# THE BLOOD VOLUMES OF SOME REPRESENTATIVE MOLLUSCS*

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The reports of measurements of the blood volume of molluscs found in the literature are few in number and represent, in most cases, incidental observations rather than results of specific investigations. Leitch (1916) obtained by the bleeding of *Planorbis* a volume of blood which was 33% of the tissue weight. In 1931, Borden determined the blood volume of *Planorbis corneus* using the Welcker method and obtained a value of 58% of the total tissue weight. This higher value is to be expected, since it is impossible to remove all the blood from an animal by simple bleeding. In 1950, Martin & Huston, using the inulin dilution method, reported the blood volume of *Aplysia californicus* to be 83·3% of the total weight. This is not surprising since *Aplysia* has a very extensive haemocoele, and more than half the body weight in blood can be drained in a few minutes from a single incision into the animal.

Prosser & Weinstein (1950) determined the blood volume of two fresh-water molluscs, *Lampsilis ventricosa* and *Amblema costata*, and found a very low value. In terms of percentage of total wet tissue weight, their average values for experiments done with sodium thiocyanate and with T-1824 are very close to 8%. These results are not verified by the brief report of Potts (1954) who, in studying the rate of urine production in *Anodonta cygnea*, found a mean inulin space of four animals to be 55% of the wet tissue weight.

Since the values which have been reported are so few in number and are so wide in range a more extensive study of the subject appeared to be of value. The purpose of this investigation was to measure the total volume of circulating fluid in members of various groups of molluscs. From the results obtained the extent of variability within certain species and, to some extent, within the phylum can be ascertained.

## GENERAL METHODS

Blood volumes were determined by measuring the dilution of a known quantity of material injected into the circulating fluid. In order to facilitate rapid mixing of the

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material, it is desirable to make injections directly into the heart or a major blood vessel. When it was impossible to use this technique, as was the case in about half the forms investigated, the injections were made directly into the haemocoele.

Table 1. *Summary of experimental procedures*

| Animal | Wet wt. without shell (g.) | Vol. of initial normal blood sample (ml.) | Material injected | Vol. injected (ml.) | Vol. of each serial sample (ml.) | Sampling interval (min.) | Total length of experiment (min.) |
|---|---|---|---|---|---|---|---|
| *Cryptochiton stelleri* | 421–877 | 5–7 | Inulin, 0·4% in blood | 5 | 0·5 | 30–60 | 300 |
| *Aplysia californicus* | 146–411 | 2–5 | Rabbit Hb 3% in blood | 7–20 | 2 | 15–30 | 60–235 |
| | | | Inulin, 0·5% in blood | 6–10 | 1–2 | 15–30 | 180–400 |
| | | | 0·05–0·10% silver proteinate in blood | 8–15 | 1–2 | 15–30 | 85–400 |
| *Archidoris* sp. | 50–62 | 0·5–1·0 | Inulin, 0·5% in sea water | 1–2 | 0·2–0·5 | 20–30 | 240–360 |
| *Achatina fulica* | 41–74 | 1·0 | Inulin, 0·5–1% in distilled water | 1 | 0·2 | 15–20 | 130–270 |
| *Arion ater* | 10·0–43·7 | 0·15 | Inulin, 4% in distilled water | 0·20 | 0·05 | 10–60 | 120–480 |
| *Mytilus californianus* | 39–122 | 1–1·5 | Inulin, 1% in sea water | 0·5–1·0 | 0·2 | 15–30 | 180–250 |
| *Margaritana margaritifera* | 31–49 | 0·5–1·0 | Inulin, 1% in distilled water | 0·5–1·0 | 0·15–0·20 | 15 | 120–180 |
| | | | T-1824 bound to rabbit serum | 0·5–1·0 | 0·15–0·20 | 15 | 120–180 |
| *Octopus hongkongensis* | 4550–22500 | 7–10 | T-1824, 0·1–0·375 in sea water | 3–15 | 1·0–2 | 15–30 | 135–540 |
| | | | HgS, 2·3% in sea water | 10 | 1·0 | 30 | 450–495 |
| | | | Inulin, 4–5% in sea water | 8–100 | 1·0–2 | 15–30 | 360–540 |

A number of agents were tested as to their applicability in blood volume determinations. These included various dyes, haemoglobin, haemocyanin, mild silver proteinate, sucrose, and inulin. The most satisfactory material used was inulin as it did not appear to complex with any portion of the circulating fluid and was only slowly excreted. The material to be injected was prepared by diluting a stock aqueous solution with blood from animals of the same species or with a fluid of approximately the same osmotic concentration as the circulating fluid. At the beginning of the experiment enough normal blood was withdrawn for preparation of blanks and standards. The volume of test solution injected approximately replaced the amount of blood removed for this purpose. Samples of blood were taken at appropriate intervals over a period of time which preliminary experiments had shown to be sufficient for uniform distribution. Before taking each sample a quantity of blood larger than the dead space of the sampling tube was removed and subsequently re-injected. In Table 1 are summarized the specific details concerning the experimental procedure followed for each form investigated.

Standard analytical procedures were used in the determination of the concentra-

tion of the materials employed. For those analyses which required a protein-free filtrate, the proteins were precipitated by the Somogyi method (1930). The concentration of various dyes and other coloured materials were determined by comparison of the optical density of the experimental samples to those of standard solutions of known concentration which were prepared simultaneously. Inulin was analysed by the Harrison method (1942) and the anthrone method (Morris, 1948). For the determination of inulin in samples from animals with a relatively high blood sugar, a yeast digestion was employed with the Harrison method and a NaOH digestion (Little, 1949) with the anthrone method to remove the interfering sugar. The instruments used in different laboratories included the Beckman DU spectrophotometer, the Klett–Summerson photoelectric colorimeter and the Cenco Photelometer.

For the calculation of blood volume the general procedure was to plot the optical density of the samples against time. Since the concentration of the injected material decreases exponentially with time, semi-logarithmic graph paper was used to obtain a linear relationship. The line obtained was extrapolated back to zero and the concentration in grams per millilitre equivalent to the optical density at zero time was determined from a standard curve. The volume of fluid into which the injection had been made originally was obtained by dividing the weight of the injected material by the concentration at zero time. This volume of fluid was then expressed as percentage of the wet weight of the animal without shell.

Blood volumes, cell water volumes and total water volumes are expressed as 'mean ± standard deviation %' of the wet weight of the animal or tissue.

## ANIMALS AND SPECIAL METHODS

### A. *Placophora*

The volume of circulating fluid was determined in the large chiton, *Cryptochiton stelleri*. The animals which occur in abundance along the shores of Puget Sound were collected at approximately 3-month intervals. They were maintained at 8–10° C. in 150 gal. aquaria through which filtered sea water was recirculated at a rate of approximately 1 l. per minute per aquarium. A description of the sea-water system in which these animals were kept may be found in Thompson (1935). Under these conditions the animals survived in healthy condition for as long as 2 years.

