031
Do	do	20	do	7.6	1.0032
Do	do	20	do	7.3	1.0033
Do	do	20	Dead	7.6	1.0027
Do	do	20	do	7.5	1.0029
Do	do	20	do	7.3	1.0037

POTASSIUM CHLORIDE, 0.25 PER CENT; PLUS CALCIUM CHLORIDE, 0.25 PER CENT [1]

Actinonais carinata	River mucket	4	Heart beating	7.5	1.0030
Do	do	4	do	8.0	1.0030
Do	do	4	do	7.5	1.0035
Lampsilis siliquoidea	Fat mucket	24	Moribund	7.3	1.0041
Do	do	24	Heart beating	7.5	1.0042
Actinonais carinata	River mucket	24	Moribund	7.3	1.0042
Do	do	24	do	7.5	1.0043
Do	do	24	do	7.3	1.0043

[1] Made up in distilled water.

NOTE.—"Moribund," under condition of animal, designates a mussel in which the heart was not beating when shell was opened but an animal still responding to tactile stimulation of the foot or mantle.

MAGNESIUM SALTS

The rôle of magnesium salts in the blood of animals is not so well understood as that of some of the other salts, but as magnesium compounds occur in the blood of fresh-water mussels and also in the water (often in relatively large amounts), in which these animals live, a series of mussels were tested in solutions of magnesium salts. The data for these series are given in Table 15 and Figure 12.

As far as determined by these series, the adjustment to magnesium salts, if they be present in amounts which can be tolerated, is made rather quickly. Mussels

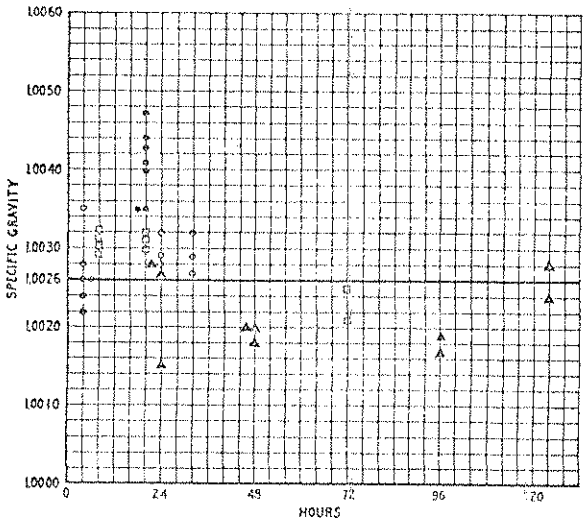

FIGURE 12.—Specific gravity of the blood of fresh-water mussels in tap water plus magnesium salts. All animals with valves propped open. Black circle, 1 per cent magnesium sulphate; circle 0.5 per cent magnesium sulphate; black triangle, 0.25 per cent magnesium sulphate; and square 0.5 per cent magnesium chloride

transferred to 1.00 per cent solution of magnesium sulphate showed the same rather abrupt rise in specific gravity of the blood during the first 24 hours as that noted for the salts of sodium and potassium, and 0.50 per cent solutions of magnesium sulphate and magnesium chloride produced lesser elevations of the blood specific gravity. Beyond the first 24 hours, the results suggest a return to normal blood specific gravity or below, although the data are scant. From the reactions of the mussels to the various solutions of magnesium salts used, the toxicity of magnesium compounds seems much less than that of either potassium or sodium salts, but it must be remembered in this connection that the permeability of living cells to magnesium salts is quite different from that to either potassium or sodium salts.

TABLE 15.—*Changes in specific gravity and pH of the blood of fresh-water mussels in solutions of magnesium salts*

MAGNESIUM SULPHATE, 1.00 PER CENT

Scientific name	Common name	Hours of exposure	Condition of animal	pH	Specific gravity
Quadrula trapezoides	Washboard	4	Heart beating	7.7	1.0022
Do	do	4	do	8.1	1.0024
Do	do	4	do	8.1	1.0026
Fusconaia ebena	Niggerhead	20	do	7.2	1.0035
Do	do	20	do	7.7	1.0035
Do	do	20	do	7.7	1.0041
Megalonaias gigantea	Washboard	20	Moribund	8.1	1.0044
Do	do	20	Heart beating	7.9	1.0047
Quadrula trapezoides	do	20	do	7.5	1.0040
Do	do	20	do	7.5	1.0043

MAGNESIUM SULPHATE, 0.50 PER CENT

Megalonaias gigantea	Washboard	4	Heart beating	8.0	1.0026
Do	do	4	Moribund	8.1	1.0028
Do	do	4	Heart beating	7.7	1.0035
Do	do	24	Moribund	7.7	1.0028
Do	do	24	Heart beating	8.1	1.0029
Do	do	24	Moribund	8.0	1.0032
Do	do	28	Heart beating	7.7	1.0027
Do	do	28	do	7.7	1.0029
Lampsilis anodontoides	Yellow sand-shell	28	Moribund	7.7	1.0032

MAGNESIUM SULPHATE, 0.25 PER CENT

Plagiola lineolata	Butterfly	24	Heart beating	8.1	1.0028
Megalonaias gigantea	Washboard	24	do	8.1	1.0027
Fusconaia undata	Pig toe	24	do	8.0	1.0015
Megalonaias gigantea	Washboard	48	do	8.1	1.0018
Do	do	48	do	8.1	1.0020
Do	do	48	Moribund	8.1	1.0020
Do	do	96	Heart beating	8.1	1.0017
Plagiola lineolata	Butterfly	96	do	8.1	1.0019
Quadrula trapezoides	Washboard	124	do	8.5	1.0024
Unio popeii	Pope's purple	124	do	7.9	1.0028

MAGNESIUM CHLORIDE, 0.50 PER CENT

Lampsilis siliquoides	Fat mucket	8	Heart beating	7.9	1.0029
Do	do	8	do	7.8	1.0030
Do	do	8	do	7.9	1.0031
Do	do	8	do	8.0	1.0032
Do	do	20	do	8.0	1.0030
Do	do	20	do	7.8	1.0032
Actinonais carinata	River mucket	20	Dead	8.1	1.0031
Do	do	72	Heart beating	8.1	1.0021
Do	do	72	do	7.7	1.0025

NOTE.—"Moribund," under condition of animal, designates a mussel in which the heart was not beating when shell was opened, but an animal still responding to tactile stimulation of the foot or mantle.

CALCIUM SALTS

As calcium salts play such an important part in the life activities of the fresh-water mussels, even to limiting the distribution of these animals very largely to regions where calcium salts are readily available in the water, calcium salts have been made the basis of a more detailed series of studies, which are only summarized in part here. In Table 16 and Figure 13 individual data for a series of calcium tests are presented. These will suffice to show the main points in relation to the blood.

The responses of the mussels living in calcium-salt solutions were very quickly adjusted, so that if the solutions were not too strong the mussels behaved much as in ordinary fresh water. The specific gravity of the blood of animals transferred to the stronger solutions rose during the first 24 to 48 hours, after which the readings range about the normal blood specific gravity. Four high cases (see fig. 13), one at

192 hours, one at 168 hours, and two at 144 hours, seem to be exceptions to this statement in the series given. These animals present an interesting complication as all were moribund. Moribund animals in calcium solutions displayed the same rise in blood specific gravity and loss of blood alkalinity as moribund individuals in any other series. This suggests that the buffering of acid products in the body of the mussel draws on calcium in the body rather than that in the environment.

TABLE 16.—*Specific gravity and pH of the blood of fresh-water mussels in solutions of calcium salts*

CALCIUM CHLORIDE, 1.00 PER CENT

Scientific name	Common name	Hours of exposure	Condition of animal	pH	Specific gravity
Lampsilis anodontoides	Yellow sand-shell	24	Dead		
Do	do	24	do		
Do	do	24	do		
Do	do	24	Heart beating	7.5	1.0058
Do	do	24	do	7.3	1.0055
Amblema costata	Three-ridge	24	Dead		