In preparation for an experiment each animal was removed from the sea water, blotted dry and weighed. In this form, blood samples were taken through a plastic catheter placed in the haemocoele. For insertion of the catheter, a needle of smaller diameter was forced through the body wall in a region below the middle shell plates. The plastic catheter stiffened with an internal wire was then pushed through the opening, the internal wire was withdrawn and the tube plugged to prevent bleeding. This technique resulted in a leak-proof opening into the haemocoele through which fluid could be injected or withdrawn. Blood samples were often expressed from the catheter under pressure, not from action of the heart but from contraction of the body wall. Occasionally blood contaminated with eggs was obtained; such an animal was omitted from the report. Many specimens were dried to constant weight at 105° C. and the total body water calculated after correcting for the shell weight.

## B. *Gastropoda*

### (1) *Opisthobranchia–Tectibranchiata*

The sea hare, *Aplysia californicus*, was collected at low tide at about 2-week intervals on the Southern California coast in the vicinity of Corona del Mar. They were maintained on a diet of algae and kept in a running sea-water aquarium until needed.

At the beginning of the experiments the animals were wiped dry, weighed, and then a multiply perforated plastic catheter was inserted through an opening made in the body wall. The tube was tied in place with a ligature which caught up a small portion of the tissue. Since this animal is able to contract the body so as to close off a surprising length of tubing it was not until catheters of half body length were inserted that successful sampling became routine. Since there was a loss in weight due to the release of fluid from glands in the handling procedure and from the escape of blood during cannulation of the animal, another weight determination was made before the injection of the experimental solution. The weight determined at this time, corrected for the contents of the gut, was used subsequently in calculation of the blood volume. The injections were made into the ctenidial vessel, and samples were taken from the catheter in the body wall.

At the end of the experimental period the body wall was cut and the volume of blood which could be drained from the animal was determined. The contents of the digestive tract were removed and weighed and the animals dried to constant weight at 105° C.

### (2) *Opisthobranchia–Nudibranchiata*

The sea lemon, *Archidoris* sp., was the animal used as a representative of the nudibranch molluscs. Specimens were collected intertidally along the shores of San Juan Island and were maintained in the all-glass sea-water system of the Friday Harbor laboratories.

The technique used for cannulation was the same as that described for *Aplysia*. However, in this form injections were made and samples subsequently withdrawn from the catheter in the haemocoele. Consequently, before the experimental samples were taken, the blood of the catheter was thoroughly mixed with the blood of the haemocoele by flushing it in and out several times. The amazingly effective reflexes of the animal sometimes resulted in contractions of the body wall which succeeded in shutting off all the apertures in the cannula. However, the animal would relax eventually and samples could then be taken successfully. At the end of the experiment the animals were bled and dried to constant weight in an oven at 105° C.

### (3) *Pulmonata*

The giant African snail, *Achatina fulica*, was collected in the vicinity of Kaneohe Bay on the island of Oahu, Territory of Hawaii. The animals were kept in glass terraria provided with a sod bottom and given water and fruit *ad lib.*

The animals used in these determinations were anaesthetized with ether vapour in a closed container, the process taking about ½ hr. When the animals were sufficiently relaxed two stitches with linen thread were taken close together through the body wall slightly posterior to the genital aperture. Between the stitches a small opening was made and the soft plastic cannula inserted through the opening into the haemocoele. The cannula was tied securely in place to prevent leakage of blood. In about 30 min. the animals appeared to have recovered from the anaesthesia and began to crawl about in the customary fashion. At this time the injection was made into the haemocoele, and then at subsequent intervals of time blood samples were removed from the same cannula. At the end of an experiment

the shell, and any eggs that might be present were removed, the free blood was drained off, and the remainder of the tissue dried to constant weight.

The slug, *Arion ater*, was collected for the most part on the campus of the University of Washington, in Seattle, and at Friday Harbor. The individuals were maintained in terraria with an ample supply of water, lettuce and oatmeal.

Collection of blood and injection of materials were made through a plastic catheter which was inserted through the lateral body wall in the middle region of the animal. In this form it was not necessary to tie the catheter in place. If the animals were cooled to about 8° C. before the insertion of the catheter, the production of mucus and general body movements were decreased, making the operation easier.

In the early experiments, the dry weights of the animals were not determined. However, they were obtained for later experiments by the drying of the specimens to constant weight in an oven at 105° C.

### C. *Pelecypoda*

#### *Filibranchiata*

The mussel, *Mytilus californianus*, was collected intertidally along the breakwater south of the entrance to Gray's Harbor, Washington. Specimens were kept in the Seattle sea-water system described earlier.

Preparation for the experiments was made without anaesthesia. After scraping away part of the hinge ligament, always working between known positions of the muscles, it was possible to break away small pieces of shell with a surgical rongeur or bone forceps. The larger part of the heart and most of the aorta were exposed by this method. It was necessary to remove the shell carefully to prevent damage to the body wall or muscles. The animal was weighed and then placed in a container of sea water of such depth as to leave only the exposed area free for injection and sampling. Operated animals which were not used for experimentation survived well in the laboratory and frequently would begin to deposit shell.

After such preparation a sharp 1 in. hypodermic needle of 26 or 27 gauge was introduced directly into the anterior aorta, and anchored in place with a piece of modelling clay resting on the shell of the animal. The test solution, coloured with T-1824, was injected into the aorta and the preparation carefully examined for leaks. During the course of the experiment, blood samples were withdrawn at appropriate intervals of time. At the completion of the experiment the animal was weighed and the volume of blood drained from multiple cuts in the region of the heart determined. The animal was removed from the shell and the weight of the shell recorded as well as the wet weight of the tissue. The dry weight of the tissue was determined after drying at 105° C.

Specimens of *Margaritana margaritifera* were obtained from the shallow waters of the Sammamish river in King County, Washington, about halfway between the origin of the river in Lake Sammamish and its termination in Lake Washington. They were kept in about 4 in. of water in a cement aquarium table through which Seattle tap water circulated at a slow rate. This water is chlorinated from time to time and does not contain much plankton. Experimental animals were not kept longer than a month before use, though some left in this aquarium survived for several months.

In preparation for an experiment a triangular section of a valve in the area of the heart and between the attachments of the adductor muscles was prised out after circumscribing the area with hacksaw cuts. The opening was extended if necessary by chipping away additional pieces of the shell. The animal was weighed and returned to a container through which fresh tap water circulated.

Since it is difficult to see the aorta in this form, a sharp 26 or 27 gauge hypodermic needle

was thrust through the pericardial sac into the lumen of the ventricle and supported firmly by a clamp. Into the end of the needle was inserted an adapter of small internal diameter to which was connected a 3 in. length of plastic tubing (1 mm. I.D.). Injections were made and samples were taken from the end of the plastic tubing. The use of this sampling device was advantageous, since it had a very small dead space and eliminated any movements during sampling which might destroy the fragile preparation. At the close of the sampling period weight and volume determinations were made similar to those for *Mytilus*.

### D. *Cephalopoda*

Specimens of the large octopus in Puget Sound, probably *Octopus hongkongensis* Hoyle (Pickford, personal communication), were taken from their dens at low tide or captured on the rocks in shallow water. They were kept in large aquaria either at the Friday Harbor Laboratories of the University of Washington, or in the sea-water system at Seattle which has already been described. In most instances the animals ate the crabs or fish offered to them and could be kept indefinitely. In a few cases the animals would not accept food during the week or two which preceded their use in the experimental work.

Surgical provision was made for obtaining blood samples, usually the evening before the determination. The octopus was anaesthetized in about 30 gal. of aerated sea water by the gradual addition of 95% ethyl alcohol to a final concentration of about 2·5%. In 15–30 min. relaxation of the animal was obtained, at which time one of the branchial hearts could be drawn out of the mantle cavity without damage to the animal. An opening was made through the wall of the branchial heart and a soft rubber catheter passed through the lumen of the heart and into the lateral vena cava. It was secured with a ligature which enclosed a complete ring of tissue. The external end of the tube was closed with a solid glass stopper, the animal weighed and returned to normal sea water. In a short time the normal respiratory movements were regained, as well as muscular control. To prevent the animal from disturbing the tube, it became the practice to tie the tentacles in a net bag immediately after recovery from anaesthesia.

The next day, in preparation for an experiment, a small bore plastic tube long enough to hang over the edge of the aquarium was connected to the rubber catheter and the net bag was removed from the animal. Solutions were injected and blood samples were taken through the same tube which was adequately rinsed both for complete injection and to insure the taking of representative blood samples.

## RESULTS

### A. *Placophora*

Early experiments on *Cryptochiton* showed that a linear relationship between time and the logarithm of the concentration of the injected material was not obtained until 1–2 hr. had elapsed. The period of time necessary for this evidence of uniform distribution will be referred to subsequently as the equilibration time. In order not to remove too much of the injected materials before mixing had occurred, the first sample was taken after approximately an hour had elapsed. In Fig. 1 are given data from a typical experiment on the species. The long periods required for distribution of the injected material probably indicate a sluggish circulation in this animal. Although samples were taken for a long period, the possibility exists that not all the injection fluid entered regions through which free circulation occurred. It is thought that this might account for some of the variability in the results obtained. Since the

weight of the valves which make up the shell, and the total body water, were not determined for each experimental animal, these quantities were determined for a series of five normal animals. The valves averaged 5·2% of the total wet weight, and the total body water averaged 85·1 ± 2·2% of the wet tissue weight.

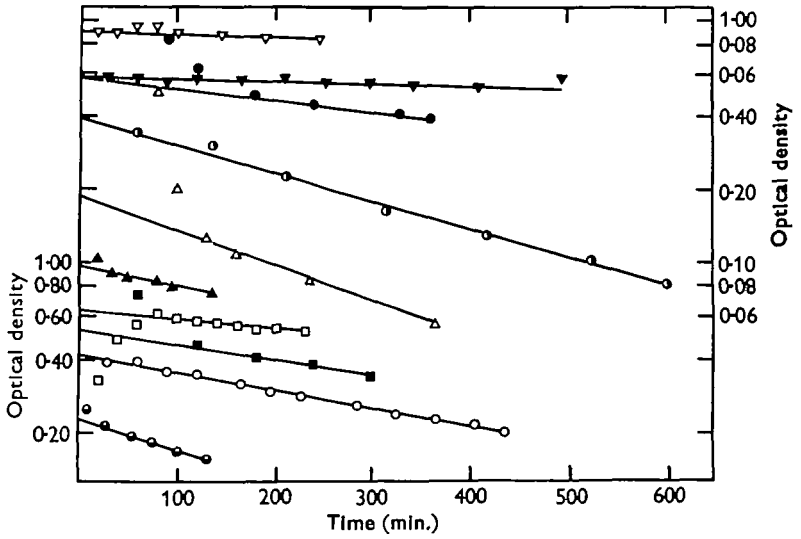


Fig. 1. Typical dilution curves following the injection of inulin into the circulatory system of the following animals: Group A. Use scale on right ordinate. Group B. Use scale on left ordinate. A: ▽, *Aplysia californicus*; ▼, *Octopus hongkongensis* (HgS); ●, *Octopus hongkongensis* (inulin); ◑, *Arion ater*; △, *Archidoris* sp. B: ▲, *Achatina fulica*; □, *Mytilus californianus*; ■, *Cryptochiton stelleri*; ○, *Octopus hongkongensis* (T-1824); ◒, *Margaritana margaritifera*.

In Table 2 the blood volumes of fifteen animals are given. The average blood volume was 43·8 ± 8·6%. Also included in this table are results of blood-protein determinations and values for cellular water. The protein was analysed by a micro-Kjeldahl method (Ballentine & Gregg, 1947). The percentage cellular water was calculated in the following manner. Since there is an open circulatory system in *Cryptochiton*, the volume of cellular water was obtained by subtracting the blood water from the total volume of water in the animal. To the volume of cellular water was added the dry weight of the animal, corrected for the shell and the salt and protein content of the blood, if this dry weight was significantly large. This sum theoretically should be equivalent to the wet weight of the cells in the tissues. The volume of cellular water was then expressed as percentage of the wet weight of the cells. The cellular water for this animal was 76·6 ± 3·4%. A discussion of the cellular water of this and the other forms studied will be deferred until later in the paper.

Table 2. *Inulin volume, blood protein and cellular water of* Cryptochiton stelleri

| Wet wt. without shell (g.) | Blood vol. (ml.) | Blood vol. as % wet wt. without shell | Blood* water (ml.) | Blood protein g./100 ml. | Dry wt. without shell (g.) | Corrected† dry wt. (g.) | Cellular water as % wet tissue wt. |
|---|---|---|---|---|---|---|---|
| 421 | 250 | 59·4 | 246 | 0·97 | 62·6 | 52·7 | 68·1 |
| 475 | 230 | 48·5 | 224 | 2·47 | 70·8 | 58·2 | 75·5 |
| 484 | 266 | 54·9 | 259 | — | 72·1 | 58·8 | 72·2 |
| 495 | 204 | 41·2 | 199 | — | 73·8 | 63·6 | 77·8 |
| 578 | 185 | 32·0 | 180 | 2·55 | 86·2 | 75·9 | 80·4 |
| 649 | 292 | 45·0 | 283 | 2·94 | 96·6 | 79·3 | 77·3 |
| 666 | 367 | 55·1 | 354 | 3·58 | 99·3 | 75·2 | 74·0 |
| 670 | 222 | 33·1 | 217 | — | 99·7 | 88·6 | 79·9 |
| 712 | 308 | 43·2 | 300 | — | 106·0 | 90·6 | 77·1 |
| 726 | 381 | 52·5 | 372 | — | 108·2 | 89·2 | 73·3 |
| 753 | 273 | 36·3 | 266 | 2·25 | 112·2 | 97·8 | 79·3 |
| 753 | 254 | 33·7 | 247 | 2·19 | 113·7 | 100·5 | 79·6 |
| 763 | 352 | 46·2 | 346 | 0·92 | 113·8 | 100·0 | 75·1 |
| 871 | 333 | 38·3 | 325 | — | 129·8 | 113·2 | 78·7 |
| 877 | 329 | 37·5 | 318 | 3·03 | 129·2 | 109·4 | 79·7 |
| Mean | — | 43·8 | — | — | — | — | 76·6 |
| S.D. | — | 8·6 | — | — | — | — | 3·4 |

* Blood volume corrected for the salt and protein content of the blood. The volume occupied by 3 g. salt was assumed to be 1 ml. The partial specific volume of the protein was assumed to be 0·74 ml./g.
† Corrected for the salt and protein content of the blood. In those cases where the blood protein was not determined, was assumed to be 2·0 g./100 ml. The salt concentration was assumed to be 3·0 g./100 ml.