CALCIUM CHLORIDE, 0.50 PER CENT

Anodonta limneana	Southern floater	24	Heart beating	7.5	1.0037
Do	do	48	do	8.1	1.0032
Do	do	48	Dead		
Lampsilis anodontoides	Yellow sand-shell	72	Heart beating	7.6	1.0027
Do	do	96	do	7.3	1.0029
Amblema costata	Three-ridge	168	Moribund	7.0	1.0015
Lampsilis anodontoides	Yellow sand-shell	192	Heart beating	7.6	1.0032
Do	do	216	do	7.3	1.0031
Do	do	244	do	7.7	1.0033
Do	do	268	do	7.4	1.0034

CALCIUM CHLORIDE, 0.25 PER CENT

Lampsilis anodontoides	Yellow sand-shell	24	Heart beating	7.7	1.0026
Do	do	24	do		1.0043
Do	do	48	do	8.0	1.0030
Do	do	48	do	7.9	1.0012
Do	do	48	do	7.6	1.0054
Do	do	72	do	7.4	1.0026
Amblema costata	Three-ridge	96	do	7.9	1.0022
Lampsilis anodontoides	Yellow sand-shell	120	Dead		
Do	do	120	Heart beating	7.8	1.0037
Do	do	144	Moribund	7.6	1.0039
Do	do	144	do	7.6	1.0043
Do	do	168	Heart beating	8.0	1.0029
Do	do	192	do	7.5	1.0025
Do	do	192	Moribund	7.1	1.0047
Do	do	216	Heart beating	8.1	1.0017
Do	do	244	do	8.1	1.0011
Do	do	244	do	8.1	1.0021
Do	do	268	do	8.2	1.0023
Do	do	268	do	8.2	1.0030
Do	do	268	do	8.0	1.0033
Lampsilis fallaciosa	Slough sand-shell	408	do	8.1	1.0017
Obliquaria reflexa	Three-horned warty-back	408	do	7.9	1.0022
Tritogonia verrucosa	Buckhorn	408	do	7.4	1.0027

CALCIUM CHLORIDE, 0.10 PER CENT

Lampsilis fallaciosa	Slough sand-shell	72	Heart beating	7.8	1.0033
Tritogonia verrucosa	Buckhorn	96	do	7.7	1.0024
Lampsilis fallaciosa	Slough sand-shell	412	do	7.8	1.0013

The calcium salt solutions were conspicuously less toxic than any of the other groups of salts studied, judging by the survivals and by the small number of moribund individuals found. In connection with the toxic action of the calcium salts it must be stated, however, that these observations on adult mussels do not apply to the glochidia. Adult females were found to tolerate solutions of calcium salts which greatly lengthened the closing reaction time of the glochidia which these same mussels were carrying

in their marsupia. In some cases glochidia, from the marsupia of mussels which had been carried successfully for several days in solutions of calcium salts, were so reduced in sensitivity that 10 per cent sodium chloride solution was required to excite these glochidia to the closing response, although the glochidia appeared otherwise to be in splendid condition.

Blood sugar and blood ash determinations were made on the blood of mussels from both the calcium-salt series and the sodium-salt series. In both groups the blood sugar was found to vary within the same limits as those defined for the normal animals, but the blood ash in both sodium and calcium series was higher than in normal mussels. The increase in blood ash shows that in the calcium and sodium series the mussels during the period of high-blood specific gravity, actually had more inorganic solids in their blood. Whether this rise in ash content was due to the loss of water from the blood, thereby producing a concentration of the salts already in the blood, or whether this high ash content represented a movement of salts into the blood, was