## B. *Gastropoda*

### (1) *Opisthobranchia*

(*a*) Aplysia californicus. The tectibranch, *A. californicus*, looks and feels like a bag of water. In specimens of a group of twenty-four animals, the mean fluid volume which could be drained off, expressed as percentage of body weight, was 60%, varying from 51 to 71%. It is, therefore, not surprising that analysis of the blood volume by dilution techniques yielded values higher than those of the other molluscs investigated.

A number of different agents were employed for the blood-volume determinations in *Aplysia* and the results obtained are presented in Table 3. Inulin gave a mean blood volume of $79{\cdot}3 \pm 3{\cdot}1\%$; haemoglobin gave $76{\cdot}2 \pm 4{\cdot}4\%$; silver proteinate gave $73{\cdot}1 \pm 6{\cdot}6\%$. In Fig. 1 there is plotted the result of a typical experiment with inulin.

The volume of fluid in which inulin was distributed was greater than that for the other agents. The variability of the data, however, prevents these differences from being highly significant. The difference of the means determined by inulin and by silver proteinate are at the borderline of significance with $P$ of 0·04. The differences between the means of inulin and haemoglobin, and haemoglobin and silver proteinate are not statistically significant. The larger molecules may not penetrate into all of the spaces in which inulin is distributed. The pericardial sac and the lumen of the kidney are the most likely areas, but tests of the inulin content of the fluids of these spaces were not included while the work was in progress, and the animals are not now available for further tests.

Table 3. *Inulin, silver proteinate and haemoglobin volumes, total body water and cellular water of* Aplysia californicus

| Animal wet wt. (g.) | Blood vol. (ml.) | Blood vol. as % wet wt. | Blood* water (ml.) | Dry wt. (g.) | Corrected† dry wt. (g.) | Body water as % wet wt. | Cellular water as % wet tissue wt. |
|---|---|---|---|---|---|---|---|
| | | | | Inulin | | | |
| 183·5 | 148 | 80·9 | 147 | 12·7 | 8·3 | 93·0 | 74·1 |
| 220·8 | 178 | 80·6 | 176 | 16·4 | 11·0 | 92·6 | 72·1 |
| 237·5 | 189 | 79·6 | 187 | 15·8 | 10·2 | 93·4 | 77·2 |
| 264·5 | 222 | 83·8 | 220 | 18·4 | 11·7 | 93·1 | 68·9 |
| 255·5 | 192 | 75·2 | 190 | 20·8 | 15·0 | 92·0 | 74·9 |
| 270·5 | 223 | 82·4 | 221 | 18·9 | 12·2 | 93·0 | 71·5 |
| 330·7 | 250 | 74·2 | 248 | 25·7 | 18·2 | 92·2 | 75·9 |
| 387·0 | 301 | 77·8 | 298 | 26·4 | 17·3 | 93·2 | 78·3 |
| Mean | — | 79·3 | — | — | — | — | 74·2 |
| S.D. | — | 3·1 | — | — | — | — | 3·0 |
| | | | | Silver proteinate | | | |
| 146·0 | 91 | 62·3 | 90 | 12·7 | 10·0 | 91·4 | 81·3 |
| 184·0 | 144 | 78·3 | 143 | 14·0 | 9·1 | 92·4 | 73·7 |
| 236·0 | 163 | 69·0 | 161 | 16·4 | 11·5 | 93·0 | 83·6 |
| 236·5 | 188 | 79·5 | 186 | 17·0 | 11·3 | 92·8 | 74·7 |
| 267·0 | 200 | 75·0 | 198 | 19·9 | 13·9 | 92·5 | 78·0 |
| 286·0 | 174 | 62·0 | 172 | 23·4 | 17·8 | 91·7 | 82·1 |
| 308·8 | 232 | 75·1 | 230 | 27·8 | 20·8 | 91·0 | 71·1 |
| 330·7 | 256 | 76·0 | 253 | 25·6 | 18·0 | 92·3 | 74·3 |
| 333·7 | 269 | 80·6 | 266 | 24·1 | 16·1 | 92·8 | 73·0 |
| Mean | — | 73·1 | — | — | — | — | 76·9 |
| S.D. | — | 6·6 | — | — | — | — | 3·3 |
| | | | | Haemoglobin | | | |
| 260·0 | 188 | 72·1 | 186 | 18·7 | 13·1 | 89·0 | 80·8 |
| 270·5 | 189 | 70·0 | 187 | 18·9 | 13·2 | 93·0 | 83·6 |
| 280·0 | 220 | 78·5 | — | — | — | — | — |
| 290·6 | 222 | 76·5 | 220 | 21·1 | 14·5 | 92·7 | 77·4 |
| 349·0 | 255 | 73·0 | — | — | — | — | — |
| 554·5 | 285 | 80·4 | 282 | 25·0 | 16·5 | 92·9 | 74·3 |
| 411·0 | 341 | 83·0 | — | — | — | — | — |
| Mean | — | 76·2 | — | — | — | 92·4 | 79·1 |
| S.D. | — | 4·4 | — | — | — | 1·0 | 3·5 |

* Corrected for the volume of the salt only.
† Corrected for the weight of the salt only.

Since *Aplysia* has a shell of negligible size it was relatively easy to obtain other data on each specimen of this mollusc. The mean of the total water content of the twenty-one animals was $92{\cdot}4 \pm 1{\cdot}0\%$. The mean of the percentage cellular water differed for inulin, silver proteinate and haemoglobin, the values being $74{\cdot}2 \pm 3{\cdot}0\%$, $76{\cdot}9 \pm 3{\cdot}3\%$, $79{\cdot}1 \pm 3{\cdot}5\%$, respectively

(*b*) Archidoris. The nudibranch *Archidoris* appears to have a more solid structure than *Aplysia*. This may be due in part to the nature of the integument, or perhaps to the body fluids being under greater hydrostatic pressure, for the percentage of total body water in *Archidoris*, $92{\cdot}4 \pm 1{\cdot}3\%$, is the same as that for *Aplysia*.

Table 4. *Inulin volume, total body water and cellular water of* Archidoris *sp.*

| Animal wet wt. (g.) | Blood vol. (ml.) | Blood vol. as % wet wt. | Blood* water (ml.) | Dry wt. (g.) | Corrected† dry wt. (g.) | Body water as % wet wt. | Cellular water as % wet tissue wt. |
|---|---|---|---|---|---|---|---|
| 50·6 | 28·6 | 56·4 | 28·3 | 4·22 | 3·36 | 91·7 | 84·3 |
| 51·2 | 32·3 | 63·1 | 32·0 | 3·44 | 2·47 | 93·1 | 86·5 |
| 53·1 | 30·3 | 57·1 | 30·0 | 3·43 | 2·52 | 93·5 | 88·7 |
| 54·6 | 38·4 | 70·3 | 38·0 | 5·03 | 3·88 | 90·8 | 74·9 |
| 57·8 | 42·5 | 73·6 | 42·1 | 3·85 | 2·58 | 93·2 | 82·1 |
| 62·1 | 44·8 | 72·2 | 44·4 | 4·50 | 3·15 | 92·0 | 80·7 |
| Mean | — | 65·5 | — | — | — | 92·4 | 82·9 |
| S.D. | — | 7·0 | — | — | — | 1·3 | 4·4 |

* Corrected for the volume occupied by the salt only.
† Corrected for the weight of the salt only.

By simple drainage a volume of blood approximately one-fourth the body weight could be obtained readily. This is much less than that from *Aplysia*, but more than that from other molluscs. Consequently, the relatively high percentage blood volume of $65{\cdot}5 \pm 7{\cdot}0\%$, was to be expected. The experimental data for this animal are presented in Table 4, and a typical experimental curve is given in Fig. 1.

The cellular water, $82{\cdot}9 \pm 4{\cdot}4\%$, is a higher value than was obtained for other forms reported, but may only represent a sampling error as the number of animals reported on is small.

(2) *Pulmonata*

(*a*) Achatina fulica. The giant African snail, *A. fulica*, appears to have an effective circulation since the equilibrium time was relatively short. The results of a typical experiment are given in Fig. 1, and the values of body weight, blood volume, etc., are given in Table 5. The blood volumes for eight animals was $40{\cdot}3 \pm 5{\cdot}6\%$, the body water $86{\cdot}4 \pm 3{\cdot}3\%$, and the cellular water $77{\cdot}1 \pm 5{\cdot}0\%$.

Although the animals used in the experiments were supplied with water, the possibility existed that the extent of hydration differed from animal to animal and

Table 5. *Inulin volume, total body water and cellular water of* Achatina fulica

| Wet wt. without shell (g) | Blood vol. (ml.) | Blood vol. as % wet wt. without shell | Dry wt. (g.) | Body water as % wet wt. without shell | Cellular water as % wet tissue wt. | Blood protein (g./100 ml.) |
|---|---|---|---|---|---|---|
| 41·0 | 15·8 | 38·6 | 6·90 | 83·2 | 72·6 | 0·60 |
| 44·5 | 15·8 | 35·5 | 7·10 | 84·0 | 75·2 | 1·63 |
| 45·1 | 23·5 | 52·2 | 4·80 | 89·5 | 77·8 | 0·55 |
| 45·8 | 18·5 | 40·4 | 8·00 | 82·4 | 70·8 | 0·15 |
| 62·3 | 28·6 | 45·8 | 6·95 | 89·0 | 79·4 | 0·36 |
| 69·8 | 24·6 | 35·2 | 10·05 | 85·7 | 77·7 | — |
| 71·8 | 25·0 | 34·8 | 5·50 | 92·4 | 88·2 | — |
| 73·8 | 28·6 | 39·8 | 11·45 | 84·6 | 74·8 | — |
| Mean | — | 40·3 | — | 86·4 | 77·1 | 0·45 |
| S.D. | — | 5·6 | — | 3·3 | 5·0 | — |

that this might account for some of the variability in the results. Animals which were kept in the laboratory with a restricted amount of water available lost about 10% of their weight the first day. However, the snails appeared to adjust to the desiccation and in some cases lost only about 20% of the original body weight after 9 days without water.

The range in total body water of the animals used in these experiments was about 10%. Nevertheless, the correlation coefficient, *r*, between percentage blood volume and percentage body water was only 0·26, a value which implies no direct relationship in the range tested.

The amount of protein in the blood of five animals was determined by a micro-Kjeldahl method and a mean of 0·46 g. of protein in 100 ml. of blood was obtained. The individual results are given in Table 5.

(*b*) Arion ater. A slug may lose as much as one-fifth of its initial wet weight during a 3–4 hr period. Therefore, care was taken during an experiment to keep the environment saturated with water and to minimize the amount of handling. To detect any changes in weight, the animals were weighed at the beginning and end of an experiment, and only the results for those animals which showed negligible weight losses (less than 5% of body weight) are reported in Table 6.

Table 6. *Inulin volume, total body water and cellular water of* Arion ater

| Animal wet wt. (g.) | Blood vol. (ml.) | Blood vol. as % wet wt. | Dry wt. (g.) | Body water as % wet wt. | Cellular water as % wet tissue wt. |
|---|---|---|---|---|---|
| 10·00 | 3·45 | 34·5 | — | — | — |
| 11·57 | 2·88 | 24·9 | — | — | — |
| 11·57 | 3·95 | 34·2 | — | — | — |
| 11·70 | 5·00 | 43·6 | — | — | — |
| 11·80 | 5·88 | 50·2 | — | — | — |
| 13·30 | 6·33 | 47·5 | — | — | — |
| 14·05 | 3·37 | 24·0 | — | — | — |
| 12·20 | 7·02 | 57·5 | 1·00 | 91·8 | 80·7 |
| 13·72 | 4·71 | 34·3 | 1·64 | 87·0 | 81·8 |
| 21·95 | 6·46 | 29·4 | 2·64 | 88·0 | 83·0 |
| 22·83 | 8·00 | 35·0 | 3·52 | 84·5 | 76·3 |
| 43·72 | 10·88 | 24·9 | 8·55 | 80·3 | 74·0 |
| Mean | — | 36·6 | — | 86·3 | 79·0 |
| S.D. | — | 10·4 | — | 3·8 | 3·4 |

There was a considerable amount of variability in the blood volumes obtained in the early experiments, the values ranging from 24·0 to 50·2 %. In order to ascertain whether this variability was attributable to the degree of hydration of the animal, dry-weight determinations were made in subsequent experiments. The coefficient of correlation, *r*, between blood volume and body water was found to be high at a value of 0·81 for the later experiments. However, due to the small number of experiments, this *r* is not reliable and the question is not settled.

The mean blood volume for all the animals reported was 36·6 ± 10·4%, the mean total body water was 86·3 ± 3·8%, and the cellular water 79·0 ± 3·4%. The variability in the results may not be extraordinary as the animal is terrestrial and does not possess a protective shell.

## C. *Pelecypoda*

*Filibranchiata*

(*a*) Mytilus californianus. In *M. californianus* after the injection of the test material into the anterior aorta equilibrium was reached in a relatively short time. Data from a typical experiment are presented in Fig. 1, and the results of the blood-volume determinations, as well as other pertinent data, are given in Table 7. The average blood volume is $50{\cdot}8 \pm 7{\cdot}6\%$, the total body water is $88{\cdot}9 \pm 0{\cdot}36\%$, and the cell water is $79{\cdot}7 \pm 3{\cdot}6\%$.