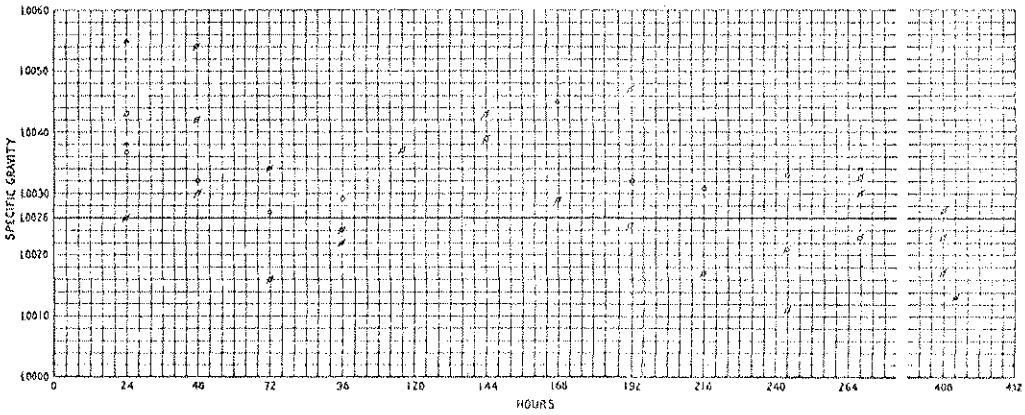

FIGURE 13.—Blood specific gravity of mussels living in solutions of calcium salts. Black circle, 1 per cent calcium chloride; circle, 0.5 per cent calcium chloride; scored circle, 0.25 per cent calcium chloride; scored black circle, 0.1 per cent calcium chloride

not determined. In either event, however, the tissues of the animal were subjected to a blood of higher salt concentration.

EFFECTS OF EXPOSURES TO AIR

AT ORDINARY TEMPERATURES

Sudden changes in stream level may leave fresh-water mussels stranded out of water, but ordinarily the low-water stages come so slowly that littoral forms like the slough sand-shell, *Lampsilis fallaciosa*, and species which move in and out of shallow water as the yellow sand-shell, *Lampsilis anodontoides*, may easily keep ahead of the receding water. For mussel species living on bars in deeper water, exposure to the air is a more or less remote possibility in their normal-life activities.

The work attendant on propagation of mussels has made it necessary to expose mussels to the air, and often these animals are shipped long distances without water. To determine the effect of removal from water and exposure to air on the mussel, using the blood as an index, a series of 50 mussels was taken directly from the river to the laboratory, wiped dry and spread out separately so that there could be no accumulation of water below or around the mussels. Every opportunity was offered, therefore, for these mussels to "dry out" at room temperature.

Blood samples were taken at intervals and only those mussels which were closed at the time the sample was to be taken were used. The determinations of specific gravity for the blood from the 28 individuals which survived this treatment are given in Table 17 and Figure 14.

During the first four hours of exposure to air the rise in blood specific gravity became evident. This may have resulted from either the loss of water from the blood, or from the addition of calcium salts to the blood to buffer down the products of

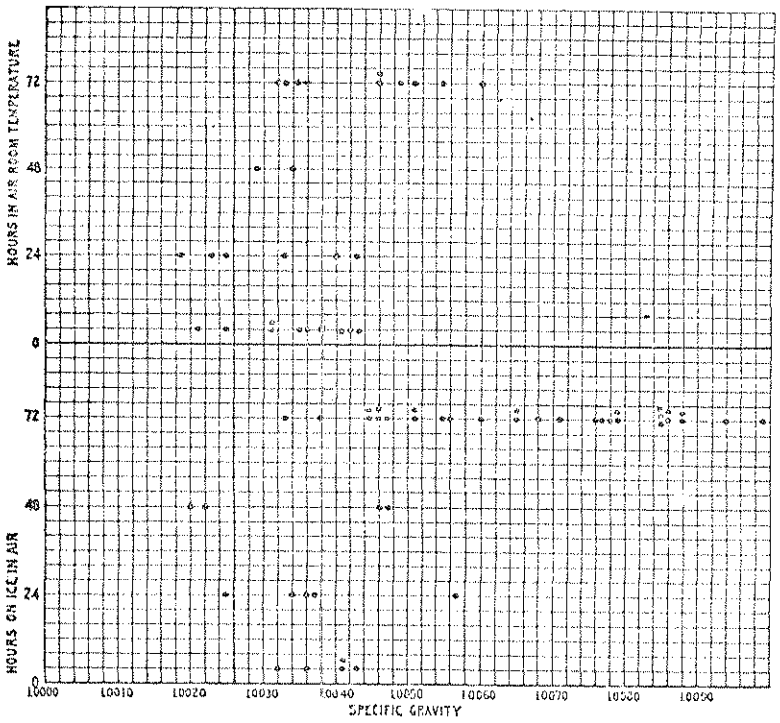

FIGURE 14.—Blood specific gravity of mussels exposed to air. Upper half, in air at room temperature; lower half, in air on ice

respiration in the animal now deprived of its regular supply of fresh water; or both factors may have contributed to the rise in specific gravity.