Table 7. *Inulin volume, total body water and cellular water of* Mytilus californianus

Series A

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|
| Measured, wet wt. without shell (g.) | Measured blood vol. (ml.) | (2/1 × 100) blood vol. as % wet wt. | Measured dry wt. (g.) | (1 × mean of 13), dry wt. calculated from data of Series B (g.) | (1 × mean of 15), dry wt. corrected for blood salt (g.) | (2 − 1 % of 2), blood water* (ml.) | (1 − [5 + 7]), cellular water (ml.) | (8 + 6), wet cell wt. (g.) | (8/9 × 100), cellular water as % wet cell wt. |
| 38·8 | 21·0 | 53·8 | 6·7 | 4·27 | 3·68 | 20·8 | 13·73 | 17·41 | 78·26 |
| 39·8 | 24·6 | 61·6 | 4·9 | 4·38 | 3·78 | 24·4 | 11·02 | 14·80 | 74·46 |
| 48·0 | 27·3 | 56·7 | 8·6 | 5·28 | 4·55 | 27·0 | 15·72 | 20·27 | 77·55 |
| 58·3 | 37·6 | 64·5 | 4·4 | 6·41 | 5·55 | 37·2 | 14·69 | 20·24 | 72·57 |
| 74·6 | 30·8 | 41·3 | 12·3 | 8·21 | 7·10 | 30·5 | 35·89 | 42·99 | 83·48 |
| 78·3 | 34·8 | 44·4 | 14·8 | 8·61 | 7·45 | 34·5 | 35·19 | 42·64 | 82·52 |
| 84·4 | 39·2 | 46·4 | 11·9 | 9·28 | 8·01 | 38·8 | 36·32 | 44·33 | 81·93 |
| 95·6 | 42·0 | 44·0 | 21·9 | 10·52 | 9·09 | 41·6 | 43·48 | 52·57 | 82·70 |
| 116·0 | 53·2 | 45·8 | 18·0 | 12·78 | 11·02 | 52·7 | 50·52 | 61·57 | 82·05 |
| 122·4 | 60·6 | 49·5 | 14·6 | 13·46 | 11·63 | 60·0 | 48·94 | 60·57 | 80·80 |
| Mean | — | 50·8 | — | — | — | — | — | — | 79·7 |
| S.D. | — | 7·6 | — | — | — | — | — | — | 3·56 |

Series B

| 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|
| Measured wet wt. without shell (g.) | Measured dry wt. (g.) | (12/11 × 100), dry wt. as % wet wt. | (12 − 0·03[11/2]), dry wt. without blood salt (g.) | (14/11 × 100), corrected dry wt. as % wet wt. |
| 34·20 | 4·65 | 13·6 | 4·13 | 12·1 |
| 35·12 | 3·99 | 11·4 | 3·46 | 9·8 |
| 35·35 | 3·59 | 10·1 | 3·05 | 8·6 |
| 40·93 | 4·60 | 11·2 | 3·98 | 9·7 |
| 43·68 | 5·04 | 11·5 | 4·38 | 10·0 |
| 45·64 | 4·37 | 9·6 | 3·68 | 8·1 |
| 50·92 | 5·34 | 10·5 | 4·56 | 8·9 |
| 55·13 | 5·79 | 10·5 | 4·95 | 9·0 |
| Mean 42·61 | — | 11·0 | — | 9·5 |

* Blood water is derived from blood volume on the assumptions that the blood protein of *Mytilus* is negligible (less than 0·1 %), and that the volume occupied by 3 g. of salt is 1 ml.
Dry weight is derived from measured dry weight by subtracting the salt content, assumed to be 3 %, of the blood estimated from Series A to be about 50 % of the wet body weight.

It will be noted that two series of experiments are presented. The blood-volume determinations were performed on animals recently brought to the laboratory, and work was completed before a decision had been reached to compute cellular water using a corrected dry weight. No attention had been paid to clearing the gut, a factor which is thought to account for the variability of the dry-weight measurements. Since the blood-volume measurements were technically quite demanding a new series was not performed. Instead, animals which had been kept in the

laboratory for several months were dissected and dried. The corrected dry weights obtained from these animals were then applied to the data derived from the first series. Errors are undoubtedly introduced by this process and it must be kept in mind that the values set forth for cellular water are not more than estimates.

(*b*) Margaritana margaritifera. In *M. margaritifera* the equilibration time was as short as 15 min. Generally, however, 30 min. elapsed before a linear relationship was established. This rapid equilibration may have been the result of the direct injection of the test material into the heart which insures the most rapid distribution through the circulatory system.

Table 8. *Inulin and T-1824 volumes, total body water and cellular water of* Margaritana margaritifera

| Wet wt. without shell (g.) | Blood vol. (ml.) | Blood vol. as % wet wt. without shell | Dry wt. (g.) | Body water as % wet wt. without shell | Cellular water as % wet tissue wt. |
|---|---|---|---|---|---|
| 31·4 (T-1824) | 16·6 | 52·8 | 4·3 | 86·3 | 71·0 |
| (Inulin | 16·2 | 52·6 | 4·3 | 86·3 | 71·6 |
| 32·5 (inulin) | 13·0 | 40·0 | 4·8 | 85·5 | 75·5 |
| 34·8 (inulin) | 13·9 | 40·0 | 4·1 | 88·2 | 80·3 |
| 36·6 (T-1824) | 19·0 | 51·9 | 4·7 | 87·2 | 73·3 |
| (Inulin) | 20·0 | 54·7 | 4·7 | 87·2 | 71·7 |
| 38·2 (inulin) | 16·6 | 43·4 | 4·9 | 87·2 | 77·3 |
| 38·4 (inulin) | 17·8 | 46·3 | 3·9 | 89·9 | 81·0 |
| 39·2 (T-1824) | 24·4 | 62·2 | — | — | — |
| (Inulin) | 19·6 | 50·0 | — | — | — |
| 44·0 (inulin) | 22·5 | 51·2 | 4·2 | 90·4 | 80·4 |
| 44·5 (inulin) | 18·5 | 41·6 | 5·7 | 87·1 | 78·1 |
| 48·6 (inulin) | 25·0 | 51·4 | 4·6 | 90·5 | 80·5 |
| Mean | — | 49·0 | — | 88·0 | 76·4 |
| S.D. | — | 6·0 | — | 1·5 | 3·8 |

The data obtained in a typical experiment are presented in Fig. 1, and the results for all the experiments reported are summarized in Table 8. The blood volume was found to be $49{\cdot}0 \pm 6{\cdot}0\%$. In three of the specimens, successful experiments were performed in which inulin and T-1824 bound to rabbit serum were injected simultaneously. Both substances appeared to measure the same space although they differ in chemical properties and the size of the molecule.

The volume of blood which could be drained from the visceral mass at the end of an experiment ranged from 30 to 50% of the volume determined by the dilution method. In a few experiments the concentration of inulin in the drained blood was determined in order to ascertain whether there was a significant contamination of the drained blood with water. In these cases the concentration of inulin in the drained blood was within the range of concentration of the experimental blood samples.

The total body water was relatively high, $88{\cdot}0 \pm 1{\cdot}5\%$, and the cellular water was $76{\cdot}4 \pm 3{\cdot}8\%$.

## D. *Cephalopoda*

*Octopoda*

(*a*) Octopus hongkongensis. Most of the measurements of the blood volume of the octopus were made with T-1824. It was possible to use this material since the octopus has a high blood protein, about 10 g. of protein per 100 ml. of blood, to which the dye was bound. To substantiate the T-1824 estimate of the size of this compartment, colloidal HgS was also used as a test material. The two substances appear to measure the same fluid compartment, but differ in the rate of loss from the circulating fluid, the HgS being lost at the slower rate. Representative curves of experiments with these two substances are given in Fig. 1. In Table 9 are summarized the data for the octopus, the blood volume being $5{\cdot}8 \pm 1{\cdot}0\%$. The percentage total water was determined for three animals and was found to be $82{\cdot}5\%$ of the body weight. Using this value, the cell water is approximately $77\%$ of the wet tissue weight.