The specific gravity of the blood continued to rise throughout the test. It was noted that those mussels which remained closed survived, while those which opened the valves and thereby lost some or all of the water which had been retained between the valves when the animal closed, as it was being taken from the river, soon succumbed.

The pH value of the blood remained near pH 7.7 in most of the animals from which samples were taken, but all moribund animals were rejected in this series.

TABLE 17.—*Specific gravity and pH values for the blood of fresh-water mussels exposed to the air*

IN AIR AT ROOM TEMPERATURE, 24°

Scientific name	Common name	Hours of exposure	Specific gravity	pH
Tritogonia verrucosa	Buckhorn	4	1.0037	7.7
Do	do	24	1.0043	7.9
Fusconaia ebena	Niggerhead	72	1.0033	7.7
Do	do	72	1.0055	7.9
Do	do	72	1.0050	7.8
Do	do	72	1.0055	7.7
Do	do	72	1.0035	7.8
Fusconaia undata	Pig toe	4	1.0042	7.9
Do	do	4	1.0031	7.7
Do	do	24	1.0033	7.8
Do	do	72	1.0040	8.0
Do	do	72	1.0046	7.9
Amblema costata	Three-ridge	4	1.0021	7.7
Do	do	4	1.0031	7.8
Do	do	24	1.0019	7.5
Do	do	48	1.0020	7.7
Do	do	48	1.0034	7.7
Do	do	72	1.0016	7.5
Amblema rariplicata	Blue point	72	1.0052	7.9
Quadrula pustulosa	Pimple back	4	1.0030	7.7
Do	do	4	1.0043	7.7
Do	do	24	1.0036	7.6
Quadrula metanevra	Monkey face	4	1.0035	7.8
Megalonaias gigantea	Washboard	4	1.0041	7.6
Strophitus rugosus	Squaw foot	24	1.0025	7.7
Actinonais carinata	River mucket	4	1.0025	7.7
Do	do	24	1.0023	7.7
Lampsilis higginsii	Higgins eye	72	1.0051	7.7

IN AIR, ON ICE

Fusconaia ebena	Niggerhead	24	1.0057	7.7
Do	do	72	1.0065	7.6
Do	do	72	1.0076	7.5
Fusconaia undata	Pig toe	4	1.0043	7.6
Do	do	24	1.0037	7.5
Do	do	48	1.0047	7.6
Do	do	72	1.0056	7.7
Do	do	72	1.0077	7.7
Do	do	72	1.0079	7.7
Amblema costata	Three-ridge	4	1.0041	7.6
Do	do	24	1.0036	7.6
Do	do	48	1.0046	7.5
Do	do	72	1.0055	7.7
Amblema rariplicata	Blue point	72	1.0055	7.7
Quadrula pustulosa	Pimple back	4	1.0032	7.8
Do	do	24	1.0034	7.6
Do	do	48	1.0022	7.4
Do	do	72	1.0045	7.6
Do	do	72	1.0046	7.6
Do	do	72	1.0048	7.6
Do	do	72	1.0045	7.6
Do	do	72	1.0056	7.8
Do	do	72	1.0058	7.6
Quadrula metanevra	Monkey face	72	1.0035	7.7
Do	do	72	1.0051	7.7
Do	do	72	1.0051	7.7
Do	do	72	1.0050	7.6
Do	do	72	1.0078	7.7
Do	do	72	1.0065	7.7
Do	do	72	1.0086	7.5
Do	do	72	1.0089	7.5
Do	do	72	1.0069	7.8
Quadrula nodulata	Warty-back	72	1.0047	7.7
Do	do	72	1.0071	7.5
Do	do	72	1.0085	-------
Do	do	72	1.0094	-------
Megalonaias gigantea	Washboard	4	1.0041	7.6
Do	do	24	1.0025	7.5
Do	do	48	1.0020	7.5
Obliquaria reflexa	Three-horned warty-back	72	1.0033	7.9
Do	do	72	1.0066	7.7
Do	do	72	1.0088	7.5
Proptera laevissima	Paper shell	72	1.0045	7.8
Actinonais carinata	River mucket	4	1.0030	7.8
Do	do	72	1.0046	7.7