Table 9. *Inulin volume, T-1824 volume and* HgS *volume of* Octopus hongkongensis

| Animal wet wt. (g.) | Test material | Blood vol. (ml.) | Blood vol. as % wet wt. | Extracellular vol. (ml.) | Extracellular vol. as % wet wt. | Extracellular equilibration time (min.) |
|---|---|---|---|---|---|---|
| 7,730 | T-1824 | 588 | 7·6 | — | — | — |
| 4,550 | T-1824 | 217 | 4·8 | — | — | — |
| 10,430 | T-1824 | 624 | 6·0 | — | — | — |
| 6,360 | T-1824 | 374 | 5·9 | — | — | — |
| 12,955 | T-1824 | 759 | 5·9 | — | — | — |
| 22,500 | T-1824, raffinose | 1272 | 5·6 | 5460 | 24·3 | 165 |
| 8,640* | T-1824, inulin | 492 | 5·7 | 1612 | 18·7 | 210 |
| 8,750 | T-1824, inulin | 492 | 5·6 | 1776 | 20·3 | 225 |
| 14,900 | T-1824, inulin | 884 | 5·9 | 4550 | 30·5 | 180 |
| 8,520 | T-1824, inulin | 649 | 7·6 | 3450 | 40·4 | 85 |
| 8,640* | HgS | 374 | 4·3 | — | — | — |
| 14,780 | HgS | 943 | 6·4 | — | — | — |
| 8,175* | HgS, inulin | 375 | 4·6 | 1835 | 22·5 | 125 |
| 19,550 | Inulin | — | — | 5970 | 30·6 | 195 |
| 4,890 | Inulin | — | — | 1775 | 36·5 | 120 |
| Mean | — | — | 5·8 | — | 28·0 | — |
| S.D. | — | — | 1·0 | — | 7·3 | — |

* Same animal.

In one experimental animal it was possible to make several measurements. In the first experiment, T-1824 as used with a resultant blood volume of $5{\cdot}7\%$. Three days later, a repeat determination was made with colloidal HgS, and a blood volume of $4{\cdot}3\%$ was obtained. A month later, another measurement was made with HgS with a value of $4{\cdot}6\%$ being obtained. The animal seemed perfectly normal, the only change noted was a loss of weight from 8640 to 8175 g., although the animal had continued to eat regularly.

For comparison with the volumes obtained with T-1824 and HgS, the volume of

fluid in which inulin was distributed was measured. In some cases the inulin was administered simultaneously with the other agents, and at other times independently. The average value obtained for the inulin space was $28{\cdot}0 \pm 7{\cdot}3\%$. The experimental data are summarized in Table 9 and a typical experiment is shown in Fig. 1.

There has been anatomical evidence for a closed circulatory system in cephalopods, but experimental verification has been lacking. From the results that have been obtained, it is believed that inulin measures the total extracellular fluid, while T-1824 and HgS measure the blood volume. Since the inulin space was 28·0% and the blood volume 5·8%, the tissue fluid compartment of the octopus is approximately 22% of the wet tissue weight.

The coefficient of correlation, $r$, between the time necessary for equilibrium and the size of the extracellular space was $-0{\cdot}71$. In testing the significance of this value a $P$ of 0·05 was obtained even though the number of samples was small. Consequently, it appears likely that an inverse relationship exists between the two values.

## DISCUSSION

The results of the determinations of blood volume in representative molluscs show a wide range of values within the phylum from 5·8% in *Octopus* to about 75% in *Aplysia*. Even if the total extracellular fluid of *Octopus*, about 28%, is considered to be the better figure for comparison with the representatives of the other classes, the range is still a very wide one and illustrates great diversity within the phylum.

The low blood volume of 5·8% reported for *Octopus* is obviously a different order of magnitude from any other of the forms studied. Only in this species was there a great difference in the volumes of fluid in which materials of different molecular size were distributed. The dye T-1824, which complexes with the protein of the blood and colloidal HgS (a material of colloidal particle size), appeared to measure the blood volume, while inulin distributed in a considerably larger space, probably the extracellular fluid. Since on a percentage basis the volume of the extra-cellular fluid of the *Octopus* is lower than the blood volumes of the other forms, it follows that a closed circulatory system containing a high concentration of respiratory pigment separated from a tissue fluid compartment may meet greater metabolic demands than an open circulatory system with a larger total volume of fluid.

Robertson (1953) reported a value of 33% for the extracellular fluid of the cephalopod *Sepia*, but with the reservation that this might be an overestimate due to the possible entrance of the test material, sucrose, into muscle cells. It was noted in the course of the work reported here that there was an inverse relationship between the size of the extracellular space measured by inulin and the time required for equilibration. This observation would appear to indicate differences in permeability of tissues from animal to animal, so that a high permeability and a rapid attainment of equilibrium would also permit the entrance of inulin into other spaces, and a higher apparent extracellular fluid volume. A part of the variability of the results with this substance may rest on these unexplained differences in permeability. Penetration of inulin into other spaces will be discussed later in this paper.

The groups which show total blood volumes next larger in size to those of the

cephalopods are the pulmonate gastropods. Since both *Achatina* and *Arion* have open circulatory systems and are terrestrial, the differences expected between their average blood volumes, 40·3 and 36·6%, respectively, would not be very great. When the *t* test was applied to the data for these forms, it was found that the difference was not large enough to be statistically significant given the variability encountered in the measurements.

The mean blood volume of 44% obtained for the most primitive of the animals included in this study, *Cryptochiton*, is somewhat larger than those of the pulmonates. This value is statistically significantly different from that of *Arion*, but not from that of *Achatina*.

In order of increasing blood volume, the next species encountered are in the class Pelecypoda. *Mytilus*, with a blood volume of 51%, and *Margaritana* at 49%, show a striking similarity in spite of one being a marine and the other a fresh-water animal. Since the animals are similar in size, shape and morphology, perhaps this should be expected. The difference between the means of these two forms is not statistically reliable, but the blood volumes of these animals are significantly different from all the others reported here.

The results obtained with *Mytilus* and *Margaritana* confirm the findings of Potts (1954) who determined the inulin space of the fresh-water pelecypod *Anodonta cygnea* to be 55% of the wet weight without shell. In view of this similarity in blood volume of one marine and two fresh-water pelecypods, and the general agreement of these results with those on the other molluscan forms, it appears likely that the value of 8% of the wet tissue weight reported for two other fresh-water pelecypods by Prosser & Weinstein (1950) may be too low. Low values might be obtained under a number of different circumstances. Because they injected through a hole in the shell, without visual confirmation that the tip of the needle lay within the heart, it is possible that the material was injected into some fluid other than the circulating blood. On the other hand, we have noted that too rapid an injection of test material into the circulatory system resulted in impairment of heart action. It is possible that in their experiments the injected material entered the blood, but was mixed with a relatively small part of the total volume of the circulatory system in the 30 min. of the experiment. As confirmatory evidence for the low value Prosser & Weinstein (1950) cite the recovery from the heart at the conclusion of the experiment of fluid amounting to 96% of the volume measured by T-1824. In none of the species studied in our work was it possible to recover quantities of this order of magnitude, and it is our opinion that such a recovery is rather an indication of error than a satisfactory accounting for the volume of blood of the animal.