NEAR FREEZING

The use of ice in connection with shipments of mussels has been accepted rather generally because the melting ice bathes the mussels continuously in a limited amount of water, without the disadvantages of a water shipment. Accordingly 60 mussels were packed on ice in perforated containers immediately after the animals were taken from the river. The containers were so prepared that no water could accumulate under or around the mussels, but they were kept moist by the constantly melting ice. Blood samples from these mussels were taken at intervals and the data are presented in Table 17 and Figure 14 in conjunction with the data from the other air series.

During the first 48 hours only those animals which were closed were used for samples, and these were found on opening to have lost most of the water which was included between the valves at the time the mussels were taken from the river. At the end of 72 hours exposure to air on ice, all animals still showing tactile responses to stimulation of the foot or mantle were sacrificed for samples. At this time practically all of the mussels were gapping slightly and the water from the inside of the shell had been lost.

In this group of animals kept on ice in air for 72 hours a blood specific gravity of 1.0099 was recorded for a specimen the monkey face, *Quadrula metanevra*. This was the highest blood specific gravity found in any of these studies on the blood of fresh-water mussels. All of the individuals surviving the ice treatment for 72 hours yielded blood with a specific gravity well above the average blood specific gravity for normal mussels, and several records were unusually high. The pH value of the blood of these mussels of the ice series was less alkaline than normal, and in the main the mussels gave the combined picture of blood concentration and low alkalinity which was exhibited by moribund mussels throughout the various tests.

The ice tests were repeated several times on other series of mussels, and it was always noted that as the animal became chilled there was a tendency for the adductor muscles to relax slightly. As the muscles relaxed the shells gapped apart more or less, and the water included between the valves at the time the animal was closed was lost more or less completely. The rise in blood specific gravity in mussels exposed to air seemed therefore, in part at least, to be associated with loss of this water from between the shells. Once the mussel became so numbed that its valves began to gap open, the soft parts of the animal were exposed to the direct action of the air, if the water between the valves were lost. There seemed to be no mechanism to maintain the concentration of the blood at the normal level while the soft parts lost water to the air.

To test this interpretation of these results, four large southern floaters, *Anodonta limneana*, were prepared by cutting a window in the shell of each in the region of the heart, as described in a previous section. Each mussel was then mounted on its side with the uncut valve down, in a glass jar, and water added until the uncut valve and the opening between the two valves were submerged. In this way the mantle cavity of the mussel was filled with water, but water could not enter the shell-window which was above the water level at all times. A thermometer was inserted through the window and supported so that the bulb of the thermometer remained in the pericardial cavity, registering therefore the temperature of the fluid surrounding the heart. The glass vessel was then covered to eliminate disturbing air currents and to reduce evaporation from the mussel to the minimum,

and each animal held at 20° C. for six hours before the initial blood sample was taken. The blood samples were drawn directly from the heart, as in previous tests, with a fine dental needle mounted on a Leur syringe.

By adding ice to the water in the jar, the temperature of the animal as determined from the pericardial fluid, was reduced at the rate of 5° C. in 30 minutes. After the mussel's body temperature had been reduced to 0° C. and the last sample taken, the mussel was slowly returned to a temperature of 15° C. to 20° C. where it was held until the following morning (approximately 24 hours after the first sample was drawn), when samples were drawn again during a second reduction of body temperature similar to that of the first day. In the case of mussel C, samples were taken at the beginning and at the end of a third temperature reduction during the third day. After the last samples were taken each mussel was held at room temperature for 24 hours as a check on its condition. None of the mussels of this series died during the 24 hours following the termination of these tests.

The data from this series are given in Table 18.