The largest blood volumes were found in the representatives of the opisthobranchs, *Archidoris* having a mean value of 65% and *Aplysia* one of approximately 75%. The difference between the means is statistically significant and the blood volumes of these animals are reliably different from those of all the other animals investigated. These blood volumes are so large that it must be inferred that the blood is meeting not only the ordinary demands upon a circulatory system, but some other requirement of these animals. The idea that water may serve as a

structural element goes back at least to the arguments for the gonocoel theory advanced by Hatshek (Goodrich, 1945). The representatives of the group studied in this work demonstrated a very definite control over the distribution of the blood in the body. It will be recalled that until a multiply perforated tube was inserted in the haemocoel for more than half the body length every opening in it could be closed off by contraction of the animal's body wall. The animal demonstrated fine control of its body form in other respects. A large *Aplysia* depleted of much of its supply of blood soon took on a normal form and could be recognized by inspection as having been a larger animal only by the relatively large size of the ctenidium. It may also be pointed out that this group feeds on marine plants which are relatively voluminous for the amount of dry digestible nutrients. On this poor diet, a large body size would be perhaps of competitive value, and a large volume of water would represent a cheap way of attaining size without increasing the metabolic requirements exorbitantly.

The differences in blood volume obtained with various substances in *Aplysia* may serve to illustrate the statement of Potts (1954) that it is likely that inulin determines not only the circulating blood volume, but also the pericardial and renal coelomic space in *Anodonta*. Measurements with silver proteinate gave 73%, with inulin, 79%—a difference which amounts to 6% of the wet body weight. This might be due to failure of the silver proteinate to penetrate into these spaces. The relative size of this error would then vary from species to species, depending upon the size of these spaces compared to the blood volume.

Further evidence for the penetration of inulin into other spaces is found in the experiments on excretion in the *Octopus* (Harrison, 1954) and in *Achatina fulica* (Martin, Harrison & Stewart, 1953). In these forms it is known that inulin penetrates the renal space, and appears after a lag period in the urine in approximately the same concentration as that found in the blood. Robinson & McCance (1952) have stated well the difficulties in using single injections of inulin in mammals when excretion is rapid. In contrast, the excretion by molluscs is very slow. Loss of inulin by this route may be compensated by the extrapolation of the dilution curve back to the time of injection. However, the entrance of inulin into the pericardial and renal spaces may account in part for the early, non-linear aspect of the dilution curve. In view of all the evidence at hand it may be well to regard all the volumes measured with inulin as somewhat greater than the true blood volume.

In addition to the differences in blood volume from species to species, there was also a considerable variation in the amount of time necessary for equilibration. This is probably due to a number of factors, including the region of injection, the effectiveness of the circulatory system, and the amount of movement of the body. In an inactive form such as *Cryptochiton*, the injection had to be made into the haemocoel, and slow equilibration might be expected. In contrast, when the injection was made directly into the heart and the animal showed considerable bodily activity, as in *Margaritana*, the equilibration would be rapid. Slow equilibration made necessary a longer extrapolation with a consequent multiplication of any errors due to differences in permeability.

Another aspect of the data is yet to be considered. The total body water ranged in value from 82·5 to 92·4%. The *t* test was used to determine the significance of the differences in the mean total water contents. The mean body water of *Aplysia* and *Archidoris*, both 92·4%, is reliably different from all of the others. The difference between *Mytilus* at 84·6% and *Octopus* at 82·5%, is significant, while that between *Mytilus* and *Margaritana*, 88%, is probably significant—$P = 0{\cdot}02$. None of the remaining comparisons was reliably different.

The parameter to be considered in conclusion is the cellular water. For each species a computation of the percentage of cellular water has been made and the data presented in the tables for individual animals. This computation can be obtained readily, since for most molluscs there is an open circulatory system and the shell, if present, is easily freed from the body tissues.

The range of values of cellular water is from 76·4 to 82·9% and differences between the values are hardly significant. Possible sources of error have been pointed out to which it should be added that no correction was applied to the data for the connective tissue of the animals. Even though these values should be considered only as approximations, it is of interest to compare them with some of those reported in the literature. The water content of the tissues of molluscs is not well known. Balland's (1898) values are those generally cited, but this author made no effort to distinguish extracellular from intracellular water, and other reports on molluscan tissue suffer from the same difficulty. On the other hand, Ephrussi (1933), working with echinoderm eggs and larvae, corrected for extracellular water in his determinations. In 40 hr. larvae of *Paracentrotus lividus* he found the cellular water amounted to 78·8% of the total wet weight. In the vertebrate group of animals it is only in recent years that the results of computations of the water content of mammalian cells have been in general agreement from laboratory to laboratory. Chanutin & Ludewig (1939) calculated the intracellular water of rat muscle fibres to be 73·2%. Lowry & Hastings (1942) are in essential agreement, their value being 74%. These figures are further confirmed by a 74·2% value for dog muscles (Muntwyler, Mellors, Mautz & Mangun, 1940) and of 75·1% for cat muscle which can be calculated from the data of Crismon, Crismon, Calabresi & Darrow (1943). But most of these values rest on the debatable assumption that there is no chloride in the cells and so the values may also be considered to be estimates. Computations for certain other mammalian tissues are available, but since these computations only demonstrate the expected variation from mammalian tissue to tissue, they will not be cited here. The water content of the various living cells reported here is, as might be expected, quite comparable with the others.

One of the objectives of this study was to examine the normal variability in blood volume in representatives of this phylum of animals. A number of factors have been discussed which may be sources of error in the results. However, within one species, these errors should be reasonably consistent, and the range of variability of blood volume is thought not to be due entirely to technical faults, but as reflecting the inability of these animals to regulate their blood volume with a high degree of stability.

SUMMARY

1. Individuals of *Cryptochiton stelleri*, as representatives of the class Placophora, distributed inulin in blood volumes yielding a mean of 43·8% of the wet body weight without shell. Mean cellular water was estimated to be 76%.

2. The two opisthobranch gastropods examined were found to have very large mean blood volumes. *Aplysia californicus* distributed haemoglobin in 76·2% and mild silver proteinate in 73·1% of the wet body weight. Inulin was distributed in a significantly larger space averaging 79·3% of the body weight, which probably included pericardial and renal spaces. *Archidoris* sp. distributed inulin in 65·4% of the wet body weight. Mean cellular waters were 74–79% in *Aplysia* and 83% in *Archidoris*.

3. Two pulmonate gastropods were studied with inulin which was distributed in a mean space 40·3% of the wet body weight without shell of *Achatina fulica*, and 36·6% of the wet body weight in *Arion ater*. The computed cellular waters were 77 and 79%, respectively.

4. Excellent agreement was shown between a marine pelecypod, *Mytilus californianus*, and a fresh-water pelecypod, *Margaritana margaritifera*, of similar body size and form. Inulin in the former was distributed in 50·8%, and in the latter inulin and T-1824 in 49% of the wet body weight without shell. The cellular water contents were 80 and 76%, respectively.

5. In a single representative of the Cephalopoda—*Octopus hongkongensis*, it was possible to demonstrate with T-1824 and with HgS a blood volume averaging 5·8% of the wet body weight, constituting a fluid space distinctly different from the tissue fluid space. Inulin was distributed in the entire extracellular space amounting to 28% of the wet body weight. The cell water which was calculated from the mean values obtained was 77%.

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