TABLE 18.—*Specific gravity of the blood of fresh-water mussels at various temperatures*

Individual	20° C.	15° C.	10° C.	5° C.	0° C.
A first day	1.0022	1.0012	1.0016	1.0022	1.0010
A second day		1.0017	1.0022	1.0021	
B first day	1.0020	1.0012	1.0012		
B second day		1.0016	1.0014	1.0018	
C first day		1.0005	1.0008	1.0010	
C second day	1.0005	1.0005	.0015	1.0021	1.0018
C third day	1.0010			1.0015	
D first day	1.0010	1.0012	1.0014	1.0012	
D second day	1.0015	1.0017	1.0021	1.0016	1.0019

The figures given in Table 18 show that the specific gravity of the blood did not rise to the levels attained by the blood from the mussels of the ice series in which no precaution was taken to prevent undue loss of water by the mussel. In fact, there was little or no concentration of the blood, as measured by the specific gravity, in these floaters which were protected from air currents and loss of water, although they were lowered to zero centigrade.

The changes in the blood specific gravity of the mussels when held in air are evidently a matter of water loss to a large extent, as the mussel seems to have no way to maintain the blood concentration level when water is removed from a large portion of the body. The changes in the alkalinity were not evident in the blood until the animal became moribund as the result of the water loss.

SUMMARY

Blood from 27 species of North American fresh-water mussels was analyzed and the values of the various characteristics and constituents of normal fresh-water mussel blood determined. These values have been summarized in Table 19.

TABLE 19.—*Summary of the characteristics and constituents of normal fresh-water mussel blood*

	Minimum	Average	Maximum		Minimum	Average	Maximum
Specific gravity	1.0003	1.0026	1.0078	Calcium chloride, per cent	0.0087	0.0270	0.1060
Total solids, per cent	.3436	.4260	.4965	pH	7.4	7.9	8.5
Total ash, do	.1256	.1539	.2820	Blood gases:			
Organic material, do	.1190	.2721	.3145	Oxygen, volumes per cent	.16	.30	.89
Blood sugar, milligrams	7	32	93	Carbon dioxide, do	.09	.43	.81
Sodium chloride, per cent	.0310	.1090	.2950	Nitrogen, do	1.08	1.34	1.80

These values collectively show the blood of fresh-water mussels to be very low in solids as compared with the blood of other animals, both fresh-water and marine. The blood of fresh-water mussels is more alkaline than that of most animals, and varies over a rather wide range on the alkaline side of neutrality; in this respect the blood of fresh-water mussels being comparable to that of other mollusca as noted by Giersback (1891).

Although the blood of fresh-water mussels was found to contain only very small quantities of inorganic salts, it was demonstrated by means of physiological tests on living preparations of the foot and of the heart of fresh-water mussels, that these salts, even though present in the blood in low concentrations, are essential for the life activities of the mussels; and that these salts are balanced against each other as in the higher animals. The activity of the heart and of the foot of these fresh-water mussels ceased promptly if the proportions or the quantities of these salts in the blood were increased or diminished beyond rather narrow limits.

The fresh-water mussels were found to be very sensitive to changes in the salt content of the water in which they were living. When small quantities of common salt or various other inorganic salts were added to the surrounding water, the specific gravity and salt content of the blood of the mussels changed rapidly in a few hours. Similarly if the mussels were transferred from ordinary river water to distilled water, the specific gravity and salt content of the blood of the mussels were lowered. From these series of tests it was demonstrated that the blood of the fresh-water mussel varies with and in the direction of the concentration of the salts in the water in which it is living; and as these changes take place rapidly, they suggest that the restriction on an osmotic balance between the blood of the mussel and the fluid surrounding the mussel is slight. Since the fresh-water mussel is very limited in its locomotion, this facile modification of the blood by the environment makes the fresh-water mussel particularly susceptible to changes in water composition resulting from the introduction of various industrial wastes into the mussel-bearing streams.

Exposure to air either at ordinary temperatures or on ice, caused the specific gravity of the blood of fresh-water mussels to rise, indicating a concentration of the blood. This concentration of the blood, which rapidly reached a critical level, was accelerated if the mussel lost the water which was retained inside of the shell when the animal was removed to the air. As mussels packed on ice were soon so numbed that the adductor muscles relaxed sufficiently to allow the shells to gape open and the water to drain out, mussels so packed succumbed more rapidly than those which were packed in moist sphagnum or other damp material.

The alkalinity of the blood was reduced and the specific gravity rose in moribund fresh-water mussels.

The blood can be used as an index of the physiological condition of fresh-water mussels.

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